NANOSUSPENSIONS OF POORLY SOLUBLE DRUGS FOR ORAL ADMINISTRATION

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To my Rafa and Nuni

with plenty of love and gratitude

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ABBREVIATIONS

BCS	Biopharmaceutics drug Classification System
USP/NF	United States Pharmacopoeia/National Formulary
KI	potassium iodide
I_2	iodine
KI_3	tri iodide complex
CMC	critical micelle concentration
RH	relative humidity
Eq.	equation
v.h.p.	*via humida paratum*
ppm	part per million
PEG	liquid polyethylene glycol
CT	combination technology
SPF	sun protection factor
SEDS	solution enhanced dispersion by the supercritical fluids
MDI	metered dose inhaler
RESAS	rapids expansion from supercritical to aqueous solution
RESSAS	rapid expansion of supercritical solution into aqueous solution
SFL	Spray Freezing into Liquid
EPAS	evaporative precipitation into aqueous solution
TCR	T-cell receptor
FDA	Food and Drug Administration Office (US Government)
RES	Reticulo endothelial system
CFCs	Chlorofluorocarbons
YMP	Yucatan micropig
AD6	cloricromene hydrochloride
NSAID	nonsteroidal anti-inflammatory drug
CoQ10	coenzyme Q10
UV A	Ultra Violet A
HLB	hydrophilic lipophilic balance
PVA	polyvinyl alcohol
SDS	sodium dodecyl sulfate

PVP	polyvinyl pyrrolidone
LD	Laser Diffractometry
PSD	particle size distribution
LLS	laser light scattering
PIDS	Polarized Intensity Diffraction Scattering
PCS	Photon Correlation Spectroscopy
PI	Polydispersity Index
ZP	zeta potential
SEM	scanning electron microscopy
HPLC	high performance liquid chromatography
SD	spray drying process
FD	freeze drying process
DSC	differential scanning calorimetry
PXRD	powder x-ray diffraction
HPH	high pressure homogenization
BM	ball milling
µS	micro Siemens
GIT	Gastro Intestinal Tract
ROS	reactive oxygen species
TA	thermal analysis

Chapter 1
INTRODUCTION, AIMS AND OBJECTIVES

1. INTRODUCTION, AIMS AND OBJECTIVES

1.1 Introduction

An increasing number of newly developed drugs are poorly soluble; in many cases drugs are poorly soluble in both aqueous and organic media excluding the traditional approaches of overcoming such solubility factors and resulting in bioavailability problems. An alternative and promising approach is the production of drug nanosuspensions to overcome these problems. The major advantages of this technology are its general applicability to most drugs and its simplicity.

In addition, poorly water soluble drugs are specially challenging, as they cannot achieve dissolution and therefore they have a very difficult pass through the dissolving fluid to contact the absorbing mucosa and to be absorbed. If the dissolution process of the drug molecule is slow, due to the physicochemical properties of the drug molecules or formulation factors, then dissolution may be the rate-limiting step in absorption and will influence drug bioavailability. This is the case of class II drugs, e.g. ibuprofen, rutin, hesperidin and coenzyme Q10 (according to the Biopharmaceutical drug Classification System BCS). For this specific kind of drugs, micronization [1-4], nanonization [5-7], complexation (e.g. cyclodextrins) [8-13], preparation of liposomes [14-16] and amorphous solid dispersions [17-21] have been proposed to increase the rate of dissolution and especially the drug bioavailability after oral administration for systemic drug absorption. A nanosuspension consists of drug nanocrystals, stabilizing agents and a liquid dispersion medium. The dispersion media can be water, aqueous solutions or non-aqueous media. The terms drugs nanocrystals implies crystalline state of the discrete particles.

In this study, the production of nanosuspensions on a laboratory scale is presented, special features such as increased saturation solubility and dissolution velocity are discussed, and special applications are highlighted, for example, nanocrystal-loaded solid dosage forms for oral delivery such as tablets, capsules, effervescent tablets and pellets. The possibilities of large

scale production — the prerequisite for the introduction of a delivery system to the market — are also discussed.

In addition, drug nanosuspension is dried to obtain drug nanocrystals powder. Physicochemical feature of drug nanocrystals will be investigated and drug nanocrystals incorporated into solid dosage form. Moreover dissolution velocities of drug nanocrystals–loaded solid dosage form are compared to marketed product.

1.2 Aims and objectives

1.2.1 Formulation development

- Formulate poorly soluble drugs nanosuspensions suitable for orally application using various stabilizer.
- Investigate the chemical (drug content) and physical (nanoparticles size distribution) stability of the formulated nanosuspensions.
- Investigate the improvement in dissolution rate and saturation solubility of the prepared poorly soluble nanocrystals.

1.2.2 Solid dosage form of poorly soluble drugs

- Prepare Rutin and Hesperidin tablets containing drug nanocrystals using the excipient tablet for direct compression method such as Avicel PH 101, AcDiSol, Explotab.
- Prepare coenzyme Q10 capsules using simple filler capsules equipment and avicel PH 102 and lactosa as filler excipient.
- Incorporation of ibuprofen nanocrystals into pellets and effervescent granule using simple methods for lab scale.
- Characterize the products to identify the improvement in dissolution obtained using these techniques.

1.2.3 *In vitro* evaluation

- Perform the *in vitro* characterization of the nanosuspensions with respect to particle size analyses, morphology, physical stability, and investigate the influence of physicochemical characteristics of different nanosuspensions such as degree crystallinity of nanocrystals, kinetic solubility and dissolution behavior.

- Evaluate the drug release from the solid dosage forms (tablet, capsules, pellet and effervescent granule) and compared to marketed solid dosage form.

Chapter 2
LITERATURE REVIEW

2. LITERATURE REVIEW

2.1. Solubility

One of the primary physicochemical considerations in preparing pharmaceutical solutions is the solubility of the drug in a suitable solvent. Solubility may be defined as the maximum concentration of a substance that may be completely dissolved in a given solvent at a given temperature and pressure. When both solute and solvent are liquids, the term miscibility rather than solubility may be used to describe the affinity between the liquids [22].

The solubility of a substance may be described in a variety of ways. The USP/NF generally expresses the solubility in terms of the volume of solvent required to dissolve 1 gram of the drug at a specified temperature (eg. 1 g acetylsalicylic acid in 300 ml H_2O, 5 ml ethanol at 25°C). Other references may use more subjective terms to describe solubility, such as those given in the following table from *Remington's* [22, 23].

Table 2.1: The descriptive terms of approximate solubility of substances

Descriptive terms	Parts of solvent needed for 1 part solute
Very soluble	< 1
Freely soluble	1-10
Soluble	10-30
Sparingly soluble	30-100
Slightly soluble	100-1000
Very slightly soluble	1000-10,000
Practically insoluble or insoluble	> 10,000

Liquids which form a homogenous system when mixed in any proportion are said to be miscible (e.g. water and ethanol). Those in which only certain volume ratios produce homogenous mixtures are said to be miscible in certain proportions (e.g. water and chloroform). Immiscible liquids will not produce a homogenous solution in any proportions (e.g. water and olive oil).

The aqueous solubility of all drugs is of interest to us, since it is only in the form of an aqueous solution that a drug can be absorbed into the general circulation to exert a therapeutic effect.

2.1.1. Thermodynamic solubility and kinetic solubility

From a thermodynamic or chemical point of view, each substance has only one solubility value at a specific temperature, pressure and volume. This value is defined as the saturated concentration of the substance in solution when it is in equilibrium with its most stable solid state structure. In the literature, the term 'solubility' is often used loosely to mean the metastable or kinetic solubility, rather than the solubility at this thermodynamically stable state. The apparent or kinetic solubility, i.e. the concentration of the material in solution at apparent equilibrium (supersaturation), decreases to the level of the true or thermodynamically stable solubility, after an infinite equilibrium time [24, 25].

It should be noted that the term 'apparent solubility' has also been used in the literature to discuss a change in solubility due to ionic interaction. The result of such inter-ionic interactions may be that the actual or effective number of ions is no longer the same as that calculated based on the concentration of the solution. This is the reason why sometimes it is necessary to replace concentration with activity, which is the effective concentration. The term apparent solubility used here refers to a deviation from the thermodynamic stability which is caused due to the existence of disordered structure. On the other hand the solubility of sparingly soluble salts is often expressed or discussed in terms of the solubility product, which is an equilibrium constant describing the equilibrium between a sparingly soluble compound and a saturated solution of its ions. The numerical value of the solubility product of a salt can thus be regarded as a quantitative statement of the limit of solubility of the salt [24, 25].

2.1.2. Solubility enhancement

Poor water solubility of drugs is normally associated with low oral bioavailability in body. Saturation solubility enhancement is the most interesting method to overcome this problem. In order to obtain increased solubility for drugs with poor water solubility, it is necessary to alter the

formulation to facilitate solubilization. There are many classical approaches for solubilization of poorly soluble drugs. These technologies are commonly used as primary strategies. The choice conventional solubilization method will depend upon how the drug can be solubilized, stability in the system and biocompatibility of the vehicle for a given delivery route. For solid dosage form, it may be possible to modify the solid phase to enhance dissolution.

2.1.2.1 Solid phase modification

It is important to always consider, that for any raw drug substance or formulation, only one solid phase is thermodynamically stable for a given set environmental condition. The most stable solid will have the lowest free energy and correspondingly the lowest solubility. The crystal forms with higher free energy may exhibit an apparent solubility that is higher than the true equilibrium solubility for the system. An apparent solubility increase can occur anytime the starting solid material is not the most stable for the given system. However a metastable crystal will produce only a transient increase in solubility. The most stable crystal will eventually precipitate and the apparent solubility gained will diminish until the thermodynamic equilibrium solubility is reached. The degree of superaturation and of duration will depend on the characteristics of starting material and on the nucleation rate and the growth kinetics of the stable form. Consequently, an inherent difficulty in working with metastable systems is that the kinetics of conversion often cannot be predicted or controlled [26-28].

By virtue of having a higher apparent solubility, a metastable crystal will have an increased dissolution rate compared to the more stable form. The change in mass, M, as a function of time, t, for a solute is directly proportional to its apparent solubility, S_{app}, according to:

$$dM/dt = K \times A(S_{app} - C) \qquad \textit{equation 1}$$

where A is the solvent accessible surface area, C is the concentration of the solute in solution, and K is a constant that includes the diffusion coefficient of the solute and hydrodynamic properties of system [26, 27]. This equation

describes that the larger the apparent solubility, the greater is the dissolution rate.

It may be practical at time to use a solid material that gives a higher apparent solubility and/or increase in the dissolution rate in order to enhance bioavailability. As example, polymorph B of chloramphenicol palmitate, which can produce a solubility that is roughly two times that of polymorph A, gives higher blood levels in vivo. It showed that as the percentage of polymorph B increases in the dosage form, there is a linier increase in the blood concentration of chloramphenicol palmitate [28].

Through alteration of the solid state form it is possible to increase the apparent solubility of a drug. However, the practical use of a higher energy solid form is limited due to physical and chemical issues. Significant investigations must be made in order to assure that a dosage form using metastable crystals will maintain integrity throughout the product shelf life [28].

2.1.2.2 Salt formation and pH control

a. Salt formation

The aqueous solubility of non electrolytes is nearly always affected in some way by the addition of an electrolyte. Salting-out is the precipitation of organic solutes from aqueous solution by the addition of an electrolyte or salt. This is attributed to competition between solute molecules for the solvent and is dependent upon the size and valence of the ions. Salting-in is the increase in solubility of an organic solute upon addition of an electrolyte [26-28]. The mechanism of this phenomenon is poorly understood and it is rarely encountered. An example is the group of proteins called globulins which are more soluble in dilute salt solutions than in water [26-29]. Complex ion formation occurs when an insoluble solute reacts with a soluble substance to form a soluble complex. An example is the addition of the soluble potassium iodide (KI) to the insoluble iodine molecule (I_2) to form a soluble triiodide complex (KI_3).

b. pH control

According to the Henderson-Hasselbalch equation, the relationship between pH, pK_a, and relative concentrations of a weak acid and its salt is as follows:

$$[A^-] = \frac{K_a[HA]}{[H^+]} \qquad \textit{equation 2}$$

where $[A^-]$ is the molar concentration of the salt (dissociated species) and [HA] is the concentration of the undissociated acid.

