STUDY OF MICROPARTICLE PREPARATION BY THE SOLVENT EVAPORATION METHOD USING FOCUSED BEAM REFLECTANCE MEASUREMENT (FBRM)

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To my beloved wife and my lovely children

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TABLE OF CONTENTS

1. INTRODUCTION

1.1. Controlled release drug delivery

Controlled release drug delivery systems are being developed to address many difficulties associated with traditional methods of administration. Controlled release drug delivery employs devices such as polymer-based disks, rods, pellets, or microparticles that encapsulate drug and release it at controlled rates for relatively long periods of time. Such systems offer several potential advantages over traditional methods of administration. First, drug release rates can be tailored to the needs of a specific application; for example, providing a constant rate of delivery or pulsatile release. Second, controlled release systems provide protection of drugs, especially proteins, that are rapidly destroyed by the body. Finally, controlled release systems can increase patient compliance.

While a variety of devices have been used for controlled release drug delivery. Polymeric microparticles are one of the most common types and hold several advantages. Microparticles can encapsulate many types of drugs including small molecules, vaccines, proteins, and nucleic acids (Azevedo et al., 2006; Feng et al., 2006; Little et al., 2005). Microparticles offer a method to deliver macromolecules by a variety of routes and effectively control the release of such drugs. In addition, many factors including the type of polymer, the polymer molecular weight, the copolymer composition, the nature of any excipients added to the microparticle formulation (e.g. for stabilization of the therapeutics), and the microparticle size can have a strong effect on the delivery rates.

Polymers have been used to control the drug release rate from the formulations. Polymers can bind the particles of a dosage form and also change the flow properties. Extensive applications of polymers in drug delivery have been increased because polymers offer unique properties which so far have not been attained by any other materials. Polymers are macromolecules having very large chains, contain a variety of functional groups, can be blended with other low and high molecular weight materials. Understanding the basic concepts of polymers needed for further understanding of drug products and designing of better delivery systems. Advances in polymer science have opened up possibilities for using a wide variety of polymeric materials as drug delivery systems (Leong and Langer, 1988; Wang et al., 2002).

The controlled release of theophylline (THEO) after a lag time was achieved with developed formulation for chronotherapeutic delivery using guar gum microspheres of theophylline prepared by emulsification technique. Coating of microspheres was performed using solvent evaporation method with pH sensitive Eudragit® polymers. The

chronotherapeutic based colon-targeted drug delivery system of theophylline (THEO) exploiting pH-enzyme sensitive property was prepared for the prevention of episodic attack of asthma in early morning. The pH dependent solubility behavior of Eudragit and gelling properties of guar gum are found to be responsible for delaying the release (Soni et al., 2011).

1.1.1. Polymer combination

1.1.1.1. Polymer combination for oral administration

Oral administration continues to be the most popular route for the delivery of all medicinal agents. The ease of delivery, lack of pain associated and cost effectiveness has made oral dosage forms (ODF) the most commonly used products for drug administration. Use of polymers as carriers in drug delivery has been well established. The development of drug delivery systems for newer drugs and design of new dosage forms for existing drugs poses newer challenges in terms of formulation needs and release profiles desired. Blends or combination of polymers are used to modulate drug release. The release profiles from polymer blends or combination depend on the interactions between the constituents at molecular levels, which govern the blend or combination morphology. The combination of polymer is more effective as compared to single polymer.

Mennini et al. (2012) designed the combined polymer system comprised of ternary complexation with hydroxypropyl-β-cyclodextrin and PVP (polyvinylpyrrolidone), to increase drug solubility, and vectorization in chitosan-Ca-alginate microspheres, to exploit the colon-specific carrier properties of these polymers. This polymer used in a multiparticulate system, for colon-specific delivery of celecoxib for both systemic (in chronotherapic treatment of arthritis) and local (prophylaxis of colon carcinogenesis) therapy. The combined effect of four formulation variables, i.e. % of alginate, CaCl2, and chitosan and time of cross-linking on microsphere entrapment efficiency and drug released after 4 h in colonic medium were investigated. These results indicated that the proposed jointed use of drug cyclodextrin complexation and chitosan-Ca-alginate microsphere was highly effective.

Release behaviors of vancomycin from combination of poly electrolyte complex of pectin–chitosan was studied by Bigucci et al. examined. Moreover, the particular composition of these complexes improved vancomycin availability at alkaline pH on the bases of an enzyme-dependent degradation as confirmed from release studies performed in

presence of beta-glucosidase (Bigucci et al., 2008). Further this group extended the research by preparing hydrogels system of vancomycin using pectin and chitosan. Their study suggested that pectin/chitosan microspheres were able to limit the release of vancomycin under acidic conditions and release it under simulated colonic conditions, confirming their potential for a colon specific drug delivery system (Bigucci et al., 2009; Shukla and Tiwari, 2012).

Combination of Lectin-conjugated chitosan-Ca-alginate microparticles (MPs) loaded with acid-resistant particles of 5-fluorouracil (5-FU) for efficient local treatment of colon cancer were prepared by Glavas et al. Microparticles were prepared by a novel one-step spray-drying technique and after wheat germ agglutinin (WGA) conjugation. The retention of biorecognitive activity of WGA after covalent coupling to MPs was confirmed by haemagglutination test. Functionalized MPs showed excessive mucoadhesiveness in vitro, due to the positive surface charge, pH-dependent swelling of the matrix and lectin–sugar recognition (Glavas et al., 2009). Laroui et al. studied nanoparticles (NPs) to deliver an anti-inflammatory tripeptide Lys-Pro-Val (KPV) to the colon and assessed its therapeutic efficacy in a mouse model of colitis. To target KPV to the colon, loaded NPs (NP-KPV) were encapsulated into a polysaccharide gel containing 2 polymers: alginate and chitosan. The effect of KPV-loaded NPs on inflammatory parameters was determined in vitro as well as in the dextran sodium sulfateinduced colitis mouse model. The studied suggested that by using NPs, KPV can be delivered at a concentration that is 12,000-fold lower than that of KPV in free solution, but with similar therapeutic efficacy (Laroui et al., 2010).

A combination polymer comprising polyelectrolyte complex (PEC) consist of porous chitosan (CS) hydrogel microsphere of ibuprofen were prepared via either wet phase-inversion or ionotropic crosslinking with sodium tripolyphosphate (Na^+-TPP) and dextran sulfate (DS). The CS/TPP/DS microspheres resisted hydrolysis in strong acid and biodegradation in enzymatic environments. The swelling kinetics for CS microspheres was close to Fickian diffusion, whereas those for CS/TPP and CS/TPP/DS were non-Fickian. The release profiles of ibuprofen from CS/TPP/DS microspheres were slow in simulated gastric fluid (SGF, pH 1.4) over 3 h, but nearly all of the initial ibuprofen content was released in simulated intestinal fluid (SIF, pH 6.8) within 6 h after changing media. Overall the results indicated that CS/TPP/DS microspheres could successfully deliver a hydrophobic drug to the intestine without drug degradation in the stomach, and hence

could be potential candidates as an orally administered colon drug delivery system (Lin et al., 2005).

Compressing the microsponges followed by coating with combination pectin:HPMC of Eudragit S-100 based microsponges bearing dicyclomine for colonic delivery was developed by Jain et al. (2010). The colon-specific tablets were prepared by. In vitro release studies exhibited that compression-coated colon-specific formulations started releasing the drug at the sixth hour corresponding to the arrival time at colon. The study presented a new approach for colon-specific drug delivery.

Polymer combination comprising Eudragit L100 (EuL)-coated chitosan (Ch)-succinylprednisolone (SP) conjugate microspheres (Ch-SP-MS/EuL), were developed. Efficacy of these microspheres was dose-dependent and the greatest in the order Ch-SP-MS/EuL > Ch-SP-MS > prednisolone (PD) alone, and Ch-SP-MS/EuL showed excellent recovery of colitis states. Toxicity was the greatest in the order PD > >Ch-SP-MS > Ch-SP-MS/EuL. Ch-SP-MS and Ch-SPMS/EuL reduced significantly the thymic atrophy caused by PD. It was demonstrated that Ch-SP-MS/EuL enhanced effectiveness of PD and reduced toxic side effects of PD greatly. Also, these results established the prediction by previous in vitro and in vivo studies (Onishi et al., 2008).

The Eudragit-S-100 coated chitosan as polymer combination microspheres for 5-ASA and camylofine dihydrochloride for the treatment of ulcerative colitis was prepared by Dubey et al. In vivo data showed that microspheres delivered most of its drug load (76.55 ± 2.13%) to the colon after 9 h, which reflects its targeting potential to the colon. The study suggested that orally administered microspheres of both drugs can be used together for the specific delivery of drug to the colon and reduce symptoms of ulcerative colitis (Dubey et al., 2010).

Multilayer shells of combination polymer of chitosan (CHI)/sodium alginate (ALG) and poly(diallyldimethylammonium chloride) (PD)/sodium poly(styrenesulfonate) (PSS) were formed on the IBU-loaded PHBV microparticles using layer-by-layer self-assembly by Wang et al. (2007). This drug-loaded microspheres produced core–shell microparticles for sustained drug release. The ibuprofen (IBU)-loaded poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV) microparticles were fabricated by conventional solvent evaporation method of oil-in-water type. The in vitro release experiments revealed that, as for the microparticles with three CHI/ALG bilayer shells, the initial burst release of IBU from the microparticles was significantly suppressed and the half release time was

prolonged to 62 h from 1 h for the microparticles without coverage. The present combination for encapsulating drug-loaded microparticles demonstrates an effective way to prolong the drug release with reduced initial burst.

Malamataris and Avgerinos (1990) reported indomethacin release from combination polymer Eudragit RS/RL microspheres. Microspheres were prepared by employing two emulsion systems with phase distributions in the opposite order (o/w and w/o) with various combinations of polymers (Eudragit RS/RL) as well as different polymer/drug ratios. The release of indomethacin from the microspheres is biphasic except for those with polymer/drug ratio 0.5. The release rate constants of the fast initial as well as slow terminal phase decrease with increase in polymer content, particularly for the insoluble Eudragit RS. The times when both the particular release mechanisms are taking place with greater probability increase with polymer/drug ratio and with Eudragit RS content.

1.1.1.2. Polymer combination for parenteral administration

The parenteral administration route is the most effective and common form of delivery for active drug substances with metabolic bio-availabilities drug for which the bio-availability in limited by high first pass metabolism effect of other physicochemical limitation and for drugs with a narrow therapeutic index. For this reason, whatever drug delivery technology that can reduce the total number of injection throughout the drug therapy period will be truly advantageous not only in terms of compliance, but also for potential to improve the quality of the therapy. Such reduction in frequency of drug dosing is achieved, in practice, by the use of specific formulation technologies that guarantee that the release of the active drug substance happens in a slow and predictable manner.

For several drugs, depending on the dose, it may be possible to reduce the injection frequency from daily to once or twice monthly or even less frequently. In addition to improving patient comfort, less frequent injection of drugs in the form of depot formulation smoothes out the plasma concentration time profiles by eliminating the peaks and valleys. Such smoothing out of the plasma profiles has the potential to not only boost the therapeutic benefit but also to reduce unwanted events and side effects. The release can either be continuous or pulsatile depending on the structure of the device and the polymer characteristics, continuous release profiles are suitable to generate on infusion like plasma level time profile in the systemic circulation without the necessity of hospitalization.

Generally, biodegradable polymers are used for the preparation of parenteral controlled drug delivery system as it get degraded in the body and hence does not require removal from the body. Biodegradable polymers investigated for controlled drug delivery are polylactide/polyglycolide polymers, polycaprolactone, polyorthoesters, natural polymers etc.

Potential injectable urethral bulking agent of PCL microparticle-dispersed PLGA solutions have been published by Heang Oh et al. (2006). The mixture solutions were prepared by mixing polycaprolactone (PCL) microparticles (diameter, 100-200 μm; fabricated by a temperature-induced phase transition method) and poly(DL-lactic-co-glycolic acid) (PLGA) solution (dissolved in tetraglycol to 10 wt%) with different PCL microparticle to PLGA solution ratio. The mixture solution was solidified by the precipitation of PLGA when the solution was contact with water. In contact with water, the PCL microparticles exhibited a well-packed structure entrapped in a solidified porous PLGA matrix, which can effectively prevent the microparticle migration in the body and retain its initial volume even after PLGA matrix degradation. The PCL microparticle-dispersed PLGA solution may be a good candidate as an injectable bulking agent for the treatment of urinary incontinence owing to its good injectability, volume retention potential as well as biocompatibility.

Parenteral microparticles of poly(DL-lactic-co-glycolic acid) (PLGA) 50:50 and poly(ethylene glycol) (PEG) blends with entrapped model drug compounds to investigate the effect of blend ratio on release kinetics has been fabricated by Cleek et al. Two model drugs (FITC-IgG and FITC-dextran) were entrapped using a double-emulsion-solvent-extraction technique with high efficiency. In vitro release studies showed that the initial burst effect was dependent on the PLGA/PEG blend ratio. Moreover, the release rate increased in direct relation to PEG content for up to 28 days. A linear release profile was obtained for microparticles loaded with FITC-IgG for initial PEG weight fractions. A biphasic release profile was obtained for FITC-dextran loaded microparticles with rates dependent on the PEG content. These results demonstrate the feasibility of modulating the release profile of entrapped compounds in biodegradable microparticles by adjusting the PLGA/PEG blend ratio (Cleek et al., 1997).

Tanaka et al. (2008) have prepared microporous foams of polymer blends of two biodegradable polyesters, poly(L-lactic acid) (PLLA) and poly(ε-caprolactone) (PCL) via thermally induced phase separation method. The phase behaviors of the solutions of the

polymer blends in 1,4-dioxane containing water were similar, which would be due to the similar solubility parameters of the two polymers. However, the porous structure of the polymer blends dependent on the blend ratio under the same conditions other than the ratio. Hepatic cells (HepG2 cells) were cultured on the porous polymer blends. The cells invaded into the scaffold of PCL and PLLA–PCL-blend (1:4) by 1.0 and 1.5 mm, respectively, while they grew near the surface of the PLLA foams. Blending of biodegradable polyesters has the potential of controlling the porous structure of biodegradable foams.

Formulations of the blends for controllable release of bovine serum albumin (BSA) which are based on the balance among the hydration rate of the chitin phase and degradation of chitin/PLA and PLGA phase developed by Long Mi et al. (2003). These biodegradable microspheres were prepared by polymers blending and wet phase-inversion methods. The parameters such as selected non-solvents, temperature of water and ratio of polylactide to polyglycolide were adjusted to improve thermodynamic compatibility of individual polymer (chitin and PLGAs or chitin/PLA), which affects the hydration and degradation properties of the blend microspheres. Triphasic pattern of drug release model is observed from the release of protein from the chitin/PLGAs and chitin/PLA microspheres: the initially fast release (the first phase), the following slow release (the second phase) and the second burst release (the third phase). A chitin/PLGA 50/50 microsphere is novel and interesting, and may be used as a protein delivery system.

Graves et al. (2004) have used two different PLGA samples (Resomer 502 and Resomer 506), either alone or in combinations to prepare microcapsules. Microcapsules were prepared using a double emulsion solvent evaporation technique. The efficiency of encapsulation increased significantly when a mixture of 1 part Resomer 506 and 7 parts Resomer 502 was used to prepare the microcapsules. In contrast, irrespective of the relative ratio of Resomer 502/Resomer 506, the median particle size of the microcapsules showed similar distribution pattern. The glass transition temperature (Tg) decreased significantly as the amount of Resomer 502 was increased in the formulation. The presence of Resomer 502 at lower concentration, along with Resomer 506, initially reduced the burst effect. However, incorporation of a higher amount of Resomer 502 increased the burst effect. Drug release from these microcapsules continued over 80 days. Efficiency of encapsulation increased significantly when Resomer 506 was mixed with Resomer 502 at a

ratio of 1:7. Blending of Resomer 502 with Resomer 506 reduced the glass transition temperature, which resulted in higher amount of drug release.

The incorporation of the poly(methacrylate) which enabled the fine tuning of the particle size and the release kinetics; was developed by the encapsulation of the antiretroviral efavirenz (EFV) within pure PCL, pure Eudragit® RS 100 and PCL/Eudragit® RS 100 and of blend particles using two different methods (nanoprecipitation and emulsion/solvent diffusion/evaporation). Results showed that the greater the poly(methacrylate) content, the less pronounced the burst effect and the more sustained the release (Seremeta et al. 2013). Regardless of the polymer composition and the production technique, all the systems displayed remarkably high encapsulation efficiency and drug payload. On the other hand, nanoprecipitation resulted in smaller particles and narrower size distribution patterns. These data together with the greater simplicity, reproducibility and eventually scalability in an industrial setup, make this method more advantageous than the traditional emulsion one.

Spherical reservoir-type microcapsules composed of poly(ethylene adipate) (PEAD) and 20% poly-scaprolactone (PCL II), poly(hydroxyl butyrate-hydroxyvalerate) (P(HB-HV)); 10.8% HV) 20% PCL II and a blend of P(HB-HV)/PEAD 20% PCL II containing bovine serum albumin (BSA; surrogate protein)-loaded agarose have been fabricated using a double emulsion technique with solvent evaporation by Atkins (1997). P(HB-HV) and PEAD microcapsules had microporous and smooth surfaces. Irrespective of the fabrication polymer, microcapsules were generated in high yield (>75%) and BSA incorporation had no significant effect on microcapsule size distribution (8-200 μm). The loss of BSA, both by partitioning into aqueous continuous phase and through the micropores of P(HB-HV) microcapsules as BSA-loaded agarose during the precipitation of the fabrication polymer concomitant with solvent evaporation, resulted in low encapsulation efficiencies (< 15%). In all cases BSA release could be monitored for up to 26d and the amount and duration of BSA release from P(HB-HV) 20% PCL II microcapsules was influenced by micropore number and diameter, and by the extent of reservoir loading, while BSA release from smooth PEAD microcapsules was assumed to be the result of an increase in membrane porosity.

Bramfeldt and colleagues (2008) have recently reported binary blends which were prepared from poly(ε-caprolactone) (PCL), and P(CL-co-D,L-lactic acid)-P(ethylene glycol)-P(CL-co-D,L-lactic acid) co-polymers, where the D,L-LA content in the side

chains varied from 0 to 70 mol%. Blend discs were fabricated by melt-molding, and the effect of blend composition on hydrolytic degradation was studied. Variations in medium pH were monitored, and morphological changes were observed using scanning electron microscopy. Blending of these co-polymers was found to constitute a simple means by which intermediate rates of water absorption and mass loss were obtained, compared to those observed in pure co-polymer preparations. In one of the blends, prepared from the two components containing 70 or 0 mol% D,L-LA in the side chains and thereby exhibiting large differences in degradation rate, hydrolysis resulted in the formation of a porous material over time. Furthermore, all blend samples maintained their initial shape throughout the study. Such materials may be interesting for further investigations for applications in cellular therapy and controlled release.

Doulabia et al. (2013) have prepared chitosan/polyethylene glycol fumarate (chitosan/PEGF) blend films as wound dressings and to evaluate the influence of composition ratio on the blending properties of the films. Blending chitosan with PEGF obviated the brittleness of neat chitosan film. Film topography performed by atomic force microscopy illustrated that blending could increase and control the surface roughness of the neat film. Controlled water solubility, swelling, wettability and surface tension of the blend films were also evaluated. Physical properties as well as antibacterial activity assessments showed that among different compositions, the film comprising 80 wt% chitosan and 20 wt% PEGF is a suitable candidate for biomedical applications as a wound dressing material.

Bovine serum albumin (BSA) as a model protein drug was encapsulated with a microparticle based on the blend of poly(D,L-lactic-co-glycolic acid) (PLGA) and poly(L-lactide)-g-oligo(ethylene glycol) (PLLA-g-oligoEG) by W/O/W double emulsion method (Cho et al., 2001). Drug loading efficiency increased with increase in the graft frequency of oligoEG in the graft copolymer in the blend. The release of BSA was found to be more efficient for microparticles based on the blend than on the PLGA, which is due to the faster protein diffusion through the swollen phase of the hydrogel-like structure. The microparticles based on the blend showed a slower degradation and a lower pH shift compared to that of PLGA.

Poly-(3-hydroxybutyrate) (P(3HB)) is a biodegradable and biocompatible polymer that has been used to obtain polymer-based drug carriers. Bidone et al. (2009) used two strategies for prolonging ibuprofen (IBF) release from P(3HB)-based microspheres were

tested: blending with poly(D,L-lactide)-b-polyethylene glycol (mPEG-PLA); and obtaining composite particles with gelatin (GEL). IBF-loaded microspheres were prepared by an oil-in water (O/W) emulsion-solvent evaporation method. SEM micrographs showed particles that were spherical and had a rough surface. A slight decrease of the crystallinity degree of P(3HB) was observed only in the DSC thermogram obtained from unloaded-microspheres prepared from 1:1 P(3HB):mPEG-PLA blend. For IBF-loaded microspheres, a reduction of around 10 °C in the melting temperature of P(3HB) was observed, indicating that the crystalline structure of the polymer was affected in the presence of the drug. DSC studies also yielded evidence of the presence of a molecular dispersion coexisting with a crystalline dispersion in the drug in the matrix. Similar results were obtained from X-ray diffractograms. In spite of 1:1 mPEG-PLA:P(3HB) blends having contributed to the reduction of the burst effect, a more controlled drug release was provided by the use of the 3:1 P(3HB):mPEGPLA blend. This result indicated that particle hydration played an important role in the drug release. On the other hand, the preparation of P(3HB):GEL composite microspheres did not allow control of the IBF release (Bidone et al., 2009).

Blanco-Príeto et al. (2004) developed Vapreotide which was microencapsulated into end-group capped and uncapped low molecular weight poly(lactide) (PLA) and poly(lactide-coglycolide) (PLGA) by spray-drying and coacervation. Microspheres were prepared from single and blended (1:1) polymer types. Spray-drying and coacervation produced microspheres in the size range of 1–15 and 10–70 μm, respectively, and with encapsulation efficiencies varying between 46% and 87%. In vitro release of vapreotide followed a regular pattern and lasted more than 4 weeks, time at which 40–80% of the total dose were released. Microspheres made of 14-kDa end-group uncapped PLGA50:50 or 1:1 blends of this polymer with 35 kDa end-group uncapped PLGA50:50 gave the best release profiles and yielded the most sustained plasma levels above a pre-defined 1 ng/ml over approximately 14 days. In vitro/in vivo correlation analyses showed for several microsphere formulations a linear correlation between the mean residence time in vivo and the mean dissolution time ($r = 0.958$) and also between the amount released between 6 h and 14 days. For several other parameters or time periods, no in vitro/in vivo correlation was found. This study demonstrates that controlled release of the vapreotide is possible in vivo for a duration of a least 2 weeks when administered i.m. to rats. These results constitute a step forward towards a twice-a-month or once-a-month microsphere-formulation for the treatment of acromegaly and neuroendocrine tumors.

Paclitaxel-loaded biodegradable drug delivery systems manufactured from poly(lactic-*co*-glycolic acid) (PLGA) are known to release the drug at extremely slow rates. Jackson et al. (2007) investigated paclitaxel-loaded microspheres composed of blends of PLGA with low molecular weight ampipathic diblock copolymers by solvent evaporation method. The encapsulation and release of a series of poly(ε-caprolactone) (PCL)- or poly(d,l-lactic acid) (PDLLA)-*co*-methoxypolyethylene glycol (MePEG) diblock copolymers was measured using quantitative gel permeation chromatography. The PCL- and PDLLA-based diblock copolymers encapsulated at high efficiency and were miscible in PLGA microspheres (30–120 μm size range). The burst phase of paclitaxel release was increased up to 20-fold by the inclusion of diblock copolymers in PLGA microspheres. Approximately 10% of the more hydrophobic PCL-based copolymers released from the microspheres in a short burst over 3 days followed by very slow release over the following 10 weeks. Only the PDLLA-based copolymer released from the PLGA microspheres in a controlled manner over 10 weeks. All microspheres containing PEG were found to have more hydrophilic surfaces (as measured by contact angle) with improved biocompatibility (reduced neutrophil activation) compared to PLGA only microspheres. These results indicate that low molecular weight polyester-based diblock copolymers may be effectively encapsulated in PLGA microspheres to increase paclitaxel release (probably through a micellization process) and improve biocompatibility.

1.1.2. Drug combination

Combination products also known as fixed dose combinations are combinations of two or more active drugs produced in a single dosage forms. They provide the advantages of combination therapy while useful to improve adherence and can simplify procurement, storage and distribution of medicines. Fixed dose combination drugs are an important approach to addressing the management of both chronic and acute diseases. Many consider combination therapy in whatever form to be essential to the treatment of hypertension, cancer and other cardiovascular conditions, as well as major infectious diseases such as HIV, tuberculosis (TB) and malaria and to the prevention of drug resistance. In pharmaceutical technology, there are several combination drugs for oral and parenteral administration have been developed by some researchers.

Combination of lipophilic steroidal drugs ethinyl estradiol (EE) and drospirenone (DRSP) drug-delivery systems and its in vitro release study has been developed by Nippe

and General (2012). EE and DRSP poly(lactic-co-glycolic acid) (PLGA) microparticles and organogels containing EE and DRSP microcrystals were prepared and characterized with regard to properties influencing drug release. EE PLGA microparticles were prepared by a single oil-in-water solvent extraction/evaporation technique, and DRSP PLGA microparticles were prepared by a double emulsion solvent evaporation technique. The morphology and release kinetics of DRSP PLGA microparticles indicated that DRSP is dispersed in the polymer. The in vitro release profiles correlated well with in vivo data. Although DRSP degradation is known to be acid-catalyzed, DRSP was relatively stable in the PLGA matrix. Aqueous DRSP PLGA microparticle suspensions were combinable with EE PLGA microparticles and EE poly(butylcyanoacrylate) (PBCA) microcapsules without interacting. EE release from PLGA microparticles was faster than DRSP release; EE release is assumed to be primarily controlled by drug diffusion. Liquid-filled EE PBCA microcapsules were shown to be more robust than air-filled EE PBCA microcapsules; the bursting of microcapsules accelerating the drug delivery was therefore delayed. The drug release profile for DRSP organogels was fairly linear with the square root of time. The system was not combinable with EE PBCA microcapsules. In contrast, incorporation of EE PLGA microparticles in organogels resulted in prolonged EE release. The drug release of EE and DRSP was thus approximated.

PLGA (RG502H) microspheres containing combination of TRAMP tumor lysate and CpG oligonucleotide have been prepared. The lysates and toll like receptor (TLR) ligands were microencapsulated by spray drying. In this study, lysed TrampC2 cells or ovalbumin expressing EG.7 thymoma cells or prostate tumors were co-encapsulated with CpG oligodeoxynucleotides. PLGA microspheres with co-encapsulated tumor lysates and CpG oligodeoxynucleotides (CpG-ODN) as well as microencapsulated polyI:C in order to elicit anti-tumor responses. Immunization of mice with such mixtures of microspheres yielded substantial cytotoxic T cell (CTL) responses and interfered with tumor growth in TRAMP mice, a pre-clinical transgenic mouse model of prostate carcinoma, which has previously resisted dendritic cell-based therapy. As an important step towards clinical application of PLGA microspheres, we could show that γ-irradiation of PLGA microspheres sterilized the microspheres, without reducing their efficacy in eliciting CTL and anti-tumor responses in subcutaneous tumor grafts. Since PLGA is approved for clinical application, sterilized PLGA microspheres containing tumor lysates and TLR

ligands hold promise as antitumor vaccines against prostate carcinoma in humans (Mueller et al., 2012).

This system allows locally releasing single and/or combinations of anticancer drugs doxorubicin hydrochloride (Dox), 5-fluorouracil (5FU) and leucovorin calcium (LC), simultaneously by a controllable way from injectable in situ forming hydrogels derived from hyperbranched poly(amine-ester) (HPAE) macromers developed by Zhang et al. (2010). The hydrogel solutions with 0.05 wt% APS had proper solidification time at room temperature and at body temperature, and were chosen for further drug delivery tests. Behaviors of drug release can be controlled by the drug-loading methods or/and the C=C modification degree of macromers loaded with the drug molecules. The drug release period could be prolonged when the drug was loaded into the macromers with high content of C=C. The HPAE macromers exhibited good biocompatibility which was evaluated in L929 and MCF7 cell lines using MTT cell proliferation assay. The swelling behavior and degradation of HPAE hydrogels in vitro were also examined. These results suggest that the HPAE hydrogels hold great potential for use as injectable systems for locally delivering single and/or multiple drugs in chemotherapy of cancer.

Hussain group (2002) has used a model antisense oligonucleotides (AODNs) targeting the epidermal growth factor receptor and the commonly used cytotoxic agent, 5-fluorouracil (5-FU) which encapsulated in poly (lactide-co-glycolide) (P(LA-GA)) microsphere for co-delivery of these agents. Both agents were either co-entrapped in a single microsphere formulation or individually entrapped in two separate microsphere formulations and release profiles determined in vitro. Using a double emulsion method for preparing the P(LA-GA) microspheres suitable entrapment and sustained release over 35 days was observed in both types of formulation. Release of AODN and 5-FU from all formulations appeared to be biphasic. However, the release rates of the two agents were significantly slower when co-entrapped as a single microsphere formulation compared to those obtained with the separate formulations.

Raula et al. (2013) successfully prepared combination drug particle assemblies where nano-sized budesonide crystals were embedded in salbutamol sulphate containing solid microparticles for inhalation therapy. The budesonide nanosuspensions were prepared by a wet milling which were mixed then with salbutamol sulphate, mannitol (bulking material) and leucine (coating material) for the preparation of micron-sized particles by an aerosol flow reactor wherein leucine formed a rough coating layer on particle surface.

These two technologies enabled incorporation of two drugs with very different solubility into a single particle. Importantly, these particles were gas-phase coated with leucine which provided excellent aerosolization properties without any coarser carrier particles conventionally used in dry powder inhalers. The budesonide remained crystalline in the ”nanos-in-micros” particles and performed concomitant dissolution with salbutamol sulphate. Complete dissolution of budesonide nanocrystals from the particles took place within 20 min with the same rate as salbutamol sulphate.

Poly-(DL-lactic-co-glycolic) acid (PLGA) nanoparticles containing rifampicin and isoniazid have been prepared by a double emulsion solvent evaporation spray-drying technique and coated with polyethylene glycol (PEG 1% v/v). The PLGA nanoparticles had a uniform size distribution and positive zeta potential. In vitro/in vivo assays were performed to evaluate the pharmacokinetic and pharmacodynamic performance of these nanoparticles following nanoencapsulation process. The results demonstrated the potential for the reduction in protein binding of these drugs by protection in the polymer core. Sustained drug release over seven days were observed for these drugs following once-off oral administration in mice with subsequent drug distribution of up to 10 days in the liver and lungs for rifampicin and isoniazid, respectively. It was concluded that spray-dried PLGA nanoparticles demonstrate potential for the improvement of tuberculosis chemotherapy by nanoencapsulation of anti-tuberculosis drugs (Booysena et al., 2013).

In another study, Sharma et al. (2001) performed inhalable poly(DL-lactic acid) microparticles containing two of the first-line anti-tuberculosis (TB) drugs, isoniazid and rifampicin. PLA microparticles were prepared by a combination of solvent extraction and evaporation. They were prepared and tested for (i) phagocytosis by mouse macrophages, (ii) administration as a dry powder inhalation to rats, and (iii) targeting alveolar macrophages with high drug doses when administered to rats. These were tested for uptake by murine macrophages in culture and resultant intracellular drug concentrations determined by high performance thin-layer chromatography (HPTLC). The extent of microparticle delivery in vivo was examined by flow-cytometry. Drug concentrations in the blood and in alveolar macrophages were estimated by high-performance liquid chromatography after oral, vascular, intratracheal, and inhalation administration. Inhalable microparticles could be prepared and were taken up by cultured macrophages. Large numbers of particles could be delivered to the bronchiopulmonary system through a 2-min exposure to fluidized particles. The intracellular drug concentrations resulting from

vascular delivery of soluble drugs were found to be lower than those resulting from particle inhalation. Inhalable microparticles containing multiple anti-TB drugs offer promises of dose and dosing-frequency reduction, toxicity alleviation, and targeting macrophages-resident persistent mycobacteria.

Nicotinic acid (NA) sustained-release pellets combined with immediate release simvastatin (SIM) was successfully prepared by the double ethyl cellucose (EC) polymer and SIM milled suspension layering on the NA pellets in a bottom-spray fluidized bed coater. The uncoated pellets were prepared by extrusion–spheronization and the double EC films were coated in a bottom-spray fluidized bed coater. SIM was milled by wet grinding and then the milled suspension was layered on the coated pellets. Results showed that coated with 1.5% subcoating and 1% outer coating composed of EC and polyvinyl pyrrolidone K30 (PVP_{K30}). NA release behavior was very similar to the reference (NER/S; SIMCOR, Abbott) in different media. And SIM was delivered more rapidly than that of the reference, while the SIM layer had no influence on NA release. During 6-month storage at 40 ^{o}C/75% RH, the two drugs exhibited stable dissolution behavior. In the compound pellets, NA dissolution behavior was controlled by double EC coating, and SIM was uniformity in the procedure and stable with the effect of light magnesium oxide. More significantly, a satisfied method to prepare pellets containing more than one drug had been developed in the fluid bed coater, especially there was a large difference between the doses of the two drugs (Zhao et al., 2010).

Modified-release multiple-unit tablets of loratadine and pseudoephedrine hydrochloride with different release profiles were prepared from the immediate-release pellets comprising the above two drugs and prolonged-release pellets containing only pseudoephedrine hydrochloride. The immediate-release pellets containing pseudoephedrine hydrochloride alone or in combination with loratadine were prepared using extrusion–spheronization method. The pellets of pseudoephedrine hydrochloride were coated to prolong the drug release up to 12 h. Both immediate- and prolonged-release pellets were filled into hard gelatin capsule and also compressed into tablets using inert tabletting granules of microcrystalline cellulose Ceolus KG-801. The in vitro drug dissolution study conducted using high-performance liquid chromatography method showed that both multiple-unit capsules and multiple-unit tablets released loratadine completely within a time period of 2 h, whereas the immediate-release portion of pseudoephedrine hydrochloride was liberated completely within the first 10 min of

dissolution study. The drug release profile of the multiple-unit tablets was found to be closely similar to that of the multiple-unit capsules, indicating that compression did not alter the release profiles of drugs. The findings of the present study suggest that multiple-unit tablet systems could be applied to deliver multiple drugs with different release profiles in the treatment of certain diseases (Zeeshan et al., 2010).

1.2. Preparation techniques of microparticles for drug delivery

Microparticle refers to a particle with a diameter of 1–1000 μm, irrespective of the precise interior or exterior structure. Within the broad category of microparticles, ”microspheres” specifically refers to spherical microparticles and the subcategory of ”microcapsules” applies to microparticles which have a core surrounded by a material which is distinctly different from that of the core. The core may be solid, liquid, or even gas. Microparticle is usually assumed that a formulation described as a microparticle is comprised of a fairly homogeneous mixture of polymer and active agent, whereas microcapsules have at least one discrete domain of active agent and sometimes more. Some variations on microparticle structures are given in Fig. 1. As the domains and subdomains of active agent within microcapsules become progressively smaller, the microcapsules become microparticles.

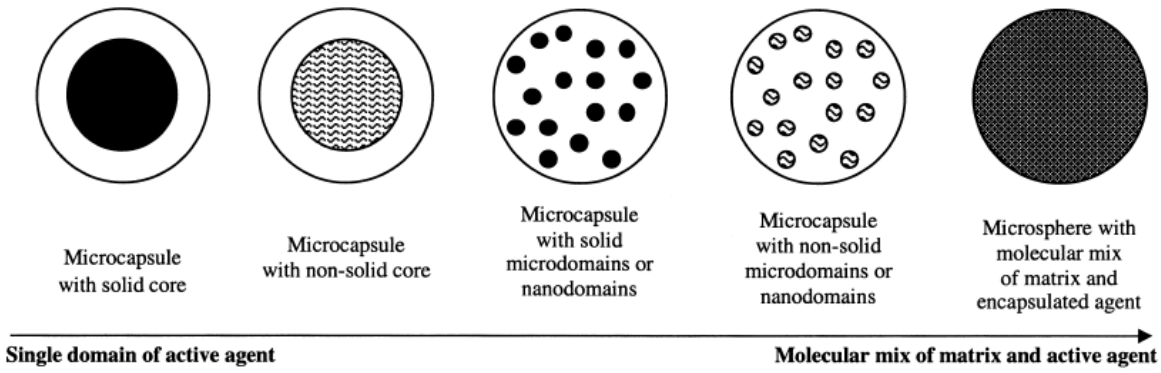

Fig. 1. Variations of microparticle formulations (Birnbaum and Peppas, 2004)

There are innumerable methods for preparing microparticles for use in various applications. The method employed to encapsulate the drug in the polymeric device must meet the following requirements (Jalil and Nixon, 1990a, 1990b): 1) The stability and biological activity of the drug must not be affected by the processing parameters employed in the fabrication of drug-loaded microparticles; 2) The yield of microparticles should have

the desired size range and the drug encapsulation efficiency should be high; and 3) The particle quality and the drug release profile should be reproducible.

1.2.1. Particle precipitation by non solvent addition (Coacervation)

In this method microparticles are produced by dispersing either the solid crystal particles or an aqueous solution of the drug in an organic solution of polymer, followed by a phase separation by adding a second organic solvent in which the polymer is not soluble (defined here as a non solvent). The water soluble drug naferalin acetate for example, has been incorporated in DL-PLG microparticles using this method (Sanders et al., 1984, 1985). An aqueous solution of the drug was emulsified in a polymer solution of DL-PLG and dichloromethane to produce a W/O emulsion. The addition of a non solvent resulted in precipitation of the polymer around the aqueous solution of drug to form microparticles. The addition of a large volume of the non solvent completes the extraction of the polymer solvent and hardens the microspheres. A similar method has been used for incorporation of oxytetracycline, but in this case the solid drug particles were suspended in the organic polymer solution (Vidmar et al., 1984). The particles produced by this method have a wide size distribution, which is not desirable for their intended clinical use. Microparticles produced by this method also tend to aggregate to a high degree. The outcome of this method can be altered by changing preparation parameters such as the drug: polymer ratio, the polymer solvent, the stirring rate, the temperature at which the process takes place, or the volume or type of non solvent.

1.2.2. Particle precipitation by solvent partitioning

In this method, a solution or suspension of the drug in the polymer/organic solvent solution is slowly injected into a stream of mineral oil. Since the organic solvent is soluble in the oil, but the drug and the polymer are not, coprecipitation of the drug and polymer occurs as the mixture partitions into the oil. The outcome will depend on the solubility of the drug. If the drug is soluble in the polymer solution, the drug and polymer precipitate together. If the drug is suspended in the polymer solution, the polymer will precipitate around the solid drug particles.

Hydrocortisone has been incorporated into polylactide polymer microparticles using this method. The microparticles were however, relatively large. The particle size ranged from 144 μm-412μm, depending on the flow rate and the diameter of the needle

through which the drug/polymer mixture was injected (Leelarasamee et al., 1988). With this method, preparation parameters that affect the size of the microparticles include the diameter of the needle, the drug:polymer ratio, the flow rate of the mineral oil, and the choice of polymer solvent.

1.2.3. Spray drying

In this technique the drug is dissolved or suspended in the organic polymer solution and the resultant mixture is spray dried to form microspheres (Bodmeier and Chen, 1988). The advantage of this technique is that water soluble and insoluble compounds can be incorporated into the spheres, in contrast to the single O/W emulsion evaporation system which is unsuitable for water soluble compounds. Progesterone and theophylline have been incorporated into polylactide microparticles using this method (Bodmeier and Chen, 1988).

This system is, however, associated with some drawbacks. For example, needleshaped crystals were formed when caffeine was incorporated using this method into a polylactide polymer, possibly the result of incompatibility between the drug and the polymer (Bodmeier and Chen, 1988). Similarly, fibres may be formed because of insufficient dispersion force to break up the polymer solution. The choice of organic solvent is also important; the polymer should be dissolved in solvents such as methylene chloride, ethyl acetate or an expensive fluorinated (hexafluroisopropanol) solvent, because these solvents evaporate quickly in the heated air in the drying phase and because the polymers used are often insoluble in common organic solvents.

Furthermore, since the particles are exposed to a large volume of heated air during the extraction step, the stability of oxidation-sensitive or thermolabile drugs may be affected. Although nitrogen would avoid oxidation of the drugs if substituted for air in this phase, the heat conductivity of nitrogen is less than that of air, which would affect the outcome. Particles of 5 to 125 μm in diameter are produced using this method.

1.2.4. Supercritical fluid extraction method

Micronisation and particle size reduction are fascinating areas in pharmaceutical technology that have been used to overcome problems involving the solubility or targeting of the drug. Conventional size reduction methods required crystallisation of the substances before the process could proceed. During this phase, the crystals can grow in size uncontrollably. When mechanical force is used to reduce the size of the crystals, the

particles often develop charged surfaces and become more cohesive. Other disadvantages associated with crystallisation include (1) the processes are time-consuming and costly, (2) the resultant particle size distribution is polydispersed with a wide size range, and (3) toxic organic solvents are used in the crystallisation process and residual solvent in the recrystallised drugs may exceed the authorised levels.

The use of supercritical fluids as extraction media is a promising alternative for the formation of microparticles of drugs and pharmaceutical excipients (Eckert et al., 1996; Fredriksen et al., 1997; Hanna et al., 1995; Marr and Gamse, 1999; McHugh and Krukonis, 1994; Subramaniam et al., 1997; York, 1999). Pioneering studies on the production of microparticles of biodegradable polymers using different supercritical fluid extraction methods have been reported in the literature (Bleich et al., 1993, 1996; Bodmeier et al., 1995; Pablo et al., 1993; Thies and Müller, 1998; Tom et al., 1993). There are two main reasons for using this technique. Firstly, the selective solvating power of supercritical fluids makes it possible to separate a particular component from a multicomponent mixture. Secondly, the favourable mass transfer properties and high solubility of solvents in the supercritical fluid make the drying of the microparticles rapid and efficient with low level of residual solvent as requested by the authorities (Folker et al., 1996; Shariati and Peters, 2003).

1.2.5. Solvent evaporation method

The solvent evaporation method has been used extensively to prepare polymeric microparticles containing different drugs (Jalil and Nixon, 1990a; Lewis et al., 1984; Suzuki and Price, 1985; Wang et al., 1999). Several variables have been identified which can influence the properties of the microparticles, including drug solubility, internal morphology, solvent type, diffusion rate, temperature, polymer composition and viscosity, and drug loading (Benoit et al., 1996; Bodmeier and McGinity, 1988a, 1988b; Bodmeier et al.,1994; Jalil and Nixon, 1990a, 1990b; Jaraswekin et al., 2007). The effectiveness of the solvent evaporation method to produce microspheres depends on the successful entrapment of the active agent within the particles, and thus, this process is most successful with drugs which are either insoluble or poorly soluble in the aqueous medium which comprises the continuous phase (Bodmeier and McGinity, 1987). Many types of drugs with different physical and chemical properties have been formulated into polymeric systems, including anti cancer drugs (Abraham et al., 2010; Boisdron-Celle et al., 1995;

Verrijk et al., 1992), narcotic agents (Mason et al., 1976), local anesthetics (Lalla and Sapna, 1993), steroids (Cowsar et al., 1985; Giunchedi et al., 1995), and fertility control agents (Beck et al., 1981; O'Hern et al., 1993). There are different methods to prepare microparticles by solvent evaporation method. The choice of the method that will give rise to an efficient drug encapsulation depends on the hydrophilicity or the hydrophobicity of drug.

1.2.5.1. Single emulsion process

This process involves oil-in-water (O/W) emulsification. The O/W emulsion system consists of an organic phase comprised of a volatile solvent with dissolved polymer and the drug to be encapsulated, emulsified in an aqueous phase containing a dissolved surfactant. For insoluble or poorly water-soluble drugs, the oil-in-water (O/W) method is frequently used. This method is the simplest and the other methods derive from this one. It consists of four major steps (Fig. 2): (1) dissolution of the hydrophobic drug in an organic solvent containing the polymer; (2) emulsification of this organic phase, called dispersed phase, in an aqueous phase called continuous phase; (3) extraction of the solvent from the dispersed phase by the continuous phase, accompanied by solvent evaporation, transforming droplets of dispersed phase into solid particles; and (4) recovery and drying of microspheres to eliminate the residual solvent.

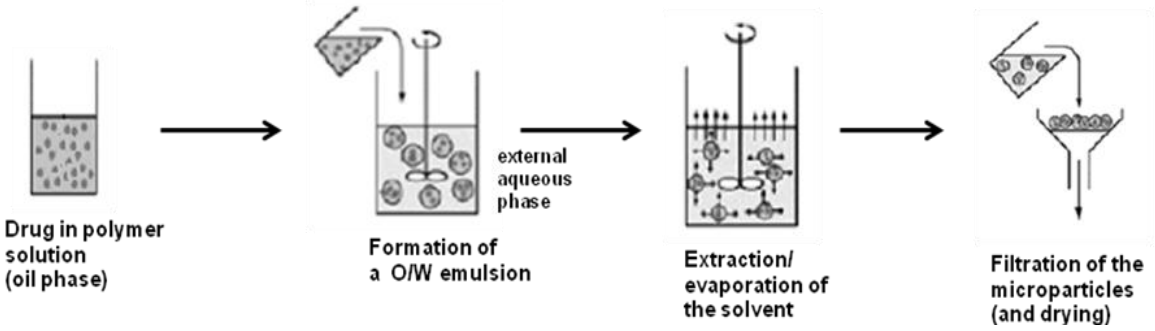

Fig. 2. Schematic overview of the four principal process steps in microsphere preparation by solvent evaporation (O/W)

Most systems that use oil in water emulsions to prepare microparticles consist of an organic phase comprised of a volatile solvent with dissolved polymer and the drug to be encapsulated, emulsified in an aqueous phase containing dissolved surfactant (Fig. 3). A surfactant is included in the aqueous phase to prevent the organic droplets from coalescing once they are formed.

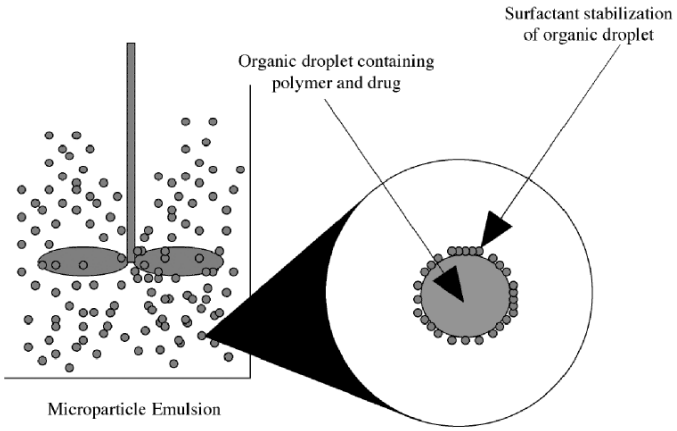

Fig. 3. Encapsulation using oil-in-water emulsion technique (Birnbaum and Peppas, 2004)

The polymer-solvent-drug solution is emulsified (with appropriate stirring and temperature conditions) to yield an O/W emulsion. The emulsion is created by using a propeller or magnetic bar for mixing the organic and aqueous phases. As seen in the Fig. 3, surfactants are used to stabilize the dispersed phase droplets formed during emulsification and inhibit coalescence. Surfactants are amphipathic in nature and will align themselves at the droplet surface promoting stability by lowering the free energy at the interface between the two phases. The surfactant also confers resistance to coalescence and microsphere flocculation. PVA is one of the widely used surfactants for producing the biodegradable and non biodegradable polymeric microparticles. Once the emulsion is formed, it is subjected to solvent removal by either evaporation or extraction process to solidify the polymer droplets. In the case of solvent removal by evaporation, the emulsion is maintained at a reduced pressure or at atmospheric pressure and the stir rate is reduced to enable the volatile solvent to evaporate.

The organic solvent leaches out of the droplet into the external aqueous phase before evaporating at the water-air interface. In the case of extraction, the emulsion is transferred to a large quantity of water or other quench medium, into which the solvent associated with the oil droplets is diffused out. The rate of solvent removal by extraction depends on the temperature of quench medium, ratio of the emulsion volume to quench medium and the solubility characteristics of the polymer, the solvent and the dispersion medium. A high extraction result will result in formation of particles with a high porosity that could lead to undesirable drug release profiles (Arshady, 1991; Jeyanthi, 1996). The solvent removal method by extraction is faster (generally <30 minutes) than the

evaporation process and hence the microspheres made by this method are often more porous in comparison to those made by solvent evaporation method. One of the disadvantages of the O/W emulsification process is the poor encapsulation efficiency with moderately water-soluble drugs. The drug diffuses out or partitions from the dispersed oil phase into the aqueous continuous phase and microcrystalline fragments of the hydrophilic drugs get deposited on to the microsphere surface (Cavalier et al., 1986) and dispersed in the polymer matrix. This results in poor trapping of the hydrophilic drug and initial rapid release of the drug (burst effect) (Jalil and Nixon, 1990b; Jones et al., 1995). The oil/water emulsification process is thus widely used to encapsulate lipid-soluble drugs. In order to increase the encapsulation efficiency of water soluble drugs, an oil-in-oil emulsion method was developed (Tsai, 1986). In this method, the drug may be dissolved or suspended in the oil phase before being dispersed in another oil phase. A water-miscible organic solvent like acetonitrile is employed to solubilise the drug in which PLGA or PLA are also soluble. This solution is then dispersed in oil such as light mineral oil in the presence of an oil soluble surfactant like sorbitan oleate (Span) to yield the O/O emulsion. Microparticles are finally obtained by evaporation or extraction of the organic solvent from the dispersed oil droplets and the oil is washed off by solvents like *n*-hexane. This process is also referred to as water-in-oil (W/O) emulsion method (Jalil and Nixon, 1990a; O'Hagan et al., 1994).

1.2.5.2. Double emulsion process

The O/W method is not suitable for the encapsulation of high hydrophilic drugs. There are two main reasons: (1) the hydrophilic drug may not be dissolved in the organic solvent; (2) the drug will diffuse into the continuous phase during emulsion, leading to a great loss of drug. Four other alternative methods have been proposed and therefore make it possible to encapsulate the hydrophilic drugs (Li et al., 2008).

1. The w/o/w double emulsion method: the aqueous solution of hydrophilic drug is emulsified with organic phase (w/o emulsion), this emulsion is then dispersed into a second aqueous solution forming a second emulsion (w/o/w double emulsion);
2. the o/w co-solvent method: when the drug is not soluble in the main organic solvent, a second solvent called co-solvent is necessary to dissolve the drug;
3. the o/w dispersion method: the drug is dispersed in form of solid powder in the solution of polymer and organic solvent;

4. the o/o non-aqueous solvent evaporation method: the aqueous phase is replaced by oil (such as mineral oil).

A double emulsion process is usually employed for drugs not soluble in an organic solvent. A solid-in-oil-in-water emulsion (S/O/W) process could be used to encapsulate a drug provided its form is of small size. The size of the drug crystal should be at least an order of magnitude smaller than the desired microparticle diameter in order to avoid large bursts associated with dissolution of larger crystals. Smaller crystals will be homogeneously distributed throughout the organic droplets formed in emulsion. Hydrophilic drugs (cisplatin, doxorubicin) have been encapsulated using this method. The problem with encapsulating hydrophilic drugs is the loss of drug to the external aqueous phase during the formation of the microparticle. Along with the loss of drug to the external phase, the remaining drug may migrate to the surface of the droplet before solidifying. To minimize these problems, the organic droplets should be solidified into microparticles as quickly as possible following their formation (Thies, 1992). This is achieved by using a viscous organic solution of polymer and drug and a large secondary volume of water that attracts the organic solvent into the aqueous phase immediately, thus leaving the microparticle with the encapsulated drug. The viscous dispersed phase minimizes the volume of organic solvent, facilitating its quick removal from the droplet and also makes it more difficult for the solid drug particles/crystal to migrate to its surface, resulting in a more homogeneous distribution of the drug within the particle.

Another alternative to encapsulate hydrophilic drugs is to employ the water-in-oil-in water (W/O/W) emulsion process (Fig. 4). An aqueous solution of the drug is added to an organic phase consisting of the polymer and organic solvent with vigorous stirring to form the first W/O emulsion. This emulsion is then dispersed in another aqueous phase containing more surfactant to form the W/O/W emulsion. A number of hydrophilic drugs like the peptide leuprolide acetate, a lutenizing hormone-releasing hormone-releasing hormone agonist (Okada, 1994; Toguchi, 1992), vaccines (Azevedo et al., 2006; Feng et al., 2006; Little et al., 2005; O'Hagan et al., 1991; Singh, 1995;), proteins/peptides (Herrmann and Bodmeier, 1998; Reithmeier et al., 2001) and conventional molecules (Ghaderi et al., 1996; Mandal and Tenjarla, 1996; Meng et al., 2003; Pérez et al., 2000, 2003) have been successfully encapsulated by this method. The problem with this type of emulsion occurs when the inner emulsion is not sufficiently stabilized, resulting in loss of aqueous droplets containing drug to the external aqueous phase. The choice of surfactants

that can be used to stabilize the inner emulsion is limited to materials that will dissolve in the organic solvent. Typically, the fatty acid esters of polyoxyethylene or sorbitan are used due to their high solubility in organic solvents and good biocompatibility.

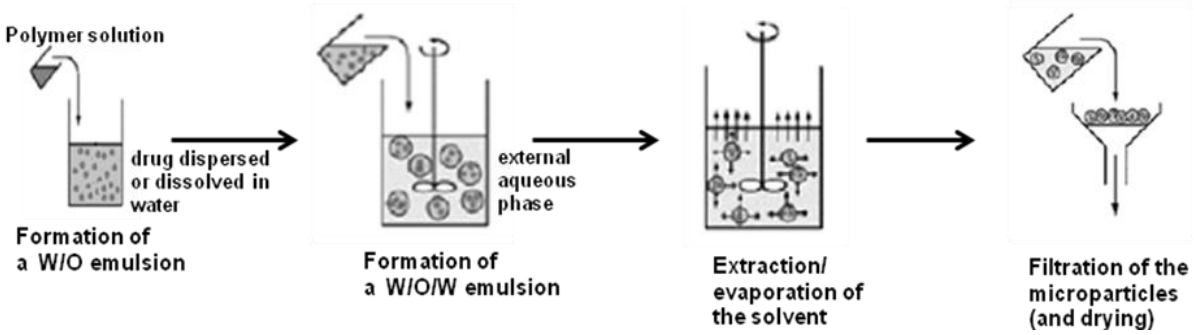

Fig. 4. Schematic overview of the four principal process steps in microsphere preparation by solvent extraction/evaporation (W/O/W)

1.3. Preparation of microparticles by solvent evaporation method

1.3.1. Materials

1.3.1.1. Dispersed phase

Polymer

The microparticles preparation method is a governing factor in the encapsulation and release of therapeutics. In addition, a complicated array of factors including the type of polymer, the polymer molecular weight, the copolymer composition, the nature of any excipients added to the microparticles formulation (e.g., for stabilization of the therapeutics), and the microparticles size can have a strong impact on the delivery rates.

First, the type of polymer used in microparticles preparation and the way in which the polymer degrades obviously affect drug release rates. Depending on the rate of hydrolysis of their functional groups, polymers can be broadly categorized into two types: surface eroding and bulk eroding (Burkersroda et al., 2002; Kang et al., 2012; Kumar et al., 2002; Tabata et al., 1993; Tamada and Langer, 1993; Wagdare et al., 2011). Bulk eroding polymers, such as PLG, readily allow permeation of water into the polymer matrix and degrade throughout the microparticles matrix. In contrast, surface eroding polymers, such as polyanhydrides, are composed of relatively hydrophobic monomers linked by labile bonds. In this way, they are able to resist the penetration of water into the polymer bulk, while degrading quickly into oligomers and monomers at the polymer/water interface via hydrolysis (Saltzman, 2001).

Bulk eroding polymer microparticles are often characterized by a ”burst” of drug as much as 50% of the total drug load (O’Donnell and McGinity, 1997) released during the first few hours of incubation, followed by a slow, diffusion controlled release and sometimes a third phase in which the remaining drug is released quickly as a result of severe degradation of the polymer matrix. In microparticles composed of surface eroding polymers, drug is released primarily at the surface as the polymer breaks down around it. Erosion of such polymers usually proceeds at a constant velocity (Gopferich and Langer, 1993; Kanjickal et al., 2004). If the drug of interest is homogeneously dispersed throughout a microparticles, the highest rate of release will occur at the beginning. As time proceeds, the surface area of the sphere and the release rate decrease asymptotically.

Polymer molecular weight can affect polymer degradation and drug release rates. As one might expect, an increase in molecular weight decreases diffusivity and therefore drug release rate (Alonso et al., 1994; Katou et al., 2008; Le Corre et al., 1994; Liggins and Burt, 2001; Mabuchi et al., 1994; Yang et al., 2001). In addition, a major mechanism for release of many drugs is diffusion through water filled pores. Then, it will be formed as polymer degradation generates soluble monomers and oligomers that can diffuse out of the particle. These small products are formed more quickly upon degradation of lower molecular weight polymers. The decrease in release rates with increasing polymer molecular weight appears to hold for small molecules, peptides, and proteins (Blanco and Alonso, 1998; Mehta et al., 1996). However, molecular weight typically has little effect on release rates from surface eroding polyanhydride microspheres (Hanes et al., 1996; Tabata and Langer, 1993). The co-monomer ratios in many copolymers can also affect release rates. Most often, increasing the content of the more rapidly degrading monomer increases the release rate (Lin et al., 2000; Shen et al., 2002; Spenlehauer et al., 1989). Similarly, when drug release is controlled by polymer erosion, release rate typically increases with higher concentration of the smaller and/or more soluble monomer (Tabata and Langer, 1993). However, the effect of the copolymer composition can be complicated by differences in the polymer phase behavior or the thermodynamics of the encapsulated drug (Kipper et al., 2002).

Solvent

Li et al. (2008) have reported that for the technique of microparticles preparation by solvent evaporation, a suitable solvent should meet the following criteria:

(1) able to dissolve the chosen polymer;
(2) should be poorly soluble in the continuous phase;
(3) have a high volatility and a low boiling point;
(4) have low toxicity.

Earlier chloroform was frequently used, but due to its toxicity and low vapour pressure, it is gradually replaced by dichloromethane. Dichloromethane (methylene chloride) is the most common solvent for the encapsulation using solvent evaporation technique because of its high volatility, low boiling point and high immiscibility with water (Li et al., 2008). Its high saturated vapour pressure compared to other solvents (at least two times higher) promises a high solvent evaporation rate, which shortens the duration of preparation of microparticles. However this solvent is confirmed carcinogenic according to EPA (Environmental Protection Agency) data and the researchers are making great efforts to find less toxic replacements.

