Preparation and characterization of ophthalmic fixed dose combination nanosuspensions

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Selbstständigkeitserklärung

Hiermit erkläre ich, dass ich diese Arbeit selbständig verfasst habe und keine anderen als die angegebenen Quellen und Hilfsmittel in Anspruch genommen habe.

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1 Introduction

Approximately 70–90% of new drug molecules belong to classes II and IV of the Biopharmaceutical Classification System (BCS) and are classified as poorly soluble [1]. Approaches to improve drug solubility include the use of cosolvents, salt formation, complexation, and particle size reduction [2].

Nanosuspensions are colloidal dispersions of drug crystals having a size below 1 µm, dispersed in an aqueous vehicle [3]. Nanosuspensions provide a unique tool for formulation of BCS class II and IV drugs, especially for high dose formulations of immediate action [4].

In contrast to other nanosized systems such as liposomes and solid lipid nanoparticles that act as drug carriers, a significant advantage of drug nanocrystals is their simplicity. Nanosuspensions are carrier-free, thus allowing for high doses and a rapid onset of action [5]. Since solvents are usually avoided during nanocrystal processing also the risk of toxicity from these sources is typically not a concern. In a suspension, the dispersed crystals are also less likely undergoing chemical degradation as only a small fraction of the drug is dissolved in the continuous phase and hence susceptible to degradation [6].

One of the foremost reasons for such a widespread interest in nanosuspensions is their ability to increase saturation solubility and dissolution rates of poorly soluble drugs. Nanosuspensions manifest these unique properties because of their small size and high surface area. The kinetics of drug crystal dissolution can best be described using the Noyes-Whitney equation with surface area being directly proportional to the dissolution rate [3]. Dissolution rate is also inversely proportional to the thickness of the diffusion layer. It has been experimentally proven in several studies that the smaller the particle size, the smaller the diffusion layer thickness and the higher the dissolution rate [7]. The Prandtl equation describes the effect of surface curvature on boundary layer thickness where the greater the surface curvature, the thinner the boundary layer [8]. Small crystals have larger surface curvature as compared to large crystals and therefore boundary layer thickness is relatively small in the case of nanocrystals.

In addition, the higher surface curvature of small crystals also increases the saturation solubility of the drug as given by the Gibbs–Thomson or Kelvin effect [9]. According to this

theory, smaller crystals exhibit a more convex surface which is not favourable energetically. Therefore these crystals have higher dissolution pressure and show higher equilibrium solubility, also described by the Ostwald–Freundlich equation. It predicts that smaller crystals have a higher solubility than larger ones . Increased saturation solubility in turns increases the concentration gradient of the dissolved drug between the drug surface and the bulk solution, which results in an increased dissolution rate due to increased drug diffusion from the surface into the bulk solution, as per Fick's first law.

A direct consequence of the increase in saturation solubility and fast dissolution rates of pharmaceutical nanocrystals is their improved pharmacokinetics in terms of increased rate and extent of absorption, rapid onset of action and better clinical efficacy [10]–[13].

The physical instability of drug nanocrystals has however continuously impeded their success. The high surface energy caused by the small particle size renders the system thermodynamically unstable, often resulting in undesirable phenomena such as aggregation, Ostwald ripening and sedimentation [14]. An increase in particle size counteracts the benefits of nanosuspensions as it reduces dissolution rate and saturation solubility and hence bioavailability. Therefore, drug nanocrystals require considerable knowledge to counteract their physical instability.

1.1 Physical stability of nanosuspensions

Suspended crystals exhibit Brownian motion and so collide, agglomerate and aggregate due to inter-particle attractive- and van der Waals forces [15]. Aggregation can be observed during the preparation and storage of nanosuspensions and broadens the particle size distribution [16].

Ostwald ripening or crystal growth is a phenomenon in which the crystal size increases due to solubility differences between smaller and larger crystals. Smaller crystals have a higher saturation solubility than larger crystals due to their higher free surface energy. This results in a concentration gradient between the crystals, resulting in diffusional mass exchange and a growth of larger crystals to the expense of smaller ones [17].

Temperature fluctuation affects this phenomenon. As the temperature rises, proportionately more drug molecules dissolve from smaller crystals which, upon cooling recrystallize on the surface of existing larger crystals [18]. To maintain the benefits of nanosuspensions, aggregation and ripening must be prevented by employing stabilizers [19].

Sedimentation is also frequently observed as nanosuspension instability. Nanocrystals tend to settle under the influence of gravity. This phenomenon is governed by Stoke's law, which depends on particle diameter, density of the dispersed phase and viscosity of the dispersion medium. The sedimentation behaviour of nanocrystals can be categorized as flocculation and deflocculation. Flocculated suspensions generally show rapid but loose sedimentation and can be easily re-dispersed. Flocculation results from attractive interactions between drug crystals and expresses in a fractal structure in very dilute systems or gel like structure in more concentrated suspensions. Flocculation of nanosuspensions may occur by polymer bridging, charge neutralization, polymer–crystal surface complex formation or a combination of these mechanisms [20]. In contrast, deflocculated suspensions exhibit slow, compact sedimentation. Sedimentation can be accepted in a nanocrystal formulation if the sediment is easily re-dispersed. Irreversible sedimentation or caking can cause serious fluctuations in dosing and must therefore be avoided. For this reason, the inhibition of nanocrystal sedimentation is vital to nanosuspensions [19].

Morphology of nanocrystals is another factor that determines nanocrystal stability. Amorphous forms, which are relatively unstable compared to crystalline forms, exhibit higher solubility. However, drug nanocrystals in amorphous forms have a high possibility of nucleating with each other, causing an increase in drug particle size [21].

Therefore, drug nanocrystals require sophisticated preparation and formulation approaches that assure the physical stability of nanosuspensions [22].

1.2 Preparation of nanosuspensions

Nanosuspensions can be prepared by particle formation (bottom-up) or by particle size reduction (top-down). Bottom-up approach refers to the building up of the nanocrystals from molecular solutions by precipitation [23]. There are different variations of this technique, such as solvent–antisolvent, supercritical fluid process, emulsion-solvent evaporation and spray drying. Bottom-up processes can be carried out at ambient temperatures, and therefore heat sensitive materials can be processed easily. One requirement that limits the applicability of this technique is however that the drug shows sufficient solubility in a non-aqueous and water miscible solvent.

The top-down approach consists of breaking down bigger crystals into smaller ones by either wet bead milling, high pressure homogenization or microfluidization. Since solvents are usually avoided during top-down approaches, the risk of toxicity from these sources is typically not a concern which makes it particularly interesting as preparation approach. Also, theoretically every drug can be subjected to a top-down approach.

During microfluidization, a drug suspension is channelled through specialized chambers under high pressure and velocity. The chamber geometry divides the suspension into two different streams which at a certain point impinge against each other. In this impingement area, particle size reduction is facilitated via high energy impact, cavitation, and shear forces [24]. Main drawbacks of microfluidization concern the specialized equipment and the high sensitivity to operational parameters [25].

High pressure homogenization involves the passage of a microsuspension through a small aperture under high pressure at high velocities. When the suspension emerges from the narrow aperture there is a drop in velocity and an increase in pressure. This generates high energies which are together with turbulent flow and high shear stress the reason for crystal fracture [26]. One disadvantage of this technique is that the initial drug should be micronized before processing to prevent blockage of the narrow orifice during operation [8].

The process of wet bead milling involves a milling chamber filled with milling beads, milling media including a stabilizer and drug and rotating the milling shaft at high speed. High shear forces are generated in the milling chamber due to the impact of the milling media. Attrition

between crystals and milling media causes crystal fracture. Among the different techniques used for the preparation of nanocrystals, wet bead milling is considered an attractive approach that permits relatively easy scale-up with respect to industrial pharmaceutical nanosuspension production [27]. The process also gives high degrees of freedom to optimize it for different drugs, such as milling speed and time, but also milling bead size and concentration.

Dual centrifugation was then introduced as miniaturized milling approach based on wet bead milling but with samples sizes down to 0.5 mL. It facilities milling by an additional rotation of the milling slurry and beads during a classical centrifugation run [28]. The small-scale milling eases screenings in early development.

1.2.1 Influence of preparation parameters on stability

Critical parameters in wet bead milling include drug amount, number and size of the milling beads, milling speed and time and temperature. Criticality may however vary considerably. Typically, the amount of drug in the milling chamber is rather low, from 2 - 30% (w/v), while the fraction of milling beads is generally higher with 10 – 50% (w/v) [29]. Time and speed vary dependent on the individual set up and the target particle size [30].

Two concepts however seem to be used most frequently. Processes are either characterized by low milling speeds and long milling times or high milling energy and short milling times. Milling times ranging from 30 min to up to 5 d were reported [31], [32]. Colombo *et al.* explained this with drug powder characteristics such as the brittleness of the drug being nanocomminuted, which explained different milling efficiencies for the drugs dexamethasone and tacrolimus [33]. This may however limit the utilization of the method because very long milling times may lead to increased contamination risks, unwanted degradation or stability problems during milling [34].

During milling, two opposite processes take place: fragmentation of material into smaller crystals or crystal growth through interparticle collisions [35]. The occurrence of these two opposite phenomena is dependent on the process parameters. After a certain time point particle size reaches a steady-state and continuing the milling does not further decrease the particle size. In some cases an increase in milling time even led to an increase in particle size and heterogeneity of the material [32].

In milling, a typical assumption is that the rate of break-up of crystals is proportional to the rate of collision of the crystals with the milling beads [35]. At smaller bead sizes, the rate of size reduction is increased, an observation that is attributed to increased collision frequency of the small beads with the crystals due to the larger number of beads [36].

Bead milling is usually done using high-energy mills. These high energy processes face the risk of high temperatures during preparation [37]. The formation of hydrophobic interactions between the nanosuspension and stabilizer is a negative entropic process. Thus, the higher the temperature of the nanosuspension, the more thermodynamically unfavourable the system stability becomes. The tendency of aggregation is therefore enhanced at higher temperatures [38]. Heat development is inevitably encountered during wet bead milling due to high energy input but usually circumvented by using colling units and short processing times [39].

In addition, retaining the crystallinity of drug crystals during processing is beneficial for drug stability [40]. However, bead milling can occasionally and significantly damage the crystallinity of the crystals [41]. Compared to its crystalline form, the amorphous drug form is characterized by enhanced risk of Ostwald ripening [42]. The number and location of amorphous regions is also very difficult to control [23]. However, unlike in dry milling processes, during wet bead milling of crystalline drugs the water may behave as an inhibitor of the formation of amorphous material due to the reduced glass-transition temperature [43].

Maximum stability was achieved when stabilizers were present during wet bead milling. As the total surface area of the particles in a nanosuspension is typically orders of magnitude larger compared to a microsuspension, nanosuspensions need careful evaluation on the stabilizer type and concentration to achieve physical stability.

1.3 Formulation considerations

Many studies have investigated possible mechanisms to stabilize drug nanocrystals using stabilizers. The specific interactions between stabilizers and drug molecules have however not been all explained and various aspects including drug-, dispersion medium- and stabilizer related factors must be considered before choosing a stabilizer.

The stability of a nanosuspension is mostly assessed experimentally for short- and long-term stability under various storage conditions. Physical stability is often described in terms of particle size, polydispersity and zeta potential. Many studies additionally stress nanosuspensions by storage at 4 °C or hyperthermal at 40 °C [13], [14].

1.3.1 Influence of drug properties on stability

Although theoretically every drug can be subjected to a top-down approach by wet bead milling, some drugs are poor candidates for nanosuspensions with respect to achieving the attributes required to form a stable nanosuspension.

Log P is generally used to indicate the degree of hydrophilicity and hydrophobicity of drugs. Drugs with high log P values formed highly stable nanosuspensions, explained by the attraction and adsorption between the hydrophobic drug surface and the hydrophobic functional groups of the stabilizers [45].
Drug enthalpy also directly correlated with nanosuspension stability [42]. Drugs with low enthalpy values were poor candidates to form a suspension, irrespective of the stabilizer used. The most likely candidate for bead milling was a drug with high hydrophobicity and enthalpy. Drugs that were strongly hydrophobic had the advantage that stabilizers covered the nanocrystal surface more easily than they did with hydrophilic drug nanocrystals. Under the premise that stabilizers wet crystal surfaces, also drugs with high cohesion were more likely to form stable nanocrystal suspensions [46].

The interaction between drug and stabilizer is crucial for physical stability and is mainly dependent on the acting surface energies [47]. Hydrophilic polymers are generally used to hydrate nanocrystal surfaces as they interact strongly with surrounding water molecules [16].

It was additionally observed for high molecular weight polymers, that drugs with surface energies like the stabilizer efficiently formed stable nanocrystals [48].

1.3.2 Influence of dispersion medium properties on stability

As defined by the Stokes–Einstein equation, high viscosity reduces crystal diffusion rate, thereby stabilizing the nanosuspension. For example, among cyclosporin A nanosuspensions prepared with various polymers using wet bead milling, poly(vinyl alcohol) (PVA)-stabilized nanosuspensions resulted in the smallest particle size and displayed no sedimentation [49]. Introduction of cellulose-based polymers on the other hand could not fully inhibit cyclosporin A sedimentation. The superiority of PVA-stabilized nanosuspensions could be attributed to the increased milling efficiency and resulting smaller particle size and increased viscosity at no shear.

Employing high molecular weight polymers in the outer phase after milling was reported to effectively increase kinetic stability [50]. Suspending agents such as hydroxypropylmethylcellulose (HPMC) or polycarbophil increased the viscosity of the system and enabled the formation of a stereospecific blockade between the nanocrystals [51].

The sedimentation rate is also described by Stokes' law which indicates the important role of particle size, medium viscosity and density difference between medium and dispersed phase [52], [53]. Therefore, matching drug and medium density is a widely used approach to alleviate sedimentation problems upon storage [28], [32].

Marlowe et. al reported drug uniformity as a percent of declared concentration, comparing a loteprednol gel and a generic prednisolone acetate suspension [51]. Loteprednol concentrations were consistent over the whole dosing study. Dose uniformity of the prednisolone acetate drops was however highly variable across all the samples analysed, when not shaken prior to expressing drops. The difference between the two formulations was the suspensions bulk viscosity. The net effect of the viscosity adapting suspensions was that suspended drug crystals did not settle in the bottle due to the presence of polycarbophil, a viscosity agent frequently employed to hinder sedimentation [20]. Despite the increased physical stability, gels or high viscosity formulations in general however face the problem of blurred vision upon instillation and hence poor patient compliance [55].

If sedimentation is unavoidable, flocculated systems might be used. In contrast to deflocculated systems and potential caking, flocculated crystals settle as loose aggregates instead of as individual crystals [56]. The loose aggregates have a larger apparent particle size compared to the single crystals and thus a higher sedimentation rate. The loose structure of the rapidly settling flocs however contains a significant amount of entrapped medium, resulting in a large flocculation volume, that can be easily broken and re-dispersed by simple agitation.

Engstrom *et al.* have attempted to take advantage of this phenomenon to achieve stable nanosuspensions via a novel design of flocs structure [57]. Thin film freezing was used to produce nanorods which were found to be highly stable when dispersed, with no apparent sedimentation observed for 1 year. Due to the strong attractive van der Waals forces at the contact sites between the particles, primary nanorods were stacked together rapidly as an open structure, with no collapsing observed.

Although sedimentation reduces the integrity of nanosuspensions, the reported studies examining such issues in aqueous nanosuspensions are very scarce. This might be since surfactants are generally used in most of the nanosuspensions to inhibit agglomeration which alleviates sedimentation issues and that size reduction already significantly reduces the sedimentation rate compared to microsuspensions [58].

Nanosuspensions basically consist of the drug, the external aqueous phase comprising the stabilizer, and a reagent that has a proven history of safe use for the intended application. Buffering or isotonicity agents can be added, provided they are compatible with the formulation and do not disrupt physical stability.

It was reported that at increased electrolyte concentrations, the stabilizer layer extended from an emulsion interface, apparently by desorption of loop contacts of hydrophilic stabilizer segments [59]. The extension of the stabilizer layer toward the aqueous phase was accompanied by a reduction in the fraction of bound polymer segments. Washington *et al.* proved that low concentrations of electrolyte in the outer phase caused the poly(propylene oxide)(PPO)-poly(ethylene oxide)(PEO)-block surfactant Poloxamer 188 (P188) to extend from an emulsion interface. At optimum concentrations, maximum extension of the PEO chains toward the outer phase increased the physical stability of the emulsion. At concentrations

above a critical threshold, chain collapse of P188 was however observed due to osmotic stress. Thus, electrolytes may change the stabilizer adsorption behaviour.

Dependent on the system, also the pH of the dispersion medium significantly affected electrostatic and steric stabilization in nanosuspensions, especially where ionic stabilizers were employed [60]. At the isoelectric point, nanosuspensions may face physical instabilities because of the absence of ionic charges to prevent aggregation. Hence, the pH of the dispersion medium must be above or below the pI to obtain electrostatic nanosuspension stability [61].

1.4 Stabilizer choice

In the case of aqueous systems, it is a straightforward approach to select stabilizers given that water-based stabilizing moieties are well known. However, selecting the anchor groups that interact strongly with the drug surface can be challenging due to the high individuality of interactions between drug crystal and stabilizer.

During wet bead milling, a dynamic environment exists, always pending between stabilizer adsorption and the development of new surface areas during drug crystal breakage. A higher rate of surface adsorption results in more efficient processing times and improved nanosuspension stability. For the top-down preparation of nanosuspensions, the particle size of a drug then usually decreases to a steady state with time, dependent also on the stabilizer choice [62]. The selection of proper steric and electrostatic stabilizers is hence considered critical in nanosuspension formulation.

Stabilizers differ in the way they obtain physical stability. Steric stabilization can generally be achieved by the adsorption of hydrophobic stabilizers with long chains onto the surface of the drug, thus limiting drug movement and reducing the entropy between two crystals. Electrostatic stabilization occurs due to repulsion between drug nanocrystals. In this case, when a stabilizer is adsorbed onto the drug surface, an electrical double layer is formed by hydrophilic moieties of the stabilizer that form electric charges around the drug. At a certain distance, two identically charged layers repel each other and the two crystals separate, ultimately preventing aggregation and crystal growth.

Surfactants as a group of stabilizers, consist of both a hydrophilic part and a hydrophobic part. When a surfactant is dissolved in water, the hydrophobic part of the surfactant adsorbs onto the hydrophobic drug surface while the hydrophilic segment orients toward the aqueous environment. Therefore, the surfactant replaces water molecules on the drug surface, decreasing the surface tension and wetting the drug crystal surface. Surfactants can be adsorbed onto crystal surfaces but also retain them within micelles and bilayers by weak van der Waals forces, hydrogen bonds, electrostatic interactions, and ionic interaction [63].

Polymers as large molecular weight compounds composed of many repeating monomers, connected by covalent or chemical bonds are another group of stabilizers. They act as steric barrier by the same mechanism as non-ionic surfactants. The hydrophilic and hydrophobic regions are however less defined, resulting in loops and coils that adsorb on the crystal surface or stretch to the outer medium. When two crystals approach each other, the stretched chains overlap with those of the adjacent crystals. This causes the chains to dehydrate, which generates a repulsive force, ultimately repelling the crystals from each other and stabilizing the nanosuspension [48].

Considering those two groups and their ionic nature, a wide range of stabilizers of different origins can be chosen from for the preparation and stabilization of ophthalmic nanosuspensions.

Ionic surfactants stabilize nanocrystals via electric repulsion with the hydrophilic part of the surfactant having electric charges [2]. Only two ionic surfactants were used for ophthalmic nanosuspensions (Table 1.1).

Table 1.1 Ionic surfactants and polymers used in ophthalmic formulations

Ionic stabilizers	Examples	Reported use
Surfactants	Benzalkonium chloride	[64]
	Sodium dodecyl sulfate	[65]
Polymers	Chitosan	[66], [67]
	Sodium carboxymethylcellulose	[68], [69]

The cationic surfactant benzalkonium chloride (BAK) is present in most ophthalmic multi-dose formulations as preservative and hence an interesting option to use as stabilizer. Due to its ocular irritancy, only low concentrations are however be acceptable [70]. It was nevertheless successfully used in the preparation of dexamethasone acetate nanosuspensions [64]. Physical instabilities were however observed upon long-term storage. Those were attributed to the high variability in alkyl groups of BAK ranging from C8 to C18 having a distinct impact on the hydrophobicity of the surfactant.

Sodium dodecyl sulfate (SDS) as anionic surfactant was also successfully used as stabilizer for many applications in different indications [27], [71], [72]. The use of SDS is however moderately toxic to the eye and the use in ophthalmic formulations is therefore restricted [73]. It was however successfully used in the preparation of ibuprofen nanosuspensions [65]. The increased ibuprofen solubility however led to immediate Ostwald ripening of the nanosuspension.

Chitosan as cationic polymer possesses antibacterial properties and a mucoadhesive character [67]. It was already successfully used in ocular formulations as viscosity agent, permeability enhancer and in micro- and nanoparticles and gels [74]–[76]. However, no evidence was found using chitosan as stabilizer for nanosuspension. This might be due to the high viscosity of the polymer, limiting its applicability at reasonable concentrations during wet bead milling.

Also sodium carboxymethylcellulose (Na CMC) was typically employed as mucoadhesive agent but was also reported as stabilizer for nanocrystals [69]. As it has the potential to work as steric and electrostatic stabilizer, Na CMC has a high potential for the preparation of nanosuspensions. Compared to a range of different stabilizers, NaCMC was however the poorest candidate in milling curcumin nanocrystals [68]. It also needed significantly more homogenisation cycles for smaller particle sizes due to increased milling medium viscosity.

Although the electrostatic stabilizing effect of ionic stabilizers is effective, the presence of an oppositely charged material in the dispersion medium can attenuate the stabilizing effect of the ionic surfactants by reducing the repulsive force [2].

In contrast, non-ionic surfactants are not significantly affected by the oppositely charged ions as they stabilize the drug nanocrystals through steric stabilization [29].

Table 1.2 Non-ionic surfactants and polymers used in ophthalmic formulations

Non-ionic stabilizers	Examples	Reported use
Surfactants	tri-block poly(ethylene oxide)-poly(propylene oxide) (Poloxamer)	[24], [45], [77]
	Tween	[78]
	Tyloxapol	[79], [80]
Polymers	Hydroxyethylcellulose	[78]
	Hydroxypropylmethylcellulose	[40], [62], [72]
	Hydroxypropylcellulose	[27]
	Poly(vinyl pyrrolidone)	[14], [81]
	Poly(vinyl alcohols)	[82]

Non-ionic stabilizers attach to crystal surfaces through hydrophobic interactions and create a steric barrier around crystals [30]. Block copolymers, such as di-block and tri-block copolymers, consist of hydrophobic and hydrophilic segments and thus are highly defined in their adsorption pattern and the way they facilitate physical stabilization [2].

Tri-block copolymers have one soluble and two insoluble segments, or two soluble and one insoluble segments. Poloxamer is an example of a synthetic tri-block copolymer with the hydrophobic PPO in the centre and the hydrophilic PEO group on the periphery. Poloxamer was successfully used in a wide range of ophthalmic nanosuspensions [24], [45], [77].

The interaction of a nanocrystalline drug and poloxamer depends on the balance between the PPO and PEO chain lengths. In particular, the hydrophobicity as defined by the PPO chains is the main driving force for the immobilization of the polymers on the surface of the drug nanocrystals. On the other hand, the PEO segment forms a layer around the nanocrystals and provides steric hindrance to prevent aggregation and growth of the nanocrystals. Poloxamers commonly used for nanocrystal stabilization are Poloxamer 407 (P407) and P188, which differ in their molecular weights [12].

Di-block copolymers, such as Tween-type surfactants, have one soluble and one insoluble polymeric segment each [86]. Tween 80 was successfully used as stabilizer for different drugs [78]. In contrast there were also reports, where Tween 80 was unsuccessful in the

preparation of nanosuspensions of six different drugs [77]. It must however be noted that no concertation optimization was performed and that surfactant concentration takes significant impact on the stabilizing ability.
Tyloxapol as the only liquid polymer in this group is of alkyl aryl polyether alcohol type and was successfully used to produce brinzolamide nanosuspensions [79], [80].
Among non-ionic surfactants, Poloxamers may have the strongest stabilizing effect since poloxamers have larger molecular weights, resulting in a more effective steric barrier [87].

Polymers on the other hand have been extensively explored for improved residence time and bioavailability of a drug given in the form of eye drops. There use as stabilizer was less frequently reported, however gaining interest in recent years. This might be due to its beneficial effect on overall eye drop characteristics (e.g. residence time, bioavailability) and their inherent potential to interact with the drug crystal surface and thus facilitate stabilization. High viscosity however imparts their use in milling aids [69]. Still, polymers were in some cases described as superior compared to surfactants due to their higher rigidity in its polymer chains which rendered them more effective in the steric stabilization [88]. Also they mostly do not increase the drug solubility, a driving factor for crystal growth [14].

Synthetic non-ionic polymers such as PVA and polyvinylpyrrolidone (PVP) have been investigated as possible stabilizers for drug nanocrystals. Nanosuspensions stabilized with 0.2% PVA prevented crystal growth by fully covering the crystal surface of hydrocortisone acetate and maintaining the particle size at 0.20 µm [89], [90].
PVP, has been reported to stabilize a teniposide nanosuspension for up to 3 months [81].

Semisynthetic polymers are another large group of stabilizers prepared by chemically modifying naturally occurring monomers such as HPMC, hydroxypropylcellulose (HPC) and hydroxyethylcellulose (HEC).
HPMC is classified as E, F, K grade according to the viscosity and its methoxyl and hydroxypropyl content. Verma *et al.* demonstrated the effective use of HPMC E15, in preparation and short-term stabilization of ibuprofen nanosuspensions [72]. Lurasidone hydrochloride nanosuspensions were also stabilized using HPMC, which formed hydrogen bonds between lurasidone hydrochloride molecules and the abundant HPMC hydroxyl

groups [108]. These hydrogen bonds led to a high affinity to the drug surface and effectively inhibited crystal growth and aggregation.
HPC was successfully used to stabilize mefenamic nanocrystals with a mean particle size of only 0.17 µm [91]. THPC was more efficient compared to other polymers which was attributed to the specific attraction of HPC to mefenamic, which resulted in a larger stabilizer amount adsorbed on the drug crystal surface.
HEC was so far used in various ophthalmic applications such as in dorzolamide ocular inserts or ketorolac tromethamine films [92], [93]. The fact that it was not employed as stabilizer for nanocrystals might be due to increased viscosities, as also reported for other high molecular weight polymers described before.

Overall, all stabilizers exhibit different stabilizing effects depending on the adsorption affinity of the stabilizers to the surface of the drug nanocrystals. Therefore, it is important to choose the appropriate stabilizer based on the specific physico-chemical properties of the drugs [49].

1.4.1 Influence of stabilizer properties on stability

A stabilizer, which covers the surface of the nanosuspension to produce stabilization, is an essential component of the formulation. The hydrophobic chains adsorbed on the surface of the nanosuspension result in a dynamically rough surface that prevents coalescence via repulsive entropic forces.

To improve the stability of drug nanocrystals, stabilizers should have sufficient affinity to the drug surface [94]. In the case of poorly soluble drugs exhibiting high hydrophobicity a more hydrophobic stabilizer seemed favourable [78]. Simultaneously, the hydrophilicity of stabilizers is critical as most drug nanocrystals are dispersed in aqueous media. The hydrophilic moieties of stabilizers contribute to inhibiting the interactions among the stabilized drug nanocrystals and should be maximally extended toward the aqueous phase. Therefore, stabilizers need to have a suitable balance between hydrophilicity and hydrophobicity [2].
Non-ionic stabilizers with high molecular weights were additionally expected superior to low molecular weight ones as they provide sufficient steric stabilization [94]. Polymeric stabilizers with molecular weights below 5,000 g/mol could not prevent crystal attraction, whereas

molecular weights above 25,000 g/mol were reported to induce bridging due to the excessive chain lengths of the stabilizers [22].

In contrast, Choi *et al.* showed, that small molecular weight stabilizers resulted in faster processing times and also smaller particle sizes in the preparation of ibuprofen nanosuspensions [48]. This was attributed to the faster adsorption isotherm and increased agility during wet bead milling. Faster adsorption was also proven in other studies and rendered a unique advantage of especially surfactants as stabilizers [14].

Certain characteristics of surfactants need however to be considered as for example the appropriate concentration. At optimal concentrations, adsorption affinity of the surfactant to the drug surface is maximized [38]. At insufficient stabilizer concentrations the stabilizer can however not efficiently cover the crystal surfaces. If drug crystals then attach to the same stabilizer molecule, aggregation, and bridging may occur [4].

It is however important to note that nanosuspension stability is not proportional to stabilizer concentration. An excessive concentration of stabilizer may result in Ostwald ripening. Also, concentration of amphiphilic stabilizers above the critical micellar concentration (CMC) may cause the formation of micelles that do not contribute to the stabilization of the nanocrystals. To prepare paclitaxel nanocrystals, P188 was selected as stabilizer [38]. To further stabilize the nanosuspensions against thermal-induced aggregation, additional P188 was added. However, the resulting formation of micelles decreased the nanosuspension stability. The performed desorption experiment suggested different surfactant adsorption affinities for the nanosuspension surface below and above the CMC. Below the CMC, monomers bound to the nanosuspension surface with high affinity, but above the CMC, low-affinity surfactant-aggregates readily left the surface upon dilution. Transmission electron microscopy revealed that, instead of forming a thicker layer on the nanocrystal surface for better stabilization, more micelles began to form in solution at higher surfactant concentrations.

Micelle formation plays a critical role also in the thermal stability of the prepared nanosuspensions as the micelles begin to compete for surface adsorption so that the total adsorption at the interface may decrease as the micelles become more numerous. Therefore, a concentration higher than the CMC resulted in less net surfactant adsorption, which destabilized the nanosuspension and thereby contributed to increased particle sizes [95].

Furthermore and at stabilizer concentrations high above the plateau of the adsorption isotherm, electrostatic stabilizers could cause a decrease in the diffuse layer, leading to a decreased zeta potential and, consequently, decreased physical stability [27].

Drug solubility is also affected by the type of stabilizer and its concentration. When the stabilizer solution alters drug solubility, the risk of Ostwald ripening increases [72]. Surfactants should therefore be used at concentrations below the CMC. However and as discussed above, an inadequate amount of stabilizer would not provide complete coverage of the drug molecule surface, which is necessary to provide a steric repulsion between the nanocrystals in suspension [96].

Diffusion, convection, adsorption, and desorption of stabilizers occur simultaneously with mechanical fracturing and the generation of the new surface area upon wet bead milling [49]. Van Eerdenbrugh *et al.* examined surface stabilization during the production of drug nanocrystals using 13 different stabilizers and nine different drugs [78]. They used both polymers and surfactants. Overall, the surfactants gave the best results in stabilizing the nanosuspensions. This was explained as being caused by the low viscosity and high surface activity of the surfactants. The cellulose ethers under investigation increased viscosity of the media to such an extent that the feasible concentrations of these materials were very low and their stabilizing effect poor. The deficiency in milling with high molecular weight polymers was hence not only based on the higher viscosity but also in the resulting feasible concentration range. Using low molecular weight polymers or surfactants, higher concentrations compared to high molecular weight polymers could be used.

Two different terms of stability must however be considered in the drug-stabilizer-dispersion interaction. First, the ability of fast and efficient adsorption and stabilization during wet bead milling and second, long-term storage stability.
A high viscosity medium might be inefficient for production of small crystals, but according to the Einstein equation, the diffusion velocity of crystals is lower at high viscosities, highlighting the potential use of polymers for achieving long-term physical stability.

Regarding storage stability, strong adsorption at full coverage and a long desorption time scale are required, in addition to steric repulsion [48]. Once absorption is complete, hydrogen bonding and electrostatic interactions may additionally form and strengthen the absorption. Surfactants are usually small molecules and as a result their interfacial films are more dynamic as compared to polymers which generally exhibit irreversible adsorption. Verma *et al.* were able to show, that the morphology and arrangement of adsorbed HPMC was completely uncoiled and adsorbed in an open extended conformation on an ibuprofen surface. P188 on the other hand adsorbed in globule-like structures and also showed preferential adsorption along the crystal's atomic steps [88]. Multiple scans of the same area further proved the migration of the adsorbed P188, where HPMC did not reveal any tendency for migration or dislocation of the adsorbed polymer. This could give a reason on why P188 was an unsuitable stabilizer for storage stability in previously conducted studies by the same working group [14]. Adsorbed polymer layers are normally also more robust and can prevent or slow down the attachment and detachment of drug molecules at the surface of dispersed crystals and hence counteracts Ostwald ripening. Polymers have been known to prevent crystal growth in a number of cases [89].

Polymers adhere to the drug surface, without micellization and hence do not alter drug solubility [48]. Drug solubility in the dispersed phase was already mentioned to play a crucial role in the dynamics of Ostwald ripening and needs consideration to achieve long-term stability. Conventional small-molecular weight surfactants may excessively increase the solubility of hydrophobic drugs. According to the Lifshitz–Slyozov–Wagner theory, Ostwald ripening is directly related to the concentration of the dispersed phase in the system. For use in the long-term stabilization of nanosuspensions, a stabilizer should hence have little effect on drug solubility.

For example, indomethacin nanosuspensions formulated with denatured food proteins displayed superior stabilization and significantly smaller particle size than nanosuspensions prepared with common stabilizers. As Tween 80 and P188 significantly increased indomethacin solubility also Ostwald ripening was more pronounced [85]. Also other studies indicated that an increase in the intrinsic solubility of a drug mediated by stabilizers such as Tween 80 induced Ostwald ripening and agglomeration [72].

Polymers like PVP K30 and HPMC, that minimally affected ibuprofen solubility resulted in lower mean particle sizes compared to stabilizers that significantly increased ibuprofen solubility. Therefore, only stabilizers that have a minimal or negligible effect on drug solubility should be used to prepare nanosuspensions.

With this discrepancy between the use of surfactants and stabilizers in the preparation and physical stabilization of nanosuspensions, recent research focused on the introduction of stabilizer combinations.

Combining ionic and non-ionic stabilizers offers the advantages of static and steric stabilization to achieve a synergistic effect and was hence frequently employed as a stabilising strategy [97].

Recent modelling and experimental investigations suggested that the combined use of non-ionic cellulosic polymers such as HPC, HPMC and anionic surfactants can have synergistic stabilization effects on some drug nanosuspensions [98]–[101].

Bilgili et. al investigated, whether such a strategy was general enough to be applied to multiple drugs in a comparative study including five drugs and two stabilizers, employed in combination and as single stabilizers [102]. The group examined whether the interactions between non-ionic cellulosic polymers and anionic surfactants significantly affected the apparent breakage kinetics, zeta potential and polymer adsorption. In general, using SDS, in addition to HPC, gave smaller particle sizes after milling than in the absence of SDS, independent of the drug. The combination further led to electrosteric stabilization of the drug nanosuspensions due to higher electrostatic repulsive forces expressed in a higher zeta potential between the crystals as well as enhanced steric hindrance from the adsorbed polymer, which was facilitated by the presence of HPC.

Another study proved the concept by employing a combination of arginine hydrochloride and HPC resulting in both steric and electrostatic stabilization to prevent aggregation by creating different energy barriers where HPC alone was not capable of maintaining physical stability [103].

PVA-stabilized nitrendipine nanosuspensions also exhibited superior physical stability when combined with chitosan [82]. It was concluded that the increase in zeta potential and hence enhanced electrostatic repulsion of chitosan was the main reason for increased physical stability. Furthermore, chitosan provided a steric stabilization effect because its deposition on

the surface of the nanosuspensions further increased the physical stability of the prepared nanosuspensions.
Ionic systems are however more sensitive to environmental changes such as pH shifts and are not considered as optimal choice for some formulations [61].

It was observed for high molecular weight polymers, that drugs with surface energies like the stabilizer efficiently formed stable nanocrystals. If the surface energy however significantly differed from that of the drug, addition of a surfactant was beneficial [29]. This implies that also a combination of non-ionic polymers and non-ionic surfactants could increase nanosuspension stability. This is further supported by observations made using atomic force microscopy (AFM) [88]. While ibuprofen was successfully milled using P188, storage stability was poor due to fluctuations of P188 on the crystal surface. HPMC on the other hand remained attached to the ibuprofen surface also upon storage. Combining a surfactant and a polymer might hence facilitate excellent milling efficiency governed by the surfactant and increased storage stability by the polymer.
As proposed elsewhere, trapped surfactant micelles by the polymer chains in a stabilizer combination provided not only an increased steric barrier but also hindered the free micelles to fluctuate on and from the crystal surface [104]. This approach showed an interesting basis for non-ionic stabilizer combinations and could ultimately increase storage stability.

1.5 Characterization of nanosuspensions

Particle size and particle size distribution (PSD) are the key parameters for evaluating the physical stability of nanocrystals. Photon correlation spectroscopy (PCS) laser diffraction (LD) and Coulter counter are the most used instruments to determine the PSD.
PCS is widely used to for nanosuspensions. The mean particle size and the width of the PSD indicated as polydispersity index (PDI) are the typical measured parameters of this technique. A PDI value of 0.1 to 0.25 indicates a narrow size distribution while a PDI greater than 0.5 refers to a broad distribution [8]. The narrow measurement range of 1 nm to 1 µm however limits PCS. Due to the small sample size, large aggregates or dust particles can disturb the size determination if the main population is significantly smaller in size [105].
LD owns a much wider detection range from 10 nm to 2,000 µm. Typical LD characterization parameters are d10, d50 and d90, indicating the respective percentage of crystals below the given size. Due to its broad detection range, LD is especially suitable for tracking the physical stability of nanosuspensions over time. However, LD is limited to determine the relative PSD of a population. A Coulter counter on the other hand, measures the absolute number of crystals per volume unit for the different size classes.