Changes in solubility brought about by alterations of the solvent pH can be predicted by the pHp equation. The pHp is the pH below which an acid or above which a base will begin to precipitate.

The Henderson-Hasselbalch equation can be simplified to,

$$S_T = S_W(1 + 10^{(pH-pK_a)}) \qquad \textit{equation 3}$$

where, S_W = the molar solubility of the undissociated acid , S_T = the total molar concentration of the salt form of the drug initially added

When the concentrations of salt and acid are equal, the pH of the system equals the pKa of the acid. As the pH decreases, the concentration of the molecular acid increases and that of the salt decreases. This has some interesting implications regarding the aqueous solubility of the acid, since the undissociated form is much less soluble than its salt. Of further interest, therapeutically, is the fact that it is the undissociated acid (HA) that more readily penetrates biological tissues to exert a therapeutic effect [26-29]. Thus, in formulating the product, some balance must be made between the more soluble salt form and the biologically active acid and factors other than pKa and pH must be considered (e.g. safety and comfort).

By analog to the weak acid the total solubility is described by:

$$S_T = S_W(1 + 10^{(pK_a - pH)}) \qquad \textit{equation 4}$$

where the pK_a refers to HA^+.

In addition, one approach is to alter the polarity of the solute by shifting it between its molecular (undissociated) and ionic (dissociated) states. A shift

toward the ionic form improves solubility of the solute in water and other polar solvents. A shift toward the molecular species improves solute solubility in non-polar solvents. Such shifts may be produced by altering the pH of the solution (or using the salt form of the compound) [26-29].

2.1.2.3 Co-solvents

Solute molecules are held together by certain intermolecular forces (dipole-dipole, induced dipole-induced dipole, ion-ion, etc.), as are the molecules of the solvent. In order for dissolution to occur, these cohesive forces of like molecules must be broken and adhesive forces between solute and solvent must be formed.

The solubility of a drug in a given solvent is largely a function of the polarity of the solvent. Solvents may be considered polar, semi-polar or non-polar. Polar solvents will dissolve ionic and other polar solutes (i.e. those with an asymmetric charge distribution or like dissolves like), whereas, non-polar solvents will dissolve non-polar molecules. Semi-polar solvents (e.g. alcohols and ketones) may induce a certain degree of polarity in non-polar molecules and may thus act to improve the miscibility of polar and non-polar liquids. The relationship between polarity and solubility may be used in practice to alter the solubility of a drug in a pharmaceutical solution [26-29].

Another approach is to mix solvents of different polarities to form a solvent system of optimum polarity to dissolve the solute. Such solvents must, obviously, be miscible. This method is referred to as solvent blending or cosolvency and uses the dielectric constant as a guide to developing the cosolvent system. Since many solvents may be toxic when ingested, most solvent blends are limited to mixtures containing water, ethanol, glycerin, propylene glycol, polyethylene glycol 400 or sorbitol solution. The list is somewhat expanded for solutions for external application.

There are many pharmaceutical substances which are non-polar or which are weak acids and bases whose ionized salt forms are unstable in solution. In order to dispense solutions of these substances, we must derive a solvent of appropriate polarity (or non-polarity). Practically speaking, this is a fairly simple problem to solve. Solutions are prepared containing varying concentrations of ethanol or acetone in water, ranging from 0 to 100%. The

required concentration of drug is added to each solution and the solutions are refrigerated overnight, then viewed for precipitation [28].

From this information it is possible to formulate a vehicle, substituting other solvents, which is of the necessary polarity and is pharmaceutically elegant. These calculated values of the dielectric constant are only approximate. Interactions between multiple solutes and solvents may increase or decrease solubility. Nonetheless, the use of the dielectric constant in formulating solvent systems gives us a simple and scientific approach to estimating our needs. It is, therefore, a useful tool [28, 29].

2.1.2.4 Solubilization by micelles

Surfactants are molecules with distinct polar and non polar regions. Most surfactant consists of a hydrocarbon segment (usually in the form of long aliphatic chain segment) connected to a polar group. The polar group can be anionic (such as carboxylate, sulfate or sulfonate), cationic (such as ammonium, trialkylammonium or pyridinium), zwitterionic (such as glycine or carnitine) or nonionic such as polyethylene glycol, glycerol or sugar. Due to the differences in properties of the polar and non polar region, surfactants tend to accumulate and orient at interfaces so that each region of the surfactant interacts with a separate phase. The polar portion of the surfactant will associate with the more polar phase (especially if it is water) and the nonpolar portion of the surfactant will remain in the more non polar surfactant solvent. In water, as the concentration of surfactant increases above a critical value, its molecules self associate into soluble structures called micelles. The concentration at which they begin to form is called the critical micelle concentration (CMC) [26, 27, 29].

These micelles are normally spherical with the nonpolar regions of surfactant molecules gathered in the center (core) and surrounded by a shell of the polar region which are in contact with the water. A nonpolar drug, which is squeezed out of water, can locate within the micelle core. A semipolar drug can locate between or partially within the core and the polar shell. Since the micelles are soluble in water any drug that is incorporated into the micelle will also be soluble in the aqueous system. If the monomers of surfactant in solution do not affect the solubility of the solute, then the solute concentration

will remain constant (at the intrinsic solubility, SW) until the CMC. After the CMC the solute concentration will increase with increasing surfactant (micelle) concentration. A simple equation representing a solute's total solubility, ST, in a surfactant system is

$$ST = SW + \kappa (C_{surf} - CMC) \qquad \textit{equation 5}$$

Where C_{surf} is the total concentration of the surfactant and κ is the solubilization capacity. The solubilization capacity reflects the number of surfactant molecule that are required to solubilize a single solute molecule [26, 27, 29].

2.1.2.5 Complexation [26, 27]

Complexation is association between two or more molecules to form a nonbonded entity with well defined stoichiometry. The two types of complexation are most useful for increasing the solubility of drugs in aqueous media. Stacking complexes are formed by the overlap of the planar regions of aromatic molecules, while the inclusion complexes are formed by the insertion of the nonpolar region of one molecule into the capacity of another molecule (or group of molecules). The mathematical description for the equilibrium constant of a 1:1 complex, $K_{1:1}$, is defined by

$$K_{1:1} = [SL]/[S][L] \qquad \textit{equation 6}$$

Where S is the concentration of the free solute, L is the concentration of free ligand, and [SL] is the concentration of the solute/ligand complex. The equilibrium constant is also commonly referred to as the stability constant or complexation constant [27].

From the above it can be seen that, as the stability constant of a 1:1 complex increases, the slope will increase until the value converges to unity for a strong complex in which one ligand molecule solubilizes one solute molecule. The initial segment of the curve in fig 2.1 illustrates this. The linear region will continue until the solubility of the complex itself is reached, at which point the total solubility of the solute remains constant as indicated by the central segment of the curve.

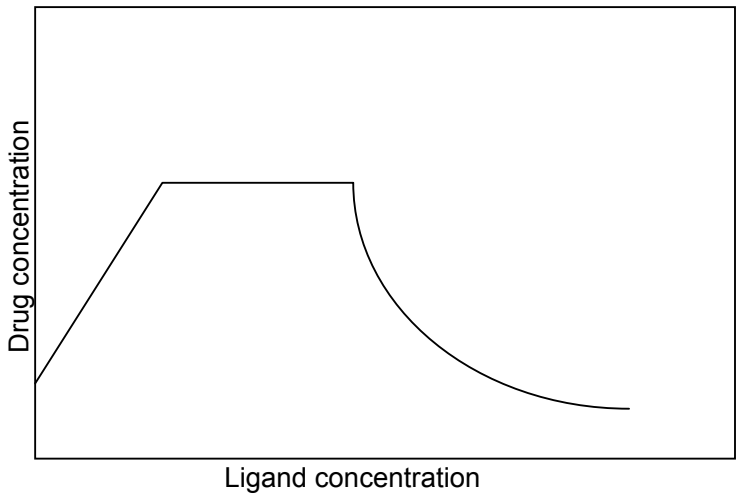


Fig. 2.1 General solubilization profile for complexation

A plateau is analogous to maximum solubility of a solute as described previously. Further addition of the complexing agent can result in a reduction of the concentration of the free solute and leveling of the curve at the solubility of the pure complex as illustrated by the final segment in the curve in **Fig. 2.1.**
An inclusion complex is produced by inclusion of a non polar molecule or the nonpolar region of a molecule (known as the guest) into the nonpolar cavity of another molecule or group of molecules (known as the host). When the guest molecule enters the host molecule, the contact between water and the nonpolar region of the both is reduced. Thus inclusion phenomena are the result of the same driving force that produces micellization, self-association, and stacking, namely the squeezing out from water of non polar moieties.
The most commonly used host molecules are the cyclodextrins. These cyclic oligomers of glucose are relative soluble in water and have cavities large enough to accept nonpolar portions of common drug molecules. The natural occurring cyclodextrins contain 6, 7 and 8 glucopyranose units and are termed α, β, and γ, respectively. The size of the cavacity in the cyclodextrin is the major factor in determining which guest solute will be most acceptable for complexation. In general, alkyl groups will fit well into the cavity of the α-cyclodextrin. The β-cyclodextrin are most well suited for accepting single

aromatic rings and the γ-cyclodextrin has a large enough cavity to accommodate larger hydrocarbons such as pyrene [27].

The degree to which a solute molecule will be solubilized by a cyclodextrin molecule will depend on several properties. The solute molecule must have a significant nonpolar portion in order to be squeezed out of the water and into the cyclodextrin cavity. Since the interior dimensions of a given cyclodextrin are fixed, a significant part of molecule (or whole molecule) must then fit inside the cyclodextrin. The intermolecular interactions between the two molecules will determine the strength of the complex. The aliphatic compounds are preferentially solubilized by α-cyclodextrin, while the aromatic ring compounds are best solubilized by β-cyclodextrin. In addition, γ-cyclodextrins may increase solubilization of the fused ring compounds [27].

2.1.2.6 Solid dispersions

Solid dispersions have been developed five decades ago. In 1961, Sekiguchi and Obi developed a practical method whereby many of the limitations of the bioavailability enhancement of poorly water-soluble drugs can be overcome. This method, which was later termed solid dispersion, involved the formation of eutectic mixtures of drugs with water-soluble carriers by the melting of their physical mixtures. Sekiguchi and Obi suggested that the drug was present in a eutectic mixture in a microcrystalline state [21, 30]. Later, Goldberg et al. demonstrated that the entire drug in a solid dispersion might not necessarily be present in a microcrystalline state; a certain fraction of the drug might be molecularly dispersed in the matrix, thereby forming a solid solution. In either case, once the solid dispersion was exposed to aqueous media and the carrier dissolved, the drug was released as very fine, colloidal particles. Because of greatly enhanced surface area obtained in this way, the dissolution rate and the bioavailability of poorly water-soluble drugs were expected to be high [21, 31].

But time to time, it has been found that there are principal limitations regarding the development of solid dispersion/solution methods. Problems limiting the commercial application of solid dispersion involve its method of preparation, reproducibility of its physicochemical properties, its formulation

into dosage forms, the scale up of the manufacturing processes, and the physical and chemical stability of drug and vehicle.