Ethyl acetate shows promising potential as a less toxic substitute of dichloromethane. But due to the partial miscibility of ethyl acetate in water (4.5 times higher than that of dichloromethane), microspheres cannot form if the dispersed phase is introduced directly into the continuous phase. The sudden extraction of a big quantity of ethyl acetate from the dispersed phase makes the polymer precipitate into fibre like agglomerates (Freytag et al., 2000). Li et al. (2008) have also recomended that to resolve this problem created by the miscibility of solvent with water, three methods can be used:

(1) the aqueous solution is pre-saturated with solvent (Bahl and Sah, 2000);
(2) the dispersed phase is first emulsified in a little quantity of aqueous solution; after the formation of drops this emulsion is poured into a large quantity of aqueous solution (Freytag et al., 2000);
(3) the dispersed phase is emulsified in a little quantity of aqueous solution, the solution is agitated and the solvent evaporates leading to solidification of microspheres (Sah, 1997).

After using the aforementioned methods, the microspheres are manufactured successfully with ethyl acetate. However, the microspheres prepared by dichloromethane are spherical and more uniform, while the use of ethyl acetate results in particles which appear to be partly collapsed (Herrmann and Bodmeier, 1998). The drug encapsulation efficiency reduces significantly compared to the microspheres made by dichloromethane according to Herrmann and Bodmeier (1998). The author assumed that it is due to the high solubility of

ethyl acetate in water, leading to the loss of drug. Based on this assumption, Li et al. (2008) make a further assumption that it is due to two main causes: (1) more drug is entrained into the continuous phase by the higher mass flux of solvent, which is driven by the diffusion from the dispersed phase into the continuous phase; (2) the big quantity of solvent present in the continuous phase increases the solubility of drug in the continuous phase, facilitating the diffusion of drug into the continuous phase.

Li et al. (2008) have also reported that ethyl formate also shows interesting results. Sah (2000) has succeeded in manufacturing microspheres of PLGA with ethyl formate. The author had observed that the evaporation rate of ethyl formate in water was 2.1 times faster than that of dichloromethane although ethyl formate possesses a lower vapour pressure and a higher boiling point. This phenomenon is explained by the fact that more molecules of ethyl formate are exposed to the air–liquid interface because of its higher water solubility. His work proved that water immiscibility of a solvent is not an absolute prerequisite for making an emulsion. More experiments have to be carried out to confirm the promising use of ethyl formate (Li et al., 2008).

In summary, less toxic solvents have been tested and show a promising future. But there are not enough results to compare the quality of microspheres prepared by different solvents. Dichloromethane is still the most used solvent because it evaporates fast, shows high drug encapsulation efficiency and produces microspheres with spherical and more uniform form.

Alternative components

In certain cases, other constituents are added in the dispersed phase such as co-solvent and porosity generator.

Co-solvent is used to dissolve the drug that is not totally soluble in the solvent in the dispersed phase (Graves et al., 2006; Hsu and Lin, 2005; Li et al., 2008; Luan et al., 2006; Reithmeier et al., 2001). Organic solvents miscible with water such as methanol and ethanol are the common choices.

Porosity generator, called also porosigen or porogen, is used to generate the pores inside the microspheres, which consequently increases the degradation rate of polymer and improves drug release rate (Li et al., 2008). Organic solvents such as hexane, which do not dissolve poly(lactic acid) and poly(lactic-co-glycol acid) can be incorporated into microspheres to form pores (Li et al., 2008; Spenlehauer et al., 1986). Incorporating

Sephadex (cross-linked dextran gel) into insulin–PLA microspheres significantly increases microsphere porosity (Li et al., 2008; Watts et al., 1990). Appropriate amount of *n*-heptane added in the ethylcellulose/dichloromethane emulsion for encapsulation of aspirin also increases the porosity. However, if an excess of *n*-heptane is introduced, microspheres with high porosity leads to a very low drug encapsulation efficiency (Li et al., 2008; Yang et al., 2000a).

1.3.1.2. Continuous phase

Surfactant

Li and colleagues (2008) have recently reported that the surfactant, also called tensioactive agent, is frequently employed for the dispersion of one phase in another immiscible phase and for the stabilization of obtained emulsion. It reduces the surface tension of continuous phase, avoids the coalescence and agglomeration of drops and stabilizes the emulsion. A suitable surfactant should be able to give microparticles a regular size and a small size distribution, guaranteeing a more predictable and stable drug release. Before choosing the type of surfactant and its concentration, it is important to know the polarity of the two immiscible phases, the desired size of microparticles and the demand on the sphericity of microparticles. Surfactants for emulsions are amphiphilic. That means one part of the molecule has more affinity to polar solutes such as water (hydrophilic) and the other part has more affinity to non-polar solutes such as hydrocarbons (hydrophobic). When it is present in an emulsion, the surfactant covers the surface of drops with its hydrophobic part in the drop and its hydrophilic part in the water (Li et al., 2008).

Li and colleagues (2008) informed there are four different types of surfactant classified by the nature of the hydrophilic part of molecule: anionic, cationic, amphoteric and non-ionic.

(1) The anionic surfactants produces a negative charge in the aqueous solution. They have a relatively high HLB (hydrophile–lipophile balance) level because they are prone to be hydrophilic.
(2) The cationic surfactants on the contrary release a positive charge in aqueous solution.
(3) The amphoteric surfactants behave as anionic in alkali pH and as cationic in acid pH.
(4) Non-ionic surfactants have no charge.

For the most used emulsion of dichloromethane/water, typical stabilizers include: non-ionic: partially hydrolyzed PVA (polyvinyl alcohol), methylcellulose (Berchane et al., 2006; Lee et al., 1999), tween (Yang et al., 2000a) and span (Jalil and Nixon, 1990a); anionic: sodium dodecyl sulphate (SDS); cationic: cetyltrimethyl ammonium bromide (CTAB).

Among these surfactants, partially hydrolyzed PVA is mostly used because it gives the smallest microspheres (Jeffery et al., 1991). The increase of surfactant concentration reduces the size of microspheres (Jeffery et al., 1993; Sansdrap and Moës, 1993; Carrio et al., 1995; Yang et al., 2001). The addition of surfactant lowers the surface tension of the continuous phase and the diminution of the latter one decreases the particles size. However, due to the critical micelle concentration (CMC), the surface tension cannot decrease infinitively. When surfactant concentration reaches a certain level, the solution surface is completely loaded. Any further additions of surfactant will arrange as micelles and the surface tension of the aqueous phase will not decrease any more (Li et al., 2008).

Antifoam

Besides the surfactant, the antifoam is sometimes added into aqueous phase in the case of strong agitation because the foaming will disturb the formation of microspheres. When the stirring speed increases, more air is entrained and forms foam. So anti foams of silicon and non silicon constituents are used to increase the rate at which air bubbles are dissipated (Berchane et al., 2006; Li et al., 2008; Torres et al., 1998).

1.3.2. Operating conditions in microparticles preparation by solvent evaporation method

1.3.2.1. Agitation and size prediction

Li et al. (2008) also reported that agitation is one of the most important parameter for controlling the size of microparticles after the physicochemical properties of materials. Many other factors linked to agitation have also an impact on the size of microparticles, such as: the geometry of the vessel, the number of impellers and their position and the ratio of impeller's diameter compared to the vessel's diameter (Li et al., 2008; Maa and Hsu, 1996). There is a great number of correlations that predict the size and distribution of the size of the drops in an emulsion of two immiscible liquids (Maa and Hsu, 1996). The correlations take into account two aspects: (1) The physical properties of materials, such as

the density of continuous phase and the interfacial tension. (2) The factors linked to agitation (Li et al., 2008).

It is clear that increasing the agitation rate decreases the average size of microparticles, as it is confirmed in the literature (Mateovic et al., 2002; Yang et al., 2000b). On the other hand, it is reported that an increase in the volume of the dispersed phase decreases the size of microparticles (Jeffery et al., 1991, 1993; Jeyanthi et al., 1997; Li et al., 2008) while in some other studies, no great influence was observed (Sansdrap and Moës, 1993).

1.3.2.2. Temperature and pressure

The solvent evaporation rate can be accelerated either by increasing the temperature of the continuous phase (Li, 1994; Li et al., 2008; Miyazaki et al., 2006) or by reducing the pressure in the vessel or reactor (Izumikawa et al., 1991; Chung et al., 2001, 2002; Meng et al., 2004). However, there are several drawbacks in the case of elevated temperature: the recovered total mass decreases; the size distribution shifts toward the larger size; the drug encapsulation efficiency decreases and the morphology becomes coarser (Freitas et al., 2005). Moreover, the temperature should not be too high so as not to disnature the drug and not to reach the boiling point of solvent. Therefore, applying a reduced pressure seems to be a better choice (Li et al., 2008).

Li and colleagues (2008) informed that in the work of Meng et al. (2004), bovine hemoglobin loaded PELA (poly(d,l-lactic acid)-co-poly(ethylene glycol)) microspheres were prepared by W/O/W emulsion method under atmospheric pressure (AP) and under reduced pressure (RP) (30 kPa). The solidification time was shortened from 240 min to 40 min by applying a reduced pressure. Similar observations have been reported in the work of Yang et al. (2000a) and Chung et al. (2001). Reduced pressure (RP) can improve the drug encapsulation efficiency in most cases. In the work of Izumikawa et al. (1991), progesterone loaded poly(l-lactide) microspheres prepared using the O/W solvent evaporation technique were studied. They found that drug encapsulation efficiency was greater for microspheres that have been prepared using solvent evaporation at a reduced pressure (the RP method) of 200 mmHg than at atmospheric pressure (the AP method) of 760 mmHg. This argument is supported by the results in the work of Meng et al. (2004): the bovine hemoglobin encapsulation efficiency increases with the decrease of solidification time. However, others studies have contradictory results. The encapsulation

efficiency of lidocaine (Chung et al., 2001) or albumin (Chung et al., 2002) in PLA microspheres prepared under a reduced pressure is lower than those prepared at atmospheric pressure. The surface morphology of the microspheres examined by scanning electron microscopy indicates a porous and rough surface for the microspheres made using the AP method (Izumikawa et al., 1991). Conversely, the microspheres made using the RP method have an apparent smooth surface. The microspheres prepared under different pressure have a similar size according to Meng et al. (2004). This result is not in agreement with the results of Chung et al. (2001, 2002), in which the microspheres prepared under reduced pressure have a smaller size than those prepared under atmospheric pressure. The influence of pressure on the size of the microspheres is not clear because of the insufficient studies.

Since reduced pressure increases the evaporation rate, it should be as low as possible. But once the pressure is lower than the saturated vapour pressure of the solvent at a given temperature, the solvent begins to boil. The formation of bubbles can destroy the drops of dispersed phase, so the reduced pressure needs to be kept higher than the saturated vapour pressure of the solvent at a given temperature. The same analysis can be done for the temperature that is to be kept below the boiling point at a reduced pressure (Li et al., 2008).

1.3.3. Incorporation of bioactive compounds in microparticles preparation by solvent evaporation method

Bioactive compounds may be added to the solution of the matrix material by either codissolution in a common solvent, dispersion of finely pulverised solid material or emulsification of an aqueous solution of the bioactive compound immiscible with the matrix material solution (Herrmann and Bodmeier, 1998). Co-dissolution may require a cosolvent to fully dissolve the drug in the matrix containing solvent. Dispersion of the solid or dissolved bioactive material in the matrix containing solution may be achieved by ultrasonication (Maa and Hsu, 1999), impeller or static mixing (Lyons and Wright, 2002; Yoon and Deng, 2004), high speed rotor–stator mixing (Maa and Hsu, 1997) or microfluidisation (Maa and Hsu, 1999).

The microencapsulation of hydrophilic compounds by dispersion of their aqueous solution in an organic solution of the matrix material was more efficient with finer W/O emulsions, i.e., at a lower ratio of bioactive material droplet size to microsphere diameter

(Maa and Hsu, 1997; Rafati et al., 1997). For the entrapment of bovine serum albumin (BSA) into poly(methyl methacrylate) (PMMA) microspheres, a ratio of less than 1:10 was suggested to yield protein loadings of >80% (Maa and Hsu, 1997). A higher target load of bioactive material is likely to decrease the encapsulation efficiencies of proteins and peptides in PLGA (Blanco et al., 1994; Lamprecht et al., 2000; Rafati et al., 1997; Yang et al., 2001) and increase the 24-h (″burst″) drug release (Sah et al., 1994: Yang et al., 2001). Although some studies report the opposite, e.g., an increase in entrapment efficiency of ovalbumin (OVA) from 40% to 98% with an increase in actual OVA content from 7% to 16% (w/w) (Jeffery et al., 1993; Marchais et al., 1996). Increasing the volume fraction of the internal aqueous phase lowered the encapsulation efficiency due to droplet coalescence and increased probability of contact between the internal drug solution and the external extraction phase resulting in drug loss (Herrmann and Bodmeier, 1995; Uchida et al., 1995). In analogy, entrapment of solid protein particles also improved with decreasing particle size (Al-Azzam et al., 2002; Maa and Hsu, 1997; Li et al., 1999). The particle size of drug powders can be reduced by either micronisation of the drug powder prior to its dispersion, or during the dispersion step itself (Constantino et al., 2001; Tracy, 1998), or by the use of excipients which are coformulated with the drug so that the blended material dissolves in the matrix's solvent (Morita et al., 2000).

For efficient encapsulation of drugs dissolved in an aqueous phase to be dispersed in an organic matrix solution, stabilisation of the resulting W/O emulsion may be required. When drug-free microparticles were prepared from emulsions consisting of plain water and PLA dissolved in dichloromethane (DCM) (Nihant et al., 1994), increasing amounts of BSA added to the water as a surfactant stabilised the emulsions and decreased the pore sizes in the resulting microspheres; the latter observation was ascribed to the finer water droplets that were entrapped and left a corresponding void in the matrix. The addition of a surfactant (poloxamer) to the organic phase was found to be much less efficient. Similarly, the model substance indigocarmine was more efficiently entrapped with increasing BSA concentrations in the inner water phase (Schugens et al., 1994). Other substances, e.g., gelatin (Ogawa et al., 1988), poly(vinyl alcohol) (PVA) (Yang et al., 2001), ovalbumin (Blanco et al., 1997) or combinations of sorbitan esters and polysorbates (Soriano et al., 1995), have also been reported for the stabilisation of such W/O emulsions. The selection of stabilisers for the W/O emulsion has to be made with caution, as coencapsulated

surfactants can adversely affect drug encapsulation efficiency and release (Blanco and Alonso, 1998; Schugens et al., 1994).

1.4. Particle sizing measurements in pharmaceutical applications

1.4.1. Off-line particle sizing methods

1.4.1.1. Laser diffraction

Laser diffraction (LD) is the most applied technique for the particle size measurement of pharmaceutical powders and granules. It can be used as an in-process method (Ma et al., 2000; Silva et al., 2013) or as an off-line method. A dispersed sample passes through a beam of monochromatic light causing light scattering, which is measured as a function of scattering angle by a multi-element detector. As the scattering pattern, i.e., scattered intensity as a function of scattering angle, is largely particle size dependent, it follows that particle size information can be extracted from the experimentally determined pattern. Older instruments mainly rely on the Fraunhofer approximation to derive particle size information from the scattering pattern, while recent LD particle size analyzers are based on Mie's theory (Silva et al., 2013; Rawle, 1993).

In laser diffraction, particle size distributions are calculated by comparing a sample's scattering pattern with an appropriate optical model. Traditionally two different models are used: the Fraunhofer Approximation and Mie Theory. The Fraunhofer approximation was used in early diffraction instruments. It assumes that the particles being measured are opaque and scatter light at narrow angles. As a result, it is only applicable to large particles and will give an incorrect assessment of the fine particle fraction. Mie Theory provides a more rigorous solution for the calculation of particle size distributions from light scattering data. It predicts scattering intensities for all particles, small or large, transparent or opaque. Mie Theory allows for primary scattering from the surface of the particle, with the intensity predicted by the refractive index difference between the particle and the dispersion medium. It also predicts the secondary scattering caused by light refraction within the particle, this is especially important for particles below 50 microns in diameter, as stated in the international standard for laser diffraction measurements. Nowadays, the ISO13320 standard for LD particle size analysis acknowledges the superiority of Mie's theory (Jones, 2003; Silva et al., 2013). LD particle size analyzers that use Mie's theory (e.g., Mastersizer® S) base their particle size calculation on the assumption that particles are spherical, which is rarely true. This is a solution to deal with

the fact that the only shape that can be described by a single dimension is the sphere. LD results are generally presented as a volume-weighted particle size distribution. Thus, LD results reporting that the median value (D_{50}) of a volume-based PSD is 100 μm means that particles with a size up to 100 μm account for 50% of the measured sample volume. Alternatively, a number-weighted distribution can be extracted, depending on the analyzer's software (Silva et al., 2013).

Laser diffraction is a non-destructive, non-intrusive method that can be used for either dry or wet samples. As it derives particle size data using fundamental scientific principles there is no need for external calibration; well-designed instruments are easy to set up and run, and require very little maintenance.

1.4.1.2. Sieve analysis

Silva et al. (2013) have reported that before the introduction of LD, sieving used to be the most commonly applied sizing method, and it is still widely used for the determination of particle size because of its inexpensiveness. It is described in the European Pharmacopoeia (2009) that sieve size is the ''size of the aperture measured perpendicular to the wire through the center of the opening.'' The mass of material that is retained on a specific sieve is weighted and presented as a percentage of the total assayed material. Therefore, a mass-based PSD is generated. The results are generally presented as a cumulative mass distribution. In this case, a median (D_{50}) of 100 μm indicates that 50% of the total weight of the measured material is constituted by particles that would pass through a sieve with 100 μm apertures. It is acknowledged that for a particle to pass through a sieve, it must have two dimensions smaller than the sieve size. This is why it can be assumed that sieve analysis separates particles according to their second largest dimension. Some of the described disadvantages of sieve analysis are as follows: test sieves require regular care in order to maintain their performance, their cleaning must be careful as vigorous brushing may distort sieve openings, it is not possible to perform sieve analysis on sprays or emulsions, measurement of dry powders with sizes under 38 μm is very difficult as electrostatic charges may cause loss of material (wet sieving may be a solution but this technique provides very poor reproducibility and is difficult to carry out), and cohesive or agglomerated materials are problematic to measure as they form aggregates that will not pass through the sieve's aperture (Rawle , 1993; Silva et al., 2013). Sieve analysis also requires a relatively large amount of sample and, as a consequence, is

not appropriate for costly materials or materials of which only small quantities are available. Samples can be eroded due to attrition during the analysis making sieving unsuitable for these materials. Measurement times and operating methods (e.g., shaking) need to be standardized as the longer the measurement is performed, the smaller the obtained particle size is as particles have time to orient themselves to fall through the sieve. This is particularly important when dealing with odd-shaped particles which are difficult to sieve and may generate peculiar results. For instance, measuring the particle size of needle-like or rod-like particles by means of sieve analysis might not be the best choice. Additionally, there is an increase in the risk of particle erosion as sieving time increases (Silva et al., 2013).

1.4.2. In-process particle sizing methods

1.4.2.1. Photometric stereo imaging

Particle size measurement by imaging is most commonly carried out with microscopy or other optical camera-based systems by measuring single particles, which are dispersed in air or a suitable liquid. Such examples are numerous, but the most interesting are the recent advances in image-based dynamic particle sizing that have taken image-based sizing to a new level enabling measurement of a large number (up to several millions) of particles using automated systems.

Another studied technique was photometric stereo imaging. In photometric stereo imaging the idea is to vary the direction of incident illumination between successive images while the viewing direction is held constant. The technique was first introduced by Woodham on 1980 (Sandler, 2011). The photometric stereo imaging unit Flashsizer3D® (FS3D®) consists of a monochrome CCD camera connected to a metal cuvette with a glass window and a computer. The tool is equipped with a sampling unit that allows online measurements. Two light sources, positioned relative to each other at an angle of 180^{o}, illuminate the sample, and two digital images of the sample are obtained. A gray-scale value between 0 (black) and 255 (white) is attributed to each individual pixel, and the shading effects expose the topography of the surface (Sandler, 2011; Silva et al., 2013).

The resulting gradient fields obtained with this setup contain direct information about surface normal in xz plane and indirect information about surface normal in yz plane. Line integration is used in the horizontal direction to obtain a three dimensional surface. The cumulative error that is typical for line integration-based methods is removed

with a high pass filter. It is assumed that the sample surface is approximately straight on larger scale, due to the fact that samples lay against a straight glass surface of the cuvette glass window. The high pass filter is constructed from a moving average low pass filter. Therefore, peaks on the 3D surface are assumed to be particles, and the projected volume-based (V) particle size is then calculated from the area of the peaks in the xy direction:

$$d = \sqrt{(a)} \cdot c \tag{1}$$

$$V = d^3 \tag{2}$$

where d is the diameter of the particle, a is the area of the peaks and c a calibration constant, calibrated by default with pellets (Sandler, 2011). If the shape of the particles to be measured differs significantly from spherical, the calibration constant can be changed accordingly to the particles to be measured (O'Sullivan et al., 2003; Silva et al., 2013). This imaging unit allows the acquisition of a volume-based PSD and related D values of the particles captured in each image. The size classes can be defined by the user. The FS3D® system has been used for the measurement of powders (Soppela et al., 2011), granules (Sandler, 2011), and pellets (Burggraeve et al., 2011) showing the potential of this technique as a fast particle size analyzer for various types of material.

1.4.2.2. The Eyecon® particle sizing technology

The Eyecon® particle sizing technology was also for the particle size measurement in pharmaceutical (Silva et al., 2013; Soppela et al., 2011). This is a very recent 3D-imaging system that allows the determination of the PSD for moving particles using a flash imaging technique (Fig. 5). The Eyecon® particle sizing technology uses a unique combination of LED illumination sources coupled with a state of-the-art image capturing device and licensed particle sizing algorithms to acquire sample images and size distribution profiles in real time. The equipment can either be used offline or in-process. During measurements, a powerful short light pulse is created and provided that the particle movement during this pulse is negligible a sharp image without blurring is captured. The particles are illuminated with red, green, and blue LEDs from different angles. The color on the surface of the particle is captured in an image, and for each individual pixel, a map of the surface height is built (Silva et al., 2013). Furthermore, using image gradient data an ellipse is fitted on the particle edges, and its maximum and minimum diameters are

obtained. These are used to calculate the average aspect ratio (AAR) of particles as an indicator of their sphericity by means of the following equation:

$$AAR = \frac{D_{max}}{D_{min}} \tag{3}$$

where D_{max} represents the maximum measured diameter and D_{min} the minimum measured diameter. Each captured image is analyzed by Eyecon® resulting in a group of ellipses. Results can either be computed using only the current image or also include data from previous images and are presented as a histogram.The D values are calculated by ordering particles in order of ascending relative mass. Firstly, the total mass is computed, and then, an iterative algorithm adds up starting with the smallest of the particles. As the running total reaches 10%, 25%, 50%, 75%, and 90% of the total mass, the diameter of the last added particle is recorded as being the D_{10}, D_{25}, D_{50}, D_{75}, and D_{90} diameter, respectively (Silva et al., 2013). The Eyecon® particle sizing technology can be used both on-line and in bench-top format to measure particle size and shape.

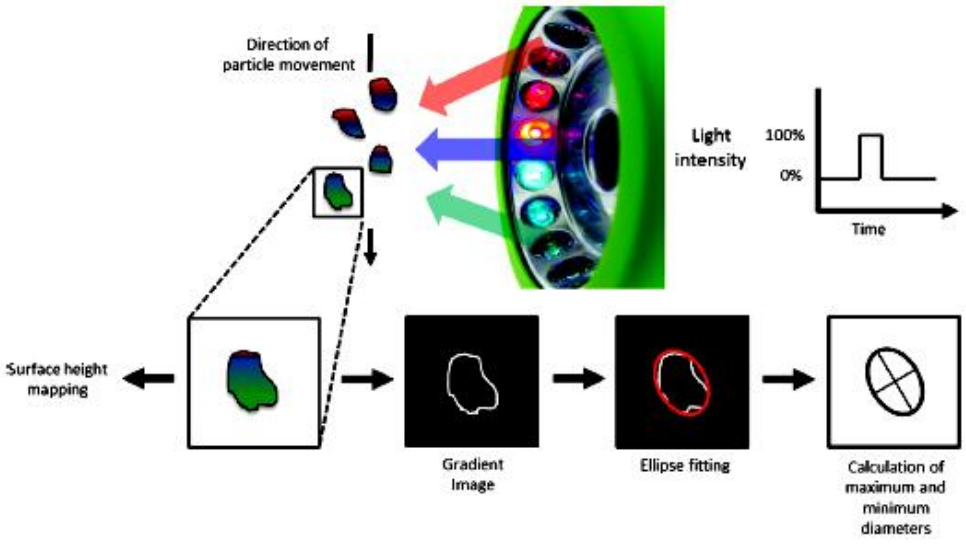

Fig. 5. Working principle of the Eyecon® equipment (Silva et al., 2013)

1.4.2.3. Methods based on chord length measurements

Spatial filtering velocimetry

Spatial filter velocimetry (SFV) is a method similar to focused beam reflectance measurement (FBRM) as they both use chord length distribution to express particle size. Both techniques project a laser beam onto the moving particles (Burggraeve et al., 2010). However, FBRM uses the backscattered light and converts it to size measurement, while

SFV uses the generated shadow (Burggraeve et al., 2010). During SFV measurements, particles pass through a laser beam and cast shadows onto a linear array of optical fibres. In that way, a burst signal is generated, which is proportional to particle velocity. As the particles pass through the beam, a secondary pulse is generated by a single optical fibre. Knowing the time of the pulse and the velocity of the moving particles, the chord length can be calculated (Burggraeve et al., 2010). The measurement cell of the SFV probe is equipped with sapphire windows that are kept clean through the use of an internal compressed air supply system, which prevents fouling of the windows. The internal airflow also ensures the dispersion of highly concentrated particles and optimizes the movement of these particles through the measurement zone (Burggraeve et al., 2010; Dillow et al., 1999). The measurement results are reported in various ways, e.g. sieve distribution (as fraction and passage), volume distribution, number distribution, velocity distribution, etc. (Petrak, 2002; Petrak and Rauh, 2006; Schmidt-Lehr et al., 2007). Närvänen et al. (2008) compared three different particle size measurement techniques: sieve analysis, laser diffraction and offline SFV to model process parameters of a fluid bed granulation process. The SFV results were the most consistent among the three studied techniques. Another study by the same group showed that the in-line particle size data measured via SFV could be used to monitor different process phenomena and process failure (Närvänen et al., 2009). However, particle size determination was influenced by size segregation in the fluid bed: the in-line technique underestimated and the at-line method overestimated the final granule size. Lipsanen et al. (2008) showed that the pressure difference over the upper filters (indication of blockage of filters) and the fluidization parameter correlated well with the in-line particle size measurements.

A system based on the SFV principle is the Parsum® IPP70 probe (Silva et al., 2013). The Parsum® IPP70 system is able to report size after converting the raw CLD to a number or volume-based PSD performed by an algorithm in the system's software. SFV has already been suggested for particle size monitoring in fluidized bed processes (Jager et al., 1990), mixing and coating, high shear wet granulation, dry granulation, and spray-drying (Hobbel et al., 1991; Petrak et al., 2011).

Focused beam reflectance measurements (FBRM)

The process analytical technologies (PAT) are measurement techniques that are spreading in laboratory and industrial applications where they provide reliable, on-line

information about the evolution of a process. Among these, the FBRM offers a possibility to implement on-line monitoring of the evolution of a crystalline population by tracking changes in the number as well as in the geometry of the particles in the considered system.

FBRM is another process analytical tool designed for measuring chord lengths. The FBRM consists of a focused laser beam rotating at a constant velocity that scans the particles located in front of the probe's sapphire window (Li and Wilkinson, 2005; Ruf et al., 2000; Silva et al., 2013; Worlitschek et al., 2005; Wynn, 2003). When the light emitted by the laser hits a crystal/particle, the sensors included in the probe record and analyze the backscattered signal. It measures the light that is reflected and propagated back through the probe when a tightly-focused laser beam, rotating at a high speed (2–8 m/s), hits a particle (Fig. 6a). The collected data can be defined, as shown in Fig. 6, as the distance between two edges of the particle; the FBRM calculates this distance by multiplying the rotating speed of the laser by the time of the corresponding backscattering signal. The chord length is then calculated by multiplying the duration of reflection with the laser beam's scan speed. FBRM D600T measurements can be performed in highly concentrated particle systems as a scraping system is installed on the probe's sapphire window, keeping it clean and preventing probe fouling during in-process measurements. FBRM D600T is a count-based technique which means that the sizing results are presented by the FBRM D600T software (iC FBRM® 4.0 software) as a number based chord length distribution (number of particles measured within a chord length class). This software also allows the extraction of D values from these distributions and of size (chord length) classes. As mentioned previously, size results are usually presented as a number, length, area, or volume-based distribution. However, the FBRM D600T system results are presented as a raw chord length frequency distribution and can be transformed into a 1/lengthweighted, length-weighted, square-weighted, or cubic-weighted chord length frequency distribution (Czapla et al., 2010; Li and Wilkinson, 2005; Ruf et al., 2000; Silva et al., 2013; Worlitschek et al., 2005; Wynn, 2003). The weighing method to use depends on the aim of the measurement. If there is the necessity of detecting slight changes in the fraction of smaller particles, no weighing or length-weighing will emphasize these rather than the larger ones. On the other hand, if the interest lies on detecting small changes in the larger particles square and cubic weighing emphasize the coarser particles at the same time making the detection of changes in the smaller size range more difficult. It is described that the raw chord length data are

similar to a length based PSD since the probability of a certain chord length being detected is proportional to the linear dimension of a particle.

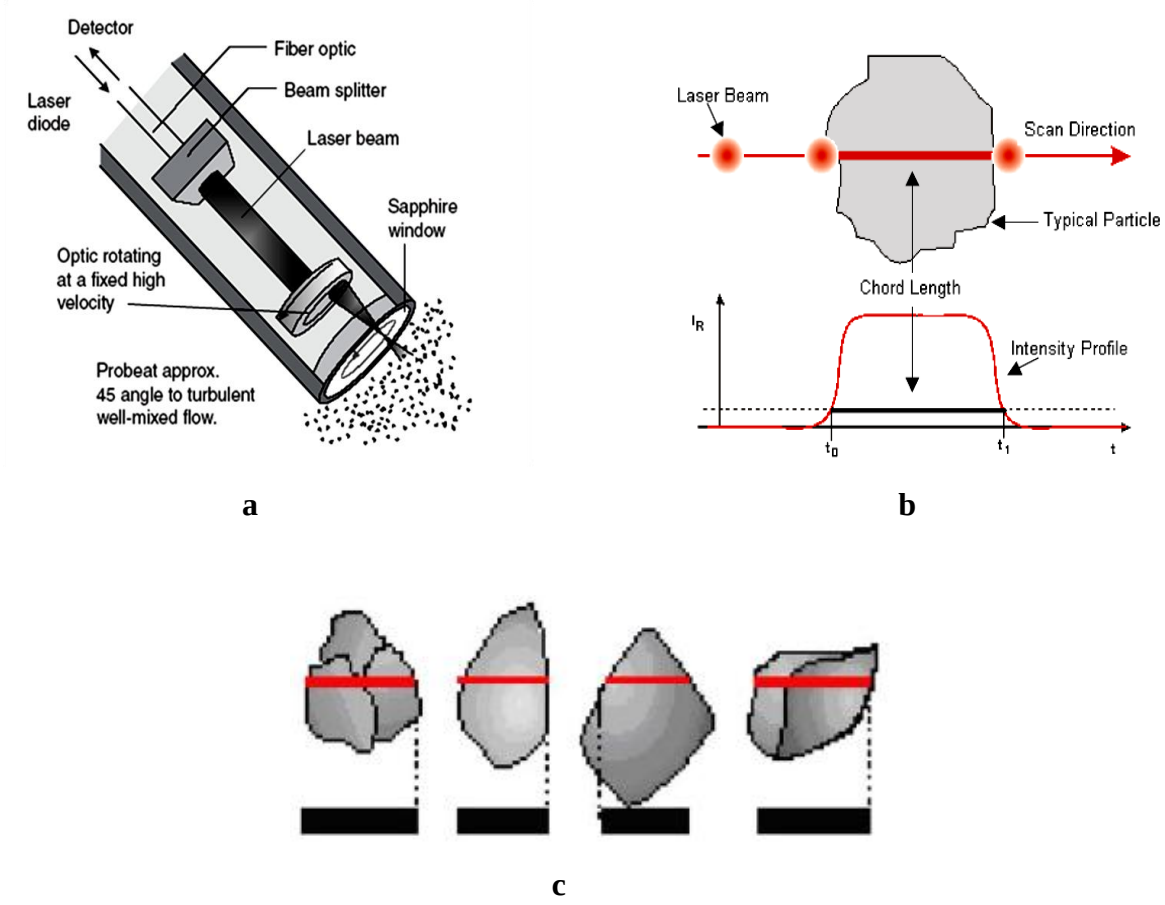

Fig. 6. FBRM probe description (from Lasentecs®) (a), Measurement of a particle chord length using the FBRM probe (b), Examples of chord lengths (from Lasentecs®) (c)

The FBRM present several advantages such as it provides on-line and real time analysis of the particles or crystalline population. Errors due to pretreatment of the system such as sampling or dilution, which are frequently implemented with other measurement tools, is eliminated as the probe is directly inserted in the slurry. It is a robust instrument which presents a short analytical time, a high resolution for a wide range of particle size (0.25-4,000 μm), that can be used in high concentrated solutions, and within harsh conditions which is often the case in pharmaceutical processes. Chemometrics related to the use of this device are heavily based on the particle shape (Maaβ et al., 2011; Martos et al., 2010; Ruf et al., 2000; Wynn, 2003).

The relationship between particles and chords is most easily developed by specifying how particles are expected to cause chords. This can then be reversed, so that the underlying particle size distribution can be deduced from a reported chord length

distribution. Wynn (2003) reported the relationship developed here is based on several assumptions:

- All particles have the same shape.
- When particles are scanned by the laser beam, they have random orientations.
- There is a ”scanning depth”: when a particle is crossed by the beam at a separation less than this depth, its reflection will be detected as a chord. If a particle is further than this depth from the probe window, any reflected light is not detected. This depth will be related to the focussing of the beam and the way that the instrument discriminates between background light and reflected light from a chord. The scanning depth is assumed to be independent of particle size.
- When a chord is traced across a particle’s silhouette, the detected length is the entire length of the chord. This is the assumption of a ”two-dimensional” chord (Hobbel et al., 1991), which relies on the scanning depth of the beam being larger than the particle. (The alternative would be a detection of some smaller part of the particle. Sometimes Lasentec® probe can be used in this way deliberately, detecting the chord lengths of the fine-scale structure of particles.)
- The path of the beam across the particle is effectively a straight line, with negligible ”spot width” compared to the particle size.
- The chord length is the time taken for the beam to cross the particle, divided by the linear speed of the beam’s rotation.
- Particles are scanned only once at each visit to the window.
- The rate at which particles enter the scanning zone is not size dependent; the time averaged particle size distribution (PSD) in the scanning zone is the same as the PSD to be measured.

1.5. Application of FBRM as an in-process analytical technology

Focused beam reflectance measurement (FBRM) is another process analytical tool designed for measuring chord lengths. FBRM technique offers unique advantages for a variety of industrial applications where in-situ monitoring of particle size or solid concentration is needed. Due to its principles of measurement, the FBRM probe can be readily inserted on-line or into a reaction vessel without the need of installing a pre-dilution side-stream as is required for other on-line particle sizing tools (Hobbel et al., 1991; Jager et al., 1990; Monnier et al., 1996). FBRM has already been successfully

applied for suspensions and crystallization processes (Abbas et al., 2002; Barrett and Glennon, 1999, 2002; Worlitschek et al., 2004; Yu et al., 2005) and has also been studied for fluid bed granulation in comparison with other PAT tools (Burggraeve et al., 2013; Fonteyne et al., 2013; Hu et al., 2008). Polymorphic transition monitoring (Jia et al., 2008), flocculation process design (Kirwan, 2009), control of particle disruption (Kougoulos et al., 2005), and solubility measurements (Kim et al., 2005) are some other applications where the use of FBRM has already been reported.

Abu Bakar et al. (2009) have been conducted the capability evaluation of a seeded batch cooling crystallization with a temperature cycling method to produce a narrow crystal size distribution and grow a desired polymorphic form of sulfathiazole crystals. The study used FBRM, and attenuated total reflectance ultraviolet/visible (ATR-UV/vis) spectroscopy for the in situ monitoring and control of the process. Based on the FBRM readings, the process was driven using a feedback control approach that employs alternating cycles of heating and cooling phases so that the number of counts, corresponding to the number of seed particles, is maintained, whilst the square-weighted chord length distribution, indicating the dynamic progress of the growth of the seeds in the system, is increased. Results of the experiments show that the temperature cycling method promoted Ostwald ripening, which helped in accelerating the growth and enhancing the size uniformity of the product. The method also has a good prospect to be implemented for the control of polymorphic purity. Seeds of Form I and Form II could be grown from *n*-propanol and water, respectively. Form I seeds in water were first transformed into Form II and/or swamped by nuclei of Form II, before the growth of the newly formed crystals took place. Seeds of Form II and Form III in *n*-propanol, however, were not able to grow at all. This study confirmed that the nucleation and growth of sulfathiazole crystals are solvent-mediated, and the insight into these phenomena was captured very well by the *in situ* monitoring tools.

In another study, Yuan et al. (2009) have conducted to measure hydrocortisone (HC) crystal particle size during crystallization process by focused beam reflectance measurement (FBRM). The particle size and the shape of the resultant HC crystals can be clearly seen that when FBRM was applied to the monitoring of the crystallization process, crystal product with a larger particle size and a much narrower particle size distribution was produced. In addition, the shape of the crystals under control was also more regular. One of the key points for understanding and utilizing FBRM data in a production

environment is to take advantage of the knowledge provided on the process variables, e.g., detecting a nucleation event, growth, agglomeration and changes in the crystal size distribution. The online monitoring of the particle size by direct installation of a FBRM probe into the crystallization process helped indicate the crystal quality, and thus, improve the control of crystallization processes.

Polymorphic transformation of pravastatin sodium in a mixture of isopropanol and water was studied by use of online focused beam reflectance measurement (FBRM) and particle vision measurement (PVM). It is shown that the form A polymorph transformed to the stable polymorph, form B. It was speculated that in the transformation process there was an agglomeration and breakage phenomenon. The transformation mechanism was identified as solution-mediated phase transformation. Influences of temperature, solvent composition, and stirrer speed on the transformation process were examined. It can be seen from the FBRM monitoring results that higher temperature, larger ratio of water to isopropanol, and higher stirrer speed can increase the transformation process (Jia et al., 2008).

The application of focused beam reflectance measurement (FBRM) was studied in a larger scale PLGA microparticle preparation process for monitoring changes of the particle size and the particles' surface properties (Vay et al., 2012). Further understanding how these parameters determine the chord length distribution (CLD) was gained by means of single object measurements and data of monodisperse microparticles. It was evaluated how the FBRM signal is influenced by the surface characteristics of the tested materials and the measuring conditions. Particles with good scattering properties provided comparable values of CLD and particle size distribution. Translucent particles caused an overestimation of the particle size by FBRM, whereas the values for transparent emulsion droplets were too low. Despite a strong dependence of FBRM results on the optical properties of the samples, it is a beneficial technique for online monitoring of microparticle preparation processes. The study demonstrated how changing reflection properties can be used to monitor structural changes during the solidification of emulsion droplets and to detect process instabilities by FBRM. The transformation of the emulsion droplets into solid particles can be detected by a change in the FBRM signal. FBRM is a strong tool to provide new insights into the microparticle formation in a solvent removal process.

In another study, Zidan et al. (2010) have employed both Lasentec FBRM and PVM for online monitoring of the chord length distributions of microparticle

manufacturing by O/W single emulsion method using cyclosporine A (CyA) as a model drug and a medium viscosity poly(lactide-co-glycolide) (PLGA) as a matrix forming biodegradable polymer. The qualitative application of the Lasentec focused beam reflectance (FBRM) system for online monitoring of microparticle size distribution was demonstrated. Lasentec particle vision and measurement (PVM) images were also employed to follow up the steps of microparticle formation and ripening. As indicated from the chord count data, FBRM was sensitive to the amount of the solid materials and the number of microparticles formed. Linear relationships with good correlations were obtained between polymer, drug, and surfactant levels. Upon organic solvent evaporation, PVM imaging detected various stages of microemulsion droplets, sheath formation, and solidification with subsequent microparticle hardening. This study illustrated the utility of FBRM and PVM in monitoring the progress of particle formation during drug encapsulation.

Focused beam reflectance measurement (FBRM) has been used to monitor the rate of particle generation from a dissolving tablet. Wilson et al. (2012) have developed a new experimental approach which links tablet disintegration and dissolution into a single measurement framework. FBRM measures the number and size of particles which are generated and subsequently evolve during tablet disintegration. Determination of the number and size of particles generated reveals an aspect of tablet behavior which is entirely overlooked by conventional measurement approaches. This new measurement strategy enables them to consider tablet disintegration and dissolution as processes with reaction rates, rather than as simple measures of extents. The consideration of disintegration and dissolution in terms of reaction rates enables them to build the observable tablet performance data into a mechanistic model. The mechanistic framework they chose is the population balance approach, and this has enabled them to incorporate disintegration and dissolution into a single coherent model. Wilson et al. have used this new measurement and modeling approach to describe and understand the performance of two variants of a single formulation which were engineered to give different dissolution behavior. The model shows that the differences in the dissolution performance of the two variants can be described by more fundamental parameters, i.e. differences in initial particle sizes of the dispersed particles and erosion rates of the tablets.

Yu and Erickson (2008) studied the impact of the solid concentration of aqueous polyvinylchloride (PVC) suspensions and refractive index of dispersing medium on chord

length distribution and particle counts using FBRM. It was found that the chord length distribution increased with the solid concentration in the diluted region, but decreased as the concentration became greater than 1.1%. It was suspected that the initial increase of C_{50} was due to the reduction of the laser penetration depth with increasing solid concentration. The decrease of C_{50} at high solid concentration was probably due to fines crowding the measurement window. The total particle counts were found to increase initially with the solid concentration, tapering off at high concentrations. The influence of the optical property of the dispersing medium on the FBRM measurement was evaluated by suspending the PVC particles in dispersants with varying refractive indices. It was shown that when the PVC particles were dispersed in the dispersant with similar refractive index, the FBRM probe failed to measure the chord length and particle counts properly, yielding significantly smaller chord length and lower particle counts. The results were corroborated by subsequent microscopy studies. The median average values obtained with FBRM and laser diffraction at various solid concentrations were compared, and the square-weighted median of the chord length distribution agreed the best with that of the particle size data.

FBRM has been integrated into flocculation study to examine the comparative performance of process hydroxamate and polyacrylate flocculants on Bayer process bauxite residue slurry. The study has focused on clarity and aggregate strength as key performance indicators. Based on FBRM data, before flocculant addition there is a maximum number of chord counts being measured and the mean square-weighted chord length is at a minimum, indicative of the well dispersed slurry particles. With addition of flocculant the chord length distribution shifts to the right, together with an increase in the mean square-weighted chord length. The significant reduction in the number of chord length counts and increase in the mean square-weighted chord length corresponds to aggregation of the primary slurry particles into larger sized aggregates containing many primary particles. At the maximum in the mean square-weighted chord length, the chord length distribution shows a minimum in the number of counts and the distribution is as far to the right as possible, indicating maximum flocculation. With continued time, the aggregates begin to shear and breakdown with the continued mixing. The mean square-weighted chord length decreases and the chord length distribution begin to grow in counts and shifts back towards the left. FBRM has proved to be a very powerful tool for investigating and comparing the flocculation performance of process hydroxamate and polyacrylate flocculants with Bayer process bauxite residue slurry. The key difference in

polyacrylate and hydroxamate flocculation is the way the aggregates develop post-shear, with hydroxamate aggregates exhibiting a greater degree of post-shear aggregation (Kirwan, 2009).

Al Nasser et al. (2011) have used FBRM as an inline monitoring technique. It is utilized to obtain real-time data of the calcium carbonate deposition on a surface. This research is focused on understanding the behaviour of calcium carbonate agglomeration and scaling in the presence and absence of electronic antifouling (EAF). The calcium carbonate crystals are obtained by standard precipitation and agglomeration processes and the formation and deposition of the crystals is monitored using an inline technique known as focused beam reflectance measurement (FBRM). The experimental work has been designed to understand the effect of EAF on precipitation, agglomeration and scaling of calcium carbonate at given calcium ion concentration and solution temperature. The scaling is characterized by measuring the rate of number of crystals deposited on a surface of the FBRM which, unlike mass measurement as used by previous workers, makes this method unique.

1.6. Objectives

The purposes of this work were:

(1) to investigate effect of type of solvent and type of method on ethyl celluloce 4 cp (EC) microparticle preparation by solvent evaporation method using a focused beam reflectance measurement (FBRM).

(2) to investigate effect of type of polymer on preparation process of polymeric microparticles and microparticle blends by an oil-in-water emulsion method using a focused beam reflectance measurement (FBRM).

(3) to investigate effect of stirring speed, volume of external aqueous phase and polymer concentration on ethyl celluloce 4 cp (EC) microparticle preparation by solvent evaporation method using a focused beam reflectance measurement (FBRM).

(4) to investigate and characterize incorporation a lipophilic and a hydrophilic drugs within non-biodegradable EC microparticle blends using solvent evaporation method.

(5) to investigate effect of various formulation and processing parameters which used in preparation of microparticle blends on the polymeric microparticle blends contained the same drug (propranolol HCl) and contained drugs with different solubility (propranolol HCl and carbamazepine) which prepared by solvent evaporation method.

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Model drugs

Propranolol hydrochloride and carbamazepine (Carl Roth GmbH & Co. KG, Karlsruhe, Germany).

2.1.2. Polymers

Ethyl cellulose (Ethocel® Standard 4 Premium, Colorcon Ltd, Kent, UK), ethyl acrylate methyl methacrylate copolymer (Eudragit® RS 100 and Eudragit® RL 100, Evonik Röhm GmbH, Darmstadt, Germany), Poly(D,L-lactide-co-glycolide) (Resomer® RG503H, Boehringer-Ingelheim Pharma GmbH & Co. KG, Ingelheim, Germany), Poly(ε-caprolactone) (PCL; Mn approx. 10000) (Sigma-Aldrich Chemie GmbH, Steinheim, Germany).

2.1.3. Surfactant

Polyvinyl alcohol (PVA, Mowiol® 40–88, Kuraray Europe GmbH, Frankfurt, Germany).

2.1.4. Solvents

Dichloromethane, methanol, ethyl acetate, chloroform and ethanol (Carl Roth GmbH & Co. KG, Karlsruhe, Germany).

2.1.5. Chemicals

Sodium chloride, sodium hydroxide, potassium dihydrogen phosphate (Carl Roth GmbH & Co. KG, Karlsruhe, Germany)

2.2. Methods

2.2.1. Online monitoring of ethyl cellulose (EC) microparticles preparation process by focused beam reflectance measurement (FBRM): Effect of organic solvent type and preparation method

2.2.1.1. Viscosity measurement

7.5% w/v EC 4 cp solution in dichloromethane, dichloromethane:methanol (1:1), chloroform and ethyl acetate as well as the W/O emulsion from each solvent were analyzed using an Ostwald viscometer type 50111/Ia, instrument constant: K = 0.05152 mm^2/s^2 (Schott-Geräte GmbH, Hofheim, Germany) at 25 ^oC (n = 3). The viscosity were calculated as follows:

$$\nu = K.t \quad (4)$$

ν : kinematic viscosity (mm^2/s or cSt)

K : instrument constant (mm^2/s^2)

t : flow time (s)

2.2.1.2. Solubility determination

The solubility of ethyl cellulose 4 cp was determined in four different solvents (dichloromethane, dichloromethane:methanol (1:1), chloroform and ethyl acetate) by adding ethyl cellulose 4 cp in a glass vial to 1 ml solvent. The vials were sealed and shaken at 37 ^oC and 75 rpm (horizontal shaker GFL 3033, Gesellschaft für Labortechnik GmbH, Burgwedel, Germany) for 24 h (n = 3) to assure saturation. 1 ml of the saturated solution was filtered and the solvent was evaporated at room temperature. After filtration the mass of ethyl cellulose was determined by an analytical balance (Analytical Balance Sartorius Research A200S, Sartorius GmbH, Göttingen, Germany).

2.2.1.3. Microparticle preparation

Solvent evaporation methods based on the formation of either W/O/W or O/W emulsions were applied to prepare microparticles. In W/O/W multiple emulsion method, an aqueous solution was emulsified into a solution of the polymer in either dichloromethane, chloroform, ethyl acetate or dichloromethane:methanol (1:1) (7.5% w/v) by probe sonication (Sonoplus® HD 250, Bandelin Electronic GmbH & Co. KG, Berlin, Germany) for 30 s under ice-cooling. The resulting primary W/O emulsion was dispersed into an external aqueous phase (800 ml 0.25% PVA solution). The emulsion was stirred for

4 h at 500 rpm with a propeller stirrer (Heidolph Elektro GmbH & Co. KG, Kelheim, Germany). After 4 h, the microparticles were separated from the external aqueous phase by wet sieving followed by washing with 200 ml deionized water, desiccator-drying for 24 h and storage in a desiccator.

For the O/W-dispersions, a solution of polymer in either dichloromethane, chloroform, ethyl acetate or dichloromethane:methanol (1:1) (7.5% w/v) was dispersed into an external aqueous phase (800 ml 0.25% PVA solution). The emulsion was stirred for 4 h at 500 rpm with a propeller stirrer. The subsequent process steps were similar to W/O/W method.

2.2.1.4. Online chord length distribution analysis

FBRM probe (Lasentec® FBRM D600T, Mettler Toledo AutoChem, Inc., Redmond, WA, USA) was immersed and positioned in the emulsification vessel (O/W and W/O/W emulsions mentioned above) to ensure good flow against the probe window and hence allowing a representative sample of the particle system to be measured (Fig. 7). The measurement range of the FBRM D600T probe is 0.25 - 4000 μm. A propeller stirrer (Heidolph Elektro GmbH & Co. KG, Kelheim, Germany) was employed beside the probe with speed 500 rpm for 4 hours. FBRM measurements were performed every 10 seconds, during a period of 4 hours. All batches were measured in triplicate. The size information was extracted through the iC FBRM® 4.0 software (Mettler Toledo AutoChem, Inc., Redmond, WA, USA).

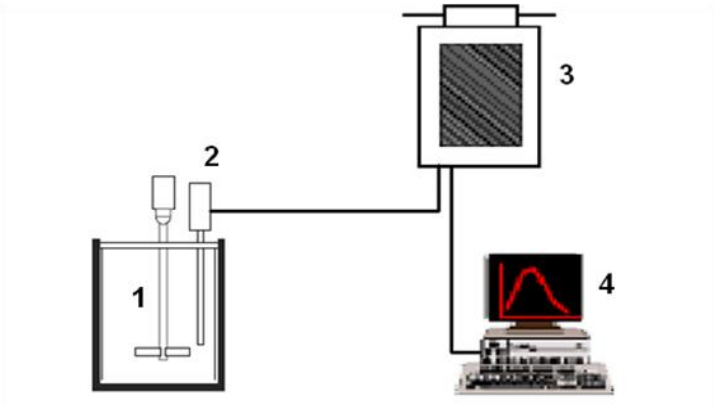

Fig. 7. Schematic drawing of probe positioning relative to the impeller (1. Propeller stirrer; 2. Lasentec® FBRM probe; 3. Processing unit; 4. PC monitoring the particle size distribution on-line)

2.2.1.5. Microparticle characterization

Optical microscopy

Microparticles were spread on microscope slides and observed with an optical light microscope (Axiotrop 50, Carl Zeiss AG, Jena, Germany) equipped with an image analysis system (INTEQ Informationstechnik GmbH, Berlin, Germany) consisting of a digital camera (type MC1) and the EasyMeasure® software (version 1.4.1).

Scanning electron microscopic studies

The external morphology of microparticles was analysed by scanning electron microscopy (SEM). For surface imaging, the microparticles were fixed on a sample holder with double-sided tape. All samples were coated under an argon atmosphere with fine gold to a thickness of 8 nm (SCD 040, Bal-Tec GmbH, Witten, Germany) in a high-vacuum evaporator. Samples were then observed with a scanning electron microscope (S-4000, Hitachi High-Technologies Europe GmbH, Krefeld, Germany).

2.2.2. Online monitoring of preparation process of polymeric microparticles and microparticle blends by focused beam reflectance measurement (FBRM): Effect of polymer type

2.2.2.1. Viscosity measurement

7.5% w/v solution of Ethocel 4 cP, Eudragit® RS 100, Eudragit® RL 100, PLGA (Resomer® RG503H) and poly(ε-caprolactone) in dichloromethane were analyzed using an Ostwald viscometer type 50111/Ia, K = 0.05152 mm^2/s^2 (Schott-Geräte GmbH, Hofheim, Germany) at 25 ^oC (n = 3). The viscosity were calculated as follows:

$$\nu = K.t \quad (5)$$

ν : kinematic viscosity (mm^2/s or cSt)

K : instrument constant (mm^2/s^2)

t : flow time (s)

2.2.2.2. Solubility determination

The solubility of five different polymers were determined in dichloromethane by adding polymer in a glass vial to 1 ml dichloromethane. The vials were sealed and shaken at 37 ^oC and 75 rpm in horizontal shaker for 24 h (n = 3) to assure saturation. 1 ml of the saturated solution was filtered and the solvent was evaporated at room temperature. After filtration the mass of polymer was determined by an analytical balance.

2.2.2.3. Microparticles preparation

Polymeric microparticles

The solvent evaporation method based on the formation of O/W emulsion was used to prepare microparticles. In the O/W-dispersion method, a solution of the polymer in dichloromethane (7.5% w/v) was dispersed into an external aqueous phase (800 ml 0.25% PVA solution). The emulsion was stirred for 4 h at 500 rpm with a propeller stirrer (Heidolph Elektro GmbH & Co. KG, Kelheim, Germany). After 4 h, the microparticles were separated from the external aqueous phase by wet sieving followed by washing with 200 ml deionized water, desiccator-drying for 24 h and storage in a desiccator.

Microparticle blends

The first and second oil phase were prepared by O/W method. A first oil phase was a solution of Ethocel® 4 cp in dichloromethane (7.5% w/v). Five different second oil phase

were 7.5% w/v solution of Ethocel® 4 cp, Eudragit® RS 100, Eudragit® RL 100, PLGA (Resomer® RG503H) and poly(ε-caprolactone) in dichloromethane. The first oil phase and on of the second oil phase were dispersed in an external aqueous phase (800 ml 0.25% PVA solution), with dispersion time intervals (DTI) of 0 and 60 min, and stirred for 4 h at 500 rpm with a propeller stirrer to allow microparticle hardening. The subsequent process steps were similar to the above process.

2.2.2.4. Online chord length distribution analysis

FBRM probe (Lasentec® FBRM D600T, Mettler Toledo AutoChem, Inc., Redmond, WA, USA) was immersed and positioned in the emulsification vessel (O/W emulsions) to ensure good flow against the probe window and hence allowing a representative sample of the particle system to be measured (Fig. 7 and Fig. 8). The measurement range of the FBRM D600T probe is 0.25 - 4000 μm. A propeller stirrer (Heidolph Elektro GmbH & Co. KG, Kelheim, Germany) was employed beside the probe with speed 500 rpm for 4 hours. FBRM measurements were performed every 10 seconds, during a period of 4 hours. All batches were measured in triplicate. The size information was extracted through the iC FBRM® 4.0 software (Mettler Toledo AutoChem, Inc., Redmond, WA, USA).

2.2.2.5. Microparticle characterization

Optical microscopy

Microparticles were spread on microscope slides and observed with an optical light microscope (Axiotrop 50, Carl Zeiss AG, Jena, Germany) equipped with an image analysis system (INTEQ Informationstechnik GmbH, Berlin, Germany) consisting of a digital camera (type MC1) and the EasyMeasure® software (version 1.4.1).

Scanning electron microscopic studies

The external morphology of microparticles was analysed by scanning electron microscopy (SEM). For surface imaging, the microparticles were fixed on a sample holder with double-sided tape. All samples were coated under an argon atmosphere with fine gold to a thickness of 8 nm (SCD 040, Bal-Tec GmbH, Witten, Germany) in a high-vacuum evaporator. Samples were then observed with a scanning electron microscope (S-4000, Hitachi High-Technologies Europe GmbH, Krefeld, Germany).

2.2.3. Online monitoring of ethyl cellulose (EC) microparticles preparation process by focused beam reflectance measurement (FBRM): Effect of stirring speed, volume of external aqueous phase and polymer concentration

2.2.3.1. Microparticles preparation

Conventional microparticles

The solvent evaporation method based on the formation of O/W emulsion was used to prepare microparticles. In the O/W-dispersion method, a solution of the polymer in dichloromethane (7.5% w/v) was dispersed into an external aqueous phase (800 ml 0.25% PVA solution). The emulsion was stirred for 4 h at 500 rpm with a propeller stirrer (Heidolph Elektro GmbH & Co. KG, Kelheim, Germany). After 4 h, the microparticles were separated from the external aqueous phase by wet sieving followed by washing with 200 ml deionized water, desiccator-drying for 24 h and storage in a desiccator.

For investigating the effect of volume of external aqueous phase, a solution of EC in dichloromethane (7.5% w/v) was dispersed into an external aqueous phase (400 ml and 800 ml, 0.25% PVA solution). The emulsion was stirred for 4 h at 200 rpm with a propeller stirrer. The subsequent process steps were similar as mentioned above.

Microparticle blends

The first and second oil phase were prepared by O/W method. A first and second oil phase was a solution of Ethocel® 4 cp in dichloromethane (7.5% w/v). The first oil phase and on of the second oil phase were dispersed in an external aqueous phase (800 ml 0.25% PVA solution), with dispersion time intervals (DTI) of 60 min, and stirred for 4 h at 200 rpm to 500 rpm and 500 rpm to 200 rpm with a propeller stirrer to allow microparticle hardening. The subsequent process steps were similar to the above process.