Zeta potential is a predictor of the physical stability especially of electrostatically stabilized nanosuspensions [64]. It is a measure of the electric charge at the shear plane of the crystal and indicates the physical stability of a colloidal system [68]. When the absolute value of the zeta potential of a drug nanocrystal is small, attractive forces between the crystals exceed the electrostatic repulsion, resulting in crystal aggregation. A nanosuspension is expected physically stable when it has a zeta potential of above 30 mV or below – 30 mV [106]. Nanocrystals with adequate zeta potential were less likely to aggregate due to the high inter-particulate electrostatic repulsion [107].

Although all the above techniques provide indication on the physical stability, they do not have the capability in evaluating crystals morphology. This might be especially interesting to differentiate between the primary particle size and the apparent particle size of for example particle aggregates, which would not be detectable using PCS, LD or a Coulter counter.

As direct visualization techniques, scanning electron microscope, transmission electron microscope and atomic force microscope (AFM) are widely used for assessment of crystals morphology. It is however very challenging and time-consuming to measure a significant number of crystals to achieve a statistically relevant PSD using these techniques. In addition, they usually require additional sample preparation such as coating that could be invasive to the crystals, potentially causing changes in crystals properties.

Besides visualization, AFM introduced a new concept in stabilizer screening. The AFM measurements performed by Verma *et al.* showed that some polymeric stabilizers had extensive surface absorption on an ibuprofen surface, as opposed to the inadequate surface absorption with PVP and poloxamer [88]. These results correlated well with their stabilizing performances. This finding confirmed the significance of AFM in providing a scientific rationale for stabilizer selection. The measurable interfacial strength could serve as a perquisite for predicting wettability, aggregation and Ostwald ripening of prepared suspensions.

Recently, a size characterization tool called nanoparticle tracking analysis (NTA) was introduced to acquire the size of particles by determining their diffusion coefficient. It is therefore often found as supplementary method for PCS as they fraction the same size definitions [105]. NTA however calculates the diffusion coefficient based on the movements of individual particles in successive optical video images and therefore combines both, mathematical and optical methods.

The possibility to measure existing forces between crystals and surfaces is also relevant regarding for example stabilizer adhesion processes. Colloidal probe microscopy, derived from AFM to measure the force exhibited between drug crystals and stabilizer. It is commonly used to measure interaction forces acting between colloidal particles and planar surfaces in solution by force measurements [108].

Static contact angle measurements also give a good indication on surface energies between drug and stabilizer [48]. The smaller the contact angle, the higher the wettability. The contact angle of a stabilizer solution can be measured by compressing a small amount of powder to form a disk. Drugs with small contact angles and satisfactory wettability were more likely to

result in stable nanosuspensions [46]. Several studies determined contact angle as the standard for stabilizer selection [109], [110].

Another physical instability that needs observation is sedimentation. The traditional method to evaluate sedimentation is by visual observation over time. By measuring the volume of the settled crystal layer relative to the total suspension volume within a specific time, a dimensionless parameter known as sedimentation volume can be obtained. Other approaches to evaluate sedimentation include laser backscattering and near infrared transmission [111]. Sedimentation behaviour can also be determined by analytical centrifugation [112]. In this technique, a space and time related extinction profile is generated in relation to the sedimentation of dispersed crystals. Employing this technology, sedimentation velocity can be calculated under accelerated conditions.
The structure of sediment can be assessed by redispersion of the suspension, where easily re-dispersed suspensions indicate loose flocs while a dense cake is hard to be broken by manual shaking.

In most cases a combination of several methods was employed to generate a full picture of the physical stability of nanosuspensions.

1.6 Ophthalmic fixed dose combination nanosuspensions

Eye drops are the most frequently prescribed form of ocular treatment [113]. This is due to several advantages of topical instillation such as non-invasive and easy administration, high patient compliance and immediate action. Despite their convenience, it is generally accepted that due to complex ocular barriers, that only 5% of the administered drug reaches its target due to complex anatomy of the eye. Moreover, only a small volume of about 30 µL can be instilled in the eye. As a result, concentrated solutions must be administered, and frequent instillations become necessary to reach efficacy [114].

Monotherapy has dominated drug development for the past few decades but is no longer considered sufficient for a growing range of diseases. Pathogenesis of retinal diseases for example is characterized by a multifactorial etiology, involving complex interactions of metabolic, functional, genetic and environmental factors [115]. Combination therapy for such diseases, employing a treatment using more than one drug, is common in clinical practice. Fixed-dose combinations (FDC) provide a clinically tested and dose-adjusted safe format for combination therapy. FDC is a term introduced by the US Food and Drug Administration (FDA) and is defined as “a drug product in which two or more separate drug components (active pharmaceutical ingredients) are combined in a single dosage form” [116].

FDCs could improve efficacy, safety and adherence in the treatment regimen of retinal diseases. With the benefit of reduced dosing compared to individual eye drops, preservative and excipient concentrations are minimized and medical therapy may become more effective and tolerable over long-term [117].

Potential drawbacks of an FDC are the loss in flexibility of individualized patient care and timing of administration. Evidence for example suggested that evening administration of a prostaglandin analogue is preferably, so there is controversy as to which is the best dosing time point for these FDCs [118].

Following the market development for FDCs, the emerging trend becomes however visible, with 56 approvals of ophthalmic FDCs within the last 19 years over a broad field of ophthalmic indications (Table 1.3). Compared to 229 approval licences for single agent products, a fraction of 22% of the e.g. whole UK market for ophthalmic accounted for FDCs [119].

Table 1.3 Summary of approved licenses by indication for ophthalmic FDC products

Indication	Active Ingredients	Tradename (inter alia)	Dosage Form
Glaucoma	2% Dorzolamide 0.5% Timolol	Cosopt	Solution
	0.05% Latanoprost 0.5% Timolol	Xalacom	Solution
	0.03% Bimatoprost 0.5% Timolol	Ganfort	Solution
	0.0015% Tafluprost 0.2% Timolol	Taptiqon	Solution
	0.2% Brimonidine 0.5% Timolol	Combigan	Solution
	0.004% Travoprost 0.5% Timolol	DuoTrav	Solution
	0.005% Latanoprost 0.02% Netasurdil	Rocklatan	Solution
	1.0% Brinzolamide 0.2%Brimonidine tartrate	Simbrinza	Suspension
	1.0% Brinzolamide 0.5% Timolol maleate	Azarga	Suspension
Antibiotic and anti-inflammatory	10,000 units/mL Polymyxin B 0.1% Trimethoprim	Polytrim	Solution
	0.05% Dexamethasone 0.5% Framycetin 0.005% Gramidicin	Sofradex	Solution
	0.1% Dexamethasone 0.3% Tobramycin	Tobradex	Suspension

Table 1.3 Summary of approved licenses by indication for ophthalmic FDC products (cont.)

Indication	Active Ingredients	Tradename (inter alia)	Dosage Form
Pupillary	1% Lidocaine 0.31% Phenylephrine 0.02% Tropicamide	Mydrane	Injectable solution
Allergy	0.5% Antazoline 0.05% Xylometazoline	Otrivine-Antistin	Solution

In the last decades considerable effort has been made to develop especially new intraocular pressure (IOP) lowering FDCs as it often includes the use multiple drugs [120]. The 2% dorzolamide and 0.5% timolol FDC solution was the first successful glaucoma FDC released commercially in the US and in Europe in 1998 by Merck & Co Inc (Whitehouse Station, USA). FDC therapy in glaucoma management has gained immense popularity.

According to the superiority of FDCs in the treatment of glaucoma, a majority of FDC products contains the well-established beta-blocker timolol in combination with either carbonic anhydrase inhibitors or prostaglandin analogues, as those actives act trough different mechanisms. Timolol is most often present as timolol maleate, which forms a solution.

In general, the major share of FDC products are solutions. To this date only three FDC suspensions found approval from the FDA (Azarga, Simbrinza, Tobradex; Table 1.3). It is worth noting, that all three FDC suspensions contain the surfactant Tyloxapol as stabilizer. Simbrinza and Azarga further comprise Carbopol 974 as viscosity agent for kinetic stabilization.
What becomes however evident, is that FDC suspension so far referred to a combination of one ingredient being suspended and the other being in solution.
This has obvious advantages in formulation design. As stated before, stabilization of a compound is a multifactorial, time-consuming and mostly empirical process. If only one of the two compounds is suspended, focus can be set on physical stabilization of the suspended compound.

Based on the latest development for new drugs, approximately 70-90% are however classified as poorly soluble, arising the need of FDC suspensions with both drugs suspended.

1.6.1 Special considerations on the stability of fixed dose combination nanosuspensions

So far, no approach was presented to combined two insoluble drugs into FDC micro- or nanosuspensions. It was recognized that for drug crystals, particle size has a significant effect on dissolution rate and hence bioavailability [121]. Therefore, an FDC suspension might be most efficient in the form of an FDC nanosuspension.

Testing for the individual PSD within the FDC is inevitable to ensure the physical stability during formulation development. Despite its importance, only very little information is provided regarding FDC suspensions.
PSD determination using light scattering was developed for an FDC suspension containing three suspended drugs with different refractive indices. To ensure that data represent the unbiased PSD of the FDC, it was found that the results obtained using the average refractive index of the three components agreed with the results obtained from the simultaneous microscopic PSD determination [122]. The method was however restricted to the joint PSD and could not differentiate the individual PSD of the components. This limits the method for the use in FDC nanosuspension where the annotation to the single drugs would be needed to track individual physical stability within an FDC suspension.

An entity sensitive method was developed using NTA, where silica and polystyrene beads were distinguished in a mixture based on their different refractive indices [123]. This approach was however restricted to highly monodisperse and significantly different size populations between the two entities of more than 0.2 µm. Also it did not determine the PSD but was limited to sole differentiation. In an FDC nanosuspension, particle size target would be the same for both drugs. Also, physical instabilities usually end in multimodal PSD. Although powerful, this method would therefore be of limited use for FDC nanosuspensions due to the overlapping particle sizes of the two compounds and the potential polydispersity of the FDC nanosuspension in the case of physical instability.

Besides the lack of measurement techniques, formulation development also becomes evidently more complex in FDC nanosuspensions. As seen for individual drug formulations, the stabilization process is highly dependent on individual drug-stabilizer interactions. The degrees of freedom and interaction sites within an FDC suspension for stabilizer interaction are doubled. Also potential synergistic or antagonistic effects would need consideration.

The choice of stabilizer significantly impacts milling efficiency [124]. With the addition of a second drug in FDCs, the milling process steps as well as different stabilizers have to be assessed to give a physically stable system. With regard to processing, the two drugs can be either milled together or separately followed by combination of the individual drug nanosuspensions.

Milling two drugs together is the simpler approach requiring fewer steps, however there is less control regarding the particle size of the individual drugs. Combined milling of the two drugs must consider potential effects of higher drug volume fractions during milling. Also, different powder characteristics of the drugs, such as brittleness, must be considered which determined the needed milling time [33]. Therefore, an optimum between particle size reduction and the risk of agglomeration due to excessive milling times must be balanced for both drugs in one approach.

Separate milling on the other hand allows the preparation of nanosuspensions with the desired properties (e.g., particle size) and more freedom regarding milling conditions (e.g., time, speed and stabilizer choice and concentration). The challenge with this approach could be the maintenance of the individual particle properties after combining them into an FDC.

Drug solubility is affected by the stabilizer, which might become critical as solubility occurs under dynamic equilibrium, from simultaneous dissolution and precipitation of crystals. In the case of wet bead milling, higher volume fractions of up to 30% solid fraction might be considered to alter milling efficiency [125]. This might need higher surfactant concentrations, which in turn causes a higher solubilization of the drug. Drug solubility was reported to potentially increase exponentially with surfactant concentration [126]. Upon dilution and combination of the individual milling slurries, drugs might thus recrystallize caused by the abrupt changes in stabilizer concentration, giving the risk to lose the nanosuspension's integrity.

To summarize on the preparation and physical stabilisation of FDC nanosuspensions, different parameters must be considered. Aside the preparation approach, also stabilizer type and concentration must be optimized. To further assure long-term stability, also dispersion medium related factors might need to be considered.

1.7 Objectives

The purpose of this work was to characterize ophthalmic fixed dose combination nanosupensions produced by wet bead milling. The specific goals were the

1. Investigation of critical stabilizer properties for wet bead milling and storage stabilization of loteprednol and nepafenac individual nanosuspensions.
2. Analytical method development for the particle size determination of and dissolution from fixed dose combination nanosuspensions.
3. Identification of critical process and formulation parameters in the production of fixed dose combination nanosuspensions.
4. Optimization of the long-term physical stability of fixed dose combination nanosuspensions.

2 Materials and Methods

2.1 Materials

2.1.1 Active pharmaceutical ingredients

Dexamethasone, micronized (Caesar & Loretz GmbH, Linden, Germany), nepafenac, micronized (Fox Chemicals GmbH, Pfinztal, Germany), loteprednol, micronized (Crystal Pharma S.A.U., Valladolid, Spain)

2.1.2 Excipients

Benzalkonium chloride (Merck KGaA, Darmstadt, Germany), Chitosan 100,000–300,000 g/mol (Themo Fisher Scientific PLC, Waltham, USA), Hydroxyethylcellulose (HEC) Natrosol HX and M, Hydroxypropylcellulose (HPC) Klucel EF and HF (Ashland Global Holdings Inc., Wilmington, USA), Hydroxypropylmethylcellulose (HPMC) Methocel E50, E4M, F4M, K4M (Colorcon Inc., Harleysville, USA), Poloxamer Pluronic 188 (P188), 338 (P338), 407 (P407) (BASF SE, Ludwigshafen, Germany), Poly(acryl acid) Carbopol® 974P NF (Lubrizol Corporation, Wickliffe, USA), Poly(vinyl)alcohol (PVA) Mowiol 4-88 (Carl Roth GmbH + Co. KG, Karlsruhe, Germany), Poly(vinyl)pyrrolidone (PVP) Kolliphor K30 (BASF, Ludwigshafen, Germany), Sodium carboxymethylcellulose (NaCMC) Blanose CMC 7LP (Ashland, Global Holdings Inc., Wilmington, USA), Sodium dodecyl sulfate (Merck KGaA, Darmstadt, Germany), Tween 80 (Croda International PlC, Snaith, United Kingdom), Tyloxapol (Labochem S.A., Athens, Greece), glycerol (Merck KGaA, Darmstadt, Germany), sodium chloride (NaCl) (Carl Roth GmbH + Co. KG, Karlsruhe, Germany)

2.2 Methods

2.2.1 Milling of single drug nanosuspension

5.0% (w/v) drug was suspended in 1.25% or 5% (w/v) stabilizer solution and homogenized using a Vortexer (1min, 2,000 min^{-1}). Dual centrifugation was performed using a ZentriMix 380 R (Andreas Hettich GmbH und Co KG, Tuttlingen, Germany). 0.5 mL of the pre-treated drug suspension and milling beads (1:2% (v/v), 0.1 or 0.3 mm, Yttrium-stabilized) (Sigmund Lindner GmbH, Warmensteinach, Germany) were added into 2 mL disposable vials and milled (1,500 rpm, cooling 0 °C, up to 3 h). The slurry was separated from beads by vacuum filtration through a 0.05 mm sieve and simultaneously rinsed with 1:10 medium. Nanosuspension final concentration was 0.5% drug and 0.5% or 0.125% stabilizer. Nanosuspensions were stirred for 15 min prior to initial measurement.

2.2.2 Milling of fixed dose combination nanosuspension

FDC nanosuspensions were either prepared by separate or combined milling. Separate milling was performed as described for single drug nanosuspensions. 5% slurries of the single drugs were combined after milling and upon dilution, resulting in nanosuspensions containing 0.5% nepafenac, 0.5% loteprednol and 0.1% stabilizer. Combined milling was performed with both drugs present during milling at 5% each. Dilution was as described for single drug nanosuspensions, resulting in a final concentration of 0.5% nepafenac, 0.5% loteprednol and 0.5% stabilizer.

2.2.3 Particle size determination

Volumetric particle size distribution was determined using the laser diffractometer Mastersizer 2000 (Malvern Panalytical Ltd, Malvern, United Kingdom) equipped with a Hydro 200 µP (20 mL) or Hydro 2000 S (120 mL) measurement chamber. Sample was thoroughly shaken and added dropwise to the measurement chamber and assessed for particle size (Table 2.1). For selective dissolution, a predefined sample volume was added to the measurement chamber to allow for complete nepafenac dissolution (Table 2.1). Dispersant and drug refractive index were selected on best residual fit. Data was reported as volumetric distribution or as distribution percentiles d10, d50 and d90 (n=3, mean ± SD).

Table 2.1 Laser diffraction parameters for individual drugs and joint and selective PSD of the FDC nanosuspension

Parameter	Nepafenac	Loteprednol	Joint PSD FDC	Selective PSD Loteprednol in FDC	
				HydroS	Hydro µS
Sample RI	1.640	1.570	1.606	1.640	
Sample iRI	0.001				
Dispersant	Saturated solution nepafenac	MilliQ water	Saturated solution nepafenac	MilliQ water	
Obscuration, %	2 - 6			1.5	
Sample volume, mL	n.a.			0.2	0.06
Stirring speed, rpm	3,5000 / 1,000			3,500	1,000
Sonication time, min	0.5			10	
Sonication intensity, %	25			100	25
Dispersant RI	1.330				
Dispersant iRI	0.001				
Background correction, s	6				
Measurement time, s	6				

2.2.4 Zeta Potential

Nanosuspension Zeta potential was measured using a Zetasizer Nano ZS-90 instrument (Malvern Panalytical Ltd, Malvern, United Kingdom). Samples were diluted 1:10 in tonicity adjusted MilliQ-water (50 µS/cm) prior to measurement (1 mL, folded capillary cell, 25 °C, 1.002 mPas, Smoluchowski equation, n=3, mean ± SD).

2.2.5 Light microscopy

Samples were examined on an objective slide under polarized light microscope (Axioscope, Carl Zeiss Microscopy GmbH, Jena, Germany) and images were processed using the software Zen (Carl Zeiss Microscopy GmbH, Jena, Germany).

2.2.6 Surface tension

Surface tension was resolved using DataPhysics dynamic contact angle meter and tensiometer DCAT 21 (DataPhysics Instruments GmbH, Filderstadt, Germany). 1.5 mL of surfactant solution was transferred to a measuring plate. The probe (cylindrical plate, platinum-iridium, outer diameter 6.6 mm) was automatically immersed in the sample where exerted tensile force that was captured by the integrated weighing system. Measurement was completed when standard deviation was below 0.03 mN/m for at least 5 consecutive data points.

2.2.7 Viscosity

Viscosity was determined using a Brookfield Ametek DV3T extra rheometer (Spindle SC4-18, 150 rpm, 20 °C) (Ametek Brookfield Inc., Berwyn, United States).

2.2.8 Static contact angle measurement

Contact angle was determined with sessile drop analysis. Compacts of 13 mm diameter were manually prepared by direct compression using a hydraulic press (4,000 kg, 5min) (Specac Ltd., Orpington, UK). A stabilizer-solution droplet was deposited by a syringe above the sample surface and an image was taken from the profile using a high resolution macroscope (Inteq Informationstechnik GmbH, Berlin, Germany). Images were processed by the software EasyMeasure (Inteq Informationstechnik GmbH, Berlin, Germany). Contact angle was determined using a protractor analysis software (Sketch up, Trimble Inc., Sunnyvale, USA).

2.2.9 Hydrodynamic radius

Hydrodynamic radius was determined using dynamic light scattering (DLS) (Zetasizer Nano ZS-90, Malvern Panalytical Ltd., Malvern, United Kingdom). Samples were diluted 1:20 in MilliQ water and 1 ml was transferred to a square cuvette for measurement (25 °C, n=3, 1.002 mPas).

2.2.10Adsorbed layer thickness

Adsorbed layer thickness was derived from the approximation of the Gouy-Chapman theory as described elsewhere [127]. Samples were diluted 1:10 in 0, 2, 4, 6, 8 and 10 mM NaCl solutions using the same equipment as for the determination of zeta potential (1 mL, folded capillary cell, 25 °C, 1.002 mPas, Smoluchowski equation, n=3, mean ± SD).

2.2.11 Solubility

An excess amount of loteprednol was added to the medium and thoroughly shaken. The samples were incubated at room temperature for at least 48 h on a horizontal shaker (HS501 IKA Rührerwerke, Germany). Saturated solutions were filtered (0.22 µm membrane filter) and diluted 1:1 in mobile phase. Drug concentrations were determined by HPLC at 254 nm using an in-house analytical method.

2.2.12 Drug brittleness

The evaluation of the apparent elastic moduli of micronized drug powders and of the reference materials were performed using direct compression (Korsch EK0, Korsch AG, Berlin, Germany) equipped with an 11 mm flat tooling and as described elsewhere [33].

2.2.13 Dissolution

Nanosuspension dissolution was performed under non sink conditions by *in situ* UV measurements with Sirius inForm (50 mL, PBS pH 7.4, 37 °C, 200 – 700 nm) (Sirius Analytical Instruments Ltd., Forest Row, UK). A 1 mm of cell path length was selected to compensate for any particle scattering effect and to achieve suitable absorbance ranges. Amount of drug dissolved in the medium was calculated using the equipment's software (Sirius® inForm Refine), considering the molar extinction coefficient, which was in advance determined through the UV-metric MEC/pKa-assay of Sirius® inForm. A Tyndall-Rayleigh scattering

correction was applied to the recorded spectra to exclude scattering of undissolved particles [128].

2.2.14 Accelerated stability study

5 mL of nanosuspension was stored in 5 mL crimped glass vials at 40 °C and 75%rH for up to two months. Stability was assessed at predetermined time intervals in terms of particle size distribution.

2.2.15 Sedimentation behaviour via Space and Time related Extinction Profiling

Sedimentation analysis was performed via analytical centrifugation (LUMiSizer®, LUM GmbH, Berlin, Germany). The so-called STEP-technology captured space and time related extinction profiles, in relation to the sedimentation of dispersed crystals. Analytical centrifugation was performed on undiluted aqueous nanosuspension (4,000 rpm, 865 nm, 120 profiles every 5s). For evaluation, all profiles were plotted in one overview graph with changes in transmission indicated by a change of the colour from red to green over measurement time. Sedimentation velocity was calculated based on sedimentation profiles using front tracking analysis.

2.2.16 Physical appearance and resuspendability

Physical appearance was documented prior shaking upright and headlong and after redispersion. Samples tending to sediment were undertaken a redispersibility test. Redispersibility was measured as number of inversions needed to obtain a homogeneous suspension. Inversions were completed manually and every five inversions sample homogeneity was assessed. Homogeneity was defined as the absence of sediment and particle streaks when turned upside down.

3 Results and Discussion

3.1 Critical stabilizer properties for wet milling and storage stabilization of loteprednol nanosuspensions

In this chapter, physical stability of the nanosuspensions was defined in terms of milling efficiency and prevention of crystal growth and agglomeration during short-term storage following wet bead milling for the individual drugs.

Several factors are described as critical for dispersion- and milling efficiency, especially stabilizer type, concentration and molecular weight. Loteprednol was milled in the presence of either 1.25% or 5% non-ionic surfactant. Milling at higher surfactant concentration generally resulted in smaller loteprednol particle sizes (Figure 3.1). 5% surfactant allowed complete coverage of the freshly formed drug crystal surfaces during milling, thus preventing reaggregation and crystal growth which counteract milling efficiency.

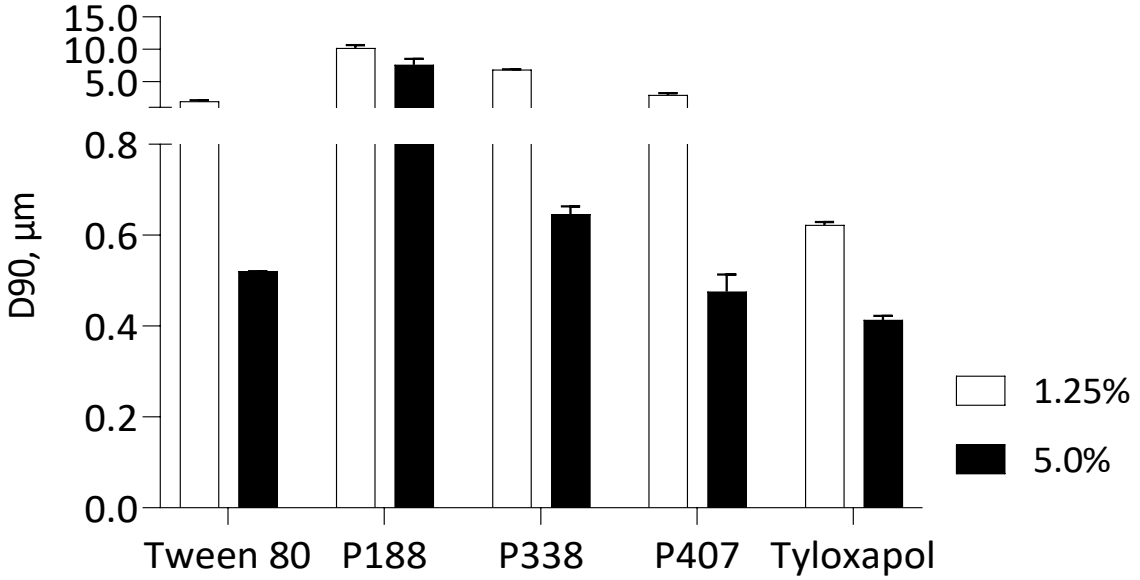

Figure 3.1 Stabilizer type and concentration-dependent (1.25% vs. 5%) loteprednol milling efficiency expressed as particle size d90

Another key factor was the amphiphilicity of the stabilizer and whether it had sufficiently long hydrocarbon chains. While 5% Poloxamer 188 (P188) resulted in a multimodal distribution with a µm-fraction, Poloxamer 338 (P338) and Poloxamer 407 (P407) resulted in nearly monomodal loteprednol nanosuspensions with P338 leaving a minor µm-fraction and broader distribution compared to P407 (Figure 3.2).

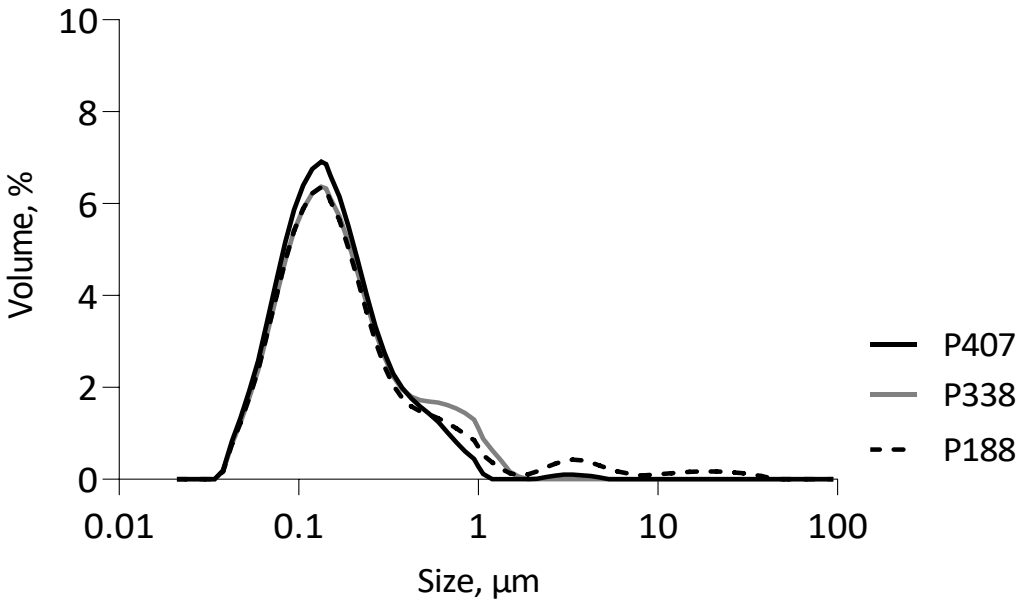

Figure 3.2 Volumetric PSD of loteprednol milled with different Poloxamer grades at 5%

The three poloxamer grades differ in molecular weight, increasing from P188 to P338 and P407. Secondly, the poly(ethylene oxide) (PEO) content is higher in P188 and P338 compared to P407, leaving P407 as most hydrophobic (Table 3.1). This reflected in milling efficiency, which was improved with larger molecular weight and higher poly(propylene oxide) (PPO) fraction and thus hydrophobicity. Including milling results and HLB values of Tween 80 and Tyloxapol of 15 and 13 [129], a general relationship between hydrophobicity and loteprednol milling efficiency was attributed with a correlation factor of 0.68. Stabilizer hydrophobicity was previously reported to increase adsorption affinity toward the hydrophobic crystal surface [78].

Table 3.1 Poloxamer grade characteristics and effect on loteprednol contact angle

Poloxamer grade	Molecular weight, g/mol	PEO,%	HLB [129]	Surface tension, mN/m^2	Loteprednol contact angle, °	Loteprednol d90, µm
188	8,000	80	29	46.4	41	7.25
338	12,700	83	27	41.2	38	0.65
407	12,700	73	22	38.5	28	0.55

A decrease in the cohesive force within the surfactant or an increase in adhesive force between the surfactant and loteprednol resulted in increased milling efficiency in Poloxamer systems [130]. This observation could also be expanded to systems containing Tween 80 and Tyloxapol. With surface tensions of 38.8 and 37.5 mN/m^2, respectively, overall correlation between surface tension and milling efficiency was 0.87. This led to the general conclusion that surfactant hydrophobicity and surface tension were critical in terms of milling efficiency.

PPO chains of poloxamer orient toward the crystal surface, a sufficient PPO fraction was crucial for interaction. This was also reflected in an indirect relationship between PPO fraction and contact angle of the different Poloxamers with loteprednol of 28° vs. 38° and 41°, as determined using static contact angle measurements. The lower the contact angle, the higher the wetting, which in turn indicated proper affinity between surfactant and crystal surface. Interestingly, loteprednol and Tyloxapol contact angle of 40° was higher compared to other systems with inferior milling efficiencies. Drug-stabilizer contact angle could hence not solely explain the steady state values of particle sizes resulting from different stabilizers. This emphasized, that efficiency of a particular stabilizer depended not only on general characteristics but also on its potential for interaction with the drug. Same conclusions were also reported after milling seven drugs with two different stabilizers [48].

Due to the relatively high viscosity of the solutions of non-ionic and high molecular weight polymers, 4-fold lower stabilizer concentrations were applied compared to the surfactants. In contrast to the surfactants under investigation, different high molecular weight polymers used as stabilizer left a significant µm-fraction of about 50% of total volume and ending far below the milling efficiency of e.g., the surfactant P407 (Figure 3.3).

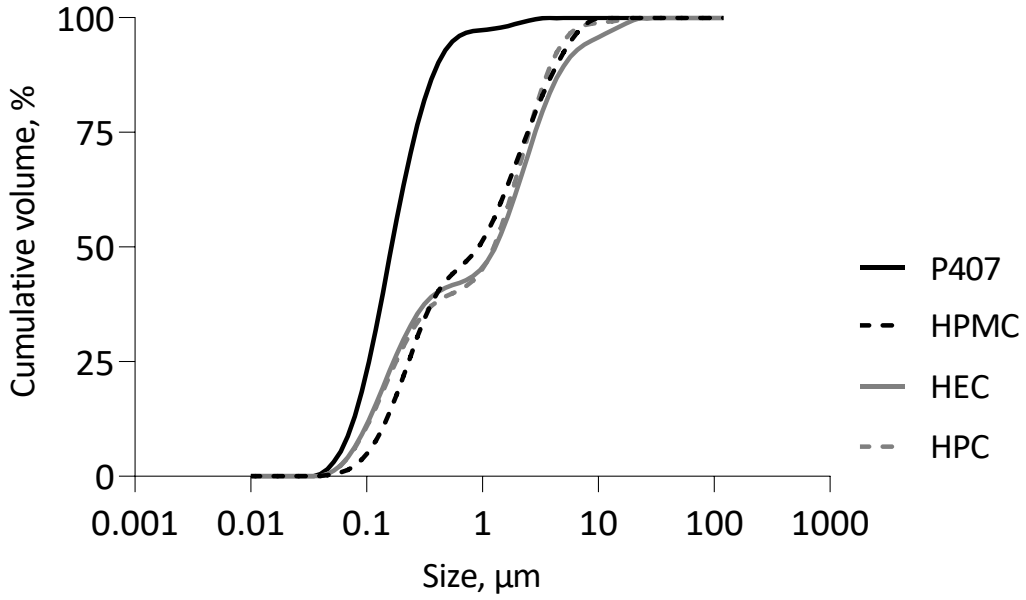

Figure 3.3 Cumulative volumetric PSD of loteprednol milled with 1.25% of different cellulose ethers or 5% P407

At constant process conditions, loteprednol milling efficiency was determined solely by the stabilizer characteristics. As described before, the lower the surface tension, the better the crystal wetting and stabilizer adsorption upon milling as immediate adsorption hinders the freshly developing crystal surfaces to reaggregate. This explained the general superiority of surfactants over high molecular weight polymers which reduced the systems surface tension only insufficiently (Figure 3.4). While the most successful surfactants in terms of loteprednol milling efficiency, Tyloxapol and P407, yielded surface tensions of below 30 mN/m^2, polymers could only decrease surface tension from 72 mN/m^2 (water) to about 50 mN/m^2.

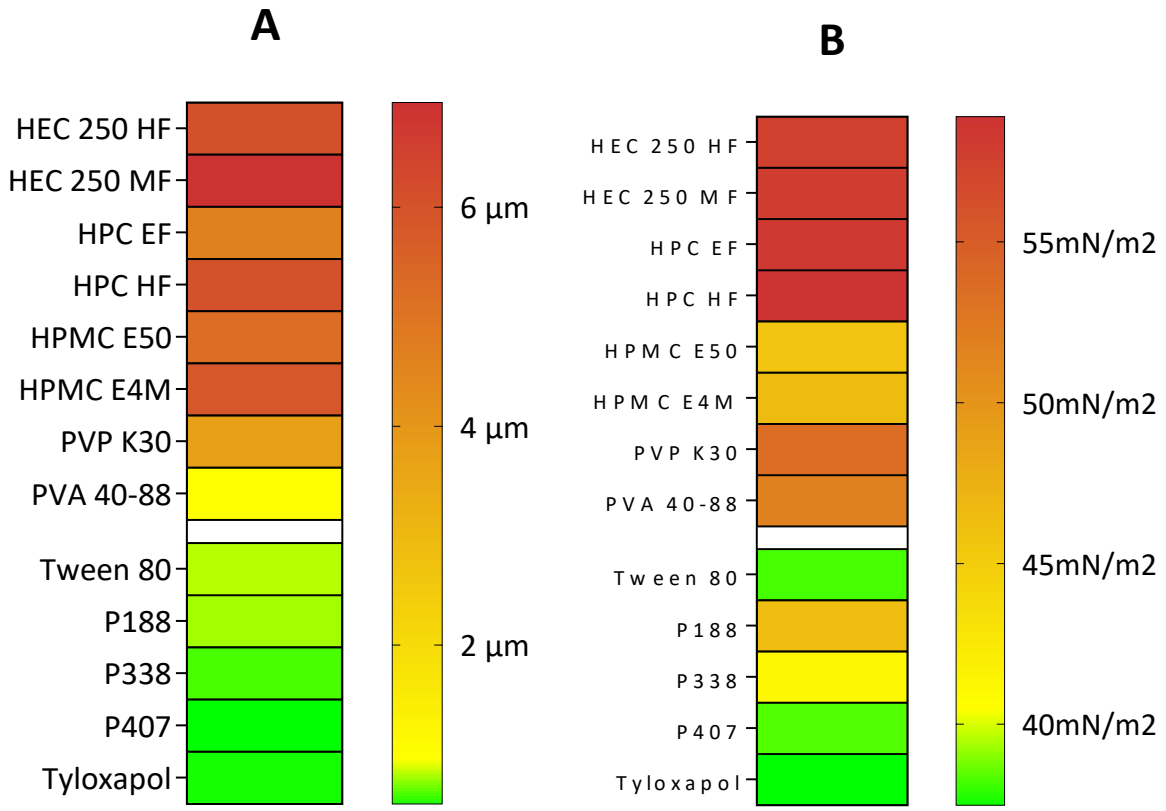

Figure 3.4 Heat map of stabilizer-dependent loteprednol milling efficiency expressed as particle size d90 milled with (A) 1.25% polymer or 5% surfactant and (B) corresponding stabilizer solution surface tension

The behaviour of surfactants differed from that of non-ionic polymers also in their effect on milling media viscosity. Lower viscosity increases transferred energies during milling and might also explain superior milling efficiency of surfactants over polymers [78], [131]. While 5% P407 resulted in a viscosity of around 4 mPas, 1.25% HPMC E4M had a significantly increased viscosity of 400 mPas at low shear. Even lower viscosity grades such as HPMC E50 still had a distinct effect on medium viscosity. Considering the shear thinning behaviour of the polymers in use, milling was still feasible but crucial initial wetting and transfer of stressing energy might be reduced at high viscosities.

Non-ionic, high molecular weight polymers still offer a broad range of various characteristics, leaving them as interesting stabilizers.

Comparing different HPMC types, no significant effect was however observed in terms of loteprednol milling efficiency. The three polymers differ in the degree of substitution, with

F4M having the lowest degree of substitution and in terms of the hydroxymethyl and hydroxypropyl content. The latter determines the hydrophobicity of the polymer. It seems, that an increase of methoxy content by 5% as for K4M and E4M did not improve HPMC adsorption on the crystal surface. In contrast to HPMC, HPC is even more hydrophobic expressed in a total solubility parameter of 21.3 $MPa^{1/2}$ compared to 22.7 $MPA^{1/2}$ [132]. Other than expected, there was no effect on milling efficiency. This emphasized, that slight changes in hydrophobicity were not sufficient to change the adsorption pattern of high molecular weight polymers.