2.2. Dissolution velocity

The dissolution process of solids in liquids has been described by Hildebrand and Scott [32] and [33] as involving three steps: (1) the removal of a molecule from the solute; (2) creation of a hole in the solvent; and (3) insertion of the solute molecule into the solvent (i.e. solute–solvent interaction). This interaction between the solute and the solvent is obviously dependent on the physical and chemical nature of the two participating molecules. The processes involved in the dissolution of hydrophobic materials, which have low aqueous solubility, will differ from those affecting the dissolution of hydrophilic substances. The amount of energy required to remove a molecule of a sparingly soluble drug from the solute particle is lower for a hydrophobic drug than for a hydrophilic drug (such as those composed of inorganic salts). The intermolecular bonds in inorganic salts are so strong that a large amount of energy is required to disassociate the discrete molecules. However, when the individual solute molecules are liberated, a hydrophilic salt molecule (or ion) is more likely to interact with water than a hydrophobic drug molecule. Thus, the main factor affecting the solubility and dissolution of a hydrophobic drug is the limited energy released when a drug molecule is bonded to the solvent [29]. In contrast, the main barrier to dissolution of a sparingly soluble hydrophilic substance appears to be disruption of the strong intermolecular forces [24].

2.2.1 Theory of Dissolution

When a solid dosage form is introduced into a beaker of water or into the gastrointestinal tract, the drugs begin to pass into solution from the intact solid. Unless the solid dosage form is a continguous polymeric device, the solid matrix also disintegrates into granules and these granules deaggregate in turn into fine particles. Disintegration, deaggregation, and dissolution may occur simultaneously with the release of a drug from its delivery form. These steps are separated for clarification as depicted in **Fig 2.2** [26].

The effectiveness of a solid dosage form in releasing of its drug for systemic absorption depends somewhat on the rate of disintegration of the dosage form and deaggregation of the granules. Ordinarily of more importance, however, is the dissolution rate of the solid drugs. For class II drugs of the Biopharmaceutical Classification System (BCS), frequently dissolution is the limiting or rate controlling step in absorption of poorly soluble drugs, because it is often the slowest of the various step involved in release of the drugs from its dosage forms and passage into systemic circulation [26].

The rate at which the solid dissolves in the solvent was proposed in quantitative terms by Noyes and Whitney in 1897 and elaborated subsequently by other coworkers [34]. The equation may be written as

$$\frac{dM}{dt} = \frac{DS}{h}(C_s - C) \qquad \textit{equation 7}$$

or

$$\frac{dC}{dt} = \frac{DS}{Vh}(C_s - C) \qquad \textit{equation 8}$$

in which *M* is the mass of the solute dissolved in time t, *dM/dt* the mass rate of dissolution (mass/time), *D* the diffusion coefficient of the solute in solution, *S* the surface area of the exposed solid, *h* the thickness of the diffusion layer, C_s the solubility of the solid (i.e., the concentration of a saturated solution of the compound of the solid and at the temperature of the experiment) and *C* the concentration of solute in the bulk solution and at time t. The quantity *dC/dt* is the dissolution rate and *V* the volume of solution.

In dissolution or mass transfer theory, it is assumed that an aqueous diffusion layer or stagnant liquid film of thickness h exist at the surface of solid undergoing dissolution, as observed in **Fig. 2.3**. This thickness h represents a stationary layer of solvent in which the solute molecule exist in concentrations from Cs to C. Beyond the static diffusion layer, at x greater than h, mixing occurs in the dissolution and the drug is found at a uniform concentration, C, throughout the bulk phase [26].

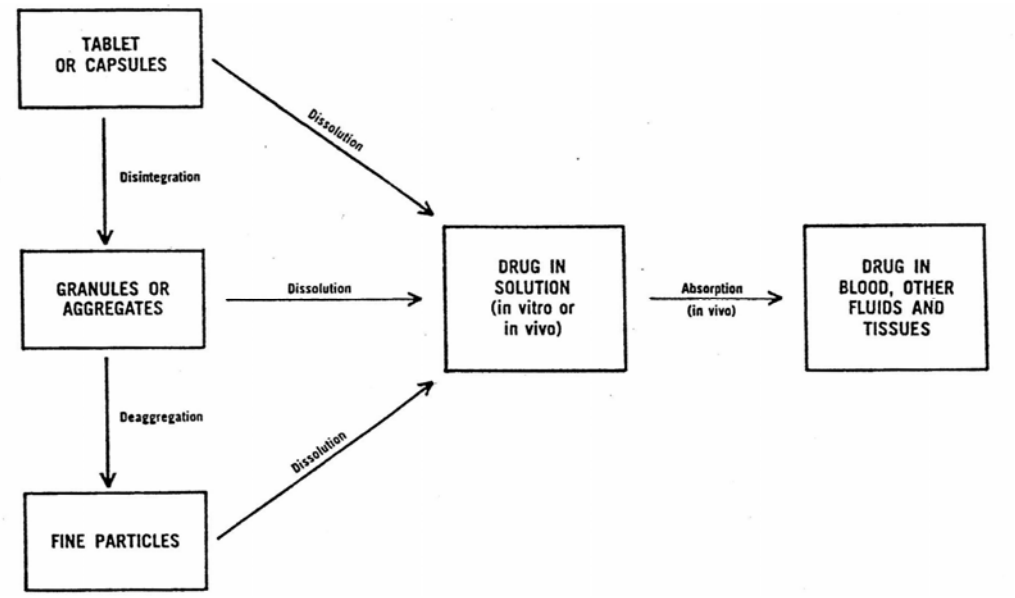


Fig. 2.2 Disintegration, deaggregation and dissolution stages as a drug leaves a tablet or granular matrix after [26]

At the solid surface-diffusion layer interface, x = 0, the drug in the solid is equilibrium with the drug in the diffusion layer. The gradient or change in concentration with distance across the diffusion layer is constant as shown by the straight downward-sloping line. This is the gradient represented in the equation by the term (C_s-C)/h. When C is considerably less than the drug's solubility, Cs, the system is represented by *sink condition*, and concentration C may eliminated from equations. The equation then becomes

$$dM/dt = DSC_s/h \qquad \textit{equation 9}$$

For a drug powder consisting of uniformly sized particles, it is possible to derive an equation that expresses the rate of dissolution based on the cube root of the weight of the particles. The radius of the particle is not assumed to be constant. The equation is known as the Hixson-Crowell cube root law [35], and presented as:

$$M_0^{1/3} - M^{1/3} = \kappa t \qquad \textit{equation 10}$$

where M_0 is the original mass of the drug particles, M mass particles at time t and **κ** is the cube root dissolution rate constant.

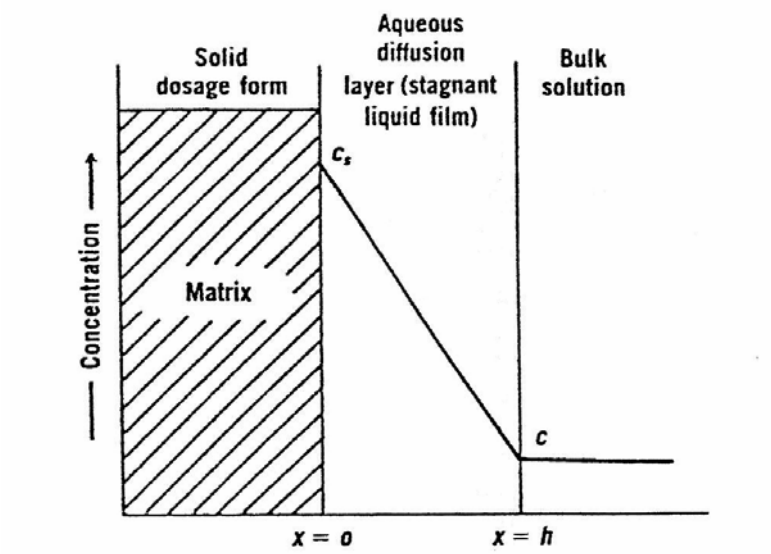


Fig. 2.3 Dissolution of the drug from a solid matrix, showing the stagnant diffusion layer between the dosage form surface and the bulk solution, after [26]

2.2.2 Biopharmaceutical Classification System (BCS)

The BCS is scientific framework for classifying drug substances based on their aqueous solubility and intestinal permeability. When combined with the dissolution of the drug product, the BCS takes into account three major factors that govern the rate and extent of drug absorption from solid oral dosage forms: dissolution, solubility, and intestinal permeability. According to the BCS, drug substances are classified as follows [36] :

Class I : High Solubility - High Permeability
Class II : Low Solubility - High Permeability
Class III : High Solubility - Low Permeability
Class IV: Low Solubility - Low Permeability

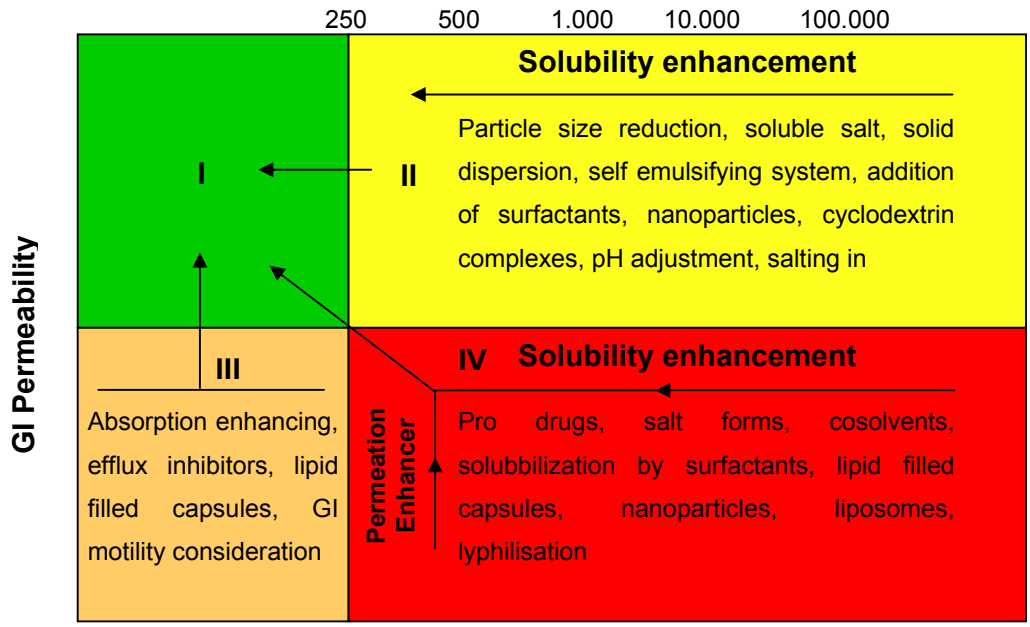


Fig. 2.4 Possibilities of shifting the solubility-dissolution characteristics from a very poorly soluble drug to Dose: Solubility ratio (D:S) within the range of values encountered in the human GI tract (D:S > 250 ml), after [37]

The Biopharmaceutical Classification System (BCS) groups poorly soluble compounds as Class II and IV drugs, compounds which feature poor solubility and high permeability, and poor solubility and poor permeability, respectively. Drug substances are considered highly soluble when the largest dose of a compound is soluble in <250mL water over a range of pH from 1.0 to 7.5; highly permeable compounds are classified as those compounds that demonstrate >90 per cent absorption of the administered dose. In contrast, compounds with solubilities below 0.1mg/mL face significant solubilisation obstacles, and often even compounds with solubilities below 10mg/mL present difficulties related to solubilisation during formulation [37].