2.2.3.2. Online chord length distribution analysis

FBRM probe (Lasentec® FBRM D600T, Mettler Toledo AutoChem, Inc., Redmond, WA, USA) was immersed and positioned in the emulsification vessel (O/W emulsions) to ensure good flow against the probe window and hence allowing a representative sample of the particle system to be measured (Fig. 7 and Fig. 8). The measurement range of the FBRM D600T probe is 0.25 - 4000 μm. A propeller stirrer (Heidolph Elektro GmbH & Co. KG, Kelheim, Germany) was employed beside the probe

with speed 200 rpm and 500 rpm for 4 hours. FBRM measurements were performed every 10 seconds, during a period of 4 hours. All batches were measured in triplicate. The size information was extracted through the iC FBRM® 4.0 software (Mettler Toledo AutoChem, Inc., Redmond, WA, USA).

2.2.3.3. Microparticle characterization by scanning electron microscopy

The external morphology of microparticles was analysed by scanning electron microscopy (SEM). For surface imaging, the microparticles were fixed on a sample holder with double-sided tape. All samples were coated under an argon atmosphere with fine gold to a thickness of 8 nm (SCD 040, Bal-Tec GmbH, Witten, Germany) in a high-vacuum evaporator. Samples were then observed with a scanning electron microscope (S-4000, Hitachi High-Technologies Europe GmbH, Krefeld, Germany).

2.2.4. Slow release of propranolol HCl from ethyl cellulose based microparticle blends

2.2.4.1. Microparticle preparation

Microparticle containing propranolol HCl or carbamazepine

Drug loaded microparticles based on ethyl cellulose were prepared using an oil-in-water (O/W) and a water-in-oil-in-water (W/O/W) solvent evaporation method. The drug loaded systems contained either one drug only (propranolol HCl or carbamazepine). For the O/W method, 300 mg of ethyl cellulose were dissolved in 3 ml dichloromethane. 43 mg carbamazepine were dissolved within this organic phase. The organic phase was then emulsified into 800 ml aqueous PVA solution (0.25% w/v) containing 0.5 M NaCl and NaOH at pH 12. The emulsion was stirred for 4 h at 500 rpm with a propeller stirrer (Heidolph Elektro GmbH & Co. KG, Kelheim, Germany) to allow microparticle hardening.

For the W/O/W method, 43 mg propranolol HCl were dissolved in 0.25 g purified deionized water. Propranolol HCl aqueous solution was first emulsified by probe sonication (Sonoplus® HD 250, Bandelin Electronic GmbH & Co. KG, Berlin, Germany) for 30 s under ice-cooling into 3 ml dichloromethane containing 300 mg of ethyl cellulose. This first emulsion (W/O) was then dispersed into 800 ml aqueous PVA solution (0.25% w/v) containing 0.5 M NaCl and NaOH at pH 12. A W/O/W emulsion was formed by extensive stirring with a propeller stirrer for 4 h at 500 rpm to allow microparticle hardening. In all cases, after 4 h the microparticles were separated from the external aqueous phase by wet sieving (stainless steel test sieves ISO 3310 - 40, 70, 100 and 160 µm) followed by washing with 200 ml deionized water, desiccator-drying for 24 h and storage in a desiccator.

Microparticle blends

Microparticle blends containing propranolol HCl

The first and second primary emulsion were prepared by W/O/W method. A first primary emulsion containing propranolol HCl was prepared as follows: 43 mg propranolol HCl were dissolved in 0.25 g purified deionized water. Propranolol HCl aqueous solution was emulsified by probe sonication for 30 s under ice-cooling into 3 ml dichloromethane containing 300 mg of ethyl cellulose. Four different second primary emulsion were prepared: (1) The same formulation as the first primary emulsion, (2) 3 ml

dichloromethane containing 300 mg of ethyl cellulose, but with a drug loading twice as high as the first primary emulsion, (3) 3 ml dichloromethane containing 300 mg of ethyl cellulose and (4) 3 ml dichloromethane. The first primary emulsion containing propranolol HCl and on of the second primary emulsion phases were dispersed in an external aqueous phase (800 ml aqueous PVA solution [0.25% w/v] containing 0.5 M NaCl and NaOH at pH 12), with dispersion time intervals (DTI) of 0 and 60 min, and stirred for 4 h at 500 rpm with a propeller stirrer to allow microparticle hardening. The subsequent process steps were similar to the above process.

Microparticle blends containing propranolol HCl and carbamazepine

The first primary emulsion containing propranolol HCl (W/O/W) and second primary oil phase containing carbamazepine (O/W). For the W/O/W method, 43 mg propranolol HCl were dissolved in 0.25 g purified deionized water. Propranolol HCl aqueous solution was first emulsified by probe sonication for 30 s under ice-cooling into 3 ml dichloromethane containing 300 mg of ethyl cellulose. This gave the first primary emulsion containing propranolol HCl. For the O/W method, 300 mg of ethyl cellulose were dissolved in 3 ml dichloromethane. 43 mg carbamazepine were then dissolved in this organic phase. This process produced the second primary oil phase containing carbamazepine. Following, the first primary emulsion containing propranolol HCl and the second primary oil phase containing carbamazepine were dispersed in an external aqueous phase (800 ml aqueous PVA solution [0.25% w/v] containing 0.5 M NaCl and NaOH at pH 12), with dispersion time intervals (DTI) of 0 and 60 min, and stirred for 4 h at 500 rpm with a propeller stirrer to allow microparticle hardening. The subsequent process steps were similar to the preparation of microparticle containing single drug process.

2.2.4.2. Determination of the actual drug loading and encapsulation efficiency

Microparticles (10 mg) were extracted in 1 ml methanol, followed by agitation in a horizontal shaker (IKA HS 501 digital horizontal Shaker, Janke & Kunkel GmbH & Co. KG IKA Labortechnik, Staufen, Germany) for 2 h (n = 3). 0.1 ml of methanol extract was diluted in 10 ml of pH 7.4 phosphate buffer. The polymer was separated from aqueous solution by filtration using filter paper (Whatman®, GE Healthcare UK Limited, Buckinghamshire, UK). Propranolol HCl and/or carbamazepine concentration in the obtained aqueous solution was determined by UV-spectrophotometry at wavelengths of 289 nm and 285 nm, respectively (HP 8453 UV-Vis spectrophotometer, Agilent

Technologies Deutschland GmbH, Waldbronn, Germany). The actual drug loading and encapsulation efficiency were calculated as follows:

Actual drug loading (%) = (drug mass in microparticles/mass of microparticles) x 100 % (6)

Encapsulation efficiency (%) = (actual drug loading/theoretical drug loading) x 100 % (7)

For microparticle blends, the amounts of incorporated propranolol HCl and carbamazepine were determined UV-spectrophotometrically by simultaneously measuring at wavelengths of 227 and 285 nm. The subsequent process steps were similar to the above process.

2.2.4.3. Particle size analysis

Particle size mean and size distribution of the microparticles were measured by focused beam reflectance measurement. FBRM probe (Lasentec® FBRM D600T, Mettler Toledo AutoChem, Inc., Redmond, WA, USA) was immersed and positioned in the emulsification vessel (WO/W and O/W emulsions mentioned above) to ensure good flow against the probe window and hence allowing a representative sample of the particle system to be measured (Fig. 8). The measurement range of the FBRM D600T probe is 0.25 - 4000 μm. In these experiments, FBRM measurements were performed every 10 seconds, during a period of 4 h. All batches were measured in triplicate. The size information was extracted through the iC FBRM® 4.0 software (Mettler Toledo AutoChem, Inc., Redmond, WA, USA).

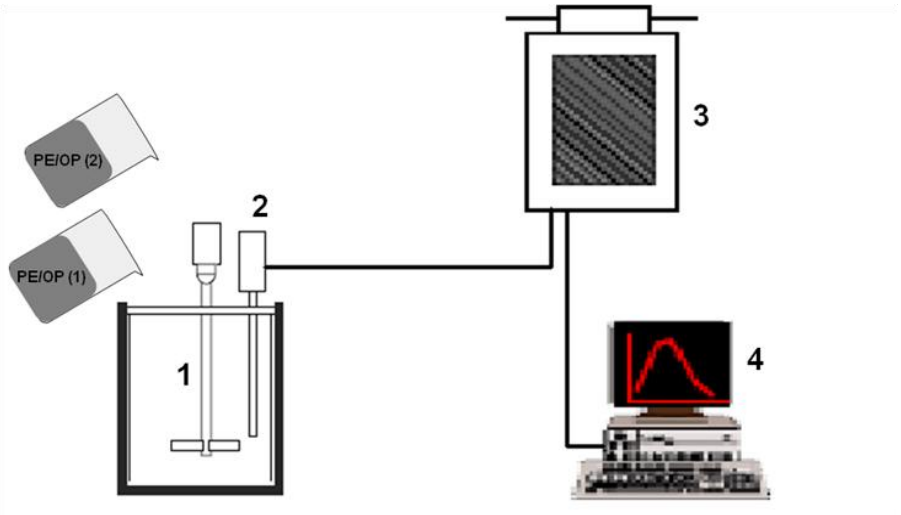

Fig. 8. Schematic drawing of probe positioning relative to the impeller (1. Propeller stirrer; 2. Lasentec® FBRM probe; 3. Processing unit; 4. PC monitoring the particle size distribution on-line). PE: Primary Emulsion, OP: Oil Phase

2.2.4.4. Microparticle characterization

Optical microscopy

Microparticles were spread on microscope slides and observed with an optical light microscope (Axiotrop 50, Carl Zeiss AG, Jena, Germany) equipped with an image analysis system (INTEQ Informationstechnik GmbH, Berlin, Germany) consisting of a digital camera (type MC1) and the EasyMeasure® software (version 1.4.1).

Scanning electron microscopy

The external and internal morphology of microparticles was analysed by scanning electron microscopy (SEM). For surface imaging, the microparticles were fixed on a sample holder with double-sided tape. To investigate the inner structure, the particles were spread on transparent tape and then cut with a razor blade. All samples were coated under argon atmosphere with gold to a thickness of 8 nm in a high-vacuum (SCD 040, Bal-Tec GmbH, Witten, Germany). Samples were then analysed on the scanning electron microscope (S-4000, Hitachi High-Technologies Europe GmbH, Krefeld, Germany).

2.2.4.5. In vitro drug release studies

10 mg microparticles/microparticle blends (particle size: < 70 µm) were placed in 10 ml pH 7.4 phosphate buffer (USP XXIV) and shaken at 37 ^{o}C in a horizontal shaker (GFL 3033, Gesellschaft für Labortechnik GmbH, Burgwedel, Germany) at 75 rpm. At predetermined time points, 1 ml samples were withdrawn and replaced with 1 ml fresh medium each 7 days, filtered and analyzed. Propranolol HCl and/or carbamazepine concentration was detected UV spectrophotometrically at wavelengths of 289 nm and 285 nm, respectively (n = 3) (HP 8453 UV-Vis spectrophotometer, Agilent Technologies Deutschland GmbH, Waldbronn, Germany).

For microparticle blends, the concentration of propranolol HCl and carbamazepine were determined UV-spectrophotometrically by simultaneously measuring at wavelengths of 227 and 285 nm (n = 3).

2.2.5. Microparticle blends system for controlled delivery of propranolol HCl and carbamazepines: Influence of the formulation and processing parameters

2.2.5.1. Microparticle preparation

Conventional Microparticles containing propranolol HCl or carbamazepine

Drug loaded microparticles based on ethyl cellulose/PLGA Rg502H/polycaprolactone were prepared using an oil-in-water (O/W) and a water-in-oil-in-water (W/O/W) solvent evaporation method. The drug loaded systems contained either one drug only (propranolol HCl or carbamazepine). For the O/W method, 300 mg of ethyl cellulose were dissolved in 4 ml dichloromethane. 60 mg carbamazepine were dissolved within this organic phase. The organic phase was then emulsified into 800 ml aqueous PVA solution (0.25% w/v) containing 0.5 M NaCl and NaOH at pH 12. The emulsion was stirred for 4 h at 500 rpm with a propeller stirrer (Heidolph Elektro GmbH & Co. KG, Kelheim, Germany) to allow microparticle hardening.

For the W/O/W method, 60 mg propranolol HCl were dissolved in 0.25 g purified deionized water. Propranolol HCl aqueous solution was first emulsified by probe sonication (Sonoplus® HD 250, Bandelin Electronic GmbH & Co. KG, Berlin, Germany) for 30 s under ice-cooling into 4 ml dichloromethane containing 300 mg of ethyl cellulose. This first emulsion (W/O) was then dispersed into 800 ml aqueous PVA solution (0.25% w/v) containing 0.5 M NaCl and NaOH at pH 12. A W/O/W emulsion was formed by extensive stirring with a propeller stirrer for 4 h at 500 rpm to allow microparticle hardening. In all cases, after 4 h the microparticles were separated from the external aqueous phase by wet sieving (stainless steel test sieves ISO 3310 - 70 µm) followed by washing with 200 ml deionized water, desiccator-drying for 24 h and storage in a desiccator.

Microparticle blends

Microparticle blends containing propranolol HCl

The first and second primary emulsion were prepared by W/O/W method. The first and second primary emulsion containing propranolol HCl was prepared as follows: 60 mg propranolol HCl were dissolved in 0.25 g purified deionized water. Propranolol HCl aqueous solution was emulsified by probe sonication for 30 s under ice-cooling into 4 ml dichloromethane containing 300 mg of ethyl cellulose. The first primary emulsion

containing propranolol HCl and on of the second primary emulsion phases were dispersed in an external aqueous phase (800 ml aqueous PVA solution [0.25% w/v] containing 0.5 M NaCl and NaOH at pH 12), with dispersion time intervals (DTI) of 0, 5, 30 and 60 min, and stirred for 4 h at 500 rpm with a propeller stirrer to allow microparticle hardening. The subsequent process steps were similar to the above process.

Microparticle blends containing propranolol HCl and carbamazepine

The first primary emulsion containing propranolol HCl (W/O/W) and second primary oil phase containing carbamazepine (O/W). For the W/O/W method, 60 mg propranolol HCl were dissolved in 0.25 g purified deionized water. Propranolol HCl aqueous solution was first emulsified by probe sonication for 30 s under ice-cooling into 4 ml dichloromethane containing 300 mg of ethyl cellulose. This gave the first primary emulsion containing propranolol HCl. For the O/W method, 300 mg of ethyl cellulose were dissolved in 4 ml dichloromethane. 60 mg carbamazepine were then dissolved in this organic phase. This process produced the second primary oil phase containing carbamazepine. Following, the first primary emulsion containing propranolol HCl and the second primary oil phase containing carbamazepine were dispersed in an external aqueous phase (800 ml aqueous PVA solution [0.25% w/v] containing 0.5 M NaCl and NaOH at pH 12), with dispersion time intervals (DTI) of 0, 5, 30 and 60 min, and stirred for 4 h at 500 rpm with a propeller stirrer to allow microparticle hardening. The subsequent process steps were similar to the preparation of microparticle containing single drug process.

2.2.5.2. Determination of the actual drug loading and encapsulation efficiency

Microparticles (10 mg) were extracted in 1 ml methanol, followed by agitation in a horizontal shaker (IKA HS 501 digital horizontal Shaker, Janke & Kunkel GmbH & Co. KG IKA Labortechnik, Staufen, Germany) for 2 h (n = 3). 0.1 ml of methanol extract was diluted in 10 ml of pH 7.4 phosphate buffer. The polymer was separated from aqueous solution by filtration using filter paper (Whatman®, GE Healthcare UK Limited, Buckinghamshire, UK). Propranolol HCl and/or carbamazepine concentration in the obtained aqueous solution was determined by UV-spectrophotometry at wavelengths of 289 nm and 285 nm, respectively (HP 8453 UV-Vis spectrophotometer, Agilent Technologies Deutschland GmbH, Waldbronn, Germany). The actual drug loading and encapsulation efficiency were calculated as follows:

Actual drug loading (%) = (drug mass in microparticles/mass of microparticles) x 100 %
Encapsulation efficiency (%) = (actual drug loading/theoretical drug loading) x 100 %.

For microparticle blends, the amounts of incorporated propranolol HCl and carbamazepine were determined UV-spectrophotometrically by simultaneously measuring at wavelengths of 227 and 285 nm. The subsequent process steps were similar to the above process.

2.2.5.3. Microparticle characterization

Optical microscopy

Microparticles were spread on microscope slides and observed with an optical light microscope (Axiotrop 50, Carl Zeiss AG, Jena, Germany) equipped with an image analysis system (INTEQ Informationstechnik GmbH, Berlin, Germany) consisting of a digital camera (type MC1) and the EasyMeasure® software (version 1.4.1).

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The external and internal morphology of microparticles was analysed by scanning electron microscopy (SEM). For surface imaging, the microparticles were fixed on a sample holder with double-sided tape. To investigate the inner structure, the particles were spread on transparent tape and then cut with a razor blade. All samples were coated under argon atmosphere with gold to a thickness of 8 nm in a high-vacuum (SCD 040, Bal-Tec GmbH, Witten, Germany). Samples were then analysed on the scanning electron microscope (S-4000, Hitachi High-Technologies Europe GmbH, Krefeld, Germany).

2.2.5.4. In vitro drug release studies

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For microparticle blends, the concentration of propranolol HCl and carbamazepine were determined UV-spectrophotometrically by simultaneously measuring at wavelengths of 227 and 285 nm (n = 3).

3. RESULTS AND DISCUSSION

3.1. Online monitoring of ethyl cellulose (EC) microparticles preparation process by focused beam reflectance measurement (FBRM): Effect of organic solvent type and preparation method

3.1.1. Introduction

Preparation of microparticles by solvent evaporation is widely used in pharmaceutical industry. It can be applied for encapsulation of a broad range of substances, from simple drugs to proteins and DNA (Bodmeier and Mc Ginity, 1988; Freitas et al., 2005; Jeyanthi et al., 1996; O'Donell and McGinity, 1997; Yan et al., 1994). There are several variation of the solvent evaporation technique that have been developed to get efficient drug encapsulation for hydrophilic and hydrophobic drugs. For insoluble or poorly water-soluble drugs, the oil-in-water (O/W) method is frequently used. While for water soluble drugs, the water-in-oil-in-water (W/O/W) method is preferred (Freitas et al., 2005; O'Donell and McGinity, 1997; Yamuda et al., 2001; Yan et al., 1994; Yeo et al., 2004).

In case of the preparation of polymeric microparticles for sustained drug release by solvent evaporation technique, the solidification rate is a decisive factor for their release behaviour. A very slow hardening of the emulsion droplets leads to the diffusion of the drug substance out of the droplets and encapsulation efficiency becomes low. Solidification rate of polymeric microparticles during solvent evaporation process was influenced solubility of polymers in organic solvents and solubility organic solvent in water, which in turn affects microparticle properties such as particle size, drug incorporation, matrix porosity, solvent residues and initial burst (Jeyanthi et al., 1997; Maa and Hsu, 1997; Mehta et al., 1994, 1996; Sansdrap and Moes, 1993). Various types of solvent with different physical properties (such as miscibility or solubility, volatility, boiling point, reactivity, viscosity, etc.) have been used to prepare polymeric microparticles. Earlier chloroform was frequently used, but due to its toxicity and low vapour pressure, it is gradually replaced by dichloromethane. Dichloromethane is the most common solvent for the encapsulation using solvent evaporation technique because of its high volatility, low boiling point and high immiscibility with water (Li et al., 2008). Ethyl acetate shows promising potential as a less toxic substitute of dichloromethane. But ethyl acetate had high solubility in water, leading to the loss of drug and resulting low encapsulation efficiency (Li et al., 2008). Additionally, organic solvents miscible with

water such as methanol and ethanol was frequently used as co-solvent. They are used to dissolve the drug that is not totally soluble in the solvent in the dispersed phase (Graves et al., 2006; Hsu and Lin, 2005; Luan et al., 2006; Reithmeier et al., 2001). Additionally, physical properties of solvent would affect optical properties or opacity level from obtained microparticles. Information about the polymeric microparticles hardening rate from these solvents is not available. It is necessary to know the effect of solvent properties on the polymeric microparticle hardening time.

To date very little work has been carried out on the online monitoring of microparticles during their formation. A technique for on line monitoring of microparticle preparation process by solvent evaporation method is the focused beam reflectance measurement (FBRM). FBRM is based on reflection measurement and strongly depending on the optical properties of particle. It does not require sampling that could affect the actual particle size distribution due to breakdown or aggregation. FBRM measures and monitores the chord length distribution (CLD), particle size mean and chord count of microparticles in real time, which is affected by the geometry, size, and number of particles under analysis (Boxall et al., 2010; Dowding et al., 2001; Heath et al., 2002; Kail et al., 2009; Kirwan, 2009; Kougoulos et al., 2005; Leba et al., 2010; Wynn, 2003; Yu and Erickson, 2008). Additionally, the measurement principle of FBRM differs fundamentally from other established particle sizing methods. The FBRM instrument consists of a probe which is placed into the reaction vessel or process stream. Within the probe, a laser is focused on the surface of a sapphire window by a set of rotating optics. When the laser encounters a particle, the light is reflected back to the detector. The reflected beam signal provides information on the number of particles present and one-dimensional information on the size of the particles, referred to as chord lengths, as a function of time (Boxall et al., 2010; Kail et al., 2009; Kirwan, 2009; Leba et al., 2010; Wynn, 2003). So, the transformation of the emulsion droplets into solid microparticles can be monitored by Focused beam reflectance measurement (FBRM).

FBRM has already been successfully applied for suspensions and crystallization processes (Abbas et al., 2002; Barrett and Glennon, 1999; Yu et al., 2005) and has also been studied for fluid bed granulation in comparison with other PAT tools (Burggraeve et al., 2013; Hu et al., 2008). Polymorphic transition monitoring (Jia et al., 2008), flocculation process design (Kirwan, 2009), control of particle disruption (Kougoulos et al., 2005), solubility measurements (Kim et al., 2005), tablet disintegration and dissolution

(Wilson et al., 2012) are some other applications where the use of FBRM has already been reported. Furthermore several studies using the FBRM for investigation of emulsion systems (Dowding et al., 2001; Turner et al., 2009) were published. Zidan et al. (2010) have studied the used of FBRM in order to monitor a solvent evaporation process for microparticle preparation with different polymer concentration and different drug loading. They found the transformation of the emulsion droplets into solid particles can be detected by a change in the FBRM signal. Vay et al. (2012) used FBRM in solvent evaporation process and found out that the transformation of the emulsion droplets into solid particles was influenced by stirring speed and temperature. Interestingly, in most studies reported so far in the literature, only little knowledge is yet available on the online monitoring by FBRM, especially on microparticle formation by solvent evaporation method. Online monitoring of microparticle formation with different solvent and method using FBRM has not been reported yet. Based on this fact, the current study aimed to employ FBRM for online monitoring of solidification rate of microparticles and particle size/distribution of the emulsion droplets/hardened microparticles manufactured by O/W and W/O/W methods using various solvent.

3.1.2. Results and discussion

3.1.2.1. Microparticles morphology and size

SEM photomicrograph of ethyl cellulose (EC) microparticles which prepared by O/W and W/O/W are shown in Fig. 9 and Fig. 10. EC microparticles prepared by O/W method were spherical with smooth surfaces, non porous and no aggregation when using dichloromethane or dichloromethane/methanol (1:1) as solvent (Fig. 9. a3 and b3). When chloroform was used spherical microparticles with rough surface and non porous were obtained (Fig. 9.c3), ethyl acetate as solvent produces spherical microparticles with hole on the surface (donut shape) (Fig. 9.d3). While, EC microparticles prepared by W/O/W method were spherical with rough and smooth surface. A rough surface with golf ball like shape of microparticles was obtained from dichloromethane (Fig. 10.a3), while a rough surface with micropores was obtained from chloroform solvent (Fig. 10.c3). The use of the co-solvent dichloromethane/methanol (1:1) and system of ethyl acetate resulted in donut shaped microparticles with smooth surface (Fig. 10.b3 and d3).

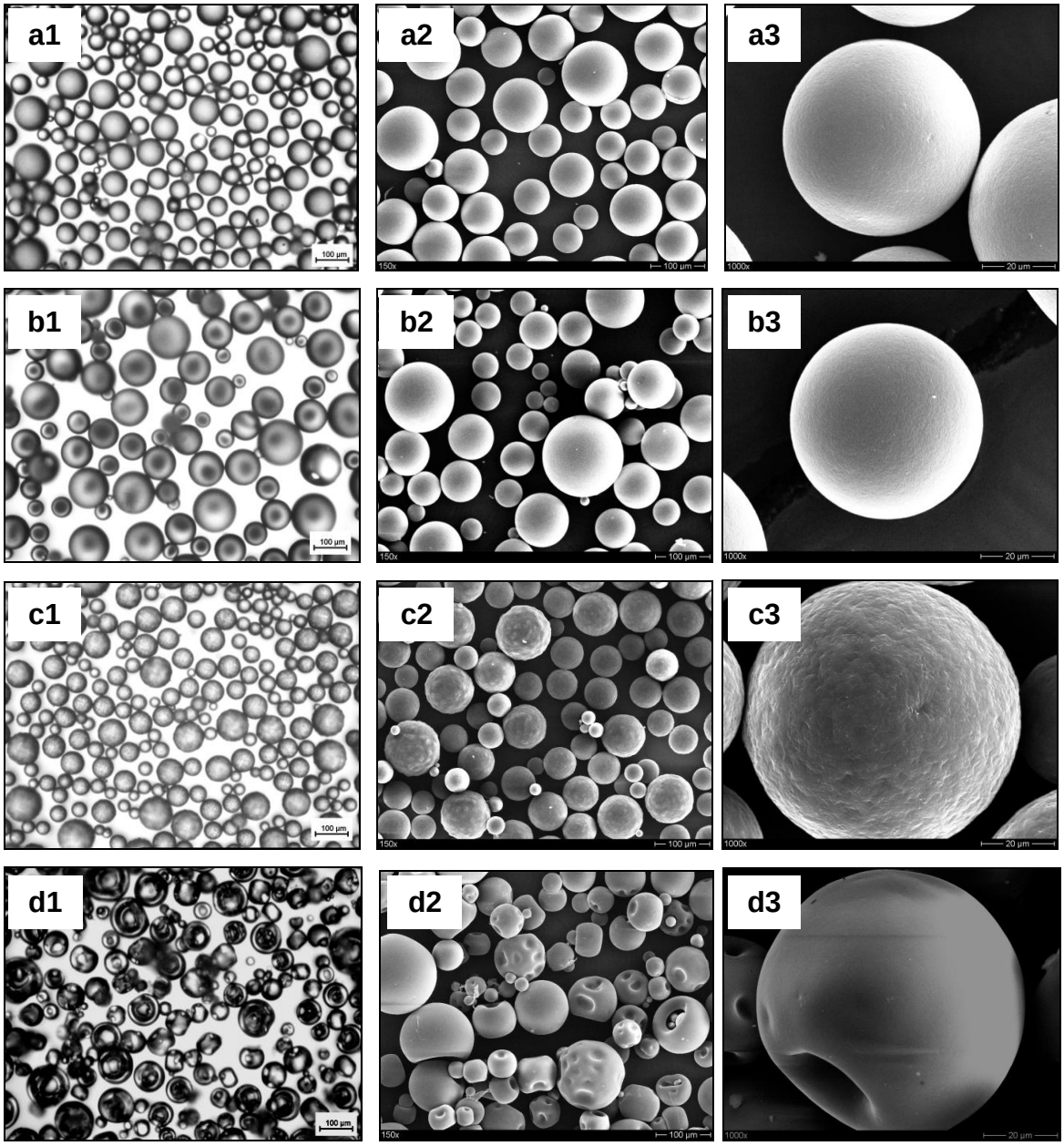

Fig. 9. Optical microscopy pictures (1) and SEM Photomicrographs (2. lower magnification; 3. higher magnification) of ethyl cellulose based microparticles which prepared by O/W (a. dichloromethane; b. dichloromethane:methanol (1:1); c. chloroform; d. ethyl acetate)

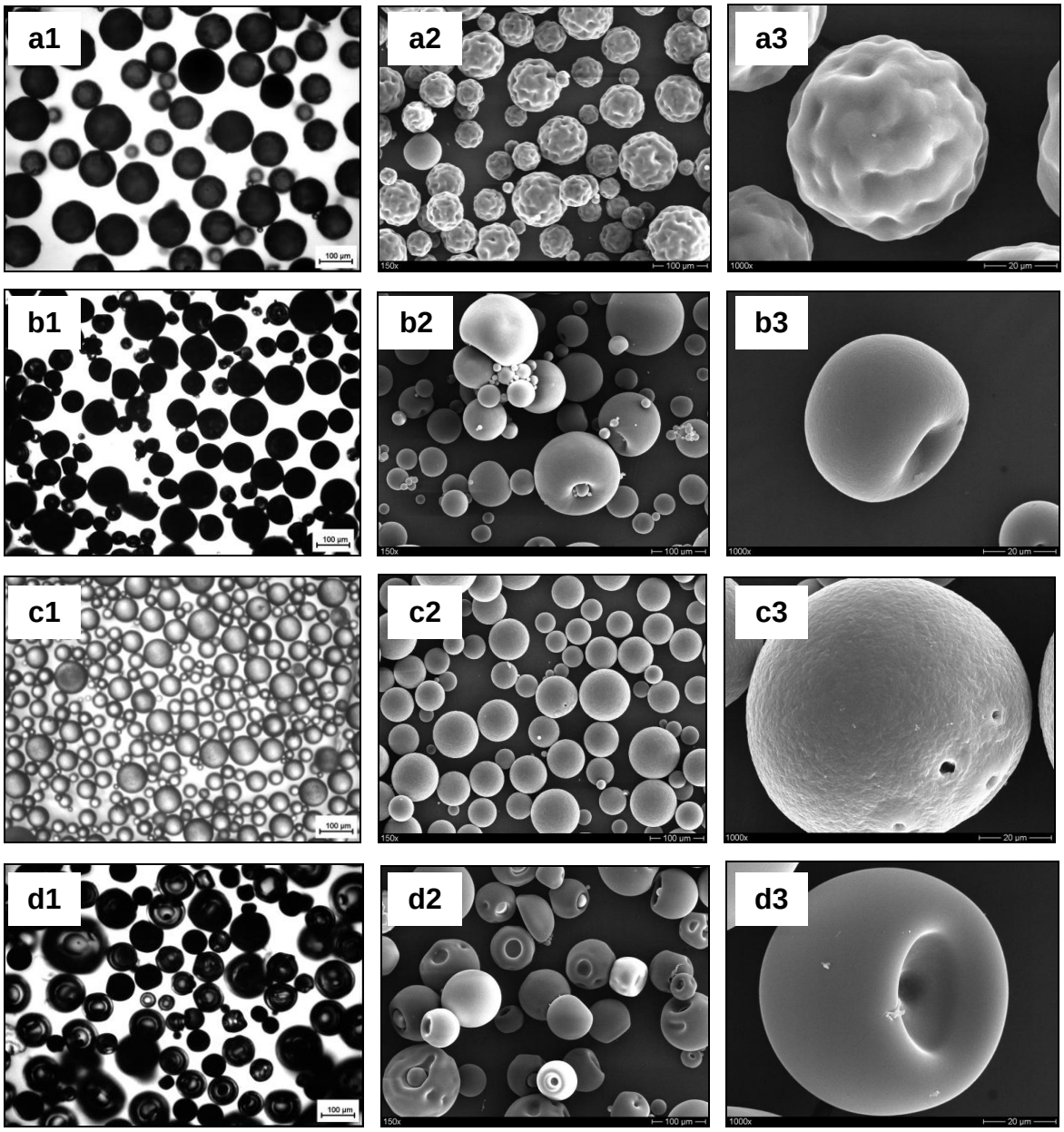

Fig. 10. Optical microscopy pictures (1) and SEM Photomicrographs (2. lower magnification; 3. higher magnification) of ethyl cellulose based microparticles which prepared by W/O/W (a. dichloromethane; b. dichloromethane:methanol (1:1); c. chloroform; d. ethyl acetate)

The different external structure of microparticles which prepared by O/W and W/O/W method could be explained with the different rates of polymer precipitation. Ethyl acetate has a higher water solubility (approx. 8.7%) compared to chloroform (approx. 0.8%), dichloromethane (approx. 1.3%) and dichloromethane/methanol (1:1) (approx. 1.3%:completely miscible) (Cleland et al., 1997). It will therefore diffuse rapidly into the

external aqueous phase leading to polymer precipitation at the droplet surface and the formation of polymeric shell with still large amounts of solvent being present. The solidified droplet surface inhibits further droplet shrinkage, consequently the rest of solvent is extracted by external penetration of the aqueous phase inside the embryonic microparticles, hence the porous and hollow nature of the microparticles is formed. Due to the low solubility of chloroform, dichloromethane and dichloromethane/methanol (1:1) in water, the liquid polymer droplets slowly shrink and the internal solvent diffuse out in a repetitive cycle of dissolution and re-solidification of the polymer instead of the external phase penetration. Therefore, the resulting microparticles had a dense structure and no pores on the external surface. While highly porous microparticles were prepared by a double emulsion (W/O/W). It was caused by the inner aqueous phase. The aqueous droplets are precursors of pores and are the result of phase separation occurring in the organic phase during the hardening of the microparticles.

Table 1. Effect of solvent type and preparation method on EC solubility and viscosity of EC solution

Solvent	**Solubility (g/ml) (± SD)**	**Viscosity (cSt) (± SD)**	
		O/W	**W/O/W**
dichloromethane	0.86 (± 0.03)	10.31 (± 1.14)	17.52 (± 1.25)
dichloromethane/methanol (1:1)	0.64 (± 0.06)	13.92 (± 1.03)	15.74 (± 1.17)
chloroform	1.06 (± 0.04)	9.08 (± 0.94)	9.71 (± 1.06)
ethyl acetate	0.79 (± 0.02)	11.45 (± 1.06)	14.85 (± 1.21)

Solubility of organic solvent in water influenced the morphology of microparticles. Mean diameter of microparticles ranged from 59 to 133 μm (FBRM) (Table 2). Generally, the microparticles prepared with the W/O/W method were larger than those prepared by O/W method, which can be attributed to higher viscosity of primary W/O emulsion (Table 1) than organic phase (polymer solution).

Table 2. Effect of solvent type and preparation method on particle size mean of microparticles

Solvent	Particle size mean (µm) (± SD)			
	FBRM		Optical microscope	
	O/W	W/O/W	O/W	W/O/W
dichloromethane	83.24 (± 5.28)	133.28 (± 4.36)	88.78 (± 7.64)	137.51 (± 5.82)
dichloromethane/methanol (1:1)	92.17 (± 4.15)	124.41 (± 6.17)	95.06 (± 8.57)	129.64 (± 6.59)
chloroform	59.31 (± 6.47)	60.25 (± 5.35)	61.39 (± 5.18)	64.15 (± 3.95)
ethyl acetate	73.11 (± 5.13)	113.39 (± 3.19)	77.46 (± 8.25)	116.75 (± 7.35)

3.1.2.2. Online monitoring of ethyl cellulose microparticles formation

Microparticle formation and hardening rate

In this study, FBRM was used for a qualitative online monitoring of the shift in the chord length distributions (CLD) at various stages of microparticle formation and ripening at a certain agitation rate. The chord length was correlated to changes in the particle size, hardening rate, and particle properties. Particle sizing measurements were performed during stirring and solvent evaporation on eight microparticle batches at 500 rpm agitation rate for 4 hours. The curve of square weighted mean chord length of microparticles which prepared by O/W and W/O/W method show as a function of time (Fig. 11a and Fig. 12a). Both methods, the O/W as well as the W/O/W lead to a different square weighted mean chord length profile during solvent evaporation method. Initially as the organic polymer solution was emulsified, FBRM detected larger droplet size mean of all type of solvents, by increasing the process time the particle size decreased followed by a plateau size where the square weighted mean chord length was constant for all solvent.

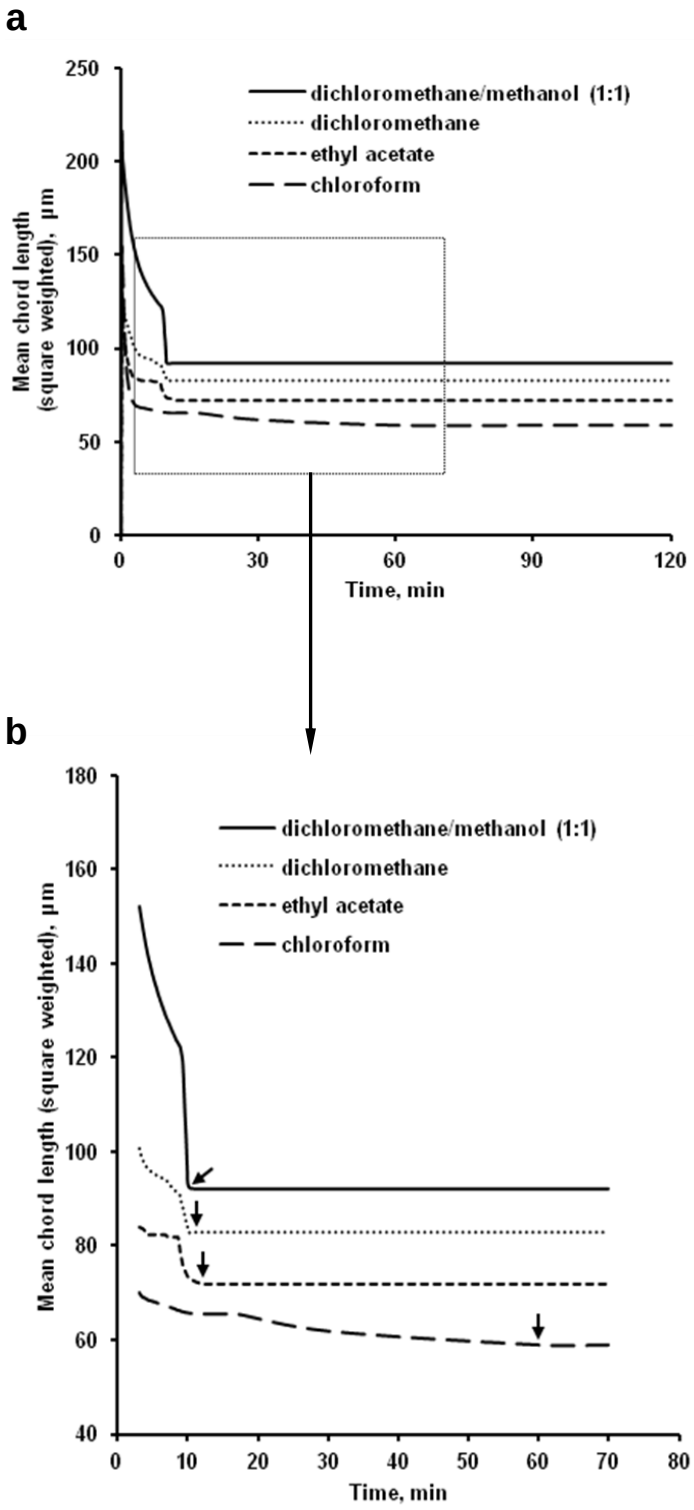

Fig. 11. Effect of solvent type on square weighted mean chord length during microparticles formation by O/W [(a) whole process and (b) hardening time of microparticle; arrow (↓): starting time of microparticle hardening]

The hardening rate of microparticles was influenced solvent evaporation rate and solvent miscibility. Methanol and ethyl acetate are water miscible, while dichloromethane and chloroform are water immiscible (Vay et al., 2011). The droplets shrinkage can be separated in two phase when using dichloromethane, dichloromethane/methanol (1:1) and ethyl acetate as solvent (O/W and W/O/W). A phase of rapid shrinkage where the initial droplet size decreased markedly within 9 min, was followed by a discontinued or slow shrinkage phase where no further pronounced shrinkage could be observed (Fig. 11a and Fig. 12a). It reveals that the solvent was rapidly extracted in the first (rapid) phase and that in the second, discontinued or slow phase, the embryonic microparticle droplets became solid microparticles. Solvent chloroform, the droplet size reduction continued up 60 min (O/W) and 90 min (W/O/W) indicating that the embryonic microparticle droplets became solid microparticles between 50-60 min and 80-90 min (Fig. 11a and Fig. 12a). Steps of microparticles formation after the emulsification of the primary emulsion into the external aqueous phase consist of (1) solvent diffusion from the droplets surface into the external aqueous phase as well as (2) exposure of the droplets surface to water and partial penetration of water into the droplets. The rate of organic solvent diffusion primarily depends on its solubility in the aqueous phase. Solvent loss from the surface of the droplets results in an increase in the polymer concentration at the surface. At this time the solvent concentration inside the droplets is still high and a solvent concentration gradient is established from the surface back towards the center of the droplets. When the minimum concentration for polymer precipitation is reached, phase separation and polymer precipitation start from the droplet surface and lead to formation of a polymeric shell through which the further solvent transport occurs. The polymeric shell formation stops further droplet shrinkage. The flux of solvent and non-solvent (water) across the droplet surface is subsequently hindered and the phase separation proceed at a slower rate towards the center of the droplet. Additionally, the loss of solvent from the droplet increases the droplets viscosity which decreases the rate of mass transfer. The precipitation stops when the center of the droplet is reached.

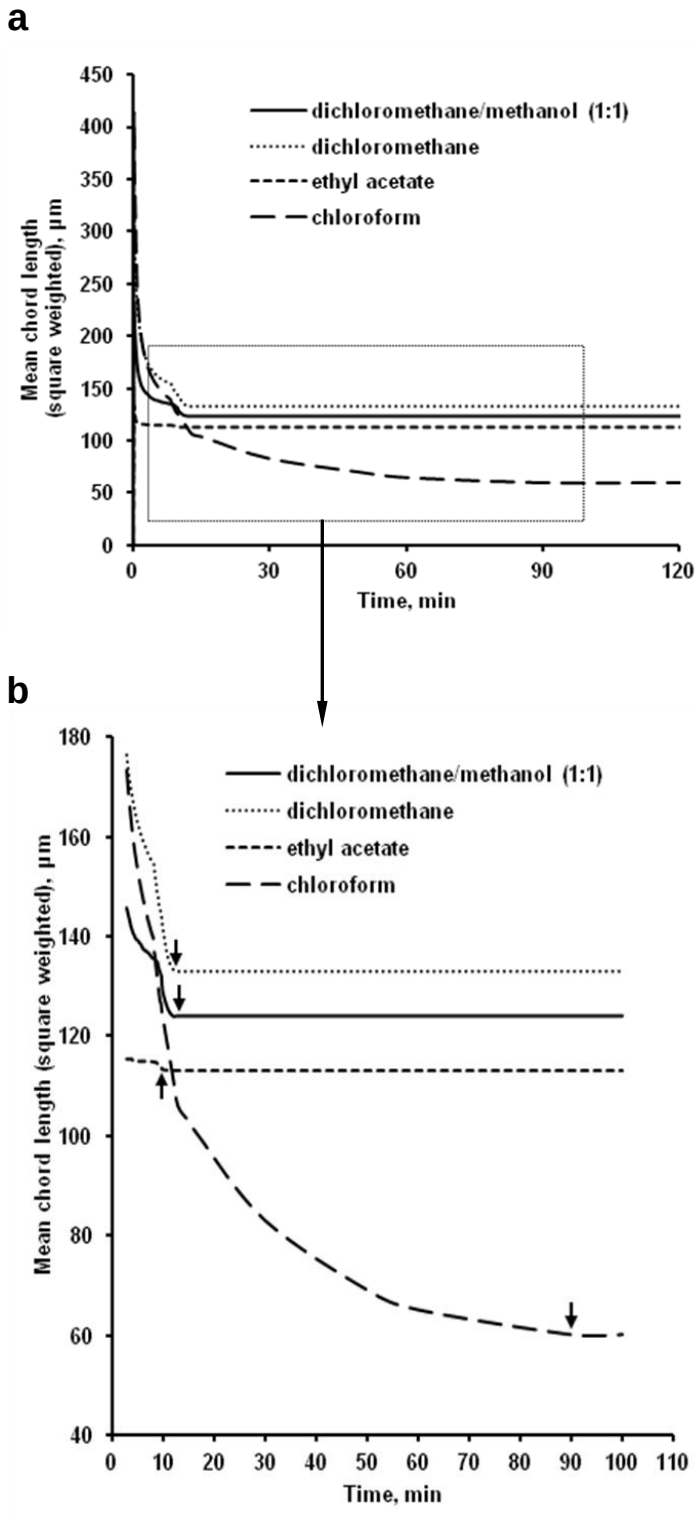

Fig. 12. Effect of solvent type on square weighted mean chord length during microparticles formation by W/O/W [(a) whole process and (b) hardening time of microparticle; arrow (↓): starting time of microparticle hardening]

For all solvent, when skin layer on the microparticle surface which already been formed. Start to perform segregation processes inside the droplets which is recorded then as embryonic microparticles. Due to phase separation the optical properties of the previously transparent emulsion droplets change to opaque. Thus the increase of the FBRM signal has to be assumed as a changes in opacification and marks the microparticles solidification occured. In all cases the conversion of liquid droplets into solid microparticles was fast. At the latest about 10-12 min after feeding the emulsion into the vessel the square weighted mean chord length had reached its final value when using dichloromethane, dichloromethane:methanol (1:1) and ethyl acetate. This period of the process has therefore the greatest impact on the resulting microparticles morphology and should be the main target for measures to control the microparticle properties. Based on FBRM data, the transformation of the emulsion droplets into solid microparticles for O/W method were occured at 10.5 min (dichloromethane), 10 min (dichloromethane/methanol (1:1)), 60 min (chloroform), and 12 min (ethyl acetate) (Fig. 11b and Table 3). Whereas the W/O/W method, the transformation of the emulsion droplets into solid microparticles were occured at 11.5 min (dichloromethane), 12 min (dichloromethane/methanol (1:1)), 90 min (chloroform) and 10 min (ethyl acetate) (Fig. 12b and Table 3).

Table 3. Effect of solvent type and preparation method on hardening time of microparticles

Solvent	**Hardening time (min)**	
	O/W	**W/O/W**
dichloromethane	10.5	11.5
dichloromethane/methanol (1:1)	10	12
chloroform	60	90
ethyl acetate	12	10

For O/W method, the square weighted mean chord length of microparticles (dichloromethane/methanol (1:1)) were larger than the others (Fig. 11a). Whereas for W/O/W method, the square weighted mean chord length of microparticles (dichloromethane) were larger than the others (Fig. 12a). It is influenced by the physical properties of the solvent and the viscosity of the polymer solution and primary W/O emulsion. High viscosity of emulsion droplets resulted in reduced dispersibility of the organic phase in the aqueous medium and then in larger microparticles (Moldenhauer and

Nairn, 1992; Murtaza, 2012; Nilkumhang and Basit, 2009). The viscosity data for ethyl cellulose as polymer solution and its solubility of ethyl cellulose in each solvent can be seen in Table 1. Based on Table 1, viscosity of primary W/O emulsion each solvent higher than viscosity of EC solution. Since the primary W/O emulsion was prepared by a sonication lead to a little bit of solvent to evaporate. It increases viscosity of primary W/O emulsion, especially the solvent quickly evaporates (e.g. dichloromethane and dichloromethane/methanol (1:1)).

In case of fast solvent extraction such as dichloromethane, dichloromethane/methanol (1:1) and ethyl acetate, the polymer solidifies rapidly on the droplet surface, which, as a result, becomes a certain degree diffusely scattering. (Fig. 11a and Fig 12a). The square weighted mean chord length of microparticles remain constant all over the extraction period which indicates, that the optical properties do not change for the entire duration of the process. Thus the increase of the FBRM signal has to be assumed as a changes in opacification and marks the microparticle solidification occured. This period of the process has therefore the greatest impact on the resulting particle morphology.

In case of slow extraction solvent such as chloroform, during the first 60 and 90 min the square weighted mean chord length is considerably smaller than the value which is measured for the primary emulsion. Subsequently, it drops down to a value between 154 to 59 µm (O/W) (Fig. 11a) and 407 to 60 µm (W/O/W) (Fig. 12a) which remains nearly constant until the end of the extraction process. This phenomenon could be observed for all batches with decelerated solvent removal irrespectively of the method by which the extraction rate was decreased. Most likely it causes also the apparent droplets expansion immediately after feeding the emulsion into the extraction medium. In case of slow solvent extraction no instantaneous formation of a skin layer on the microparticle surface is to be expected.

It is often stated that the square weighted mean chord length or median of the chord length distribution meets best the volume weighted mean or median of the diameter distribution obtained by other particle sizing techniques (Yu and Erickson, 2008; Heath et al., 2002). Thus, the square weighted CLD or its median will be mainly used for comparison with volume weighted particle size distributions (PSD) derived from other methods. In addition, based on microscopy data, the square weighted mean of a chord length distribution is better estimation for mean particle diameter (Table 2).

The trend of chord counts during solvent evaporation process

The curve of chord count of microparticles show as a function of time (Fig. 13 and Fig. 14). Both methods, the O/W and the W/O/W show similar chord count profile during solvent evaporation method. When using dichloromethane, dichloromethane/methanol (1:1) and ethyl acetate as solvent revealed initially, as the organic polymer solution was emulsified, FBRM detected lower chord counts for all solvents with increasing process time, the chord counts increased, followed by a plateau phase where the chord counts remain constant for all solvent. When, using chloroform, after organic polymer solution was emulsified, FBRM detected an increase in chord counts followed by a reduction phase where the chord counts decreased. The reduction phase was probably reflected either the agglomeration or coalescence of the fine droplets or microparticles into larger ones. A plateau phase follows the reduction phase indicating no further changes as the shearing stress continued. This plateau phase was indicative of hardening of the polymeric shell of the microparticles (Kougoulos et al., 2005; Zidan et al., 2010).

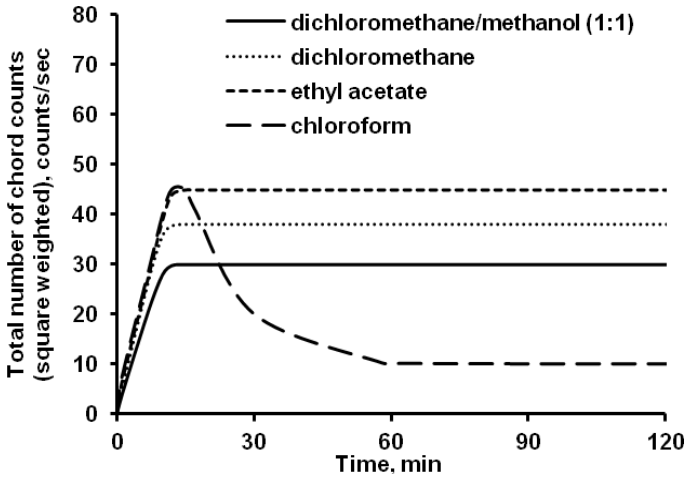

Fig. 13. Effect of solvent type on the number of chord counts (square weighted) during solvent evaporation process (O/W method)

In O/W method, the chord counts of EC microparticles from dichloromethane were lower than that from ethyl acetate. It is due to microparticles with larger size. Contrary, solvent chloroform give smallest square weighted mean chord length but the chord counts is not the highest. It is due to dichloromethane, cosolvent dichloromethane/methanol (1:1) and ethyl acetate produce more opaque microparticles (Fig. 9.a1,b1,d1), while chloroform produces slightly translucent microparticles (Fig. 9.c1). In W/O/W method, solvent

chloroform gave smallest square weighted mean chord length but the chord counts was the lowest. While for ethyl acetate, the square weighted mean chord length slightly larger than chloroform, and the chord counts observed by the FBRM probe is the highest. Ethyl acetate as solvent produce the highest chord counts.

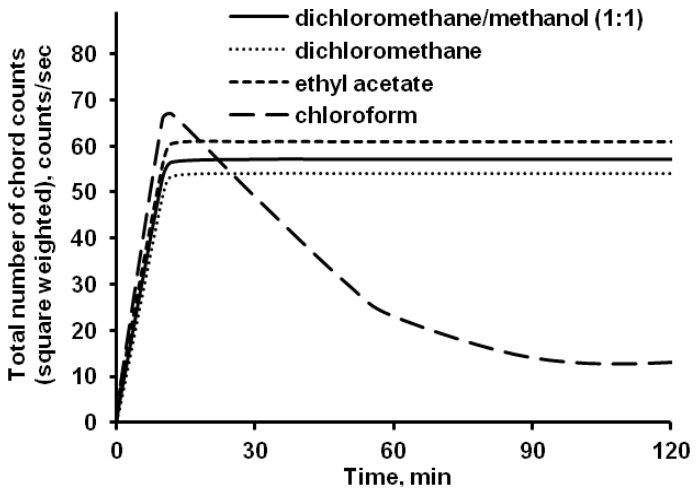

Fig. 14. Effect of solvent type on the number of chord counts (square weighted) during solvent evaporation process (W/O/W method)

This result can be attributed to the microparticle properties. Ethyl acetate produces more opaque microparticles (Fig. 10.d1). If the laser beam hits a opaque microparticles, the signal scattered to detector will be higher intensity than translucent and transparent microparticles. This effect is also reflected by the mean of the CLD and chord counts (Heath et al., 2002; Kougoulos et al., 2005; Vay et al., 2012; Yu and Erickson, 2008). The square weighted mean chord length measured with FBRM was much better. Chloroform produces slightly translucent microparticles (Fig. 10.c1). If the laser beam hits a translucent microparticles multiple reflections within the microparticles occured and the whole sphere lights up (Vay et al., 2012; Yu and Erickson, 2008). Due to the absorbance of the translucent microparticles, the backscattered signal is lower intensity than opaque microparticles, which results in a lower degree of chord lenght. It leads to low chord counts. FBRM signals which are strongly determined by the optical properties of the microparticles. The explanations about this phenomenon resulting from opaque and slightly translucent microparticles (W/O/W) given with the O/W process also apply here.

The EC microparticles which prepared using chloroform as solvent produce slight translucent of microparticles. It is due to slow solvent diffusion from droplets into water

phase. So, a gradual shrinkage of the droplet into solid microparticles was observed remain translucent.

The chord length distributions and cumulative volume chord length distributions

The chord length distributions of microparticles prepared by O/W and W/O/W method with different solvent shown in Fig. 15 and Fig. 16. As expected, larger microparticles gave longer chord lengths, and lower peak of particle number due to the decreased number of microparticles.

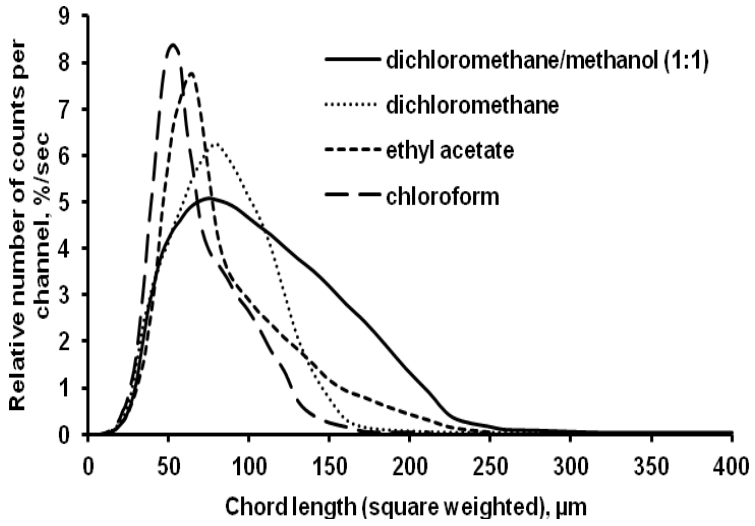

Fig. 15. Effect of solvent type on the square weighted chord length distributions [O/W method, at 4 hours stirring time]

In both cases it was observed that with increasing viscosity of polymer solution the square weighted mean chord length increased. This is caused by the more rapid solidification process occurring at the surface of embryonic microparticle droplets which resist in extensive shrinkage of embryonic microparticles droplets. Viscosity of polymer solution (organic phase) was lower than primary W/O emulsion. So, they produced smaller microparticles. They therefore have smaller square weighted mean chord length and narrow chord length distributions compared microparticles prepared by W/O/W method.

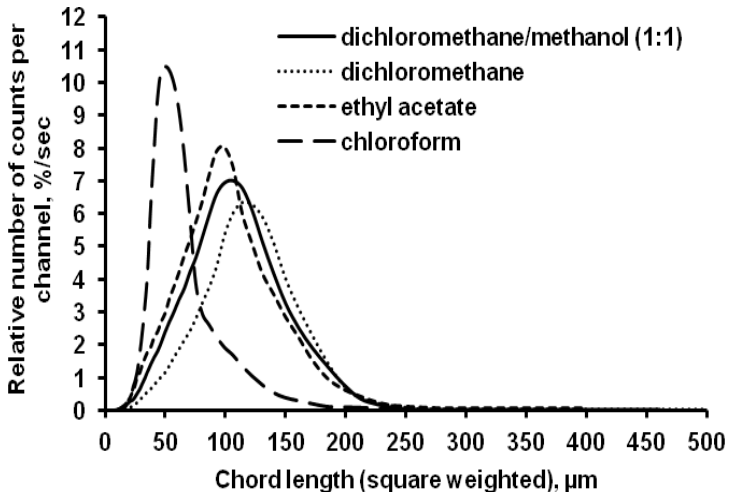

Fig. 16. Effect of solvent type on the square weighted chord length distributions [W/O/W method, at 4 hours stirring time]

The cumulative volume square weighted chord length distributions of microparticles prepared by O/W and W/O/W method with different solvent shown in Fig. 17. For microparticles which have smaller square weighted mean chord length, they will have much more particles with small size fraction and the fine and intermediate microparticles.