Different molecular weights may also influence polymer adsorption by changing the stretching behaviour of the polymer in solution, resulting in different steric protection effects [133]. Polymers of higher molecular weight were therefore expected to provide better stabilization. This was contrary to what was seen for HPMC and HPC systems, where lower molecular weight polymers showed higher loteprednol milling efficiency (Figure 3.5).
Adsorption occurs over the complete milling time and hence also kinetic aspects of adsorption need to be considered [134]. The higher the molecular weight, the slower the diffusion rate and the lower the rate of adsorption. This would mean, that longer processing times would be required for higher molecular weight polymers to achieve smaller particle sizes. Longer milling times however often induce reaggregation due to extended energy input [135]. Also, for loteprednol, the milling time of 3 h was the maximum before an increase in particle size was observed again.

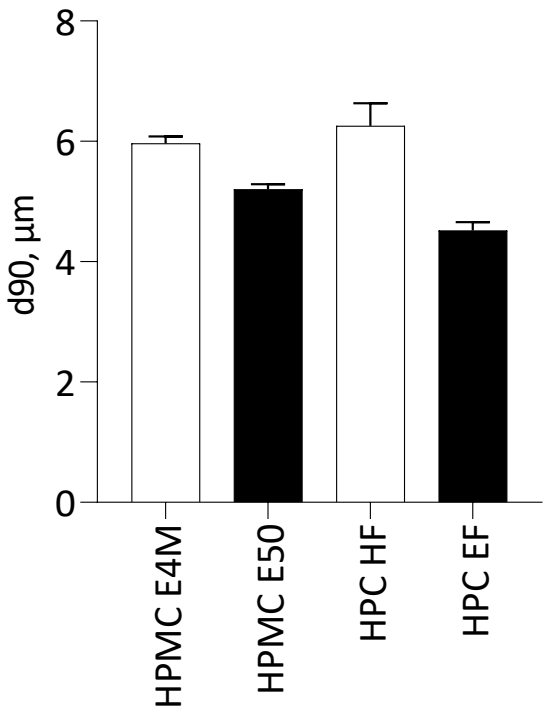

Figure 3.5 Loteprednol milling efficiency expressed as particle size d90 after milling with 1.25% of high (white) and low (black) molecular weight HPMC or HPC

Of all systems investigated, particle size of loteprednol was the smallest for P407 systems with a d90 of about 550 nm. Smaller particle sizes may however be favourable in terms of dissolution rate. Particle sizes down to below or around 200 nm were successfully reached for other drug nanosuspensions [28], [77], [136], [137]. Aside small particle sizes, also the width of the particle size distribution (PSD), expressed as span, could be improved. The closer the sizes of small crystals are to the largest, the lower the concentration gradient, a driving factor for crystal growth. It was therefore considered, to further tune loteprednol particle size by altering P407 conformation. Additives suitable for ophthalmic use are limited, but tonicity agents are frequently used to assure isotonicity. Loteprednol was therefore milled with P407 and the frequently used tonicity agents glycerol and NaCl.

A direct correlation between loteprednol milling efficiency and glycerol concentration was found for P407 systems (Figure 3.6). P407 resulted in a broad loteprednol PSD with a span of 3.7. Introduction of glycerol to the milling medium up to 10% increased milling efficiency constantly, resulting in a monomodal particle size distribution with a d90 of about 200 nm and

a span of 1.6. Above 10% glycerol, milling efficiency was again lost, leaving a d90 of 570 nm at 20% glycerol which was very close to the untuned system.

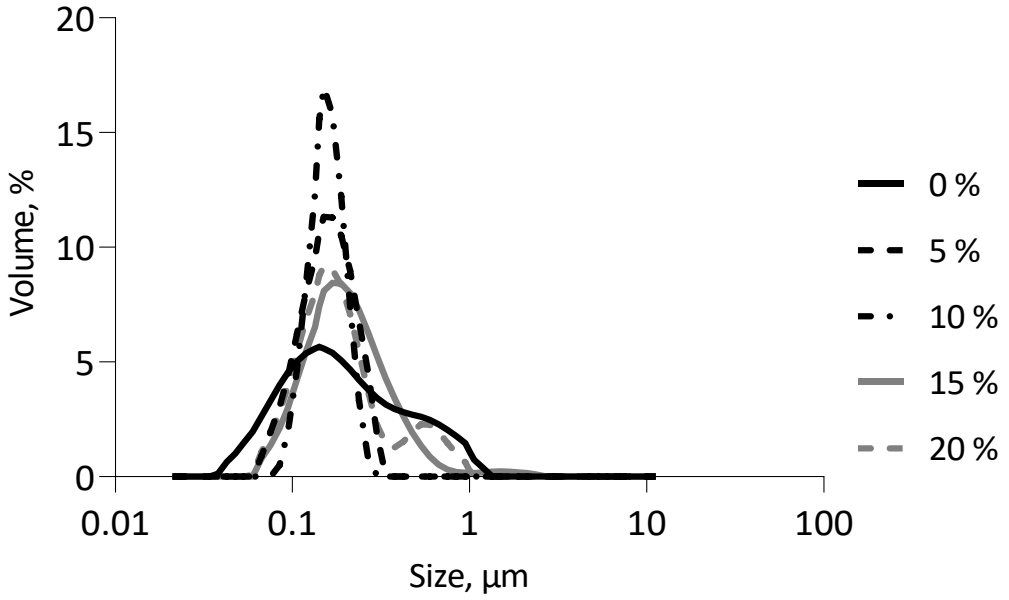

Figure 3.6 Volumetric PSD of loteprednol milled in the presence of 5% P407 and 0-20% glycerol

Washington *et al.* stated that adsorption of Poloxamer always included PEO chains co-adsorbing on the drug surface resulting in a significant population of polymer loops [138]. It was hence proposed that low concentrations of glycerol caused the detachment of PEO from the crystal surface and a denser loteprednol crystal coverage by PPO chains. This would increase the surface coverage and thus stabilization.

Electrolyte concentrations above a critical threshold however destabilize colloidal systems containing Poloxamer due to chain collapse under the osmotic stress of electrolytes. As less PPO chains are available after chain collapse, also milling efficiency at high glycerol concentrations was lost [139].

This indicated that a change in polymer conformation caused the increased milling efficiency. The measured radius of gyration of P407 in water of 5.6 nm was in very good agreement with experimental data for a PEO homopolymer of comparable molecular weight [140]. Looking at the P407 conformation as a function of glycerol concentration, a structural change of P407 became apparent (Figure 3.7). Radius increased up to 9 nm with increasing glycerol concertation, suggesting a stretching of the polymer chains. A significant increase of P407 size

was then observed at 20% glycerol, indicating the full collapse and aggregation of the P407 chains due to osmotic stress. The onset of collapse was already observed at lower concentrations of 10 and 15%, however, most of the Poloxamer was still in the stretched state.

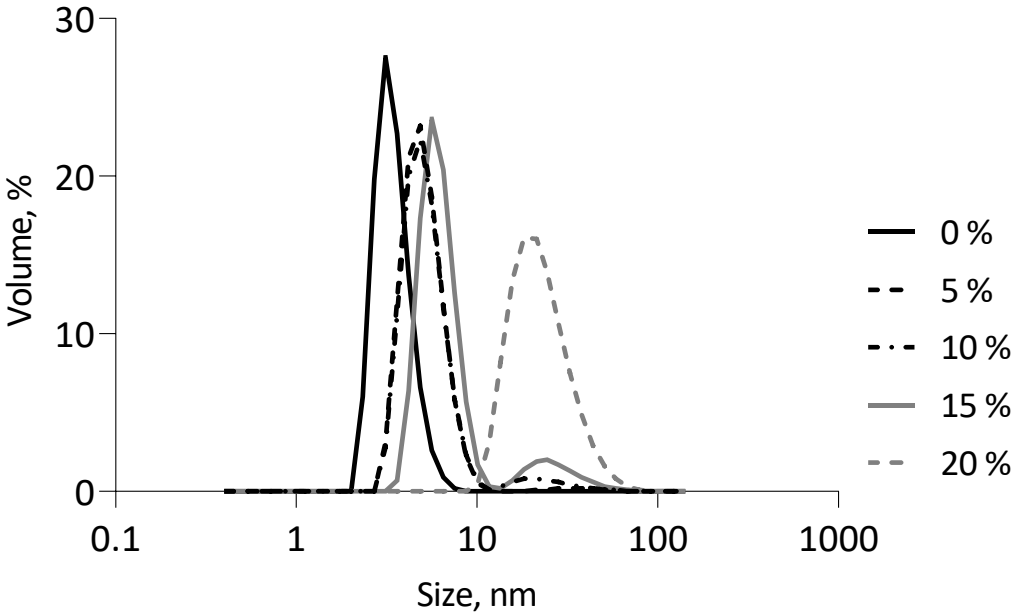

Figure 3.7 Volumetric PSD of 5% P407 as a function of 0 – 20% glycerol in water

NaCl was additionally tested as it is also frequently found as electrolyte in ophthalmic formulations. Compared to glycerol, NaCl required reduced concentrations to induce the same effect in P407 conformation (Figure 3.8). P407 size gradually increased suggesting structural changes with a maximum extension of 9 nm which was in good accordance with findings from glycerol. Chain collapse was also observed in NaCl systems, with a gradual shift of P407 size toward 20 nm at 12% NaCl.

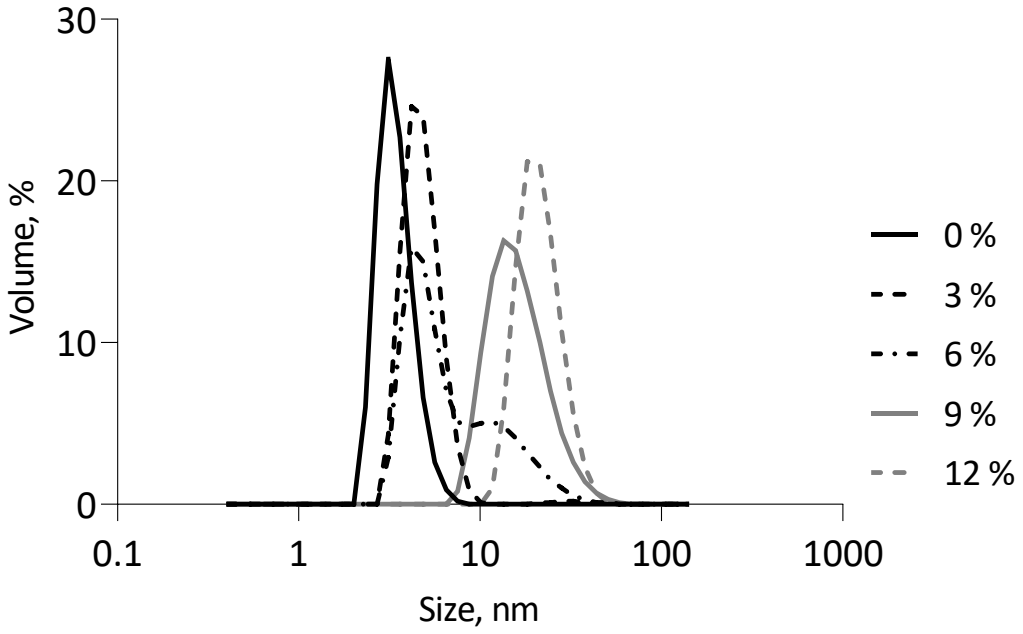

Figure 3.8 Volumetric PSD of 5% P407 as a function of 0 – 12 % NaCl in water

As shown for glycerol and NaCl systems, loteprednol milling efficiency could be tuned via environmental effects and P407 conformational changes (Figure 3.9).

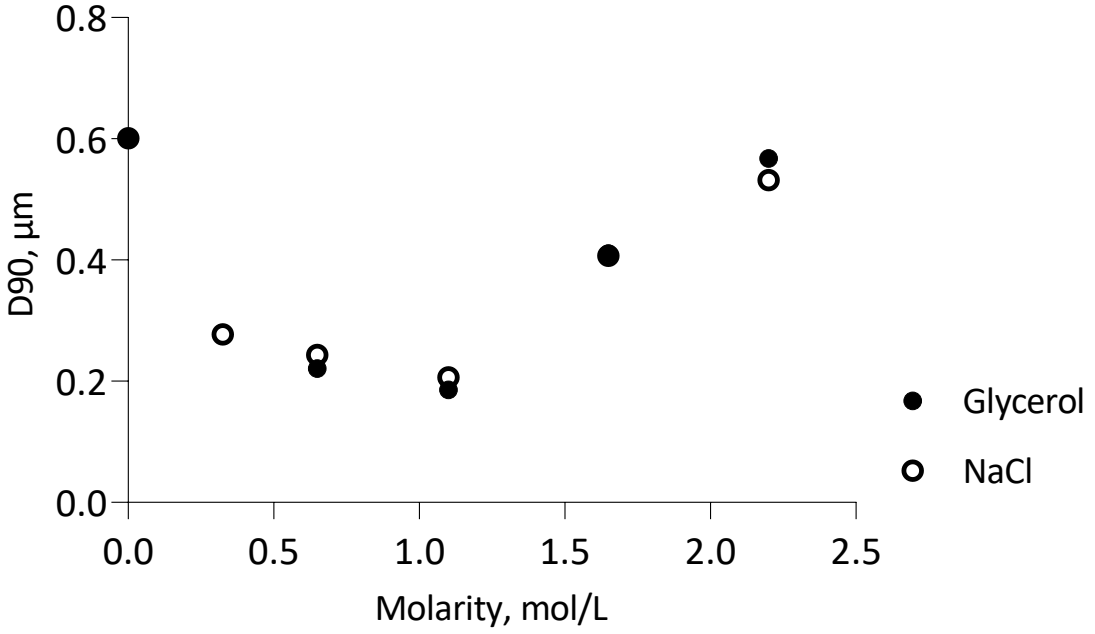

Figure 3.9 Influence of glycerol and NaCl concentration on loteprednol milling efficiency expressed as particle size d90 milled with 5% P407

As proven for P188-stabilized emulsions, increasing the temperature or electrolyte concentration had the effect of PEO detachment from the interface and elongation toward the aqueous medium. Ultimately it reduced solvent quality for the PEO blocks, resulting in a shift of segment density toward the surface of emulsion droplets [59]. This implied an environmentally induced structural rearrangement for this copolymer and is in very good agreement with the observations during loteprednol milling.
As electrolytes led to detachment of PEO adsorption contacts, loteprednol was covered by PPO blocks alone. This implied, that a denser adsorption pattern was generated during milling in the presence of electrolytes. The other parameter determining steric stabilization is the well-solvated tail segment which extends into the bulk medium. At optimum osmotic agent concentrations, crystal coverage by PPO segments and steric stabilization increased by maximally elongated PEO chains, thus improving milling efficiency.

To further support the findings for P407, the small angle neutron scattering spectrum of the loteprednol nanosuspensions could be measured at increased glycerol or NaCl concentrations. Using the volume fraction profile from the scattering data Washington *et al.* were able to determine physical changes of P188-stabilized oil droplets in the presence of NaCl [59].

Surface activity was described as crucial factor in terms of milling efficiency for all stabilizers and correlated well with surface tension. Surface tension and contact angle were determined for 10% glycerol and 3% NaCl, which were the systems with the highest milling efficiency. However, surface tension was not changed in the presence of glycerol or NaCl, emphasizing the effect of glycerol and NaCl on the specific loteprednol-P407 affinity (Table 3.2). Also loteprednol contact angle was significantly lowered to complete wetting in the presence of 3% NaCl again indicating an increase in loteprednol-P407 affinity. This was not observed for the glycerol-containing system.

Table 3.2 Influence of milling medium composition on loteprednol contact angle and corresponding milling medium surface tension

	Surface tension, mN/m^2	Loteprednol contact angle, °
Water	72.2	64
P407	38.5	28
P407 + 10% glycerol	38.7	41
P407 + 3% NaCl	39.8	0
Water + 10% glycerol	Not determined	76
Water + 3% NaCl	Not determined	59

Steric stabilization arises because of polymer adsorption onto the surface of particles and is highly dependent on the affinity of the stabilizer for the crystal surface.

Electrostatic stabilization is a different approach, which is based on formation of repulsive Coulomb forces between the charged colloidal crystals. With charged surfactants, the stabilization is based on electrostatic effect and differs from that of non-ionic stabilizer systems. Loteprednol was milled with an anionic surfactant sodium dodecyl sulfate (SDS) and polymer sodium carboxymethylcellulose (NaCMC) as well as with a cationic surfactant benzalkonium chloride (BAK) and the polymer chitosan (Figure 3.10).

Comparing the anionic systems, SDS showed improved milling efficiency compared to Na CMC, due to above mentioned superiority of surfactants in terms of wetting and the negative effect of polymers on milling medium viscosity.

Also the cationic surfactant BAK resulted in a loteprednol nanosuspension. In contrast, to what was generally observed for polymers, the cationic polymer chitosan was highly efficient in terms of loteprednol milling efficiency. This was explained by the benefit of chitosan acting as both, an electrostatic and steric stabilizer to facilitate particle size reduction.

In contrast to previous results, increasing the surfactant concentration led to a loss of loteprednol milling efficiency of up to 40% for both ionic surfactants. Optimized concentrations should be used for each surfactant, as excess surfactant is associated with increased solubility . This increase again induced ripening process and explained the loss of milling efficiency [72].

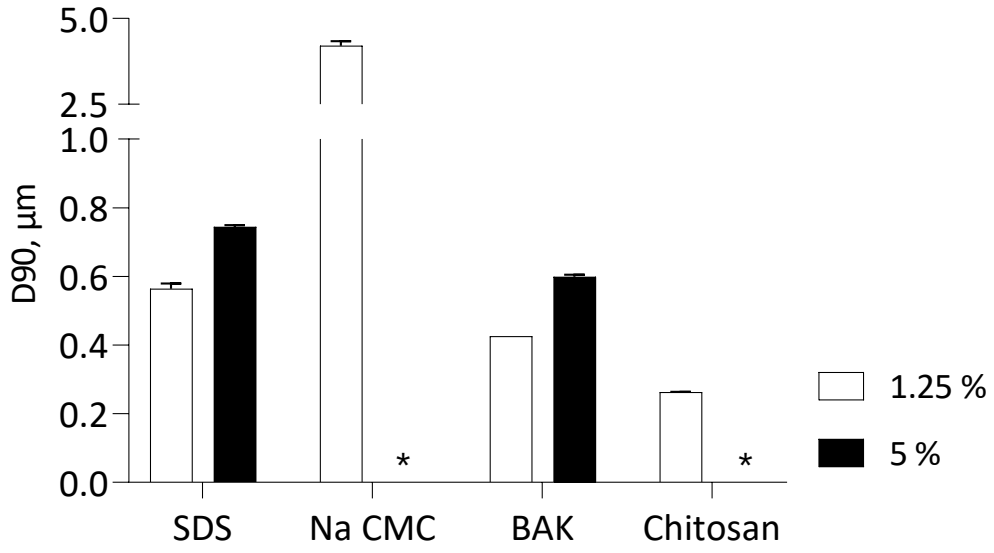

Figure 3.10 Ionic stabilizer type and concentration-dependent (1.25% vs. 5%) loteprednol milling efficiency expressed as particle size d90, *not determined due to high viscosity

Nanosuspensions prepared by wet bead milling must be physically stable during milling and during storage to ensure adequate shelf-life, depending on the intended final dosage form. Investigations were therefore expanded to storage stability. Loteprednol nanosuspensions were stored at 40 °C and 75%RH over a two-month period. As described before, surfactants were the best candidates to obtain loteprednol nanosuspensions. Storage stability of those suspensions revealed controversial effects.

Poloxamer insufficiently stabilized loteprednol, especially pronounced at low concentrations of 0.125%, where nanosuspension integrity was lost within the first weeks (Figure 3.11). Crystal growth was observed irrespective of initial loteprednol size and poloxamer grade. This was attributed to insufficient coverage of the loteprednol surface and hence poor stabilization. As expected, crystal size ran into a plateau at around 10 µm which complied with the original drug crystal size and hence the thermodynamically stable state. Subsequent agglomeration of the loteprednol microcrystals was observed upon storage (Figure 3.12).

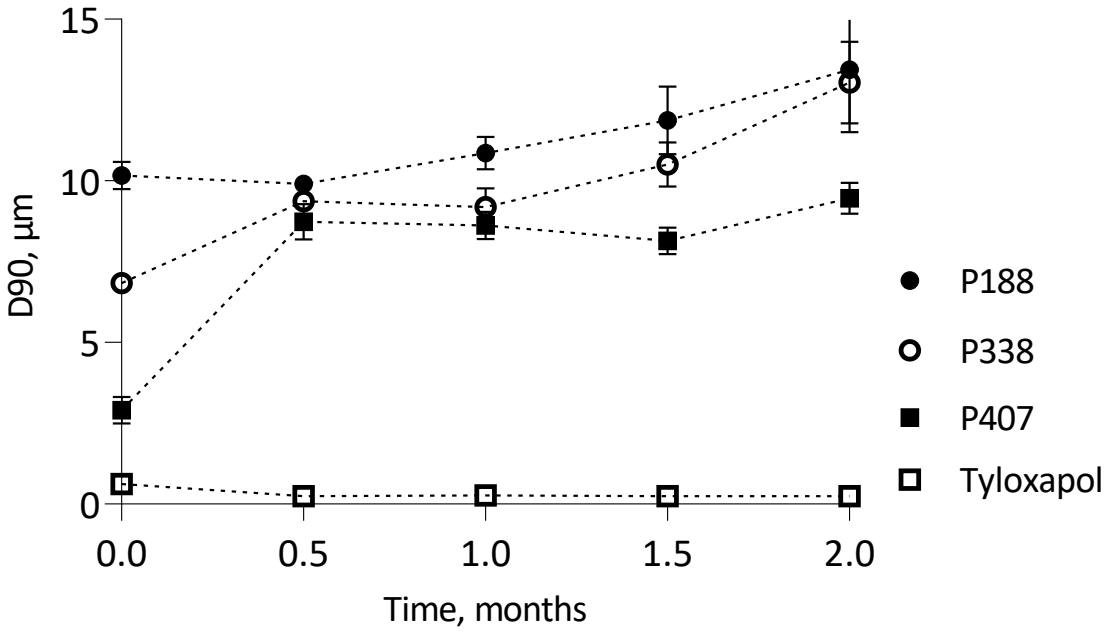

Figure 3.11 Evolution of loteprednol particle size d90 in dependence of surfactant type at 0.125% stored at 40 °C/75%RH

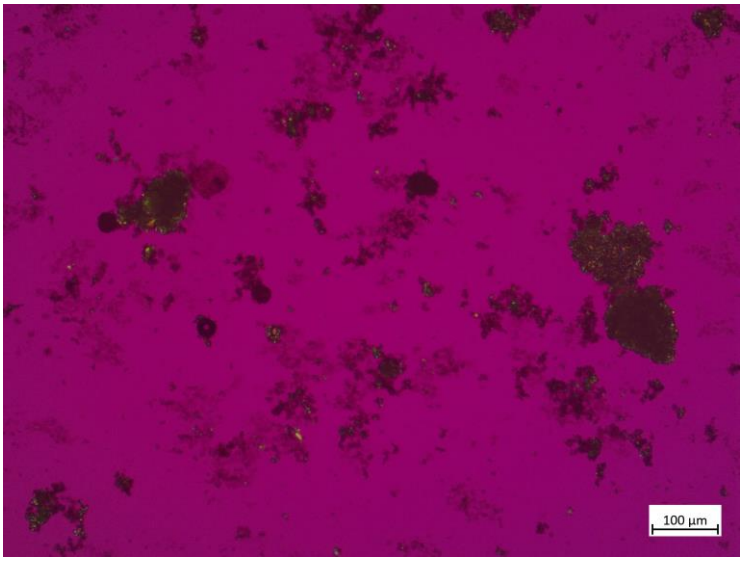

Figure 3.12 Microscopic appearance of 0.5% w/v loteprednol stabilized with 0.125% P188 after 6-month storage at 40 °C/75%RH

Even at optimized concentration of 0.5%, integrity was only prolonged toward two months for the more hydrophilic P188 and P338 (Figure 3.13). 0.5% P407, with the highest PPO fraction of all poloxamers investigated, resulted in comparably better physical stability, but still with a growth rate of about 2.5 µm/month.

Tyloxapol-stabilized systems on the other hand remained completely physically stable. This was surprising since loteprednol solubility was 25 times higher in 0.5% Tyloxapol compared to 0.5% P407. Solubility is known as driving factor for Ostwald ripening [21]. One must however consider, that even at comparably elevated loteprednol solubility in the presence of Tyloxapol of 12.6 µg/mL, only 0.01% was in solution which might still be negligible in terms of ripening.

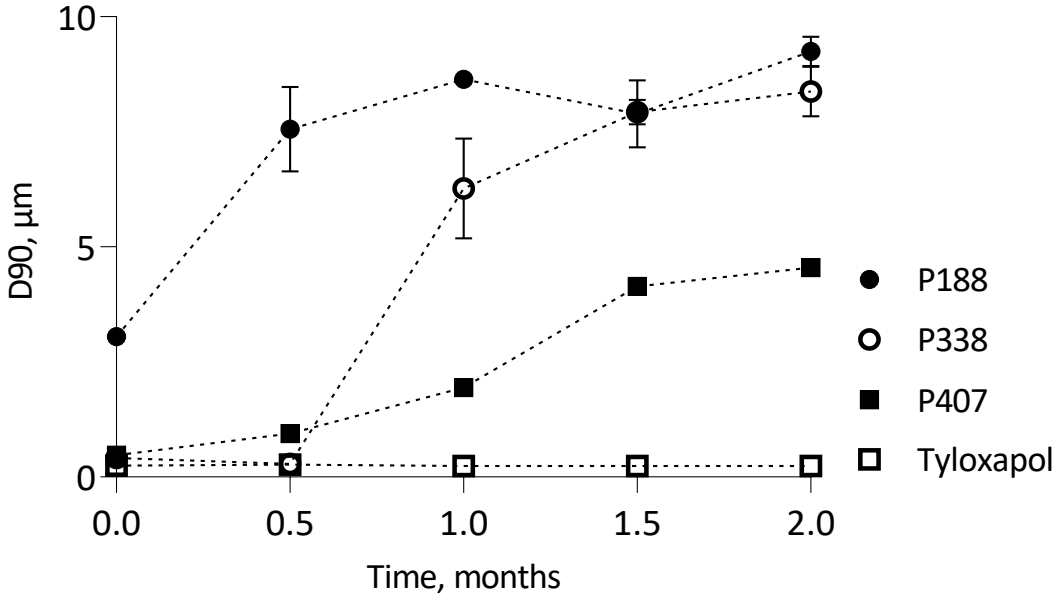

Figure 3.13 Evolution of loteprednol particle size d90 in dependence of surfactant type at 0.5% stored at 40 °C/75%RH

Verma *et al.* faced similar stability issues upon storage for poloxamer-stabilized ibuprofen [88]. Particle size increase was partially contributed to increased solubility, but also to the adsorption behaviour of poloxamer. Multiple scans of the same area using atomic force microscopy proved the migration of the adsorbed poloxamer on the crystal surface which was not revealed for other stabilizers. With migration, sites without stabilizer were exposed and prone to aggregation and crystal growth. This could give an insight on why poloxamers were useful as milling aid but were not able to maintain physical stability.

Same applied to the tuned loteprednol systems containing 1% glycerol or 0.6% NaCl together with 0.5% P407. While milling efficiency was significantly increased at 1 M glycerol or NaCl, storage stability was governed by initial particle size and P407 adsorption rather than surrounding environment (Figure 3.14).

The diluted and undiluted systems were compared. The diluted system referred to 0.5% loteprednol as final formulation. The undiluted system refers to the 10-fold concentrated milling slurry which was also stored to keep glycerol and NaCl concentration and hence P407 conformation as during milling.

As loteprednol solubility increased linearly with P407 concentrations, the untuned system only containing P407 behaved comparable in the diluted vs. undiluted state.

Interestingly, also the tuned system increased in loteprednol particle size after dilution, but less by almost a factor of five. This was attributed to the smaller initial particle size and narrow size distribution. A narrower particle size range minimizes the gradient between drug concentration and the surrounding environment. As a result, ripening was reduced.

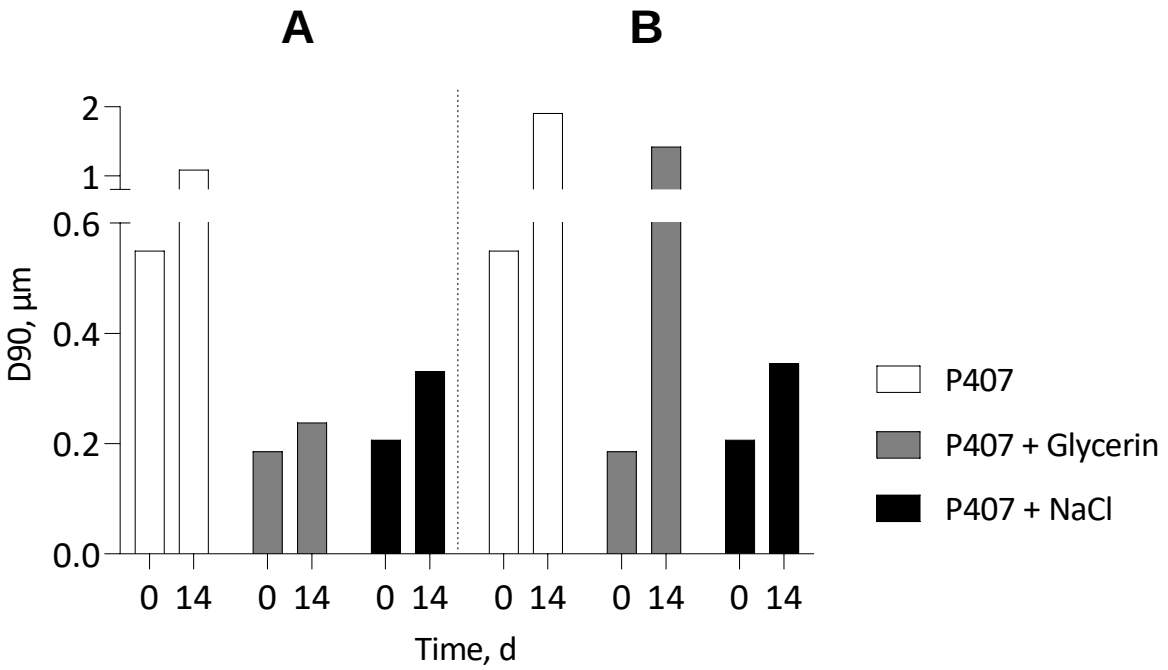

Figure 3.14 Evolution of loteprednol particle size d90 stabilized with 0.5% P407 in the presence of glycerol or NaCl prior and after 14 d storage at 40 °C/75%RH; (A) Diluted = 1:10 in water (1% glycerol, 0.6% NaCl), (B) Undiluted = 10-fold milling concentrate (10% glycerol or 6% NaCl)

In the undiluted system containing glycerol or NaCl, P407 affinity toward the crystal surface should be increased due to the phenomena described before. Loteprednol particle size d90 in the presence of 10% glycerol was about 30% smaller compared to the untuned system, while NaCl counteracted particle growth with an 80% lower d90. Glycerol was a potent solvent for loteprednol with a solubility of 68 µg/mL in the undiluted system. As NaCl did not alter

loteprednol solubility, particle size increase was defined by initial particle size and therefore comparable to the diluted system. Apparently, the change in P407 conformation could increase milling efficiency but did not affect P407 adsorption upon storage.

This emphasized, that milling efficiency might not be a predictor for storage stability. Also, conformational changes could not compensate for the inefficient stabilization of P407.

Despite lower milling efficiency and a concise bimodal distribution, loteprednol particle size remained stable over time if high molecular weight polymer were employed as stabilizer (Figure 3.15). Irrespective of non-ionic polymer species, particle sizes only differed by initial milling efficiency. Normally, broader particle size distributions and hence solubility gradients emphasize physical instabilities which highlighted the advantageous use of high molecular weight polymers in storage stability [141].

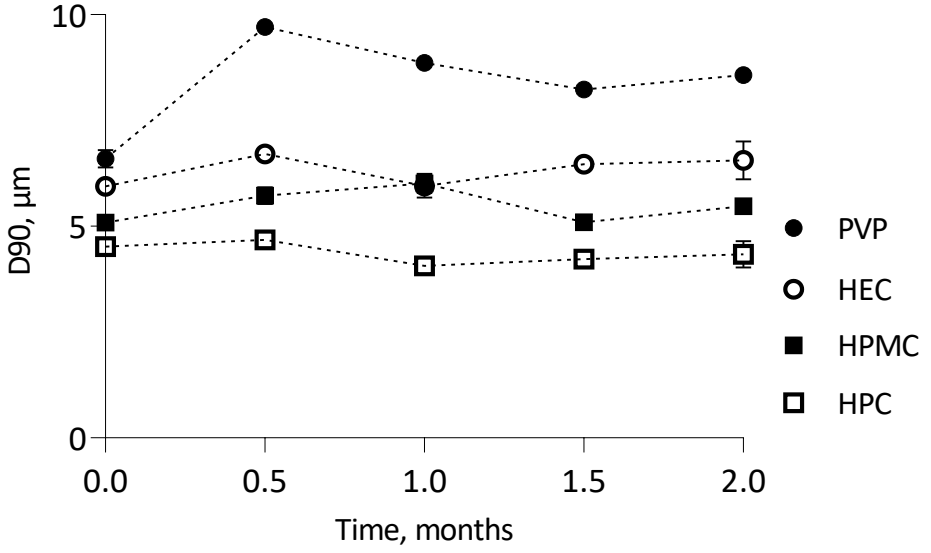

Figure 3.15 Evolution of loteprednol particle size d90 stabilized with 0.125% non-ionic polymers stored at 40 °C/75%RH

Solely looking at the d90 of the size distribution could give the impression of a loteprednol microsuspension. This is due to the distinct bimodal distribution of those systems, which distorted the d90 of the distribution toward 5 µm. All polymers however had a distinct nm-fraction as can be better seen from the cumulative volume distributions (Figure 3.16).

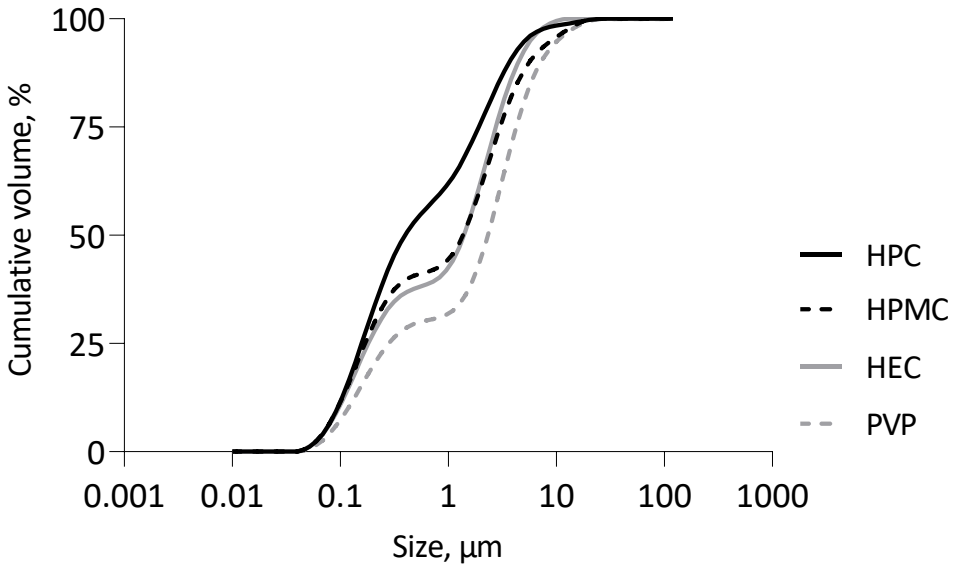

Figure 3.16 Cumulative volume distribution of loteprednol stabilized with 0.125% non-ionic polymers after 2-month storage at 40 °C/75%RH

Especially smaller crystals are lost to Ostwald ripening upon storage. By looking solely at the nm-fraction of loteprednol nanosuspensions, it could be shown that also this sensitive fraction remained stable over time (Figure 3.17). PVP was not included in this observation as the transition from nanocrystals to microcrystals did not allow to cut off the µm-fraction using the instrument's software.