2.2.3 Dissolution of tablets, capsules, and granules

A number of methods for the in vitro and in vivo testing of dosage form have been suggested. The purpose of the in vitro dissolution study is to provide a fast and inexpensive method that correlates to the performance of the dosage form in human subjects. The Hansen paddle equipment and a research apparatus provide a convenient system as shown in **Fig. 2.5** [26]. Its design

has advantages in research and product formulation. The paddle equipment of this figure is currently known as the United States Pharmacopoeia (USP) dissolution apparatus 2 and rotating basket apparatus (not shown) is referred to as USP dissolution apparatus 1 [22].

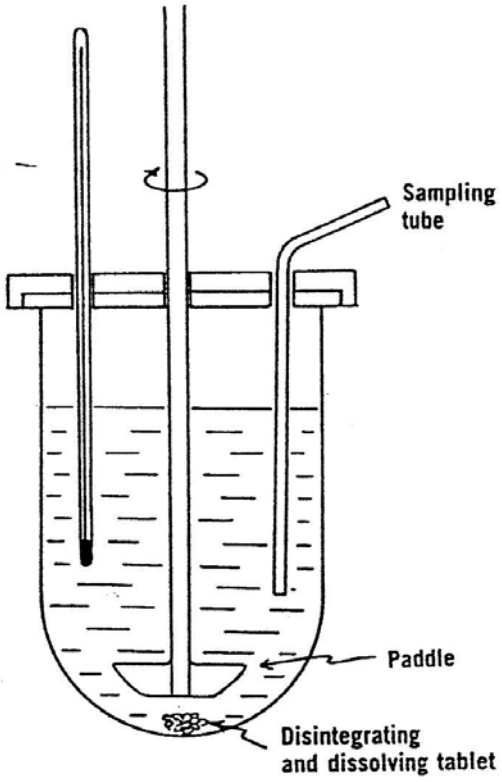


Fig 2.5 Dissolution apparatus: Hansen paddle equipment for granules and tablets, after [26]

Dissolution profiles can be described investigating two products (6 units each), the test product and the reference product. To use the mean dissolution values from both curves, the percent coefficient of variation at the earlier time points should not be more than 20% and at other time points should not be more than 10%. Only one measurement should be considered after 85% dissolution of both of the products. The dissolution measurements of the test and reference products should be made under exactly the same conditions [26].

2.2.4 Dissolution & Particle Size

In order for a drug to have its effect after oral administration it must go into solution and then diffuse through the gut wall into the body. The first step in that process is the disintegration of the dosage form followed by dissolution of the active ingredient. Dissolution of a pure substance follows the Noyes Whitney Equation [34], $dC/dt = kS (C_s-C_x)$ where k is the dissolution rate constant. One way to increase the dissolution rate of poorly soluble drugs is to increase the surface available for dissolution. This is done by reducing the particle size or by dividing the dosage form into two smaller tablets or capsules, with a larger combined surface area. Other ways include increasing the disintegration rate and deaggregation. Influence of particle size reduction on dissolution rate is discussed detail in the next section.

2.3 Solid Dosage Forms

2.3.1 Tablet

The compressed tablet is one of the most popular dosage forms today. About one-half of all prescriptions dispensed are tablets. Usually one considers a compressed tablet as an oral medication; however, tablets have many other uses. The sublingual tablet, the pellet, the troche, and the vaginal insert are manufactured by the same procedure as an oral tablet.

Tablets are solid dosage form containing medical substances with or without suitable diluents. They may be classified, according to the method of manufacture, as molded tablets or compressed tablets [22, 38, 39].

There are three methods of commercially making compressed tablets [38-41]:

2.3.1.1 The direct compression method

A compressible vehicle is blended with the medicinal agent, and if necessary, with a lubricant and a disintegrant, and then the blend is compressed. Substances that are commonly used as directly compressible vehicles are: anhydrous lactose, dicalcium phosphate (Emcompress), granulated mannitol, microcrystalline cellulose (Avicel), compressible sugar (Di-Pac), starch (Sta-Rx 1500), hydrolyzed starch (Celutab), and a blend of sugar, invert sugar, starch and magnesium stearate [39, 41].

2.3.1.2 The dry granulation method (slugging method)

The ingredients in the formulation are intimately mixed and precompressed on heavy duty tablet machines. The slug which is formed is ground to a uniform size and compressed into the finished tablet [39].

2.3.1.3 The wet granulation method [39, 40]

This method has more operational manipulations, and is more time-consuming than the other methods. The wet granulation method is not suitable for drugs which are thermolabile or hydrolyzable by the presence of water in the liquid binder. The general steps involved in a wet granulation process are:

- The powdered ingredients are weighed and mixed intimately by geometric dilution.
- The granulating solution or binder is prepared.
- The powders and the granulation solution are kneaded to proper consistency.
- The wet mass is forced through a screen or wet granulator.
- The granules are dried in an oven or a fluidized bed dryer.
- The dried granules are sieved to a suitable size for compression.
- A lubricant and a disintegrating agent are mixed with the granules.
- The granulate is compressed into the finished tablet.

2.3.1.4 Methods Used to Compound Tablets

The only limitation of commercially manufactured tablets is that they are available only in fixed dosage strengths and combinations. To provide the flexibility of compounded formulations, pharmacists can extemporaneously prepare molded and compressed tablets for their patients. Molded tablets are compounded using a tablet triturate mold. Compressed tablets can be made using a pellet press or a single-punch tableting machine.

a. Molded Tablets [39, 42]

Molded tablets are generally prepared by mixing the active drug with lactose, dextrose, sucrose, mannitol, or some other appropriate diluent that can serve as the base. This base must be readily water soluble and should not degrade during the tablet's preparation. Lactose is the preferred base but mannitol adds a pleasant cooling sensation and additional sweetness in the mouth.

b. Single Punch Tablet Machines [39, 43, 44]

Tableting machines are commonly used in pharmaceutical industry. They are high-speed machines that create thousands of tablets in a small time period. The compounding pharmacist uses a variation of these machines. It is called a single-punch tablet press and makes one tablet at a time. A "punch" has two pieces of casted tubular metal. The bottom metal piece has a small cavity in one end of the tube; the top metal piece has one end that is tapered into a small rod that will just fit into the small cavity in the other piece. The rod does not go all the way to the bottom of the cavity, but leaves a small gap. The punch is fitted into a press so that when the handle is depressed and released, the rod goes into and then comes out of the bottom piece. To make a tablet, the powder material is placed into the bottom piece, and the handle is depressed and released. The powders are compressed and occupy the size of the gap designed in the punch. Punches come in many sizes which allow the production of tablets of different sizes and compression strengths. But each punch is a matched set; it is not possible to interchange the top and bottom pieces of different punches. Compressed tablets can be made using a tablet press. The ingredients may include binders (e.g., acacia), lubricants (e.g., stearic acid), colors, and flavors. The powder mixture is prepared; the desired quantity of mixture is weighed, and then pressed with a single-punch tablet machine.

2.3.1.5 Evaluation of Tablets

Tablets are evaluated by a variety of methods [26, 39, 45, 46].

a. Analytical determination of tablet content

This probably will not be done due to the requirement of specialized equipment. However, the weight variation of the tablets can be measured by weighing each individual tablets and determining the percent difference from the intended amount. In case of content uniformity, constant weight guaranties a constant drug content. Guidelines in the USP 24/NF19 Supplement 1 indicate that each tablet "shall be not less than 90% and not more than 110% of the theoretically calculated weight for each unit."

b. Tablet hardness

The tablets must be hard enough to withstand mechanical stress during packaging, shipment, and handling by the consumer. Section <1216> of the USP 24/NF19 outlines a standard tablet friability test applicable to manufactured tablets. Most compounding pharmacy would not have the apparatus specified in Section <1216>. However, there are several hand operated tablet hardness testers that might be useful. Examples of devices are the Strong Cobb, Pfizer, and Stokes hardness testers. The principle of measurement involves subjecting the tablet to an increasing load until the tablet breaks or fractures. The load is applied along the radial axis of the tablet. Oral tablets normally have a hardness of 4 to 8 or 10 kg; however, hypodermic and chewable tablets are much softer (3 kg) and some sustained release tablets are much harder (10-20 kg).

c. Tablet disintegration

There are commercially available disintegration and dissolution apparatuses. Most pharmacists in a pharmacy shop will not have this equipment. However, a simple disintegration apparatus can be made. Start by supporting a 10 mesh screen about 2 inches above the bottom of a 1000 ml beaker. Fill the beaker with 1000 ml of water, add a stirring bar, and place the beaker on a magnetic stirring plate. Stir at a moderate speed. Drop the tablets onto the mesh screen and record the time needed for the tablets to disintegrate. A reasonable disintegration time should be between 15 and 30 minutes, although the time will depend on the product, the stirring speed, etc.

d. Drug dissolution from the tablet

Disintegration time determination is a useful tool for production control, but disintegration of a tablet does not imply that the drug has dissolved. A tablet can have a rapid disintegration time yet be biologically unavailable. The dissolution rate of the drug from the primary particles of the tablet is the important factor in drug absorption and for many formulations is the rate-limiting step. Therefore, a dissolution time of the drug is more indicative of the availability of a drug from a tablet than the tablet disintegration test. Even though this is an important parameter to measure, most pharmacies do not have the equipment needed to conduct these kinds of tests.

2.3.2 Effervescent tablets

Effervescence is defined as the evolution a gas from a liquid as the result of a chemical reaction. Effervescent mixtures have been used medicinally for many years. For example effervescent powder was used as salina cathartics were already available since the eighteenth century and were subsequently listed in official compendia as compound effervescent powders. Effervescent tablets are a convenient, easy to use pre-measured dosage form. They cannot spill as can powdered preparations. They can be individual packaged to exclude moisture, thereby avoiding the problem of product instability of the contents during storage.

Several investigators have studied changes in the bioavailability of a drug when delivered from an effervescent tablet. The classical example is effervescent aspirin. It has been indicated that significant differences in the absorption kinetics of aspirin were observed between effervescent and conventional or enteric coated tablets and that the differences could be attributed to gastric emptying rate and rapid tablet disintegration. Another investigator reported increased bioavailability of phenylbutazone from effervescent dosage form [47, 48].

2.3.2.1 Raw material

In generally, very important for effervescent product are the adsorption/desorption isotherm of the raw material and consequently, the moisture content. To avoid a premature effervescent reaction in the dosage form, substances with low moisture content will have to be used. The aqueous solubility is another important property of the substances used in effervescent products. It is also important to use raw materials that are easily wetted.

a. Acid source

The acidity for the effervescent reaction can be obtained from three main sources: acids, acid anhydrides, and acid salts. Traditional sources of acidic materials are the organic acids, citric and tartaric acid; however, some acid salts are also used [47, 48].

Citric acid. Citric acid is obtained as a monohydrate or a hydrate. A variety of particle size grade are available, i.e. colorless, translucent crystals, or white,

granular to crystalline powders. It is odorless and has a strong acidic taste. Citric acid is freely soluble in water and alcohol.

Tartaric acid. Tartaric acid is soluble in less than 1 part of water and 1 part in 2.5 of alcohol. It absorbs significant amounts of moisture at relative humidities up to about 65%, but at relative humidities above 75%, it is deliquescent.

Ascorbic acid. Ascorbic acid occurs as white to light yellow crystalline powder or colorless crystals with a sharp acidic taste and no odor. It is not hygroscopic. Upon to exposure to light, it gradually darkens. Ascorbic acid is freely soluble in water (1g in about 3 ml) and soluble in absolute alcohol (1g in 50 ml).

Other acids. Fumaric acid is a white, odorless or nearly odorless, crystalline powder. Nicotinic acid is soluble 1 part in 55 to 60 part water, a low solubility that complicates the formulation. Acetylsalicylic acid (aspirin), malic acid, succinic acid are other acids which have been mentioned in connection with effervescent products.

b. Sources of carbon dioxide

Both carbonate and bicarbonates are used as carbonate sources.