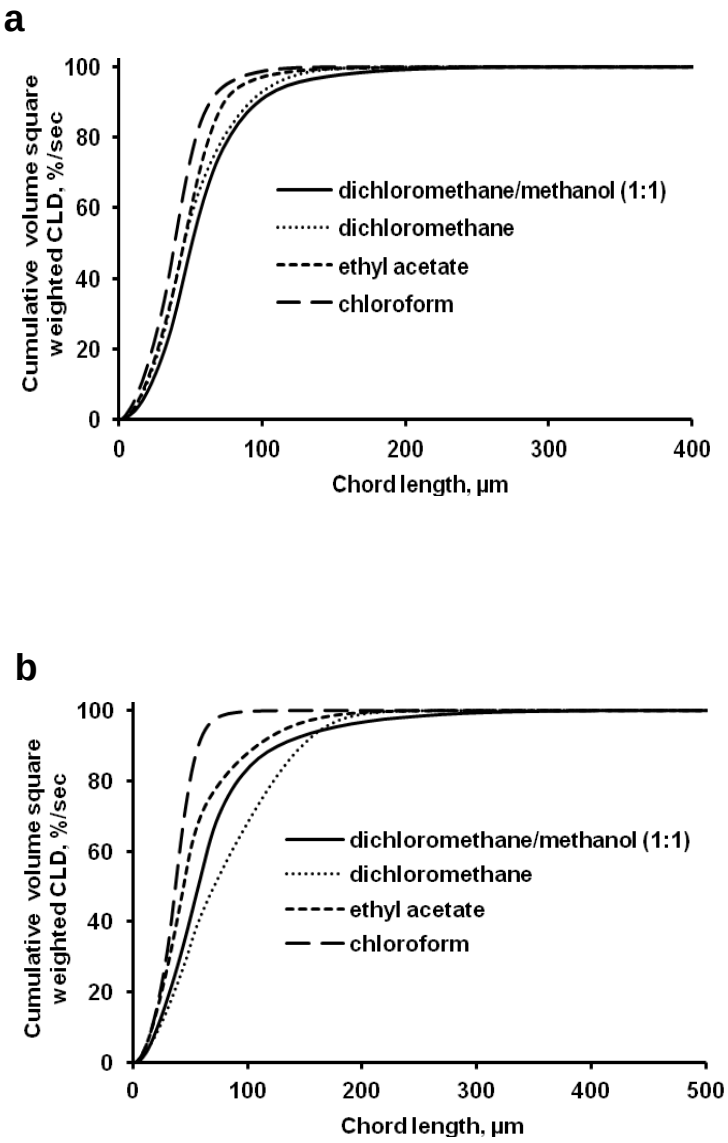

Fig. 17. Comparison of the square weighted chord length distribution for various solvent obtained by the FBRM method [(a) O/W and (b) W/O/W; at 4 hours stirring time]

The cumulative volume for microparticles smaller than 100 µm which prepared by O/W method for dichloromethane, cosolvent dichloromethane/methanol (1:1), chloroform and ethyl acetate are 92 %/sec, 90 %/sec, 97 %/sec and 95 %/sec, respectively (Fig. 17a). While, the cumulative volume for microparticles smaller than 100 µm which prepared by W/O/W method for dichloromethane, cosolvent dichloromethane/methanol (1:1), chloroform and ethyl acetate are 75 %/sec, 84 %/sec, 99 %/sec and 88 %/sec, respectively (Fig. 17b).

3.1.3. Conclusion

The FBRM can be used for on line monitoring of effect of solvent type and preparation method type on CLD, chord counts, square weighted mean chord length and hardening rate of microparticles from emulsion droplets until become solid microparticles during solvent evaporation process. The hardening rate of ethyl cellulose microparticles formation which used dichloromethane, dichloromethane/methanol (1:1) and ethyl acetate by O/W and W/O/W methods was not so different. Based on FBRM data, the transformation from the emulsion droplets into solid particles in solvent evaporation process when using dichloromethane, ethyl acetate and dichloromethane:methanol (1:1) by O/W and W/O/W methods occured within the first 10-12 minutes after feeding the emulsion into the external aqueous phase, while using chloroform within 60 (O/W) and 90 (W/O/W) minutes.

Different solvents and methods in preparation of microparticles gave different microparticles properties. Preparation of microparticles using dichloromethane, dichloromethane:methanol (1:1) and ethyl acetate as solvent produced more opaque microparticles than chloroform which produced slightly translucent microparticles (O/W and W/O/W). FBRM signal was influenced by surface characteristics and optical properties of microparticles. As indicated microparticles with good scattering properties provided comparable values for the CLD and chord count and also good estimation of particle size. This study also found that larger microparticles gave longer chord lengths, and lower peak of particle number. The microparticle CLD and transformation process was strongly influenced by solvent type and type of preparation method. Despite a strong dependence on the optical properties of the samples, FBRM is the most beneficial technique for online monitoring of microparticle preparation processes.

3.2. Online monitoring of preparation process of polymeric microparticles and microparticle blends by focused beam reflectance measurement (FBRM): Effect of polymer type

3.2.1. Introduction

Solvent evaporation method is a popular technique for the encapsulation of drugs within polymeric microparticles. Water insoluble polymers are usually used as encapsulation matrix for these microparticles. Microparticles have been prepared with a wide range of polymers and polymer blends (Alhnan and Basit, 2011; Saralidze et al., 2010). The solidification rate of polymeric microparticles is an important parameter influencing the particle size, the encapsulation efficiency and the initial burst in microparticulate systems. A very slow hardening of the emulsion droplets can lead to a diffusion of the drug substance out of the droplets and precipitation in the external phase. The precipitation kinetics of the polymer solution droplets will not only be affected by the affinity between solvent and external phase, but also by their phase ratio. According to Mehta et al., solubilities of polymers in organic solvents determine the solidification rate of the polymers during the microparticle preparation process, which in turn affects microparticle properties such as drug incorporation, matrix porosity, and solvent residues (Mehta et al., 1996).

Various types of polymer with different physical properties (such as biodegradable, non-biodegradable, permeable, etc.) have been prepared microparticles. They are poly(ϵ-caprolactone), poly(lactic-co-glycolic acid), Eudragit® RS 100, Eudragit® RL 100 and ethyl cellulose microparticles (Chen et al., 2000b, 2009; Gibaud et al., 2004; Jeyanthi et al., 1997; Li et al., 1995; Murtaza, 2012; Sinha et al., 2004; Souza et al., 2011; Trapani et al., 2007). Poly(ϵ-caprolactone) (PCL) and poly(lactic-co-glycolic acid) (PLGA) are biocompatible and biodegradable polyesters (Chen et al., 2000a, 2000b; Gibaud et al., 2004; Ray, et al., 2003). Eudragit® RS 100 and Eudragit® RL 100 are copolymers of ethyl acrylate, methyl methacrylate with low contents of a methacrylic acid ester with quaternary ammonium groups (Trapani et al., 2007). Eudragit® RL 100 is more permeable than Eudragit® RS 100, as the amount of co-trimethylammonioethyl methacrylate chloride in Eudragit® RL 100 is higher than in Eudragit® RS 100. Ethyl cellulose is a nonbiodegradable hydrophobic polymer (Duarte et al., 2006; Moldenhauer and Nairn, 1992; Pearnchob and Bodmeier, 2003; Rekhi and Jambhekar, 1995; Saravanan and

Anupama, 2011). Information about the microparticle hardening rate from these polymers is not available. It is necessary to know the effect of polymer properties on the polymeric microparticle hardening time.

To date very little work has been carried out on the online monitoring of microparticles during their formation. In a solvent evaporation process, solidification of the emulsion droplets and particle size changes occur after emulsifying the organic inner phase into the external aqueous phase (Freitas et al., 2005; Li et al., 1995; O'Donell and McGinity, 1997). The transformation of the emulsion droplets into solid microparticles can be monitored by focused beam reflectance measurement (FBRM). FBRM is a laser based technique, which offers the advantage of in-line measurement of the chord length distribution (CLD) of dispersed particles inside a flowing fluid, without the need of installing a pre-dilution side-stream, as required for other online particle sizing tools. It does not require sampling that could affect the actual particle size distribution due to breakdown or aggregation. FBRM measures a CLD, which is affected by the geometry, size, and number of particles under analysis (Boxall et al., 2010; Dowding et al., 2001; Heath et al., 2002; Kail et al., 2009; Kirwan, 2009; Kougoulos et al., 2005; Leba et al., 2010; Wynn, 2003). The FBRM signal strongly depends on the surface properties of the measured sample, it provides an effective solution to track the process (Boxall et al., 2010; Kail et al., 2009; Vay et al., 2012; Yu and Erickson, 2008; Zidan et al., 2010). With regard to controlling such a microparticle preparation process the determination of the rate and time point of conversion from liquid droplets into solid particles is of great interest.

FBRM uses a focused beam of laser light, which scans in a circular path. As this light scans across a particle or its structure passing in front of the probe window, light is scattered in all directions. The light scattered back towards the probe is used to measure a chord length of the given particle. Typically, many thousands of chords are measured per second, providing a robust measurement that is sensitive to the change in the size or number of particles under investigation (Kail et al., 2008, 2009; Kempkes et al., 2008; Kougoulos et al., 2005; Leba et al., 2010; Simmons et al., 1999; Vay et al., 2012). FBRM does not depend on the presence of a threshold particle concentration, as soon as one particle is in the detectable size range, it will be detected. A more detailed description of the operation of the FBRM probe is available (Boxall et al., 2010; Greaves et al., 2008; Heath et al., 2002; Kirwan, 2009; Kougoulos et al., 2005; Leba et al., 2010).

A variety of applications using FBRM have been developed and reported in the literature, including crystallization control (Abbas et., 2002; Barrett et al., 2002; Yu et al., 2005), flocculation process design (Williams et al., 1992), slurry transfer (Daymo et al., 1998), monitoring of polymorphic transitions (O'Sullivan et al., 2003), particle disruption control (Kougoulos et al., 2005) and solubility measurements (Kim et al., 2005). Furthermore several studies using the FBRM for investigation of emulsion systems (Dowding et al., 2001; Turner et al., 2009) have been published. Zidan et al. have studied using FBRM in order to monitor a solvent extraction process for microparticle preparation with different polymer concentration and different drug loading (Zidan et al., 2010). They found the transformation of the emulsion droplets into solid particles can be detected by a change in the FBRM signal. Vay et al. used FBRM in solvent evaporation process and found out that the transformation of the emulsion droplets into solid particles is influenced stirring speed and temperature. Increasing in stirring speed and temperature give fast solidification rate of particle (Vay et al., 2012).

Interestingly, in most studies reported so far in the literature, online monitoring of polymeric microparticles and microparticle blends formation with different polymers using FBRM has not been reported yet. Based on this fact, the current study aimed to employ FBRM for online monitoring of solidification rate of polymeric microparticles/ microparticle blends and particle size/distribution of the emulsion droplets/hardened polymeric microparticles/microparticle blends manufactured by O/W method using various polymers.

3.2.2. Results and discussion

3.2.2.1. Microparticles morphology and size

SEM photomicrograph of ethyl cellulose (EC), Eudragit® RL 100, Eudragit® RS 100, polycaprolactone and PLGA (RG503H) microparticles which were prepared by O/W method are shown in Fig. 18 (a2-e2 and a3-e3). For this analysis, the particles were dried prior to observation by vacuum drying for 24 hours at room temperature. The surface analysis of empty microparticles (without drug) prepared by the O/W method revealed that ethyl cellulose (EC), polycaprolactone and PLGA (RG503H) microparticles were spherical with smooth surfaces and showed no aggregation. While, Eudragit® RL 100 and Eudragit® RS 100 microparticles were spherical, oval and needle shaped (mixture) with smooth surfaces and no aggregation. All microparticle surfaces did not show any pores (Fig. 18.a3-e3).

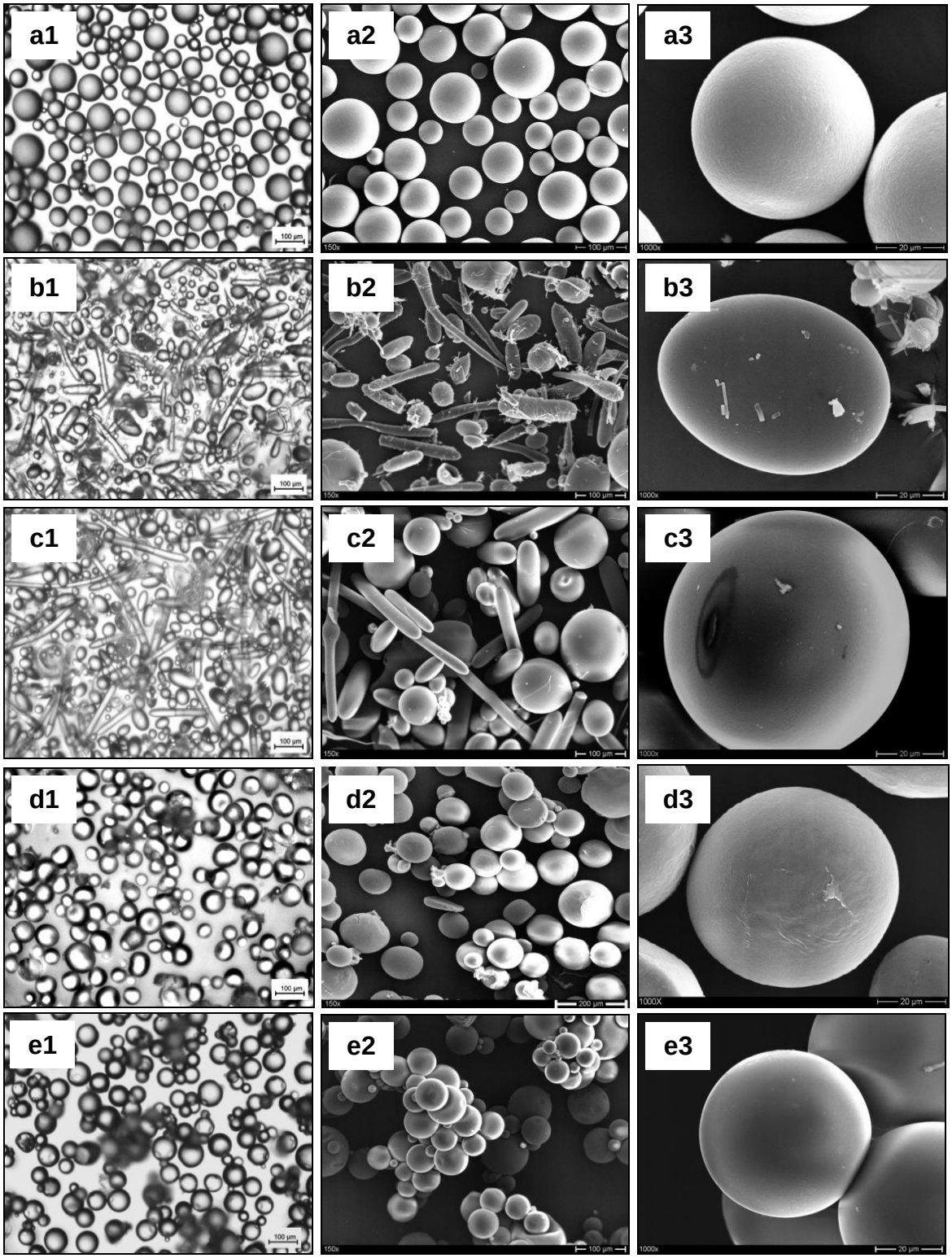

Fig. 18. Optical microscopy pictures (1) and SEM pictures of polymeric microparticles (2. at lower magnification & 3. at higher magnification) [a. Ethyl cellulose 4 cp; b. Eudragit RL 100; c. Eudragit RS 100; d. Polycaprolactone (Mw. 10000); e. PLGA (RG503H)].

The type and physical properties of polymer have influenced the opacity level of particle. All microparticles have different opacity level (Fig. 18.a1-e1). It is effect of polymer solubility in solvent dichloromethane. Polymers have high solubility in dichloromethane took longer time to solidify and stayed longer in the semi solid, the dispersed phase became more concentrated before it completely solidified. The polymer matrix is dense when it is allowed to shrink for a longer period of time. So, a gradual shrinkage of the droplet into solid microparticles was observed remain translucent. Mean diameter of polymeric microparticles ranged from 51 to 83 µm (FBRM) (Table 4). The particle size mean of microparticles which was prepared using the high viscosity of polymer organic solution were larger than those prepared with the low viscosity of polymer organic solution. This is caused by the more rapid solidification process occurring at the surface of embryonic microparticle droplets which resist in extensive shrinkage of embryonic microparticles droplets. Based on microscopy data, the square weighted mean of a chord length distribution is better estimation for mean particle diameter (Table 4).

Table 4. Effect of polymer type on solubility in dichloromethane, viscosity of polymeric solution and particle size mean of microparticles

Polymer	**Solubility (g/ml) (± SD)**	**Viscosity (cSt) (± SD)**	**Particle size mean (µm) (± SD)**	
			FBRM	**Optical microscope**
Ethyl cellulose 4 cp	0.86 (± 0.03)	10.31 (± 1.14)	83.24 (± 5.28)	88.78 (± 7.64)
Eudragit RL 100	1.04 (± 0.04)	4.72 (± 0.83)	73.42 (± 6.44)	79.62 (± 9.17)
Eudragit RS 100	1.42 (± 0.02)	3.84 (± 0.39)	59.36 (± 5.21)	63.96 (± 8.92)
Polycaprolactone (Mw. 10000)	1.89 (± 0.05)	3.15 (± 0.45)	51.29 (± 4.09)	57.86 (± 8.12)
PLGA (RG503H)	1.25 (± 0.06)	4.36 (± 0.52)	64.08 (± 3.18)	68.15 (± 6.95)

3.2.2.2. Online monitoring of polymeric microparticles formation

Methods based chord length measurements

The FBRM technique provides with measurements of chord lengths. Therefore, for a spherical particle of diameter, the instrument will ideally generate a number of values between the limit of detection and diameter. If the particle is not spherical, diameter will be the diameter of the sphere circumscribed to the irregular particle (Li et al., 2005; Silva et al., 2013).

A particle's chord length can be defined as a geometric line segment whose endpoints both lie on the surface of the particle. These analysers utilize a laser beam that crosses the particle randomly acquiring a chord length. The number of times a given chord length is measured takes the form of a probability density function. In case of spherical microparticles (ethyl cellulose, polycaprolactone and PLGA (RG503H)), the diameter is the largest chord possible and the probability of the measured chord length is independent of the microparticle orientation towards the laser beam (Fig. 19a) while for irregular and odd-shaped microparticles (Eudragit® RL 100 and Eudragit® RS 100), shape and orientation will influence the measured chord lengths (Fig. 19b and 19c). Hence, the chord length distribution (CLD) depends on both the particle size distribution (PSD) and the particle shape. FBRM utilize a laser beam for their measurements and FBRM calculates it from the laser light that is reflected back from the microparticle and propagated back through the probe.

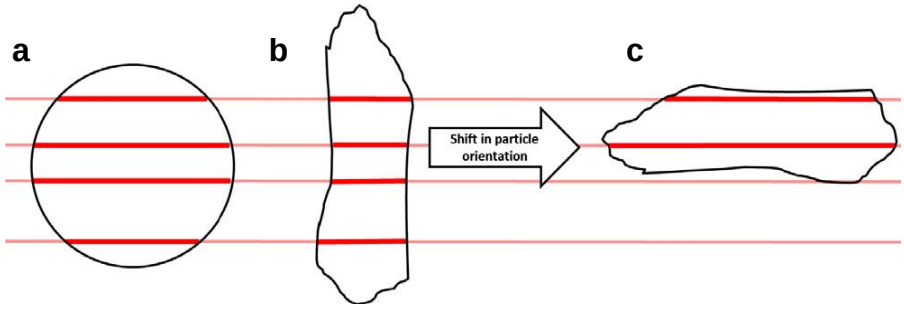

Fig. 19. Examples of the measured chord length (bold line) when a laser beam crosses (a) a spherical particle and (b and c) an irregular particle in different positions – illustration of the effect of particle orientation on the obtained chord length (Silva et al., 2013).

Microparticle formation and hardening rate

FBRM was used for a qualitative online monitoring of the shift in the chord length distributions (CLD) at various stages of microparticles ripening at certain agitation rates. The main focus of this study was to monitor microparticles formation, changes of the particle size, hardening rate, particle's properties and chord length distribution. All polymers produced particles with small size (less than 300 μm). The curve of square weighted mean chord length of polymeric microparticles show as a function of time (Fig. 20a).

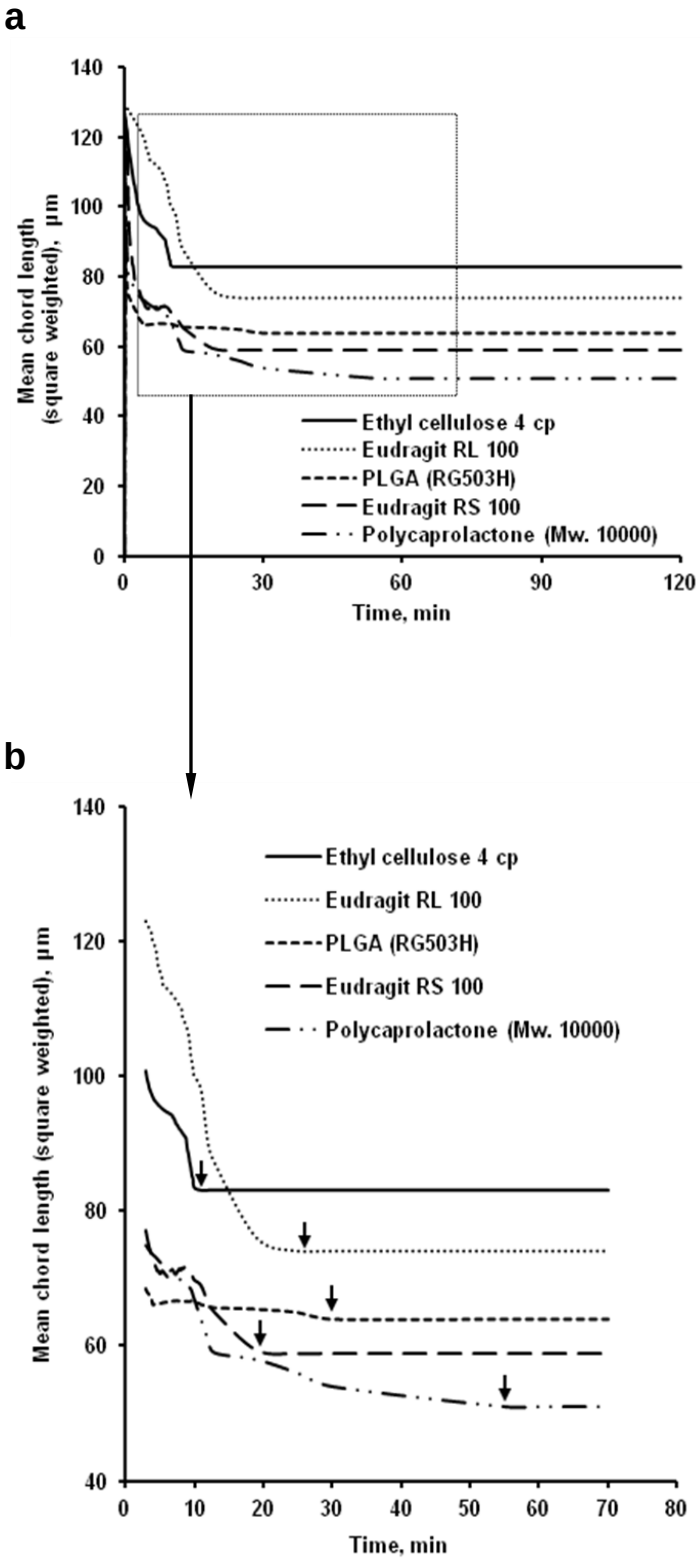

Fig. 20. Effect of polymer type on square weighted mean chord length during microparticle formation by O/W method [(a) whole process and (b) hardening time of microparticle; arrow (↓): starting time of microparticle hardening]

Various type of polymers which used in polymeric microparticles formation lead to a different square weighted mean chord length profile during solvent evaporation method. Initially as the organic polymer solution was emulsified, FBRM detected larger droplet size mean of all type of polymers, by increasing the process time the particle size decreased followed by a plateau size where the square weighted mean chord length was constant for all polymers. For ethyl cellulose, the square weighted mean chord length of microparticles were larger than the others. It is influenced by the viscosity of the polymer solution. High viscosity of emulsion droplets resulted in reduce of dispersibility of the organic phase into the aqueous medium resulting in larger particles. The viscosity data of polymers solution can be seen in Table 4.

The solidification rate of polymeric microparticles were influenced solubility of polymer in solvent. Here, solubilities of the polymers in dichloromethane were compared (Table 4). Ethyl cellulose (EC) had the lowest solubility in dichloromethane and polycaprolactone had the highest solubility in dichloromethane compared to other polymers. So, hardening rate of polycaprolactone based microparticles was slower than the others. While, ethyl cellulose giving fastest hardening rate. Whereas, Eudragit® RL 100, Eudragit® RS 100 and PLGA (RG503H) were more soluble in dichloromethane than ethyl cellulose (Li et al., 1995; Sansdrap and Moes, 1993; Yeo and Park, 2004). These properties lead to solidification rate of microparticles from these three polymers slower than that of ethyl cellulose. Polymers having relatively high solubilities in dichloromethane took longer time to solidify (Mehta et al., 1996). Since polymers having higher solubilities in dichloromethane stayed longer in the semi-solid state, the dispersed phase became more concentrated before it completely solidified, resulting in denser microparticles (Jeyanthi et al., 1997; Mehta et al., 1996).

In general, the droplets shrinkage can be separated in two phase for all polymers. A phase of rapid shrinkage where the initial droplet size decreased markedly within 9 min (EC), 15 min (Eudragit® RS 100), 20 min (Eudragit® RL 100) and 25 min (PLGA (RG503H) were followed by a discontinued or slow shrinkage phase where no further pronounced shrinkage could be observed (Fig. 20a). It reveals that the solvent was rapidly extracted in the first (rapid) phase and that in the second, discontinued or slow phase, the embryonic microparticle droplets became solid microparticles. Polycaprolactone, the droplet size reduction continued up 55 min indicating that the embryonic microparticle droplets became solid microparticles between 50-55 (Fig. 20a). Based on FBRM data, the

start of the plateau phase for all polymers showed that hardening of microparticles were occurred at 10.5 min (ethyl cellulose 4 cp), 25 min (Eudragit® RL 100), 19 min (Eudragit® RS 100), 55 min (polycaprolactone) and 30 min (PLGA (RG503H)) (Fig. 20b and Table 5).

Table 5. Effect of polymer type on hardening time of microparticles

Polymer	Hardening time (min)
Ethyl cellulose 4 cp	10.5
Eudragit RL 100	25
Eudragit RS 100	19
Polycaprolactone (Mw. 10000)	55
PLGA (RG503H)	30

In case of fast solvent extraction for EC microparticles (Fig. 20), the polymer solidifies rapidly on the droplet surface (Freitas et al., 2005; Yu and Erickson, 2008), which, as a result, becomes a certain degree diffusely scattering. The square weighted mean chord length of polymeric microparticles remains constant all over the extraction period which indicates, that the optical properties do not change for the entire duration of the process. Thus the increase of the FBRM signal has to be assumed as a changes in opacification and marks the particle solidification occured. This period of the process has therefore the greatest impact on the resulting particle morphology and should be the main target for measures to control the particle properties.

The trend of chord counts during solvent evaporation process

The curve of chord count of polymeric microparticles show as a function of time (Fig. 21). The O/W method shows ethyl cellulose chord count profile during solvent evaporation method. Initially as the organic polymer solution was emulsified, FBRM detected lower chord counts, by increasing the process time, the chord counts increased followed by a plateau phase where the chord counts constant. Whereas, for Eudragit® RL 100, PLGA (RG503H), Eudragit® RS 100 and polycaprolactone show similar chord counts profile during solvent evaporation method. Initially as the organic polymer solution was emulsified, FBRM detected lower chord counts of all type of polymers, by increasing the process time, the chord counts increased followed by decreasing the chord counts for all

polymers. It was followed by a plateau phase where the chord counts constant for all polymers.

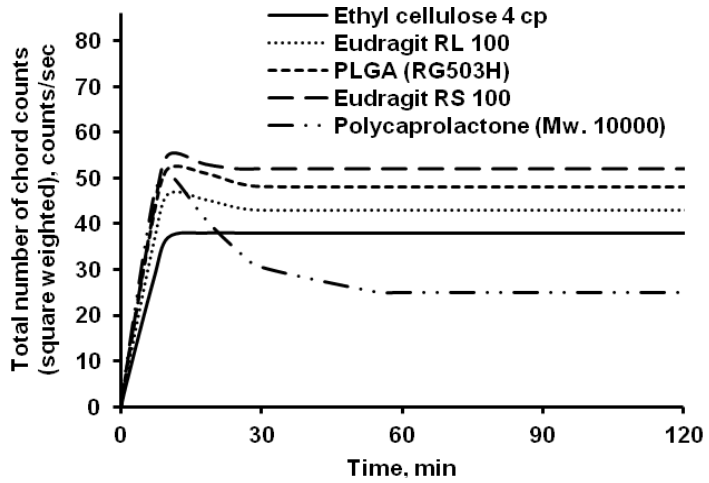

Fig. 21. Effect of polymer type on the number of chord counts (square weighted) during solvent evaporation process

The chord counts of ethyl cellulose microparticles was lower than others. It is due to production of microparticles with the largest size. The square weighted mean chord length of Eudragit® RL 100, Eudragit® RS 100 and PLGA (RG503H) were smaller than ethyl cellulose, so the chord counts was higher. Polycaprolactone gave the smallest square weighted mean chord length but the chord counts was not the highest. This result can be attributed to the particle properties. Polycaprolactone produces slightly translucent microparticles compared to other polymers (Fig. 18.d1). If the laser beam hits a translucent microparticle multiple reflections within the microparticle occured and the whole sphere lights up (Greaves et al., 2008; Vay et al., 2012; Yu and Erickson, 2008; Wu et al., 2011). While if the laser beam hits a more opaque microparticle, the signal will be scattered to detector. The opaque microparticles were measured with FBRM will give higher scattering value than translucent and transparent one. Due to the absorbance of the opaque microparticles, the backscattered signal is very high intensity, which results in a high degree of chord length. So, it leads to low chord counts. This is in agreement with Greaves et al. and was also found by Sparks and Dobbs, who concluded that only droplets or microparticles which are opaque and highly reflective (with microstructure on the

surface) give reproducible and accurate results (Greaves et al., 2008; Spark and Dobbs, 1993).

The chord length distributions and cumulative volume chord length distributions

The chord length distributions measured by FBRM for various polymers with the same concentration are different (Fig. 22). As expected, larger microparticles gave longer chord lengths and lower peak of particle number due to the decreased number of microparticles. In this case it was observed that with increasing viscosity of polymer solution the square weighted mean chord length increased and chord lengths distribution become longer. The viscosity of polycaprolactone solution (organic phase) was lower than other polymers. So, it produced smaller microparticles. It therefore have smaller square weighted mean chord length and narrow chord length distributions compared to other polymeric microparticles.

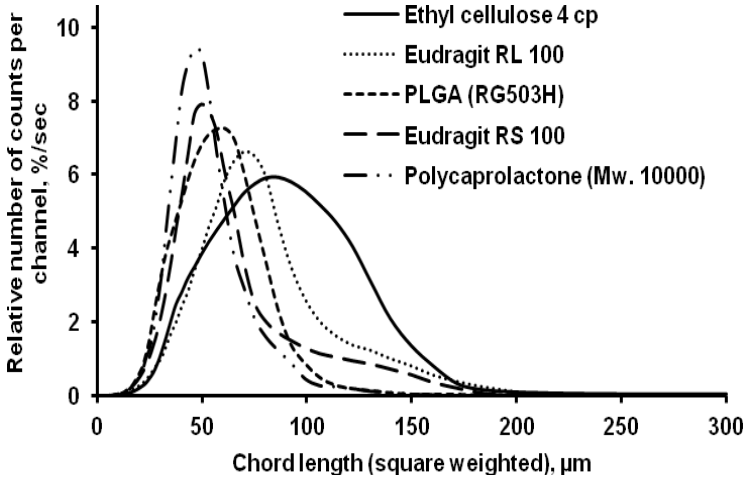

Fig. 22. Effect of polymer type on the square weighted chord length distributions (at 4 hours stirring time)

The cumulative volume square weighted chord length distributions of polymeric microparticles prepared by O/W method shown in Fig. 23. For polymeric microparticles which have smaller square weighted mean chord length, they will have much more particles with small size fraction and also the fine and intermediate microparticles.

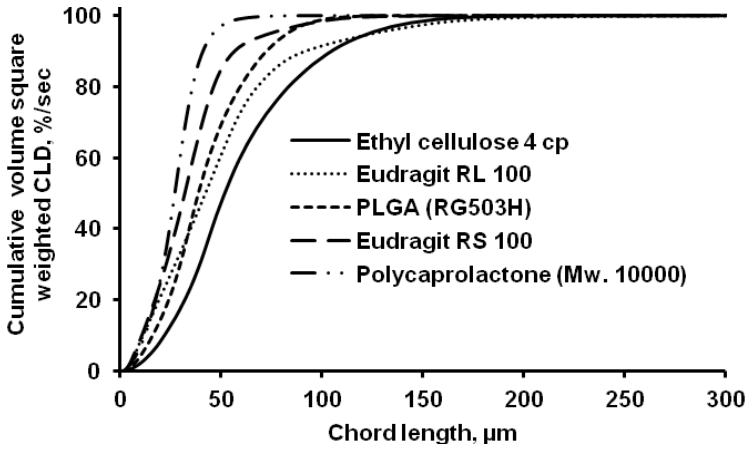

Fig. 23. Comparison of the square weighted chord length distribution for various polymer obtained by the FBRM method (O/W) [at 4 hours stirring time]

The cumulative volume value of polymeric microparticles which prepared by O/W method for microparticles smaller than 100 µm for ethyl cellulose, Eudragit® RL 100, Eudragit® RS 100, polycaprolactone and PLGA (RG503H) are 92 %/sec, 94 %/sec, 98.5 %/sec and 99.5 %/sec and 98 %/sec, respectively (Fig. 23).

3.2.2.3. Polymeric microparticle blends

In this experiment two group of microparticle blends had been prepared. The first microparticle blends was polymeric microparticle blends with DTI 0 min and the second microparticle blends was polymeric microparticle blends with DTI 60 min. The chord counts for all polymeric microparticle blends were represented as square weighted distributions. The curve of square weighted mean chord length of microparticle blends (DTI 0 and 60 min) show as a function of time (Fig. 24a and Fig. 25a).

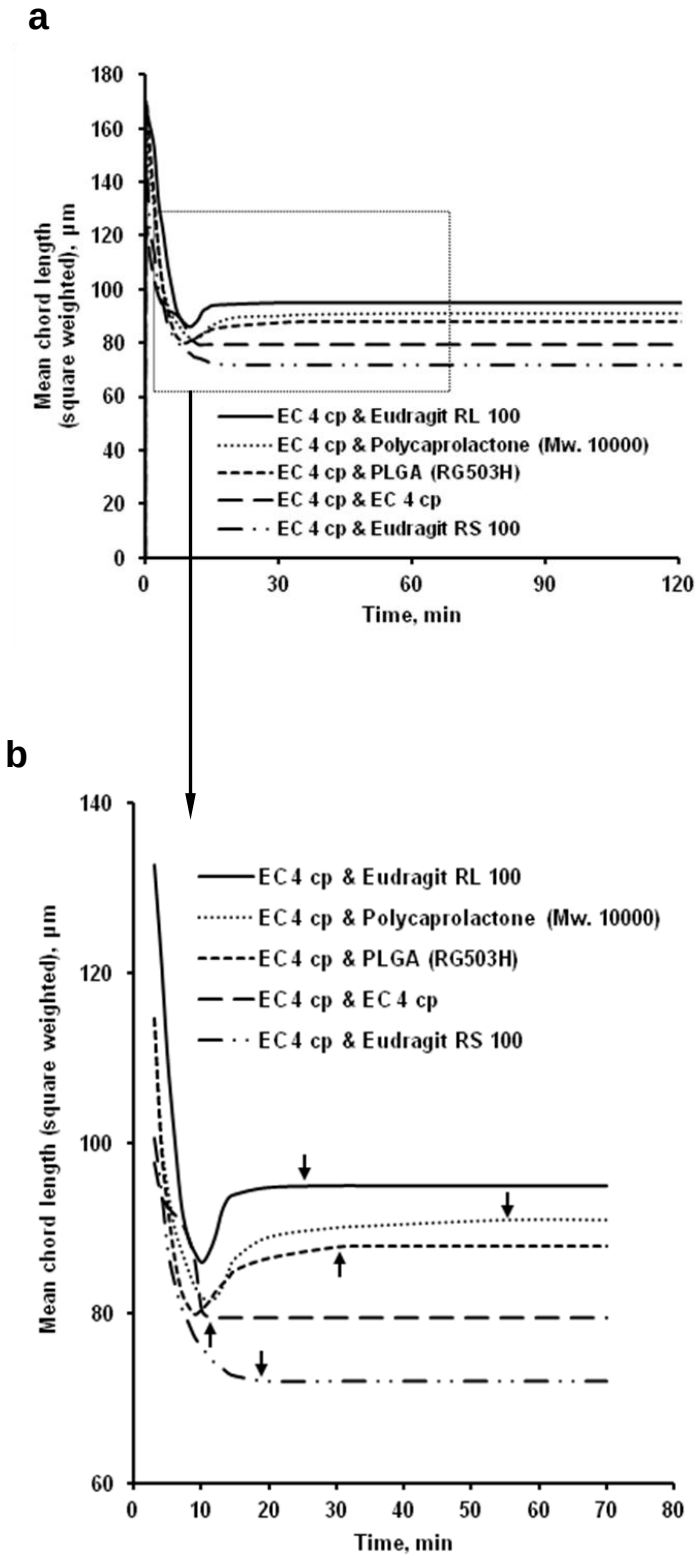

Fig. 24. Effect of polymer type in second oil phase with DTI 0 min on the square weighted mean chord length during microparticle blends formation [(a) whole process and (b) hardening time of microparticle; arrow (↓): starting time of microparticle hardening]

It was observed from FBRM data that the emulsification of second primary oil phase (DTI 0 and 60 min) into single external aqueous phase affected the partice size. Difference dispersion time interval in preparation of microparticle blends (DTI 0 and 60 min) lead to a different square weighted mean chord length profile during solvent evaporation method. For second oil phase consisted of Eudragit® RL 100, PLGA (RG503H) and polycaprolactone, the square weighted mean chord length of microparticle blends were larger than microparticle normal. While for second oil phase consisted of EC 4 cp and Eudragit® RS 100, the square weighted mean chord length of microparticle blends were smaller than microparticle normal. The second primary oil phase contained Eudragit® RL 100, PLGA (RG503H) or polycaprolactone, which contributed in enhancement of particle size. Increasing in the square weighted mean chord length corresponds to agglomeration of the microparticle blends. So the chord length distribution is as far to the right as possible compared to microparticle normal, indicating agglomeration of microparticles. Polymeric microparticle blends agglomerates were occurred after 20 min emulsification (for DTI 0 min) and after 20 min addition second oil phase into external aqueous phase containing hard EC microparticles (for DTI 60 min) (Fig. 24b and Fig. 25b).

The agglomeration process may be considered as a collision between hydrophobic particle (EC 4 cp) and oil droplets (Eudragit® RL 100, polycaprolactone and PLGA [RG503H]). These collisions lead to adhesion as a result of the formation of pendular oil bridges. Additionaly, the physical properties (viscosity and solubility) of these three polymer solutions contributes to agglomeration with EC 4 cp microparticles. Ethyl cellulose (EC) had the lower solubility in dichloromethane compared to other polymers. So, hardening rate of EC microparticles was faster than the others. While, hardening rate of Eudragit® RL 100, polycaprolactone and PLGA [RG503H] were slower than EC. It caused interaction between Eudragit® RL 100, polycaprolactone and PLGA (RG503H) with EC, when these polymer were prepared microparticle blends with EC.

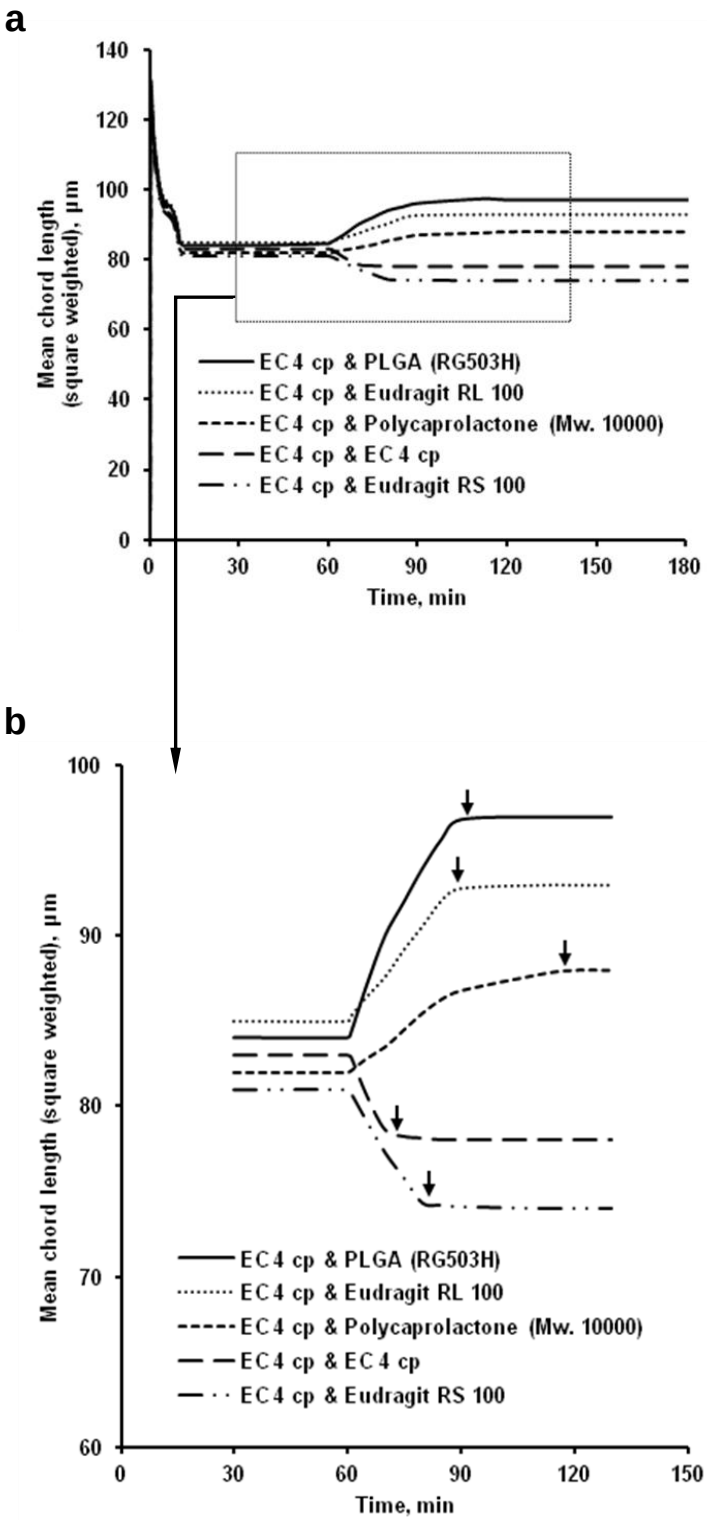

Fig. 25. Effect of polymer type in second oil phase with DTI 60 min on the square weighted mean chord length during microparticle blends formation [(a) whole process and (b) hardening time of microparticle; arrow (↓): starting time of microparticle hardening]

The number of chord counts increase significantly as the oil phase added and followed a plateau phase for microparticle blends with second oil phase containing EC 4 cp. In contrast, chord counts for second oil phase containing Eudragit® RL 100, Eudragit® RS 100, PLGA (RG503H) and polycaprolactone after increase chord counts followed by a reduction phase where the chord counts decreased. This phase was followed a plateau phase indicated that no change in the chord counts and microparticles already hard. The start of the plateau phase at FBRM data for all polymeric microparticle blends showed that hardening of the microparticle was occurred. This phenomenon is similar for both of microparticle blends with DTI 0 and 60 min. The largest square weighted mean chord length of microparticle blends will give the lowest chord count. EC-PCL microparticle blends (DTI 60 min) produced no the largest square weighted mean chord length but the chord counts was the lowest (Fig. 26a and Fig. 26b). This result can be attributed to the microparticle properties. It is due to the slight translucent of the EC-PCL microparticle blends compared to the others. If the laser beam hits a translucent microparticle multiple reflections within the particle occured and the whole sphere lights up.

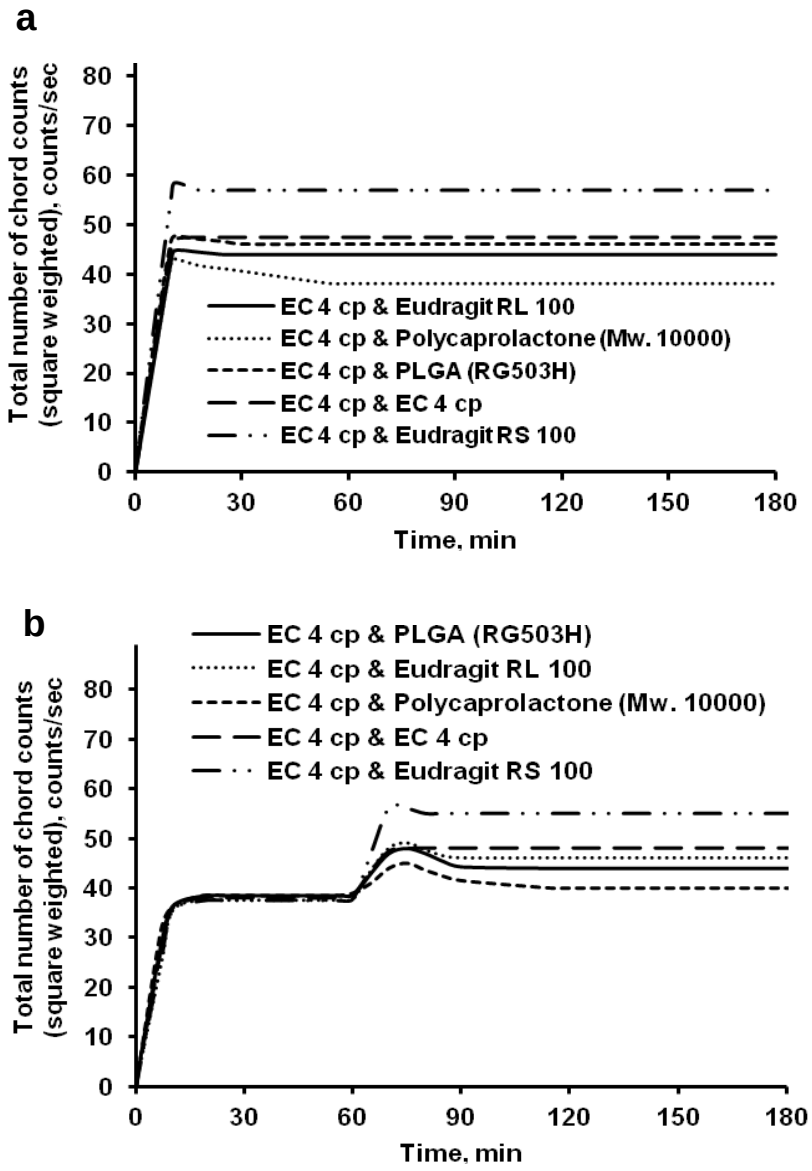

Fig. 26. Effect of polymer type in second oil phase with (a) DTI of 0 min and (b) DTI of 60 min on the number of chord counts (square weighted) during solvent evaporation process

Many microparticles are translucent to a certain degree. In this case a portion of the light penetrates also into the microparticles. Each ray is reflected a number of times at irregular angles inside the material and exits the surface at a different point and angle. In consequence a microparticle hit by the beam lights up and, as the studied microparticles are spherical or other shape and the matrix is considered to be homogeneous, the particle emits the scattered light uniformly in all spatial directions (Scheler, 2013). These light or signal will be scattered to detector with lower intensity and results in a lower degree of chord length compared to opaque particles. So, it leads to low chord counts.

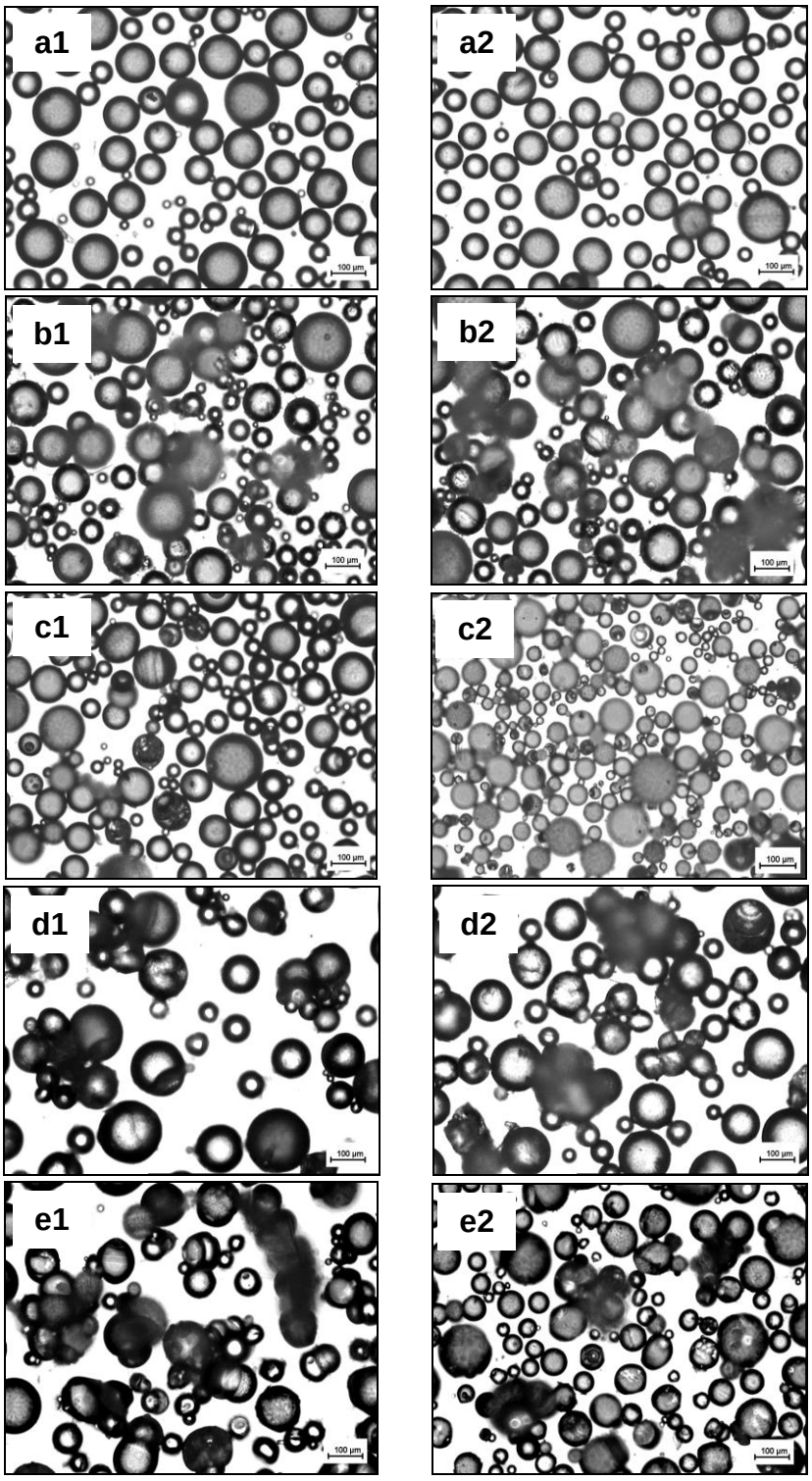

Fig. 27. Optical microscopy pictures of polymeric microparticle blends [a. EC 4 cp – EC 4 cp; b. Ec 4 cp -Eudragit RL 100; c. Ec 4 cp - Eudragit RS 100; d. Ec 4 cp - Polycaprolactone (Mw. 10000); e. Ec 4 cp - PLGA (RG503H). 1. DTI: 0 min and 2. DTI: 60 min]

Optical microscopy pictures showed that the particles have spherical shape and agglomerates for microparticle blends which prepared with DTI of 0 and 60 min. Figure 27 shows microparticle blends with DTI of 0 and 60 min. These microparticle blends have similar size with diameter range of 72.29 µm to 95.41 µm (DTI 0 min) and 74.08 µm to 97.16 µm (DTI 60 min) (Table. 6).

Table 6. Effect of dispersion time interval type on particle size mean of polymeric microparticle blends

Polymer		**Particle size mean (µm) (± SD)**	
Emulsion 1	**Emulsion 2**	**DTI 0 min**	**DTI 60 min**
EC 4 cp	EC 4 cp	79.35 (± 5.74)	78.42 (± 4.93)
EC 4 cp	Eudragit RL 100	95.41 (± 4.21)	93.27 (± 5.68)
EC 4 cp	Eudragit RS 100	72.29 (± 4.66)	74.08 (± 5.93)
EC 4 cp	Polycaprolactone	91.09 (± 5.82)	88.31 (± 5.17)
EC 4 cp	PLGA (RG503H)	88.39 (± 4.38)	97.16 (± 4.51)

Microparticle blends agglomerates are EC-Eudragit RL 100, EC-PCL and EC-PLGA (RG503H) (Fig. 27.b,d,e). The chord length distributions measured by FBRM for all batches of microparticle blends with DTI 0 and 60 min are different (Fig. 28a and Fig. 28b). These curve showed that larger square weighted mean chord length gave longer chord length distributions and lower peak of particle number due to the decreased number of microparticles.

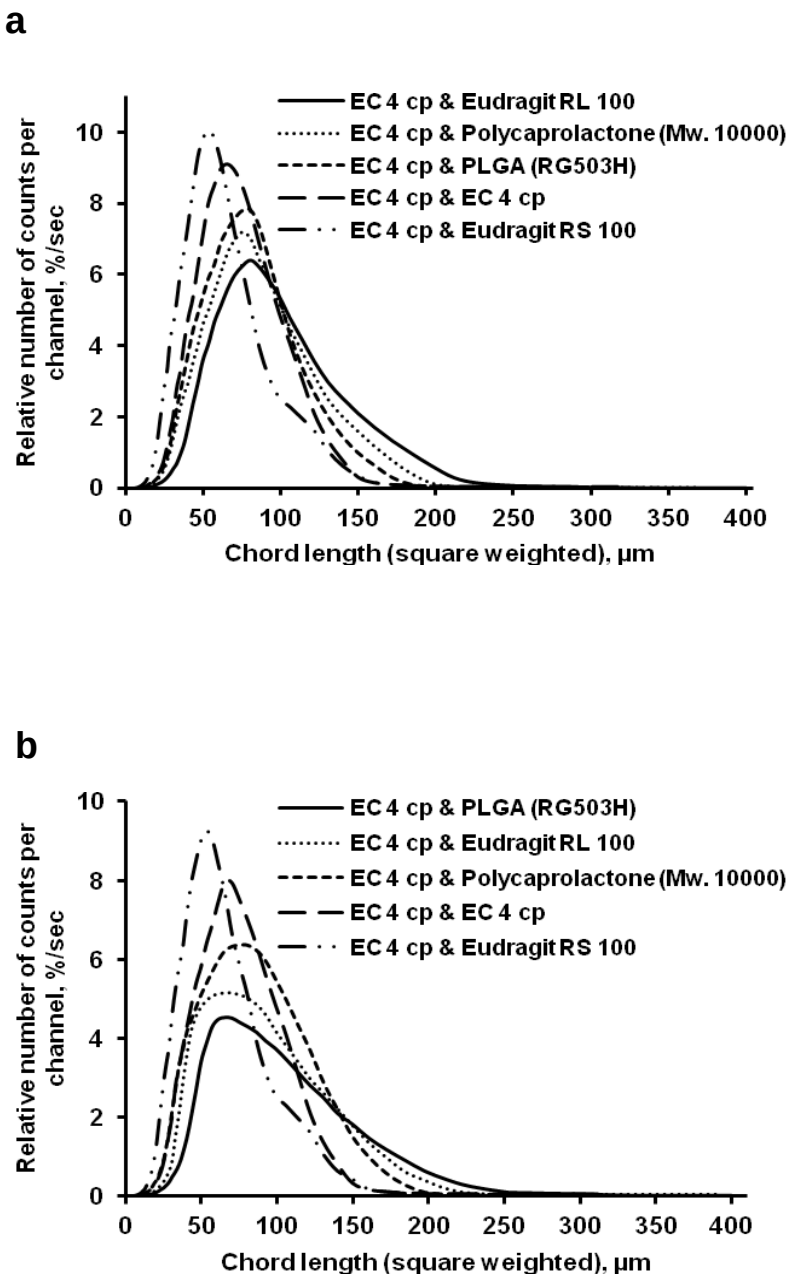

Fig. 28. Effect of dispersion time interval on the chord length distributions [(a) DTI 0 min and (b) DTI 60 min; at 4 hours stirring time]

3.2.3. Conclusion

The FBRM was successfully employed as a powerful and convenient process analyzer for the qualitative online monitoring of the shift in the microparticle chord length distribution for polymeric microparticles and microparticle blends during solvent

evaporation process. FBRM detect transformation of the emulsion droplets into solid microparticles and microparticle blends agglomeration by a change in the FBRM signal. FBRM signal was influenced by surface characteristics and optical properties of particles. Based on FBRM data, the transformation from the emulsion droplets into solid particles in solvent evaporation process when using EC 4 cp, Eudragit® RS 100 and Eudragit® RL 100 occuring within the first 10-25 minutes after feeding the emulsion into the external aqueous phase, while using PLGA (RG503H) within 30 min and polycaprolactone within 55 min. Polymeric microparticle blends agglomerates were occurred after 20 min emulsification (for DTI 0 min) and after 20 min addition second oil phase into external aqueous phase containing hard EC microparticles (for DTI 60 min). Microparticle blends agglomerates are EC - PLGA (RG503H), EC - PCL and EC – Eudragit RL 100.

Different polymers in preparation of microparticles gave different microparticles properties. Particles with good scattering properties provided comparable values for the CLD and chord count and also good estimation of particle size. The microparticle CLD, transformation process and agglomeration was strongly influenced by polymer type.

3.3. Online monitoring of ethyl cellulose (EC) microparticles preparation process by focused beam reflectance measurement (FBRM): Effect of stirring speed, volume of external aqueous phase and polymer concentration

3.3.1. Introduction

Several methods and techniques are potentially useful for the preparation of microparticles in the field of controlled drug delivery. The type and the size of the microparticles, the entrapment, release characteristics and stability of drug in microparticles in the formulations are dependent on the method used. One of the most common methods of preparing microparticles is solvent evaporation technique. The conventional emulsification solvent evaporation technique elaborated by Bodmeier and Mc Ginity (1987), Ogawa et al. (1988), Jeffery et al. (1991), and different recent variations are commonly used for encapsulation of various substances from simple pharmaceutical products to proteins and DNA (Rosca et al., 2004). Although the technique is well defined as methodology, there are only a few studies about the microparticle formation mechanism. Microparticle formation mechanism is crucial for size distribution and morphology, which in turn determine the delivery system behavior during encapsulation and release. It is well known that solvent evaporation technique is mainly a two-step process: the emulsification of a polymer solution containing the encapsulated substance, followed by particle hardening through solvent evaporation and polymer precipitation. During emulsification, the polymer solution is broken up in microdroplets by the shear stress produced either by homogenizer, sonicator or whirl mixer in the presence of a surface active agent.