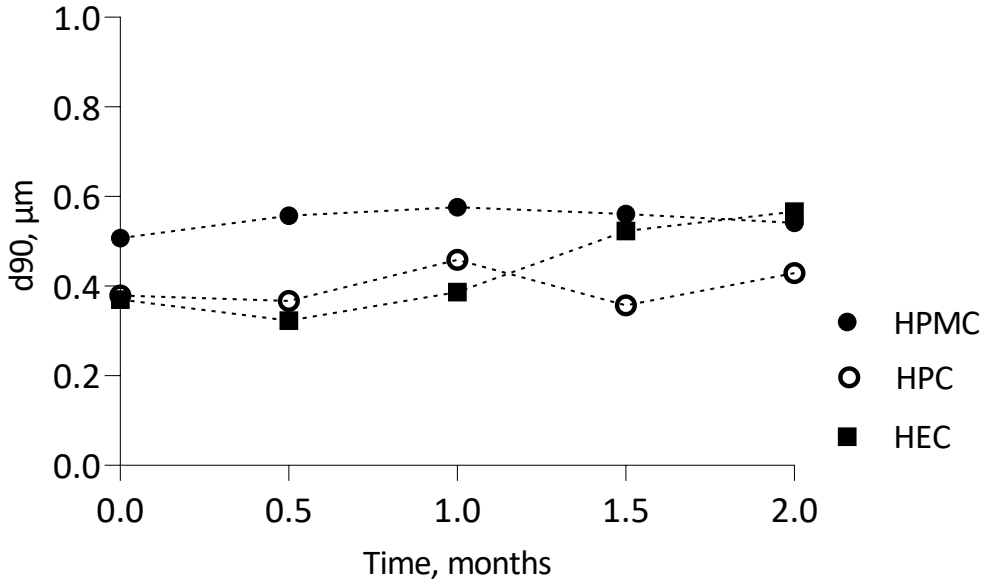

Figure 3.17 Evolution of loteprednol nm-fraction d90 stabilized with 0.125% non-ionic polymers stored at 40 °C/75%RH

Compared to P188, with an equal milling efficiency as the high molecular weight polymers, and with complete ripening, nanocrystal integrity was fully maintained for the polymers. This underlines the importance of sufficient and especially durable adsorption of stabilizer on the crystal surface to form a steric barrier. Additionally, non-ionic polymers did not alter loteprednol solubility.

Surfactants showed promising milling efficiency, but not storage stability. The opposite effect was observed for high molecular weight polymers. Other stabilizing mechanisms might be able to facilitate both. Hydrophilic molecules that contain electric charges are known to stabilize drug nanocrystals through electrostatic repulsion between crystals, thereby providing sufficient space or charge stability. This facilitates a different approach in loteprednol stabilization.

Stabilization was not substantially differing for anionic or cationic stabilizers (Figure 3.18). The SDS-containing nanosuspension showed a growth rate of 1.9 µm/month, again explained with increased loteprednol solubility in the presence of SDS. Anionic BAK-stabilized loteprednol was comparably better, faced however aggregation issues upon storage. This was elsewhere observed and attributed to the high variation in alkyl chain length within BAK and hence different hydrophobicity and affinity toward the crystal surface [64].
The ionic polymer Na CMC showed a behaviour like non-ionic polymers. While milling efficiency was low, storage stability was maintained. This could be attributed to the combination of steric and electrostatic stabilization. It was therefore surprising, that chitosan rendered the suspensions unstable.

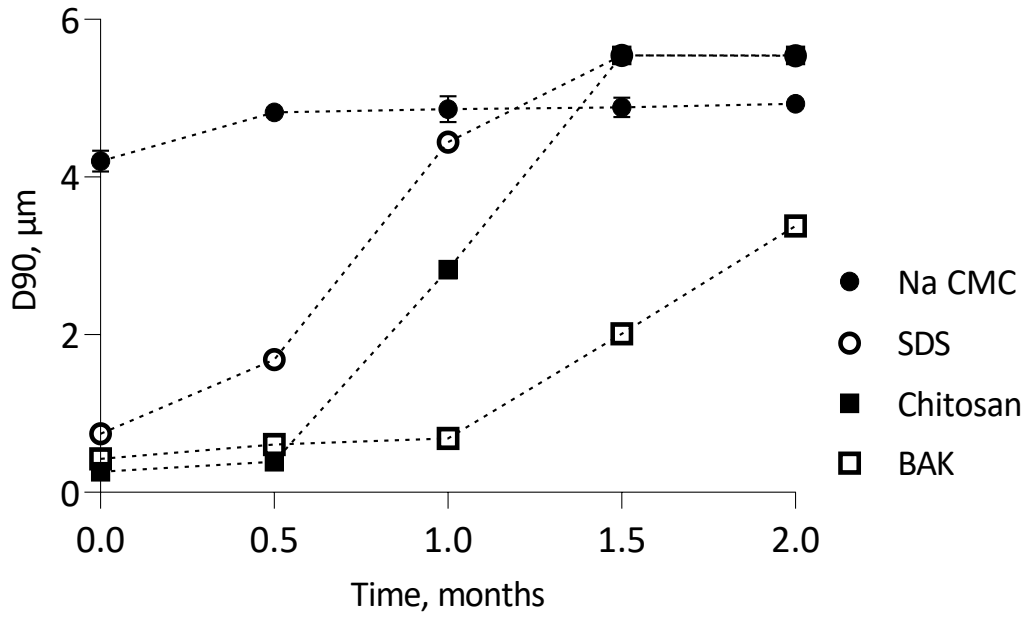

Figure 3.18 Evolution of loteprednol particle size d90 stabilized with 0.125% ionic polymers stored at 40 °C/75%RH

Neither surfactants nor high molecular weight polymers were able to yield a sufficient loteprednol milling efficiency and storage stability. The introduction of ionic stabilizers and thus the change in the stabilizing mechanism did not increase physical stability.

One single stabilizer may hence be enough, but often combinations of stabilizers can be useful. With this approach, the beneficial effects of surfactants on milling efficiency and that of high molecular weight polymers on intermediate storage stability could be utilized. For this approach, P407 was chosen as surfactant at 5% with highest milling efficiency and was paired with 1.25% HPMC E4M which showed good storage stability.

The combination of surfactant and polymer significantly increased loteprednol milling efficiency compared to the single stabilizers (Figure 3.19). This was explained by a synergistic co-adsorption of both stabilizers.

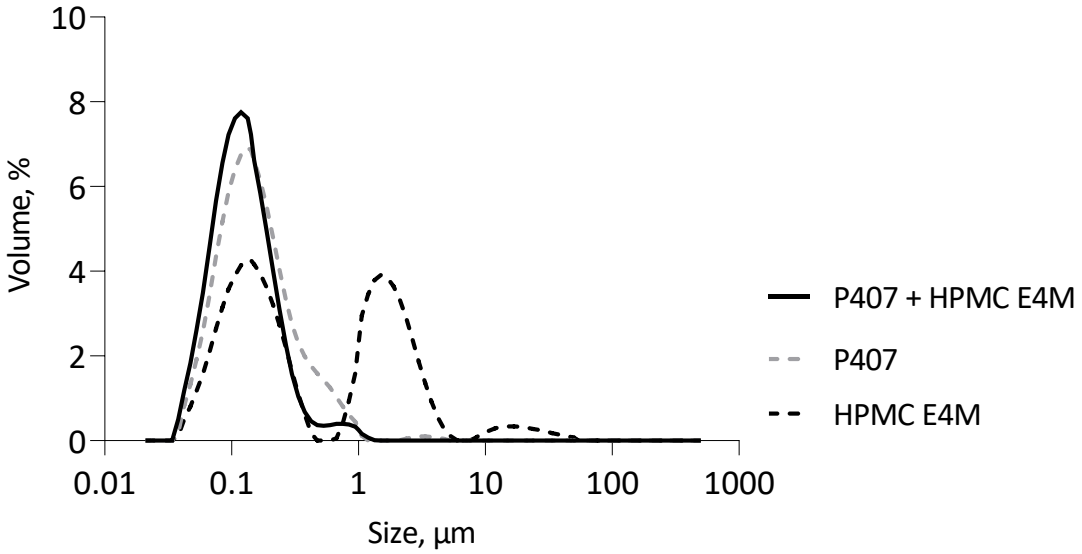

Figure 3.19 Volumetric PSD of loteprednol milled in the presence of 5.0% P407, 1.25% HPMC E4M or a combination of 5.0% P407 and 1.25% HPMC E4M

Surface tension and contact angle were already described as parameters determining milling efficiency and were in the combinational system solely defined by P407 with no change in those parameters in the presence of HPMC (Table 3.3). Therefore, it was expected that also loteprednol milling was controlled by the surfactant.

Adsorbed layer thickness gave information on the adsorption behaviour on the crystal surface, as it is defined by the expansion of hydrophilic stabilizer segments toward the aqueous phase. In the case of P407 the adsorbed layer thickness corresponded to PEO chain length and as expected was 6.8 nm [127]. Two distinct uses of HPMC were then investigated. Once HPMC was added after milling and once HPMC was used in combination with P407 during milling. In the case of HPMC added after milling in the outer phase, layer thickness did not change, suggesting no interaction between HPMC and the loteprednol nanocrystals. If however added during milling, adsorbed layer thickness was lowered to 5.3 nm, a decrease of about 30% compared to P407 alone. This decrease suggested a change in stabilizer adsorption pattern.

Table 3.3 Surface tension, contact angle and adsorbed layer thickness of 5.0% P407, 1.25% HPMC E4M or a combination thereof; adsorbed layer thickness determined for 5.0% loteprednol; "P407 + HPMC E4M": HPMC used in a stabilizer combination with P407, "outer phase HPMC E4M": HPMC added after milling with P407; adsorbed layer thickness not determined for water and HPMC E4M as no nanosuspensions were formed during milling

Stabilizer system	Surface tension, mN/m	Contact angle, °	Adsorbed layer thickness, nm
Water	72	64	Not applicable
HPMC E4M	46	69	Not applicable
P407	38	28	6.8
P407 + HPMC E4M	37	34	5.3
Outer phase HPMC E4M	37	34	6.7

It was therefore postulated that P407 determined milling efficiency whereas trapped P407 micelles by the HPMC chains provided an increased coverage and steric barrier [137]. Additionally, non-ionic polymers were expected to irreversibly attach to the crystal surface, hindering trapped P407 from fluctuating and increasing the steric barrier's strength upon storage [88].

An optimum stabilizer combination could therefore result in maximum wettability, highest milling efficiency and subsequently also maximum stability. The combinations employed could counteract crystal growth with negligible growth rates of 0.005 µm/month at accelerated conditions over 6-month storage at 40 °C/75%RH. The superior physical stability was also attributed to the crystal growth rate-limiting step from bulk diffusion to surface integration by PVP and HPMC [142]. This suggested that the polymer could additionally delay the rate of surface integration by immediate adsorption of those onto the growing crystal surface.

Optimization of loteprednol nanosuspension milling efficiency and storage stability is a complex process since it involved many factors that affect milling efficiency. This chapter focused on the identification of key stabilizer properties that had a direct outcome in the formation of a stable loteprednol nanosuspension. From these experiments, surfactant concentration, hydrophobicity, surface tension and media viscosity were concluded to have direct impact on loteprednol milling efficiency. This knowledge gave a formulation design

space from which nanosuspensions could be prepared. Additionally, this paper showed novel approaches in further tuning P407 conformation and hence loteprednol milling efficiency by addition of osmotic agents to the milling media. In contrast to milling efficiency, storage stability was critical in the presence of most surfactants. For this reason, a combination of non-ionic surfactant and high-molecular weight polymer was introduced, which ultimately stabilized the system through synergistic co-adsorption.

3.2 Critical stabilizer properties for wet milling and storage stabilization of nepafenac nanosuspensions

The quality of a resulting nanosuspension is judged by the initial particle size distribution (PSD) and its subsequent stability against agglomeration and crystal growth. In contrast to loteprednol, nepafenac was easily milled to nanocrystals also at low surfactant concentrations. Aside the most hydrophilic Poloxamer 188 (P188), all surfactants resulted in a particle size d90 of about 0.25 µm (Figure 3.20). P188 was the most hydrophilic surfactant with the lowest molecular weight of those under investigation. A sufficient hydrophobicity was hence also crucial for nepafenac stabilization.

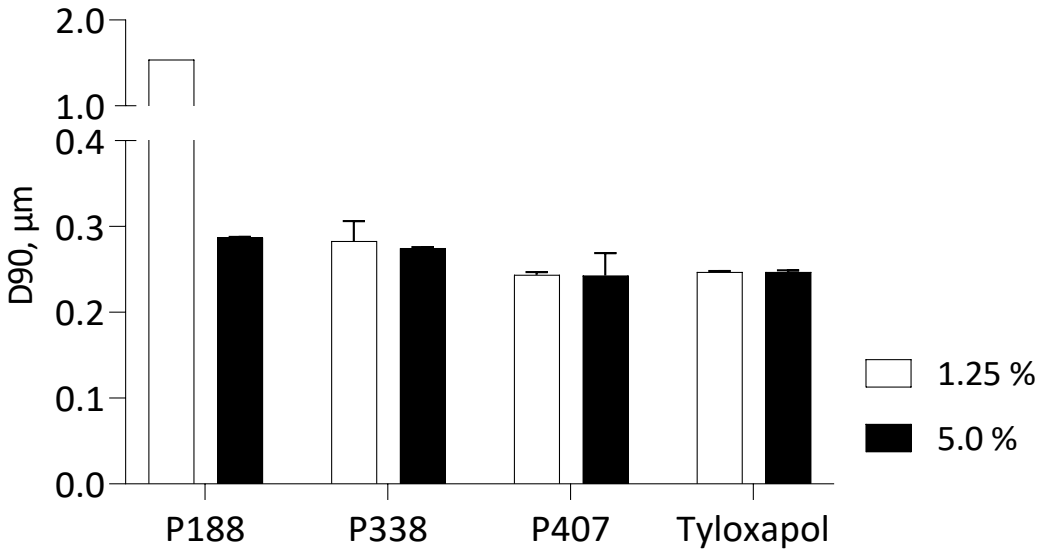

Figure 3.20 Stabilizer type and concentration-dependent (1.25% vs. 5%) nepafenac milling efficiency expressed as particle size d90

Aside milling efficiency, also storage stability was significantly better for nepafenac compared to loteprednol nanosuspensions. Even at low surfactant concentrations of 0.125% surfactant, all nanosupensions remained physically stable except for P188, most likely due to broad initial size distribution (Figure 3.21).

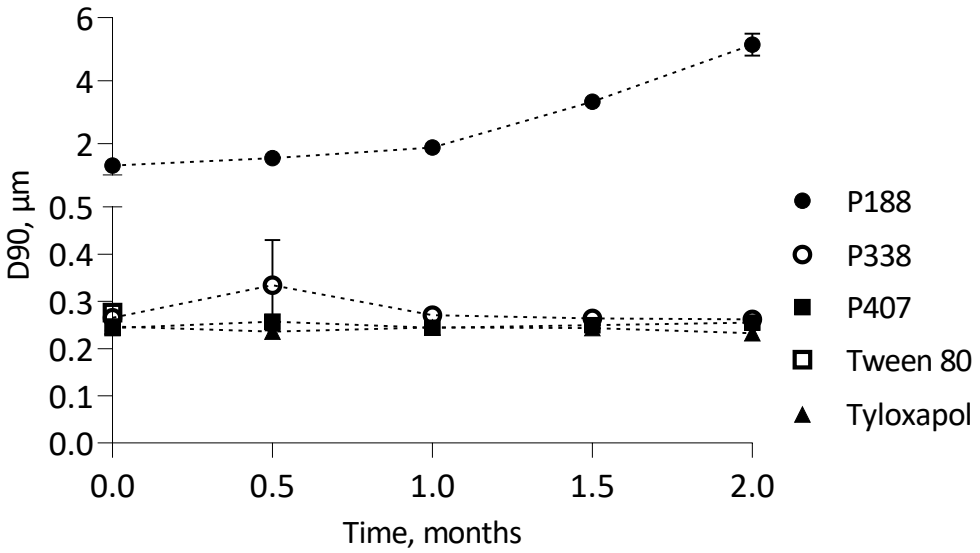

Figure 3.21 Evolution of nepafenac particle size d90 with 0.125% surfactant stored at 40 °C/75%RH

As stated before, milling efficiency is dependent on the interaction between surfactant and drug crystal. High nepafenac milling efficiency was also in accordance with contact angles of nepafenac and the different surfactants of 0° and hence complete wetting of the nepafenac surface.

Nepafenac was also successfully milled using different non-ionic high molecular weight polymers at 1.25%. Not only did all polymers facilitate high milling efficiency, but also sufficient storage stability was seen for all stabilizers for up to two months. With negligible changes in the PSD d90 of about 10 nm within 2-month storage, nepafenac was considered storage stable for all systems investigated (Figure 3.22). Compared to loteprednol, the presence of amide bonds in nepafenac might ease the formation of intermolecular and especially hydrogen bonds. Although hydrophobic interactions are relatively stronger than other weak intermolecular forces like van der Waals interactions or hydrogen bonds, the latter ones allow for an additional interaction between nepafenac and respective stabilizers.

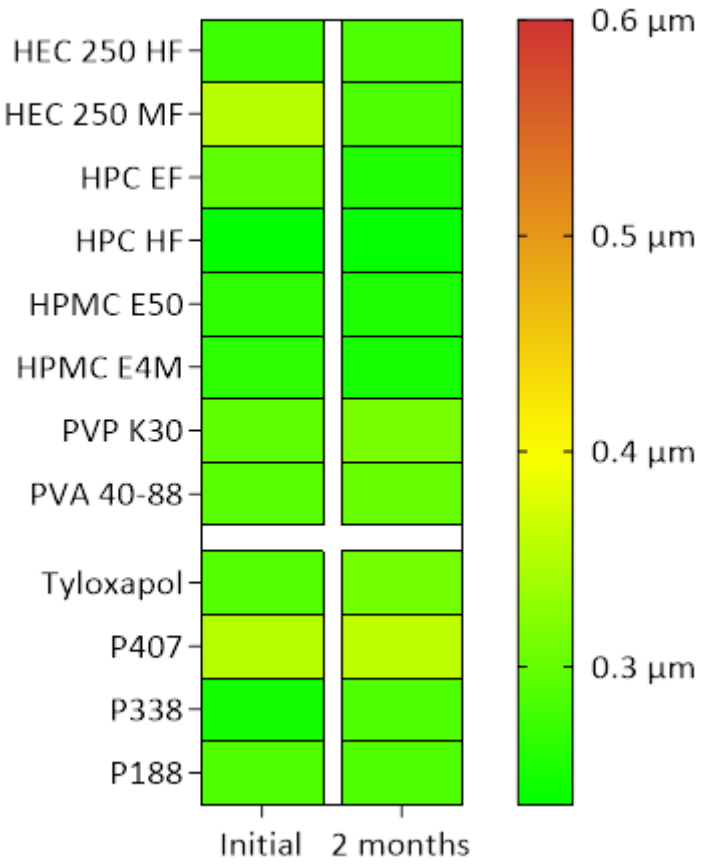

Figure 3.22 Heat map of stabilizer-dependent nepafenac milling efficiency expressed as particle size d90 milled with 1.25% polymer or 5% surfactant and nepafenac particle size d90 of the same systems after 2-month storage at 40 °C/75%RH

While non-ionic stabilizers ensure nepafenac stabilization over a broad range of stabilizers and concentrations, electrostatic stabilization was less efficient (Figure 3.24). Sodium dodecyl sulfate (SDS) as well as benzalkonium chloride (BAK) resulted in insufficient nepafenac milling efficiency with a d90 greater 2.5 µm and only small improvement at higher concentrations.

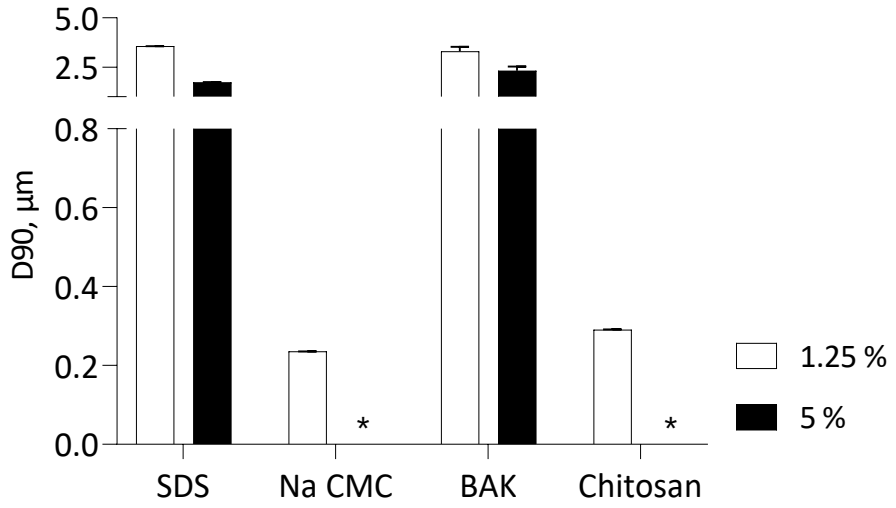

Figure 3.23 Ionic stabilizer type and concentration-dependent (1.25% vs. 5%) nepafenac milling efficiency expressed as particle size d90, * = not determined due to high viscosity

Poor milling efficiency was independent on ionic groups of the surfactant. Zeta potential expresses the electrokinetic potential in colloidal dispersions and a value below - 30 mV or above + 30 mV is generally considered to have sufficient repulsive force to attain physical stability. SDS showed an initial zeta potential of only around 20 mV (Table 3.4). When the zeta potential is this small, attractive forces may exceed repulsion forces, rendering the system unstable. In contrast to the milling experiment, BAK was expected to stabilize nepafenac as indicated in the zeta potential of 40 mV. Other factors like the significant effect of BAK on nepafenac solubility could explain this contraindication.

Table 3.4 Surfactant type and concentration-dependent zeta potential of nepafenac nanosuspensions, initial and after storage for 2-month storage at 40 °C/75%RH

Surfactant	Concentration,%	Zeta potential
SDS	1.25	- 18.8
	5.0	- 24.7
BAK	1.25	40.2
	5.0	40.2

While ionic surfactants lacked nepafenac milling efficiency, a different picture was assessed for ionic polymers, resulting in nepafenac particle sizes comparable to non-ionic polymers and

surfactants. This might be due to the stabilization effect rather being electrosteric with polymers.

Milling efficiency served very well as precursor for nepafenac storage stability. An exception was seen for ionic stabilizers. Within 2 weeks, significant crystal growth was observed in the presence of SDS, BAK and chitosan (Figure 3.24). Ripening is a multifactorial process, where also the broad initial size distribution and increased solubility must be considered. Sodium carboxymethylcellulose (Na CMC) was the only promising ionic stabilizer. This was attributed to the combination of electrostatic and steric stabilization. Furthermore, Na CMC did not alter nepafenac solubility.

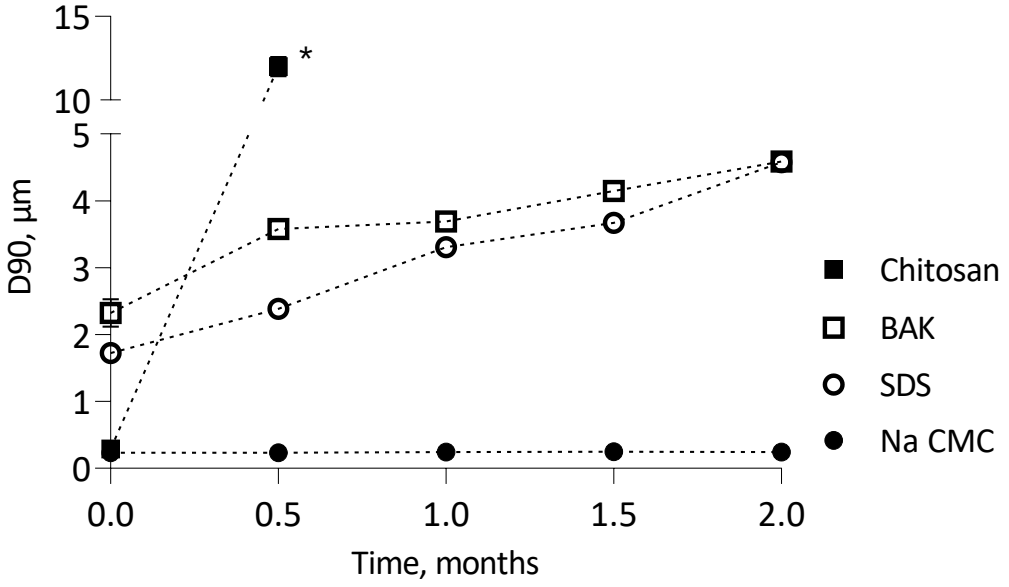

Figure 3.24 Evolution of nepafenac particle size d90 stabilized with 0.125% ionic polymers stored at 40 °C/75%RH; *PSD could not be measured due to significant crystallization

Chitosan-stabilized nepafenac suspensions formed a cake, that was not fully dispersible prior to measurement. The cake consisted of large needle-like crystals, which developed over storage time (Figure 3.25).

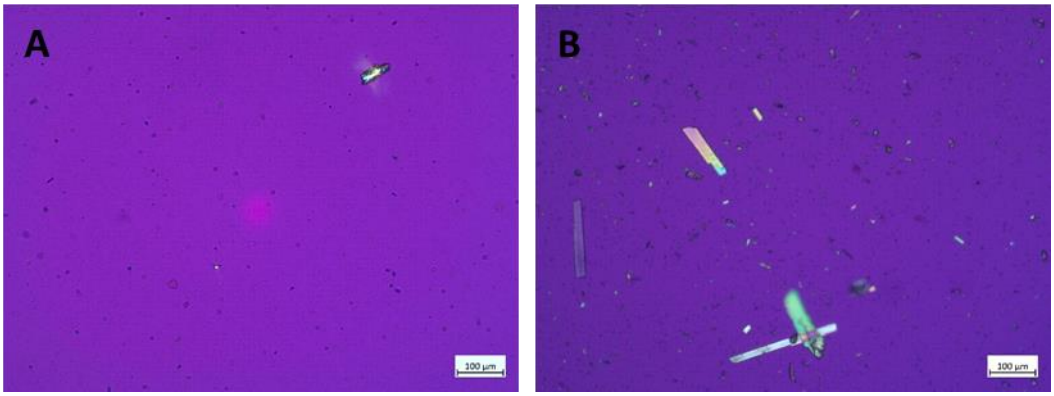

Figure 3.25 Microscopic appearance of nepafenac stabilized with 0.125% chitosan (A) after 0.5-month and (B) after 1-month storage at 40 °C/75%RH

Compared to loteprednol, nepafenac was generally less sensitive to stabilizer choice. Nepafenac could be processed to nanosuspensions irrespective of the non-ionic stabilizer type. The superiority of nepafenac over loteprednol was attributed to the stabilizer affinity and the interaction sites present for nepafenac. Nepafenac possesses more sites open for interaction with the stabilizer compared to loteprednol.

Ionic stabilization was not further pursued as the stabilizers investigated were unsuitable for both individual drugs. Non-ionic systems are also more often found for ophthalmic applications, as they mitigate the risk of irritation as sometimes observed for ionic systems [70], [143].

3.3 Analytical method development and critical preparation parameters in fixed dose combination nanosuspensions

Determination of drug particle size of aqueous nanosuspensions is vital as a quality control parameter to ensure product bioavailability, efficacy and shelf life.

Particle size distribution (PSD) could be measured for the individual loteprednol or nepafenac nanosuspensions as well as for fixed dose combination (FDC) nanosuspension. Although the characteristics of the individual PSD was transferable from single to FDC nanosuspension, it was not possible to assign the single compounds within the joint PSD (Figure 3.26). This highlights the need to develop a method for the determination of the individual drug PSD within an FDC suspension.

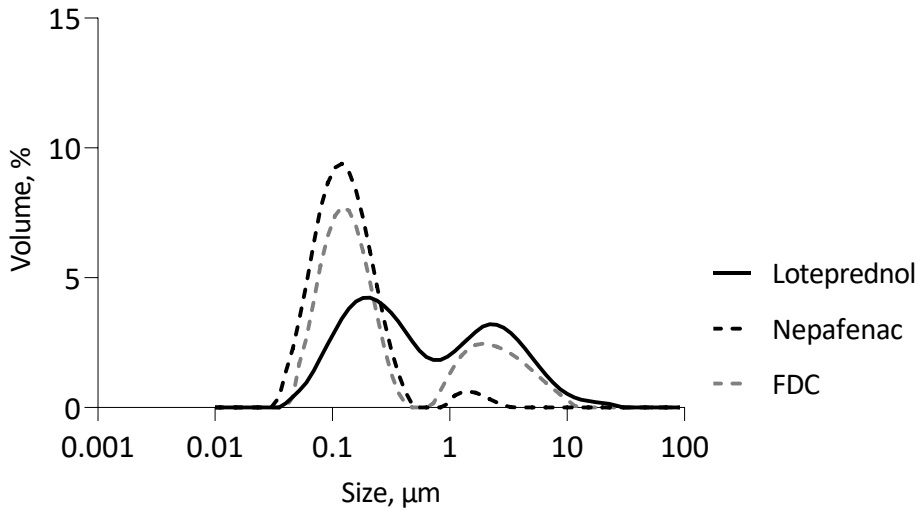

Figure 3.26 Volumetric PSD of 0.5% w/v loteprednol and 0.5% w/v nepafenac and their FDC nanosuspension with 0.5% P407 after 2-month storage at 40 °C/75%RH

The method developed was based on the different aqueous solubilities of loteprednol and nepafenac. Loteprednol was practically insoluble in water (0.5 µg/mL). The forty times higher nepafenac solubility (20 µg/mL) resulted in the complete dissolution of nepafenac nanocrystals in the volume used in the particle size measurement vessel (20 mL or 80 mL). The loteprednol particle size distribution was thus obtained after complete dissolution of nepafenac during particle size measurement of the FDC suspension.

Particle size determination using laser diffraction is sensitive to particle concentration during measurement. As the sample volume was restricted to guarantee nepafenac dissolution, some requirements of the method had to be assured. It was first verified that particle size was robust against low obscuration. For general measurements an obscuration of 5% would be targeted, but measurement was also possible at a tenth of the obscuration without fluctuations in the PSD (Table 3.5). Obscuration ranges for selective dissolution of nepafenac from the FDC would result in about 2% and were thus covered by the working range.

Secondly, also representativeness of the sample was to be tested. It had to be assured that even with small sample volumes, the sample accurately reflected the characteristics of the larger population. An unstable sample was chosen to assure that also broad size distributions could be measured unbiased (d10 = 0.06, d50 = 0.18, d90 = 3.49, FDC from Figure 3.26). In this case, the d90 was the most sensitive parameter. With a standard deviation in the d90 of ± 0.01 µm for six individual samples, representativeness and repeatability were successfully proven.

Table 3.5 Influence of obscuration range on joint particle size distribution of a loteprednol and nepafenac FDC nanosuspension

Obscuration,%	Joint PSD, percentiles		
	D10	D50	D90
0.5	0.06	0.12	0.27
1	0.07	0.13	0.24
2	0.07	0.13	0.24
5	0.07	0.12	0.23

With these requirements fulfilled, the concept of selective dissolution was proven by combining and measuring the PSD of differently sized nepafenac and loteprednol nano- (d90 = 0.24 µm) and microcrystals (d90 = 9.35 µm). This had the advantage, that the distinct entities allowed to show (a) complete dissolution of nepafenac and (b) would show any influence on loteprednol PSD. Additionally, the presence of nepafenac microcrystals was seen as a worst-case scenario, as microcrystals show a lower dissolution rate compared to nanocrystals.

In situ dissolution of nepafenac nanocrystals during particle size measurement resulted in only loteprednol microcrystals in the suspension (Figure 3.27). The PSD of loteprednol was not affected by the dissolution of nepafenac and the measurement time.

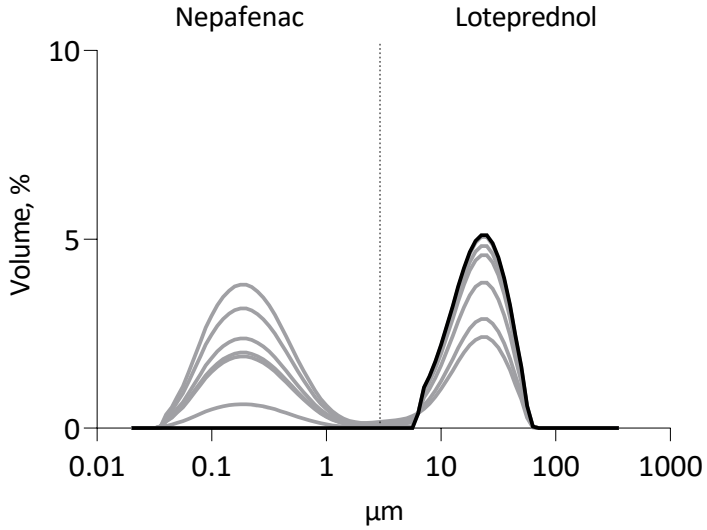

Figure 3.27 Evolution of volumetric PSD over measurement time (10 min, 1 measurement per min) of a 0.5% w/v loteprednol and 0.5% w/v nepafenac FDC suspension with 0.5% P407 performed with selective dissolution, nepafenac present as nanocrystals, loteprednol present as microcrystals

Performing the experiment with nepafenac microcrystals and loteprednol nanocrystals in turn resulted again in the dissolution of nepafenac and in suspended loteprednol nanocrystals from which the PSD was obtained (Figure 3.28). The increase in loteprednol particle size d90 from 0.24 to 0.32 µm was probably caused by the high energy sonication input, which induced crystal aggregation or ripening. A lowering of the sonication intensity was tested, which as expected slowed down nepafenac dissolution. Since slower dissolution required longer measurement times, an increase in loteprednol particle size was observed.

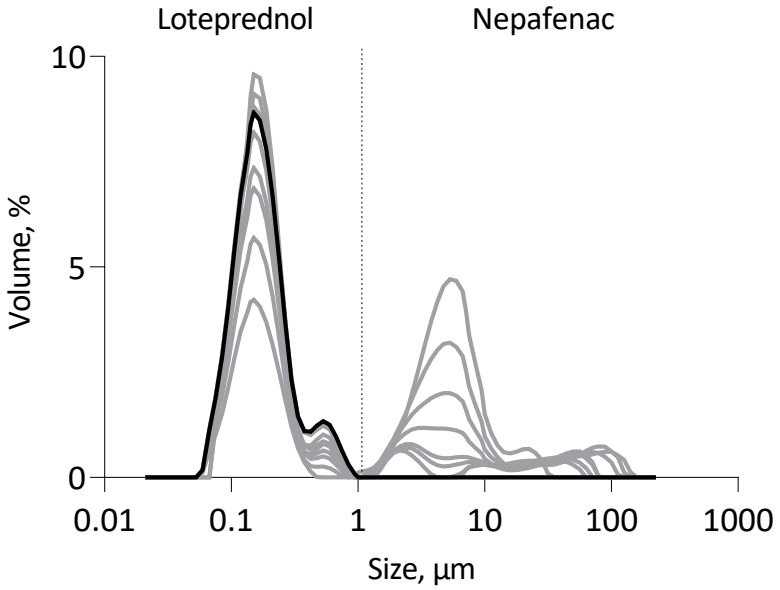

Figure 3.28 Evolution of volumetric PSD over measurement time (10 min, 1 measurement per min) of a 0.5% w/v loteprednol and 0.5% w/v nepafenac FDC suspension with 0.5% P407 performed with selective dissolution, loteprednol present as nanocrystals, nepafenac present as microcrystals

With the employed method, loteprednol particle size was determined within the FDC nanosuspension from Figure 3.26. As expected from the individual nanosuspension's PSD, the µm-fraction of the FDC could be assigned to loteprednol (Figure 3.29).

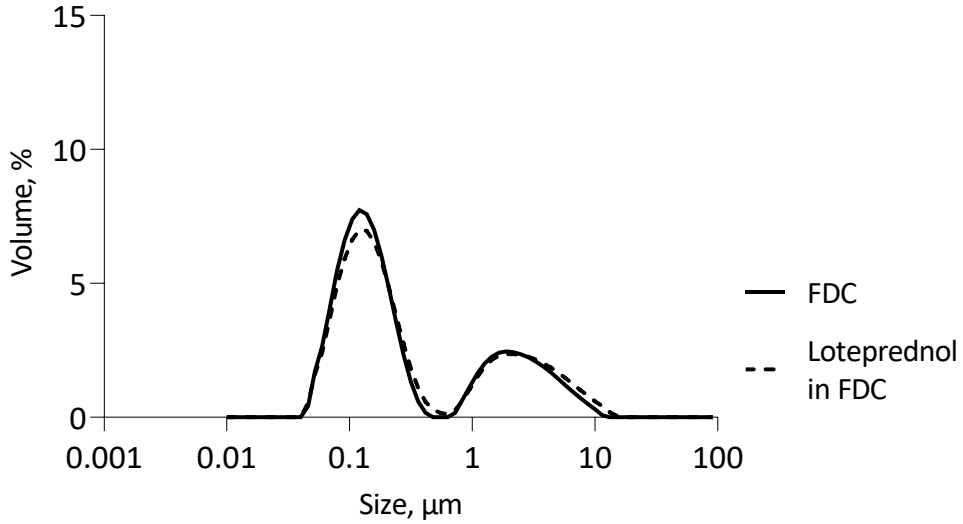

Figure 3.29 Volumetric PSD of a 0.5% w/v loteprednol and 0.5% w/v nepafenac FDC nanosuspension with 0.5% P407 after 2-month storage at 40 °C/75%RH and *in situ* loteprednol within the FDC nanosuspension performed with selective dissolution

The same approach was considered to determine nepafenac PSD within the FDC suspension. Loteprednol solubility however restricted the *in situ* dissolution. With a solubility of only 0.5 µg/mL and a concentration of 0.5% loteprednol in the suspension, the sample volume would be too small. 20% isopropanol was thus used as cosolvent to increase loteprednol solubility to facilitate *in situ* dissolution. With a resulting solubility of about 1 mg/mL, complete dissolution of loteprednol in the measurement vessel would be possible. Selective dissolution of loteprednol was however also limited by loteprednol dissolution rate, with an observed change in d90 of only 0.2 µm/min (Figure 3.30). Starting at about 10 µm, this would result in a measurement duration of about 1 h. This made the approach of loteprednol *in situ* dissolution impracticable. In addition, it would very likely have led to nepafenac aggregation over such long measurement intervals.