Sodium bicarbonate. Sodium bicarbonate is an odorless, white crystalline powder with saline, slightly alkaline taste. Sodium bicarbonate decomposes when heated to temperature over 65^{o}C. Heat treatment at temperatures from 50^{o}C will convert the bicarbonate to carbonate.

Sodium carbonate. Sodium carbonate is commercially available as an anhydrous form and as a monohydrate or a decahydrate. All forms are very soluble in water. The anhydrate is hygroscopic and slowly absorbs moisture forming the monohydrate, whereas the decahydrate effloresces.

Potasium bicarbonate and potasium carbonate are very soluble in water and less sensitive to heat in connection with drying than sodium bicarbonates [47, 48].

Potasium carbonate is a highly deliquescent powder with a strongly alkaline taste. Potasium carbonate is soluble in 1 part of water and pratically insoluble in alcohol.

Calcium carbonate. Calcium carbonate occurs as fine, white, odorless and tasteless powder or crystals. The solubility in water is less than 1 in 50.000 and insoluble in 100% ethanol or isopropanol.

Other sources, amino acid-alkali metal carbonate derivatives, for example, sodium glycine carbonate [47, 48].

Effervescent tablets and powders are mentioned in the pharmacopeias and exist as products on the market. Effervescent powder or granules are usually packed in individual dose units or laminates with protective properties. The older method of preventing the effervescent reaction during storage was to pack the acidic and alkaline components in separate wrappings [47, 48].

2.3.2.2 Processing

The manufacturing of effervescent dosage forms requires strict control of the environment factors. It is clear that it is essential to maintain RH through the plant of no more than 20%. Besides this it was desirable to maintain the temperature uniformly at 21^{o}C.

Effervescent tablets generally contain ingredients such as tartaric acid, citric acid, and sodium bicarbonate. These powders would be appropriately mixed and compressed into tablets using the same procedure as chewable tablets. They will not require a disintegrant since they will effervesce when placed into water. Compressed tablet mixtures generally contain the active drug, a diluent (e.g. lactose), a disintegrant (e.g. starch), and a lubricant (e.g. 1% magnesium stearate).

a. Wet granulation methods

In the wet method, the one molecule of water present in each molecule of acid sources (citric acid) acts as the binding agent for the powder mixture. The water is added to the non solvent (such as alcohol), which is employed as the moisturing agent, to form the pliable mass of material for granulation. In this method all of the powders may be anhydrous so long as water is added (in portions) to prepare a mass of proper consistency. The granule mass is passed through a sieve to produce granules of the desired size. When all of the mass has passed through the sieve, the granules are immediately dried at temperatures 70^{o}C to 75^{o}C and immediately transferred to containers which are then promptly and tightly sealed [47, 48].

b. Dry granulation and fusion method

Granulation by slugging-slug or large tablets are compressed using heavy-duty tablet equipment or roller compaction is suitable for materials that cannot

be wet granulated. The slugs and the material from the roller compactor are reduced to proper size. Lubrication is often necessary during slugging but not with roller compaction. The acidic and basic component may be dry-granulated separately or together [47, 48].

The fusion method differs from the wet method in the source of the binding agent. The acid source (i.e. citric acid) produces water as the binding agent during heating process. The powder of effervescent tablets is placed in an oven (or other suitable source of heat) previously heated to between 93^{o}C and 104^{o}C. The heat causes the release of the water of the crystals of the citric acid, which in turn dissolves a portion of the powder mixture, setting off the chemical reaction and consequently release of some carbon dioxide. This causes the softening of the mass of powder to become somewhat spongy, and of the proper consistency, it has to be removed from the oven and rubbed through a sieve to produce granules of desired size. In the process of wet granulation, the granules are immediately dried at temperature not exceeding 54^{o}C and immediately transferred to containers which are then promptly and tightly sealed [49].

c. Direct compression.

Some effervescent tablet products are successfully produced by direct compression (for example acetylsalicylic acid products). Direct compression normally requires careful selection of raw materials to achieve free flowing, non segregating, compressible mixtures. Some formulations of effervescent tablets containing ascorbic acid granulated with polyvinyl pyrrolidone were made by direct compression on a small scale [47, 48].

2.3.3 Pellets

Pellets are small and free flowing, spherical particulates typically created by the agglomeration of fine powder or granules of drug substances and excipients using appropriate processing equipment. Specific items often termed 'pellet' include implants [22]. Pellets have been developed in the pharmaceutical industry more than four decade ago. Pellets provide the scientist with a high degree of flexibility during the design and development of oral dosage forms. They can be divided into desired dose strengths without formulation and process change, and can also be blended to deliver

incompatible bioactive agents simultaneously or particles with different release profiles at the same site or at different sites within the gastrointestinal tract. In addition, pellets have numerous therapeutic advantages over traditional single units, such as tablets and powder-filled capsules. Taken orally, pellets generally disperse freely in the gastrointestinal tract, and consequently maximize the drug absorption, minimize local irritation of the mucosa by irritant drugs because of the small quantity of drug available in a single pellet, and reduce inter- and intra-patient variability. As the advantage of the pellets over single units become clear, the pharmaceutical industry as a whole started to devote resources to conduct research in pellet technology and, whenever possible, acquire advanced equipment suitable for the manufacture of pellets. Although a variety of techniques are available, the most commonly used pelletization processes are layering and extrusion with subsequent spheronization. Other technologies used occasionally are balling, spray congealing and drying as well as emerging technologies such as cryopelletization and melt spheronization [50].

3.3.3.1 Layering

Layering processes are probably the most well-controlled and straight forward pelletization techniques that have been used over the years. They are classified into three categories: solution, suspension and powder layering. Solution and suspension layering involve the deposition of successive layers of solution and suspension of drug substances, respectively, on started seeds which may be inert material or crystals or granules of the same drug. The factors that control coating processes apply directly to solution or suspension layering, and, as a result, require basically the same processing equipment. Over the years, conventional coating pans, fluid bed centrifugal granulators and Wurster coaters have been used to manufacture pellets by solution and suspension layering. The efficiency of the process and the quality of the pellets produced is in part related to the type of equipment employed. Although the equipment and process that provide the desired pellets can easily be identified and utilized, it is usually the availability of equipment that dictates the decision-making process.

Powder layering involves the deposition of successive layers of dry powders of drugs and/or excipients on performed nuclei or cores with the help of a binding liquid. Because powder layering involves the simultaneous application of the binding liquid and dry powder, not all the pelletization equipment that is routinely used to prepare pellets by solution or suspension layering can be employed, although the reverse is true. The main equipment-related requirement in a powder layering process is that the product container should have solid walls with no perforations in order to avoid powder loss underneath the product chamber before the powder is picked up by the wet masses of pellets that are being layered upon.

The most common pieces of layering equipment employed by the pharmaceutical industry are the standard or conventional coating pans, the Wurster coater and the centrifugal fluid-bed granulators [50].

2.3.3.2 Extrusion-Spheronization

Extrusion-spheronization as pelletization technique was developed in the early 1960s and since then has been extensively investigated and discussed. Interest in the technology is still strong as witnessed by the extent of coverage of the topic in proceeding of scientific meetings and symposia as well as in scientific literature. The technology is unique in that is not only suitable for manufacture of pellets with high drug loading, but it can also be used to produce extended-release pellets in the same step in certain situations and hence obviate the need for subsequent film coating. The most important equipment in the extrusion-spheronization processes is the extruder and the spheronizer [50].

A variety of extruders can be classified as:

a. Screw-fed extruder; the screw rotates along the horizontal axis and hence transports the material horizontally; they may be axial or radial screw extruders.
b. Gravity-fed extruders include the rotary cylinder and rotary gear extruder, which differ mainly in design of two counter rotating cylinders.
c. The ram extruder is probably the oldest type of extruders; a piston displaces and forces the material through a die at the end. These

extruders are preferentially used in the development phase, because they can also measure the reological properties of formulations.

A spheronizer, known as Marumerizer, consists of a static cylinder or stator and a rotating friction plate at the base. The stator can be jacketed for temperature control. The friction plate, a rotating disk with a characteristically grooved surface, is the most important component of the equipment.
The extrusion-spheronization process is a multiple step procedure, involving dry mixing, wet granulation, extrusion, spheronization and drying. The first step was dry mixing of nanosuspension and lactose followed by wet granulation using a coating pan, which converted the ibuprofen and lactose into a plastic mass that can be easily extruded by a twin-screw extruder. The extruded strands were transferred into a spheronizer where, when upon contact with the rotating friction plate, they were instantaneously broken into short cylindrical rods and were pushed towards and up the stationary wall of the processing chamber by centrifugal force. Due to gravity, the particles fall back to the friction plate and the cycle was repeated until the desired sphericity is achieved. Finally, they are dried either on trays or in a fluid bed-dryer prior to the further processing [50].

2.3.4 Capsule

Capsules are solid dosage forms in which the drug is enclosed within either a hard or soft soluble container or “shell”. The shells are usually formed from gelatin; however, they also may be made from starch or other suitable substances. Hard-shell capsule sizes range from No. 5, the smallest, to No. 000, which is the largest, except for veterinary sizes. However, size No. 00 generally is the largest size acceptable to patients. Size 0 of hard gelatin capsules having an elongated body (known as size OE) also are available, which provides greater filling capacity without an increase in diameter. Hard gelatin capsules consist of two, telescoping cap and body pieces. Generally, there are unique grooves or indentations molded into the cap and body portions to provide a positive closure when fully engaged, which helps prevent the accidental separation of the filled capsules during shipping and handling. Positive closure also may be affected by spot fusion (“welding”) of the cap and

body pieces together through direct thermal means or by application of ultrasonic energy. Factory-filled hard gelatin capsules may be completely sealed by banding, a process in which one or more layers of gelatin are applied over the seam of the cap and body, or by a liquid fusion process wherein the filled capsules are wetted with a hydro alcoholic solution that penetrates into the space where the cap overlaps the body, and then dried. Hard-shell capsules made from starch consist of two, fitted cap and body pieces. Since the two pieces do not telescope or interlock positively, they are sealed together at the time of filling to prevent their separation. Starch capsules are sealed by the application of a hydro alcoholic solution to the recessed section of the cap immediately prior to its being placed onto the body [51].

Capsules have several advantages as pharmaceutical dosage forms [51]:

- They dissolve and release the drugs for absorption following oral administration (as compared with tablets).
- They offer the pharmacist versitility to prepare any dose desired, and can be used to mask the unpleasant tastes, aromas, or appearance of a drug.
- They allow powders to be dispensed in an uncompressed form, thus allowing fast absorption (e.g. oral, inhalation, rectal, or to be diluted for vaginal, rectal, oral or topical use).
- They may be easier than tablets for some people to swallow.
- They can be made to alter the release rate of the drug.

Their disadvantages or limitations include the following [51]:

- They are easily tampered between active ingredients and excipients (although techniques exist for preventing this).
- They are subject to the effects of relative humidity and to microbial contamination.
- They may be difficult for some people to swallow.
- More expensive (commercially).

Table 2.2 Size and loaded volume and mean of capsules, [51]

Capsule Size	Volume (ml)	mg of lactose	mg of Aspirin
000	1.37	1340	1000
00	0.95	929	600
0	0.68	665	500
1	0.50	489	300
2	0.37	362	250
3	0.30	293	200
4	0.20	195	125
5	0.13	127	60

To aid in the selection of the appropriate size, a table, with the capacity of five common drugs for that particular size capsule, is printed in **Table 2.2**. As a guide, the relative sizes and fill capacities of capsules are given as well. By knowing the bulk density of filling material, a proper choice of the capsule size is usually made easier; however, trial and error soon develops the judgment of the beginning pharmacist.