Various formulation parameters and operating conditions influence the properties of microparticles. It is very common to vary the viscosity of the dispersed phase. Instead, the viscosity of the continuous phase is rarely modified. That is because the viscosity of the continuous phase is very close to that of water. Increasing polymer concentration or the molecular weight of polymer increases the viscosity of dispersed phase (Li et al., 2008; Yeo and Park, 2004). The size increases exponentially with viscosity. Increasing viscosity improves also the drug encapsulation efficiency. Additionally, agitation is one of the most important parameter for controlling the size of microparticles after the physicochemical properties of materials. Many other factors linked to agitation have also an impact on the size of microparticles, such as: the geometry of the vessel, the number of impellers and their position and the ratio of impeller's diameter compared to the vessel's diameter (Maa

and Hsu, 1996). There is a great number of correlations that predict the size and distribution of the size of the drops in an emulsion of two immiscible liquids (Maa and Hsu, 1996). The correlations take into account two aspects: (1) the physical properties of materials, such as the density of continuous phase and the interfacial tension, and (2) the factors linked to agitation. Increased viscosity of the drug/matrix dispersion yields larger microparticles because higher shear forces are necessary for droplet disruption (Freitas et al., 2005). Various studies reported a reduction in the mean microparticle size with decreasing continuous phase volume (Benoit et al, 1999).

The hardening rate and the mechanism of the initial emulsion or emulsion droplets transformed into the hardened microparticles by solvent elimination and polymer precipitation influence the properties of microparticles. This process determines the microparticle morphology and has important influence on the microparticle encapsulation and release behavior. Information about the microparticle hardening rate from these several parameters are not available. It is necessary to know the effect of several parameters on the polymeric microparticle hardening time.

To date very little work has been carried out on the online monitoring of microparticles during their formation. In a solvent evaporation process, solidification of the emulsion droplets and particle size changes occur after emulsifying the organic inner phase into the external aqueous phase (Freitas et al., 2005; Li et al., 1995; O'Donell and McGinity, 1997). The transformation of the emulsion droplets into solid microparticles can be monitored by focused beam reflectance measurement (FBRM). FBRM is a laser based technique, which offers the advantage of in-line measurement of the chord length distribution (CLD) of dispersed particles inside a flowing fluid, without the need of installing a pre-dilution side-stream, as required for other online particle sizing tools. It does not require sampling that could affect the actual particle size distribution due to breakdown or aggregation. The FBRM signal strongly depends on the surface properties of the measured sample, it provides an effective solution to track the process (Boxall et al., 2010; Kail et al., 2009; Vay et al., 2012; Yu and Erickson, 2008; Zidan et al., 2010). FBRM uses a focused beam of laser light, which scans in a circular path. As this light scans across a particle or its structure passing in front of the probe window, light is scattered in all directions. The light scattered back towards the probe is used to measure a chord length of the given particle (Kail et al., 2009; Kempkes et al., 2008; Kougoulos et al., 2005; Leba et al., 2010; Simmons et al., 1999; Vay et al., 2012). With regard to

controlling such a microparticle preparation process the determination of the rate and time point of conversion from liquid droplets into solid particles is of great interest.

A variety of applications using FBRM have been developed and reported in the literature, including crystallization control (Abbas et al., 2002; Barrett et al., 2002; Yu et al., 2005), flocculation process design (Williams et al., 1992), slurry transfer (Daymo et al., 1998), monitoring of polymorphic transitions (O'Sullivan et al., 2003), particle disruption control (Kougoulos et al., 2005) and solubility measurements (Kim et al., 2005). Furthermore several studies using the FBRM for investigation of emulsion systems (Dowding et al., 2001; Turner et al., 2009) have been published. Zidan et al. have studied using FBRM in order to monitor a solvent extraction process for microparticle preparation with different polymer concentration and different drug loading (Zidan et al., 2010). They found the transformation of the emulsion droplets into solid particles can be detected by a change in the FBRM signal. Vay et al. used FBRM in solvent evaporation process and found out that the transformation of the emulsion droplets into solid particles is influenced stirring speed and temperature. Increasing in stirring speed and temperature give fast solidification rate of particle (Vay et al., 2012).

Interestingly, in most studies reported so far in the literature, online monitoring of polymeric microparticles formation with several parameters using FBRM has not been reported yet. Based on this fact, the current study aimed to employ FBRM for online monitoring of solidification rate of polymeric microparticles and particle size/distribution of the emulsion droplets/hardened polymeric microparticles manufactured by O/W method using various parameters.

3.3.2. Results and discussion

3.3.2.1. Microparticles morphology and size

SEM photomicrograph of ethyl cellulose (EC) microparticles which were prepared by O/W method are shown in Fig. 29. The surface analysis of microparticles prepared by the O/W method revealed that ethyl cellulose (EC) microparticles were spherical with smooth surfaces, no pores and no aggregation. The particle size mean of microparticles which was prepared using high polymer concentration were larger than those prepared by low polymer concentration. This is caused by rapid solidification process occurring at the surface of embryonic microparticle droplets which resist in extensive shrinkage of embryonic microparticles droplets.

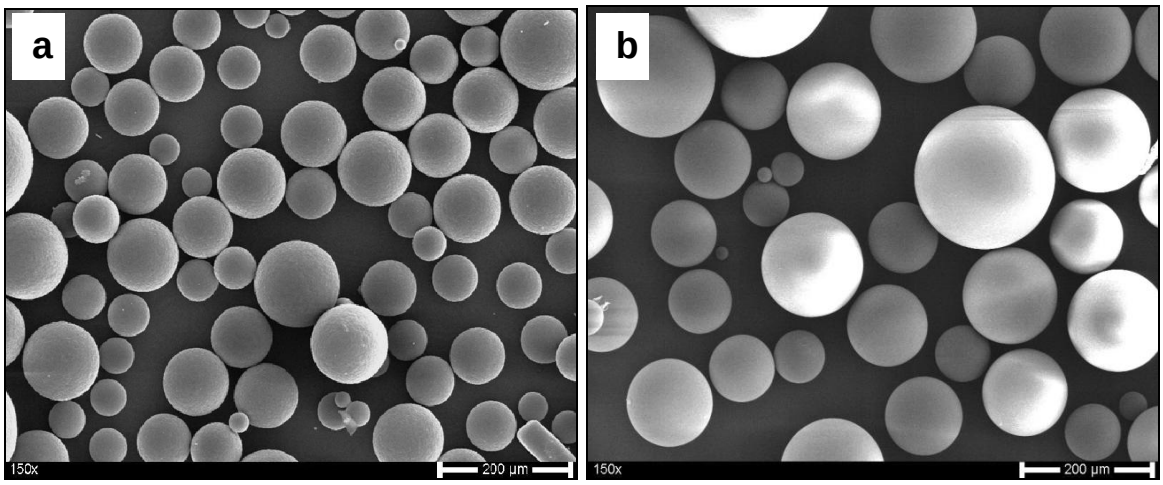

Fig. 29. SEM photomicrographs of ethyl cellulose based microparticles with different polymer concentration. (a. 7.5% (w/v) ; b. 10% (w/v))

3.3.2.2. Online monitoring of polymeric microparticles formation

Effect of stirring speed

FBRM was used for qualitative online monitoring of the shift in the chord length distributions (CLD) at various stages of microparticles ripening at certain agitation rates. The main focus of this study was to monitor microparticles formation, changes of the particle size, hardening rate, particle's properties and chord length distribution. The curve of square weighted mean chord length of polymeric microparticles shown as a function of time (Fig. 30a). Type of stirring speed which used in polymeric microparticles formation lead to a different square weighted mean chord length profile during solvent evaporation method. Initially as the organic polymer solution was emulsified, FBRM detected larger droplet size mean for each stirring speed, by increasing the process time the particle size decreased followed by a plateau size where the square weighted mean chord length was constant. For stirring speed of 200 rpm, the square weighted mean chord length of microparticles were larger than stirring speed of 500 rpm. The square weighted mean chord lengths of EC microparticles are 212 µm (200 rpm) and 83 µm (500 rpm).

Stirring speed is the most straightforward method to generate droplets of the drug/matrix dispersion in the continuous extraction phase for subsequent solvent removal. In the simplest approach, extraction phase is filled into a vessel and agitated by an impeller. The drug/matrix dispersion is then added, dropwise or all at once, under agitation at a speed sufficient to reach the desired droplet size. Obviously, the impeller speed is the main parameter for controlling the drug/matrix dispersion's droplet size in the continuous

phase. Increasing the mixing speed generally results in decreased microsphere mean size (Freitas et al., 2005; Li et al., 2008), as it produces smaller emulsion droplets through stronger shear forces and increased turbulence.

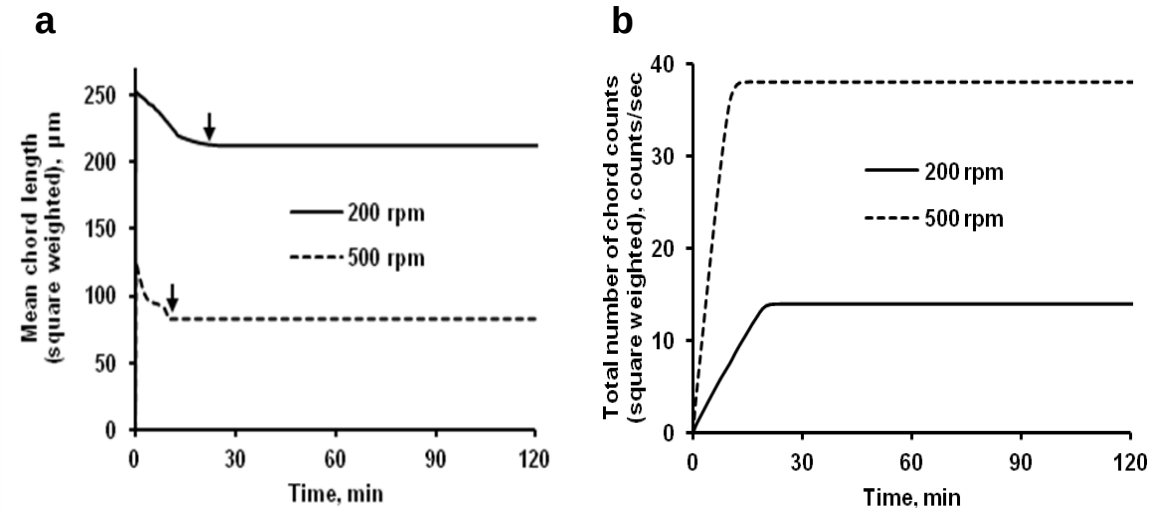

Fig. 30. Effect of stirring speed on (a) square weighted mean chord length during microparticles formation and on (b) the number of chord counts (square weighted) during solvent evaporation process. (Arrow (↓): starting time of microparticle hardening)

Size of microparticles is determined by the stirring speed. Stirring speed is the dominating factor because it provides the energy to disperse the oil phase in water. Our experimental results demonstrate that a high stirring speed yields smaller microparticles because the second emulsion is broken up into smaller droplets at a higher input power. So, yield is higher because microspheres are formed much more at a higher input power. Thus, the stirring speed needs to be optimized in order to obtain a sufficiently high yield of microspheres with a desired size distribution.

The hardening rate of microparticles influenced stirring speed. Rate of solvent removal influence the solidification rate of the dispersed phase as well as morphology of the resulting microparticles. High stirring speed have led to fast solidification of microparticles. Since, fast solvent removal rate and the polymer precipitates faster on the surface of the dispersed phase. The droplets shrinkage can be separated in two phases when applying the stirring speed of 200 rpm and 500 rpm. A phase of rapid shrinkage where the initial droplet size decreased markedly within 15 and 9 min, was followed by a discontinued or slow shrinkage phase where no further pronounced shrinkage could be

observed (Fig. 30a). It reveals that the solvent was rapidly extracted in the first (rapid) phase and that in the second, discontinued or slow phase, the embryonic microparticle droplets became solid microparticles. Based on FBRM data, the transformation of the emulsion droplets into solid microparticles were occured at 10.5 min (stirring speed of 500 rpm) and 20 min (stirring speed of 200 rpm) (Fig. 30a).

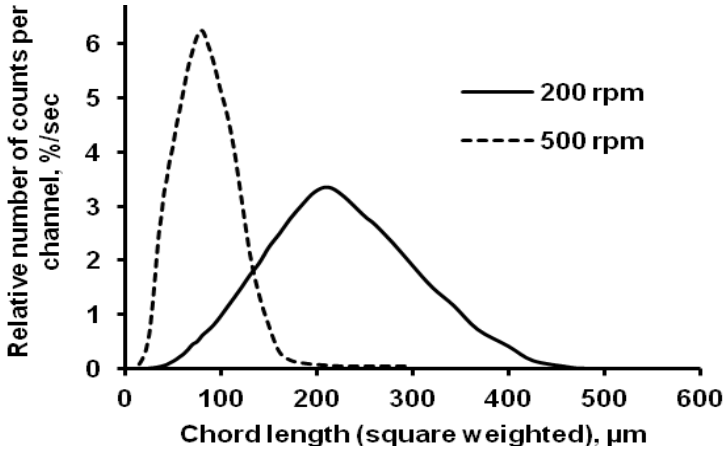

Fig. 31. Effect of stirring speed on the square weighted chord length distributions (at 4 hours stirring time)

The curve of chord count of polymeric microparticles shown as a function of time (Fig. 30b). Both stirring speed, 200 rpm and 500 rpm shows different profile of ethyl cellulose microparticles chord count during solvent evaporation method. Initially as the organic polymer solution was emulsified, FBRM detected lower chord counts, by increasing the process time, the chord counts increased followed by a plateau phase where the chord counts constant. The chord counts of ethyl cellulose microparticles were prepared by stirring speed of 200 rpm was lower than stirring speed of 500 rpm. It is due to production of microparticles with the larger size.

The chord length distributions of microparticles measured by FBRM for various stirring speed with the same volume of external aqueous phase are different (Fig. 31). As expected, larger microparticles gave longer chord lengths and lower peak of particle number due to the decreased number of microparticles. In this case it was observed that with increasing stirring speed the square weighted mean chord length decreased and chord lengths distribution become narrow. The stirring speed of 500 rpm produced smaller

microparticles than stirring speed of 200 rpm. It therefore have smaller square weighted mean chord length, narrow chord length distributions and higher peak of particle number.

Effect of volume of external aqueous phase

The hardening rate and particle size of microparticles influenced volume of external aqueous phase. An increase in the volume of the external aqueous phase (from 400 to 800 ml) resulted in an increase in microparticle size (Fig. 32a). The increase in particle size was attributed to a reduction in agitation that occurred because of a decrease in mixing efficiency associated with larger volumes. A reduction in mixing efficiency probably produced an increase in the size of the emulsion droplets formed during the preparative process, which would result in the formation of large microparticles (Benoit et al., 1999). In addition, there is relationship between the volume of the external aqueous phase and the particle size of the microparticles produced.

The droplets shrinkage can be separated in two phase when using the volume of external aqueous phase of 400 and 800 ml. A phase of rapid shrinkage where the initial droplet size decreased markedly within 30 and 15 min, was followed by a discontinued or slow shrinkage phase where no further pronounced shrinkage could be observed (Fig. 32a). It shows that the solvent was rapidly extracted in the first (rapid) phase and that in the second, discontinued or slow phase, the embryonic microparticle droplets became solid microparticles. The transformation of the emulsion droplets into solid microparticles were occured at 20 min (800 ml) and 45 min (400 ml) (Fig. 32a). The square weighted mean chord lengths of EC microparticles are 212 µm (800 ml) and 159 µm (400 ml).

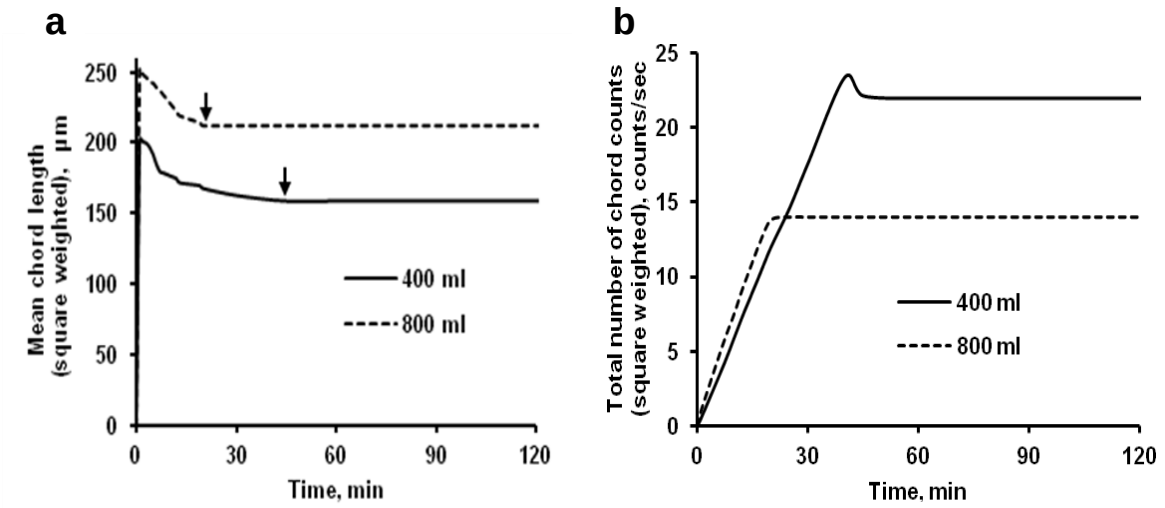

Fig. 32. Effect of volume of external aqueous phase on (a) square weighted mean chord length during microparticles formation and on (b) the number of chord counts (square weighted) during solvent evaporation process. (Arrow (↓): starting time of microparticle hardening)

The volume of external aqueous phase affects ethyl cellulose microparticles chord count during solvent evaporation method. For volume of external aqueous phase of 800 ml, initially as the organic polymer solution was emulsified, FBRM detected lower chord counts, by increasing the process time, the chord counts increased followed by a plateau phase where the chord counts constant. Whereas volume of external aqueous phase of 400 ml, initially as the organic polymer solution was emulsified, FBRM detected lower chord counts, by increasing the process time, the chord counts increased followed by decreasing the chord counts. It was followed by a plateau phase where the chord counts constant. The chord counts of ethyl cellulose microparticles prepared by volume of external aqueous phase of 400 ml was higher than volume of external aqueous phase of 800 ml (Fig. 32b). It is due to production of microparticles with the smaller size.

The chord length distributions of microparticles for various of volume of external aqueous phase are different (Fig. 33). As expected, larger microparticles gave longer chord lengths and lower peak of particle number due to the decreased number of microparticles. In this case it was observed that with increasing volume of external aqueous phase the square weighted mean chord length increased and chord lengths distribution become longer. The volume of external aqueous phase of 800 ml produced larger microparticles than volume of external aqueous phase of 400 ml. It therefore have larger square weighted mean chord length, longer chord length distributions and lower peak of particle number.

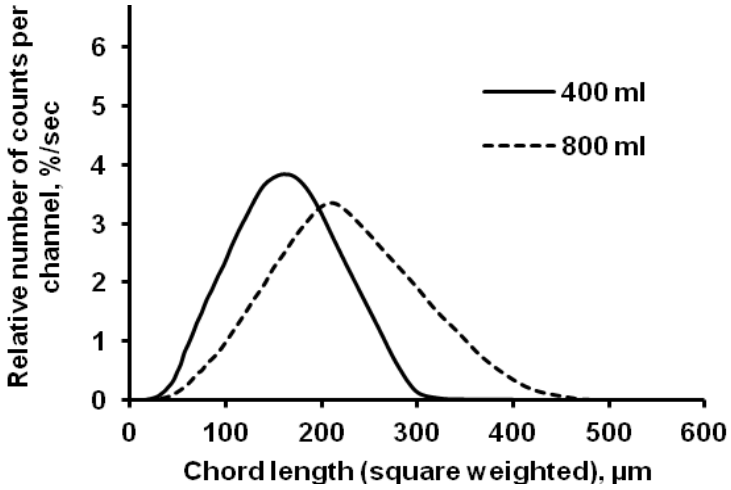

Fig. 33. Effect of volume of external aqueous phase on the square weighted chord length distributions (at 4 hours stirring time)

Effect of polymer concentration

Ethyl cellulose (EC) microparticles were prepared using different concentrations of EC (from 7.5 to 20%, w/v) by variation in the weight of polymer dissolved in dichloromethane. he eventual modifications of the solidification rate of ethyl cellulose (EC) microparticles and particle size/distribution of the emulsion droplets/hardened microparticles during solvent evaporation process has been investigated. Increasing the concentration (weight) of polymer in a fixed volume of organic solvent resulted in an increase in mean particle size. The hardening rate and particle size of microparticles was influenced by polymer concentration.

An increase in the polymer concentration (from 7.5 to 20%, w/v) resulted in an increase in microparticle size (Fig. 34a). The droplets shrinkage can be separated in two phase when using the polymer concentration of 7.5%, 10%, 15% and 20 %. A phase of rapid shrinkage where the initial droplet size decreased markedly within 9, 8, 7 and 5.5 min, was followed by a discontinued or slow shrinkage phase where no further pronounced shrinkage could be observed (Fig. 34a). It reveals that the solvent was rapidly extracted in the first (rapid) phase and that in the second, discontinued or slow phase, the embryonic microparticle droplets became solid microparticles. Based on FBRM data, the transformation of the emulsion droplets into solid microparticles were occured at 10.5 min (7.5%), 8.7 min (10%), 7.5 min (15%) and 6 min (20%) (Fig. 34a). The square weighted

mean chord lengths of EC microparticles are 83 µm (7.5%), 98 µm (10%), 128 µm (15%) and 150 µm (20%).

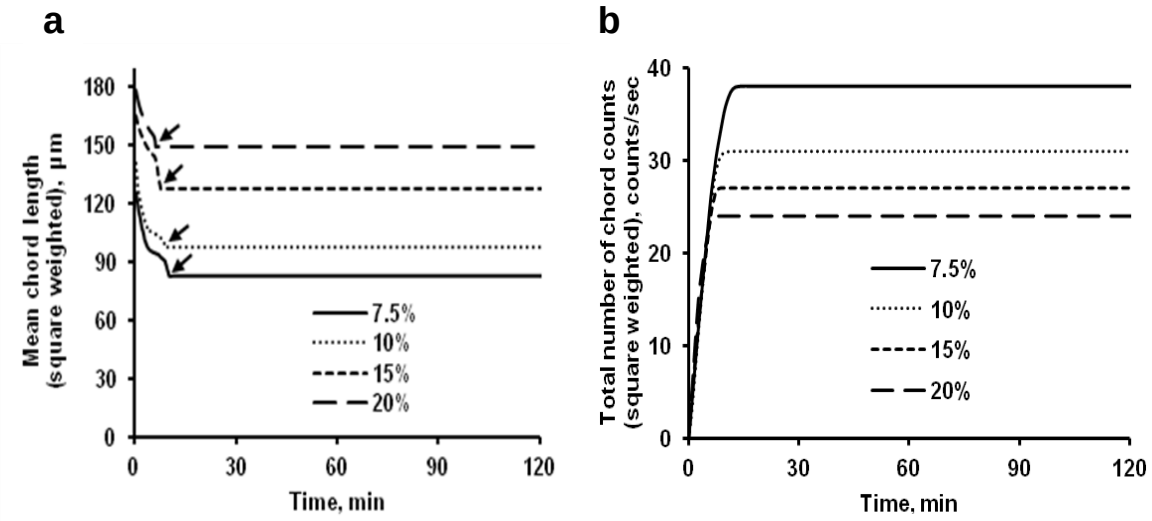

Fig. 34. Effect of polymer concentration on (a) square weighted mean chord length during microparticles formation and on (b) the number of chord counts (square weighted) during solvent evaporation process. (Arrow (↓): starting time of microparticle hardening)

Type of polymer concentration shows different profile of ethyl cellulose microparticles chord count during solvent evaporation method. For all of polymer concentration, initially as the organic polymer solution was emulsified, FBRM detected lower chord counts, by increasing the process time, the chord counts increased followed by a plateau phase where the chord counts constant. The chord counts of ethyl cellulose microparticles were increased by decreasing polymer concentration (Fig. 34b). Since low polymer concentration produced microparticles with the smaller size and high particle number. The chord length distributions of microparticles measured by FBRM for different of polymer concentration are different (Fig. 35). As expected, larger microparticles gave longer chord lengths and lower peak of particle number due to the decreased number of microparticles. In this case it was observed that with increasing polymer concentration the square weighted mean chord length increased and chord lengths distribution become longer. The polymer concentration of 20% (w/v) produced larger microparticles than others. It therefore have larger square weighted mean chord length, longer chord length distributions and lower peak of particle number.

This is in agreement with the findings of Jeffery et al. (1991), suggesting that the higher concentration of polymer in the sample may have led to an increased frequency of

collisions, resulting in fusion of semiformed particles and producing finally an overall increase in the size of the microparticles. Finally, at high polymer concentration (20%, w/v), the viscosity was so high that the efficiency of emulsion stirring was reduced and allowed the production of large particles. The viscosity increases with increasing polymer concentration. High viscosity have led to fast solidification of microparticles. When highly concentrated, the polymer precipitates faster on the surface of the dispersed phase.

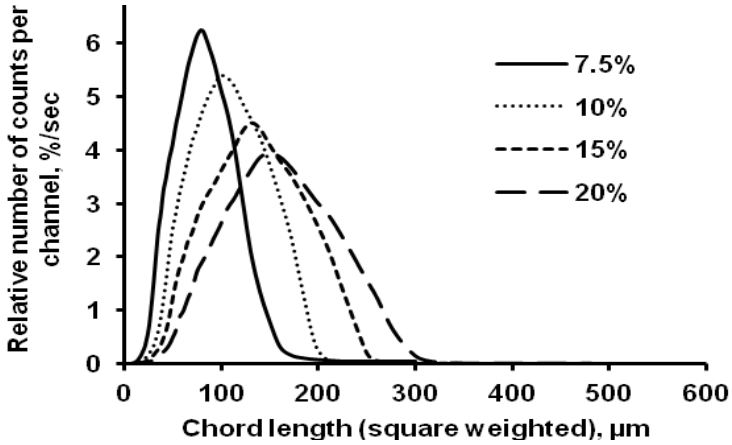

Fig. 35. Effect of polymer concentration on the square weighted chord length distributions (at 4 hours stirring time)

Effect of combinations of different stirring speeds

The impact of combination of stirring speed during solvent evaporation process by oil in water (O/W) on chord length distribution (CLD), particle size mean, solidification rate and chord count of ethyl cellulose 4 cp (EC) had been investigated using Lasentec FBRM. Morphology of EC microparticle blends were characterized by scanning electron microscopy (SEM). The surface of microparticle blends revealed that ethyl cellulose (EC) microparticle blends were spherical with smooth surfaces, no pores and no aggregation (Fig. 36). The particle size of microparticles which was prepared using high stirring speed were smaller than those prepared with low stirring speed, as it produces smaller emulsion droplets through stronger shear forces and increased turbulence (Freitas et al., 2005).

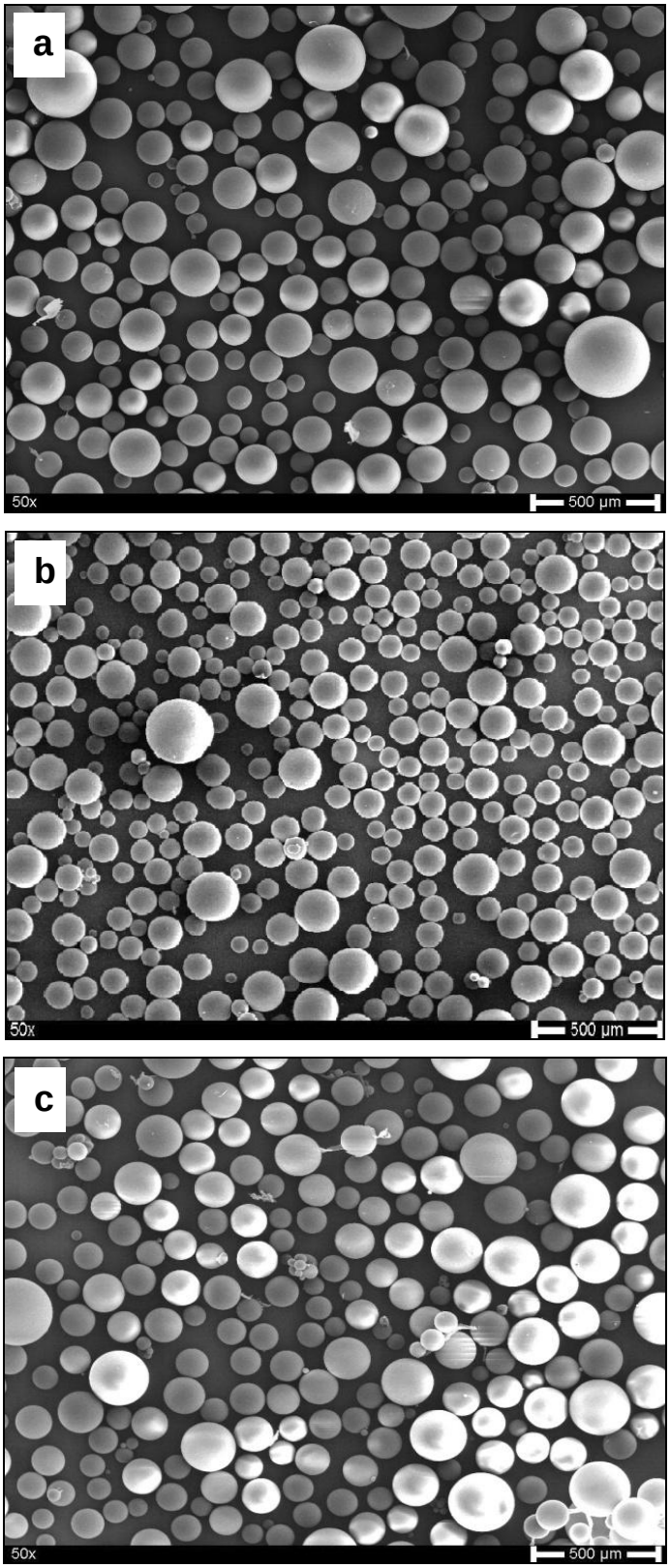

Fig. 36. SEM photomicrographs of ethyl cellulose microparticle blends with different stirring speed. (a. 200 rpm to 500 rpm; b. 500 rpm to 200 rpm; c. Microparticle blends from 200 and 500 rpm)

It was observed from FBRM data that transformation of the emulsion droplets into solid particles occured within the first 20 and 10.5 minutes, when stirring speed 200 and 500 rpm used respectively. The square weighted mean chord lengths of EC microparticles are 212 µm (200 rpm) and 83 µm (500 rpm) (Fig. 37). When these microparticles were mixed and stirred at 200 and 500 rpm, square weighted mean chord lengths of 142 µm and 139 µm, respectively (Fig. 37). The CLDs result of these various stirring speed showed that larger particle size mean gave longer CLD and a reduced count rate due to the decreased of particles number. Square weighted mean chord lengths and CLD of mixed microparticle were not so different for stirring speed 200 and 500 rpm. While the number of counts of 200 rpm was lower than that of 500 rpm (Fig. 38).

The final size of microparticle blends were influenced by combination of stirring speed. EC microparticle blends were prepared by solvent evaporation method (O/W) with stirring speed 200 rpm for first polymer formulation, and emulsification of the second polymer formulation after hardening of the first polymer formulation with stirring speed 500 rpm. The square weighted mean chord lengths of EC microparticle blends are 134 µm (Fig. 37). Whereas, EC microparticle blends were prepared with stirring speed 500 rpm for first polymer formulation, and emulsification of the second polymer formulation after hardening of the first polymer formulation with stirring speed 200 rpm, the square weighted mean chord lengths of EC microparticle blends are 122 µm (Fig. 37). Emulsification of the second polymer formulation after hardening of the first polymer formulation resulted in the formation of a blend of two separate microparticle formulation in one process when compared to two separate preparation processes followed by blending of the individually prepared microparticle formulations. This phenomenon showed good scattering properties of EC microparticles. The microparticle CLD and transformation process was strongly influenced by stirring speed.

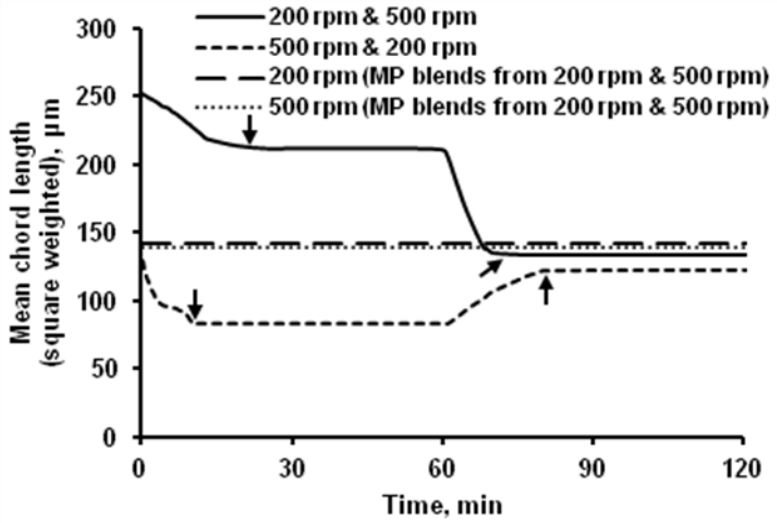

Fig. 37. Effect of different stirring speed on the square weighted mean chord length of ethylcellulose microparticle blends. (Arrow (↓): starting time of microparticle hardening)

The chord length distributions of microparticles measured by FBRM for ethyl cellulose microparticle blends (200 and 500 rpm) with different stirring speed are not different in square weighted mean chord lengths and chord length distribution, except in peak of particle number (Fig. 38). The stirring speed of 200 rpm gives lower peak of particle number than stirring speed of 500 rpm, due to the decreased number of microparticles.

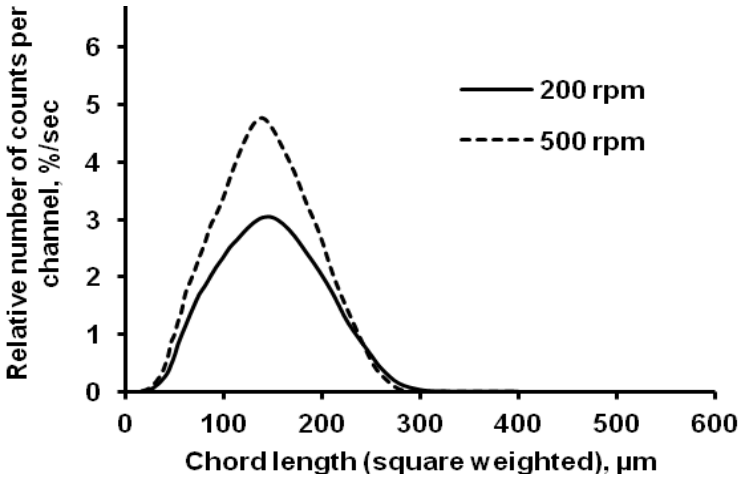

Fig. 38. Effect of different stirring speed on the square weighted chord length distributions of ethyl cellulose microparticle blends (200 and 500 rpm). (at 4 hours stirring time)

Fig. 39 shows the chord length distributions of droplets/hardened microparticles of ethyl cellulose microparticle blends at certain time during solvent evaporation process. Larger droplets/hardened microparticles gave longer chord length distribution and lower peak of particle number. Based on data in Fig. 37, at the maximum square weighted mean chord length, the chord length distribution shows a minimum number of counts and the distribution is as far to the right as possible.

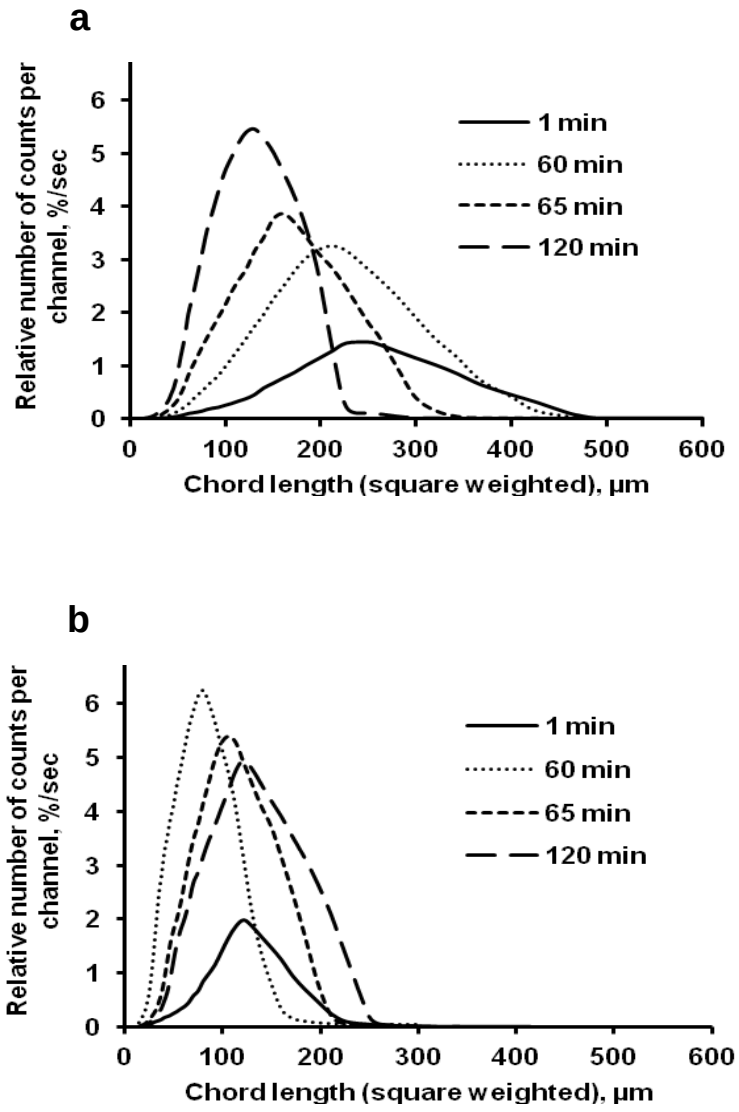

Fig. 39. Effect of different stirring speed on the square weighted chord length distributions of ethyl cellulose microparticle blends at certain time during microparticles formation. (a) 200 and 500 rpm, and (b) 500 and 200 rpm

The FBRM was successfully employed as a powerful and convenient process analyzer for the qualitative online monitoring of the shift in the microparticle chord length

distribution for polymeric microparticles and microparticle blends during solvent evaporation process. FBRM is based on reflection measurement and strongly depending on the optical properties of microparticles. FBRM measures and monitores the chord length distribution (CLD), particle size mean and chord count of microparticles in real time, which is affected by the geometry, size, and number of particles under analysis (Boxall et al., 2010; Dowding et al., 2001; Heath et al., 2002; Kail et al., 2009; Kirwan, 2009; Kougoulos et al., 2005; Leba et al., 2010; Wynn, 2003; Yu and Erickson, 2008). Additionally, the measurement principle of FBRM differs fundamentally from other established particle sizing methods. The reflected beam signal provides information on the number of particles present and one-dimensional information on the size of the particles, referred to as chord lengths, as a function of time. Despite a strong dependence on the optical properties of the samples, FBRM is the most beneficial technique for online monitoring of microparticle preparation processes.

3.3.3. Conclusion

The FBRM can be used for on line monitoring of effect of stirring speed, volume of external aqueous phase and polymer concentration on CLD, chord counts, square weighted mean chord length and hardening rate of microparticles from emulsion droplets until become solid microparticles during solvent evaporation process. Based on FBRM data, the transformation of the emulsion droplets into solid microparticles occured within the first 10.5 and 20 min when stirring speed of 500 rpm and 200 rpm; the first 20 and 45 min when volume of external aqueous phase of 800 ml and 400 ml; and the first 6, 7.5, 8.7, and 10.5 min when polymer concentration were 20%, 15%, 10% and 7.5% (w/v). The square weighted mean chord lengths of EC microparticles were 212 and 83 µm when stirring speed of 500 rpm and 200 rpm; 159 and 212 µm when volume external aqueous phase 800 ml and 400 ml; and 83, 98, 128 and 150 µm when polymer concentration were 7.5%, 10%, 15% and 20% (w/v) respectively. Furthermore the emulsification of the second polymer formulation after hardening of the first polymer formulation resulted in the formation of a blend of two separate microparticle formulation in one process when compared to two separate preparation processes followed by blending of the individually prepared microparticle formulations. The CLDs measured by FBRM showed that a larger particle size mean gave longer CLD and a lower peak of particle number due to the decreased number of microparticles. FBRM data are highly dependent on the optical

properties of materials and opacity level of microparticles. Scanning electron microscopy (SEM) data revealed that the morphology of microparticles were spherical with smooth surfaces, no pores and no aggregation.

The microparticle CLD and transformation process was strongly influenced by several parameters, such as stirring speed, volume of external aqueous phase and polymer concentration. Despite a strong dependence on the optical properties of the samples, FBRM is the most beneficial technique for online monitoring of microparticle preparation processes.

3.4. Slow release of propranolol HCl from ethyl cellulose based microparticle blends

3.4.1. Introduction

Microparticles are widely used in different applications such as the controlled release of drugs, cosmetics and chemical reagents. Several methods are potentially useful for the preparation of microparticles in the field of controlled drug delivery. One of the most common methods for preparing microparticles is the solvent evaporation method (Bodmeier and McGinity, 1988; Freitas et al., 2005; Li et al., 2008; O'Donell and McGinity, 1997). The control of the microparticle preparation processes is essential to produce a desired mean size of the microparticles, size distribution and morphology of the microparticles. The solubility properties of the drugs of the microparticles are important parameters when selecting the emulsion phases for a microparticles preparation process. A low solubility of the drugs in the continuous phase is required for obtaining a high yield. Microparticles can encapsulate many types of drugs including small molecules, proteins, and nucleic acids. Depending on the solubility of the drug, simple or multiple emulsion techniques like oil-in-water (O/W) or water-in-oil-in-water (W/O/W) methods are used (Pérez et al., 2003; Yamakawa et al., 1992; Yang et al., 2000a). The microparticle preparation method is a governing factor in the encapsulation and release of drugs. In addition, a complicated array of factors including the type of polymer, the polymer molecular weight, the copolymer composition, the nature of any excipients added to the microparticle formulation (e.g., for stabilization of the drugs), porosity, and the microparticle size can have a strong impact on the delivery rates (Herrmann and Bodmeier, 1995; Pérez et al., 2000; Yang et al., 2000b and 2001).

Polymers have been used as a main tool to control the drug release rate from the formulations. Polymers can bind the particles of a solid dosage form. Pharmaceutical polymers are widely used to achieve taste masking; controlled release (e.g., extended, pulsatile, and targeted), enhanced stability, and improved bioavailability. Non biodegradable polymers with good biocompatibility are also used as drug carriers, such as ethyl cellulose (degradable but non biodegradable). EC is a derivative of cellulose in which some of the hydroxyl groups on the repeating anhydroglucose units are modified into ethyl ether groups, largely called as non-ionic ethyl ether of cellulose. EC has extensively been used for microencapsulation due to its many versatile properties such as water insoluble but soluble in many organic solvents such as alcohol, ether, ketone and ester;

biocompatible and compatible with many celluloses, resin and almost all plasticizers; stable against light, heat, oxygen and wetness and chemicals; non-toxic; etc (Murtaza, 2012). EC is used for microencapsulation of various pharmaceuticals to stabilize them against active interactions, hydrolysis and oxidation. It also is employed as a matrix and/or coating agent to impart sustained release characteristics.

In most studies reported so far, only one drug was entrapped within controlled release microparticles at a time. Only few attempts have been made on the co-encapsulation of two drugs, especially if the latter exhibits significantly different solubility behavior. Pérez et al. (2000) have incorporated a lipophilic and a hydrophilic drug simultaneously within biodegradable, poly (ε-caprolactone)-based microparticles by solvent evaporation methods. In another study, Pérez et al. (2003) have successfully incorporated the hydrophilic drug propranolol HCl and/or the lipophilic drug nifedipine separately as well as simultaneously within non-degradable, ammonio methacrylate copolymers (Eudragit RS:RL 4:1 blends) based microparticles. They were prepared with an oil-in-water (O/W) and a water-in-oil-in-water (W/O/W) solvent evaporation method. Whereas, Nippe and General (2012) have developed a combination of lipophilic steroidal drugs ethinyl estradiol and drospirenone poly(lactic-co-glycolic acid) (PLGA) microparticles. Combination products also known as fixed dose combinations are combinations of two or more active drugs produced in a single dosage forms. They provide the advantages of combination therapy while useful to improve adherence and can simplify procurement, storage and distribution of medicines. Fixed dose combination drugs are an important approach to addressing the management of both chronic and acute diseases.

Microparticle blends containing two drugs with different solubility have not been reported yet. In the present study, the solvent evaporation method was used to incorporate a lipophilic and a hydrophilic drug within ethyl cellulose based microparticle blends. The hydrophilic drug propranolol HCl and the lipophilic drug carbamazepine were used as model drugs. Accurate particle size analysis during solvent evaporation process is a key to study microparticle blends formation from oil-in-water (O/W) and water-in-oil-in-water (W/O/W) methods. For more information about micropaticle blends formation during solvent evaporation process, FBRM can be used to provide in situ/on-line particle characterization in a wide range of applications (Dowding et al., 2001; Kail et al., 2009; Vay et al., 2012; Wu et al., 2011; Zidan et al., 2010). The great advantage of this technique is that data is acquired on-line and in real time to give particle size data and population

trends of particles in suspension, emulsion etc. (Boxall et al., 2010; Dowding et al., 2001; Kail et al., 2009; Ruf et al., 2000; Vay et al., 2012; Wu et al., 2011; Zidan et al., 2010).

The purpose of this study was to investigate effect of dispersion time interval (DTI) and formulation of second primary emulsion/oil phase on ethyl cellulose based microparticle blends contained the same drug (propranolol HCl) and contained drugs with different solubility (Propranolol HCl and carbamazepine) which prepared by solvent evaporation method.

3.4.2. Results and discussion

3.4.2.1. Morphology and particle size/distribution of microparticle blends

In this experiment two kinds of microparticle blends had been prepared. The first microparticle blends contained the same drug (propranolol HCl and propranolol HCl) and the second microparticle blends contained different drugs with different solubility (propranolol HCl and carbamazepine). The surface morphology of the microparticles was observed by scanning electron microscopy (SEM). The microparticle blends contained the same drug (propranolol HCl and propranolol HCl) revealed that the microparticles were spherical and not aggregated (Fig. 41) with diameters of 104.26 µm to 127.64 µm. For the microparticle blends prepared with second primary emulsion consisted of EC and dichloromethane or propranolol HCl, EC and dichloromethane (with DTI 60 min) appeared two population of microparticles. These microparticles show with pores and rough surface (Fig. 41c and Fig. 41d). Whereas microparticle which prepared with DTI 0 min (Fig. 41b) produced microparticles with pores.

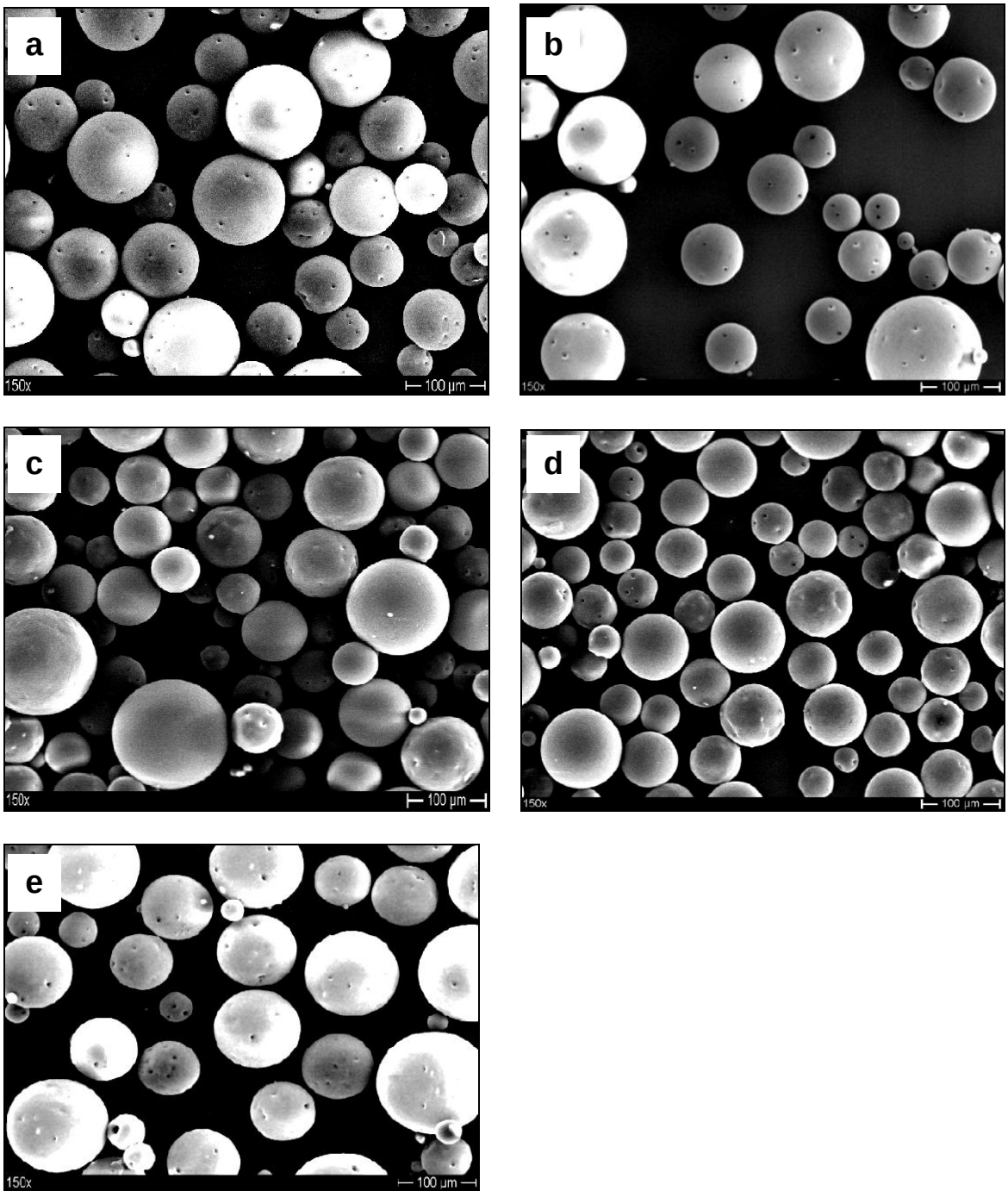

Fig. 41. SEM pictures of ethyl cellulose microparticles blend with varying dispersion time interval between primary emulsion 1 and primary emulsion 2 [(a) Pro (W/O/W); (b) Pro (W/O/W) and Pro (W/O/W), DTI: 0 min; (c) Pro (W/O/W) and Pro (W/O/W), DTI: 60 min; (e) Pro (W/O/W) and EC 4 cp (W/O/W), DTI: 60 min; (e) Pro (W/O/W) and dichloromethane, DTI: 60 min]

SEM images and optical microscopy pictures showed that propranolol HCl loaded microparticles (W/O/W) and carbamazepine loaded microparticles (O/W) have spherical shape, smooth surface (CBZ) and porous surface (Pro) (Fig. 42).

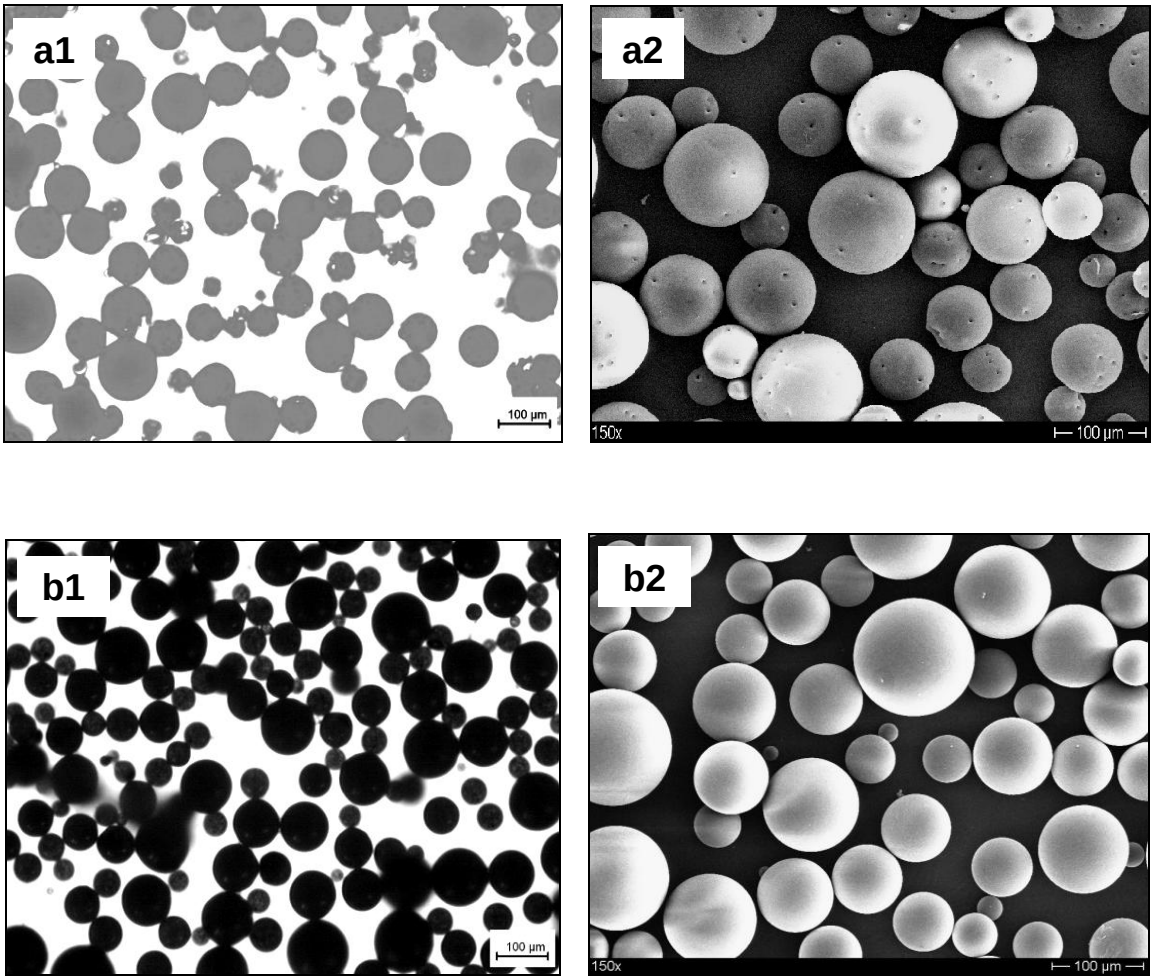

Fig. 42. Optical microscopy pictures [b1 containing dye (black)] (1) and SEM pictures (2) of ethyl cellulose microparticles. [(a) Pro (W/O/W); (b) CBZ and dye (O/W)

The surface analysis of drug-loaded microparticle blends with different drug solubility prepared by the WO/W (Pro) and O/W (CBZ) reveal that the microparticles were spherical and not aggregated (Fig. 43) with a diameter range of 113.27 µm to 122.42 µm.

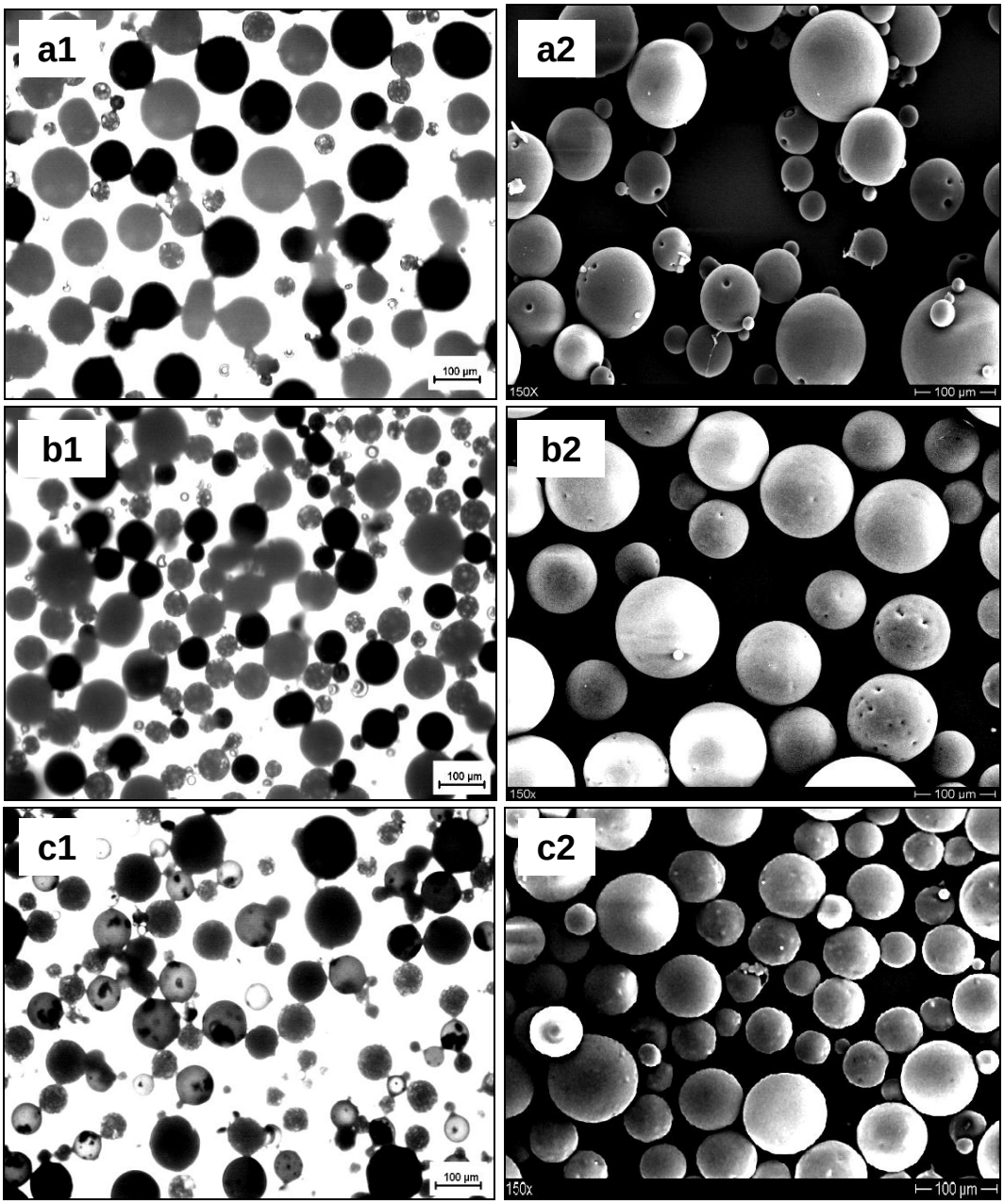

Fig. 43. Optical microscopy pictures [a1, b1 and c1 containing dye (black)] (1) and SEM pictures (2) of ethyl cellulose microparticle blends with varying dispersion time interval between primary emulsion and primary oil phase. [(a) CBZ (O/W) and Pro (W/O/W), DTI: 60 min; (b) Pro (W/O/W) and CBZ (O/W), DTI: 0 min; (c) Pro (W/O/W) and CBZ (O/W), DTI: 60 min]

Microparticle blends containing both, propranolol HCl and carbamazepine, prepared by the WO/W (Pro) and O/W (CBZ) methods with DTI 60 min appeared in two population of microparticles, smooth and rough surface (Fig. 43.c2). While microparticle which prepared with DTI 0 min (Fig. 43.b2) produced microparticles with pores and smooth surface. This phenomenon was the same for preparing microparticle blends with emulsification stage, first O/W (CBZ) and second W/O/W (Pro) with DTI 60 min produced microparticles with pores and smooth surface (Fig. 43.a2). Micropores were observed on the microparticles surface that it was propranolol HCl loaded EC microparticles. No pores were observed on the microparticles surface that it was carbamazepine loaded EC microparticles.