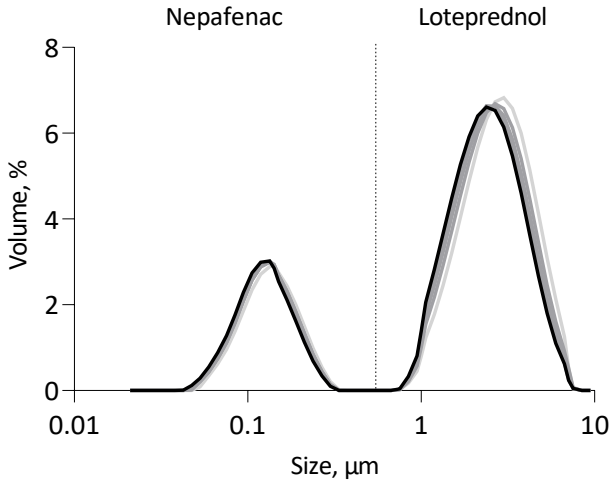

Figure 3.30 Evolution of volumetric PSD over measurement time (10 min, 1 measurement per min) of a 0.5% w/v loteprednol and 0.5% w/v nepafenac FDC suspension with 0.5% P407 performed with selective dissolution in 20% isopropanol, nepafenac present as nanocrystals, loteprednol present as microcrystals

As an alternative, a calculation-based approach was considered. Particle size determinations using laser diffractions are expressed in a relative volume distribution, making them independent on particle concentration present. Laser diffraction analysis is however based on the Fraunhofer diffraction theory, stating that the intensity of light scattered by a particle is directly proportional to its particle size. While particle size is expressed as portions of the total scattering and hence independent on concentration, light intensity as underlying raw data was concentration-dependent.

The concept was first proven using a particle standard (d50 = 500 nm). The highly monomodal PSD was measured individually from the concentration as a relative volumetric PSD (Figure 3.31 C). The underlying light scattering data however showed a concentration dependency, which was linearly dependent on sample concentration (Figure 3.31 A and B). The mean scattering intensity of each measurement was therefore used to normalize the volumetric PSD of the particle standard.

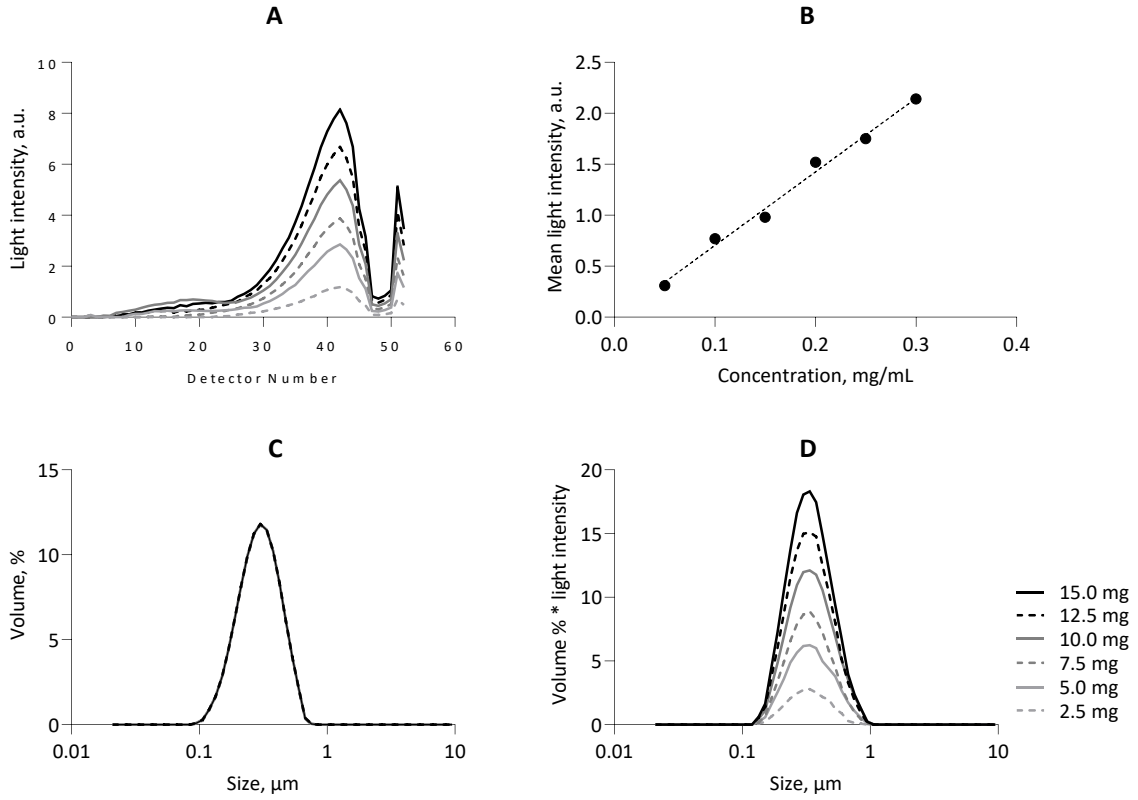

Figure 3.31 Concentration-dependent light intensity (A) raw data, (B) correlation between sample concentration and measured light intensity, (C) volumetric PSD, and (D) concentration-normalized volumetric PSD of a particle standard (d50 = 500 nm)

Therefore, raw data in form of scattered light intensity could be used to normalize and differentiate the PSD of the FDC nanosuspensions. The latter one was different for the FDC and the *in situ* loteprednol particle size of the FDC nanosuspension as the first one contained 0.5% nepafenac and 0.5% loteprednol and the second one only 0.5% loteprednol after nepafenac dissolution. Joint and selective dissolution gained PSD could hence be utilized to calculate nepafenac PSD based on the respective light scattering.

At monomodal distributions, the FDC and *in situ* loteprednol PSD look similar (Figure 3.32 A). Light intensity was however cut by half due to nepafenac being dissolved and only loteprednol remaining in the measurement chamber (Figure 3.32 B). As described above, normalization on the light intensity maximum lead to distributions, with the FDC now larger compared to the loteprednol PSD within the FDC nanosuspension. By subtracting one from the other, theoretical nepafenac PSD was gained (Figure 3.32 D).

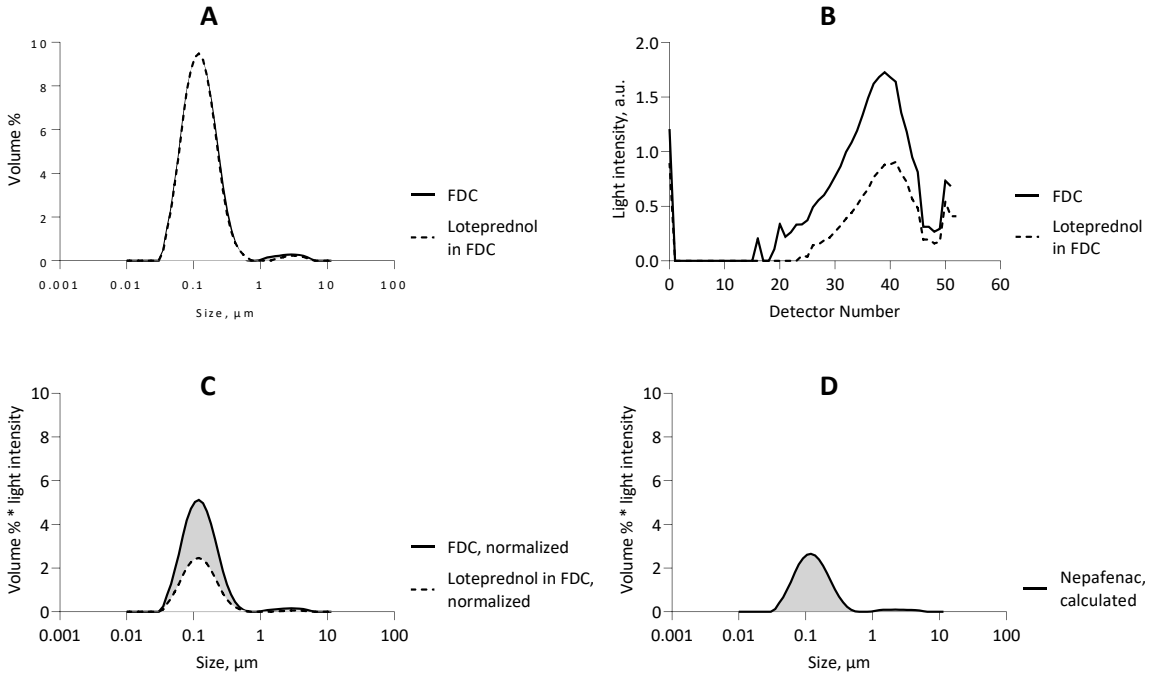

Figure 3.32 Volumetric (A) PSD, (B) light intensity raw data, (C) concentration-normalized volumetric PSD and (D) calculated nepafenac PSD of a 0.5% w/v loteprednol and 0.5% w/v nepafenac FDC nanosuspension using selective dissolution and light intensity normalization

This approach was however limited to monomodal distributions. As for monomodal distributions, also the bimodal PSD of the joint and selective-dissolution gained loteprednol PSD look similar (Figure 3.33 A). This was because it was loteprednol showing the µm-fraction. To normalize the PSD, the underlying light intensity plot was again used. It however only showed one maximum in light intensity which translated into two maxima in the volumetric PSD (Figure 3.33 B). Using the light intensity mean or maximum value would therefore not assign the second maximum of the µm-fraction to solely loteprednol. This led to a bias regarding the nepafenac distribution, which was then associated with a µm-fraction that was beforehand solely attributed to loteprednol beforehand (Figure 3.33 D).

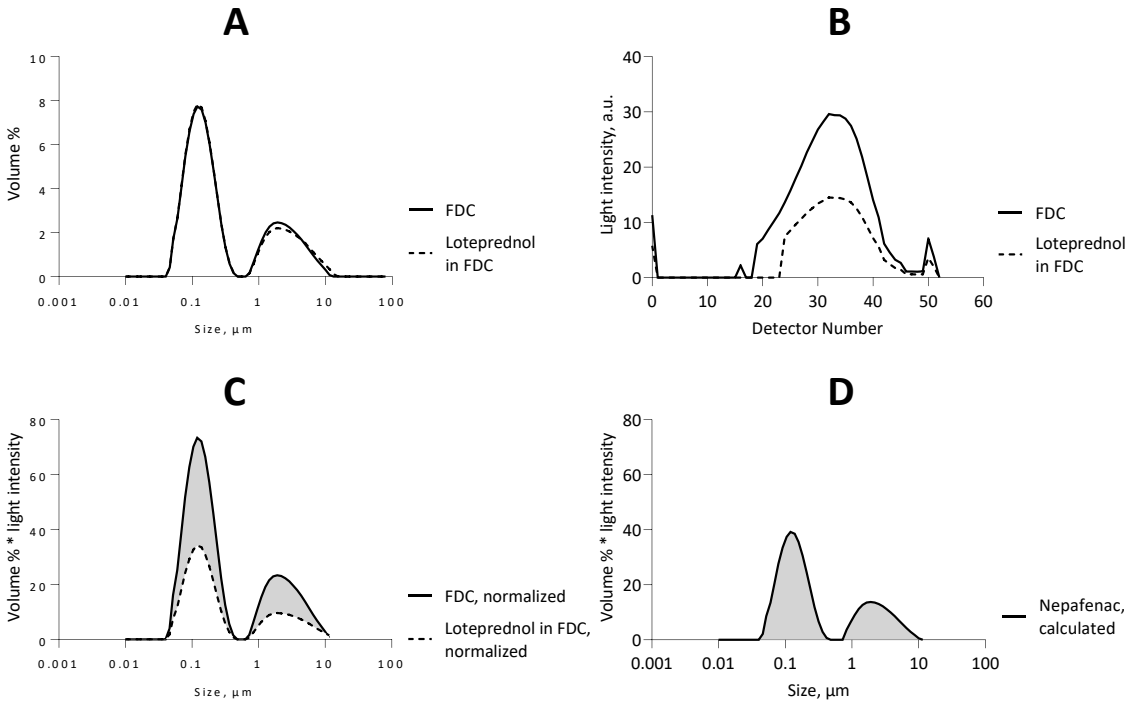

Figure 3.33 (A) Volumetric PSD, (B) light intensity raw data, (C) concentration-normalized volumetric PSD and (D) calculated nepafenac PSD of an loteprednol and nepafenac FDC using selective dissolution and light intensity normalization

The use of selective dissolution is powerful to obtain information on individual PSD within an FDC suspension. In this specific combination of loteprednol and nepafenac it was limited to one compound due to the exceptional low loteprednol solubility and dissolution rate. The novel approach of selective dissolution could give valuable insight in FDC suspension physical stability of various drugs. With the only requirement that solubility of the individual drugs is high enough, selective dissolution could be employed for all drug variations and different particle size ranges, such as nano- or microsuspensions.

As shown in the previous chapter, loteprednol was the more challenging compound in terms of milling efficiency and physical stabilization. Therefore, selective dissolution of nepafenac could give valuable information on critical preparation considerations of FDC nanosuspensions comprising loteprednol and nepafenac.

Particle size reduction increases the surface energy which in turn requires adequate stabilization by excipients and stabilizer choice significantly impacts milling efficiency [124].

The production of an FDC nanosuspensions differs from that of the individual nanosuspensions as optimal conditions must be considered for both drugs.
Regarding of processing, the two drugs can be either milled together (combined milling) or separately followed by combination of the individual milling slurries (separate milling).

Milling two drugs together is the simpler approach requiring fewer steps, however there is less control over the particle size of the individual drugs. Separate milling allows the preparation of nanosuspensions with the desired properties (e.g., particle size) and more freedom regarding milling conditions (e.g., time, speed and stabilizer choice and concentration). The challenge with this approach could be the maintenance of the individual particle properties after combining into an FDC. Both approaches were investigated. Comparable monomodal PSD were obtained for both nanosuspensions immediately after preparation (Figure 3.30).

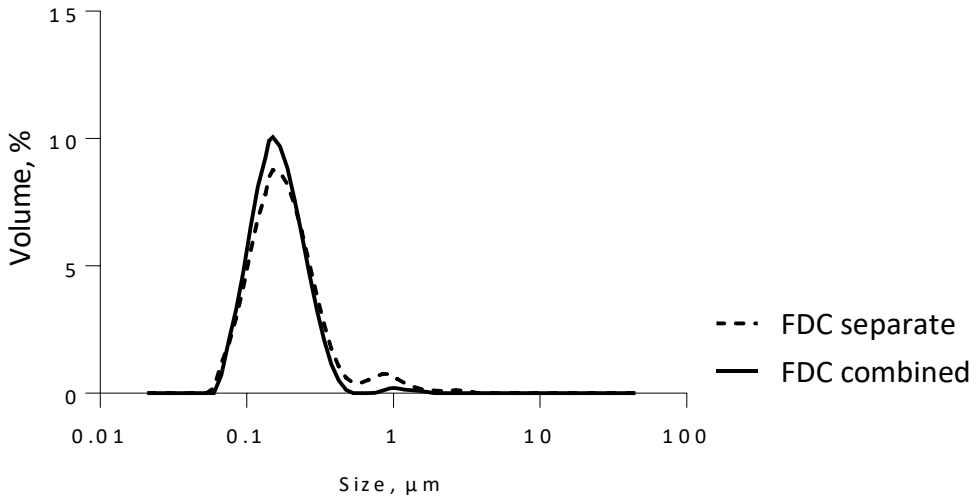

Figure 3.34 Volumetric PSD of 0.5% w/v loteprednol and 0.5% w/v nepafenac FDC nanosuspension milled in combination or separately; combined FDC: 5% w/v loteprednol and 5%w/v nepafenac with 5% P407, diluted 1:10 with water to 0.5% loteprednol, 0.5% nepafenac and 0.5% P407; separate FDC: 5% w/v loteprednol with 5% P407 and 5% w/v nepafenac with 5% P407, individual milling slurries were combined after milling and diluted with water to 0.5% loteprednol, 0.5% nepafenac and 1.0% P407

Within one day at room temperature, a µm-fraction was formed in the separate milling-FDC (Figure 3.35). Employing selective dissolution, this µm-fraction was attributed to loteprednol and thus indicated loteprednol crystal growth or recrystallization.

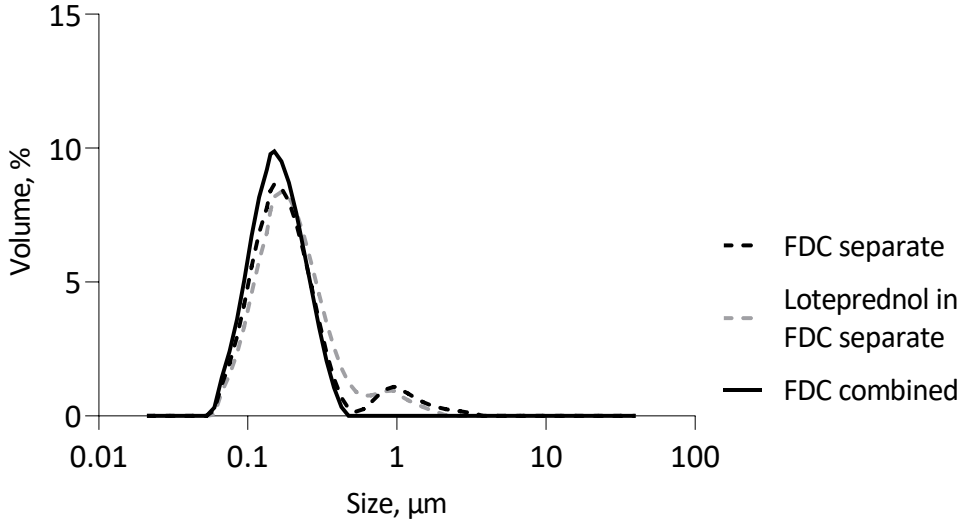

Figure 3.35 Volumetric PSD of a 0.5% w/v loteprednol and 0.5% w/v nepafenac FDC nanosuspension milled in combination or separately and combined after milling after 1 day at room temperature; loteprednol in FDC performed with selective dissolution; combined FDC: 5% w/v loteprednol and 5%w/v nepafenac with 5% P407, diluted 1:10 with water to 0.5% loteprednol, 0.5% nepafenac and 0.5% P407; separate FDC: 5% w/v loteprednol with 5% P407 and 5% w/v nepafenac with 5% P407, individual milling slurries were combined after milling and diluted with water to 0.5% loteprednol, 0.5% nepafenac and 1.0% P407

The combined milling-FDC (Figure 3.35) and the individual drug nanosuspensions (Figure 3.36) did not show changes in particles size after one day.

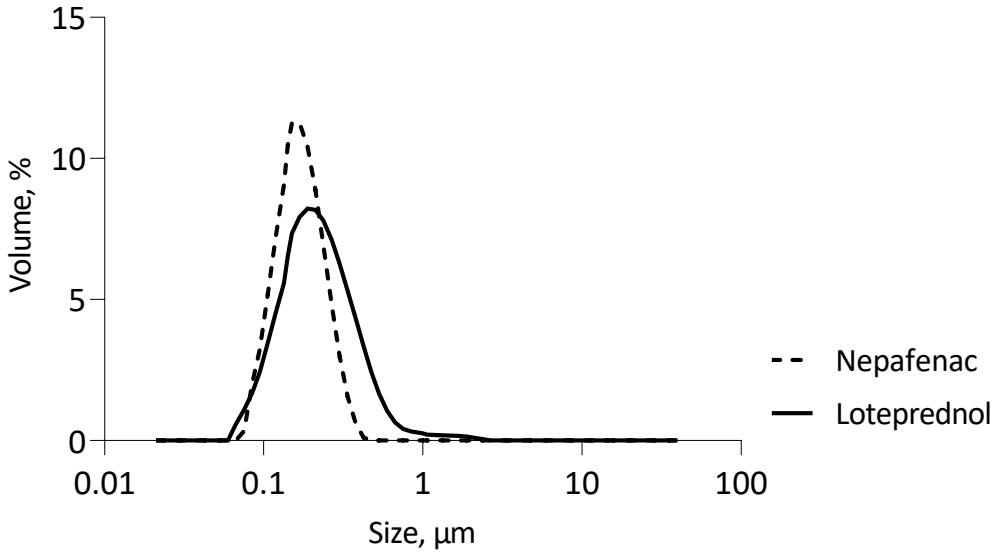

Figure 3.36 Volumetric PSD and of 0.5% w/v loteprednol and 0.5% w/v nepafenac individual nanosuspensions with 0.5% P407 after 1 day at room temperature

The reason behind loteprednol recrystallization was due to its lower solubility in the presence of nepafenac (Figure 3.37).

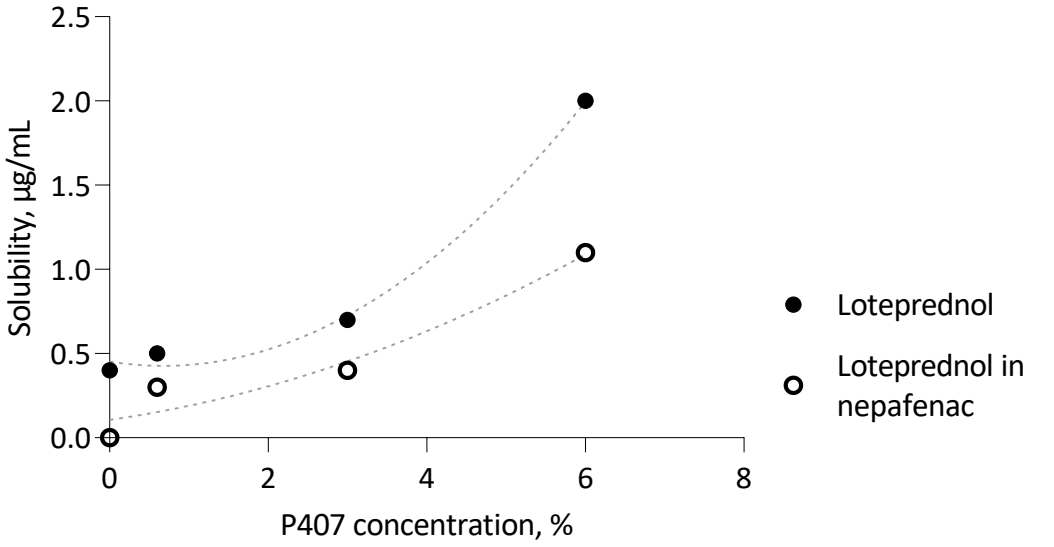

Figure 3.37 P407-dependent loteprednol solubility in water and in a saturated solution of nepafenac

All suspensions were milled 10-fold concentrated, leaving final dilution as a critical step (Table 3.6). During milling, an absolute amount of 2 µg loteprednol was dissolved, allowing dilution without recrystallization. As the solubility was at the upper maximum of what could be

dissolved, solubility could certainly become an issue. Loteprednol solubility was decreased by about a factor 2 in the presence of nepafenac (Table 3.6). This decrease resulted in recrystallization when combining the two separately milled suspensions, as loteprednol could not compensate the lower solubility upon dilution. This phenomenon was not observed when milled together as recrystallization was counteracted during milling and because solubilities were most likely already in equilibrium after milling.

Table 3.6 Absolute loteprednol amount solubilized during milling (10-fold concentrated) and after dilution (1:10) to final formulation with water in comparison for an individual loteprednol nanosuspension and within the FDC nanosuspension either being milled in combination with nepafenac or separately and combined afterwards

	Loteprednol amount dissolved, µg	
	Milling (10-fold concentrated)	Formulation (1:10 dilution)
Individual	2.0	2.0
Combined milling	1.1	1.2
Separate milling	2.0	1.2

It must also be noted that the separate milling led to an increased P407 concentration of 1% upon dilution compared to 0.5% when milled together. This was due to the need of 5% P407 during milling of the individual suspensions. It did however not significantly impact loteprednol solubility and was thus comparable to the combined milling approach and the lower P407 concentration.

As a control, loteprednol was milled in a saturated nepafenac solution with 5% P407 and did not recrystallize upon dilution (Figure 3.38). Only after 7 d, a broadening of the PSD was observed, with a µm-fraction present. This was, as stated before, attributed to the inefficient stabilization with P407.

This proved the point of critical solubility changes after separate milling and combination afterwards and emphasized the benefit of combined milling. For approaches where separate milling might be useful, such as the use of different stabilizers or milling conditions, one must consider potential solubility changes in the combination suspension.

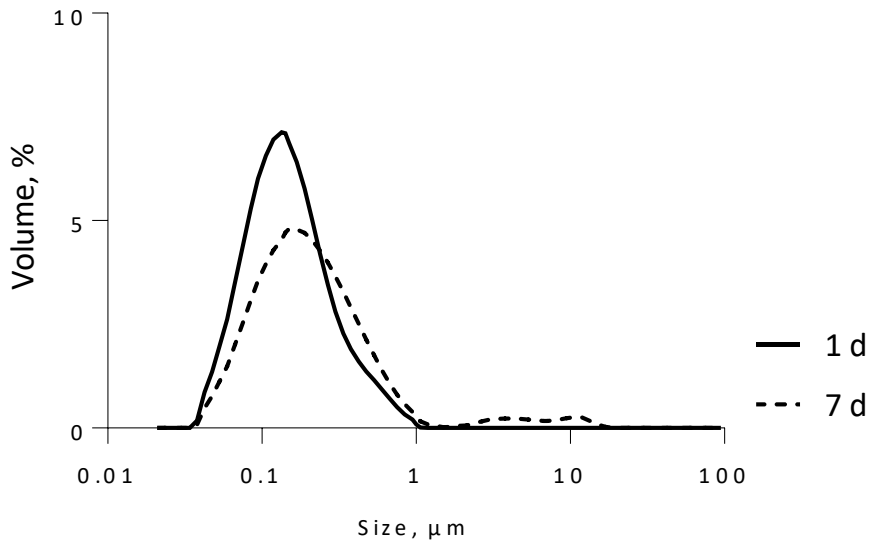

Figure 3.38 Volumetric PSD of a loteprednol nanosuspension milled in a saturated solution of nepafenac and 5% P407 compared to the loteprednol-nepafenac FDC nanosuspension milled in combination (5% loteprednol, 5% nepafenac, 5% P407), initial (A) and after 7-d storage at room temperature (B)

Another benefit from combined milling was the increased milling efficiency compared to the individual nanosuspensions (Figure 3.39). Loteprednol reached its minimum d90 at about 0.6 µm and after 1.5 h milling. A subsequent d90 increase after 3 h was attributed to large energy input and aggregation. Nepafenac showed a minimum d90 at 0.2 µm which was reached and maintained already after 1 h milling. When milled in combination, particle size d90 shifted toward lower particle sizes, with a d90 of about 0.2 µm after 1.5 h. When milled together, particle size d90 of loteprednol within the FDC was correspondingly reduced compared to the individual nanosuspension.

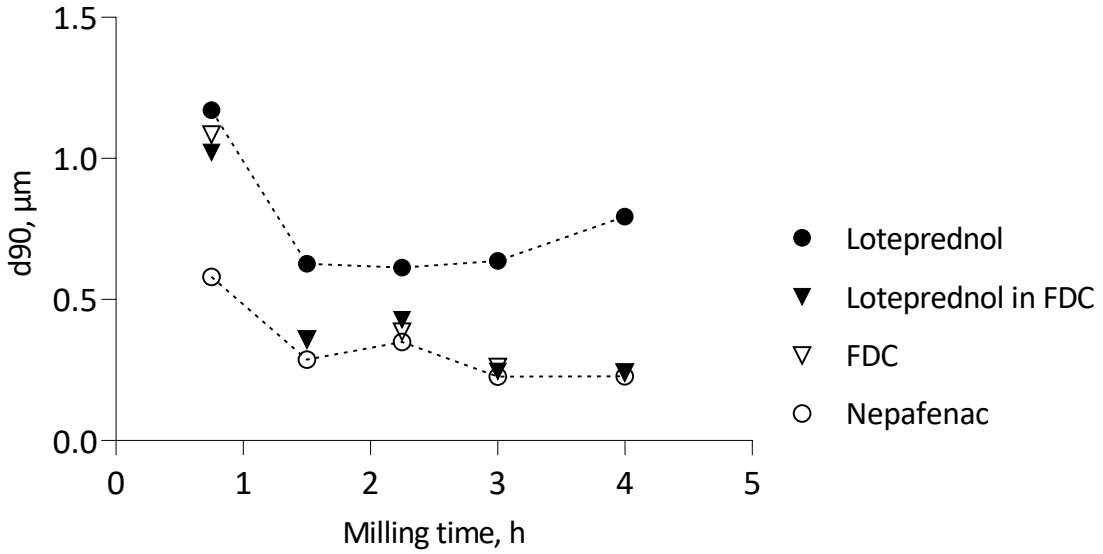

Figure 3.39 Milling efficiency expressed as drug crystal d90 as a function of milling time for 5% w/v loteprednol, 5% w/v nepafenac and 5% w/v loteprednol and 5% w/v nepafenac (milled in combination) FDC with 5% P407 and *in situ* loteprednol particle size d90 within the FDC nanosuspension performed with selective dissolution

The higher milling efficiency was hence increased when milling loteprednol and nepafenac in combination. Four requirements must be fulfilled for high milling efficiency: the number of stress events must be high enough, stress intensity must be sufficient and high viscosities and particle aggregation must be avoided. The latter two are determined by the stabilizer system and P407 was proven to fulfil those requirements for the individual compounds earlier.

Following the stressing model of Kwade, particle breakage is dependent on the energy available for stressing the particle in one event and the overall number of contacts during milling [144]. The overall number of contacts would be increased when milled in combination, as the solid fraction was doubled from 5% (individual drug) to 10% (5% of each drug).

To test this hypothesis, loteprednol was milled in the presence of increasing nepafenac concentrations and particle size decreased with increasing nepafenac concentration until reaching its minimum after about 2% nepafenac (Figure 3.41).

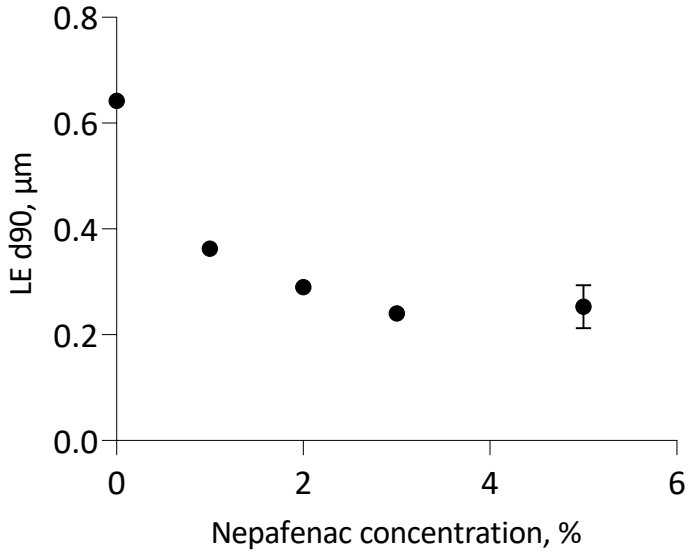

Figure 3.40 Evolution of loteprednol milling efficiency expressed as particle size d90 of 5% w/v loteprednol nanosuspensions milled in the presence of increasing nepafenac concentrations of 0 – 5% w/v, performed with selective dissolution

Not only was the d90 decreasing, but the PSD also was narrower compared to the individual loteprednol PSD (Figure 3.41). The increase in loteprednol milling efficiency was attributed to an increase in total active milling volume which is the sum of the active milling volume between two beads and the grinding media amount. The latter one was stated to be increased in the presence of nepafenac nanocrystals.

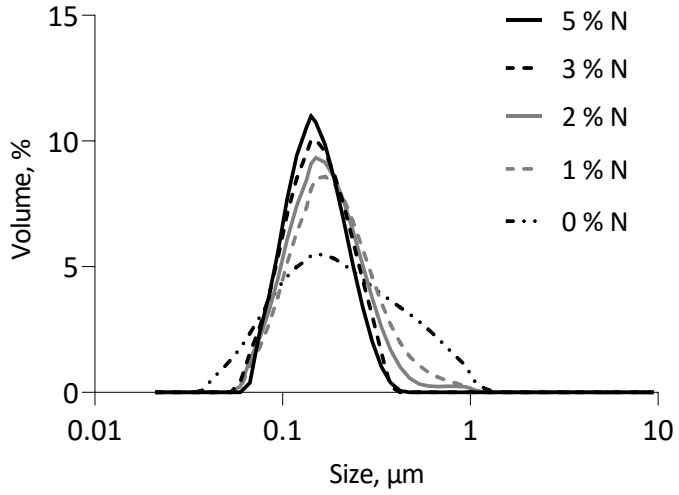

Figure 3.41 Volumetric PSD of 5% w/v loteprednol nanosuspensions milled with 5% P407 and in the presence of increasing nepafenac concentrations of 0 – 5% w/v, performed with selective dissolution

The active milling volume is dependent on the grinding medium size and amount. Therefore, loteprednol was milled with different bead sizes, 0.1 and 0.3 mm. The standard bead size for milling was 0.3 mm. The active milling volume was then calculated for these two systems and for the system of loteprednol being milled in the presence of nepafenac with 0.3 mm beads. Milling experiments were performed simultaneously.

It became obvious, that surface area and hence total active milling volume was increasing in the order from 0.3 mm to 0.1 mm beads to nepafenac and 0.3 mm beads and correlated well with the resulting loteprednol particle sizes (Figure 3.42). Nepafenac reached nm-sizes very early in the process, thus drastically increasing the total active milling volume of the system. This alteration in breaking events increased loteprednol milling efficiency until reaching its minimum at around 0.2 µm.

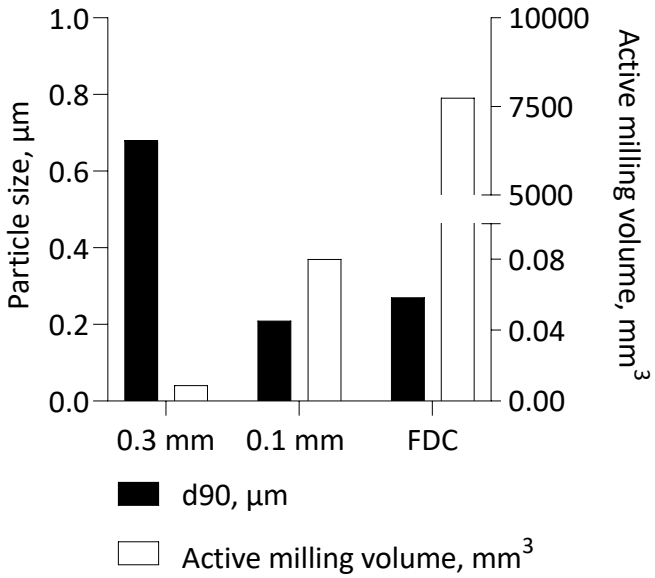

Figure 3.42 Relationship between 5% loteprednol particle size d90 and active milling volume during milling at 1:2 v/v beads with sizes of 0.3 mm, 0.1 mm and 0.3 mm + 5% w/v nepafenac (FDC)

It was crucial to obtain a system with large active milling volume early in the process. Therefore it needed a compound like nepafenac, reaching nm-sizes already within the first hour of milling. Simply increasing the volume fraction did not alter the active milling volume. Comparing different drug combinations, only the presence of nepafenac resulted in smaller particle sizes compared to the individual drugs (Figure 3.43).

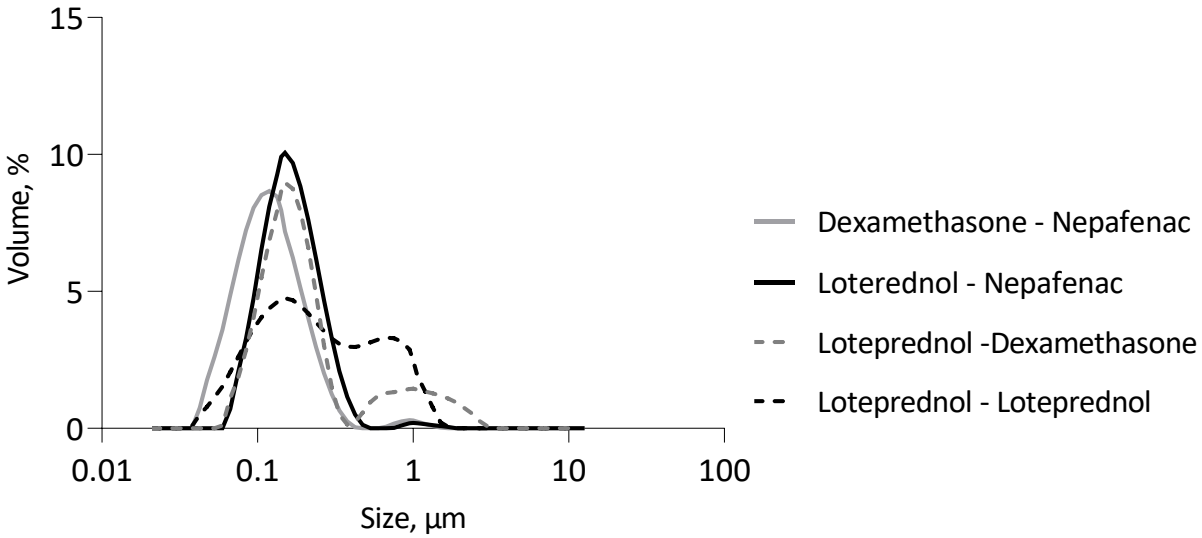

Figure 3.43 Volumetric PSD of FDC nanosuspensions comprising 0.5% w/v P407 and 0.5% w/v dexamethasone and 0.5% w/v nepafenac, 0.5% w/v loteprednol and 0.5% w/v nepafenac, 0.5% w/v loteprednol and 0.5% w/v dexamethasone or 1.0% w/v loteprednol (Loteprednol – Loteprednol)

Drug brittleness was indicated as a key parameter. The higher the drug brittleness, the easier to break the drug crystals and the faster the nanocomminution [33]. Aside the active milling volume, also the minimum energy required to break a particle needs to be considered during milling. The larger the elastic modulus, the lower the transported energy and the lower the milling efficiency [145]. As seen for single nanosuspensions, nepafenac was easily nanocomminuted resulting in a monomodal particle size distribution. Dexamethasone and loteprednol showed lower milling efficiencies and a tendency of multimodal distributions (Figure 3.44).