Hard-shell capsules typically are filled with powder, beads or granules. Inert sugar beads (nonpareils) may be coated with active ingredients and coatings that provide extended release profiles or enteric properties. Alternatively, larger dose active ingredients themselves may be formed into pellets and then coated. Semisolids or liquids also may be filled into hard-shell capsules; however, when the latter are encapsulated, one of the sealing techniques must be employed to prevent leakage.

In hard gelatin capsule filling operations, the body and cap of the shell are separated prior to dosing. In hard starch shell filling operations, the bodies and caps are supplied separately and are fed into separate hoppers of the

filling machine. Machines employing various dosing principles may be employed to fill powders into hard-shell capsules; however, most fully automatic machines form powder plugs by compression and eject them into empty capsule bodies. Accessories to these machines generally are available for the other types of fills. Powder formulations often require adding fillers, lubricants, and glidants to the active ingredients to facilitate encapsulation. The formulation, as well as the method of filling, particularly the degree of compaction, may influence the rate of drug release. The addition of wetting agents to the powder mass is common where the active ingredient is hydrophobic. Disintegrants also may be included in powder formulations to facilitate deaggregation and dispersal of capsule plugs in the gut. Powder formulations often may be produced by dry blending; however, bulky formulations may require densification by roll compaction or other suitable granulation techniques [44, 51].

In extemporaneous prescription practice, hard-shell capsules may be hand-filled; this permits the prescriber latitude of choice in selecting either a single drug or a combination of drugs at the exact dosage level considered best for the individual patient. This flexibility gives hard-shell capsules an advantage over compressed tablets and soft-shell capsules as a dosage form [44].

2.4. Nanosuspensions

2.4.1 Theoretical overview

Recently, many poorly soluble drugs have been nanonized to increase their dissolution rate, their saturation solubility and in turn to enhance their oral bioavailability [52-58]. The concept of oral nanosuspensions has been specifically used to increase the rate and extent of the absorption of drugs, which have poor and/or erratic dissolution. In the current decade, the concept of nanosuspensions could be commercially exploited by pharmaceutical companies as micronization did in the last few decades. Therefore drug nanocrystals represent an alternative to existing drug delivery technologies for poorly soluble compounds [59, 60].

Drug nanonization can be achieved through different techniques, where one distinguishes between the bottom-up and the top-down technologies. Bottom

up technologies are precipitation methods [61-64], whereas top down technologies start from coarse drug macrosuspensions. The diminution is achieved either by pearl/ball milling [5, 65], high pressure homogenization, either in water [6] or in water free or water reduced media [66], by combination technologies, e.g. precipitation and subsequent high pressure homogenization [67] or ball milling and subsequent high pressure homogenization [68]. In the literature many data are available covering the formulation and optimization of nanosuspensions [69-71]. Liversidge and Cundy have reported that if bioavailability is truly dissolution rate limited, particle size reduction can significantly improve the oral bioavailability of the drug [65]. It has been also investigated that producing nanosuspensions for oral administration leads to effective therapeutic concentrations in the blood because solubility and absorption problems in the gastrointestinal tract have been overcome by extensive size reduction [6, 52, 59, 65, 72, 73].

An outstanding feature of nanosuspensions is the increase in saturation solubility and consequently an increase in the dissolution velocity of the compound. The increase in saturation solubility can be explained by the Kelvin-Gibbs and the Ostwald–Freundlich equations. The Kelvin equation describes the vapor pressure over a curved surface of a liquid. It describes droplets in a gas phase. The vapor pressure increases with increasing curvature of the droplets, which means decreasing particle size.

It has also been postulated that the surface curvature of the dissolving solid particles will influence solubility in water. The basic theory derives from the classical Gibbs–Kelvin equation which, when adapted to the solubility of solids, is known as the Ostwald–Freundlich equation:

$$-\Delta(\Delta G)=RT\ln(S_r/S_\infty)=(2\gamma V)/r \qquad \textit{equation 11}$$

or it can be simplicity written by

$$\ln\frac{S_r}{S_\infty}=\frac{2\gamma M}{\rho r RT} \qquad \textit{equation 12}$$

where Δ(Δ*G*) is the difference in the free energy of a solution of small and large particles; S_r and S_∞ are the solubility of a spherical particle of radius *r* and the solubility of a noncurved solute surface ($r \rightarrow \infty$), respectively; *V* is the molar volume of the solute; M is molarities of the solute; ρ is density; γ is the solid–liquid interfacial tension; *R* is the universal gas constant; *T* is the absolute temperature; and *r* is the particle radius. Eq. (11) is valid for particles that have a very large surface to volume ratio and is of practical importance only for particles smaller than 1.0 μm in diameter [29, 74-76]. It has also been claimed that the surface of finely divided solids may be less regularly crystalline and more amorphous than that of well-grown crystals [76]. There are thus different reasons behind deviations from the thermodynamically stable solubility. Factors such as impurities, ion effect, particle size and crystal structure are some of the factors which may lead to such deviations.

The most important feature of nanocrystals is the increase in saturation solubility and surface area, and consequently an increase in the dissolution velocity of the compound. The law of Noyes-Whitney describes the dissolution velocity [34]:

$$\frac{dC}{dt} = \frac{DA}{h}(C_s - C_x) \qquad \textit{equation 13}$$

D is the diffusion coefficient, A is the surface area, C_s is the saturation solubility, C_x is the bulk concentration, and *h* is the so-called "diffusional distance" over which the concentration gradient occurs (note: division of the equation by the volume *v* leads to d*c*/d*t*). It is obvious that an increase in the surface area consequently increases the dissolution velocity, e.g. exploited in micronized or nanosized products. In addition, drug nanoparticles are characterized by an increase in saturation solubility C_s. According to Noyes-Whitney, the increase in C_s - in addition to enlargement surface area - further increases the dissolution velocity. The final saturation solubility achieved is, of course, compound-specific based on the differences in compound specific dissolution pressures. The dissolution velocity is reversely proportional to the diffusional distance *h*, which means that reducing *h* leads to a further increase in dissolution velocity. According to the Prandtl equation [55, 77, 78], the

diffusional distance *h* decreases for very small particles. The simultaneous increase in saturation solubility C_s and decrease in *h* leads to an increased concentration gradient $(C_s-C_x)/h$, thus enhancing the dissolution velocity in addition to the surface effect.

2.3.2 Production process

There are several production techniques to produce drug nanocrystals. Basically, one can differentiate between top down and bottom up technologies. Typically, the drug nanocrystals are generated in a liquid dispersion medium (e.g. by precipitation or a disintegration process). The obtained product from this process is a suspension of drug nanocrystals in a liquid stabilized by a surfactant or polymer (so-called 'nanosuspensions'). In contrast to micronized powders the drug nanocrystals can be administered using very different administration routes. Oral administration is possible as a suspension. More patient convenient dosage forms can be produced by transferring the liquid nanosuspensions to solid dosage forms, i.e. tablets or pellets or granulate containing capsules. In addition, because of their small size, the nanosuspensions can be injected parenteraly, especially intravenously. Intravenous injection leads 'per definition' to a 100% bioavailability [53].

2.4.2.1 Bottom-up technologies

The term "Bottom-up technology" means that one starts from the molecular level, and goes via molecular association to the formation of a solid particle. That means that we are discussing classical precipitation techniques by reducing the solvent quality, for example, by pouring the solvent into a nonsolvent or changing the temperature or a combination of both. Precipitation is a classical technique in pharmaceutical chemistry and technology. The Latin terminology is via *humida paratum (v.h.p.)*, which means being prepared via a liquid process (solutions are made to obtain a fine powder (precipitate) dispersed in a 'wet' environment. Because it is a long known process in pharmacy, a clear outline is necessary to document the innovative height of patent applications based on this process [57].

The particles generated by precipitation, as for example by Sucker are in most cases crystalline in nature; in contrast to this, the company Knoll (nowadays

owned by Abbott, the new name is “Soliqs”) created amorphous particles by a precipitation technique [19]. The product is NanoMorph^{TM}. As outlined above, an additional positive feature is a further increase in the dissolution velocity due to the amorphous character of the product. The precipitation in the amorphous form is achieved by an aqueous polymer solution. However, for a commercial product, it is necessary to preserve the amorphous character during the shelf life to avoid changes in bioavailability caused by a reduction in the dissolution velocity due to the transfer of the amorphous drug to a crystalline drug [57].

The advantage of the precipitation techniques is that relatively simple equipment can be used. For example, the solvent can be poured into the non-solvent with a constant velocity in the presence of a high-speed stirrer. Very elegant approaches are the use of static mixers or micromixers which simulate the precipitation conditions in a small volume (i.e. simulating lab scale conditions), but being simultaneously a continuous process. In the case of micromixers, scaling up can be performed in a simple way just by so-called numbering up, which means arranging many micromixers in parallel. The equipment is relatively simple, and in the case of large conventional static mixers, of relatively low cost (this is not necessarily valid for the micromixers). However, there are some major general disadvantages of the precipitation techniques.

- The drug needs to be soluble in at least one solvent (thus excluding all new drugs that are simultaneously poorly soluble in aqueous and in organic media).
- The solvent needs to be miscible with at least one non-solvent.
- Solvent residues need to be removed, thus increasing production costs.
- It is a little bit tricky to preserve the particle character (i.e. size, especially the amorphous fraction).

In general, it is recommended that a second consecutive process has to be performed for particle preservation, that is spray drying or lyophilization [57].

2.4.2.2 Top down technologies

There are basically two top-down techniques, which mean starting from a large sized powder and performing a size diminution [53, 57]:

a. Pearl Milling

Liversidge and co-workers developed the milling process to yield the so-called NanoCrystals®. The mills used by the élan Company are basically containers with small pearls, beads, or balls which can have different sizes, a typical size being 1-2 mm. The drug powder is dispersed in a surfactant solution, and the obtained suspension is poured into the mill. In the milling process, the pearls are moved, either by using a stirrer or by moving the milling container itself. The drug particles are disintegrated between the moving pearls. This process is accompanied by the erosion of milling material from the pearls, a phenomenon well known for these mills and documented in the literature [57].

The milling technique clearly has some advantages:

- simple technology
- low-cost process regarding the milling itself
- large-scale production possible to some extent (batch process).

As disadvantages, we can see:

- potential erosion from the milling material leading to product contamination
- duration of the process not being very production friendly
- potential growth of germs in the water phase when milling for a long time
- time and costs associated with the separation procedure of the milling material from the drug nanoparticle suspension, especially when producing parenteral sterile products.

b. Homogenization in water

Homogenization in water is performed either using Microfluidizers as high-pressure homogenizers (Skye Pharma Canada Inc.) or piston-gap homogenizers. In microfluidizer, a frontally collision of two fluid streams under pressure up to 1700 bar leads to particle collision, shear forces and cavitation forces. The collision chambers are designed as Y type or Z type. A major disadvantage of microfluidizers is the required long production time. In many

cases time consuming 50 to 100 passes are necessary for sufficient particle reduction [79]. The company Skye Pharma Canada Inc applies this technology to produce submicron particles of poorly soluble drugs [53, 79].

Cavitation was employed as the most important effect to diminute particles in a piston-gap homogenizer. In this homogenizer types, the dispersion (emulsion or suspension) passes a very tiny gap with an extremely high velocity. Prior to entering the gap, the suspension is contained in a cylinder with a relatively large diameter compared to the width of the following gap. In the APV LAB 40, the diameter of the cylinder is about 3 cm, it narrows to about roughly 3 - 25 µm (varies with applied pressure) when the suspension enters the homogenization gap. The cavitation and shear forces in the gap were sufficiently high to break particles which were distinctly larger than the gap width. Therefore it is possible to disrupt relatively large sized powders (up to 200 µm) [53, 57, 80].