The preparation conditions substantially affected the morphology and porosity of the microparticles. In W/O/W method, the microparticles revealed a porous inner structure caused by the inner aqueous phase. The aqueous droplets are precursors of pores and are the result of phase separation occurring in the organic phase during the hardening of the microparticles (Freiberg and Zhu, 2004; Grattard et al., 2002; Pérez et al., 2000, 2003; Siepmann et al., 2004; Yeo and Park, 2004).

Microparticle blends contained the same drug (propranolol HCl), the particle size mean of microparticles blend with DTI 60 min was larger than the microparticles blend with DTI 0 min after stirring 4 h (Fig. 44).

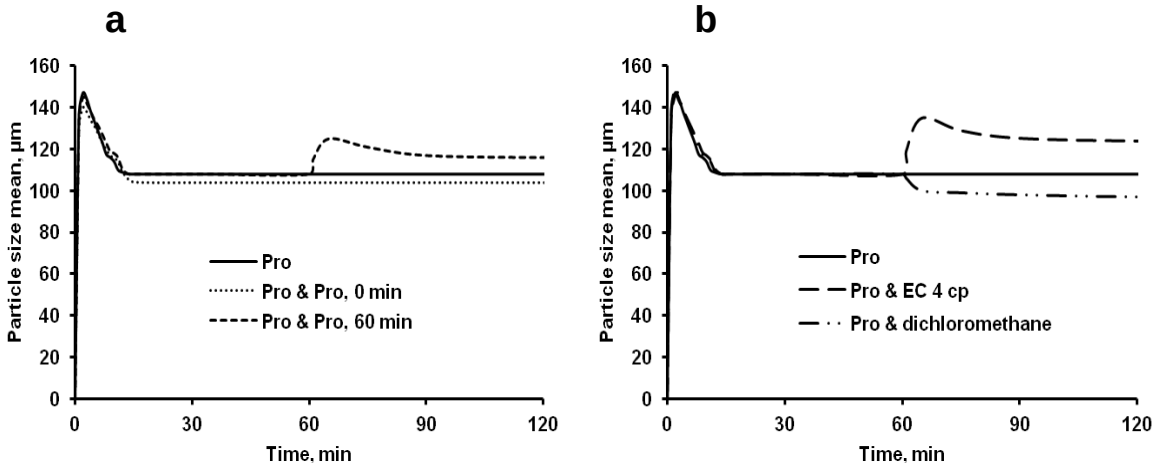

Fig. 44. Effects of dispersion time interval (a) and the primary emulsion formulation 2 (b) on particle size mean of ethyl cellulose based microparticle blends obtained by the FBRM method during the solvent evaporation process. (Primary emulsion 2 is added at time = 60 min)

When second oil phase consisted of just dichloromethane (with DTI 60 min), the particle size mean of microparticles blend was smaller than the microparticles normal (Fig. 44b). Whereas, the particle size mean of microparticles blend was larger than the others when the second primary emulsion contain EC and dichloromethane (Fig. 44b). It was observed from FBRM data that addition of second primary emulsion after stirring 60 min into single external aqueous phase affected the particle size. Addition of dichloromethane as second primary oil phase dissolved polymer on the surface of first hard particles, so produce particles in smaller size. While, addition of second primary emulsion consisted of EC and dichloromethane or EC, propranolol HCl and dichloromethane (with DTI 60 min and stirring time 4 h) had been increased the particle size mean. They contributed in enhancement of particle size, so produce particles in larger size. All batches of microparticles show different particle size distributions profile (Fig. 45). As expected the larger particles size mean gave longer particle size distribution, and a reduced count rate due to the decreased number of particles. This phenomenon is applicable to all microparticle blends. The number of particles for microparticle blends was higher than for normal microparticles, it is due to higher volume of EC solution.

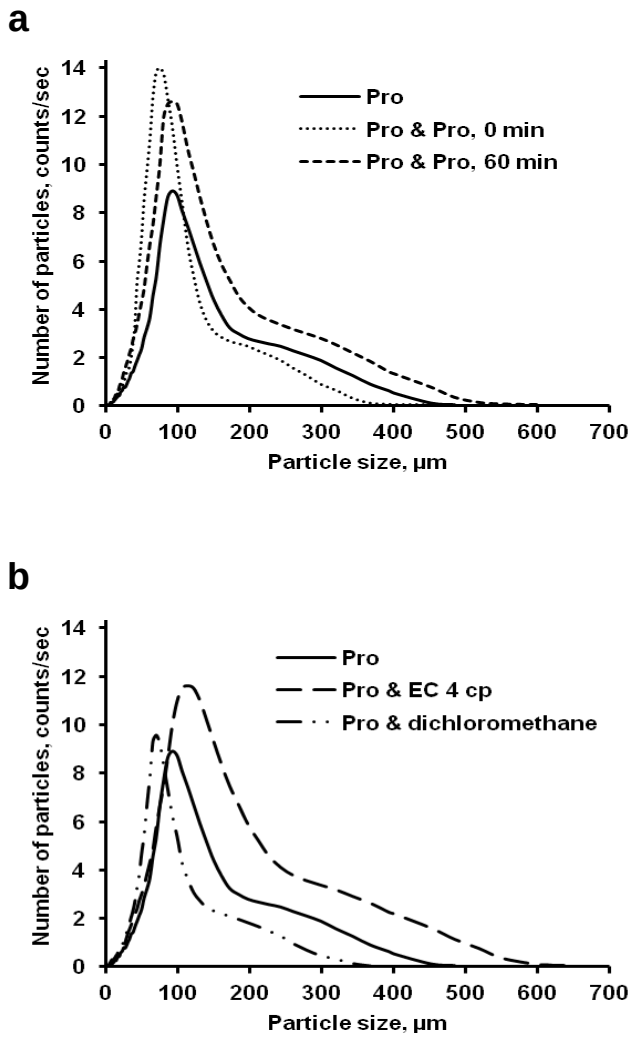

Fig. 45. Effects of dispersion time interval (a) and the primary emulsion formulation 2 (b) on particle size distribution obtained by the FBRM method for all batches of ethyl cellulose based microparticle blends (stirring time = 4 h)

For microparticle blends contained different drugs with different solubility (propranolol HCl and carbamazepine), the size of microparticle blends prepared by W/O/W (propranolol HCl) and O/W (carbamazepine) methods (with DTI 60 min and stirring time 4 h) was larger than the microparticle blends (with DTI 0 min) and microparticles normal (Fig. 46). Particle size mean/distribution before and after addition of the primary oil phase into single external aqueous phase (Fig. 46a and Fig. 46b). Based on FBRM data the addition of second primary oil phase contained EC, carbamazepine and dichloromethane (with DTI 60 min) contributed in enhancement of particle size.

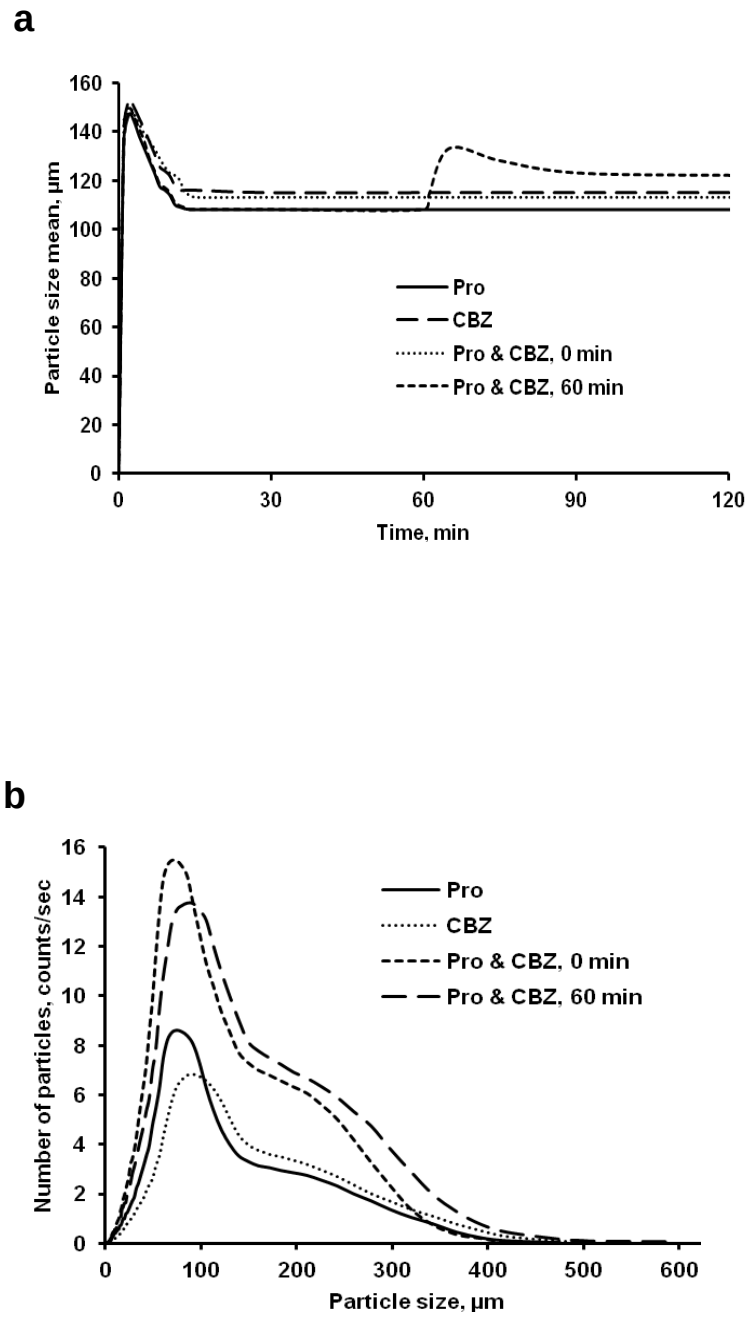

Fig. 46. Particle size mean of ethyl cellulose based microparticle blends obtained by the FBRM method (before and after primary oil phase addition) during the solvent evaporation process (primary oil phase is added at time = 60 min) (a), and Particle size distribution obtained by the FBRM method for all batches of ethyl cellulose based microparticles blend (at 4 hours stirring time) (b)

3.4.2.2. Entrapment efficiency within microparticle blends

Both microparticle blends types had a similar encapsulation efficiency (EE) for propranolol HCl. The EE was about 76.53% to 78.81% for propranolol HCl in microparticle blends containing the same drug (Table 7).

Table 7. Formulation, drug entrapments and particle size mean of microparticles (whole size)

Emulsion 1	Emulsion 2	Dispersion time interval (minute)	Actual drug loading (%) (± SD)	Encapsulation efficiency (%) (± SD)	Particle size mean (µm) (± SD)
Pro[a]	-	-	9.62 (± 0.35)	76.96 (± 2.83)	108.38 (± 4.07)
Pro[a]	Pro[a]	0	9.69 (± 0.31)	77.52 (± 2.47)	104.26 (± 3.16)
Pro[a]	Pro[a]	60	9.82 (± 0.26)	78.56 (± 2.11)	116.05 (± 5.37)
Pro[b]	No drug	60	9.85 (± 0.38)	78.81 (± 3.04)	127.64 (± 6.71)
Pro[a]	No drug	60	5.12 (± 0.29)	76.53 (± 2.32)	125.16 (± 6.08)
Pro[a]	dichloromethane	60	9.54 (± 0.38)	76.32 (± 3.04)	97.02 (± 4.25)

Pro[a]: consisted of propranolol HCl (DL 12.5%), ethyl cellulose and dichloromethane
Pro[b]: consisted of propranolol HCl (DL 25%), ethyl cellulose and dichloromethane
No drug: consisted of ethyl cellulose and dichloromethane

The encapsulation efficiency (EE) was about 77.28% to 78.64% for propranolol HCl and 96.48% to 98.64% for carbamazepine in microparticle blends containing different drugs (Table 8).

Table 8. Formulation, drug entrapments and particle size mean of microparticles (whole size)

Drug		Dispersion time interval (minute)	Actual drug loading (%) (± SD)		Encapsulation efficiency (%) (± SD)		Particle size mean (µm) (± SD)
Emulsion 1	Emulsion 2		Pro	CBZ	Pro	CBZ	
Pro	-	-	9.62 (± 0.35)	-	76.96 (± 2.83)	-	108.38 (± 4.07)
CBZ	-	-	-	12.31 (± 0.08)	-	98.48 (± 0.65)	115.09 (± 5.12)
Pro	CBZ	0	9.78 (± 0.28)	12.15 (± 0.07)	78.24 (± 2.26)	97.20 (± 0.53)	113.27 (± 4.35)
Pro	CBZ	60	9.83 (± 0.27)	12.33 (± 0.04)	78.64 (± 2.13)	98.64 (± 0.32)	122.42 (± 6.04)
CBZ	Pro	60	9.66 (± 0.31)	12.06 (± 0.11)	77.28 (± 2.51)	96.48 (± 0.84)	117.35 (± 3.25)

Pro: consisted of propranolol HCl (DL 12.5%), ethyl cellulose and dichloromethane
CBZ: consisted of carbamazepine (DL 12.5%), ethyl cellulose and dichloromethane

The difference observed in the EE of the two drugs in the microparticle blends can be explained with the different solubilities of the drugs in the aqueous continuous phase used for the two encapsulation techniques. The high solubility of the propranolol HCl in the external aqueous phase and its high volume compared to that of the internal aqueous phase (W/O/W technique) caused the leakage of the drug into the continuous phase. This leakage process is believed to happen mainly during the first minutes of emulsification since the polymer precipitates rapidly thereby decreasing leakage (Alhnan and Basit, 2011; Freiberg and Zhu, 2004; Hsu and Lin, 2005; Pérez et al., 2000, 2003). However, after the precipitation of the polymer, the propranolol HCl, due to its hydrophilic nature, still tends to diffuse through the polymeric matrix into the external aqueous phase. Beside that, the degree of ionization of the drug and the pH of the external aqueous phase are critical for the entrapment of ionizable drugs such as propranolol HCl (Pérez et al., 2000, 2003). Increasing the pH of the external phase above the pKa of the propranolol HCl results in a decrease of its solubility and, consequently, in an increase of its entrapment in the microparticles.

3.4.2.3. Release of drugs

Release of propranolol HCl from microparticle blends

The release of propranolol HCl from both EC microparticle blends in pH 7.4 phosphate buffer showed difference in release rate (Fig. 47 and Fig. 50). Different release rate of propranolol HCl which were given by microparticle blends containing the same drug which were prepared by the W/O/W method (primary emulsion 1) and W/O/W method (primary emulsion 2) (Fig. 47). The cumulative percent of propranolol HCl released from each microparticle blends (with range of actual drug loading (ADL) ≈ 4.92% to 8.76%) at pH 7.4 after 28 days is in the range of 28.95% to 73.28% (Fig. 47 and Table 9). The propranolol HCl release from microparticle blends with dispersion time interval 0 min (73.28%) was faster than 60 min (54.05%) (Fig. 47a). It is apparent from the data reported in Fig. 47 and Table 9 that the type of dispersion time interval and composition of formulation second primary emulsion affected the percent of propranolol HCl released. Porosity of the microparticles influenced drug release mechanism. This could lead to a more rapid release of propranolol HCl. The mechanism for release of propranolol HCl from ethyl cellulose matrix is diffusion through water-filled pores (Chiao and Price, 1994; Freiberg and Zhu, 2004; Grattard et al., 2002; Yeo and Park, 2004).

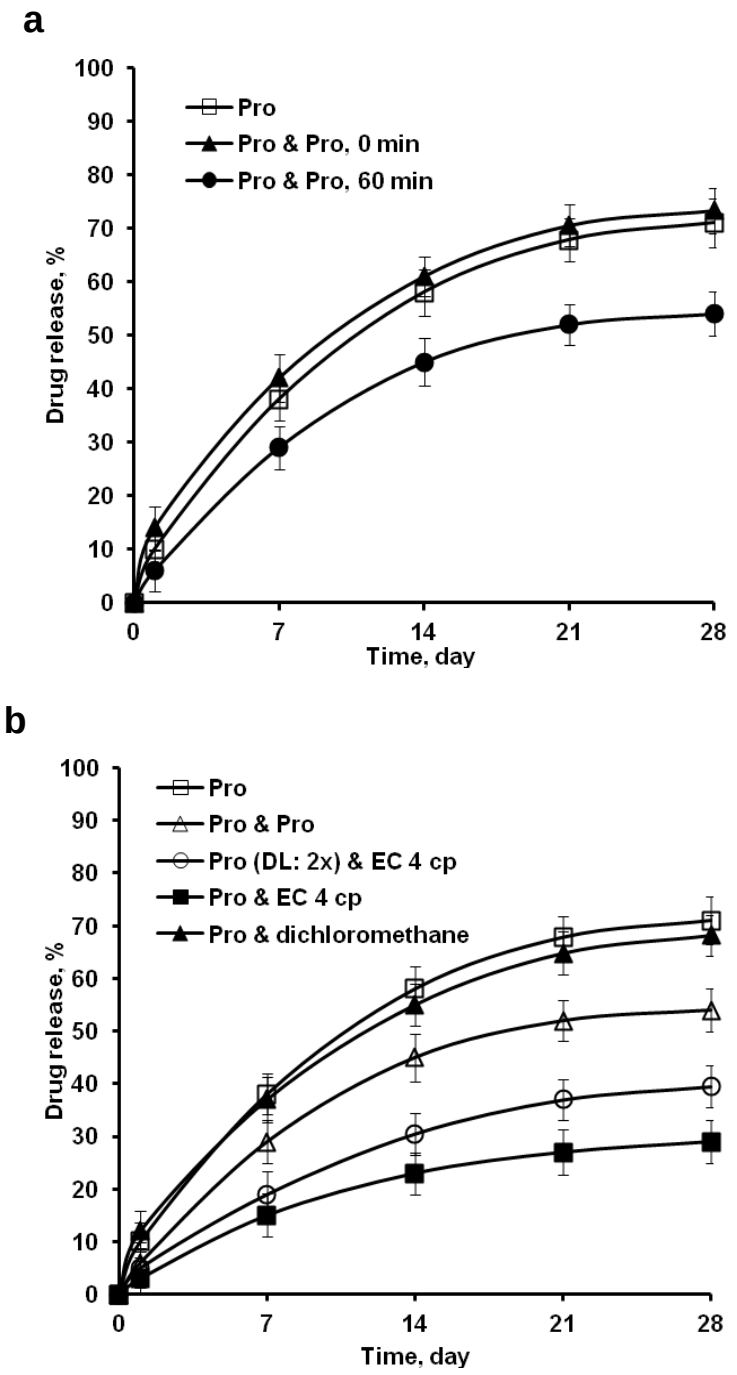

Fig. 47. Effects of dispersion time interval (a) and the primary emulsion formulation 2 (b) on propranolol HCl release from ethyl cellulose microparticle blends (phosphat buffer, pH 7.4, 37 ^{o}C, 75 rpm).

Table 9. Cumulative release of propranolol HCl from EC microparticles (particle size: < 70 µm) in phosphate buffer (pH 7.4) after 28 days

Drug		Dispersion time interval (minute)	Actual drug loading (%, w/w) (± SD)	Drug release (%) (± SD)
Emulsion 1	**Emulsion 2**			
Pro[a]	-	-	8.62 (± 0.41)	71.32 (± 4.73)
Pro[a]	Pro[a]	0	8.60 (± 0.49)	73.28 (± 4.31)
Pro[a]	Pro[a]	60	8.74 (± 0.38)	54.05 (± 4.09)
Pro[b]	No drug	60	8.76 (± 0.54)	39.48 (± 4.01)
Pro[a]	No drug	60	4.92 (± 0.43)	28.95 (± 3.86)
Pro[a]	dichloromethane	60	8.58 (± 0.37)	68.19 (± 3.93)

Pro[a]: consisted of propranolol HCl (DL 12.5%), ethyl cellulose and dichloromethane
Pro[b]: consisted of propranolol HCl (DL 25%), ethyl cellulose and dichloromethane
No drug: consisted of ethyl cellulose and dichloromethane

The propranolol HCl release was increased when particle size decreased. Clearly, microparticles size strongly affected the rate of drug release (Fig. 48). The mean diameters of five different size fraction of this microparticles were given in Table 10, ranging from 36.18 µm to 149.14 µm.

Table 10. Mean diameter and actual drug loading of different size fractions of propranolol HCl from EC based microparticle blends with different dispersion time interval (theoretical drug loading = 12.5% w/w)

Size fraction (µm)	Mean diameter (µm) (± SD)		Actual drug loading (%, w/w) (± SD)	
	0 min	**60 min**	**0 min**	**60 min**
< 41	36.18 (± 2.76)	38.46 (± 4.22)	6.92 (± 0.36)	6.95 (± 0.33)
41-70	61.05 (± 3.34)	65.18 (± 3.87)	8.97 (± 0.32)	9.14 (± 0.28)
71-100	91.77 (± 4.01)	94.25 (± 4.57)	10.04 (± 0.39)	10.12 (± 0.37)
101-160	141.46 (± 2.86)	149.14 (± 3.18)	11.21 (± 0.28)	11.39 (± 0.25)

As size decreases, the surface area-to-volume ratio of the particle increases. Thus, for a given rate of drug diffusion through the microparticles, the rate of flux of drug out of the microparticles, per mass of formulation, will increase with decreasing particle size. In addition, water penetration into smaller particles may be quicker due to the shorter distance from the surface to the center of the particle (Siepmann et al., 2004; Yang et al., 2000b,

2001). As diffusion is known to play a major role in the control of drug release from EC based microparticles, an increase in system size is a priori expected to result in reduced relative release rates. It is due to the increased length of the diffusion pathways and, thus, decreased drug concentration gradients.

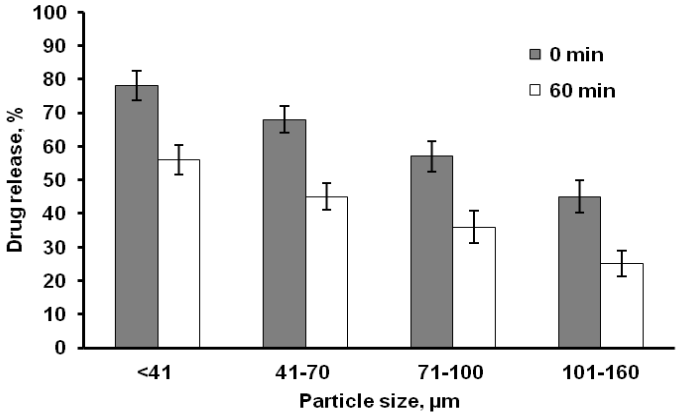

Fig. 48. Effect of the size of ethyl cellulose based microparticle blends on propranolol HCl release in phosphate buffer pH 7.4 (after 28 days)

Propranolol HCl release profile from each microparticle blends indicated there is interaction between first primary emulsion and second primary emulsion during preparation process of microparticle blends. The second primary emulsion had blocked and coated pores on the surface of hard particle from first primary emulsion (Fig. 49b. a1,b1). This hypothesa was supported by cross section of the microparticles blends (DTI 60 min) whereby the internal structure appeared reducing in the number of pores (Fig. 49b. a3,b3).

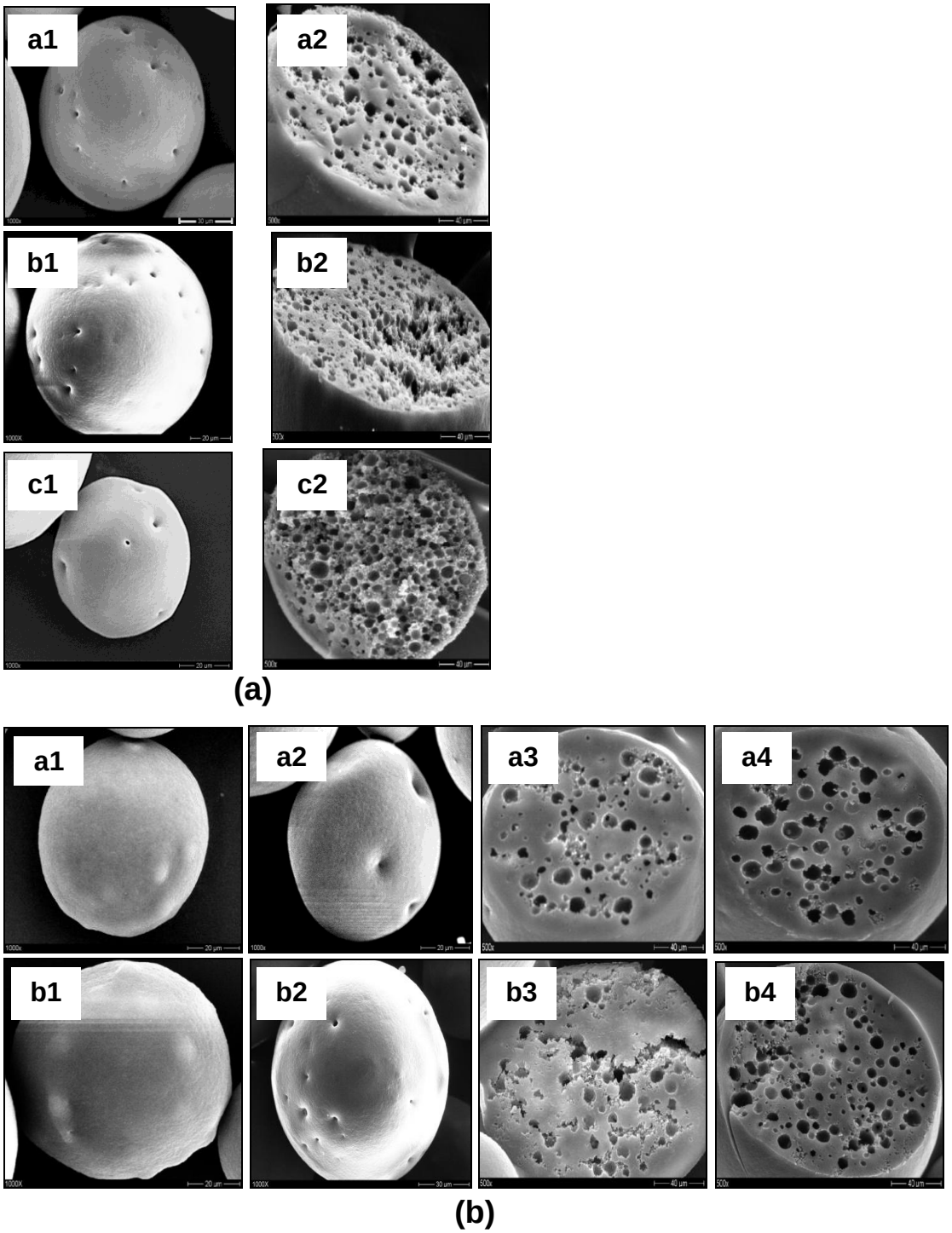

Fig. 49. SEM pictures of ethyl cellulose microparticle blends with varying dispersion time interval between primary emulsion 1 and primary emulsion 2 (higher magnification and cross-section). (a) a.1-2. Pro (W/O/W); b.1-2. Pro (W/O/W) and Pro (W/O/W), DTI: 0 min; and c.1-2. Pro (W/O/W) and dichloromethane, DTI: 60 min, and (b) a.1-4. Pro (W/O/W) and Pro (W/O/W), DTI: 60 min; and b.1-4. Pro (W/O/W) and EC 4 cp (W/O/W), DTI: 60 min

Release of propranolol HCl and carbamazepine from microparticle blends

Different release rate were observed for propranolol HCl and carbamazepine from EC microparticle blends in pH 7.4 phosphate buffer (Fig. 50).

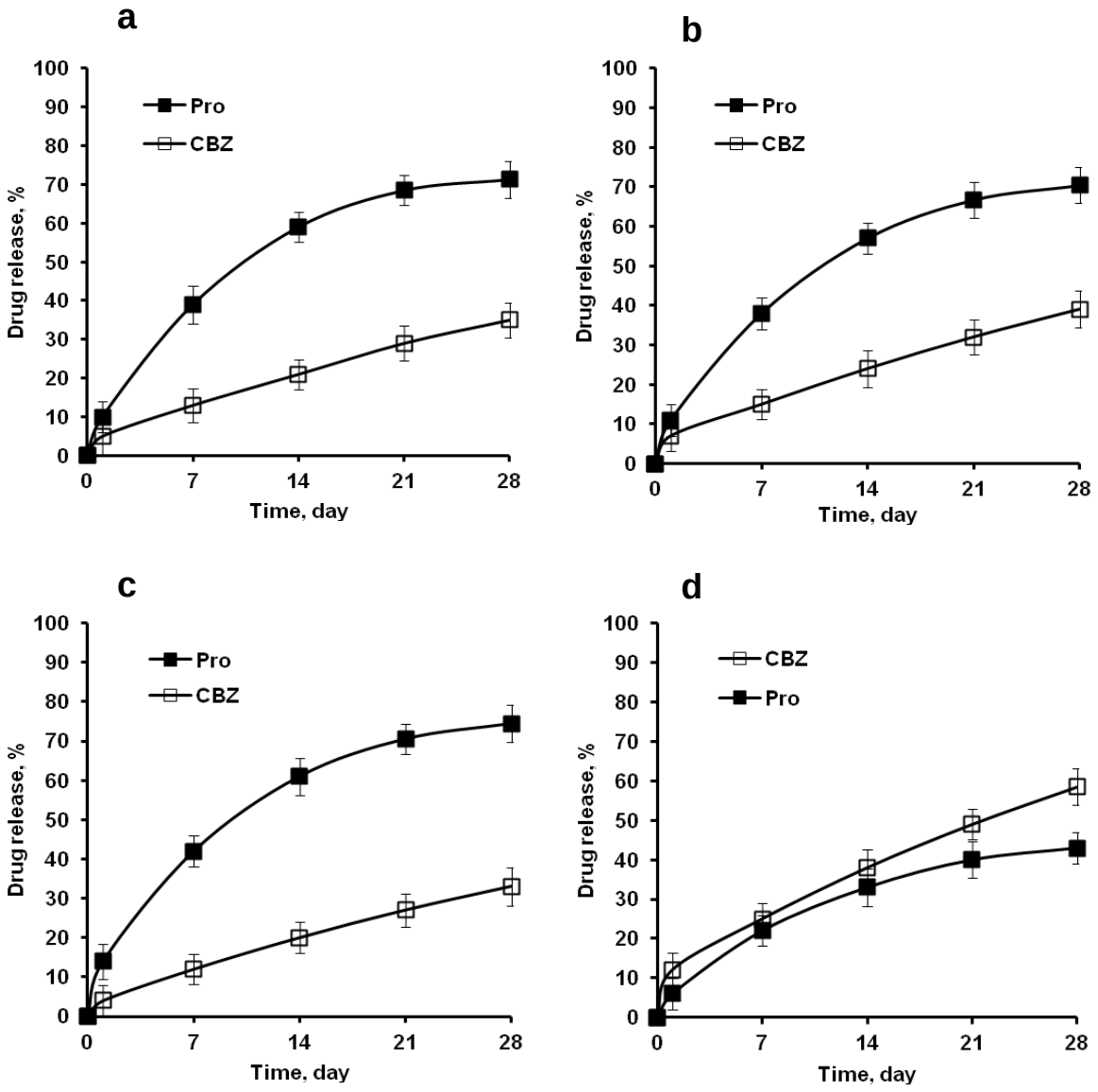

Fig. 50. Effects of the dispersion time interval between primary emulsion and primary oil phase on propranolol HCl and carbamazepine release from ethyl cellulose microparticle blends (phosphate buffer, pH 7.4, 37 ^{o}C, 75 rpm). [a. Pro (W/O/W) [single drug], CBZ (O/W) [single drug]; b. Pro (W/O/W) and CBZ (O/W), DTI: 0 min; c. CBZ (O/W) and Pro (W/O/W), DTI: 60 min; d. Pro (W/O/W) and CBZ (O/W), DTI: 60 min]

The propranolol HCl release from microparticle normal, microparticle blends (with DTI 0 min), microparticle blends (first primary oil phase (CBZ) and second primary

emulsion (Pro), DTI 60 min) were faster than carbamazepine release (Fig. 50. a-c). Whereas, propranolol HCl release (43.16%) was slower than carbamazepine release (58.72%) from EC microparticle blends (first primary emulsion (Pro) and second primary oil phase (CBZ), DTI 60 min) (Fig. 50d). Fig. 50 and Table 11 shows that the cumulative percent of propranolol HCl and carbamazepine released from each microparticle blends (the range of ADL Pro ≈ 8.59% to 8.64% and ADL CBZ ≈ 10.02% to 10.26%) at pH 7.4 after 28 days are in the range of 43.16% to 74.39% (propranolol HCl) and 33.05% to 58.72% (carbamazepine).

Table 11. Cumulative release of propranolol HCl and carbamazepine from EC microparticles (particle size: < 70 µm) in phosphate buffer (pH 7.4) after 28 days

Drug		**Dispersion time interval (minute)**	**Actual drug loading (%, w/w) (± SD)**		**Drug release (%) (± SD)**	
Emulsion 1	**Emulsion 2**		**Pro**	**CBZ**	**Pro**	**CBZ**
Pro	-	-	8.62 (± 0.41)	-	71.32 (± 4.73)	-
CBZ	-	-	-	10.26 (± 0.14)	-	35.06 (± 4.51)
Pro	CBZ	0	8.59 (± 0.34)	10.08 (± 0.08)	70.44 (± 4.48)	39.18 (± 4.65)
Pro	CBZ	60	8.64 (± 0.36)	10.19 (± 0.06)	43.16 (± 4.06)	58.72 (± 4.55)
CBZ	Pro	60	8.61 (± 0.39)	10.02 (± 0.12)	74.39 (± 4.72)	33.05 (± 4.82)

Pro: consisted of propranolol HCl (DL 12.5%), ethyl cellulose and dichloromethane
CBZ: consisted of carbamazepine (DL 12.5%), ethyl cellulose and dichloromethane

Particle size of microparticle blends influenced the rate of propranolol HCl and carbamazepine releases. The mean diameters of five different size fraction of this microparticle were given in Table 12, ranging from 38.05 µm to 154.07 µm.

Table 12. Mean diameter and actual drug loading of different size fractions of propranolol HCl and carbamazepine from EC based microparticle blends with different dispersion time interval (theoretical drug loading = 12.5% w/w)

Size fraction (µm)	Mean diameter (µm) (± SD)		Actual drug loading (%, w/w) (± SD)			
			Propranolol HCl		Carbamazepine	
	0 min	60 min	0 min	60 min	0 min	60 min
< 41	38.05 (± 3.38)	39.04 (± 3.82)	6.86 (± 0.34)	6.91 (± 0.32)	8.79 (± 0.09)	8.83 (± 0.07)
41-70	63.17 (± 4.06)	68.11 (± 4.71)	8.94 (± 0.29)	9.12 (± 0.27)	10.45 (± 0.05)	10.56 (± 0.04)
71-100	92.88 (± 5.17)	97.24 (± 5.63)	10.11 (± 0.36)	10.22 (± 0.33)	11.35 (± 0.07)	11.43 (± 0.06)
101-160	147.28 (± 4.75)	154.07 (± 5.02)	11.27 (± 0.31)	11.35 (± 0.28)	12.41 (± 0.08)	12.47 (± 0.05)

Propranolol HCl and carbamazepine release were increased with decreasing particle size (Fig. 51). In this figure show propranolol release was slower than carbamazepine release for all size fractions (with DTI 60 min). While for DTI 0 min, propranolol release was faster than the carbamazepine release for all size fractions. Clearly, microparticles size strongly affected the rate of drug release. As size decreases, the surface area-to-volume ratio of the particle increases. Thus, for a given rate of drug diffusion through the microparticles, the rate of flux of drug out of the microparticles, per mass of formulation, will increase with decreasing particle size. In addition, water penetration into smaller particles may be quicker due to the shorter distance from the surface to the center of the particle (Siepmann et al., 2004; Yang et al., 2000b, 2001). As diffusion is known to play a major role in the control of drug release from EC based microparticles, an increase in system size is a priori expected to result in reduced relative release rates. It is due to the increased length of the diffusion pathways and, thus, decreased drug concentration gradients.

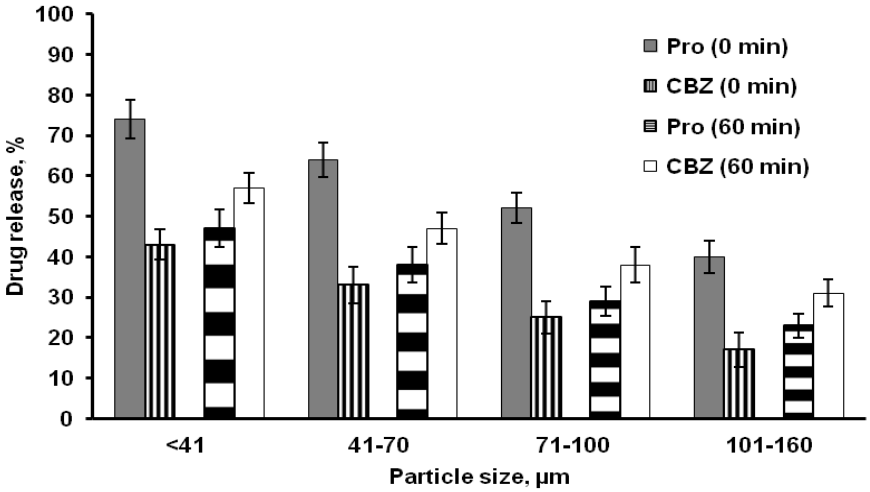

Fig. 51. Effect of the size of ethyl cellulose based microparticle blends on propranolol HCl and carbamazepine release in phosphate buffer pH 7.4 (after 28 days).

In all cases, the resulting release rate(s) of the incorporated drug(s) was/were found to be controlled over periods of at least 28 days. Furthermore, the release of carbamazepine was generally slower than that of propranolol HCl which can most probably be attributed to the much lower solubility of carbamazepine compared to propranolol HCl in the release medium (0.2 mg/ml vs. 250 mg/ml), resulting in lower concentration gradients, the driving forces for diffusion. Another reason is the fact that EC is water insoluble and also has very low permeability. Since this formulation did not contain any channeling agents, formation of pores and cracks did not occur to facilitate drug release. When propranolol HCl loaded EC microparticles were blended with second polymer organic formulation containing carbamazepine (with DTI 60 min), propranolol release became slower than carbamazepine release. This phenomenon might be attributable to the interaction of second primary oil phase (CBZ) with hard particles from first primary emulsion (Pro).

The increase of carbamazepine release from microparticle blend with the W/O/W (Pro) and O/W (CBZ) (DTI 60 min) could be due to the incorporation of carbamazepine on surface of propranolol HCl loaded microparticle. This may have reduced propranolol HCl migration to the surface of the microparticles, and its leakage in the dissolution medium as compared to the microparticles prepared with W/O/W method where the propranolol HCl is either molecularly dispersed or amorphous in the matrix. In addition, the porous

membrane observed in the case of the microparticles prepared with W/O/W method favoured a fast release of the hydrophilic propranolol HCl. The incomplete release of carbamazepine from microparticles may be the result of the hydrophobic nature of the drug and its very low water solubility.

On the contrary, the release of propranolol HCl was significantly slowed down in the case of the microparticle blends (DTI 60 min) compared to that of the microparticles normal. Only 43.16% of propranolol HCl was released from microparticle blends prepared by W/O/W (Pro) and O/W (CBZ) methods with DTI 60 min. It has to be emphasized that the propranolol HCl was inside of microparticle and carbamazepine was on outer surface of microparticle. Thus only the drug located close to the outer surface could be initially released. The release of surface associated drug creates water-filled channels that allow subsequent diffusion of the drugs located inside the microparticles. A major mechanism for release of propranolol HCl and carbamazepine are diffusion through water-filled pores.

Based on release data for each microparticle blends, it can be assumed that there is interaction between first primary emulsion (propranolol HCl) and second primary oil phase (carbamazepine) during preparation process of microparticle blends. FBRM data about particle size mean before and after addition of second primary oil phase into single external aqueous phase (Fig. 46) and surface morphology of microparticles blend (Fig. 43) have indicated it. In addition, cross section of the microparticles revealed a porous inner structure and absence of pores.

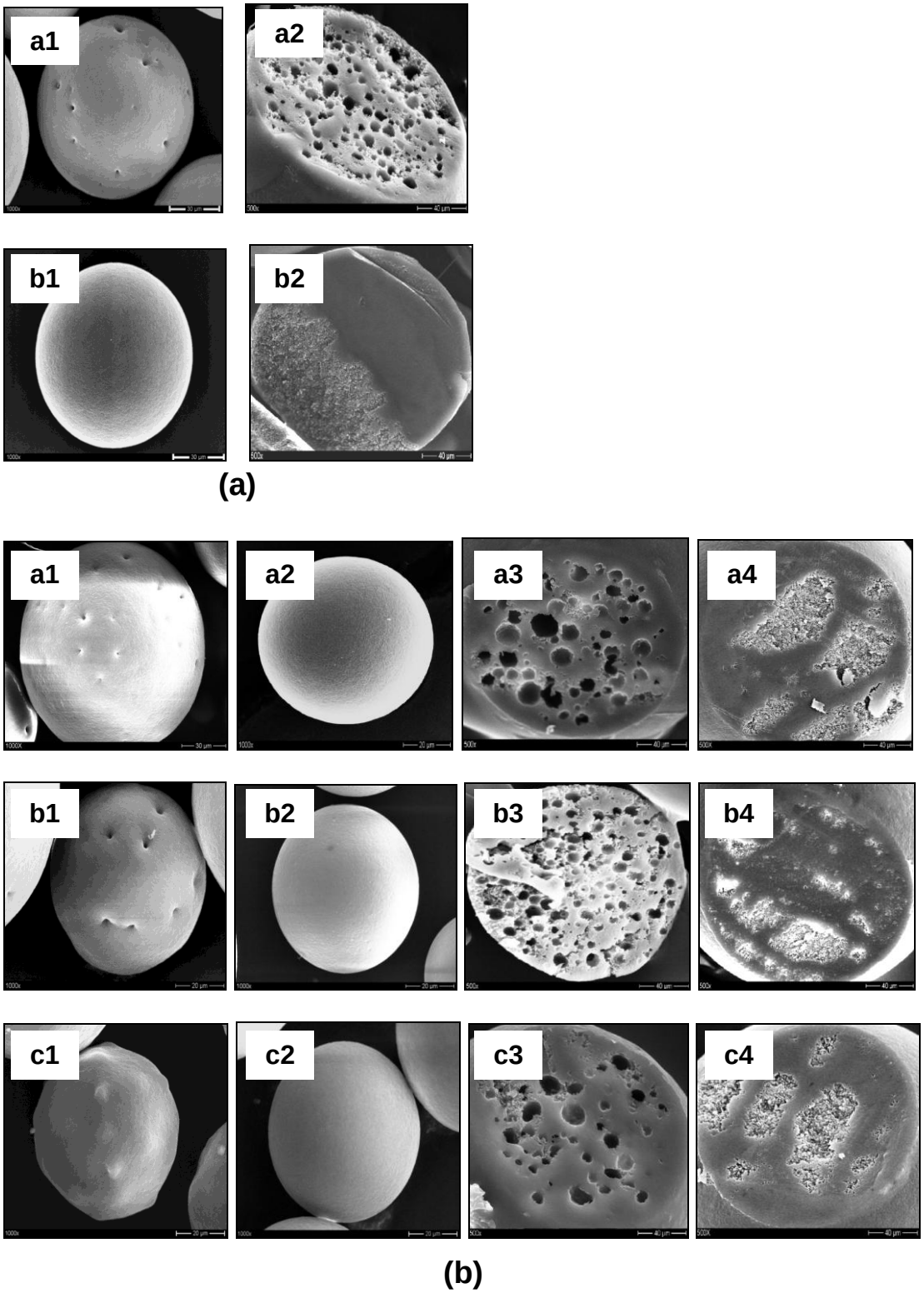

Fig. 52. SEM pictures of ethyl cellulose microparticle blends with varying dispersion time interval between primary emulsion and primary oil phase (higher magnification and cross-section). (a) a.1-2. Pro (W/O/W) and b.1-2. CBZ (O/W), and (b) a.1-4. CBZ (O/W) and Pro (W/O/W), DTI: 60 min, b. Pro (W/O/W) and CBZ (O/W), DTI: 0 min and c.1-4. Pro (W/O/W) and CBZ (O/W), DTI: 60 min

The internal structure of carbamazepine loaded EC microparticles appeared to be dense with absence of pores irrespective of the preparation technique (O/W method) (Fig. 52a. b2). A highly porous inner structure was found in EC microparticles containing propranolol HCl prepared by a W/O/W method (Fig. 52a. a2). The observed absence of pores in the present O/W method is of major importance for the underlying drug release mechanisms, because drug diffusion through water-filled cavities is much faster than through dense polymeric networks. For microparticle blends which were prepared with DTI 60 min the internal structure appeared reducing in the number of pores and size of pores (Fig. 52b. c3). This phenomenon might be attributable to the interaction of second primary oil phase (CBZ) with hard particles from first primary emulsion (Pro), whereby the second primary oil phase (CBZ) had blocked and coated pores on the surface of hard particle from first primary emulsion (Fig. 52b. c1). This hypothesa supported by optical microscopy pictures. It indicates that the emulsification stage first W/O/W (Pro) and second (CBZ/dye) resulting in two kind of microparticle blends (Fig. 43. c1). This picture showed microparticle with black plaque on the surface and black microparticles.

3.4.3. Conclusion

The novel microparticle blends containing drugs of the same solubility (e.g. propranolol HCl and propranolol HCl) or drugs of different solubility (e.g. propranolol HCl and carbamazepine) offer a high potential for controlled release drug delivery systems. For microparticle blends (with DTI 60 min) containing drugs of the same solubility gave propranolol HCl release was slower than propranolol HCl release from microparticle blends (with DTI 0 min) and microparticles normal. Whereas microparticle blends (with DTI 60 min) containing drugs of different solubility gave propranolol HCl release was slower than carbamazepine release. FBRM studies showed that particle size of microparticle from first primary emulsion (Pro) was smaller than particle size of microparticle after addition second primary emulsion (Pro) or primary oil phase (CBZ) (with DTI 60 min). It was caused second primary emulsion (Pro) or primary oil phase (CBZ) interacted with microparticles from first primary emulsion (Pro). Optical and scanning electron microscopy revealed that microparticle blends (DTI 60 min) were spherical and had two populations. These microparticle blends consisted of microparticles with pores and rough surface (microparticle blend containing the same drug) and microparticles with smooth and rough surface (microparticle blend containing different

drug solubility). This phenomenon might be attributable to the interaction of second primary emulsion or oil phase with hard particles from first primary emulsion, whereby the second primary emulsion or oil phase had blocked and coated pores on the surface of hard particle from first primary emulsion.

Type of emulsion system, type of dispersion time interval, formulation type of second primary emulsion, and emulsification stage in preparation process of microparticle blends influenced the physical properties of the microparticle blends.

3.5. Microparticle blends system for controlled delivery of propranolol HCl and carbamazepines: Influence of the formulation and processing parameters

3.5.1. Introduction

The solvent evaporation method has been used extensively to prepare polymeric microparticles containing many different drugs. Several variables have been identified which can influence the properties of the microparticles, including drug solubility, internal morphology, solvent type, diffusion rate, temperature, polymer composition and viscosity, and drug loading (Bodmeier and Mc Ginity, 1987; Jeffery et al., 1991; Ogawa et al., 1988; Rosca et al., 2004). The effectiveness of the solvent evaporation method to produce microparticles depends on the successful entrapment of the active agent within the microparticles. The solubility properties of the drugs of the microparticles are important parameters when selecting the emulsion phases for a microparticles preparation process. A low solubility of the drugs in the continuous phase is required for obtaining a high yield. Depending on the solubility of the drug, simple or multiple emulsion techniques like oil-in-water (O/W) or water-in-oil-in-water (W/O/W) methods are used (Pérez et al., 2003; Yamakawa et al., 1992; Yang et al., 2000a, 2001). For insoluble or poorly water soluble drugs, the oil-in-water (O/W) method is frequently used. While hydrophilic or water soluble drugs, the water-oil-in-water (W/O/W) method is used. Many types of drugs with different physical and chemical properties have been formulated into polymeric microparticles systems, including anti-cancer drugs, narcotic agents, local anesthetics, steroids, and fertility control agents (Li et al., 2008; Yeo and Park, 2004).

Microparticles offer greater effectiveness, lower toxicity and better stability than conventional dosage forms (Freitas et al., 2005). Microparticulate drug delivery systems are used to prolong the delivery of the drug, to improve bioavailability, to enhance stability and, to target drug to specific site (Li et al., 2008; Yang et al., 2000b; Yeo and Park, 2004). The physical properties of obtained microparticles are strongly dependant on the nature of materials and also on the parameters during the manufacturing of microparticles (O'Donnell and McGinity, 1997). A complicated array of factors including type of polymer, polymer molecular weight, polymer concentration, drug loading, type of solvent, the nature of any excipients added to the microparticles formulation (e.g., for stabilization of the therapeutics), and the microparticles size can have a strong impact on the delivery rates. Type of polymer was used in microparticles fabrication and the way in which the

polymer degrades obviously affect drug release rates. Depending on the rate of hydrolysis of their functional groups, polymers can be broadly categorized into two types: surface eroding and bulk-eroding. Polymer molecular weight can affect polymer degradation and drug release rates (Yeo and Park, 2004). As one might expect, an increase in molecular weight decreases diffusivity and therefore drug release rate. A major mechanism for release of many drugs from polymeric microparticles are diffusion through water-filled pores. In addition, polymers have been used as a main tool to control the drug release rate from the formulations. Polymers can bind the particles of a solid dosage form. Pharmaceutical polymers are widely used to achieve taste masking; controlled release (e.g., extended, pulsatile, and targeted), enhanced stability, and improved bioavailability. There are different methods to use microencapsulation by solvent evaporation technique. The choice of the method that will give rise to an efficient drug encapsulation depends on the hydrophilicity or the hydrophobicity of drug.

In most studies reported so far, only one drug was entrapped within controlled release microparticles at a time. Only few attempts have been made on the co-encapsulation of two drugs, especially if the latter exhibits significantly different solubility behavior. Pérez et al. (2000) have incorporated a lipophilic and a hydrophilic drug simultaneously within biodegradable, poly (ε-caprolactone)-based microparticles by solvent evaporation methods. In another study, Pérez et al. (2003) have successfully incorporated the hydrophilic drug propranolol HCl and/or the lipophilic drug nifedipine separately as well as simultaneously within non-degradable, ammonio methacrylate copolymers (Eudragit RS:RL 4:1 blends) based microparticles. They were prepared with an oil-in-water (O/W) and a water-in-oil-in-water (W/O/W) solvent evaporation method. Whereas, Nippe and General (2012) have developed a combination of lipophilic steroidal drugs ethinyl estradiol and drospirenone poly(lactic-co-glycolic acid) (PLGA) microparticles. Combination products also known as fixed dose combinations are combinations of two or more active drugs produced in a single dosage forms. They provide the advantages of combination therapy while useful to improve adherence and can simplify procurement, storage and distribution of medicines. Fixed dose combination drugs are an important approach to addressing the management of both chronic and acute diseases.

Microparticle blends containing two drugs with different solubility have not been reported yet. In the present study, the solvent evaporation method was used to incorporate

a lipophilic and a hydrophilic drug within polymeric microparticle blends. The hydrophilic drug propranolol HCl and the lipophilic drug carbamazepine were used as model drugs.

The purpose of this study was to investigate effect of various formulation and processing parameters which used in preparation of microparticle blends on the polymeric microparticle blends contained the same drug (propranolol HCl) and contained drugs with different solubility (propranolol HCl and carbamazepine) which prepared by solvent evaporation method.

3.5.2. Results and discussion

3.5.2.1. Encapsulation efficiency

The encapsulation efficiency (EE) of propranolol HCl and carbamazepine for all type of microparticle blends (whole size) are available in Table 13, 14, 15, 16 and 17.

Effect of type of dispersion time interval

The encapsulation efficiency (EE) was about 73.58% - 75.25% for propranolol HCl in microparticle blends containing the same drug.

Table 13. Influence of dispersion time interval between primary emulsion 1 and primary emulsion 2 on the encapsulation efficiency of propranolol HCl loaded microparticle blends (whole size, theoretical drug loading = 16.7% w/w)

Variables	Encapsulation efficiency (%) (± SD)
Dispersion time interval between primary emulsion 1 and primary emulsion 2	
0 min	73.58 (± 3.18)
5 min	73.92 (± 2.75)
30 min	74.98 (± 3.01)
60 min	75.25 (± 2.68)

The EE of propranolol HCl were 72.68% - 76.24% and carbamazepine were 90.16% - 94.72% in microparticle blends containing different drugs. Lower encapsulation efficiency was obtained at dispersion time interval 0 min. It is due to longer contact time between internal and external phase. Therefore, more drugs diffuse into the external aqueous phase.

Table 14. Influence of dispersion time interval on the encapsulation efficiency of propranolol HCl and carbamazepine loaded microparticle blends (whole size, theoretical drug loading = 16.7% w/w)

Variables	**Encapsulation efficiency (%) (± SD)**	
	Propranolol HCl	**Carbamazepine**
Dispersion time interval between primary emulsion 1 and primary emulsion 2		
0 min	72.68 (± 3.85)	90.16 (± 3.02)
5 min	73.24 (± 4.06)	90.38 (± 3.55)
30 min	75.85 (± 3.74)	94.05 (± 3.16)
60 min	76.24 (± 3.46)	94.72 (± 2.05)

Effect of polymer concentration

Microparticle blends with high propranolol HCl encapsulation efficiencies were obtained when high polymer concentration were used. Propranolol HCl encapsulation efficiencies were in the range of 63.45% - 89.24% (DTI of 0 min) and 66.36% - 91.45% (DTI of 60 min) when ethyl cellulose concentration at second primary emulsion are 5% to 20%. While, the EE was in the range 61.45% - 84.16% (DTI of 0 min) and 64.95% - 89.52% (DTI of 60 min) for propranolol HCl and 80.32% - 94.75% (DTI of 0 min) and/or 83.95% - 97.22% (DTI of 60 min) for carbamazepine in microparticle blends containing different drugs, when ethyl cellulose concentration are 5% to 15%. The contribution of high polymer concentration to the encapsulation efficiency can be interpreted in two ways. First, when highly concentrated, the polymer precipitates faster on the surface of the dispersed phase and prevents drug diffusion across the phase boundary (Rafati et al., 1997). Second, the high concentration increases viscosity of the solution and delays the drug diffusion within the polymer droplets (Bodmeier and McGinity, 1988).