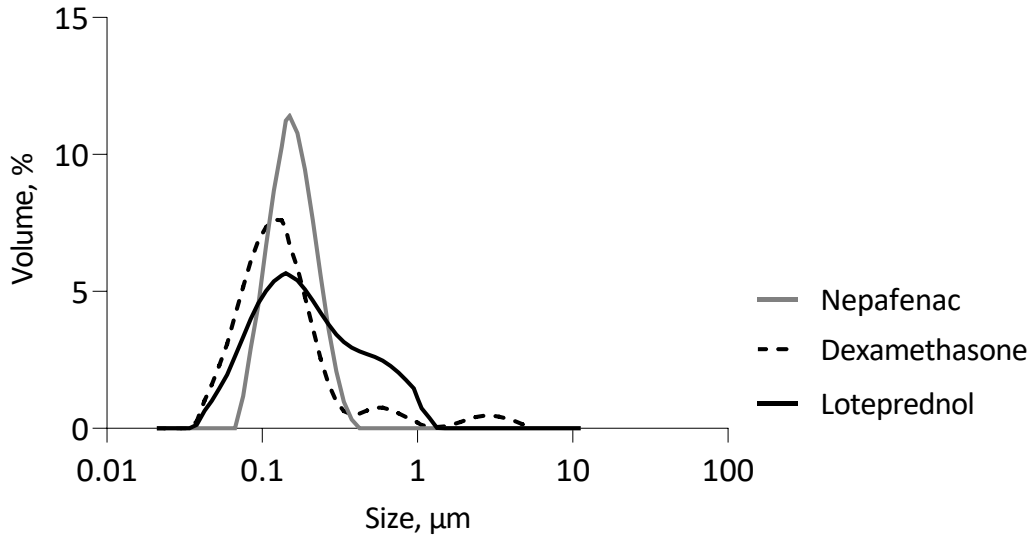

Figure 3.44 Volumetric PSD of individual nanosuspensions comprising 0.5% P407 and 0.5% drug

Brittleness of the drug powders was determined in comparison to brittle DHCP with 39 GPa and ductile MCC with 4.8 GPa. Brittleness was the highest for nepafenac with 24 GPa, whereas loteprednol and dexamethasone were less brittle with 17 and 1.5 GPa respectively. This explained not only the single milling results but also why a combination of loteprednol and dexamethasone did not result in an increased milling efficiency.

As a control, other comparably brittle drugs should be employed and proven the hypothesis of increased milling efficiency due to increased total active volume in the presence of a brittle compound.

Particle size determines dissolution rate from crystals. A positive impact on dissolution from the FDC nanosuspension was expected due to the increased milling efficiency. An *in situ* UV measurement was developed to determine the dissolution rates of nepafenac and dexamethasone from FDC micro- and nanosuspensions.

Nepafenac possesses a second absorption maximum, enabling the simultaneous UV detection of both drugs during dissolution (Figure 3.45). The approach was limited for the use of dexamethasone and nepafenac as loteprednol was not UV detectable due to the low solubility in the aqueous medium.

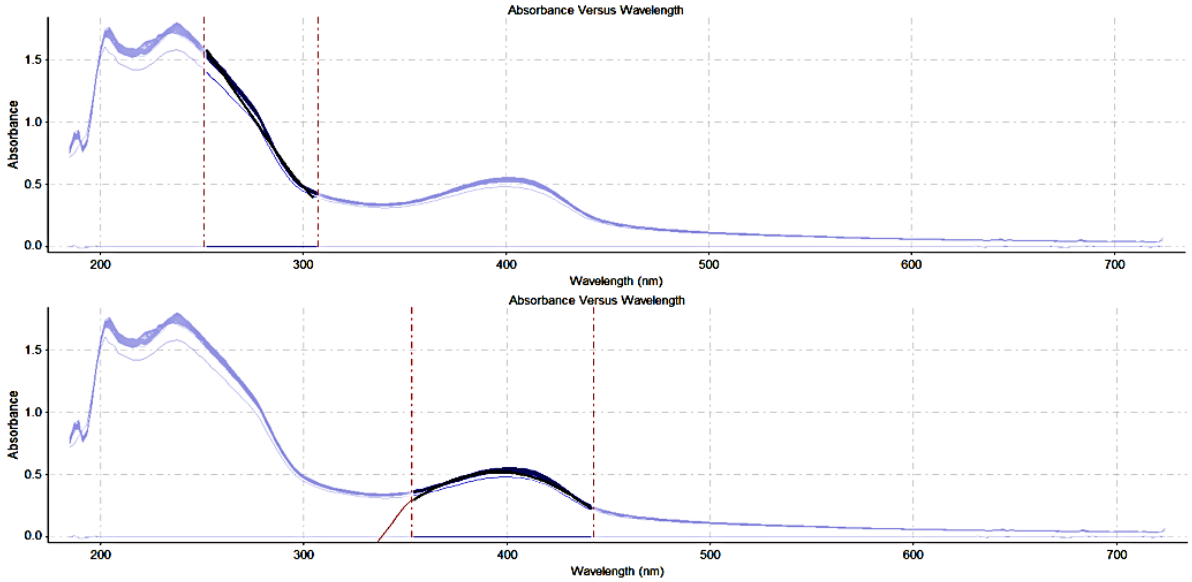

Figure 3.45 UV spectrum of a 0.5% w/v dexamethasone and 0.5% w/v nepafenac FDC nanosuspension performed using Sirius inForm; simultaneous determination of both drugs during dissolution at 250 – 300 nm (dexamethasone, top) and 350 – 450 nm (nepafenac, bottom); the dark line indicates the goodness of fit for the molar extinction coefficient for the respective drug

As can be seen in Figure 3.46 dexamethasone and nepafenac microcrystals dissolved under non-sink conditions within 2 min and 3.5 min from the FDC microsuspension until reaching their specific saturation solubility.

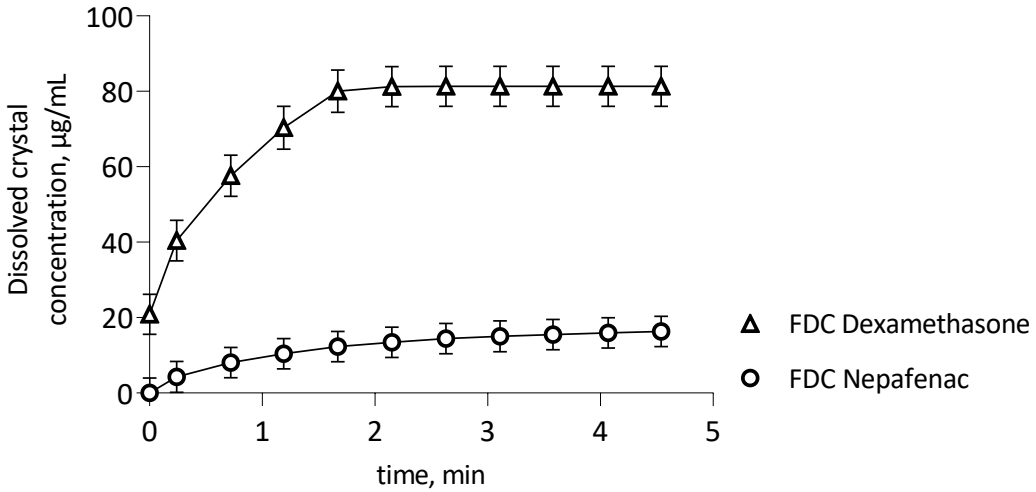

Figure 3.46 Absolute dissolution under non-sink conditions of a 0.5% w/v nepafenac and 0.5% w/v dexamethasone FDC microsuspension

Crystal size is inversely related to dissolution rate as per Noes-Whitney equation [146]. By decreasing the crystal size of both drugs from micro to nano, dissolution rate of the individual nanosuspension of nepafenac and dexamethasone were increased nearly 10-fold (Figure 3.47).

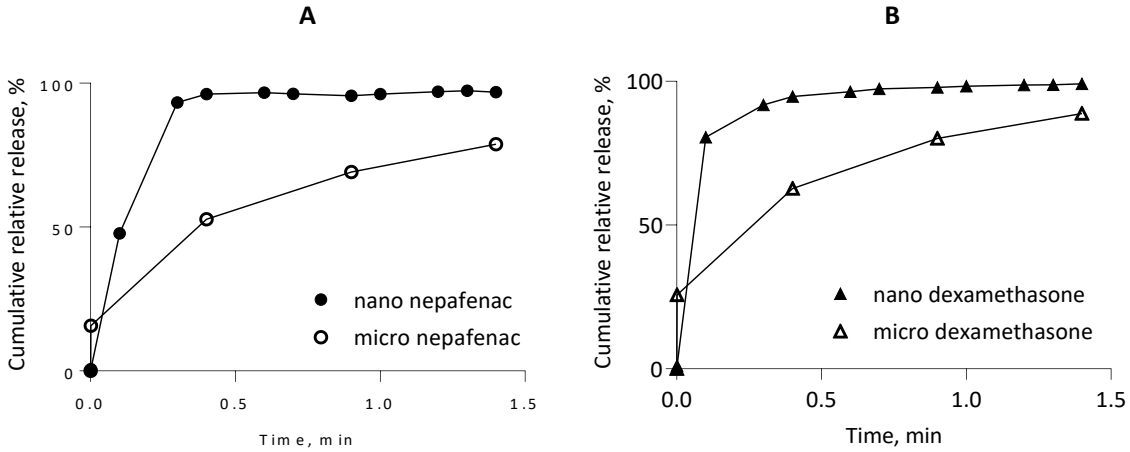

Figure 3.47 Dissolution of (A) 0.5% w/v nepafenac and(B) 0.5% w/v dexamethasone from single micro- and nanosuspensions, n=1

As has been shown in combined milling approaches, particle size could be further decreased when nepafenac and dexamethasone were milled together. While the d90 was more than halved compared to the individual nanosuspensions, also the d50 was decreased from 0.16 (dexamethasone) and 0.14 µm (nepafenac) to 0.12 µm in the FDC nanosuspension. Figure 3.48 showed the additional effect of dissolution rate when particle size d50 was further reduced by at least 0.02 µm within the FDC nanosuspension.

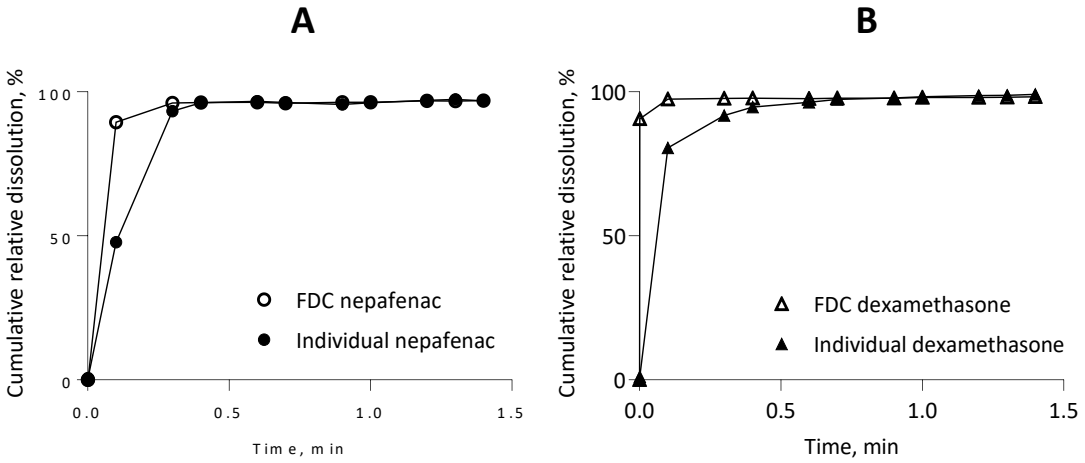

Figure 3.48 Dissolution of (A) 0.5% w/v nepafenac and (B) 0.5% w/v dexamethasone from the individual or from within the FDC nanosuspension, n=1

Several challenges arose during the preparation and characterization of FDC nanosuspensions. First, two drugs must be observed in terms of physical stability. This chapter suggested the approach of selective dissolution, successfully providing the *in situ* particle size of the individual compound within an FDC suspension. Preparation also needed special considerations. Combined milling of two drugs emerged as beneficial regarding risk of recrystallization and milling efficiency. The latter one was significantly improved in the presence of the more brittle compound nepafenac, which increased the total active milling volume during milling. Finally, beneficial effect of combined milling on dissolution was shown for the FDC nanosuspension. As combined milling of dexamethasone and nepafenac reduced particle size compared to the individual nanosuspensions, dissolution rate could be increased even further compared to the single micro- and nanosuspensions.

3.4 Preparation and optimization of long-term physical stability of fixed dose combination nanosuspensions

Stabilizer type and molecular weight are critical in terms of dispersion- and milling efficiency. While nepafenac generally resulted in monomodal nanosupensions with a particle size d90 of about 0.2 µm, loteprednol milling efficiency was dependent on different stabilizer types. There, surfactants were generally superior in terms of loteprednol milling efficiency, due to their amphiphilicity and effect on surface tension [32].

As shown previously, milling the two compounds together could increase milling efficiency for loteprednol. As nepafenac was easily milled to nanocrystals very early in the process, breaking events were increased for loteprednol based on the significantly higher total active milling volume. This effect was present in all stabilizer systems as long the two drugs were milled in combination. In the presence of surfactants, milling efficiency in the fixed dose combination (FDC) was doubled for loteprednol resulting in a particle size d90 decrease from 0.60 to 0.25 µm. Although still comprising a µm-fraction, milling efficiency was also increased for high molecular weight polymers, seen in the reduced d90 of 10 – 30% compared to the individual loteprednol suspensions (Figure 3.49). As it was already described for loteprednol individual nanosuspensions, polymer type or molecular weight did not impact milling efficiency.

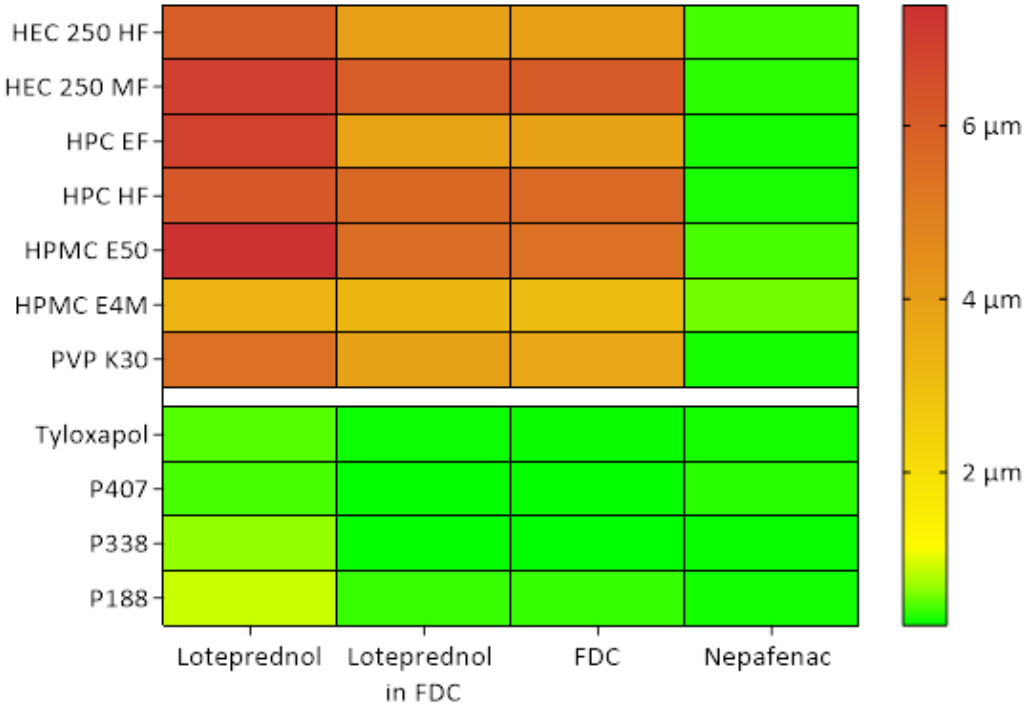

Figure 3.49 Milling efficiency expressed as particle size d90 of individual loteprednol, loteprednol within the FDC (performed with selective dissolution), loteprednol and nepafenac FDC (FDC) and individual nepafenac suspensions milled with 1.25% polymers or 5% surfactants

Aside reaching small sizes upon milling, nanosuspensions must be physically stable during storage or to ensure adequate shelf-life depending on the intended final dosage form. Surfactants were the best candidates to obtain FDC nanosuspensions based on increased loteprednol milling efficiency. Due to their drug solubilizing effect and reported unstable adsorption [88], Poloxamers however failed to keep the nanosuspensions physically stable (Figure 3.50). Poloxamer 407 (P407), with the highest poly(propylene oxide) (PPO) portion and molecular weight of the three poloxamers investigated, resulted in comparably better physical stability, but with a d90 growing at about 1.5 µm/month. Tyloxapol-stabilized systems on the other hand remained completely physically stable.

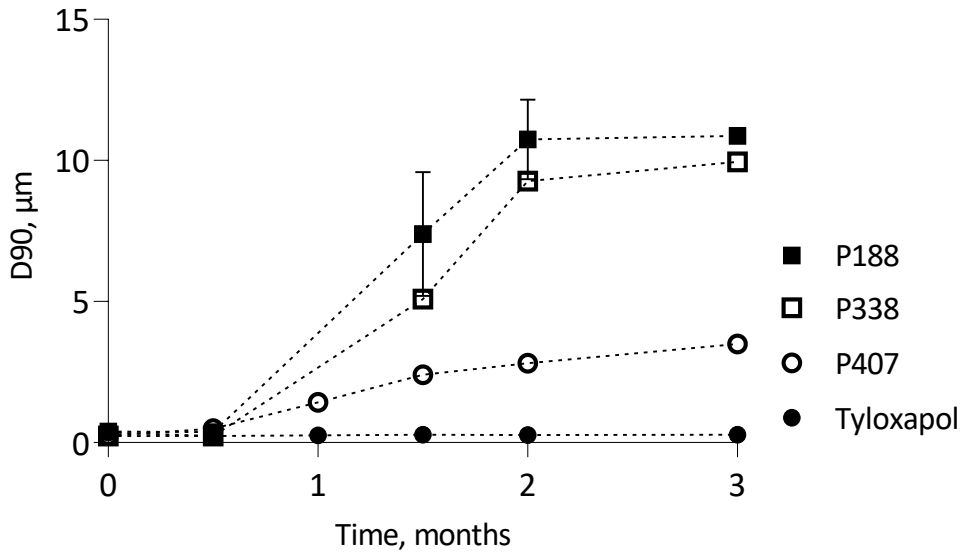

Figure 3.50 Evolution of 0.5% w/v loteprednol and 0.5% w/v nepafenac FDC nanosuspension particle size d90 in dependence of surfactant type at 0.5% during storage at 40 °C/75%RH

In contrast to surfactants, high molecular weight polymers left a clear µm-fraction in the FDC nanosuspensions represented in a d90 of 4 – 6 µm for the different polymers. The restricted diffusion and slow adsorption of these polymer left the crystal surfaces incompletely covered, resulting in a bimodal size distribution. Viscosity was also increased in these systems, reducing transferred stressed energies during milling.

However, in the case of the FDC nanosuspensions, kinetic adsorption over storage time seemed relevant (Figure 3.51). Within 2-month storage, particle sizes of all high molecular weight polymer systems neared that of surfactant-containing FDC nanosuspensions, without a µm-fraction present. This phenomenon was already described elsewhere, with a higher molecular weight resulting in longer particle size equilibration time [62]. It was explained by low adsorption kinetics of the high molecular weight polymers, which could however not fully explain the effects observed in this study.

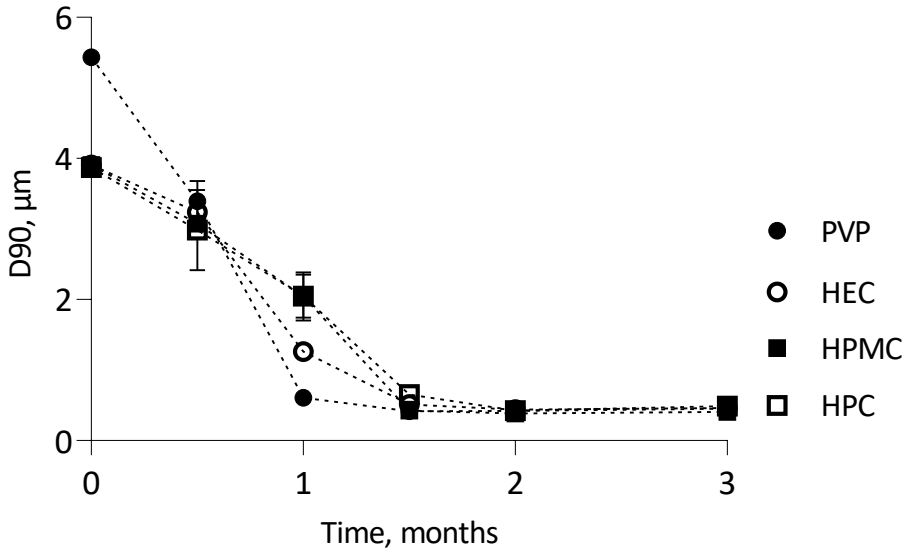

Figure 3.51 Evolution of 0.5% w/v loteprednol and 0.5% w/v nepafenac FDC nanosuspension particle size d90 stabilized with 0.125% non-ionic polymers during storage at 40 °C/75%RH

The significant decrease in d90 was observed in primary particle size. Primary particle size refers to the steady state particle size reached upon measurement. While initial particle size reached steady state at very early measurement times (1 min), measurement time increased to more than 5min with storage time, resulting however in smaller primary particle sizes. This decrease from apparent to primary particle size was associated with the µm-fraction being agglomerates, which loosened during storage and ultimately could be separated into primary particles over measurement time.

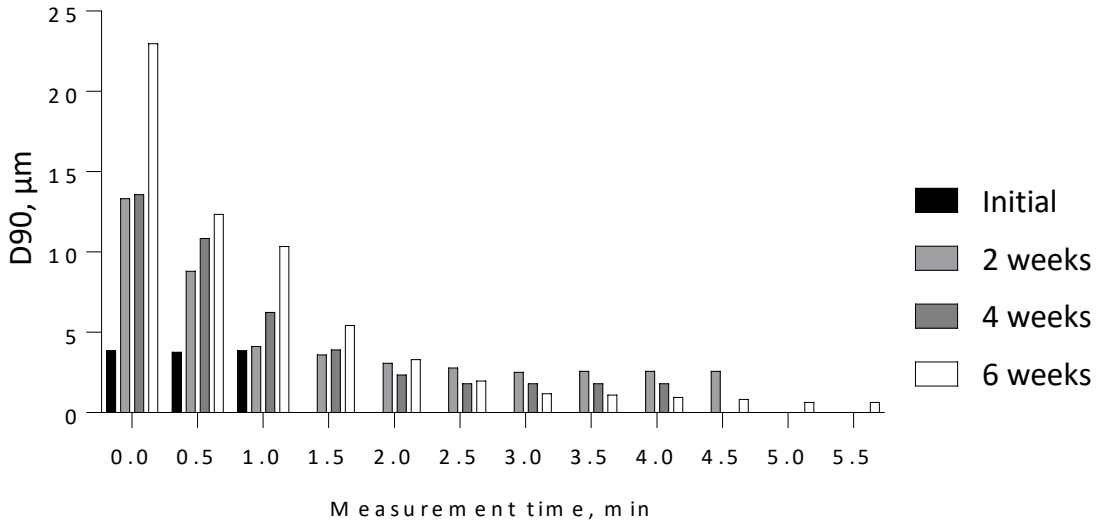

Figure 3.52 Equilibration of 0.5% w/v loteprednol and 0.5% w/v nepafenac FDC nanosuspension primary particle size d90 stabilized with 0.125% HPC HF over measurement time and in dependence of storage time (40 °C/75%RH)

The apparent µm-fraction could be attributed to loteprednol by selective dissolution. Furthermore, high molecular weight polymers also left a significant µm-fraction in the single loteprednol suspensions upon milling (Figure 3.49). In contrast to the FDC nanosuspension, individual loteprednol suspensions did however not show the unusual behaviour of particle size decrease when milled with polymers (Figure 3.17). Agglomeration and relaxation behaviour must hence be due to combined milling with nepafenac.

Non-ionic polymer stabilization is facilitated by polymer adsorption with a significant kinetic aspect due to slow movement of high molecular weight polymer chains [62]. Observing this decrease in particle size over time solely for FDC nanosuspensions might therefore be due to a contrary effect between high milling efficiency in the FDC and comparably slow polymer adsorption. Combined milling of loteprednol and nepafenac would result in smaller particle sizes compared to the single loteprednol suspensions due to the larger active milling volume upon milling. If nanocrystals were formed during milling but polymer adsorption was not rapid enough, several effects could explain the µm-crystal artifacts.

Deng at al. described the nanosized particles obtained through high-energy wet milling to be in a “far-from-equilibrium”-state right after milling [147]. This indicated also for this study, that the higher breakage rate in the presence of nepafenac resulted in loteprednol

nanocrystals, however the fresh surfaces were not readily covered with polymer and therefore remained in a “far-from-equilibrium”-state after milling. Partially covered crystals might then agglomerate or aggregate. Partially uncovered crystals were furthermore suspectable to polymer bridging due to the excessive chain lengths of the stabilizers available [94], [148].

All these phenomena are to some point reversible as they concern the apparent and not the primary particle size. A relaxation of the system by either free polymer adsorption or deagglomeration due to repulsive effects might explain the decrease in particle size over time (Figure 3.53).

It must also be noted that during milling, shear thinning behaviour of the polymers eased particle movement. After milling the system abruptly turned toward higher viscosities which hindered particle motion. This might explain the long equilibration times observed in this study.

Once equilibrium particle size was reached, the FDC nanosuspensions comprising polymers remained physically stable. In contrast to poloxamer type surfactants, polymers tend to adhere irreversibly hence facilitate long-term physical stability. However, observed post-milling relaxation must be avoided since the unpredictable size evolution during shelf-life was considered undesirable.

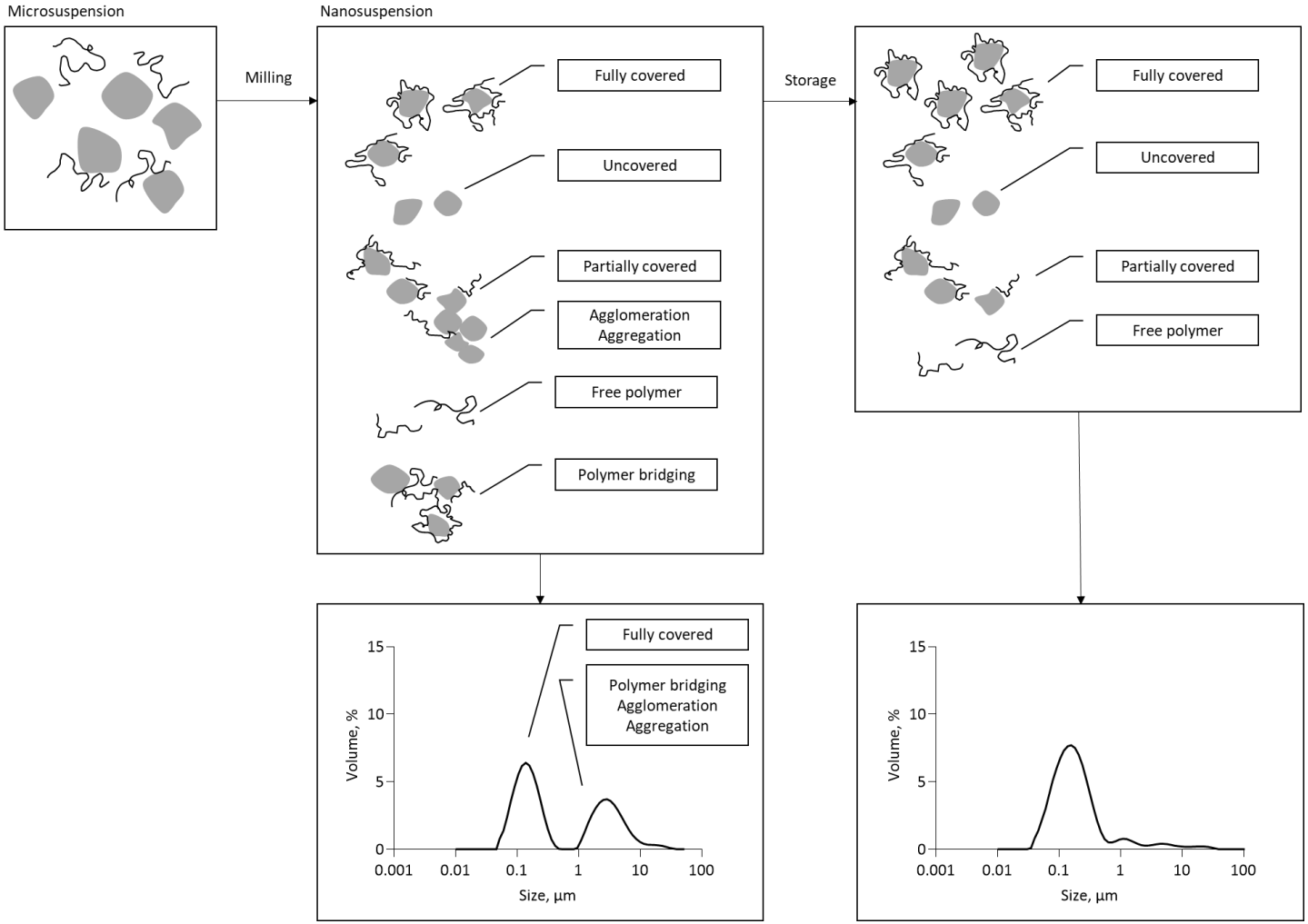

Figure 3.53 Schematic of decreasing particle size for FDC nanosuspensions over storage time by either free polymer adsorption or deagglomeration of “far-from-equilibrium”-state after milling

Physical long-term stabilization of FDC nanosuspensions is hence a major challenge. As observed before, instability of nanosuspensions may manifest by a shift to larger particle sizes due to particle growth or irreversible aggregation.

Individual and FDC nanosuspensions were prepared and stored at 40 °C/75%RH to challenge the physical stability.

P407 was an efficient milling aid for all systems; the individual drugs as well as the FDC nanosuspension. Physical stability could however not be maintained for loteprednol nanosuspensions, with a complete loss of nanocrystals within 6-month storage at 40 °C/75%RH (Figure 3.54 A). As observed before for other poloxamers, final particle size of loteprednol levelled off at the thermodynamically favourable size of the initial drug powder with a d90 of around 9 µm. Nepafenac on the other hand remained physically stable in the presence of P407. Therefore in the FDC, both fractions were present, with the µm-fraction being attributed to loteprednol using selective dissolution (Figure 3.54 C and D).

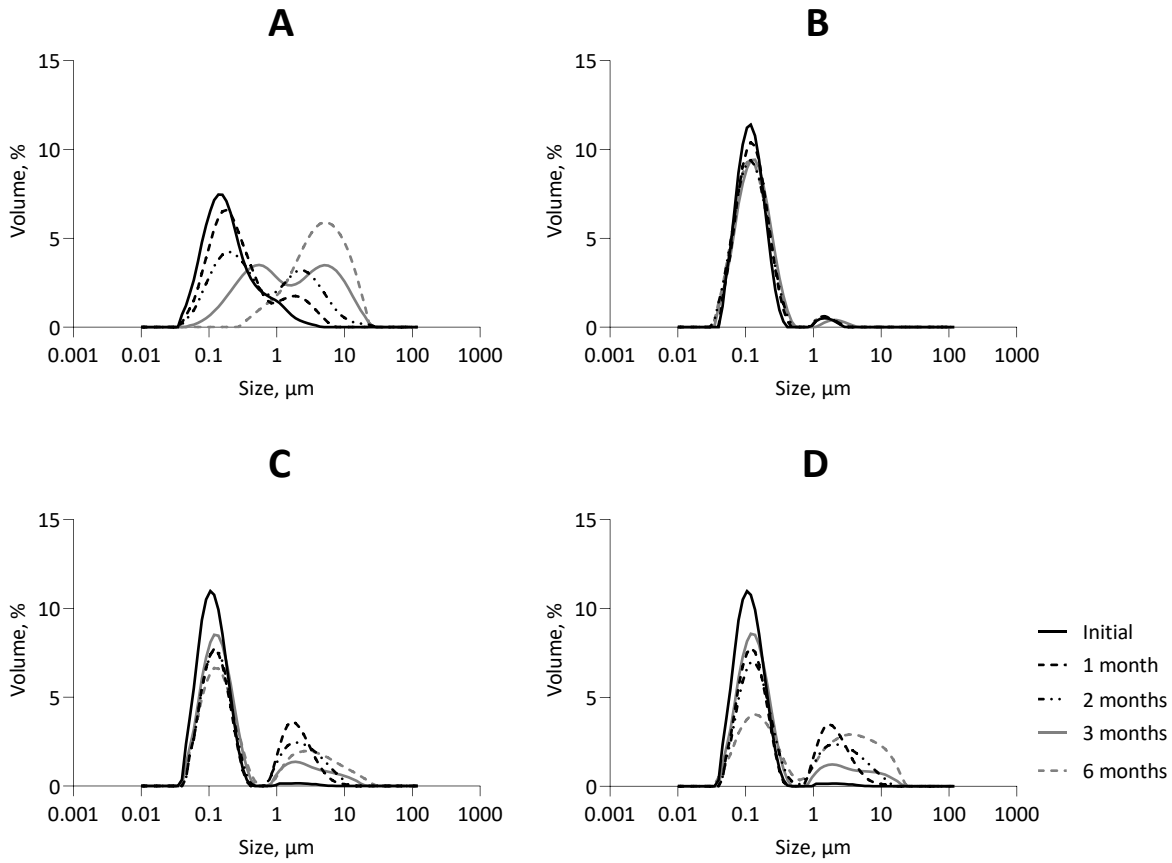

Figure 3.54 Volumetric PSD of (A) 0.5% w/v loteprednol, (B) 0.5% nepafenac, (C) 0.5% loteprednol and 0.5% nepafenac FDC nanosuspensions with 0.5% P407 stored at 40 °C/75%RH and (D) loteprednol within the FDC nanosuspension performed with selective dissolution

Growth rate of loteprednol d90 was lower within the FDC compared to the individual suspension, as can be seen from the nm-fraction still present after 6-month storage. This is due to the significantly smaller initially particle size when milled together with nepafenac.

To prove the hypothesis of smaller initial particle size slowing down the growth rate, loteprednol was prepared at two different initial particle sizes and stored as individual nanosuspension. Smaller particle sizes were obtained by decreasing the milling bead size during preparation. Crystal growth upon storage followed the same trend, only reduced by the factor of the initially higher milling efficiency (Figure 3.55).

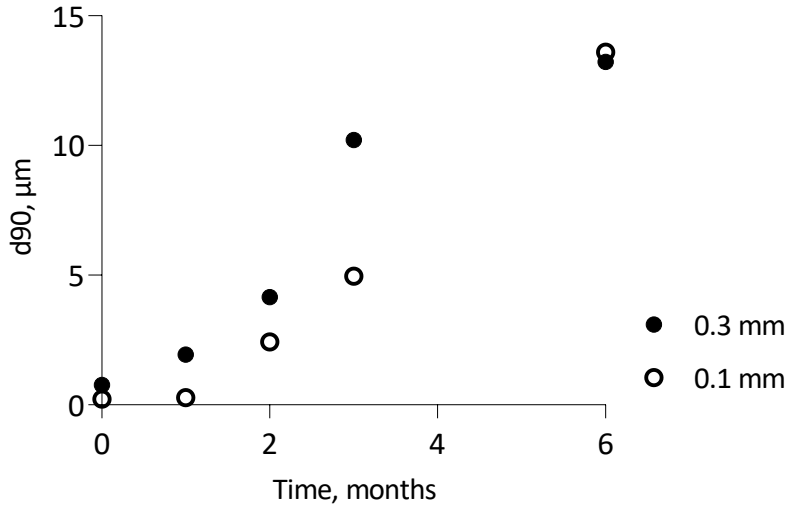

Figure 3.55 Evolution of 0.5% w/v loteprednol particle size d90 with 0.5% P407 during storage at 40 °C/75RH, milled with 0.1 mm and 0.3 mm beads (1:2 v/v)

This highlighted that milling efficiency alone (a) could not prevent crystal growth and (b) did not serve as predictor for storage stability. As mentioned in a previous chapter, stabilizer attachment and increased solubility play a crucial role in terms of long-term physical stability.

Aside stabilizer choice, other formulation parameters were thus investigated to increase the physical stability of the inherently unstable P407 system. Increased viscosity and the introduction of a stabilizer combination both were successful in preventing P407 physical instabilities (Figure 3.56). With growth rates of 0.01 µm/month and below, different degrees of freedom were given to achieve a physically stable FDC nanosuspension.