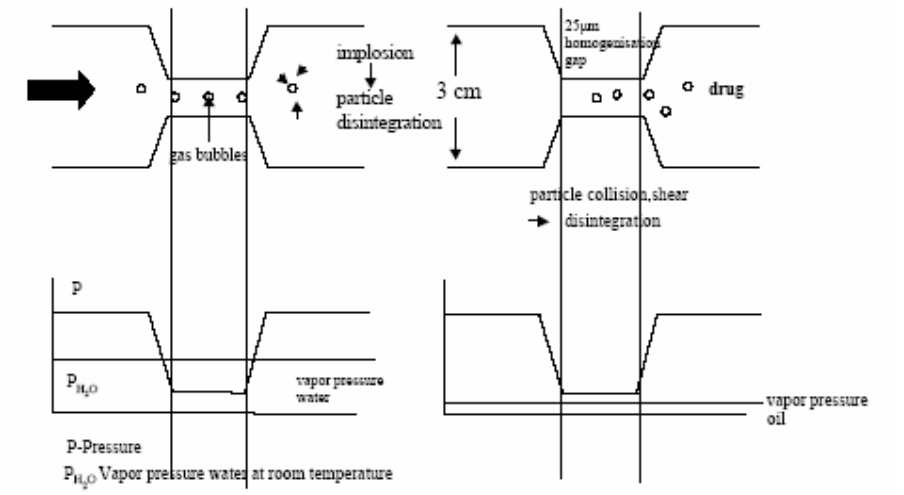


Fig. 2.6 Change of the diameter of the streaming dispersion in a piston-gap homogeniser from the cylinder containing the bulk suspension to the narrow homogenisation gap. The actual range of the static pressure as a function of the location inside the homogeniser is given in the diagrams below (left: situation for homogenisation in water, right: homogenisation in water-mixtures or water-free media, with permission after [53])

According to the law by Bernoulli, the flow volume of a liquid in a closed system per cross-section is constant. That means the reduction in the

diameter leads to a tremendous increase in the dynamic pressure and simultaneously a decrease of the static pressure when the liquid is in the homogeniser gap. A liquid boils when its vapour pressure is equal to the air/static pressure of the environment. In the gap the static pressure drops below the vapour pressure of the liquid at room temperature (**Fig. 2.6)**. Consequently, the liquid starts boiling,forms gas bubbles which implode after leaving the homogenisation gap and being again under normal air pressure conditions [53, 57].

There are advantages of the piston-gap homogenization technique:

- the effective particle diminution
- production lines can be qualified and validated
- production lines already exist in industry, for example, production of parenterals, and so on
- no contamination, for example, iron content is below 1 ppm [81]
- off-shelf equipment
- simple process
- low-cost process/low-cost equipment.

To summarize: homogenization with piston-gap homogenizers has good potential to be used as a production technique because it fulfills the key industrial features such as large scale production and various regulatory aspects [53, 57].

c. Homogenization in Water-Liquid Mixtures and Non aqueous Media

The DissoCubes® patent describes homogenization in pure water as a dispersion medium; the rationale behind - as explained in detail in the patent - is that the cavitation is seen as being responsible for the diminution of the particles. To obtain cavitation in the dispersion medium, one needs to have a high vapor pressure of the dispersion fluid at room temperature. This is fulfilled with water, but not with other liquids such as oils or liquid polyethylene glycol (PEG) 600. In addition, homogenization at higher temperatures (e.g., 80^{o}C) should be much more efficient than at room temperature because the vapor pressure of water is much higher at 80^{o}C, thus leading to more extensive cavitation [82]. The basic of the Nanopure® technology is that

homogenization was performed against these teachings, leading to a comparable or even improved product. Under the Nanopure® technology, not only drugs, but also polymers can be processed, leading to a product with a mean diameter in the nanometer range (nanoparticles) or a size of a few micrometers (both particle ranges are covered).

Nonaqueous dispersion media can be used to produce oral formulations, for example, drug nanocrystals dispersed in liquid PEG 600 or Miglyol (MCT) oil which can be directly filled into soft gelatin capsules, sealed hard gelatine capsules or HPMC capsules. Nanosuspensions in mixtures of water with water-miscible liquids (e.g., ethanol, isopropanol) can be used in the granulation process for tablet production; the solvent mixture evaporates much better than water. Drugs that are susceptible to hydrolysis in water can be prepared as non aqueous suspensions, for example ethanol, glycerol or propylene glycol [83]. Prior to intravenous injection, the glycerol drug nanosuspensions can be diluted with water for injection to yield an isotonic suspension. Alternatively, isotonic water glycerol mixtures can be directly homogenized. Previously cavitation was considered as the dominating factor for particle diminution; therefore, homogenization was only performed in pure water, and glycerol was added afterwards to avoid an impairment of particle diminution efficiency. Another option of the Nanopure® technology is the production of aqueous polymer particle dispersions with a diameter of a few micrometers or in the nanometer range. This could be an alternative to polymer dispersions (e.g. surelease, ethyl cellulose) for the coating of tablets which are now produced by using solvents. Aqueous shellac dispersions produced with Nanopure® technology have already been described [57].

2.4.2.3 Combination technology

In 2001 the Baxter Healthcare Company presented their NANOEDGE® technology at the annual meeting of the American Association of Pharmaceutical Scientists (AAPS) in Denver. NANOEDGE® is an aqueous suspension of drug nanoparticles suitable for intravenous injection. The particles are prepared basically by a high pressure homogenization process, which means either by microfluidization or homogenization in water using piston-gap homogenizers, or alternatively, by a new production process

developed by Baxter [57]. This process is called the "microprecipitation method," and is a combination of precipitation followed by a process with high energy input (e.g., homogenization). The process includes three steps:

- dissolving the organic compound in a water-miscible first solvent to form a solution
- mixing this solution with the second solvent to obtain a "pre-suspension" (=precipitate)
- adding energy to this "pre-suspension" to form particles having an average effective particle size of 400 nm - 2.0 µm.

The advantages of the Baxter method are that the problem of the precipitation technique - continuing crystal growth after precipitation - has been overcome because the particles are only an intermediate product to undergo subsequent diminution/annealing. In addition, by optimal choice of precipitation conditions, more friable particles (semicrystalline, amorphous) can be produced which allow more effective diminution in the subsequent high pressure homogenization step. This is of special importance when the available jet-milled drug is highly crystalline and simultaneously possesses a very hard crystal structure (high Mohs degree). In such cases, the direct homogenization of the jet-milled powder can require a higher number of homogenization cycles, that means longer production times. In the case of very hard crystals, the maximum homogenization pressure applicable during production (e.g. 2000 bar) might potentially lead to a maximum dispersitivity being rather close to 1 µm, and not in the preferred nanometer range [53, 57].

Another combination technology has been developed by Petersen using ball milling and subsequent high pressure homogenization (CT) [68]. With the CT technology, the performance (e.g. physical stability) of nanocrystals could be distinctly improved, as example for dermal application. Nanocrystals can increase the penetration of poorly soluble cosmetic and pharmaceutical actives into the skin. The increased saturation solubility leads to an increased concentration ingredient, thus promoting passive penetration. Molecules penetrated into the skin are very fast replaced by new molecules dissolving from the nanocrystal depot in the cream. The first four cosmetic nanocrystal products with rutin were launched by Juvena. Compared to the water-soluble

rutin glucoside, the original rutin molecule as smartCrystal formulation possesses a 500 times higher bioactivity (as measured by sun protection factor (SPF)) [68, 84]. Of course the same principle can be applied to poorly soluble pharmaceutical drugs of interest for dermal application. Dermal application of nanocrystals is protected by a US and PCT patent application [68, 84].

2.4.2.4 Other technique for production of drug nanoparticles

The intensive researches of new technologies lead to many other approaches to produce nanocrystals. There are many alternative technologies such as:

a. Precipitation with compressed fluid antisolvent

This technique uses an anti-solvent based recrystallization process. CO_2 is used as anti-solvent. The substances are atomized into a chamber containing compressed CO_2. As two liquids collide, intense atomization into micronized droplets occurs, subsequently drying of microdroplets occurs as the solvent(s) and CO_2 mix. Nanoparticles are formed because the two way mass transfer: extraction of organic solvent and CO_2 diffusion into the droplets [85].

High mass transfers rates have been successfully achieved in the solution enhanced dispersion by the supercritical fluids (SEDS). The SEDS process was patented by Hanna and York and owned by University of Bradford [86]. This technique has produced e.g. a new polymorph of flucticasone propionate, it also enabled control over the particle size and shape of formed particles. This polymorph exhibited improved drug delivery characteristics in a metered dose inhaler (MDI) formulation compared to conventional and micronized drugs [86, 87].

b. Rapid expansion from liquified-gas solution

Johnson et al. developed a process based on supercritical fluids, rapid expansion from the supercritical to the aqueous solution (RESAS). This technique [88] could produce stable nanosuspensions of poorly water soluble drugs. The principle of this process is to induce rapid nucleation of in supercritical fluids dissolved drugs in the presence of surface modifying agents resulting in particles formulation with a desirable size distribution in a very short time. The surface modifying agents serve to stabilize the formed small particles and suppress any tendency towards particle agglomeration or

particle growth while they are being formed. The rapid intimate contact with the surface modifier is achieved by having the surface modifiers dissolved in the supercritical fluid containing the dissolved drugs. A rapid intimate contact between the surface modifier and the newly formed particles inhibits the crystal growth of the newly formed particle [87, 89].

This technique successfully produced cyclosporine with a size of 500-700 nm. Cyclosporine nanocrystals could be stabilized at drug concentrations as high as 6.2 and 37.5 mg/ml in 1.0% and 5.0% (w/w) Tween 80 solution, respectively [87, 90].

c. Rapid expansion of supercritical solution into aqueous solution

In contrast to RESAS, the rapid expansion of supercritical solution into aqueous solution (RESSAS) process utilizes a supercritical fluid which is expanded into an aqueous solution containing a stabilizer. This technique was used by Türk *et al.* to produce phytosterol particles with a diameter less than 500 nm. The surfactants or stabilizers are dissolved in the aqueous phase, not in the supercritical fluid [91].

d. Cryogenic Spray Processes

The crogenic spray process is an attractive alternative to obtain microparticles and nanoparticles of poorly soluble drugs. Halocarbon refrigerants and liquid nitrogen have been used as cryogenic media in conventional spray-freezing into the vapor processes. The feed solution is atomized through a nozzle positioned at a distance above the boiling refrigerant. The droplets gradually solidify while passing through the cold halocarbon vapor, freeze completely as contact is made with the boiling refrigerant liquid. Unfortunately, this process may result in broad particle size distributions and non-micronized particles because agglomerates of the solution droplets are solidified while passing through the vapor phase and settle onto the surface of the cryogenic liquid [87, 92].

e. Spray Freezing into Liquid Process (SFL)

To overcome agglomerated particles, spray freezing into liquid was developed and patented by The University of Texas at Austin in 2001 [93] and commercialized by the Dow Chemical Company. The SFL process utilizes the atomization of feed solution containing drugs directly into a cryogenic liquid to

produce frozen nanostructured particles. The frozen particles are then lyophilized to obtain dry, freely-flowing micronized powders. The advantages of the SFL process result from intense atomization and rapid freezing rates. A high degree of atomization is achieved by spraying directly into cryogenic liquid and the ultra–rapid freezing rates prevent the phase separation of solutes within the feed solution and induce formation of amorphous structures. The intense atomization and ultra-rapid freezing rates lead to amorphous nanostructures with enhanced wetting and significantly enhanced dissolution rates [87, 93].

f. Solvent Evaporation Process-Evaporative precipitation into Aqueous Solution Process

The evaporative precipitation into aqueous solution (EPAS) applies rapid phase separation to nucleate and grow nanoparticles and microparticles of poorly water soluble drugs. The EPAS was developed and patented by the University of Texas at Austin in 2001 and commercialized by the Dow Chemical Company [87]. A drug solution in organic solvent is pumped through a tube where it is heated under pressure and then spraying through a nozzle into heated aqueous solution. This process results in an amorphous suspension. The stable aqueous drug suspension is dried by lyophilization or spray drying. A variety of hydrophilic surfactants are added to the solution to diffuse to surface of the growing particles rapidly enough to prevent growth of particles. The EPAS technique has produced cyclosporine A nanosupensions with particle a size ranging from 130 to 460 nm [87, 94].