Table 15. Influence of various variables on the encapsulation efficiency of propranolol HCl and carbamazepine loaded microparticles (whole size, theoretical drug loading = 16.7% w/w)

Variables	Encapsulation efficiency (%) (± SD)	
	Propranolol HCl	**Carbamazepine**
Polymer concentration		
5%	63.85 (± 3.12)	80.84 (± 3.05)
7.5%	72.24 (± 3.45)	91.85 (± 3.52)
15%	84.46 (± 3.35)	93.31 (± 3.22)
20%	88.75 (± 2.76)	-
Drug loading		
10%	60.86 (± 3.25)	78.74 (± 3.46)
16.7%	72.24 (± 3.45)	91.85 (± 3.52)
30%	83.55 (± 3.95)	94.83 (± 3.08)
50%	92.04 (± 3.62)	-
Solvent		
Ethyl acetate	62.75 (± 3.52)	81.14 (± 3.25)
Dichloromethane	72.24 (± 3.45)	91.85 (± 3.52)
Chloroform	53.72 (± 3.08)	66.15 (± 3.36)
Molecular weight of ethyl cellulose		
EC 4 cp	72.24 (± 3.45)	-
EC 7 cp	82.75 (± 2.95)	-
EC 10 cp	91.02(± 3.12)	-
Preparation method		
Pro (W/O/W)	72.24 (± 3.45)	-
Pro (O/W)	70.86 (± 3.36)	-
CBZ (W/O/W)	-	87.92 (± 4.06)
CBZ (O/W)	-	91.85 (± 3.52)
Polymer		
Ethyl cellulose 4 cp (EC 4 cp)	72.24 (± 3.45)	91.85 (± 3.52)
PLGA (RG502H)	71.36 (± 3.75)	86.12 (± 2.35)
Polycaprolactone (Mw. 10000)	60.92 (± 3.26)	71.75 (± 2.68)

Table 16. Influence of various variables on the encapsulation efficiency of propranolol HCl loaded microparticle blends (whole size, theoretical drug loading = 16.7% w/w)

Variables	Encapsulation efficiency (%) (± SD)	
	0 min	60 min
Polymer concentration		
5%	63.45 (± 3.93)	66.36 (± 2.55)
7.5%	73.58 (± 3.18)	75.25 (± 2.68)
15%	84.06 (± 2.46)	86.15 (± 2.14)
20%	89.24 (± 3.05)	91.45 (± 2.85)
Drug loading		
10%	62.18 (± 3.25)	65.36 (± 3.86)
16.7%	73.58 (± 3.18)	75.25 (± 2.68)
30%	84.36 (± 2.65)	87.76 (± 2.46)
50%	91.18 (± 3.18)	94.05 (± 2.72)
Solvent		
Ethyl acetate	63.36 (± 3.08)	67.75 (± 3.04)
Dichloromethane	73.58 (± 3.18)	75.25 (± 2.68)
Chloroform	54.74 (± 2.86)	58.47 (± 2.15)
Molecular weight of ethyl cellulose		
EC 4 cp	73.58 (± 3.18)	75.25 (± 2.68)
EC 7 cp	82.12 (± 3.08)	85.19 (± 2.14)
EC 10 cp	90.07 (± 3.25)	93.36 (± 2.05)
Preparation method		
Pro (W/O/W) and Pro (W/O/W)	73.58 (± 3.18)	75.25 (± 2.68)
Pro (W/O/W) and Pro (O/W)	71.72 (± 2.94)	74.12 (± 3.14)
Pro (O/W) and Pro (W/O/W)	70.54 (± 3.25)	72.85 (± 1.69)
Pro (O/W) and Pro (O/W)	70.25 (± 3.05)	71.18 (± 2.42)
Polymer		
Ethyl cellulose 4 cp (EC 4 cp)	73.58 (± 3.18)	75.25 (± 2.68)
PLGA (RG502H)	70.25 (± 3.04)	72.64 (± 2.52)
Polycaprolactone (Mw. 10000)	61.15 (± 3.02)	64.12 (± 3.14)

Table 17. Influence of various variables on the encapsulation efficiency of propranolol HCl and carbamazepine loaded microparticle blends (whole size, theoretical drug loading = 16.7% w/w)

Variables	Encapsulation efficiency (%) (± SD)			
	Propranolol HCl		Carbamazepine	
	0 min	60 min	0 min	60 min
Polymer concentration				
5%	61.45 (± 3.75)	64.95 (± 3.75)	80.32 (± 4.12)	83,95 (± 2.15)
7.5%	72.68 (± 3.85)	76.24 (± 3.46)	90.16 (± 3.02)	94.72 (± 2.05)
15%	84.16 (± 3.05)	89.52 (± 3.72)	94.75 (± 3.58)	97.22 (± 3.82)
Drug loading				
10%	60.14 (± 3.74)	61.32 (± 3.24)	78.18 (± 3.94)	80,98 (± 3.02)
16.7%	72.68 (± 3.85)	76.24 (± 3.46)	90.16 (± 3.02)	94.72 (± 2.05)
30%	83.52 (± 3.15)	88.72 (± 4.12)	93.26 (± 3.75)	96.25 (± 3.35)
Solvent				
Ethyl acetate	63.64 (± 2.14)	68.52 (± 2.16)	80.88 (± 3.94)	85.58 (± 3.18)
Dichloromethane	72.68 (± 3.85)	76.24 (± 3.46)	90.16 (± 3.02)	94.72 (± 2.05)
Chloroform	51.72 (± 3.66)	55.98 (± 4.02)	65.82 (± 3.92)	70.55 (± 2.85)
Preparation method				
Pro (W/O/W) and CBZ (W/O/W)	73.97 (± 3.75)	77.14 (± 4.05)	87.74 (± 2.92)	88.04 (± 3.35)
Pro (W/O/W) and CBZ (O/W)	72.68 (± 3.85)	76.24 (± 3.46)	90.16 (± 3.02)	94.72 (± 2.05)
Pro (O/W) and CBZ (W/O/W)	71.04 (± 4.35)	72.44 (± 2.65)	87.72 (± 4.12)	89.22 (± 2.48)
Pro (O/W) and CBZ (O/W)	70.29 (± 3.52)	71.62 (± 3.48)	91.58 (± 3.18)	93.08 (± 3.64)
Polymer				
Ethyl cellulose 4 cp (EC 4 cp)	72.68 (± 3.85)	76.24 (± 3.46)	90.16 (± 3.02)	94.72 (± 2.05)
PLGA (RG502H)	69.62 (± 3.42)	71.66 (± 2.78)	85.54 (± 3.22)	89.15 (± 4.08)
Polycaprolactone (Mw. 10000)	62.05 (± 2.26)	66.38 (± 4.42)	70.56 (± 3.14)	73.85 (± 2.88)

Effect of drug loading

Microparticle blends with high propranolol HCl encapsulation efficiencies were obtained high theoretical drug loading. Propranolol HCl encapsulation efficiencies were in the range of 62.18% - 91.18% (DTI of 0 min) and 63.36% - 94.05% (DTI of 60 min) when theoretical drug loading at second primary emulsion are 10% to 50%. The EE was in the range of 60.14% - 83.52% (DTI of 0 min) and 61.32% - 88.72% (DTI of 60 min) for propranolol HCl and 78.18% - 93.26% (DTI of 0 min) and 80.98% - 96.25% (DTI of 60 min) for carbamazepine in microparticle blends containing different drugs, when theoretical drug loading are 5% to 30%. This could be explained by increased drug

entrapped into the surface of embryonic microparticle droplets during emulsification process.

Effect of solvent type

Dichloromethane resulted in a higher encapsulation efficiency compared with chloroform or ethyl acetate, despite that dichloromethane was the best solvent for ethyl cellulose. The encapsulation efficiency (EE) was about 73.58%, 63.36% and 54.74% (DTI of 0 min) and 75.25%, 67.75% and 58.47% (DTI of 60 min) for propranolol HCl in microparticle blends containing the same drug, when dichloromethane, ethyl acetate and chloroform were used at second primary emulsion respectively. The EE was about 72.68%, 63.64% and 51.72% (DTI of 0 min) and 76.24%, 68.52% and 55.98% (DTI of 60 min) for propranolol HCl and 90.16%, 80.88% and 65.82% (DTI of 0 min) and 94.72%, 85.58%, and 70.55% (DTI of 60 min) for carbamazepine in microparticle blends containing different drugs, when dichloromethane, ethyl acetate and chloroform were used respectively. Dichloromethane has fast solvent removal rate. Additionally, dichloromethane and ethyl acetate are more soluble in water than chloroform. The miscibility of solvents is strongly related to their polarity. The high solubility allowed relatively fast mass-transfer between the dispersed and the continuous phases and led to fast precipitation of the polymer. It contributed to increase of the encapsulation efficiency.

Effect of molecular weight of ethyl cellulose (EC)

Encapsulation efficiency is affected by the molecular weight of the polymer. In general, low molecular weight polymers result in low encapsulation efficiency. This is partly because the low molecular weight polymer is more soluble in the organic solvent and undergoes slow solidification to produce more porous microparticles. It cause drug diffusion from internal phase into external phase. The encapsulation efficiency (EE) was about 73.58%, 82.12% and 90.07% (DTI of 0 min) and 75.25%, 85.19% and 93.36% (DTI of 60 min) for propranolol HCl in microparticle blends containing the same drug, when EC 4 cp, EC 7 cp and EC 10 cp were used at second primary emulsion respectively.

Effect of preparation method

Depending on the solubility of the drug, simple or multiple emulsion techniques like oil-in-water (O/W) or water-in-oil-in-water (W/O/W) methods were used. In the first

case (O/W), a lipophilic drug (carbamazepine) is dissolved together with the polymer in an organic phase, which is then dispersed into an outer aqueous phase. Upon contact, the organic solvent diffuses into the external water phase and evaporates at its surface (Pérez et al., 2003; Yamakawa et al., 1992; Yang et al., 2000a). Consequently, the polymer precipitates and entraps the drug. W/O/W techniques can be used, avoiding the direct contact of the hydrophilic drug (propranolol HCl)-containing phase with the outer water phase. An aqueous solution of the drug is emulsified into an organic phase containing the dissolved polymer. This primary W/O emulsion is dispersed into an outer aqueous phase. Upon solvent diffusion/evaporation, the polymer precipitates and incorporates the drug. Microparticle blends with high propranolol HCl encapsulation efficiencies were obtained when using the preparation method W/O/W. While, carbamazepine gave high encapsulation efficiency when using the preparation method O/W. The encapsulation efficiency (EE) was in the range of 70.25% - 73.58% (DTI of 0 min) and 71.18% - 75.25% for propranolol HCl in microparticle blends containing the same drug. While, the EE was in the range of 70.29% - 73.97% (DTI of 0 min) and 71,62% - 77.14% (DTI of 60 min) for propranolol HCl and 87.72% - 91.58% (DTI of 0 min) and 88.04% - 94.72% (DTI of 60 min) for carbamazepine in microparticle blends containing different drugs.

Effect of polymer type

Ethyl cellulose (EC 4 cp) resulted in a higher encapsulation efficiency compared with PLGA (RG502H) and polycaprolactone. The encapsulation efficiency (EE) was about 73.58%, 70.25% and 61.15% (DTI of 0 min) and 75.25%, 72.64% and 64.12% (DTI of 60 min) for propranolol HCl in microparticle blends containing the same drug, when EC 4 cp, PLGA (RG502H) and polycaprolactone were used at second primary emulsion respectively. The EE was about 72.68%, 69.62% and 62.05% (DTI of 0 min) and 76.24%, 71.66% and 66.38% (DTI of 60 min) for propranolol HCl and 90.16%, 85.54% and 70.56% (DTI of 0 min) and 94.72%, 89.15%, and 73.85% (DTI of 60 min) for carbamazepine in microparticle blends containing different drugs, when EC 4 cp, PLGA (RG502H) and polycaprolactone were used respectively.

The difference observed in the EE of the two drugs in the microparticle blends can be explained different solubilities of drugs in the aqueous continuous phase used for two encapsulation techniques. The high solubility of the propranolol HCl in the external aqueous phase and its high volume compared to that of the internal aqueous phase (W/O/W

technique) caused the leakage of the drug into the continuous phase. This leakage process is believed to happen mainly during the first minutes of emulsification since the polymer precipitates rapidly thereby decreasing leakage (Alhnan and Basit, 2011; Freiberg and Zhu, 2004; Hsu and Lin, 2005; Pérez et al., 2000, 2003). However, after the precipitation of the polymer, the propranolol HCl, due to its hydrophilic nature, still tends to diffuse through the polymeric matrix into the external aqueous phase.

3.5.2.2. Release of propranolol HCl and carbamazepine from microparticle blends

Actual drug loading of propranolol HCl and carbamazepine for all type of microparticle blends (< 70 µm) which used in release test are available in Table 18, 19, 20, 21 and 22.

Effect of type of dispersion time interval

Figure 53 shows the effect of type of dispersion time interval (DTI) on the release of propranolol HCl from microparticle blends containing the same drug in pH 7.4 phosphate buffer.

Table 18. Influence of dispersion time interval between primary emulsion 1 and primary emulsion 2 on actual drug loading of propranolol HCl loaded microparticle blends (particle size: < 70 µm)

Variables	Actual drug loading (%) (± SD)
Dispersion time interval between primary emulsion 1 and primary emulsion 2	
0 min	11.02 (± 0.31)
5 min	11.05 (± 0.36)
30 min	11.19 (± 0.27)
60 min	11.28 (± 0.24)

Microparticle blends prepared with dispersion time interval of 0 and 5 min showed similar release profile with conventional microparticle (single drug). Propranolol HCl released at pH 7.4 after 28 days for DTI of 0 min, 5 min and single drug are 80%, 83% and 85%, respectively. Whereas propranolol HCl release from microparticle blends after 28 days for dispersion time interval of 30 min and 60 min were slower (64% and 61%). Based on release data of microparticle blends, it can be assumed that there is interaction between

first hardened particles with second primary emulsions during preparation process for microparticle blends with DTI of 30 min and 60 min.

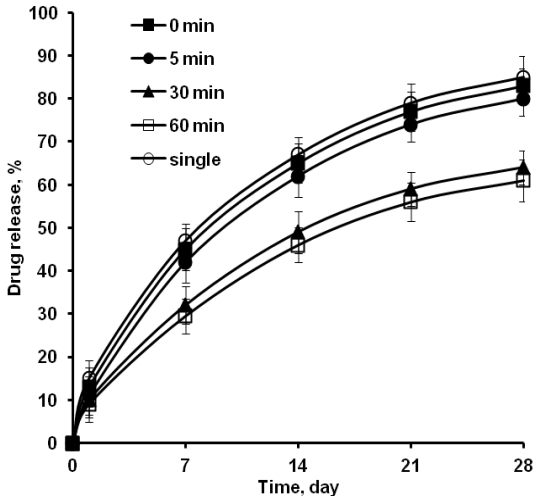

Fig. 53. Effect of type of dispersion time interval between first primary emulsion and second primary emulsion on propranolol HCl release from ethyl cellulose microparticle blends (phosphat buffer, pH 7.4, 37 ^{o}C, 75 rpm).

Figure 54 shows the effect of type of dispersion time interval (DTI) on the release of propranolol HCl and carbamazepine from microparticle blends containing different drugs in pH 7.4 phosphate buffer.

Table 19. Influence of dispersion time interval between primary emulsion and oil phase on actual drug loading of propranolol HCl and carbamazepine loaded microparticle blends (particle size: < 70 µm)

Variables	**Actual drug loading (%) (± SD)**	
	Propranolol HCl	**Carbamazepine**
Dispersion time interval between primary emulsion 1 and primary emulsion 2		
0 min	11.09 (± 0.36)	14.12 (± 0.08)
5 min	11.17 (± 0.29)	14.31 (± 0.06)
30 min	11.45 (± 0.25)	14.58 (± 0.04)
60 min	11.62 (± 0.32)	14.74 (± 0.05)

Microparticle blends were prepared with dispersion time interval of 0 and 5 min showed the similar release profile with conventional microparticles (single drug).

Propranolol HCl released at pH 7.4 after 28 days for DTI of 0 min, 5 min and single drug are 79%, 71,5% and 75%, respectively. Propranolol HCl release from microparticle blends after 28 days for dispersion time interval of 30 min and 60 min were 57% and 53.5%. Whereas carbamazepine released at pH 7.4 after 28 days for DTI of 0 min, 5 min and single drug are 46%, 51% and 44%, respectively. The carbamazepine release from microparticle blends after 28 days for dispersion time interval of 30 min and 60 min were 67% and 69%. Based on release data, for microparticle blends it can be assumed that there is interaction between first hardened particles with second primary emulsions during preparation process for microparticle blends with DTI of 30 min and 60 min.

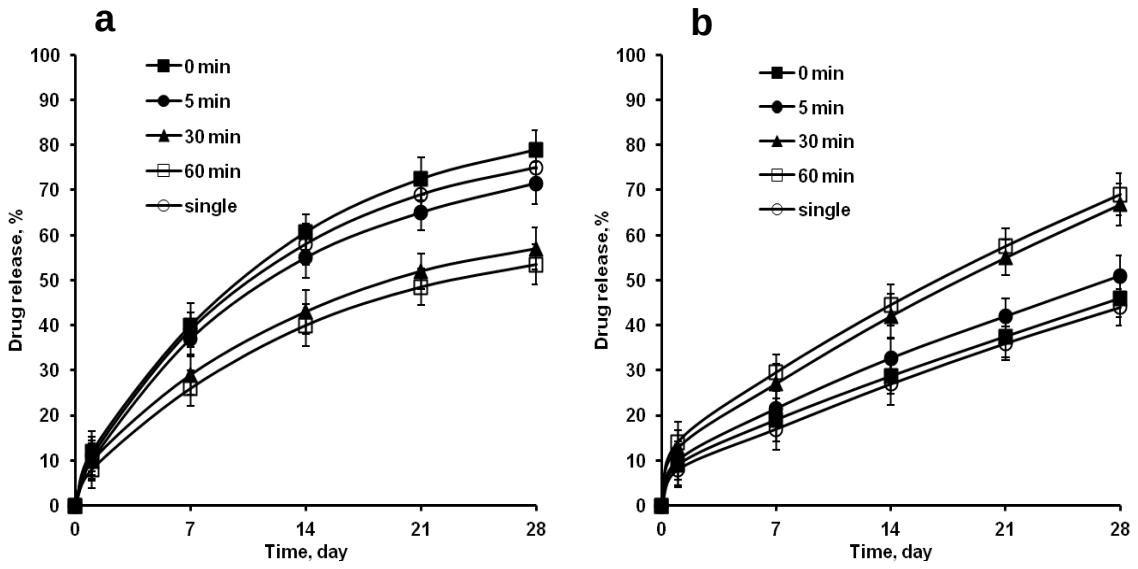

Fig. 54. Effects of dispersion time interval between primary emulsion and oil phase on propranolol HCl and carbamazepine release from ethyl cellulose microparticle blends (phosphate buffer, pH 7.4, 37 ^{o}C, 75 rpm). (a) Propranolol HCl and (b) Carbamazepine

Effect of polymer concentration

The polymer concentration plays an important role in the drug release from microparticle blends systems. A decrease in the drug release from microparticle blends systems with the increasing polymer concentration was already reported (Yeo and Park, 2004). A higher polymer concentration led to a more viscous solution, which delayed the polymer precipitation and resulted in a less porous polymer matrix with a slower drug release. In microparticle blends systems, propranolol HCl release after 28 days decreased dramatically from 71%, 60.5%, 49% and 38% (DTI 60 min) and 90.5%, 83%, 67% and

51% (DTI 0 min) with an increasing polymer solution concentration of 5%, 7.5%, 15% and 20% (w/v), respectively (Fig. 55). Propranolol HCl release from microparticle blends after 28 days for DTI of 60 min was slower than DTI of 0 min.

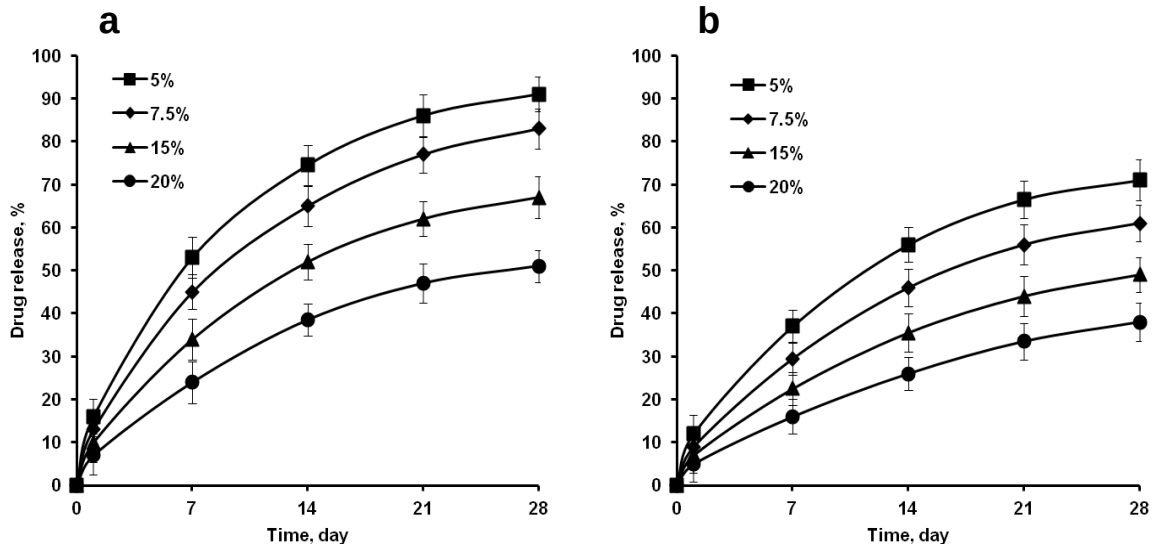

Fig. 55. Effects of polymer concentration at second primary emulsion on propranolol HCl release from ethyl cellulose microparticle blends (phosphat buffer, pH 7.4, 37 ^{o}C, 75 rpm). Dispersion time interval: (a) 0 min and (b) 60 min

Different release rate were observed for propranolol HCl and carbamazepine from EC microparticle blends with different polymer concentration (Fig. 56). Propranolol HCl and carbamazepine releases from conventional microparticle and microparticle blends (DTI of 0 min) was similar (Fig. 56). Whereas propranolol HCl release was slower than carbamazepine release from EC microparticle blends (DTI of 60 min) (Fig. 56c and Fig 56d). Propranolol HCl and carbamazepine released from each microparticle blends with different polymer concentration at pH 7.4 after 28 days are in the range of 65% - 89% (DTI of 0 min) and 39% - 69.5% (DTI 60 min) for propranolol HCl, Whereas carbamazepine are 37.5% - 28.6% (DTI of 0 min) and 55.5% - 82% (DTI 60 min).

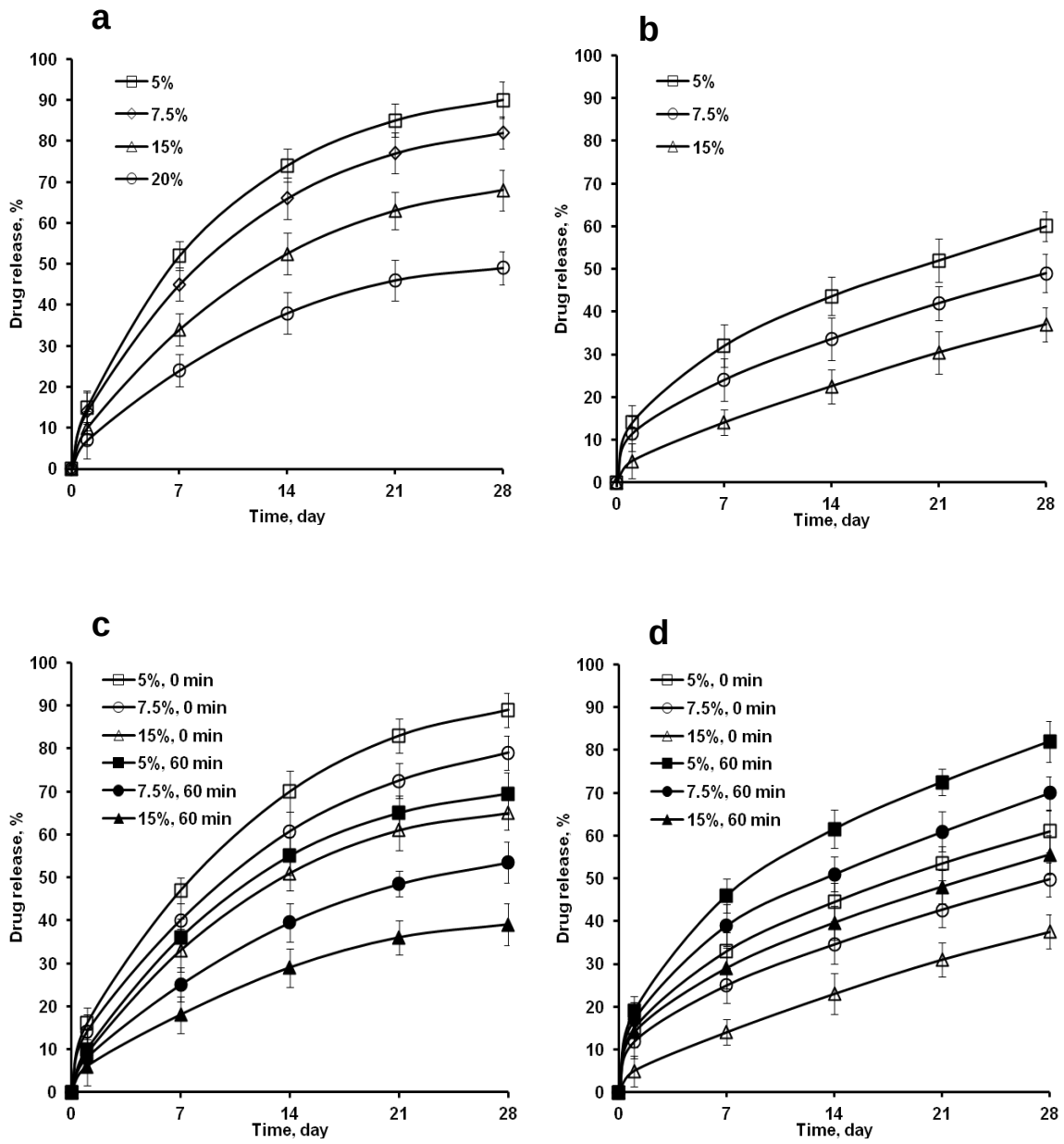

Fig. 56. Effects of polymer concentration on propranolol HCl and carbamazepine release from ethyl cellulose microparticle blends (phosphate buffer, pH 7.4, 37 ^{o}C, 75 rpm). (a and c) Propranolol HCl and (b and d) Carbamazepine

Effect of drug loading

In microparticle blends systems, release of propranolol HCl after 28 days decreased dramatically from 84%, 73%, 61% and 46% (DTI 60 min) and 97%, 90%, 83% and 59% (DTI 0 min) with an decreasing drug loading of 50%, 30%, 16.7% and 10% (w/v),

respectively (Fig. 57). The propranolol HCl release from microparticle blends after 28 days for DTI of 60 min was slower than DTI of 0 min.

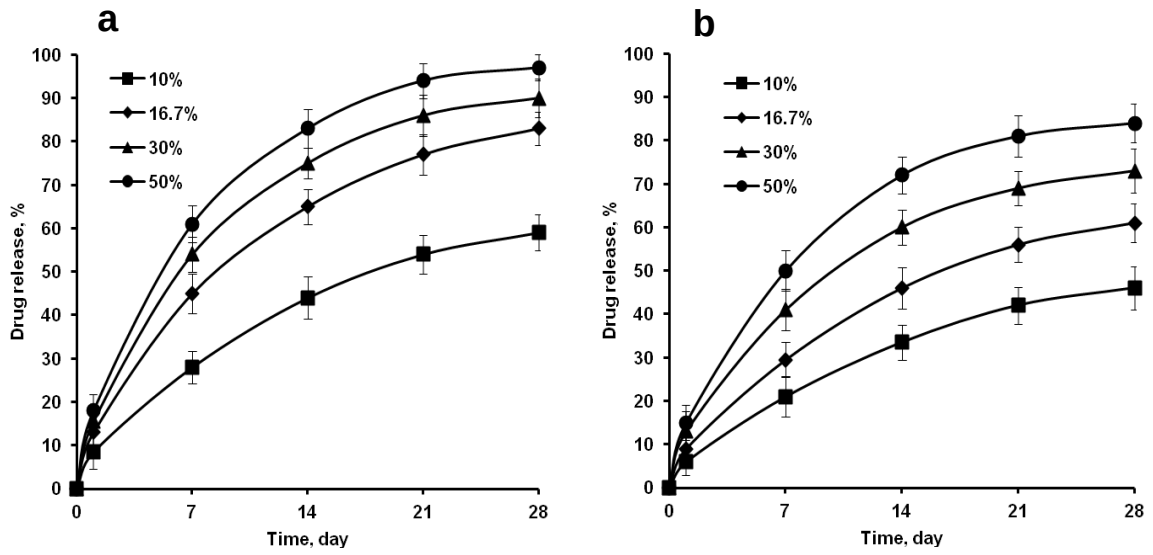

Fig. 57. Effects of drug loading at second primary emulsion on propranolol HCl release from ethyl cellulose microparticle blends (phosphat buffer, pH 7.4, 37 ^{o}C, 75 rpm). Dispersion time interval: (a) 0 min and (b) 60 min

Microparticle blends containing different drugs which prepared with different drug loading shows different drug release profile. Propranolol HCl and carbamazepine released from conventional microparticle and microparticle blends (DTI of 0 min) for each drug loading were similar (Fig. 58). Whereas propranolol HCl release was slower than carbamazepine release from EC microparticle blends (DTI of 60 min) (Fig. 58c and Fig. 58d). Propranolol HCl and carbamazepine released from microparticle blends with different drug loading after 28 days are 58%, 79% and 92% (DTI of 0 min) and 20%, 53.5% and 65% (DTI 60 min) for propranolol HCl, and 29%, 49.7% and 61% (DTI of 0 min) and 38%, 70% and 83% (DTI of 60 min) for carbamazepine with an increasing drug loading of 5%, 16.7% and 30%.

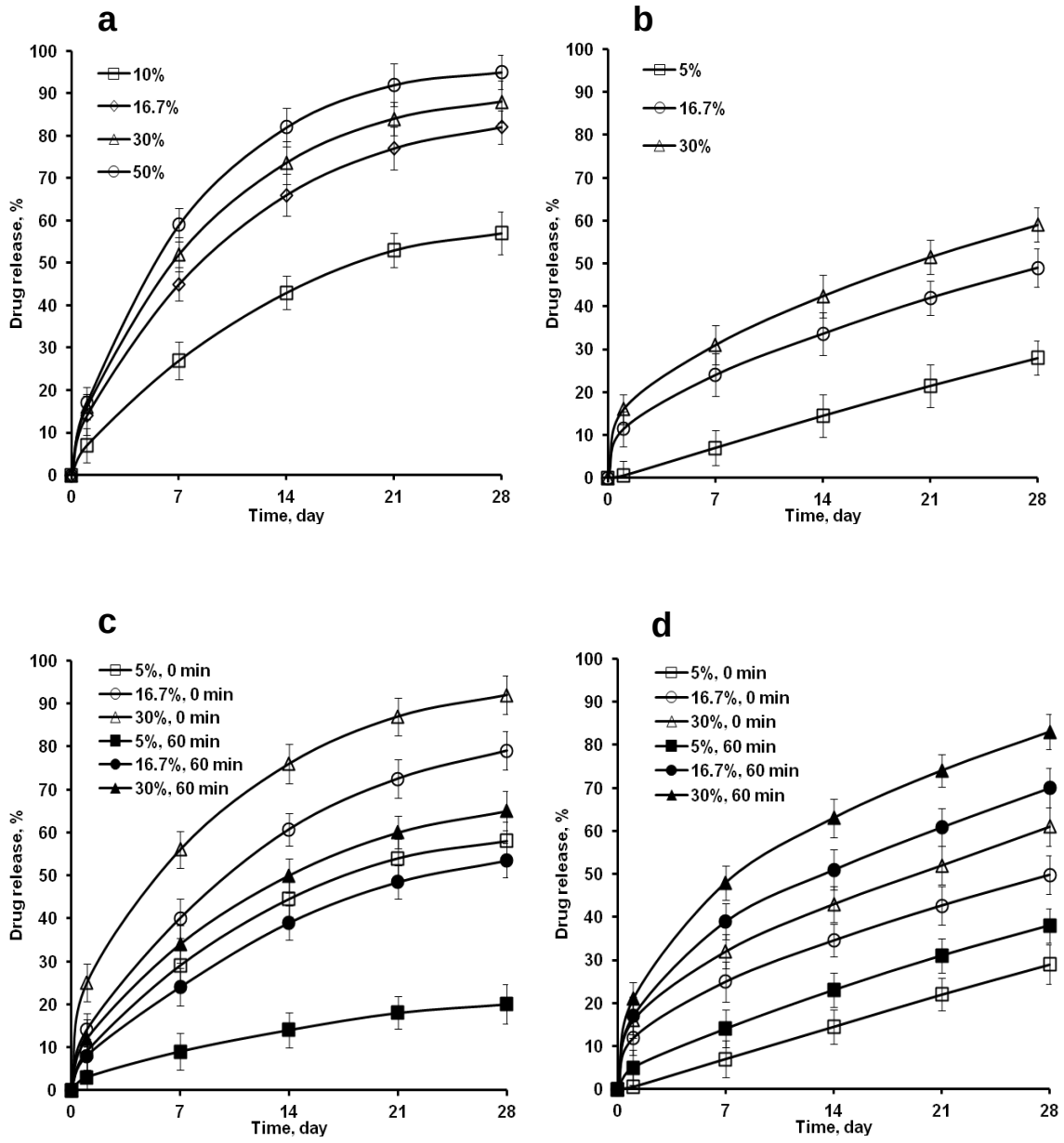

Fig. 58. Effects of drug loading on propranolol HCl and carbamazepine release from ethyl cellulose microparticle blends (phosphate buffer, pH 7.4, 37 ^{o}C, 75 rpm). (a and c) Propranolol HCl and (b and d) Carbamazepine

In many cases, the drug release rate increases with increasing drug loading (Yeo and Park, 2004). There are two possible explanations for the effect of drug loading. First, the elution of surface associated drug creates water-filled channels that allow subsequent elution of the drugs located inside the microparticles. By facilitating formation of these channels, high drug loadings lead to high initial bursts and fast release rate (Yeo and Park, 2004).

Alternatively, a large drug concentration gradient between the microparticles and the release medium may promote the high initial bursts and fast release rate (Yang et al., 2001).

Table 20. Influence of various variables on the actual drug loading of propranolol HCl and carbamazepine loaded microparticles (particle size: < 70 μm)

Variables	Actual drug loading (%) (± SD)	
	Propranolol HCl	**Carbamazepine**
Polymer concentration		
5%	9.57 (± 0.35)	12.26 (± 0.04)
7.5%	10.95 (± 0.28)	14.25 (± 0.06)
15%	12.88 (± 0.38)	14.46 (± 0.12)
20%	13.65 (± 0.25)	-
Drug loading		
10%	5.22 (± 0.31)	6.68 (± 0.15)
16.7%	10.95 (± 0.28)	14.25 (± 0.06)
30%	22.42 (± 0.36)	26.65 (± 0.08)
50%	41.05 (± 0.24)	-
Solvent		
Ethyl acetate	9.34 (± 0.32)	12.36 (± 0.14)
Dichloromethane	10.95 (± 0.28)	14.25 (± 0.06)
Chloroform	7.95 (± 0.34)	10.05 (± 0.18)
Molecular weight of ethyl cellulose		
EC 4 cp	10.95 (± 0.28)	-
EC 7 cp	12.52 (± 0.25)	-
EC 10 cp	14.16 (± 0.38)	-
Preparation method		
Pro (W/O/W)	10.95 (± 0.28)	-
Pro (O/W)	10.16 (± 0.36)	-
CBZ (W/O/W)	-	13.47 (± 0.12)
CBZ (O/W)	-	14.25 (± 0.06)
Polymer		
Ethyl cellulose 4 cp (EC 4 cp)	10.95 (± 0.28)	14.25 (± 0.06)
PLGA (RG502H)	10.35 (± 0.15)	13.04 (± 0.11)
Polycaprolactone (Mw. 10000)	8.94 (± 0.32)	10.24 (± 0.08)

Table 21. Influence of various variables on actual drug loading of propranolol HCl loaded microparticle blends (particle size: < 70 µm)

Variables	Actual drug loading (%) (± SD)	
	0 min	60 min
Polymer concentration		
5%	9.51 (± 0.26)	9.85 (± 0.36)
7.5%	11.02 (± 0.31)	11.28 (± 0.24)
15%	12.72 (± 0.34)	12.95 (± 0.22)
20%	13.52 (± 0.38)	13.76 (± 0.35)
Drug loading		
10%	5.31 (± 0.26)	5.65 (± 0.28)
16.7%	11.02 (± 0.31)	11.28 (± 0.24)
30%	22.35 (± 0.32)	24.96 (± 0.36)
50%	41.18 (± 0.35)	44.58 (± 2.72)
Solvent		
Ethyl acetate	9.46 (± 0.25)	9.85 (± 0.37)
Dichloromethane	11.02 (± 0.31)	11.28 (± 0.24)
Chloroform	7.82 (± 0.36)	8.19 (± 0.32)
Molecular weight of ethyl cellulose		
EC 4 cp	11.02 (± 0.31)	11.28 (± 0.24)
EC 7 cp	12.68 (± 0.36)	13.04 (± 0.36)
EC 10 cp	14.28 (± 0.24)	14.62 (± 0.28)
Preparation method		
Pro (W/O/W) and Pro (W/O/W)	11.02 (± 0.31)	11.28 (± 0.24)
Pro (W/O/W) and Pro (O/W)	10.26 (± 0.32)	10.59 (± 0.36)
Pro (O/W) and Pro (W/O/W)	10.18 (± 0.25)	10.45 (± 0.27)
Pro (O/W) and Pro (O/W)	10.02 (± 0.34)	10.32 (± 0.35)
Polymer		
Ethyl cellulose 4 cp (EC 4 cp)	11.02 (± 0.31)	11.28 (± 0.24)
PLGA (RG502H)	10.44 (± 0.26)	10.85 (± 0.37)
Polycaprolactone (Mw. 10000)	8.85 (± 0.28)	9.35 (± 0.24)

Table 22. Influence of various variables on actual drug loading of propranolol HCl and carbamazepine loaded microparticle blends (particle size: < 70 µm)

Variables	Encapsulation efficiency (%) (± SD)			
	Propranolol HCl		Carbamazepine	
	0 min	60 min	0 min	60 min
Polymer concentration				
5%	9.62 (± 0.26)	9.87 (± 0.35)	12.35 (± 0.06)	12.78 (± 0.09)
7.5%	11.09 (± 0.36)	11.62 (± 0.32)	14.32 (± 0.08)	14.74 (± 0.05)
15%	12.95 (± 0.24)	13.98 (± 0.26)	14.78 (± 0.03)	15.04 (± 0.06)
Drug loading				
10%	5.42 (± 0.25)	5.77 (± 3.24)	6.91 (± 0.04)	7.35 (± 0.04)
16.7%	11.09 (± 0.36)	11.62 (± 0.32)	14.32 (± 0.08)	14.74 (± 0.05)
30%	23.84 (± 0.38)	25.15 (± 4.12)	26.85 (± 0.02)	27.14 (± 0.08)
Solvent				
Ethyl acetate	9.58 (± 0.25)	10.17 (± 0.24)	12.57 (± 0.07)	12.95 (± 0.11)
Dichloromethane	11.09 (± 0.36)	11.62 (± 0.32)	14.32 (± 0.08)	14.74 (± 0.05)
Chloroform	7.94 (± 0.32)	8.35 (± 0.35)	10.24 (± 0.05)	10.68 (± 0.07)
Preparation method				
Pro (W/O/W) and CBZ (W/O/W)	11.28 (± 0.32)	11.62 (± 0.32)	13.55 (± 0.03)	13.92 (± 0.02)
Pro (W/O/W) and CBZ (O/W)	11.09 (± 0.36)	11.42 (± 0.36)	14.32 (± 0.08)	14.74 (± 0.05)
Pro (O/W) and CBZ (W/O/W)	10.76 (± 0.38)	11.16 (± 0.25)	13.72 (± 0.06)	13.98 (± 0.04)
Pro (O/W) and CBZ (O/W)	10.48 (± 0.28)	10.85 (± 0.28)	14.48 (± 0.09)	14.95 (± 0.08)
Polymer				
Ethyl cellulose 4 cp (EC 4 cp)	11.09 (± 0.36)	11.62 (± 0.32)	14.32 (± 0.08)	14.74 (± 0.05)
PLGA (RG502H)	10.51 (± 0.32)	10.98 (± 0.24)	13.28 (± 0.11)	13.52 (± 0.09)
Polycaprolactone (Mw. 10000)	9.32 (± 0.29)	9.86 (± 0.36)	10.69 (± 0.12)	10.97 (± 0.04)

Effect of solvent type

In microparticle blends systems, the cumulative release of propranolol HCl after 28 days decreased dramatically from 71.5%, 61%, and 48% (DTI 60 min) and 94%, 83% and 68% (DTI 0 min) when ethyl acetate, dichloromethane and chloroform used respectively (Fig. 59). Propranolol HCl release from microparticle blends after 28 days for DTI of 60 min was slower than DTI of 0 min.

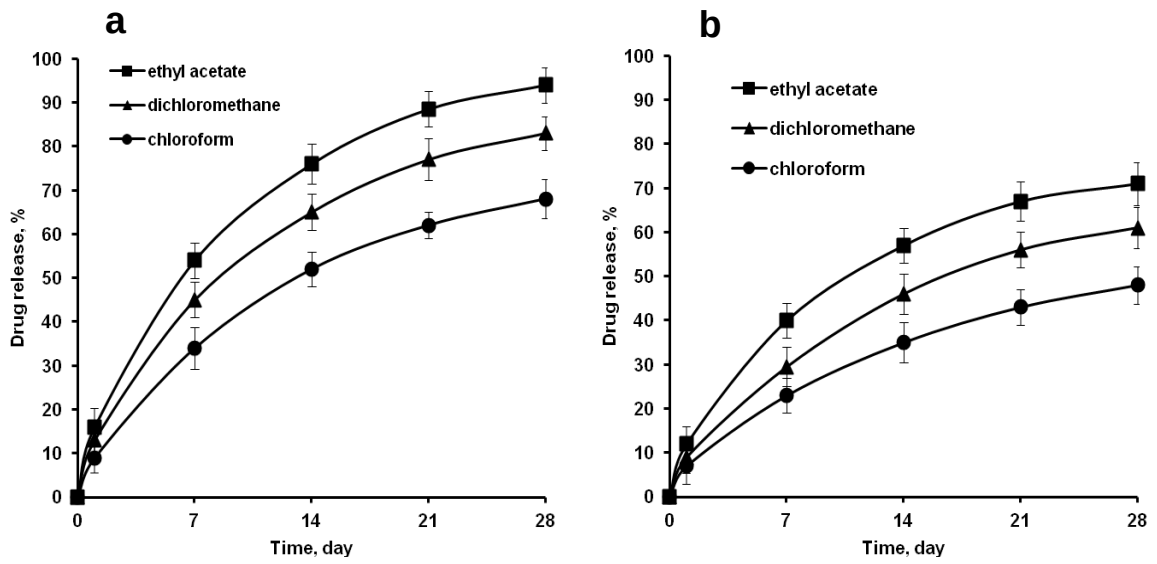

Fig. 59. Effects of solvent type at second primary emulsion on propranolol HCl release from ethyl cellulose microparticle blends (phosphat buffer, pH 7.4, 37 ^{o}C, 75 rpm). Dispersion time interval: (a) 0 min and (b) 60 min

Propranolol HCl and carbamazepine release from conventional microparticle and microparticle blends (DTI of 0 min) for different solvent type were similar (Fig. 60). Propranolol HCl release was slower than carbamazepine from EC microparticle blends (DTI of 60 min) which were prepared using different solvent type (Fig. 60c and Fig. 60d). Propranolol HCl and carbamazepine released from microparticle blends with different solvent type after 28 days are 86%, 79% and 70% (DTI of 0 min) and 61%, 53.5% and 43% (DTI 60 min) for propranolol HCl, and 58.2%, 49.7% and 44% (DTI of 0 min) and 78%, 70% and 62% (DTI of 60 min) for carbamazepine when ethyl acetate, dichloromethane and chloroform used respectively.

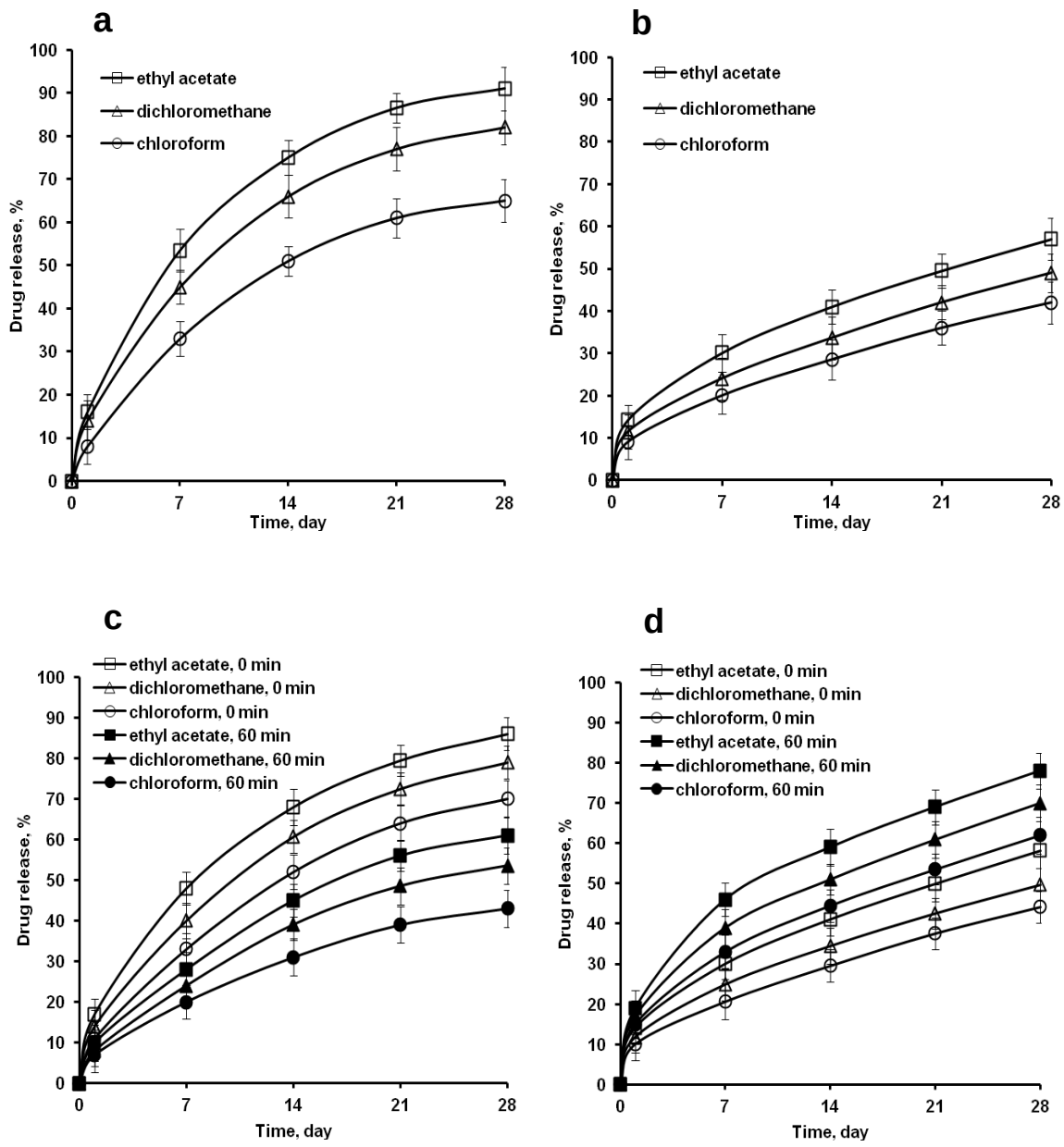

Fig. 60. Effects of solvent type on propranolol HCl and carbamazepine release from ethyl cellulose microparticle blends (phosphate buffer, pH 7.4, 37 ^{o}C, 75 rpm). (a and c) Propranolol HCl and (b and d) Carbamazepine

The different drug release profile from microparticle blends which prepared by W/O/W and O/W methods using different solvent could be explained by different rates of polymer precipitation. Ethyl acetate has a higher water solubility (approx. 8.7%) compared to chloroform (approx. 0.8%) and dichloromethane (approx. 1.3%) (Cleland et al., 1997). It will therefore diffuse rapidly into the external aqueous phase leading to polymer

precipitation at the droplet surface and the formation of polymeric shell with still large amounts of solvent being present. The solidified droplet surface inhibits further droplet shrinkage, consequently the rest of solvent is extracted by external penetration of the aqueous phase inside the embryonic microparticles, hence the porous and hollow nature of the microparticles is formed. Due to low solubility of chloroform and dichloromethane in water, the liquid polymer droplets slowly shrink and the internal solvent diffuse out in a repetitive cycle of dissolution and re-solidification of the polymer instead of external phase penetration.

Effect of molecular weight of ethyl cellulose

The propranolol HCl release from microparticle blends prepared with EC 4 cp, EC 7 cp and EC 10 cp as polymer at second primary emulsion were shown in Fig. 61. Microparticle blends were prepared by higher molecular weight EC 10 cp showed slower release. In contrast, microparticle blends prepared by lower molecular weight EC 4 cp and EC 7 cp showed a faster propranolol HCl release than EC 10 cp after 28 days. Additionally, propranolol HCl release from conventional microparticles and microparticle blends (DTI of 0 min) for each molecular weight of EC were similar (Fig. 61).

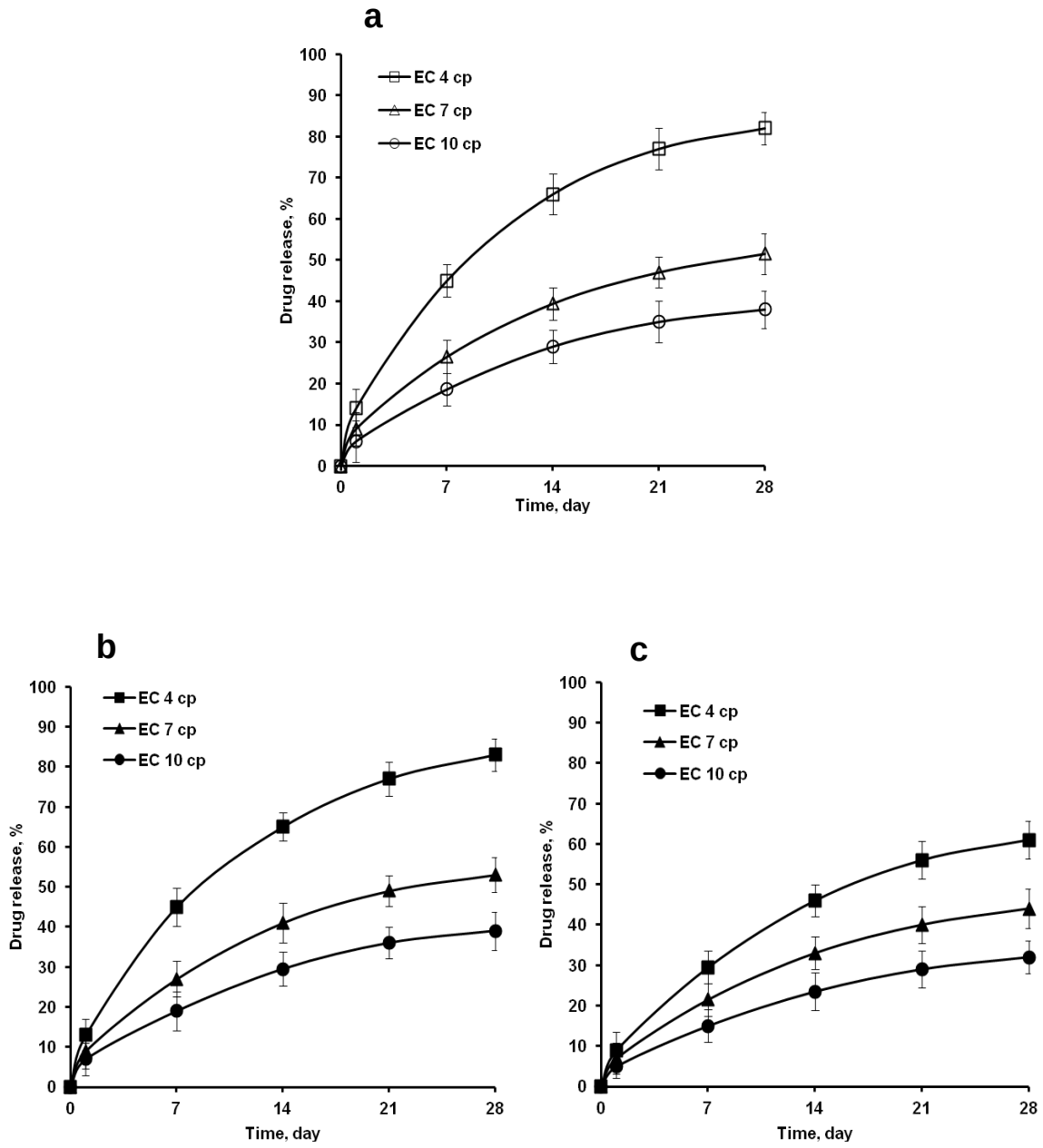

Fig. 61. Effects of molecular weight of ethyl cellulose at second primary emulsion on propranolol HCl release from ethyl cellulose microparticle blends (phosphat buffer, pH 7.4, 37 ^{o}C, 75 rpm). Dispersion time interval: (a) conventional microparticles, (b) 0 min and (b) 60 min

Low molecular weight polymer is more soluble in the organic solvent and undergoes slow solidification to produce more porous microparticles. So, low molecular weight polymers result in high burst release and fast drug release rate. Propranolol HCl release from microparticle blends after 28 days for DTI of 60 min was slower than DTI of

0 min. The cumulative release of propranolol HCl after 28 days decreased dramatically from 61%, 44%, and 32% (DTI 60 min) and 83%, 53% and 39% (DTI 0 min) when EC 4 cp, EC 7 cp and EC 10 cp used respectively (Fig. 61).

Effect of preparation method

Different release rate of propranolol HCl from microparticle blends containing the same drug which were prepared by W/O/W or O/W method (primary emulsion 1) and W/O/W or O/W method (primary emulsion 2) (Fig. 62) were obtained. Application W/O/W method for primary emulsion 1 (with DTI of 60 min) showed slow release of propranolol HCl. Whereas propranolol HCl release from all of microparticle blends (with DTI of 0 min) showed similar profiles. The cumulative percentage of propranolol HCl released from microparticle blends with different preparation method for first and second primary emulsions at pH 7.4 after 28 days are in the range of 71% - 83% (DTI of 0 min) and 56% - 80% (DTI 60 min). It is apparent from the data reported in Fig. 10 that type of preparation method and the type of dispersion time interval and composition of formulation second primary emulsion affected propranolol HCl released. Porosity of microparticles influenced drug release mechanism, which could lead to a more rapid release of propranolol HCl. The mechanism for release of propranolol HCl from ethyl cellulose matrix is diffusion through water-filled pores (Chiao and Price, 1994; Freiberg and Zhu, 2004; Grattard et al., 2002; Yeo and Park, 2004).

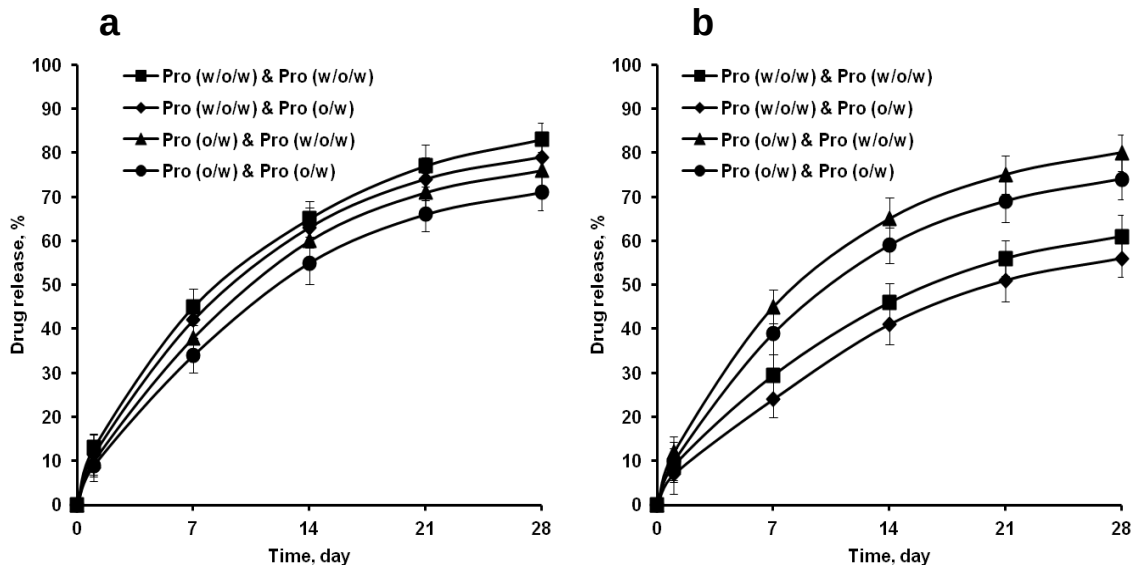

Fig. 62. Effects of method on propranolol HCl release from ethyl cellulose microparticle blends (phosphat buffer, pH 7.4, 37 ^{o}C, 75 rpm). Dispersion time interval: (a) 0 min and (b) 60 min

Different release rate were observed for propranolol HCl and carbamazepine from EC microparticle blends with different preparation method for first and second primary emulsions (Fig. 63). The propranolol HCl and carbamazepine release from conventional microparticle and microparticle blends (DTI of 0 min) for each preparation method were similar (Fig. 63). Whereas, propranolol HCl release was slower than carbamazepine release from EC microparticle blends (DTI of 60 min) (Fig. 63.c-f). Propranolol HCl and carbamazepine released from each microparticle blends with different preparation method for first and second primary emulsions at pH 7.4 after 28 days are in the range of 69% - 82% (DTI of 0 min) and 53.5% - 74% (DTI 60 min) for propranolol HCl. Whereas carbamazepine in the range of 42% - 59% (DTI of 0 min) and 47% - 85% (DTI 60 min).

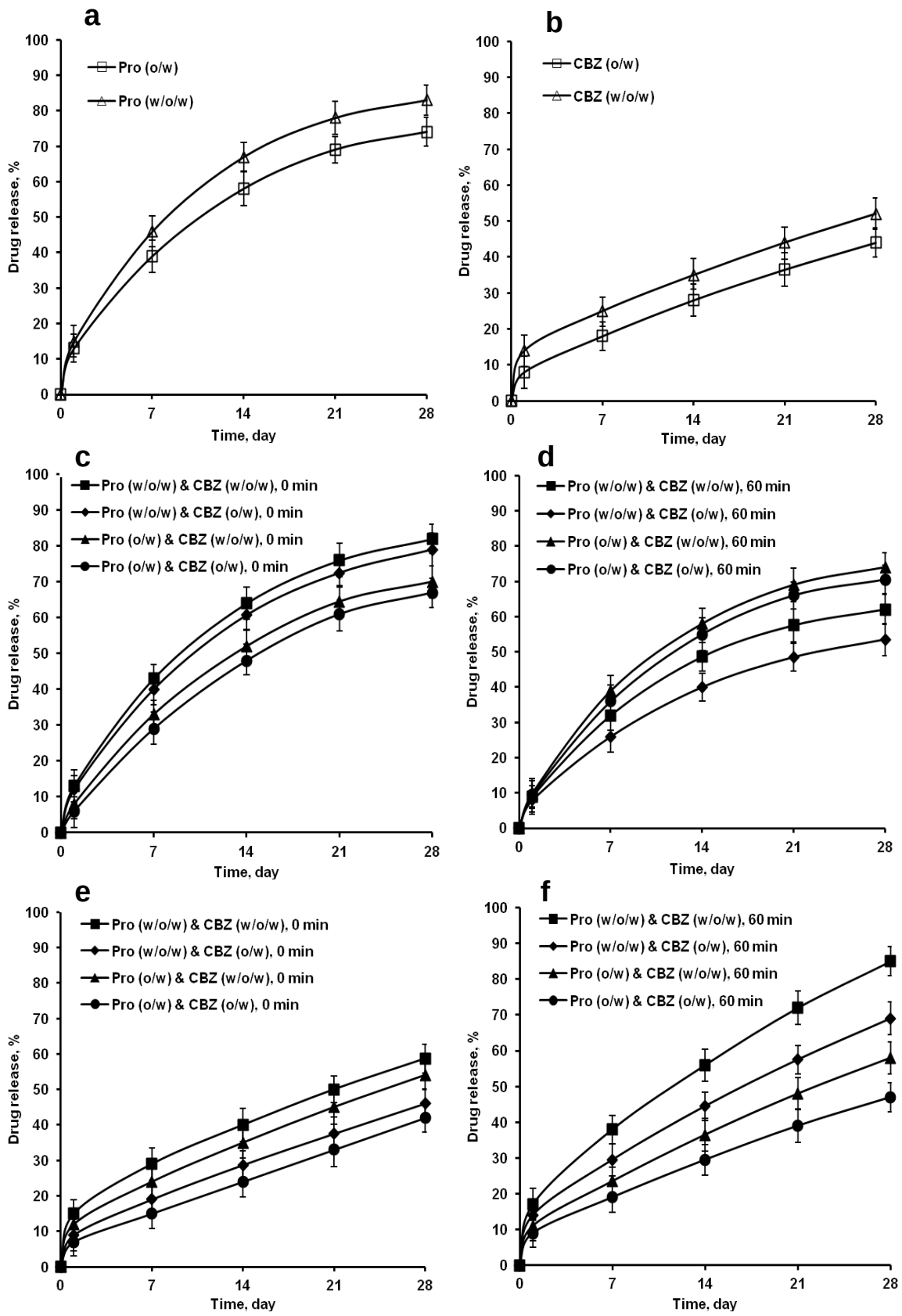

Fig. 63. Effect of preparation method on propranolol HCl and carbamazepine release from ethyl cellulose microparticle blends (phosphate buffer, pH 7.4, 37 ^{o}C, 75 rpm). (a, c and d) Propranolol HCl and (b, e and f) Carbamazepine

The preparation conditions substantially affected the morphology and porosity of the microparticles. Stability of the primary emulsion is a prerequisite for the successful encapsulation of multiple emulsions. In W/O/W method, the microparticles revealed a porous inner structure caused by the inner aqueous phase. The aqueous droplets are precursors of pores and are the result of phase separation occurring in the organic phase during the hardening of the microparticles. It influenced encapsulation efficiency and drug release mechanism.