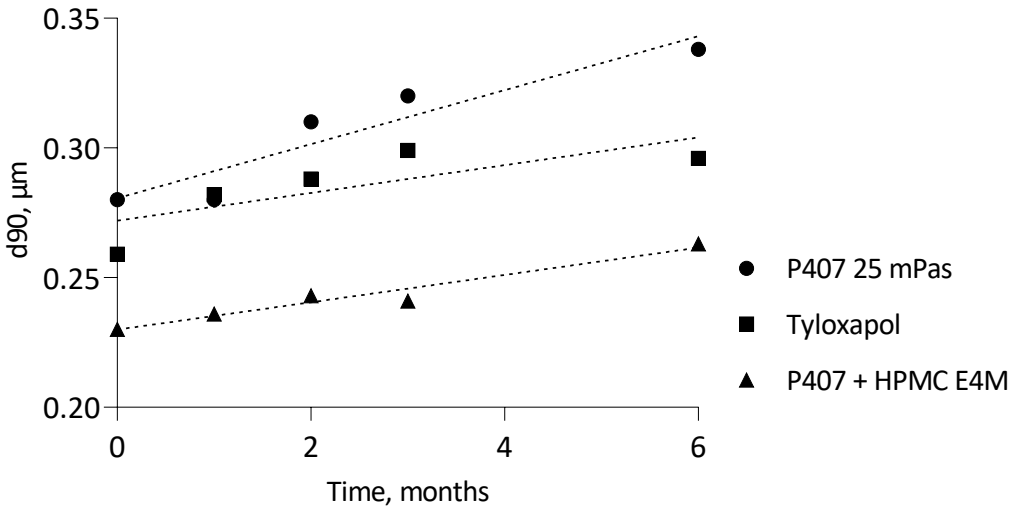

Figure 3.56 Growth rate of 0.5% w/v loteprednol and 0.5% w/v nepafenac FDC nanosuspensions with 0.5% P407 and 0.5% HPMC E4M as viscosity agent at 25 mPas, 0.5% Tyloxapol or 0.5% P407 and 0.125% HPMC E4M as stabilizer combination during storage at 40 °C/75%RH

Increasing the zero-shear viscosity of the continuous phase of an emulsion or suspension is a popular method to increase physical stability. The addition of 0.5% HPMC allowed kinetic stability to counteract thermodynamic instabilities, which rendered the system overall physically stable. Different polymers were therefore introduced to the FDC nanosuspensions, all resulting in a viscosity of 25 mPas. Positive effect of viscosity was observed irrespectively if Carbopol 974, hydroxypropylmethylcellulose (HPMC) K4M or HPMC K100M were used (Figure 3.57). This indicated that the network, rather than any specific interaction stabilized the nanocrystals.

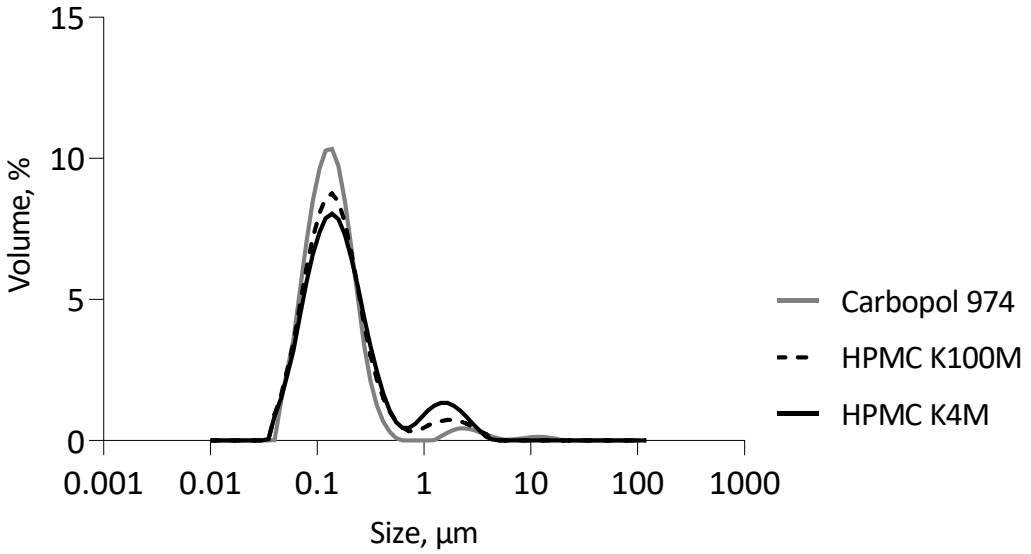

Figure 3.57 Volumetric PSD of 0.5% w/v loteprednol and 0.5% w/v nepafenac FDC nanosuspensions at 25 mPas using 0.5% P407 and 0.5% HPMC K4M, 0.2% HPMC 100M or 0.05% Carbopol 974 after 3-month storage at 40 °C/75%RH

Apart from crystal growth, also sedimentation occurred in all suspension, thus imposing the risk of inhomogeneity and unknown dose upon administration. Stokes' law states that sedimentation velocity is inversely related to viscosity and directly proportional to particle size. For particles of a given size, doubling the suspension viscosity would therefore halve the rate of sedimentation. Viscosity of the system was about 6-fold increased at 0.5% HPMC E4M and accompanied with a decrease in sedimentation velocity from 11 µm/s and 3 µm/s (Figure 3.58). Velocity reduction was however less than expected. Sedimentation was very likely more complex due to potential interactions between neighbouring particles and agglomeration that are not considered in Stokes' law.

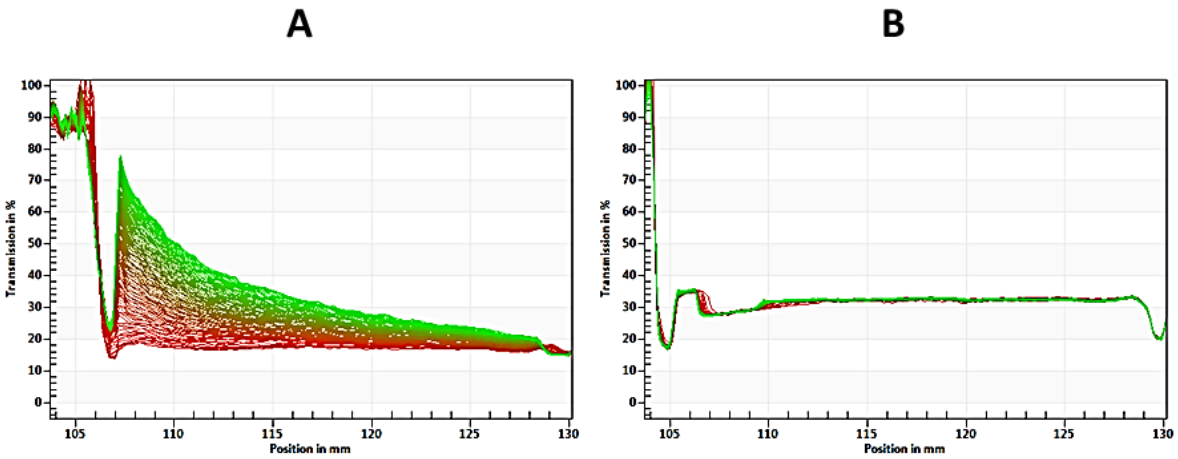

Figure 3.58 Sedimentation profile (Transmission vs. Position of cell) of (A) 0.5% w/v loteprednol and 0.5% w/v nepafenac FDC nanosuspensions in 0.5% P407 at 4 mPas and (B) 0.5% P407 and 0.5% HPMC E4M at 25 mPas

An ideal formulation would maintain the dispersed phase in a uniformly-suspended state for the lifetime of the product. Increasing the formulation's viscosity counteracted but did not fully prevent sedimentation and redispersibility was worsened compared to P407 alone, with more than 20 instead of 15 inversions needed to resuspend all crystals after 6-month storage. As crystals could not be maintained in a discrete suspended state, it was preferable to prevent tight cohesion of small particles by intentionally inducing flocculation. Although flocs settled more rapidly than individual discrete particles at increased viscosity, flocculated crystals formed a lattice that resisted complete settling and was thus less prone to compaction (Figure 3.59).

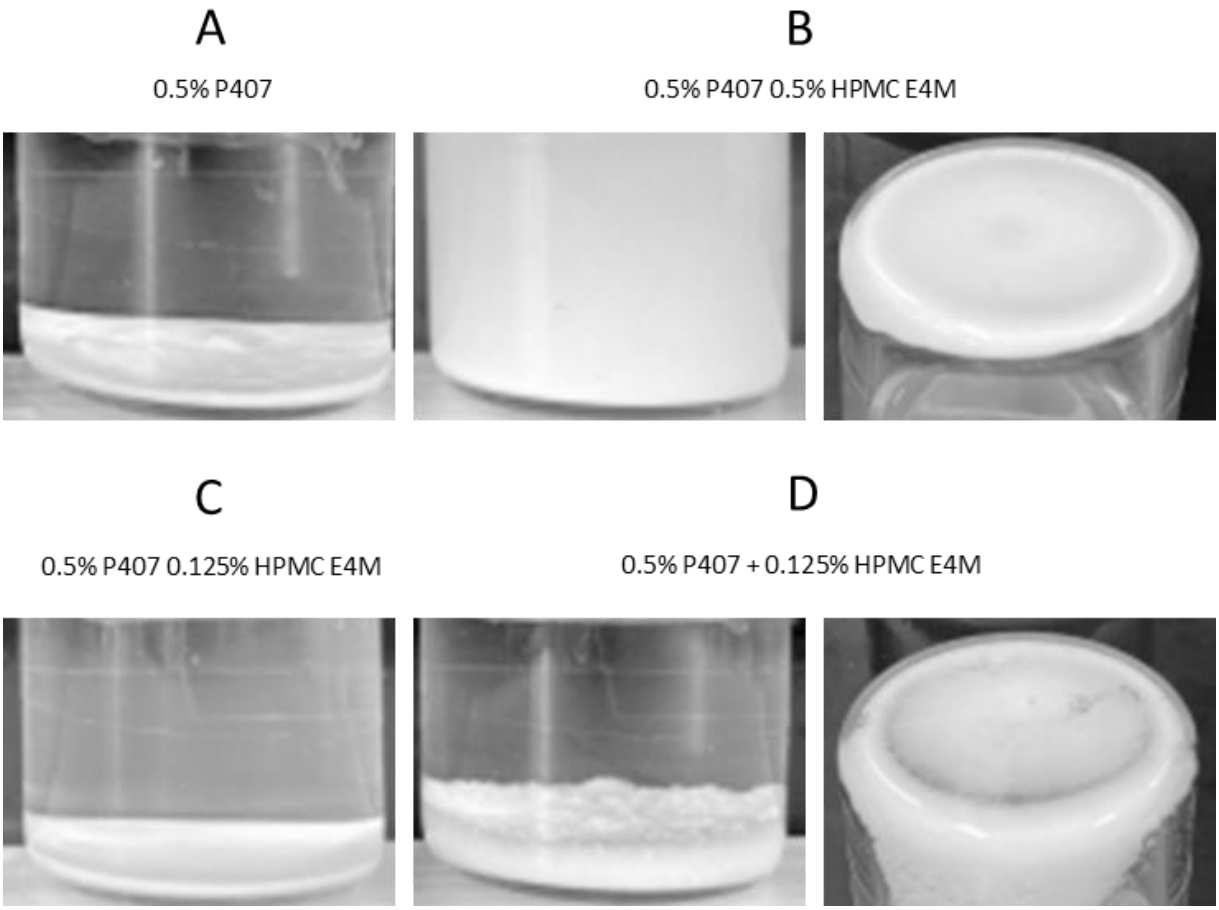

Figure 3.59 Physical appearance of 0.5 % w/v loteprednol and 0.5% w/v nepafenac FDC nanosuspensions after 6-month storage at 40 °C/75%RH; (A) 0.5% P407, (B) 0.5% P407 in 0.5% HPMC E4M (25 mPas) upright and upside-down, (C) 0.5% P407 in 0.125% HPMC E4M and (D) stabilizer combination of 0.5% P407 and 0.125% HPMC E4M upright and upside-down

With the introduction of a flocculated system, redispersibility could be decreased from 15 inversions to less than 5 inversions required to obtain a completely homogeneous suspension. Aside flocculation, also physical stability was remarkably increased compared to P407 alone. Based on stabilizer screening results, 0.125% HPMC E4M was introduced in a stabilizer combination with 5% P407. The system counteracted crystal growth, decreasing the d90 growth rate from 1.5 to even below 0.001 µm/month (Figure 3.60).

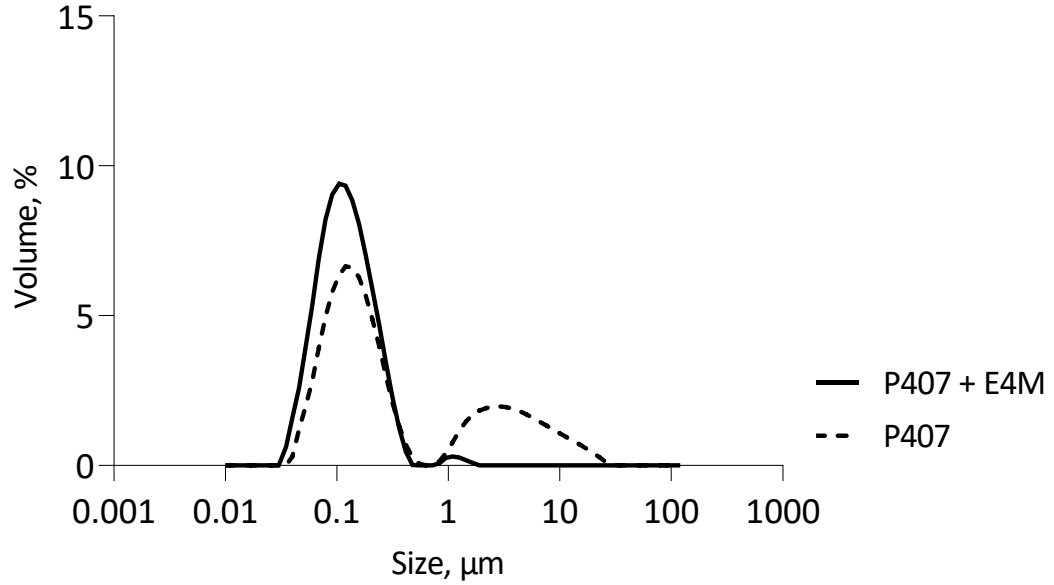

Figure 3.60 Volumetric PSD of a 0.5% w/v loteprednol and 0.5% w/v nepafenac FDC nanosuspension after 6-month storage at 40 °C/75%RH with 0.5% P407 or a stabilizer combination of 0.5% P407 and 0.125% HPMC E4M

It is important to highlight the difference between adding HPMC after or during milling. The first one did not alter physical stability, whereas the latter one maintained the FDC nanosuspension stable over the complete 6-month storage at 40 °C/75%RH (Figure 3.61).

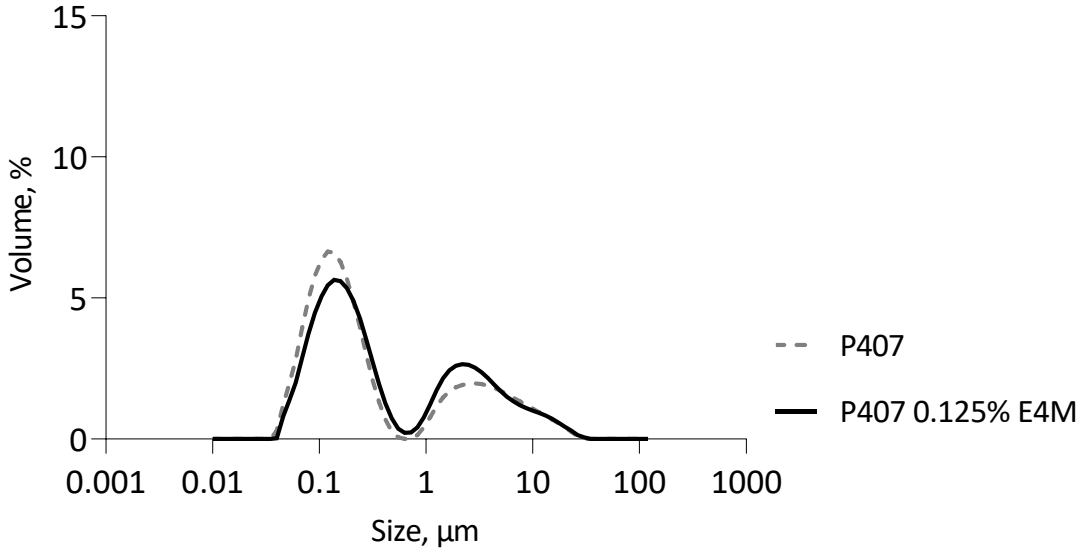

Figure 3.61 Volumetric PSD of a 0.5% w/v loteprednol and 0.5% w/v nepafenac FDC nanosuspension after 6-month storage at 40 °C/75%RH with 0.5% P407 or 0.5% P407 and 0.125% HPMC E4M in the outer phase

Flocculation by polymer bridging involves the adsorption of the polymer on crystal surfaces and the formation and growth of flocs. This explained why it was important to add the polymer during milling, as otherwise crystal sites were not available for HPMC adsorption. If added after milling, HPMC acts as a viscosity agent only, without induction of flocculation (Figure 3.59 C vs. D).

When comparing different approaches to gain a physically stable FDC nanosuspension, only one stabilizer emerged as suitable, namely Tyloxapol. While other surfactants resulted in significant ripening, polymers showed insufficient milling efficiency and unpredictable size evolution upon storage. This highlights, that other formulation approaches must be considered in many cases. Increasing the outer phase viscosity counteracted crystal growth via kinetic aspects, but worsened resuspendability. The approach of combining a surfactant and a polymer resulted in a physically stable system with small particle sizes. When milled in the presence of both stabilizers, a flocculated system which was easy to redisperse emerged.

4 Summary

Optimization of nanosuspensions produced by wet bead milling is a complex process involving many factors that affect milling efficiency. This thesis focused on the identification of key stabilizer properties that have a direct outcome in the formation of a stable nanosuspension. Furthermore, critical process parameters in the preparation of fixed dose combination (FDC) nanosuspensions were described. Finally, formulation parameters determining the long-term physical stability of FDC nanosuspensions were described.

A comprehensive stabilizer screening was initially performed on the individual drugs. Loteprednol was milled in the presence of five surfactants at two concentrations. Higher concentration generally resulted in smaller particle sizes. Surfactant hydrophobicity ($r = 0.68$) and surface activity ($r = 0.87$) were observed to positively affect milling efficiency. For PEO-PPO-PEP block polymers (Poloxamer) a sufficient PPO fraction was crucial to interact with the drug surface as indicated in decreasing contact angles at increasing milling efficiency. The best candidate for milling loteprednol was Poloxamer 407. Two factors could however be further optimized: milling to smaller particle sizes and a more monomodal particle size distribution. Poloxamer 407 milling efficiency was further tuned from a d90 of 550 to about 200 nm by introducing glycerol or NaCl to the milling media. Poloxamer 407 conformation as a function of osmotic agent concentration suggested a stretching of the polymer chains thus increasing the steric stabilization. Chain collapse due to high medium ionic strength was then also accompanied by a loss in milling efficiency.

The success rate of high molecular weight polymers on the other hand was generally limited by insufficient wetting and increased media viscosity. In contrast to surfactants, milling with high molecular weight polymers left a significant µm-fraction of about 50% of total volume, ending far below the milling efficiency of surfactants.

Under accelerated storage at 40 °C/75%RH, surfactants however only insufficiently stabilized the loteprednol nanosuspensions, with a growth rate of at least 2.5 µm/month while particle size was completely maintained for non-ionic polymers. It became apparent that a combination of 0.5% Poloxamer 407 and 0.125% HPMC E4M could significantly increase milling efficiency to a d90 of 200 nm and result in superior storage stability with a negligible

growth rate of 0.005 µm/month. This was explained by a synergistic co-adsorption of both stabilizers.

Combination therapy with two drugs being classified as poorly soluble, could be formulated as an FDC nanosuspension. Several difficulties arise during formulation development of such products, especially regarding their production and characterization. This thesis presented the approach of selective dissolution, providing the *in situ* particle size of the individual compound within the FDC. The approach was based on the difference in drug solubilities, facilitating the *in situ* dissolution of nepafenac during the laser diffraction measurement and thus facilitating the particle size determination of loteprednol within the FDC.

Two approaches were considered for the preparation of the FDC nanosuspensions, separate or combined milling of the two individual drugs. Using selective dissolution, precipitation of loteprednol was detected in the presence of the second drug nepafenac after dilution. This was attributed to critical changes in solubility after combining the two single nanosuspensions, making the approach of separate milling unfeasible for this FDC.

It became apparent that milling nepafenac and loteprednol together increased milling efficiency of the more challenging compound loteprednol by 50% for surfactants and by 10-30% for non-ionic polymers compared to the single nanosuspension. The increase in loteprednol milling efficiency was attributed to an increase in total active milling volume due the presence of nepafenac. The same effect was seen in a combination of dexamethasone and nepafenac. Nepafenac reached nm-sizes very early in the process, which seemed an important factor as a simple increase in e.g. volume fraction did not alter loteprednol or dexamethasone milling efficiency. Drug brittleness was indicated as a key parameter. Brittleness of three drug powders was determined and brittleness was the highest for nepafenac, followed by loteprednol and dexamethasone. As particle size determines dissolution rate, dissolution studies were subsequently performed for the nepafenac-dexamethasone FDC and the beneficial effect of combined milling on the dissolution of dexamethasone and nepafenac FDC nanosuspensions could be shown.

Nanosuspensions are thermodynamically unstable systems and tend to agglomerate or exhibit crystal growth upon storage. When preparing an FDC nanosuspension, both milling efficiency and storage stability were considered.

Poloxamer 407 was the best candidate to obtain a loteprednol and nepafenac FDC nanosuspension with a d90 of 240 nm. It however failed to maintain the nanosuspension physically stable upon storage at 40 °C/75%RH. In contrast, high molecular weight polymers left a clear µm-fraction in the FDC nanosuspension after milling. Kinetic adsorption seemed to however be relevant over storage time, where minimum particle sizes with a d90 around 400 nm were reached within 2-month storage. This suggested, that the increased breakage rate during combined milling resulted in loteprednol breakage, with the fresh surfaces however not readily being covered with polymer. A relaxation of the system by either free polymer adsorption or deagglomeration due to repulsive effects explained the decrease in particle size over time.

Aside stabilizer choice, other formulation parameters were investigated to increase the physical stability of the inherently unstable FDC nanosuspension. Increased viscosity and the introduction of a stabilizer combination both were successful in preventing physical instabilities and decreased crystal growth rate from 1.5 to 0.01 µm/month and below. Viscosity of the system was increased from 5 to 25 mPas which also was accompanied with a decrease in sedimentation velocity from 11 and 3 µm/s. Increasing the formulation's viscosity however worsened redispersibility after 6-month storage. As crystals could not be maintained in a discrete suspended state, flocculation was induced by a stabilizer combination of 0.5% Poloxamer 407 and 0.125% HPMC E4M. Although flocs settled more rapidly than crystals at increased viscosity, flocculated crystals formed a lattice that resisted complete settling and thus was less prone to cake formation. The stabilizer combination was therefore the formulation approach with the highest milling efficiency and best characteristics upon storage for the FDC nanosuspension.

5 Zusammenfassung

Die Optimierung von Nanosuspensionen, welche über Nassvermahlung hergestellt werden, ist komplex da viele Faktoren die Mahleffizienz bestimmen. Diese Arbeit fokussierte sich darauf, Charakteristika von Stabilisatoren aufzudecken, welche direkten Einfluss auf die Mahleffizienz haben. Des Weiteren wurden kritische Prozessparameter in der Herstellung von Kombinationsnanosuspensionen beschrieben. Abschließend wurden Formulierungsparameter, welche die physikalische Langzeitstabilität der Kombinationsnanosuspension bestimmen untersucht.

Loteprednol wurde mit fünf Tensiden zu jeweils zwei Konzentrationen vermahlen. Eine höhere Tensidkonzentration erhöhte generell die Mahleffizienz. Die Hydrophobizität ($r = 0.68$) und Öberflächenaktivität ($r = 0.87$) des Tensids waren bei der Auswahl entscheidend. Für PEO-PPO-PEO Blockpolymere war der Anteil an PPO entscheidend, wie aus Kontaktwinkelmessungen hervorging.

Poloxamer 407 war der aussichtsreichste Kandidat, um Loteprednol zu vermahlen. Mahleffizienz und die Homogenität der Partikelgröße konnten jedoch durch den Zusatz von Glyzerin und NaCl noch weiter von einem d90 von 550 zu 200 nm gesteigert werden. Poloxamer 407 zeigte in Abhängigkeit der Glyzerin- oder NaCl-Konzentration ein Streckungsverhalten der hydrophilen Seitenketten, welches die bessere sterische Stabilisierung erklärte. Ab einer kritischen Ionenstärke kam es zu einem Polymerkollaps, welcher mit einer Verringerung der Mahleffizienz einherging.

Im Gegensatz zu Tensiden zeigten Polymere eine verminderte Mahleffizienz auf Grund der geringeren Benetzung der Oberfläche und die erhöhte Viskosität der Mahllösung. Dies zeigte sich in einer signifikanten µm-Fraktion mit einem Volumenanteil von bis zu 50% und weit unter der Effizienz der Tenside.

Gelagert bei 40 °C/75%RH zeigten Tenside jedoch Defizite in der Stabilisierung, mit Kristallwachstumsraten von mindestens 2.5 µm/Monat. Die Partikelgröße der mit nicht-ionischen Polymere stabilisierten Loteprednol-Suspensionen blieb hingegen konstant. Die Kombination aus 0.5% Poloxamer 407 and 0.125% HPMC E4M konnte die Mahleffizienz signifikant steigern mit einem d90 von 200 nm. Darüber hinaus wurde auch die Lagerstabilität

mit einer vernachlässigbaren Kristallwachstumsrate von 0.005 µm/Monat deutlich verbessert. Diese wurde auf eine synergistische Ko-Adsorption der beiden Stabilisatoren zurückgeführt.
Eine Kombinationtherapie mit zwei jeweils schwer löslichen Wirkstoffen kann als Kombinationsnanosuspension formuliert werden. Hier ergeben sich Hürden während der Formulierungsentwicklung, insbesondere bezüglich Herstellung und Charakterisierung. Innerhalb dieser Arbeit wurde die Methode der selektiven Auflösung entwickelt, um die *in situ* Partikelgröße der individuellen Wirkstoffes innerhalb des Kombinationsproduktes zu bestimmen. Der Ansatz basiert auf den unterschiedlichen Löslichkeiten von Loteprednol und Nepafenac. Diese ermöglichen es, Nepafenac *in situ* aufzulösen und somit die Partikelgröße Loteprednols innerhalb der Kombinationssuspension zu messen.
Mit dieser Methode konnte nachgewiesen werden, dass Loteprednol in Anwesenheit Nepafenacs präzipitiert. Dies konnte auf eine Änderung der Löslichkeit Loteprednol zurückgeführt werden, welche nach Kombination der einzelnen Mahlansätze zur Präzipitation führte. Daher war der Ansatz der individuellen Vermahlung für diese Wirkstoffkombination nicht zulässig.
Darüber hinaus zeigte das gemeinsame Vermahlen der beiden Wirkstoffe den Vorteil, dass die Mahleffizienz Loteprednols bis zu 50% bei Tensiden und 10-30% bei nicht ionischen Polymeren anstieg. Die erhöhte Mahleffizienz wurde mit dem erhöhten totalen aktiven Mahlvolumen in Gegenwart Nepafenacs in Verbindung gebracht. Das gleiche Phänomen wurde auch in der Mahleffizienz Dexamethasons mit gesehen, wenn mit Nepafenac vermahlen. Nepafenac erreicht Nanogrößen sehr früh im Prozess, was für die erhöhte Mahleffizienz essenziell war. Eine reine Erhöhung des Volumenanteils Loteprednols oder Dexamethasons zeigte diesen Effekt nicht. Der positive Einfluss Nepafenacs wurde auf dessen Brüchigkeit zurückgeführt, welche über der Loteprednols und Dexamethasons lag. Da die Partikelgröße darüber hinaus die Auflösungsrate der Kristalle bestimmt, konnte gezeigt werden, dass sich der positive Effekt des Vermahlens auch auf die Auflösungsrate auswirkt. Diese liegt für Dexamethason und Nepafenac Kombinationsnanosuspensionen über der der einzelnen Nanosuspensionen.

Nanosuspensionen sind thermodynamisch instabile Systeme und neigen während der Einlagerung zu Agglomeration oder Kristallwachstum.
Poloxamer 407 war der vielversprechendste Kandidat in der Vermahlung der Kombinationsnanosuspension, mit einem d90 von 240 nm. Während der Einlagerung zeigte

sich das System allerdings instabil. Dem gegenüber standen Polymere, welche eine eindeutige µm-Fraktion nach der Vermahlung aufwiesen. Hier griffen jedoch kinetische Adsoprtionsprozesse welche dazu führten, dass die eingelagerten Nanosuspensionen innerhalb von zwei Wochen eine Partikelgröße von 240 nm im d90 hatten. Dies wies darauf hin, dass die erhöhte Mahleffizienz in Präsenz Nepafenacs zwar griff, die Polymeranlagerung allerdings nicht schnell genug war um die entstehenden Loteprednol-Nanokristalle ausreichend zu stabilisieren. Die darauffolgende Relaxation des Systems wurde mit der Anlagerung freien Polymers oder der Deagglomeration durch angelagerte Polymerketten erklärt.

Neben der Stabilisatorenwahl stehen noch weitere Parameter zur Verfügung, um die physikalische Stabilität einer Kombinationsnanosuspension zu erhöhen. Erhöhte Viskosität und der Einsatz einer Stabilisatorenkombination konnten die Kristallwachstumsrate von 1.5 auf 0.01 µm/Monat und darunter vermindern. Eine Erhöhung der Viskosität von 5 auf 25 mPas führte überdies zu einer Verringerung der Sedimentationsrate von 11 auf 3 µm/s. Nachteilig war die schlechtere Redispergierbarkeit nach Lagerung. Daher wurde das System durch die Kombination aus 0.5% Poloxamer 407 und 0.125% HPMC E4M kontrolliert ausgeflockt. Die Flocken setzten sich zwar schneller ab, formten jedoch nur ein loses Gitter, welches durch geringes Schütteln aufgebrochen werden konnte. Dadurch konnte gezeigt werden, dass eine Stabilisatorenkombination nicht nur zu hohen Mahleffizienzen, sondern auch zu vielversprechenden Eigenschaften der Langzeitstabilität der Kombinationssuspension führte.

6 References

[1] R. H. Müller and C. M. Keck, “Twenty years of drug nanocrystals: Where are we, and where do we go?,” *Eur. J. Pharm. Biopharm.*, vol. 80, no. 1, pp. 1–3, 2012, doi: 10.1016/j.ejpb.2011.09.012.

[2] H. Yang *et al.*, “Pharmaceutical Strategies for Stabilizing Drug Nanocrystals,” *Curr. Pharm. Des.*, vol. 24, no. 21, pp. 2362–2374, 2018, doi: 10.2174/1381612824666180515125247.

[3] L. Wu, J. Zhang, and W. Watanabe, “Physical and chemical stability of drug nanoparticles,” *Adv. Drug Deliv. Rev.*, vol. 63, no. 6, pp. 456–469, 2011, doi: 10.1016/j.addr.2011.02.001.

[4] B. E. Rabinow, “Nanosuspensions in drug delivery,” *Nat. Rev. Drug Discov.*, vol. 3, pp. 785–796, 2004, doi: 10.1038/nrd1494.

[5] A. Madni *et al.*, “Non-invasive strategies for targeting the posterior segment of eye,” *Int. J. Pharm.*, vol. 530, no. 1–2, pp. 326–345, 2017, doi: 10.1016/j.ijpharm.2017.07.065.

[6] J. Möschwitzer, G. Achleitner, H. Pomper, and R. H. Müller, “Development of an intravenously injectable chemically stable aqueous omeprazole formulation using nanosuspension technology,” *Eur. J. Pharm. Biopharm.*, vol. 58, no. 3, pp. 615–619, 2004, doi: 10.1016/j.ejpb.2004.03.022.

[7] M. Bisrat and C. Nyström, “Physicochemical aspects of drug release. VIII. The relation between particle size and surface specific dissolution rate in agitated suspensions,” *Int. J. Pharm.*, vol. 47, no. 1–3, pp. 223–231, 1988, doi: 10.1016/0378-5173(88)90235-9.

[8] V. B. Patravale, A. A. Date, and R. M. Kulkarni, “Nanosuspensions: a promising drug delivery strategy,” *J. Pharm. Pharmacol.*, vol. 56, no. 7, pp. 827–840, 2004, doi: 10.1211/0022357023691.

[9] S. Kumar, J. Shen, B. Zolnik, N. Sadrieh, and D. J. Burgess, “Optimization and dissolution performance of spray-dried naproxen nano-crystals,” *Int. J. Pharm.*, vol. 486, no. 1–2, pp. 159–166, 2015, doi: 10.1016/j.ijpharm.2015.03.047.

[10] S. X. Yin *et al.*, “Bioavailability enhancement of a COX-2 inhibitor, BMS-347070, from a nanocrystalline dispersion prepared by spray-drying,” *J. Pharm. Sci.*, vol. 94, no. 7, pp. 1598–1607, 2005, doi: 10.1002/jps.20366.

[11] Y. Wu *et al.*, “The role of biopharmaceutics in the development of a clinical nanoparticle formulation of MK-0869: A Beagle dog model predicts improved bioavailability and diminished food effect on absorption in human,” *Int. J. Pharm.*, vol. 285, no. 1–2, pp. 135--146, 2004, doi: 10.1016/j.ijpharm.2004.08.001.

[12] G. G. Liversidge and P. Conzentino, “Drug particle size reduction for decreasing gastric irritancy and enhancing absorption of naproxen in rats,” *Int. J. Pharm.*, vol. 125, no. 2, pp. 309–313, 1995, doi: 10.1016/0378-5173(95)00148-C.

[13] O. Kayser, C. Olbrich, V. Yardley, A. F. Kiderlen, and S. L. Croft, “Formulation of amphotericin B as nanosuspension for oral administration,” *Int. J. Pharm.*, vol. 254, no. 1, pp. 73–75, 2003, doi: 10.1016/S0378-5173(02)00686-5.

[14] S. Verma, S. Kumar, R. Gokhale, and D. J. Burgess, “Physical stability of nanosuspensions: Investigation of the role of stabilizers on Ostwald ripening,” *Int. J. Pharm.*, vol. 406, no. 1–2, pp. 145–152, 2011, doi: 10.1016/j.ijpharm.2010.12.027.

[15] B. P. Binks, “Particles as surfactants - Similarities and differences,” *Curr. Opin. Colloid Interface Sci.*, vol. 7, no. 1–2, pp. 21–41, 2002, doi: 10.1016/S1359-0294(02)00008-0.

[16] L. Wu, J. Zhang, and W. Watanabe, “Physical and chemical stability of drug nanoparticles,” *Adv. Drug Deliv. Rev.*, vol. 63, no. 6, pp. 456–469, 2011, doi: 10.1016/j.addr.2011.02.001.

[17] Y. Wang, X. Li, L. Wang, Y. Xu, X. Cheng, and P. Wei, “Formulation and pharmacokinetic evaluation of a paclitaxel nanosuspension for intravenous delivery.,” *Int. J. Nanomedicine*, vol. 6, pp. 1497–1507, 2011, doi: 10.2147/ijn.s21097.

[18] J. Beirowski, S. Inghelbrecht, A. Arien, and H. Gieseler, “Freeze drying of nanosuspensions, 2: The role of the critical formulation temperature on stability of drug nanosuspensions and its practical implication on process design,” *J. Pharm. Sci.*, vol. 10, pp. 4471–4481, 2011, doi: 10.1002/jps.22634.

[19] Y. Wang, Y. Zheng, L. Zhang, Q. Wang, and D. Zhang, “Stability of nanosuspensions in drug delivery,” *J. Control. Release*, vol. 172, no. 3, pp. 1126–1141, 2013, doi: 10.1016/j.jconrel.2013.08.006.

[20] M. J. Coffey, H. H. Decory, and S. S. Lane, “Development of a non-settling gel formulation of 0.5% loteprednol etabonate for anti-inflammatory use as an ophthalmic drop,” *Clin. Ophthalmol.*, vol. 7, pp. 299–312, 2013, doi: 10.2147/OPTH.S40588.

[21] L. Lindfors, P. Skantze, U. Skantze, M. Rasmusson, A. Zackrisson, and U. Olsson,

"Amorphous drug nanosuspensions. 1. Inhibition of ostwald ripening," *Langmuir*, vol. 22, no. 3, pp. 906–910, 2006, doi: 10.1021/la0523661.

[22] A. Tuomela, J. Hirvonen, and L. Peltonen, "Stabilizing agents for drug nanocrystals: Effect on bioavailability," *Pharmaceutics*, vol. 8, no. 2, p. 16, 2016, doi: 10.3390/pharmaceutics8020016.

[23] K. V. Mahesh, S. K. Singh, and M. Gulati, "A comparative study of top-down and bottom-up approaches for the preparation of nanosuspensions of glipizide," *Powder Technol.*, vol. 256, pp. 436–449, 2014, doi: 10.1016/j.powtec.2014.02.011.

[24] S. Verma, R. Gokhale, and D. J. Burgess, "A comparative study of top-down and bottom-up approaches for the preparation of micro/nanosuspensions," *Int. J. Pharm.*, vol. 380, no. 1–2, pp. 216–222, 2009, doi: 10.1016/j.ijpharm.2009.07.005.