Effect of polymer type

The polymer type plays an important role in the drug release from microparticle blends systems. High viscosity polymer solution will delayed the polymer precipitation resulted in a less porous polymer matrix hence a slower drug release. In microparticle blends systems, the cumulative release of propranolol HCl after 28 days increased dramatically from 48%, 61%, and 74% (DTI 60 min) and 75%, 83%, and 88% (DTI 0 min) when polycaprolactone (PCL), EC 4 cp and PLGA (RG503H) used respectively (Fig. 64). Propranolol HCl release from microparticle blends after 28 days for DTI of 60 min was slower than DTI of 0 min.

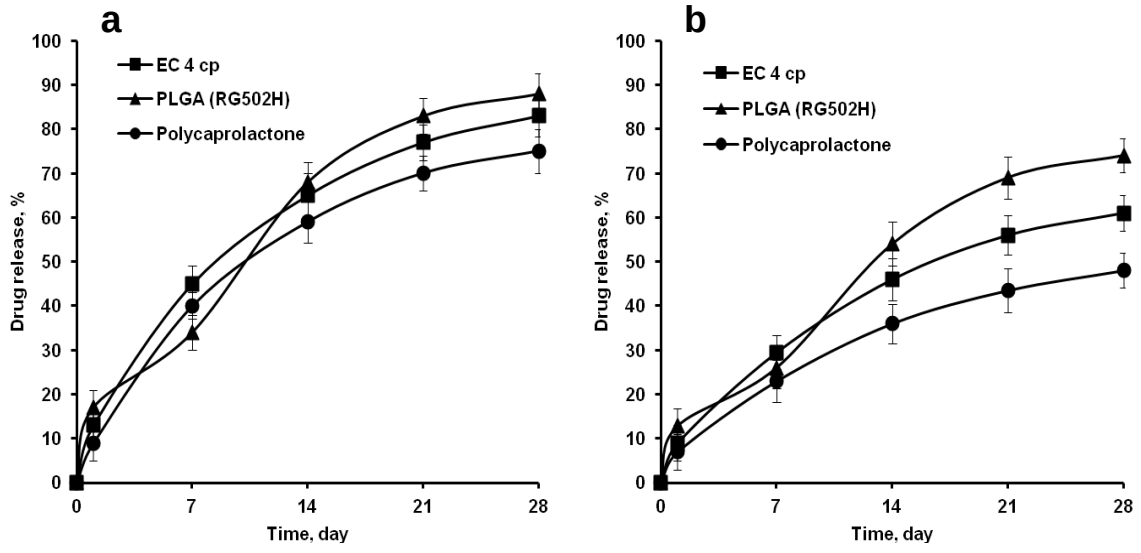

Fig. 64. Effects of polymer type at second primary emulsion on propranolol HCl release from microparticle blends (phosphat buffer, pH 7.4, 37 ^{o}C, 75 rpm). Dispersion time interval: (a) 0 min and (b) 60 min

Different release rate were observed for propranolol HCl and carbamazepine from PCL microparticle blends and PLGA microparticle blends with different dispersion time interval between primary emulsion and oil phase (Fig. 65a and Fig. 65b). Propranolol HCl and carbamazepine release from conventional microparticle and microparticle blends (DTI of 0 min) for each polymer were similar (Fig. 65). Whereas, propranolol HCl release was slower than carbamazepine from PCL microparticle blends and PLGA microparticle blends (DTI of 60 min) (Fig. 65). Propranolol HCl and carbamazepine released from PCL microparticle blends after 28 days are 68.7% (DTI of 0 min) and 33% (DTI 60 min) for propranolol HCl and 41% (DTI of 0 min) and 60% (DTI 60 min) for carbamazepine. Propranolol HCl released from PLGA microparticle blends after 28 days are 94% (DTI of 0 min) and 81% (DTI 60 min). Whereas carbamazepine are 80.5% (DTI of 0 min) and 92.5% (DTI 60 min)

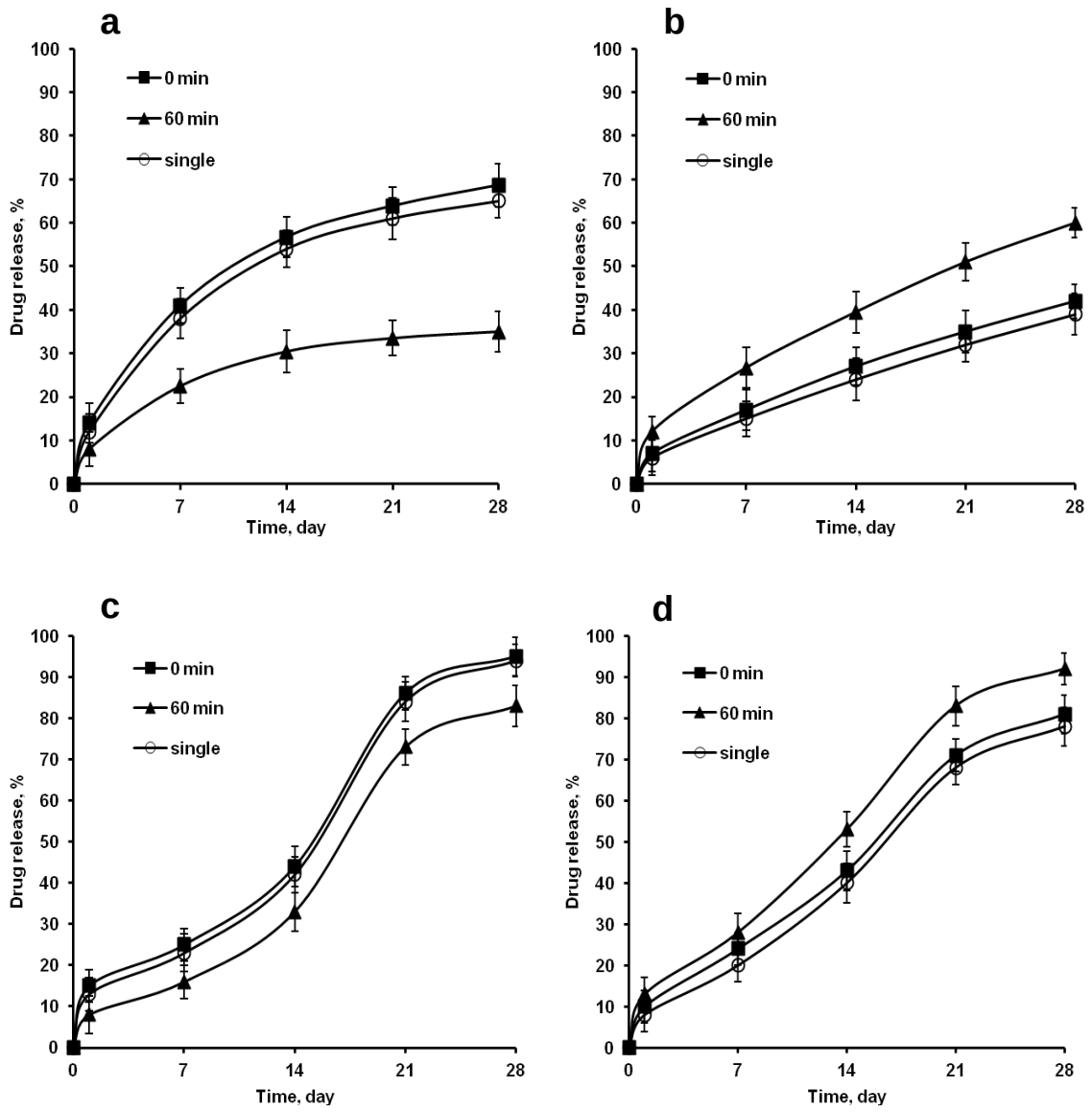

Fig. 65. Effects of dispersion time interval on propranolol HCl and carbamazepine release from polymeric microparticle blends (phosphate buffer, pH 7.4, 37 ^{o}C, 75 rpm). [(a) Polycaprolactone and (b) PLGA (RG502H) and (1) Propranolol HCl and (2) Carbamazepine]

In general, hydrophilic polymers resulted in high release rates. PLGA with relatively high glycolide content (50/50) was used in this experiment. The higher glycolide content made the polymer more hydrophilic, facilitated water uptake from release medium, and resulted in a faster release. On the other hand, slow drug release can also be attributed to the delayed hydration of the microparticles due to the hydrophobicity of polymer (e.g. ethyl cellulose and polycaprolactone).

Microparticle blends were prepared with dispersion time interval 0 and 60 min had typical release profile for propranolol HCl release and carbamazepine release. The release of carbamazepine was generally slower than that of propranolol HCl which can probably be attributed to lower solubility of carbamazepine compared to propranolol HCl in the release medium (0.2 mg/ml vs. 250 mg/ml), resulting in lower concentration gradients and driving forces for diffusion. Another reason is the fact that EC is water insoluble and also has very low permeability. Since this formulation did not contain any channeling agents, formation of pores and cracks did not occur to facilitate drug release. When propranolol HCl loaded EC microparticles were blended with second polymer organic formulation containing carbamazepine (with DTI of 60 min), propranolol HCl release became slower than carbamazepine. It has to be emphasized that the propranolol HCl was inside of microparticle and carbamazepine was on outer surface of microparticle. Thus only the drug located close to the outer surface could be initially released. The release of surface associated drug creates water-filled channels that allow subsequent diffusion of the drugs located inside the microparticles. A major mechanism for release of propranolol HCl and carbamazepine are diffusion through water-filled pores.

This phenomenon might be attributable to the specific interaction behaviors of second primary oil phase (carbamazepine) with hardened microparticles from first primary emulsion (propranolol HCl). Based on release data of microparticle blends (DTI of 60 min) it can be assumed that there is interaction between first hardened particles with second primary emulsions during preparation process of microparticle blend. The second primary emulsion or oil phase had blocked and coated pores on the surface of hardened microparticles from first primary emulsion during solvent evaporation process.

3.5.2.3. Structure of microparticle blends

The spherical shape of the microparticle blends was visualized by scanning electron microscopy (SEM). The surface analysis of drug-loaded microparticles blend revealed that the microparticles were spherical and not aggregated (Fig. 66). Microparticle blends containing the same drug and microparticle blends containing different drug produced two population of microparticles. They are microparticles with pores and rough surface (the same drugs) and microparticles with smooth and rough surface (different drugs).

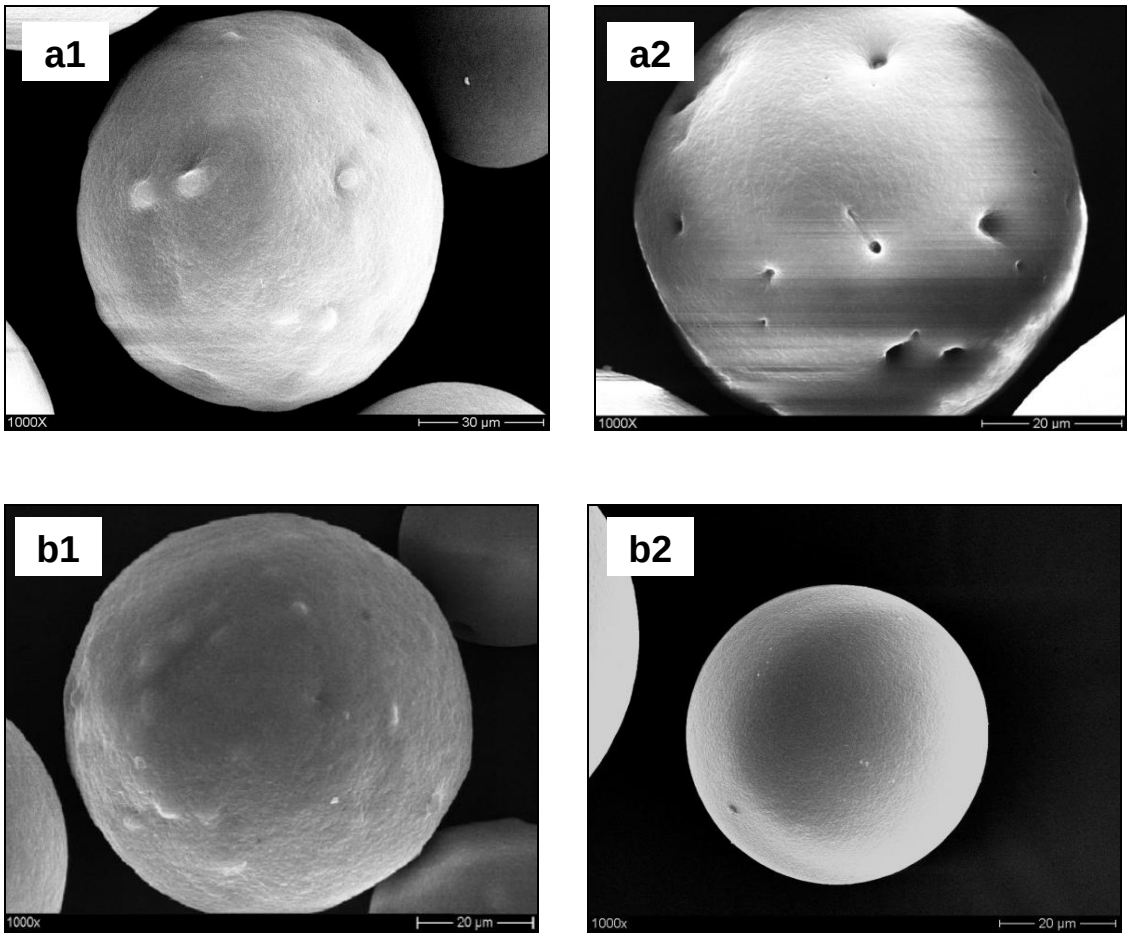

Fig. 66. SEM pictures of ethyl cellulose microparticle blends with dispersion time interval of 60 min. (a) Pro (W/O/W) and Pro (W/O/W), and (b) Pro (W/O/W) and CBZ (O/W). Propranolol HCl (a1, a2 and b1) and Carbamazepine (b2)

Based on release data for microparticle blends, it can be assumed that there is interaction between first primary emulsion (propranolol HCl) and second primary oil phase (carbamazepine) during preparation process of microparticle blends. It was confirmed by SEM study on surface morphology of microparticles blend (Fig. 66). In addition, the absence of pores on surface of microparticle blends which were prepared with DTI 60 min (Fig. 66b) might be attributable to the interaction of second primary oil phase (carbamazepine) with hardened particles from first primary emulsion (propranolol HCl), whereby the second primary oil phase (carbamazepine) had blocked and coated the pores on the surface of hard particle from first primary emulsion (Fig. 66b). This hypothesa supported by optical microscopy pictures (Fig. 67 and Fig. 68). Figure 67 shows microparticles size before and after emulsification second oil phase. Whereas Fig. 68

indicates that the emulsification stage first W/O/W (Pro) and second (CBZ/dye) resulting in two kind of microparticle blends. This picture showed microparticle with black plaque on the surface and black microparticles.

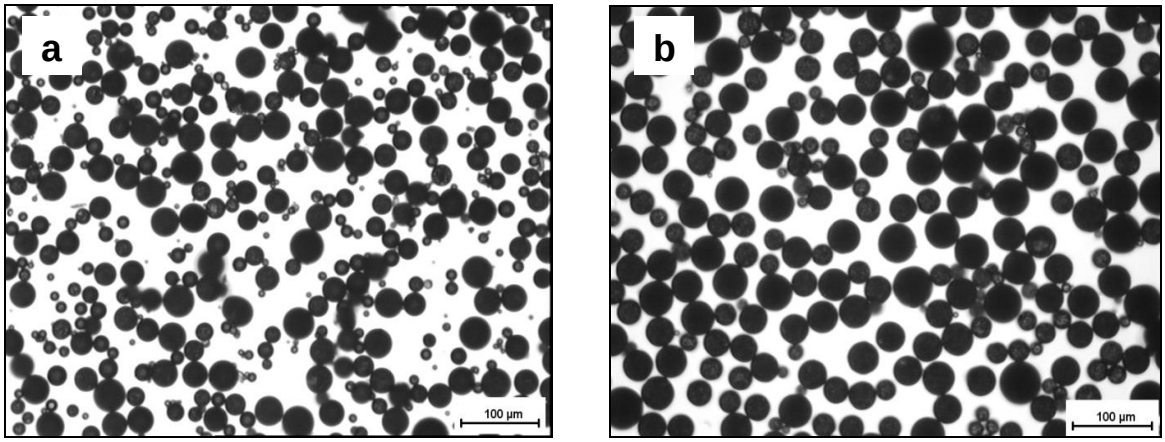

Fig. 67. Optical microscopy pictures of ethyl cellulose microparticle blends (DL 16.7%, EC 4 cp 7.5% w/v) before and after emulsification second oil phase with dispersion time interval of 60 min. (a) At stirring time of 1 hour, and (b) At stirring time of 4 hours

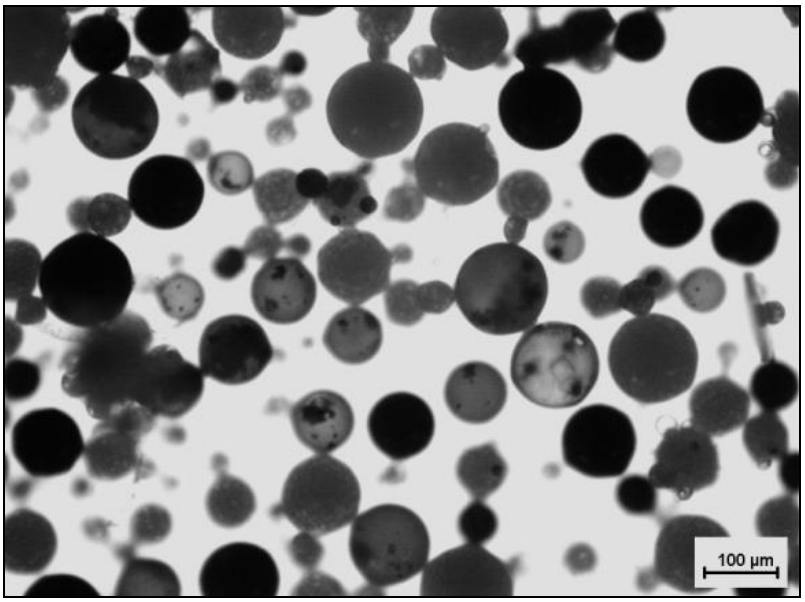

Fig. 68. Optical microscopy pictures of ethyl cellulose microparticle blends with dispersion time interval of 60 min. [Microparticles containing dye (black)]

3.5.3. Conclusion

Encapsulation efficiency, morphology of microparticles and drug release rate from polymeric microparticle blends were influenced by various formulation and processing parameters used in preparation process by solvent evaporation method. Dispersion time interval (DTI) between first primary emulsion (propranolol HCl) with second primary emulsion (propranolol HCl) or oil phase (carbamazepine) was important parameter in microparticles blends preparation process by solvent evaporation method. Dispersion time interval (DTI) influence physical properties of microparticle blends. Propranolol HCl and carbamazepine release from conventional microparticles and microparticle blends (DTI of 0 min) was similar, whereby the propranolol HCl release was faster than carbamazepine. In contrast, microparticle blends prepared with dispersion time interval (DTI) of 60 min showed a faster carbamazepine release than propranolol HCl after 28 days (for microparticle blends containing different drug solubility). Microparticle blends containing the same drug showed slow release of propranolol HCl compared with release from conventional microparticles and microparticle blends (DTI of 0 min). This phenomenon also applicable when applying various formulation parameters of microparticle blends preparation (e.g. type of dispersion time interval, polymer concentration, polymer type, polymer molecular weight, drug loading, solvent type and type of preparation method). This phenomenon might be attributable to the interaction of second primary emulsion or oil phase with hardened particles from first primary emulsion, whereby the second primary emulsion or oil phase had blocked and coated pores on the surface of hardened microparticles from first primary emulsion.

Dispersion time interval, polymer concentration, polymer type, polymer molecular weight, drug loading, solvent type and preparation method in microparticle blends preparation by solvent evaporation method influence the physical properties of microparticle blends.

4. SUMMARY

4.1. Online monitoring of ethyl cellulose (EC) microparticles preparation process by focused beam reflectance measurement (FBRM): Effect of organic solvent type and preparation method

The solidification rate of microparticle which prepared by solvent evaporation method was influenced by type of solvent and emulsion system type. These are influencing particle size, encapsulation efficiency and initial burst. A very slow hardening of the emulsion droplets leads to the diffusion of the drug substance out of the droplets and encapsulation efficiency becomes low. A preferably used technique for process monitoring is the focused beam reflectance measurement (FBRM). This approach allowed for online information in real time about different particles in the sample under investigation. The objective of this study was to investigate effect of type of solvent and method on ethyl celluloce 4 cp (EC) microparticle preparation by solvent evaporation method using a FBRM. EC microparticles were prepared by O/W and W/O/W methods using various solvents, including dichloromethane, chloroform, ethyl acetate and dichloromethane/methanol (1:1). The particle size/distribution of the emulsion droplets/hardened microparticles was followed by FBRM by monitoring the chord length distribution (CLD), particle size mean and chord count of microparticles. The morphology of EC microparticles was characterized by optical microscopy and scanning electron microscopy (SEM).

The transformation of the emulsion droplets into solid microparticles occured within the first 10, 10.5, 12, and 60 minutes (O/W), and the first 12, 11.5, 10, and 90 minutes (W/O/W) when dichloromethane/methanol (1:1), dichloromethane, ethyl acetate and chloroform were used respectively. The square weighted mean chord lengths of EC microparticles were 59, 73, 83 and 92 μm (O/W), and 60, 113, 133 and 124 μm (W/O/W) when chloroform, ethyl acetate, dichloromethane and dichloromethane/methanol (1:1) were used respectively. Larger square weighted mean chord length of EC microparticles gave lower chord counts. Solvent chloroform gave smallest square weighted mean chord length but the chord counts was no the highest (O/W and W/O/W). It is due to slight translucent of the EC microparticles produced by chloroform compared to the other solvents. The CLDs measured by FBRM showed that a larger particle size mean gave longer CLD and a lower peak of particle number due to the decreased number of microparticles. FBRM data are highly dependent on the optical properties of materials and

opacity level of microparticles. Scanning electron microscopy (SEM) data revealed that the morphology of microparticles was influenced by type of solvent and type of preparation method.

In conclusion, FBRM can be employed for online monitoring of the shift in the microparticle CLD and detect transformation of the emulsion droplets into solid microparticles during the solvent evaporation process. The microparticle CLD and transformation process was strongly influenced by solvent type and type of preparation method.

4.2. Online monitoring of preparation process of polymeric microparticles and microparticle blends by focused beam reflectance measurement (FBRM): Effect of polymer type

The objective of this study was to investigate effect of type of polymer on preparation process of polymeric microparticles and microparticle blends by an oil-in-water emulsion method using a focused beam reflectance measurement (FBRM). Polymeric microparticles and microparticle blends were prepared by an O/W- solvent evaporation method using various polymers, including ethyl cellulose 4 cp (EC), Eudragit® RS 100, Eudragit® RL 100, PLGA (Resomer® RG503H) and poly(ε-caprolactone) (PCL). The particle size mean, chord length distribution (CLD), and chord count of the emulsion droplets/hardened microparticles were monitored by FBRM. The morphology of polymeric microparticles/microparticle blends was characterized by optical microscopy and scanning electron microscopy (SEM).

The transformation of the emulsion droplets into solid microparticles occured within the first 10.5, 19, 25, 30 and 55 min and square weighted mean chord lengths are 83, 59, 73, 64 and 51 µm when EC 4 cp, Eudragit® RS 100, Eudragit® RL 100, PLGA (RG503H) and PCL were used respectively. For microparticles blends, the emulsification of first oil phase (EC 4 cp) and second oil phase (EC 4 cp/Eudragit® RL 100/Eudragit® RS 100/PLGA (RG503H)/PCL) into single external aqueous phase with dispersion time interval (DTI) of 0 and 60 min affected the partice size. The second oil phase consisted of EC 4 cp and Eudragit® RS 100 produce smaller microparticle than microparticle normal. While for second oil phase consisted of Eudragit® RL 100, PLGA (RG503H) and PCL produce larger microparticle than microparticle normal. It corresponds to agglomeration of

the microparticle blends. Microparticle blends agglomerates were occurred after 20 min emulsification both of oil phase (DTI 0 min) and after 20 min addition second oil phase into external aqueous phase containing hard EC microparticles (DTI 60 min). Microparticle blends agglomerates are EC-PLGA (RG503H), EC-PCL and EC-Eudragit® RL 100. Larger square weighted mean chord length of polymeric microparticles/microparticle blends gave lower chord counts. PCL microparticles and EC-PCL microparticle blends gave smallest square weighted mean chord length but the chord counts was no the highest. It is due to slight translucent of the PCL microparticles and EC-PCL microparticle blends compared to the other polymers. The CLDs measured by FBRM showed that a larger particle size mean gave longer CLD and a lower peak of particle number due to the decreased number of microparticles. Scanning electron microscopy (SEM) data revealed that the morphology of microparticles was influenced by type and physical properties of polymer.

In conclusion, FBRM can be employed for online monitoring of the shift in the microparticle CLD, detect transformation of the emulsion droplets into solid microparticles and microparticle blends agglomeration during the solvent evaporation process. The microparticle CLD, transformation process and agglomeration was strongly influenced by polymer type.

4.3. Online monitoring of ethyl cellulose (EC) microparticles preparation process by focused beam reflectance measurement (FBRM): Effect of stirring speed, volume of external aqueous phase and polymer concentration

Microparticle formation mechanism is crucial for size distribution and morphology, which in turn determine the delivery system behavior during encapsulation and release. It is well known that solvent evaporation method is mainly a two step process: the emulsification of a polymer solution containing the encapsulated substance, followed by particle hardening through solvent evaporation and polymer precipitation. During emulsification, the polymer solution is broken up in microdroplets by the shear stress produced either by homogenizer, sonicator or whirl mixer in the presence of a surface active agent. Based on this fact, the current study aimed to employ FBRM for online monitoring of solidification rate of ethyl celluloce 4 cp (EC) microparticles and particle size/distribution of the emulsion droplets/hardened EC microparticles manufactured by

O/W method using various parameters (e.g. stirring speed, volume of external aqueous phase and polymer concentration). The microparticles are generated by accelerated solvent elimination due to the combined effects of high solvent volatility and polymer precipitation. The solvent elimination accompanied by important shrinkage determines the hardening rate and morphology of microparticles. During the fast solvent elimination and shrinkage, the FBRM measures and monitores the chord length distribution (CLD), particle size mean and chord count of microparticles in real time, which is affected by the geometry, size, number of microparticles and optical properties of microparticles under analysis.

Based on FBRM data, the transformation of the emulsion droplets into solid microparticles occured within the first 10.5 and 20 min when stirring speed of 500 rpm and 200 rpm; the first 20 and 45 min when volume of external aqueous phase of 800 ml and 400 ml; and the first 6, 7.5, 8.7, and 10.5 min when polymer concentration were 20%, 15%, 10% and 7.5% (w/v). The square weighted mean chord lengths of EC microparticles were 212 and 83 µm when stirring speed of 500 rpm and 200 rpm; 159 and 212 µm when volume external aqueous phase 800 ml and 400 ml; and 83, 98, 128 and 150 µm when polymer concentration were 7.5%, 10%, 15% and 20% (w/v) respectively. Furthermore the emulsification of the second polymer formulation after hardening of the first polymer formulation resulted in the formation of a blend of two separate microparticle formulation in one process when compared to two separate preparation processes followed by blending of the individually prepared microparticle formulations. The CLDs measured by FBRM showed that a larger particle size mean gave longer CLD and a lower peak of particle number due to the decreased number of microparticles. FBRM data are highly dependent on the optical properties of materials and opacity level of microparticles. Scanning electron microscopy (SEM) data revealed that the morphology of microparticles were spherical with smooth surfaces, no pores and no aggregation.

In conclusion, FBRM can be employed for online monitoring of the shift in the microparticle CLD and detect transformation of the emulsion droplets into solid microparticles during the solvent evaporation process. The microparticle CLD and transformation process was strongly influenced by several parameters, such as stirring speed, volume of external aqueous phase and polymer concentration.

4.4. Slow release of propranolol HCl from ethyl cellulose based microparticle blends

The objective of this study was to investigate and characterize incorporation propranolol HCl (hydrophilic drug) and carbamazepine (lipophilic drug) within non-biodegradable EC microparticle blends using solvent evaporation method. Microparticle blends consisting of ethyl cellulose encapsulating propranolol HCl (Pro) and carbamazepine (CBZ) were prepared with a W/O/W and an O/W solvent evaporation method. Two kinds of microparticle blends had been prepared. The first microparticle blends contained the same drug (Pro and Pro) and the second microparticle blends contained drugs with different solubility (Pro and CBZ). The first Pro emulsion (W/O) and second Pro emulsion (W/O) or CBZ oil phase (O) were dispersed in an external aqueous phase, with dispersion time interval (DTI) of 0 and 60 min. The morphology of microparticle blends were characterized by optical microscopy and scanning electron microscopy (SEM). The particle size mean, chord length distribution (CLD), and chord count of the emulsion droplets/hardened microparticles were monitored by FBRM. Encapsulation efficiency (EE) and in vitro drug release in phosphate buffer (pH 7.4) were also investigated.

The resulting microparticles obtained by solvent evaporation method were spherical and two populations. The particle size mean of microparticle blends ranged from 104.26 µm to 127.64 µm (the same drug) and 113.27 µm to 122.42 µm (different drug solubility). The EE was about 76.53% to 78.81% for propranolol HCl in microparticle blends containing the same drug. The EE was about 77.28% to 78.64% for propranolol HCl and 96.48% to 98.64% for carbamazepine in microparticle blends containing different drugs. FBRM studies showed that the size of microparticle blends prepared by W/O/W (Pro) and O/W (CBZ) methods with DTI 60 min and stirring time 4 h was larger than the microparticle blends (DTI 0 min) and microparticles normal. In vitro drug release studies after 28 days, it was found that the propranolol HCl release from microparticle blends contained the same drug with DTI 60 min (54.05%) was slower than microparticle blends with DTI 0 min (73.28%) and non blend (71.32%). Whereas carbamazepine release (58.72%) was faster than propranolol HCl release (43.16%) from microparticle blends contained different drug solubility [Pro (W/O/W) & CBZ (O/W), DTI 60 min]. Based on these data, there is interaction of second primary emulsion (Pro) or oil phase (CBZ) with hard particles from first primary emulsion (Pro), whereby the second primary emulsion

(Pro) or oil phase (CBZ) had blocked and coated pores on the surface of hard particle from first primary emulsion. This blocking and coating effects of microparticles depended on the method step and DTI. This phenomenon is only applicable if the first primary emulsion is W/O/W system.

In conclusion, the novel microparticle blends containing drugs of the same solubility or drugs of different solubility offer a high potential for controlled release drug delivery systems.

4.5. Microparticle blends system for controlled delivery of propranolol HCl and carbamazepine: Influence of the formulation and processing parameters

The solvent evaporation method has been used extensively to prepare polymeric microparticles containing many different drugs. Several variables can influence the properties of the microparticles, including drug solubility, polymer type, solvent type, polymer composition and viscosity, drug loading etc. The effectiveness of the solvent evaporation method to produce microparticles depends on the successful entrapment of the active agent within the microparticles. The solubility properties of the drugs of the microparticles are important parameters when selecting the emulsion phases for a microparticles preparation process. The purpose of this study was to investigate effect of various formulation and processing parameters which used in preparation of microparticle blends on the polymeric microparticle blends contained the same drug (propranolol HCl) and contained drugs with different solubility (propranolol HCl and carbamazepine) which prepared by solvent evaporation method.

Encapsulation efficiency, morphology of microparticles and drug release rate from polymeric microparticle blends were influenced by various formulation and processing parameters used in preparation process by solvent evaporation method. Dispersion time interval (DTI) between first primary emulsion (propranolol HCl) with second primary emulsion (propranolol HCl) or oil phase (carbamazepine) was important parameter in microparticles blends preparation process by solvent evaporation method. Dispersion time interval (DTI) influence physical properties of microparticle blends. Propranolol HCl and carbamazepine release from conventional microparticles and microparticle blends (DTI of 0 min) was similar, whereby the propranolol HCl release was faster than carbamazepine. In contrast, microparticle blends prepared with dispersion time interval (DTI) of 60 min

showed a faster carbamazepine release than propranolol HCl after 28 days (for microparticle blends containing different drug solubility). Microparticle blends containing the same drug showed slow release of propranolol HCl compared with release from conventional microparticles and microparticle blends (DTI of 0 min). This phenomenon also applicable when applying various formulation parameters of microparticle blends preparation (e.g. type of dispersion time interval, polymer concentration, polymer type, polymer molecular weight, drug loading, solvent type and type of preparation method). This phenomenon might be attributable to the interaction of second primary emulsion or oil phase with hardened microparticles from first primary emulsion, whereby the second primary emulsion or oil phase had blocked and coated pores on the surface of hardened microparticles from first primary emulsion.

In conclusion, dispersion time interval, polymer concentration, polymer type, polymer molecular weight, drug loading, solvent type and type of preparation method in preparation process of microparticle blends by solvent evaporation method influence the physical properties of the microparticle blends.

5. ZUSAMMENFASSUNG

5.1. Online Beobachtungen des Herstellungsprozesses von Ethylcellulose Mikropartikeln mittels Focused Beam Reflectance Measurement (FBRM): Einfluss des organischen Lösungsmittels und der Herstellungsmethode

Die Verfestigungsrate der Mikropartikel die mittels Lösungsmittel Verdampfungsmethode hergestellt wurden, wurde durch den Lösungsmittel- und den Emulsionstyp beeinflusst. Dies beeinflusst wiederum die Partikelgröße, die Verkapselungseffektivität und die Initial hohe Freisetzung. Eine sehr langsame Verfestigung der Emulsionstropfen führt zu einer Diffusion des Arzneistoffes aus den Tröpfchen und die Verkapselungsrate sinkt. Eine üblich genutzte Technik für die Beobachtung des Prozesses ist das Focused Beam Reflectance Measurement (FBRM). Dieses Vorgehen liefert online Informationen in Echtzeit über verschiedene Partikel in der untersuchten Probe. Das Ziel dieser Untersuchung war es den Effekt des Lösungsmittels und der Herstellungsmethode von Ethylcellulose 4 cp (EC) Mikropartikeln, die durch die Lösungsmittelverdampfungsmethode hergestellt wurden, mittels FBRM zu ermitteln. EC Mikropartikel wurden durch O/W und W/O/W Emulsionsmethoden und verschiedene Lösungsmittel wie Dichlormethan, Chloroform, Ethylacetat und Dichlormethan/Methanol (1:1) hergestellt. Die Partikelgrößenverteilung der Emulsion und das Verhältnis von Tröpfchen zu Verfestigten Mikropartikeln wurde mit FBRM, durch die Beobachtung der Kreissehnenlängenverteilung (Chord Lenght Distribution CLD), mittlere Partikelgröße und Kreissehnenanzahl (Chord Count) der Mikropartikel verfolgt. Die Morphologie der EC Mikropartikel wurde mit Hilfe eines Optischen- und eines Rasterelektronenmikroskops (Scanning Electron Microscopy SEM) charakterisiert.

Die Umwandlung von Emulsionströpfchen in feste Mikropartikel trat nach den ersten 10; 10,5; 12 und 60 Minuten bei Verwendung der O/W Emulsionsmethode und in den ersten 12; 11,5; 10 und 90 Minuten bei Verwendung der W/O/W Emulsionsmethode auf, wenn entsprechend Dichlormethan/Methanol (1:1), Dichlormethan, Ethylacetat, und Chloroform eingesetzt wurden. Das quadratisch gewichtete Mittel der Sehnenlänge der EC Mikropartikel betrug 59, 73, 83 und 92 µm bei Verwendung der O/W Emulsionsmethode und 60, 113, 133 und 124 µm bei Verwendung der W/O/W Emulsionsmethode wenn entsprechend Chloroform, Ethylacetat, Dichlormethan und Dichlormethan/Methanol (1:1) eingesetzt wurden. Größere quadratische Mittel der Sehnenlänge der EC Mikropartikel ergaben niedrigeren Kreissehnenlängenzahlen. Das Lösungsmittel Chloroform ergab das

kleinste quadratische Mittel der Sehnenlänge, aber die Kreissehnenlängenzahl war nicht die höchste (O/W und W/O/W). Dies ist zurückzuführen auf die leichte Lichtdurchlässigkeit der EC Partikel die mithilfe von Chloroform hergestellt wurden, im Vergleich zu den Partikeln die mit anderen Lösungsmitteln hergestellt wurden. Die mittels FBRM gemessenen CLDs zeigten, dass eine größere durchschnittliche Partikelgröße längere CLD und einen niedrigeren Peak der Partikelanzahl, aufgrund einer reduzierten Mikropartikelanzahl, ergab. FBRM Daten sind in hohem Maße von den optischen Eigenschaften des Materials und der Trübheit der Mikropartikel abhängig. Die Daten des Rasterelektronenmikroskops zeigten, dass die Morphologie der Mikropartikel durch den Lösungsmitteltyp und die Herstellungsmethode beeinflusst wurden.

Fazit: FBRM lässt sich für die Online Beobachtung der Veränderung der Mikropartikel CLD verwenden und erfasst die Umwandlung der Emulsionströpfchen in feste Mikropartikel während des Lösungsmittelverdampfungsprozesses. Die Mikropartikel CLD und der Umwandlungsprozess wurden stark durch den Lösungsmitteltyp und die Herstellungsmethode beeinflusst.

5.2. Online Beobachtungen des Herstellungsprozesses von Polymermikropartikeln und Mikropartikelmischungen mittels Focused Beam Reflectance Measurement (FBRM): Effekt des verwendeten Polymer Typs

Das Ziel dieser Untersuchung war es die Auswirkung des verwendeten Polymers auf den Herstellungsprozess von Polymermikropartikeln und Mischungen von Mikropartikeln aus verschiedenen Polymeren, die mittels einer Öl in Wasser Emulsionsmethode hergestellt wurden, mit Focused Beam Reflectance Measurement (FBRM) zu ermitteln. Polymermikropartikeln und Mikropartikelmischungen wurden durch eine O/W – Lösungsmittelverdampfungsmethode und Verwendung verschiedener Polymere einschließlich Ethylcellulose 4 cp (EC), Eudragit® RS 100, Eudragit® RL 100, PLGA (Resomer® RG503H) und Poly(ε-caprolacton) (PCL) hergestellt. Die mittlere Partikelgröße, die Kreissehnenlängenverteilung (Chord Lenght Distribution CLD), und Kreissehnenanzahl (Chord Count) der Emulsionströpfchen/verfestigten Mikropartikel wurden mittels FBRM beobachtet. Die Morphologie der

Polymermikropartikel/Mikropartikel Mischungen wurde mit einem Optischen- und einem Rasterelektronenmikroskop bestimmt (Scanning Electron Microscope SEM).

Die Umwandlung von Emulsionströpfchen in feste Mikropartikel trat nach den ersten 10,5; 19; 25; 30 und 55 Minuten auf, und das quadratisch gewichtete Mittel der Sehnenlänge betrug 83, 59, 73, 64, und 51 µm wenn entsprechend EC 4 cp, Eudragit® RS 100, Eudragit® RL 100, PLGA (RG503H) und PCL verwendet wurde. Die Partikelgröße der Mikropartikel Mischungen wurde durch das Dispersionszeitintervall (Dispersion Time Intervall DTI) von 0 und 60 Minuten der Emulgierung der ersten öligen Phase (EC 4 cp) und der zweiten öligen Phase (EC 4 cp/Eudragit® RL 100/Eudragit® RS 100/PLGA (RG503H)/PCL) in die einfache äußere wässrige Phase beeinflusst. Mit der zweiten öligen Phase, die aus EC 4 cp und Eudragit® RS 100 bestand, erhielt man kleinere Mikropartikel als normalerweise. Während man mit der zweite ölige Phase, die aus Eudragit® RL 100, PLGA (RG503H) und PCL bestand größere Mikropartikel als normalerweise erhielt. Das ist auf Agglomeration der Mikropartikelgemische zurückzuführen. Agglomerate der Mikropartikelmischungen traten nach 20 Minuten emulgieren der beiden öligen Phasen (DTI 0 min) und nach 20 Minuten nach der Zugabe der zweiten öligen Phase in die äußere wässrige Phase, die harte EC Mikropartikel enthielt, auf (DTI 60 min). Gemischte Mikropartikel Agglomerate waren EC-PLGA (RG503H), EC-PCL und EC-Eudragit® RL 100. Größere quadratische Mittel der Sehnenlänge von Polymermikropartikel/gemischten Mikropartikeln ergaben niedrigeren Kreissehnenlängenzahlen. PCL Mikropartikel und gemischte EC-PCL Mikropartikel ergaben die niedrigsten quadratischen Mittel der Sehnenlänge jedoch waren ihre Kreissehnenlängenzahlen nicht die größten. Dies ist zurückzuführen auf die leichte Lichtdurchlässigkeit der PCL Mikropartikel und der gemischten EC-PCL Mikropartikel im Vergleich zu den anderen Polymeren. Die mittels FBRM gemessenen CLDs zeigten, dass eine größere durchschnittliche Partikelgröße längere CLD und einen niedrigeren Peak der Partikelanzahl, aufgrund einer reduzierten Mikropartikelanzahl, ergab. Die Daten des Rasterelektronenmikroskops zeigten, dass die Morphologie der Mikropartikel durch den Polymertyp und dessen Eigenschaften beeinflusst wird.

Fazit: FBRM lässt sich für die Online Beobachtung der Veränderung der Mikropartikel CLD verwenden und erfasst die Umwandlung der Emulsionströpfchen in feste Mikropartikel und Agglomeration von Mikropartikelmischungen, während des Lösungsmittelverdampfungsprozesses. Der CLD der Mikropartikel, der

Umwandlungsprozess und die Agglomeration wurden stark durch den Polymertyp beeinflusst.

5.3. Online Beobachtungen des Herstellungsprozesses von Ethylcellulose Mikropartikeln mittels Focused Beam Reflectance Measurement (FBRM): Effekt der Rührgeschwindigkeit, des Volumens der Äußeren wässrigen Phase und der Polymerkonzentration

Der Ausbildungsprozess der Mikropartikel ist ausschlaggebend für die Größenverteilung und Morphologie, was wiederum das Verhalten der Darreichungsform während der Verkapselung und Freisetzung bestimmt. Es ist bekannt das das Lösungsmittelverdampfungsverfahren vorzugsweise ein zweistufiger Prozess ist: die Emulgierung der Polymerlösung, die die zu verkapselnde Substanz enthält gefolgt von der Verfestigung durch die Lösungsmittelverdampfung und Polymerpräzipitation. Während der Emulgierung ist die Polymerlösung durch die Schubspannung die durch den Homogenisator, Ultraschallprozessor oder Wirbelmischer erzeugt wird in Gegenwart von einem Oberflächenaktivenstoff in Mikro-Tropfen zerteilt. Davon ausgehend war das Ziel dieser Studie FBRM für die Online Beobachtung der Verfestigungsrate von Ethylcellulose 4cp (EC) Mikropartikeln und die Partikelgröße/Partikelgrößenverteilung der Emulsionstropfen/ verfestigten EC Mikropartikeln die mittels O/W Methode mit verschiedenen Parametern (z.B. Rührgeschwindigkeit, Volumen der äußeren wässrigen Phase und der Polymerkonzentration) hergestellt wurden zu beobachten. Die Mikropartikel werden durch ansteigende Lösungsmittelelimination durch den kombinierten Effekt von hoher Lösungsmittelflüchtigkeit und Polymerpräzipitation gebildet. Die Lösungsmittelelimination wird begleitet von einer maßgeblichen Schrumpfung die die Härtungsrate und die Morphologie der Mikropartikel bestimmt. Während der schnellen Lösungsmittelelimination und der Schrumpfung wird die Kreissehnenlängenverteilung (CLD chord lenght distribution), die mittlere Partikelgröße und die Kreissehnenzahl der Mikropartikel in Echtzeit mittels FBRM gemessen und aufgezeichnet. Die gemessenen Größen werden beeinflusst durch die Geometrie, die Größe, die Anzahl und den optischen Eigenschaften der analysierten Mikropartikel.

Die FBRM Daten zeigen, dass bei einer Rührgeschwindigkeit von 500 und 200 rpm die Umwandlung der Emulsionstropfen in feste Mikropartikel in den ersten 10.5 bis 20

Minuten, bei einem Volumen der externen Phase von 800 und 400 ml in den ersten 20 und 45 Minuten und bei einer Polymerkonzentration von 20%, 15%, 10% und 7.5% (w/v) in den ersten 6, 7.5, 8.7 und 10.5 Minuten stattfindet. Das quadratisch gewichtete Mittel der Sehnenlänge der EC Mikropartikel betrug 212 und 83 µm bei einer Rührgeschwindigkeit von 500 und 200 rpm, 159 und 212 µm wenn das Volumen der externen wässrigen Phase 800 und 400 ml betrug und jeweils 83, 98, 128, 150 µm bei einer Polymerkonzentration von 7,5%, 10%, 15% und 20% (w/v). Des Weiteren bildete sich während des Emulgierens der zweiten Polymerformulierung nach der Verfestigung der ersten Polymerformulierung in einer Mischung von zwei separaten Mikropartikelformulierungen in einem Arbeitsschritt, was zu vergleichen ist mit zwei getrennten Herstellungsschritten und einer anschließenden Vermischung der einzeln hergestellten Mikropartikelformulierungen. Die Messungen der CLDs mittels FBRM zeigten, dass größere mittlere Partikelgrößen längere CLDs und einen niedrigeren Peak der Anzahl aufgrund von geringerer Anzahl an Mikropartikeln ergab. Die FBRM Daten sind in hohem Maße von den optischen Eigenschaften und der Lichtdurchlässigkeit des Materials abhängig. Die Ergebnisse des Rasterelektronenmikroskops zeigten, dass die Mikropartikel sphärisch waren und eine glatte Oberfläche besaßen, keine Poren hatten und nicht agglomeriert waren.

Zusammenfassend kann man sagen, dass FBRM, für die online Beobachtung der Veränderung der CLD und der Umwandlung der Emulsionstropfen in feste Mikropartikel während des Lösungsmittelverdampfungsprozesses verwendet werden kann. Die CLD der Mikropartikel und der Umwandlungsprozess wurden stark beeinflusst durch verschiedene Parameter, wie die Rührgeschwindigkeit, das Volumen der externen wässrigen Phase und der Polymerkonzentration.

5.4. Langsame Freisetzung von Propranolol HCl aus Ethylcellulose basierten Mikropartikelmischungen

Das Ziel dieser Untersuchung war es die Einarbeitung von Propranolol HCl (hydrophieler Arzneistoff) und Carbamazepin (lipophieler Arzneistoff) in nichtbioabbaubare EC Mikropartikelmischungen, die mittels Lösungsmittelverdampfungsmethode hergestellt wurden zu untersuchen und zu charakterisieren. Die Mikropartikelmischungen bestehend aus Ethylcellulose die Propranolol HCl (Pro) und Carbamazepin (CBZ) einschließt, wurden mit einer W/O/W-

und O/W- Emulsionsmethode durch Lösungsmittelverdampfung hergestellt. Zwei Arten von Mikropartikelmischungen wurden hergestellt. Die erste Mikropartikelmischung enthielt den gleichen Arzneistoff (Pro und Pro) und die zweite Mikropartikelmischung enthielt Arzneistoffe mit verschiedenen Löslichkeiten (Pro und CBZ). Die erste Pro Emulsion (W/O) und die zweite Pro Emulsion (W/O) oder CBZ Öl-Phase (O) wurden in der äußeren wässrigen Phase mit Dispersionsintervallen (Dispersion Time Intervall DTI) von 0 und 60 Minuten dispergiert. Die Morphologie der Mikropartikelmischungen wurde mit einem Optischen- und einem Rasterelektronenmikroskop bestimmt (Scanning Electron Microscope SEM). Die mittlere Partikelgröße, die Kreissehnenlängenverteilung (Chord Lenght Distribution CLD), und Kreissehnenanzahl (Chord Count) der Emulsionströpfchen/verfestigten Mikropartikel wurden mittels FBRM beobachtet. Auch die Effizienz der Verkapselung (Encapsulation efficiancy EE) und die in vitro Freisetzung in Phosphat Puffer (pH 7.4) wurden untersucht.

Die durch Lösungsmittelverdampfungsmethode entstandenen Mikropartikel waren sphärisch und in zwei Populationen geteilt. Die mittlere Partikelgröße der Mikropartikelmischungen lag zwischen 104,26 µm und 127,64 µm (bei gleichem Arzneistoff) und zwischen 113,27 µm und 122,42 µm (bei Arzneistoffen mit unterschiedlicher Löslichkeit). Die EE lag zwischen 76.53% und 78,81% für Propranolol HCl in Mikropartikelmischungen die den gleichen Arzneistoff enthielten. In den Mikropartikelmischungen die verschieden Arzneistoffe enthielten lag die EE für Propanolol HCl zwischen 77,28% und 78,64% und für Carbamazepin zwischen 96,48% und 98,64%. FBRM Untersuchungen zeigten, dass die Größe der Mikropartikel in den Mischungen die durch W/O/W- Emulsionsmethode (Pro) und O/W- Emulsionsmethode (CBZ) hergestellt wurden nach DTI 60 min und 4 Stunden Rührzeit größere waren als Mikropartikelmischungen (DTI 0 min) und normalen Mikropartikel. In vitro Freisetzungstests zeigten nach 28 Tagen eine langsamere Freisetzung von Propranolol HCl Mikropartikelmischungen die den gleichen Arzneistoff enthielten (DTI 60 min) 54,05% im Vergleich zu Mikropartikelmischungen mit DTI 0 min (73,28%) und nicht Mischungen (71,32%). Die Carbamazepin Freisetzung aus Mikropartikelmischungen mit Arzneistoffen verschiedener Löslichkeit war schneller (58,72%) als die Propranolol HCl Freisetzung (43,16%) in diesen Mikropartikelmischungen [Pro (W/O/W) & CBZ (O/W), DTI 60 min]. Basierend auf diesen Daten gibt es ein spezifisches Interaktionsverhalten der zweiten primären Emulsion (Pro) oder der Öl-Phase (CBZ) mit schon festen Partikeln der ersten

primären Emulsion, wodurch Poren auf der Oberfläche der festen Partikel durch die zweite primäre Emulsion überzogen und blockiert wurden. Das Blockieren und Überziehen der Poren sind abhängig vom DTI und dem Schritt der Methode. Dieses Phänomen ist nur dann sichtbar, wenn die erste primäre Emulsion eine W/O/W-System ist.

Fazit: Diese neuen Mikropartikelmischungen, die Arzneistoffe einer oder unterschiedlicher Löslichkeiten enthalten bieten ein hohes Potential für kontrollierte Arzneistofffreisetzungssysteme.

5.5. Micropartikel Mischungssysteme für die kontrollierte Freisetzung von Propranolol HCl und Carbamazepin: Einfluss der Formulierungs- und Prozessparameter

Die Lösungsmittelverdampfungsmethode wurde vielfältig genutzt um Polymermikropartikel mit vielen verschiedenen Arzneistoffen herzustellen. Es gibt einige Variablen wie zum Beispiel die Löslichkeit des Arzneistoffs, der Polymertyp, der Lösungsmitteltyp, die Polymerzusammensetzung, die Viskosität und die Arzneistoffbeladung etc. die die Eigenschaften der Mikropartikel beeinflussen können. Die Effektivität der Lösungsmittelverdampfungsmethode um Mikropartikel herzustellen hängt von der Erfolgreichen Einbettung des Arzneistoffs in die Mikropartikel ab. Die Löslichkeit des Arzneistoffs der Mikropartikel ist ein wichtiger Parameter bei der Auswahl der Emulsionsphase für den Herstellungsprozess der Mikropartikel. Das Ziel dieser Arbeit war es den Effekt von mehreren Formulierungs- und Herstellungsparametern die bei der Herstellung von gemischten Mikropartikeln einflussnehmen zu untersuchen. Es wurden Polymermikropartikelmischungen die den gleichen Arzneistoff (Propranolol HCl) und verschiedenen Arzneistoffe mit unterschiedlicher Löslichkeit (Propranolol HCl und Carbamazepin) enthalten und mit dem Lösungsmittelverdampfungsverfahren hergestellt wurden untersucht.

Die Verkapselungseffektivität, die Morphologie der Mikropartikel und die Arzneistofffreisetzungsrate von Polymermikropartikeln wurden beeinflusst durch mehrere Formulierungs- und Herstellungsparameter die bei der Lösungsmittelverdampfungsmethode verwendet wurden. Das Dispersionszeitintervall (DTI Dispersion time intervall) zwischen der ersten primären Emulsion (Propranolol HCl) mit der zweiten primären Emulsion (Propranolol HCl) oder Öl-Phase (Carbamazepin) war ein

wichtiger Parameter bei der Herstellung von gemischten Mikropartikeln mit dem Lösungsmittelverdampfungsverfahren. Das Dispersionszeitintervall beeinflusst die physikalischen Eigenschaften der gemischten Mikropartikel. Die Freisetzung von Propranolol HCl und Carbamazepin von konventionellen Mikropartikeln und gemischten Mikropartikeln war bei einem DTI von 0 min ähnlich, wobei die Propranolol HCl Freisetzung schneller erfolgte als die Freisetzung von Carbamazepin. Im Kontrast dazu war die Carbamazepin Freisetzung in gemischte Mikropartikel die mit einem DTI von 60 min hergestellt wurden nach 28 Tagen schnellere als die Propranolol HCL Freisetzung (für gemischte Mikropartikel, die Arzneistoffe mit unterschiedlicher Löslichkeit enthielten). Gemischte Mikropartikel die den gleichen Arzneistoff enthielten zeigten eine langsamere Propranolol HCl Freisetzung im Vergleich zu konventionell hergestellten Mikropartikeln und gemischten Mikropartikeln (DTI von 0 min). Dieses Phänomen ist ebenfalls anwendbar wenn verschiedenen Formulierungsparameter (z.B. Typ des Dispersionszeitintervalls, Lösungsmitteltyp, Herstellungsmethode) verwendet wurden. Dieses könnte zurückzuführen sein auf die Interaktion der zweiten primären Emulsion oder Öl-Phase mit erstarrten Partikeln der ersten primären Emulsion, wobei die sekundäre primäre Emulsion oder Öl-Phase Poren auf der Oberfläche von verfestigten Mikropartikeln der ersten primären Emulsion blockiert und bedeckt.

Fazit: Das Dispersionszeitintervall, die Polymerkonzentration , der Polymertyp das Molekulargewicht des Polymers, die Arzneistoffbeladung, der Lösungsmitteltyp und die Art des Herstellungsprozesses von gemischten Mikropartikeln mit dem Lösungsmittelverdampfungsverfahren beeinflussen die physikalischen Eigenschaften der gemischten Mikropartikel.

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7. PUBLICATIONS AND PRESENTATIONS RESULTING FROM THIS WORK

7.1. Publications

Muhaimin, Dickenhorst, B., Bodmeier, R., Online monitoring of ethyl cellulose (EC) microparticles preparation process by focused beam reflectance measurement (FBRM): Effect of organic solvent type and preparation method. (in preparation)

Muhaimin, Dickenhorst, B., Bodmeier, R., Online monitoring of preparation process of polymeric microparticles and microparticle blends by focused beam reflectance measurement (FBRM): Effect of polymer type. (in preparation)

Muhaimin, Dickenhorst, B., Bodmeier, R., Online monitoring of ethyl cellulose (EC) microparticles preparation process by focused beam reflectance measurement (FBRM): Effect of stirring speed, volume of external aqueous phase and polymer concentration. (in preparation)

Muhaimin, Dickenhorst, B., Bodmeier, R., Slow release of propranolol HCl from ethyl cellulose based microparticle blends. (in preparation)

Muhaimin, Bodmeier, R., Microparticle blends system for controlled delivery of propranolol HCl and carbamazepine: Influence of the formulation and processing parameters. (in preparation)

7.2. Presentations

Muhaimin, Dickenhorst, B., Bodmeier, R., Application of focused beam reflectance measurement (FBRM) in monitoring process of ethyl cellulose (EC) microparticle preparation by single emulsion solvent evaporation method: Effect of solvent type, Gesellschaft Deutscher Chemiker (GDCh) - Wissenschaftsforum Chemie, 2013, Darmstadt, Germany.

Muhaimin, Dickenhorst, B., Bodmeier, R., Focused beam reflectance measurement (FBRM) as an in-process analytical tool for the preparation of microparticles by the solvent evaporation method, 19th International Symposium on Microencapsulation, 2013, Pamplona, Spain.

Muhaimin, Dickenhorst, B., Bodmeier, R., Focused beam reflectance measurement (FBRM) to study microparticle preparation by the solvent evaporation method, Annual Meeting and Exposition of the American Association of Pharmaceutical Scientists (AAPS), 2013, San Antonio, USA.

8. CURRICULUM VITAE

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