[25] E. Kastner, R. Kaur, D. Lowry, B. Moghaddam, A. Wilkinson, and Y. Perrie, "High-throughput manufacturing of size-tuned liposomes by a new microfluidics method using enhanced statistical tools for characterization," *Int. J. Pharm.*, vol. 477, no. 1–2, pp. 361–368, 2014, doi: 10.1016/j.ijpharm.2014.10.030.

[26] S. Schultz, G. Wagner, K. Urban, and J. Ulrich, "High-pressure homogenization as a process for emulsion formation," *Chem. Eng. Technol.*, vol. 27, no. 4, pp. 361–368, 2004, doi: 10.1002/ceat.200406111.

[27] S. K. Singh, K. K. Srinivasan, K. Gowthamarajan, D. S. Singare, D. Prakash, and N. B. Gaikwad, "Investigation of preparation parameters of nanosuspension by top-down bead milling to improve the dissolution of poorly water-soluble glyburide," *Eur. J. Pharm. Biopharm.*, vol. 78, no. 3, pp. 441–446, 2011, doi: 10.1016/j.ejpb.2011.03.014.

[28] M. Hagedorn, A. Bögershausen, M. Rischer, R. Schubert, and U. Massing, "Dual centrifugation – A new technique for nanomilling of poorly soluble drugs and formulation screening by an DoE-approach," *Int. J. Pharm.*, vol. 530, no. 1–2, pp. 79–88, 2017, doi: 10.1016/j.ijpharm.2017.07.047.

[29] L. Peltonen and J. Hirvonen, "Pharmaceutical nanocrystals by nanomilling: Critical process parameters, particle fracturing and stabilization methods," *J. Pharm. Pharmacol.*, vol. 62, no. 11, pp. 1569–1579, 2010, doi: 10.1111/j.2042-7158.2010.01022.x.

[30] L. Gao, D. Zhang, and M. Chen, "Drug nanocrystals for the formulation of poorly soluble drugs and its application as a potential drug delivery system," *J. Nanoparticle Res.*, vol.

10, pp. 845–862, 2008, doi: 10.1007/s11051-008-9357-4.

[31] G. G. Liversidge and K. C. Cundy, “Particle size reduction for improvement of oral bioavailability of hydrophobic drugs: I. Absolute oral bioavailability of nanocrystalline danazol in beagle dogs,” *Int. J. Pharm.*, vol. 125, no. 1, pp. 91–97, 1995, doi: 10.1016/0378-5173(95)00122-Y.

[32] J. Lee, J. Y. Choi, and C. H. Park, “Characteristics of polymers enabling nano-comminution of water-insoluble drugs,” *Int. J. Pharm.*, vol. 355, no. 1–2, pp. 328–336, 2008, doi: 10.1016/j.ijpharm.2007.12.032.

[33] M. Colombo, S. Orthmann, M. Bellini, S. Staufenbiel, and R. Bodmeier, “Influence of Drug Brittleness, Nanomilling Time, and Freeze-Drying on the Crystallinity of Poorly Water-Soluble Drugs and Its Implications for Solubility Enhancement,” *AAPS PharmSciTech*, vol. 18, no. 7, pp. 2437–2445, 2017, doi: 10.1208/s12249-017-0722-4.

[34] M. Juhnke, D. Märtin, and E. John, “Generation of wear during the production of drug nanosuspensions by wet bead milling,” *Eur. J. Pharm. Biopharm.*, vol. 81, no. 1, pp. 214–222, 2012, doi: 10.1016/j.ejpb.2012.01.005.

[35] A. Annapragada and A. Adjei, “Numerical simulation of milling processes as an aid to process design,” *Int. J. Pharm.*, vol. 136, no. 1–2, pp. 1–11, 1996, doi: 10.1016/0378-5173(96)04465-1.

[36] A. Kwade, “A stressing model for the description and optimization of grinding processes,” *Chem. Eng. Technol.*, 2003, doi: 10.1002/ceat.200390029.

[37] N. Rasenack and B. W. Müller, “Micron-Size Drug Particles: Common and Novel Micronization Techniques,” *Pharm. Dev. Technol.*, vol. 9, no. 1, pp. 1–13, 2004, doi: 10.1081/PDT-120027417.

[38] J. Deng, L. Huang, and F. Liu, “Understanding the structure and stability of paclitaxel nanocrystals,” *Int. J. Pharm.*, vol. 390, no. 2, pp. 242–249, 2010, doi: 10.1016/j.ijpharm.2010.02.013.

[39] C. Bartos, P. Szabó-Révész, C. Bartos, G. Katona, O. Jójárt-Laczkovich, and R. Ambrus, “The Effect of an Optimized Wet Milling Technology on the Crystallinity, Morphology and Dissolution Properties of Micro- and Nanonized Meloxicam,” *Molecules*, vol. 21, no. 4, p. 507, 2016, doi: 10.3390/molecules21040507.

[40] B. Van Eerdenbrugh, G. Van den Mooter, and P. Augustijns, “Top-down production of drug nanocrystals: Nanosuspension stabilization, miniaturization and transformation

into solid products," *Int. J. Pharm.*, vol. 364, no. 1, pp. 64–75, 2008, doi: 10.1016/j.ijpharm.2008.07.023.

[41] G. Zografi and K. J. Crowley, "Cryogenic grinding of indomethacin polymorphs and solvates: Assessment of amorphous phase formation and amorphous phase physical stability," *J. Pharm. Sci.*, vol. 91, no. 2, pp. 492–507, 2002, doi: 10.1002/jps.10028.

[42] M. George and I. Ghosh, "Identifying the correlation between drug/stabilizer properties and critical quality attributes (CQAs) of nanosuspension formulation prepared by wet bead milling technology," *Eur. J. Pharm. Sci.*, vol. 48, no. 1–2, pp. 142–152, 2013, doi: 10.1016/j.ejps.2012.10.004.

[43] P. Sharma, W. A. Denny, and S. Garg, "Effect of wet milling process on the solid state of indomethacin and simvastatin," *Int. J. Pharm.*, vol. 380, no. 1–2, pp. 40–48, 2009, doi: 10.1016/j.ijpharm.2009.06.029.

[44] N. S. K. Srinivas, R. Verma, G. P. Kulyadi, and L. Kumar, "A quality by design approach on polymeric nanocarrier delivery of gefitinib: Formulation, in vitro, and in vivo characterization," *Int. J. Nanomedicine*, vol. 12, pp. 15–28, 2017, doi: 10.2147/IJN.S122729.

[45] M. George and I. Ghosh, "Identifying the correlation between drug/stabilizer properties and critical quality attributes (CQAs) of nanosuspension formulation prepared by wet bead milling technology," *Eur. J. Pharm. Sci.*, vol. 48, no. 1–2, pp. 142–152, 2013, doi: 10.1016/j.ejps.2012.10.004.

[46] P. F. Yue, Y. Li, J. Wan, M. Yang, W. F. Zhu, and C. H. Wang, "Study on formability of solid nanosuspensions during nanodispersion and solidification: I. Novel role of stabilizer/drug property," *Int. J. Pharm.*, vol. 454, no. 1, pp. 269–277, 2013, doi: 10.1016/j.ijpharm.2013.06.050.

[47] Y. Yang *et al.*, "Tumor-penetrating peptide functionalization enhances the anti-glioblastoma effect of doxorubicin liposomes," *Nanotechnology*, vol. 24, no. 40, 2013, doi: 10.1088/0957-4484/24/40/405101.

[48] J. Y. Choi, J. Y. Yoo, H. S. Kwak, B. U. Nam, and J. Lee, "Role of polymeric stabilizers for drug nanocrystal dispersions," *Curr. Appl. Phys.*, vol. 5, no. 5, pp. 472–474, 2005, doi: 10.1016/j.cap.2005.01.012.

[49] J. H. Kim, S. W. Jang, S. D. Han, H. D. Hwang, and H. G. Choi, "Development of a novel ophthalmic ciclosporin A-loaded nanosuspension using top-down bead milling

methods," *Pharmazie*, vol. 66, no. 7, pp. 491–495, 2011, doi: 10.1691/ph.2011.0358.

[50] I. Ghosh and B. Michniak-Kohn, "Influence of critical parameters of nanosuspension formulation on the permeability of a poorly soluble drug through the skin - A case study," *AAPS PharmSciTech*, vol. 14, no. 3, pp. 1108–1117, 2013, doi: 10.1208/s12249-013-9995-4.

[51] Z. T. Marlowe and S. R. Davio, "Dose uniformity of loteprednol etabonate ophthalmic gel (0.5%) compared with branded and generic prednisolone acetate ophthalmic suspension (1%)," *Clin. Ophthalmol.*, vol. 8, pp. 23–29, 2013, doi: 10.2147/OPTH.S55004.

[52] C. J. Kim, *Advanced pharmaceutics: Physicochemical principles*. 2004.

[53] A. K. K. O. N. S. G. M. Wall, "General principles of suspensions," in *Pharmaceutical Suspensions: From Formulation Development to Manufacturing*, 2010.

[54] Y. Ali and K. Lehmussaari, "Industrial perspective in ocular drug delivery," *Adv. Drug Deliv. Rev.*, vol. 58, no. 11, pp. 1258–1268, 2006, doi: 10.1016/j.addr.2006.07.022.

[55] F. A. Maulvi, K. H. Shetty, D. T. Desai, D. O. Shah, and M. D. P. Willcox, "Recent advances in ophthalmic preparations: Ocular barriers, dosage forms and routes of administration," *Int. J. Pharm.*, vol. 608, 2021, doi: 10.1016/j.ijpharm.2021.121105.

[56] A. N. Oktay, S. Ilbasmis-Tamer, A. Karakucuk, and N. Celebi, "Screening of stabilizing agents to optimize flurbiprofen nanosuspensions using experimental design," *J. Drug Deliv. Sci. Technol.*, vol. 57, 2020, doi: 10.1016/j.jddst.2020.101690.

[57] J. D. Engstrom, J. M. Tam, M. A. Miller, R. O. Williams, and K. P. Johnston, "Templated open flocs of nanorods for enhanced pulmonary delivery with pressurized metered dose inhalers," *Pharm. Res.*, vol. 26, no. 1, pp. 101–117, 2009, doi: 10.1007/s11095-008-9707-z.

[58] E. Merisko-Liversidge, G. G. Liversidge, and E. R. Cooper, "Nanosizing: A formulation approach for poorly-water-soluble compounds," *Eur. J. Pharm. Sci.*, vol. 18, no. 2, pp. 113–120, 2003, doi: 10.1016/S0928-0987(02)00251-8.

[59] C. Washington and S. M. King, "Effect of electrolytes and temperature on the structure of a poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) block copolymer adsorbed to a perfluorocarbon emulsion," *Langmuir*, vol. 13, no. 17, pp. 4545–4550, 1997, doi: 10.1021/la9701081.

[60] D. H. Napper, "ROLE OF POLYMERS IN THE STABILIZATION OF DISPERSE SYSTEMS.,"

Chem Technol Water-Soluble Polym, pp. 233–248, 1983, doi: 10.1007/978-1-4757-9661-2_14.

[61] E. Zimmermann and R. H. Müller, "Electrolyte- and pH-stabilities of aqueous solid lipid nanoparticle (SLN^{TM}) dispersions in artificial gastrointestinal media," *Eur. J. Pharm. Biopharm.*, vol. 52, no. 2, pp. 203–210, 2001, doi: 10.1016/S0939-6411(01)00167-9.

[62] J. Y. Choi, C. H. Park, and J. Lee, "Effect of polymer molecular weight on nanocomminution of poorly soluble drug," *Drug Deliv.*, vol. 15, no. 5, pp. 347–353, 2008, doi: 10.1080/10717540802039113.

[63] B. S. Sekhon, "Surfactants: Pharmaceutical and Medicinal Aspects," *J. Pharm. Technol. Res. Manag.*, vol. 1, no. 1, pp. 43–68, 2013, doi: 10.15415/jptrm.2013.11004.

[64] G. B. Romero, C. M. Keck, R. H. Müller, and N. A. Bou-Chacra, "Development of cationic nanocrystals for ocular delivery," *Eur. J. Pharm. Biopharm.*, vol. 107, pp. 215–222, 2016, doi: 10.1016/j.ejpb.2016.07.005.

[65] V. E. Bernard *et al.*, "Microcrystalline cellulose, a useful alternative for sucrose as a matrix former during freeze-drying of drug nanosuspensions - A case study with itraconazole," *Eur. J. Pharm. Biopharm.*, vol. 70, no. 2, pp. 590–596, 2008, doi: 10.1016/j.ejpb.2008.06.007.

[66] P. Quan *et al.*, "A novel surface modified nitrendipine nanocrystals with enhancement of bioavailability and stability," *Int. J. Pharm.*, vol. 430, no. 1–2, pp. 366–371, 2012, doi: 10.1016/j.ijpharm.2012.04.025.

[67] M. Singh, S. Bharadwaj, K. E. Lee, and S. G. Kang, "Therapeutic nanoemulsions in ophthalmic drug administration: Concept in formulations and characterization techniques for ocular drug delivery," *J. Control. Release*, vol. 328, no. 10, pp. 895–916, 2020, doi: 10.1016/j.jconrel.2020.10.025.

[68] H. Rachmawati, L. Al Shaal, R. H. Müller, and C. M. Keck, "Development of curcumin nanocrystal: Physical aspects," *J. Pharm. Sci.*, vol. 102, no. 1, pp. 204–214, 2013, doi: 10.1002/jps.23335.

[69] K. B. Wróblewska, B. Jadach, and I. Muszalska-Kolos, "Progress in drug formulation design and delivery of medicinal substances used in ophthalmology," *Int. J. Pharm.*, vol. 607, 2021, doi: 10.1016/j.ijpharm.2021.121012.

[70] M. F. Saettone, P. Chetoni, R. Cerbai, G. Mazzanti, and L. Braghiroli, "Evaluation of ocular permeation enhancers: In vitro effects on corneal transport of four β-blockers,

and in vitro/in vivo toxic activity," *Int. J. Pharm.*, vol. 142, no. 1, pp. 103–113, 1996, doi: 10.1016/0378-5173(96)04663-7.

[71] J. Hecq *et al.*, "Preparation and in vitro/in vivo evaluation of nano-sized crystals for dissolution rate enhancement of ucb-35440-3, a highly dosed poorly water-soluble weak base," *Eur. J. Pharm. Biopharm.*, vol. 6, no. 43, pp. 360–368, 2006, doi: 10.1016/j.ejpb.2006.05.008.

[72] S. Verma, R. Gokhale, and D. J. Burgess, "A comparative study of top-down and bottom-up approaches for the preparation of micro/nanosuspensions," *Int. J. Pharm.*, vol. 380, no. 1–2, pp. 216–222, 2009, doi: 10.1016/j.ijpharm.2009.07.005.

[73] M. E. Rowe, R.C., Sheskey, P.J. Quinn, *Handbook of Pharmaceutical Excipients.*, 6th ed. 2009.

[74] Y. Cao, C. Zhang, W. Shen, Z. Cheng, L. (Lucy) Yu, and Q. Ping, "Poly(N-isopropylacrylamide)-chitosan as thermosensitive in situ gel-forming system for ocular drug delivery," *J. Control. Release*, vol. 120, no. 3, pp. 168–194, 2007, doi: 10.1016/j.jconrel.2007.05.009.

[75] R. V. Pai, J. D. Monpara, and P. R. Vavia, "Exploring molecular dynamics simulation to predict binding with ocular mucin: An in silico approach for screening mucoadhesive materials for ocular retentive delivery systems," *J. Control. Release*, vol. 309, pp. 190–202, 2019, doi: 10.1016/j.jconrel.2019.07.037.

[76] R. S. Dave, T. C. Goostrey, M. Ziolkowska, S. Czerny-Holownia, T. Hoare, and H. Sheardown, "Ocular drug delivery to the anterior segment using nanocarriers: A mucoadhesive/mucopenetrative perspective," *J. Control. Release*, vol. 336, pp. 71–88, 2021, doi: 10.1016/j.jconrel.2021.06.011.

[77] M. L. A. D. Lestari, R. H. Müller, and J. P. Möschwitzer, "Systematic screening of different surface modifiers for the production of physically stable nanosuspensions," *J. Pharm. Sci.*, vol. 104, no. 3, pp. 1128–1140, 2015, doi: 10.1002/jps.24266.

[78] B. Van Eerdenbrugh *et al.*, "A screening study of surface stabilization during the production of drug nanocrystals," *J. Pharm. Sci.*, vol. 98, no. 6, pp. 2091–2301, 2009, doi: 10.1002/jps.21563.

[79] A. Vo *et al.*, "Factors affecting the particle size distribution and rheology of brinzolamide ophthalmic suspensions," *Int. J. Pharm.*, vol. 586, 2020, doi: 10.1016/j.ijpharm.2020.119495.

[80] A. Tuomela *et al.*, “Brinzolamide nanocrystal formulations for ophthalmic delivery: Reduction of elevated intraocular pressure in vivo,” *Int. J. Pharm.*, vol. 467, no. 1–2, pp. 34–41, 2014, doi: 10.1016/j.ijpharm.2014.03.048.

[81] S. He, H. Yang, R. Zhang, Y. Li, and L. Duan, “Preparation and in vitro-in vivo evaluation of teniposide nanosuspensions,” *Int. J. Pharm.*, vol. 478, no. 1, pp. 131–137, 2015, doi: 10.1016/j.ijpharm.2014.11.020.

[82] D. Xia *et al.*, “Preparation of stable nitrendipine nanosuspensions using the precipitation-ultrasonication method for enhancement of dissolution and oral bioavailability,” *Eur. J. Pharm. Sci.*, vol. 40, no. 4, pp. 325–334, 2010, doi: 10.1016/j.ejps.2010.04.006.

[83] A. Makhlof, Y. Miyazaki, Y. Tozuka, and H. Takeuchi, “Cyclodextrins as stabilizers for the preparation of drug nanocrystals by the emulsion solvent diffusion method,” *Int. J. Pharm.*, vol. 357, no. 1–2, pp. 280–285, 2008, doi: 10.1016/j.ijpharm.2008.01.025.

[84] N. P. Aditya, H. Yang, S. Kim, and S. Ko, “Fabrication of amorphous curcumin nanosuspensions using β-lactoglobulin to enhance solubility, stability, and bioavailability,” *Colloids Surfaces B Biointerfaces*, vol. 127, pp. 114–121, 2015, doi: 10.1016/j.colsurfb.2015.01.027.

[85] W. He, Y. Lu, J. Qi, L. Chen, F. Hu, and W. Wu, “Food proteins as novel nanosuspension stabilizers for poorly water-soluble drugs,” *Int. J. Pharm.*, vol. 441, no. 1–2, pp. 269–278, 2013, doi: 10.1016/j.ijpharm.2012.11.033.

[86] H. Rachmawati, A. Rahma, L. Al Shaal, R. H. Müller, and C. M. Keck, “Destabilization mechanism of ionic surfactant on curcumin nanocrystal against electrolytes,” *Sci. Pharm.*, vol. 84, no. 4, pp. 685–693, 2016, doi: 10.3390/scipharm84040685.

[87] B. Sinha, R. H. Müller, and J. P. Möschwitzer, “Bottom-up approaches for preparing drug nanocrystals: Formulations and factors affecting particle size,” *Int. J. Pharm.*, vol. 453, no. 1, pp. 126–141, 2013, doi: 10.1016/j.ijpharm.2013.01.019.

[88] S. Verma, B. D. Huey, and D. J. Burgess, “Scanning probe microscopy method for nanosuspension stabilizer selection,” *Langmuir*, vol. 25, no. 21, pp. 12481–12487, 2009, doi: 10.1021/la9016432.

[89] S. L. Raghavan, A. Trividic, A. F. Davis, and J. Hadgraft, “Crystallization of hydrocortisone acetate: Influence of polymers,” *Int. J. Pharm.*, vol. 212, no. 2, pp. 213–221, 2001, doi: 10.1016/S0378-5173(00)00610-4.

[90] D. Douroumis and A. Fahr, “Stable carbamazepine colloidal systems using the cosolvent technique,” *Eur. J. Pharm. Sci.*, vol. 30, no. 5, pp. 367–374, 2007, doi: 10.1016/j.ejps.2006.12.003.

[91] A. Ito, C. Konnerth, J. Schmidt, and W. Peukert, “Effect of polymer species and concentration on the production of mefenamic acid nanoparticles by bead milling,” *Eur. J. Pharm. Biopharm.*, vol. 98, pp. 98–107, 2016, doi: 10.1016/j.ejpb.2015.11.011.

[92] J. R. Franca *et al.*, “Chitosan/hydroxyethyl cellulose inserts for sustained-release of dorzolamide for glaucoma treatment: In vitro and in vivo evaluation,” *Int. J. Pharm.*, vol. 570, 2019, doi: 10.1016/j.ijpharm.2019.118662.

[93] G. Mohammadi, S. Mirzaeei, S. Taghe, and P. Mohammadi, “Preparation and evaluation of Eudragit® L100 nanoparticles loaded impregnated with kt tromethamine loaded PVA-HEC insertions for ophthalmic drug delivery,” *Adv. Pharm. Bull.*, vol. 30, no. 1, 2019, doi: 10.15171/apb.2019.068.

[94] J. Lee, S. J. Lee, J. Y. Choi, J. Y. Yoo, and C. H. Ahn, “Amphiphilic amino acid copolymers as stabilizers for the preparation of nanocrystal dispersion,” *Eur. J. Pharm. Sci.*, vol. 24, no. 5, pp. 441–449, 2005, doi: 10.1016/j.ejps.2004.12.010.

[95] H. Wang, Q. Pan, and G. L. Rempel, “Micellar nucleation differential microemulsion polymerization,” *Eur. Polym. J.*, vol. 47, no. 5, pp. 973–980, 2011, doi: 10.1016/j.eurpolymj.2010.11.009.

[96] I. Ghosh, D. Schenck, S. Bose, and C. Ruegger, “Optimization of formulation and process parameters for the production of nanosuspension by wet bead milling technique: Effect of Vitamin e TPGS and nanocrystal particle size on oral absorption,” *Eur. J. Pharm. Sci.*, vol. 47, no. 4, pp. 718–728, 2012, doi: 10.1016/j.ejps.2012.08.011.

[97] E. Bilgili, M. Li, and A. Afolabi, “Is the combination of cellulosic polymers and anionic surfactants a good strategy for ensuring physical stability of BCS Class II drug nanosuspensions?,” *Pharm. Dev. Technol.*, vol. 21, no. 4, pp. 499–510, 2016, doi: 10.3109/10837450.2015.1022788.

[98] A. Bhakay, R. Davé, and E. Bilgili, “Recovery of BCS Class II drugs during aqueous redispersion of core-shell type nanocomposite particles produced via fluidized bed coating,” *Powder Technol.*, vol. 236, pp. 221–234, 2013, doi: 10.1016/j.powtec.2011.12.066.

[99] A. Bhakay, M. Azad, E. Vizzotti, R. N. Dave, and E. Bilgili, “Enhanced recovery and

dissolution of griseofulvin nanoparticles from surfactant-free nanocomposite microparticles incorporating wet-milled swellable dispersants," *Drug Dev. Ind. Pharm.*, vol. 40, no. 11, pp. 1509–1522, 2014, doi: 10.3109/03639045.2013.831442.

[100] C. Knieke, M. A. Azad, R. N. Davé, and E. Bilgili, "A study of the physical stability of wet media-milled fenofibrate suspensions using dynamic equilibrium curves," *Chem. Eng. Res. Des.*, vol. 7, pp. 1245–1258, 2013, doi: 10.1016/j.cherd.2013.02.008.

[101] W. Zhu, F. S. Romanski, S. V. Dalvi, R. N. Dave, and M. Silvina Tomassone, "Atomistic simulations of aqueous griseofulvin crystals in the presence of individual and multiple additives," *Chem. Eng. Sci.*, vol. 73, pp. 218–230, 2012, doi: 10.1016/j.ces.2012.01.008.

[102] E. Bilgili, M. Li, and A. Afolabi, "Is the combination of cellulosic polymers and anionic surfactants a good strategy for ensuring physical stability of BCS Class II drug nanosuspensions?," *Pharm. Dev. Technol.*, vol. 21, no. 4, pp. 499–510, 2016, doi: 10.3109/10837450.2015.1022788.

[103] A. Ain-Ai and P. K. Gupta, "Effect of arginine hydrochloride and hydroxypropyl cellulose as stabilizers on the physical stability of high drug loading nanosuspensions of a poorly soluble compound," *Int. J. Pharm.*, vol. 351, no. 1–2, pp. 282–288, 2008, doi: 10.1016/j.ijpharm.2007.09.029.

[104] M. Nakach, J. R. Authelin, T. Tadros, L. Galet, and A. Chamayou, "Engineering of nano-crystalline drug suspension: Employing a physico-chemistry based stabilizer selection methodology or approach," *Int. J. Pharm.*, vol. 476, no. 1–2, pp. 277–288, 2014, doi: 10.1016/j.ijpharm.2014.09.048.

[105] A. Kim, W. B. Ng, W. Bernt, and N. J. Cho, "Validation of Size Estimation of Nanoparticle Tracking Analysis on Polydisperse Macromolecule Assembly," *Sci. Rep.*, vol. 9, pp. 26–39, 2019, doi: 10.1038/s41598-019-38915-x.

[106] G. Shetea, H. Jaina, D. Punja, H. Prajapata, P. Akotiyaa, and A. K. Bansala, "Stabilizers used in nano-crystal based drug delivery systems," *Journal of Excipients and Food Chemicals*. 2014.

[107] A. Chen *et al.*, "Dosage Form Developments of Nanosuspension Drug Delivery System for Oral Administration Route," *Curr. Pharm. Des.*, vol. 21, no. 29, pp. 4355–4365, 2015, doi: 10.2174/1381612821666150901105026.

[108] L. Wu and S. R. P. Da Rocha, "Biocompatible and biodegradable copolymer stabilizers for hydrofluoroalkane dispersions: A colloidal probe microscopy investigation,"

Langmuir, vol. 23, no. 24, pp. 12104–12110, 2007, doi: 10.1021/la702108x.

[109] J. Pardeike and R. H. Müller, "Nanosuspensions: A promising formulation for the new phospholipase A2 inhibitor PX-18," *Int. J. Pharm.*, vol. 391, no. 1–2, pp. 322–329, 2010, doi: 10.1016/j.ijpharm.2010.03.002.

[110] A. M. Cerdeira, M. Mazzotti, and B. Gander, "Miconazole nanosuspensions: Influence of formulation variables on particle size reduction and physical stability," *Int. J. Pharm.*, vol. 396, no. 1–2, pp. 210–218, 2010, doi: 10.1016/j.ijpharm.2010.06.020.

[111] M. Kuentz and D. Röthlisberger, "Rapid assessment of sedimentation stability in dispersions using near infrared transmission measurements during centrifugation and oscillatory rheology," *Eur. J. Pharm. Biopharm.*, vol. 56, no. 3, pp. 355–361, 2003, doi: 10.1016/S0939-6411(03)00108-5.

[112] D. Hespeler, D. Knoth, C. M. Keck, R. H. Müller, and S. M. Pyo, "smartPearls ® for dermal bioavailability enhancement – Long-term stabilization of suspensions by viscoelasticity," *Int. J. Pharm.*, vol. 562, no. February, pp. 293–302, 2019, doi: 10.1016/j.ijpharm.2019.03.016.

[113] E. B. Souto *et al.*, "Advanced formulation approaches for ocular drug delivery: State-of-the-art and recent patents," *Pharmaceutics*, vol. 11, no. 9, p. 490, 2019, doi: 10.3390/pharmaceutics11090460.

[114] C. Jumelle, S. Gholizadeh, N. Annabi, and R. Dana, "Advances and limitations of drug delivery systems formulated as eye drops," *J. Control. Release*, vol. 321, no. 10, pp. 1–22, 2020, doi: 10.1016/j.jconrel.2020.01.057.

[115] V. Carelli, C. La Morgia, F. N. Ross-Cisneros, and A. A. Sadun, "Optic neuropathies: The tip of the neurodegeneration iceberg," *Hum. Mol. Genet.*, vol. 26, no. 2, pp. 139–150, 2017, doi: 10.1093/hmg/ddx273.

[116] FDA, "Guidance for industry. Nonclinical safety evaluation of drug or biologic combinations," *Guid. Ind.*, no. March, pp. 1–13, 2006.

[117] R. D. Fechtner and T. Realini, "Fixed combinations of topical glaucoma medications," *Curr. Opin. Ophthalmol.*, vol. 15, no. 2, pp. 132–135, 2004, doi: 10.1097/00055735-200404000-00013.

[118] A. G. P. Konstas, L. Quaranta, A. Katsanos, I. C. Voudouragkaki, and G. N. Dutton, "Fixed Combination Therapies in Glaucoma," in *Glaucoma: Second Edition*, 2015, pp. 583–591.

[119] P. J. Morgan-Warren and J. B. Morarji, "Trends in licence approvals for ophthalmic

medicines in the United Kingdom," *Eye*, vol. 34, no. 10, pp. 1856–1865, 2020, doi: 10.1038/s41433-019-0758-7.

[120] Lichter P *et al.*, "Interim clinical outcomes in the Collaborative Initial Glaucoma Treatment Study comparing initial treatment randomized to medications or surgery.," *Ophthalmology*, vol. 108, no. 11, pp. 1943–1953, 2001.

[121] M. A. Kassem, A. A. Abdel Rahman, M. M. Ghorab, M. B. Ahmed, and R. M. Khalil, "Nanosuspension as an ophthalmic delivery system for certain glucocorticoid drugs," *Int. J. Pharm.*, vol. 340, no. 1–2, pp. 126–133, 2007, doi: 10.1016/j.ijpharm.2007.03.011.

[122] S. Toongsuwan, H. C. Chang, L. C. Li, D. Stephens, and H. Plichta-Mahmoud, "Particle size determination of a three-component suspension using a laser-scattering particle size distribution analyzer," *Drug Dev. Ind. Pharm.*, vol. 26, no. 8, pp. 895–900, 2000, doi: 10.1081/DDC-100101315.

[123] E. Van Der Pol, F. A. W. Coumans, A. Sturk, R. Nieuwland, and T. G. Van Leeuwen, "Refractive index determination of nanoparticles in suspension using nanoparticle tracking analysis," *Nano Lett.*, vol. 14, no. 11, pp. 6195–6201, 2014, doi: 10.1021/nl503371p.

[124] W. Peukert, H. C. Schwarzer, and F. Stenger, "Control of aggregation in production and handling of nanoparticles," *Chem. Eng. Process. Process Intensif.*, vol. 44, no. 2, pp. 245–252, 2005, doi: 10.1016/j.cep.2004.02.018.

[125] K. P. Krause and R. H. Müller, "Production and characterisation of highly concentrated nanosuspensions by high pressure homogenisation," *Int. J. Pharm.*, vol. 214, no. 1–2, pp. 21–24, 2001, doi: 10.1016/S0378-5173(00)00626-8.

[126] A. Beig *et al.*, "Head-To-Head Comparison of Different Solubility-Enabling Formulations of Etoposide and Their Consequent Solubility-Permeability Interplay," *J. Pharm. Sci.*, vol. 104, no. 9, pp. 2941–2947, 2015, doi: 10.1002/jps.24496.

[127] A. A. Abdelbary, X. Li, M. El-Nabarawi, A. Elassasy, and B. Jasti, "Effect of fixed aqueous layer thickness of polymeric stabilizers on zeta potential and stability of aripiprazole nanosuspensions.," *Pharm. Dev. Technol.*, vol. 18, no. 3, pp. 730–735, 2013, doi: 10.3109/10837450.2012.727001.

[128] M. Colombo, S. Staufenbiel, E. Rühl, and R. Bodmeier, "In situ determination of the saturation solubility of nanocrystals of poorly soluble drugs for dermal application," *Int. J. Pharm.*, vol. 21, no. 1–2, pp. 156–166, 2017, doi: 10.1016/j.ijpharm.2017.02.030.

[129] L. Sek, B. J. Boyd, W. N. Charman, and C. J. H. Porter, “Examination of the impact of a range of Pluronic surfactants on the in-vitro solubilisation behaviour and oral bioavailability of lipidic formulations of atovaquone,” *J. Pharm. Pharmacol.*, vol. 58, no. 6, pp. 809–820, 2010, doi: 10.1211/jpp.58.6.0011.

[130] T. F. Tadros, “Role of Surfactants in Wetting, Spreading and Adhesion,” in *Applied Surfactants: Principles and Applications*, Wiley-VCH Verlag GmbH & Co. KGaA, 2005, pp. 335–397.

[131] A. Strobel, J. Schwenger, S. Wittpahl, J. Schmidt, S. Romeis, and W. Peukert, “Assessing the influence of viscosity and milling bead size on the stressing conditions in a stirred media mill by single particle probes,” *Chem. Eng. Res. Des.*, vol. 136, no. 1, pp. 859–869, 2018, doi: 10.1016/j.cherd.2018.06.040.

[132] E. Doelker, “Swelling Behavior of Water-Soluble Cellulose Derivatives,” 1990.

[133] W. Sun, W. Tian, Y. Zhang, J. He, S. Mao, and L. Fang, “Effect of novel stabilizers-cationic polymers on the particle size and physical stability of poorly soluble drug nanocrystals,” *Nanomedicine Nanotechnology, Biol. Med.*, vol. 8, no. 4, pp. 460–467, 2012, doi: 10.1016/j.nano.2011.07.006.

[134] J. Y. Choi, C. H. Park, and J. Lee, “Effect of polymer molecular weight on nanocomminution of poorly soluble drug,” *Drug Deliv.*, vol. 15, no. 5, pp. 347–353, 2008, doi: 10.1080/10717540802039113.

[135] A. Bitterlich *et al.*, “Process parameter dependent growth phenomena of naproxen nanosuspension manufactured by wet bead milling,” *Eur. J. Pharm. Biopharm.*, vol. 92, pp. 171–179, 2015, doi: 10.1016/j.ejpb.2015.02.031.

[136] L. Peltonen, “Design space and QbD approach for production of drug nanocrystals by wet bead milling techniques,” *Pharmaceutics*, vol. 10, no. 3, 2018, doi: 10.3390/pharmaceutics10030104.

[137] J. Authelin *et al.*, “Engineering of nano-crystalline drug suspensions : Employing a physico-chemistry based stabilizer selection methodology or approach,” vol. 476, no. 1–2, pp. 277–288, 2018.

[138] C. Washington *et al.*, “Polymer bristles: adsorption of low molecular weight poly(oxyethylene)-poly(oxybutylene) diblock copolymers on a perfluorocarbon emulsion,” *Macromolecules*, vol. 33, no. 4, pp. 1289–1297, 2000, doi: 10.1021/ma990730v.

[139] R. K. Cummins, P. G., Staples, E., Penfold, J., Heenan, “The geometry of micelles of the poly(oxyethylene) nonionic surfactants C16E6 and C16E8 in the presence of electrolyte,” *Langmuir*, vol. 5, no. 5, pp. 1195–1199, 1989, doi: 10.1021/la00089a012.

[140] S. Stolnik *et al.*, “The effect of surface coverage and conformation of poly(ethylene oxide) (PEO) chains of poloxamer 407 on the biological fate of model colloidal drug carriers,” *Biochim. Biophys. Acta - Biomembr.*, vol. 1514, no. 2, pp. 261–279, 2001, doi: 10.1016/S0005-2736(01)00376-5.

[141] S. Hu and W. X. Li, “Influence of Particle Size Distribution on Lifetime and Thermal Stability of Ostwald Ripening of Supported Particles,” *ChemCatChem*, 2018, doi: 10.1002/cctc.201800331.

[142] D. D. Patel and B. D. Anderson, “Effect of precipitation inhibitors on indomethacin supersaturation maintenance: Mechanisms and modeling,” *Mol. Pharm.*, vol. 11, no. 5, pp. 1489–1499, 2014, doi: 10.1021/mp400658k.

[143] S. Datta, C. Baudouin, F. Brignole-Baudouin, A. Denoyer, and G. A. Cortopassi, “The eye drop preservative benzalkonium chloride potently induces mitochondrial dysfunction and preferentially affects LHON mutant cells,” *Investig. Ophthalmol. Vis. Sci.*, 2017, doi: 10.1167/iovs.16-20903.

[144] A. Kwade and J. Schwedes, “Breaking characteristics of different materials and their effect on stress intensity and stress number in stirred media mills,” *Powder Technol.*, vol. 122, no. 2–3, pp. 109–121, 2002, doi: 10.1016/S0032-5910(01)00406-5.

[145] S. Mende, F. Stenger, W. Peukert, and J. Schwedes, “Mechanical production and stabilization of submicron particles in stirred media mills,” *Powder Technol.*, vol. 132, no. 1, pp. 64–73, 2003, doi: 10.1016/S0032-5910(03)00042-1.

[146] A. Dokoumetzidis and P. Macheras, “A century of dissolution research: From Noyes and Whitney to the Biopharmaceutics Classification System,” *Int. J. Pharm.*, vol. 321, no. 1–2, pp. 1–11, 2006, doi: 10.1016/j.ijpharm.2006.07.011.

[147] Z. Deng, S. Xu, and S. Li, “Understanding a relaxation behavior in a nanoparticle suspension for drug delivery applications,” *Int. J. Pharm.*, vol. 351, no. 1–2, pp. 236–243, 2008, doi: 10.1016/j.ijpharm.2007.10.001.

[148] P. Liu *et al.*, “Nanosuspensions of poorly soluble drugs: Preparation and development by wet milling,” *Int. J. Pharm.*, vol. 411, no. 1–2, pp. 215–222, 2011, doi: 10.1016/j.ijpharm.2011.03.050.

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7 Curriculum vitae

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