2.4.3 Drug nanosuspension administration

2.4.3.1. Oral administration of the nanosuspensions

First choice of application is oral administration. When a drug is given orally, the bioavailability and finally its efficacy depends on the solubility and absorption in the gastrointestinal tract. In vitro, active compounds have failed in the past because their poor solubility has limited in vivo absorption and did not lead to effective therapeutic concentrations. Simple examples reflecting this problem of poor solubility combined with low absorption are the experimental compound buparvaquone and atovaquone. A way to improve bioavailability of atovaquone or buparvaquone would be to increase the

absorption rate by formulating them as a nanosuspension. Oral administration of nanosuspensions can overcome this problem because of the high adhesiveness of drug particles sticking on biological surfaces — here the epithelial gut wall — and prolonging the absorption time.

Table 2.3 Current marketed pharmaceutical products utilized nanocrystals, after [53, 95, 96]

Product	Drug compound	Company	Nanoparticle technology
RAPAMUNE®	Sirolimus	Wyeth	Elan NanoCrystals®
EMEND®	Aprepitant	Merck	Elan NanoCrystals®
TriCor®	Fenofibrate	Abbott	Elan NanoCrystals®
MEGACE® ES	Megestrol acetate	PAR Pharmaceutical	Elan NanoCrystals®
Avinza®	Morphine sulfate	King Pharmaceutical	Elan NanoCrystals®
Focalin® XR	Dexmethylphenidate hydrochloride	Novartis	Elan NanoCrystals®
Ritalin® LA	Methylphenidate hydrochloride	Novartis	Elan NanoCrystals®
Zanaflex Capsules™	Tizanidine hydrochloride	Acorda	Elan NanoCrystals®
Triglide™	Fenofibrate	First Horizon Pharmaceutical	SkyePharma IDD®-P technology

In comparison to Wellvone-treated mice, containing a micronized drug, nanosuspensions of atovaquone at equivalent doses reduced infectivity from 40% to 15% at a reduced drug concentration of only 7.5 mg/kg after oral administration [75]. These results reflects the potency of the nanosuspension technique, reducing drug load from 22.5 mg/kg (Wellvone®) to 7.5 mg/ kg, but increasing activity 2.5-fold at the same time [75]. Buparvaquone nanosuspensions provide some advantages, and in TCR-alpha-deficient mice

infected with *C. parvum* oocysts, the significance of the nanosuspensions is documented. In comparison to micronized drug powder, the infectivity score was reduced from 2.0 (negative control group, untreated) to 1.47 for micronized buparvaquone and even to 1.02 for equivalent nanosuspensions [75, 97]. Despite the substantial amount of literature data in support of nanoparticles formulations, especially for BCS Class II compounds, a relatively limited number of current drug products in the market utilize nanoparticles for oral application (**Table 2.3**).

Furthermore, the nanosuspension was able to overcome the significant food effect (~3.2×) observed with the microsuspension formulation. It is worth mentioning that, in the dog model, the Nanocrystal® suspension (120 nm) significantly outperformed a suspension of wet-milled drugs with a size of 480 nm in terms of fasted state exposure. The results from the dog model were confirmed in the clinic, with the EMEND formulation exhibiting no food effect. It is also worth mentioning that aprepitant is classified as a BCS Class II and IV compound, emphasizing that nanonizing is not confined to high permeability compounds but may also be applicable to low permeability drugs, provided dissolution is the slower step in the absorption process [96, 98].

2.4.3.2. Parenteral nanosuspension — intravenous administration route

Administration of drugs via the parenteral route is critical, i.e. usually accompanied by physical and biological problems, like production under aseptic conditions, a sophisticated protocol for safety issues, last but not least, biological problems, such as endotoxins, allergic reactions, and inconvenience for the patient. Formulation of atovaquone as a nanosuspension for i.v. use to treat murine toxoplasmosis showed a significant reduction of *Toxoplasma gondii* at a dose of 0.3 mg/kg in comparison to 30 mg/kg when given orally [75].

The nanoparticle-based product Abraxane® was approved by FDA in 2006 for intravenous administration. Abraxane® is a novel formulation consisting of lyophilized particles with 10% (w/w) paclitaxel and 90% (w/w) albumin. The particle size of the suspension is about 130 nm. In a Phase I trial, 39 patients with advanced nonhematologic malignancies were treated at dose levels from

80 to 200 mg/ml in multiple cycles of weekly 30-minute intravenous infusions. In contrast to Taxol treatment, no premedication was used in this study. The maximum tolerated dose observed from this study was higher than the commercial Taxol® formulation. Furthermore this study confirmed that the nanoparticle formulation of paclitaxel eliminates the need for premedication (since the toxic excipient Cremophor EL was not used in the formulation) [99].
Other investigates reported that particle size played an important role on pharmacokinetics and tissue distribution of oridonin nanosuspension [100]. The pharmacokinetics and tissue distribution of oridonin nanosuspensions A (particle size of about 100 nm) and nanosuspension B (particle size of about 900 nm) were studied after intravenous administration using New Zealand rabbits and Kunming mice as experimental animals, respectively. An Oridonin control solution was parallelly studied. The results showed that oridonin nanosuspension A exhibited pharmacokinetic and biodistribution properties similar to the solution due to its rapid dissolution in the blood circulation. Oridonin nanosuspension B, however, showed a high uptake in RES organs, thus exhibited a markedly different pharmacokinetic property compared to nanosuspension A [100].

2.4.3.3 Polmunary drug delivery

Many water insoluble drugs were delivered to the respiratory tract for local or systemic treatment of diseases. Unfortunately, many of these drugs are poorly soluble in aqueous solution and simultaneously insoluble in non-aqueous media. Since a number of years ago, Chlorofluorocarbons (CFCs) aerosols, was the best choice as drugs delivery for respiratory tract. But in compliance with the Montreal protocol of 1987 the use of Chlorofluorocarbons (CFCs) must be avoided. Therefore as alternative drugs delivery without CFCs, metered dose inhalers (MDI) or dry powder inhalers (DPI) were developed. The nebulized nanosuspensions produce aerosol droplets loaded with a large number of drugs nanocrystals. The respirable fraction is distinctly increased using nebulized nanosuspensions compared to nebulized microcrystals (conventional MDI). The smaller particle size of the drugs nanocrystals, the more doplets are loaded with drug. In addition, the mucoadhesive property of

nanoparticles leads to a prolonged residence time at mucosal surface of the lung [79].

Yang et al. reported that nanosuspensions of fluctasone exhibited good physical/chemical properties for pulmonary delivery. The pharmacokinetic studies after the intratracheal administration of nanosuspensions showed deep lung deposition and fast lung absorption, with solubility playing an important role in lung retention and duration of action. Overall, these studies have demonstrated that nanosuspensions can be used for pulmonary drug delivery in preclinical animal studies [101]. Superiority of the drug nanosususpension was also shown by Hernandez-Kirstein using buparvaquone nanosuspensions for pulmonary delivery system [102].

2.4.3.4 Transdermal delivery of nanosuspensions

It has been reported to use diclofenac sodium nanosuspension for transdermal delivery. The basic transdermal characteristics of the nanosuspension were evaluated using a Yucatan micropig (YMP) skin model. Diclofenac sodium nanosuspension was successfully dispersed into isopropyl myristate as a nanosized suspension via complex formation with sucrose erucate. The resultant nanosuspension increased the permeability flux of diclofenac sodium across the skin by up to 3.8-fold compared to the control. The optimal weight ratio for the highest diclofenac sodium permeation was 8.8, at which point the mean diameter of the nanosuspension was 14.4 nm [103].

2.4.3.5 Ocular delivery of nanosuspensions

Drug Nanosuspensions for ocular drug delivery system has been developed by Pignatello *et al.* [104-107]. The co-dispersion of cloricromene hydrochloride (AD6) in Eudragit RS or RL polymers resulted in nanosuspensions that showed good mean sizes for ophthalmic applications and a positive surface charge. The suspensions allowed for improved corneal adhesion and stability upon storage, particularly at low temperatures. When preparation was performed in an isotonic saline solution, the dispersion of AD6 in the polymer network protected the ester drug from the hydrolytic cleavage into the inactive and insoluble acid form. According to preliminary biological evaluation of the nanosuspensions that showed a higher drug

availability in the rabbit aqueous humor after the drug's administration in Eudragit RL nanosuspensions, AD6-loaded Eudragit Retard nanosuspensions appear to offer a promising means of improving the shelf life and bioavailability of this drug after ophthalmic application [104].

Nanosuspension as an ophthalmic delivery system has been also investigated by Kassem et al. [108]. The effect of particle size in the micron and nano size ranges as well as the effect of viscosity of the nanosuspensions on the ocular bioavailability was studied by measuring the intraocular pressure of normotensive Albino rabbits using a tonometer. The results show that compared to solution and microcrystalline suspensions, it is a common feature of the three drugs that the nanosuspensions always enhance the rate and extent of ophthalmic drug absorption as well as the intensity of drug action. In the majority of cases nanosuspensions extend the duration of the drug effect to a significant extent. The data presented confirms that nanosuspensions differ from microcrystalline suspensions and solutions as ophthalmic drug delivery systems and that the differences are statistically, highly to very highly significant. The results confirm also the importance of viscosity of nanosuspensions especially in increasing the duration of drug action [108].

Chapter 3
MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1 Materials

3.1.1 Rutin

Rutin (3-[[6-O-(6-deoxy-alpha-L- mannopyranosyl)-beta-D-glucopyranosyl] oxy]-2- (3,4-dihydroxyphenyl) -5,7-dihydroxy-4H-1-Benzopyran-4-one) the glycoside of quercetin, is a bioflavonol having an aromatic trimeric heterocyclic structure. It is a naturally occurring pigment and appear as a yellow to greenish crystalline powder with a melting point of 189^{o}C. Rutin is classified as vitamin P which increases the strength of the walls of the blood capillaries and regulates their permeability. Other compounds classified as vitamin P are catechin, citrin, eriodictin, hesperetin, hesperidin, nobiletin, quercetin, sinensetin and tangeretin. It is not a dietary essential but it is known to have the effect on capillary disorders [109].

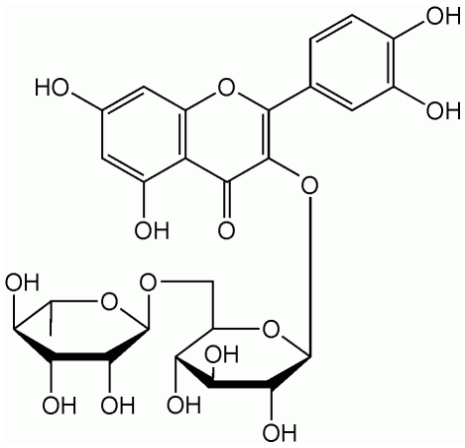


Fig. 3.1 Chemical structure of rutin

Rutin (quercetin-3-O-rutinoside, **Fig. 3.1**) is abundantly found and distributed in higher plants such as in buckwheat seed, fruits and fruit rinds, especially citrus fruits (orange, grapefruit, lemon, lime). Other important properties of rutin are significant scavenging properties on oxidizing species such as OH radical, superoxide radical, and peroxyl radical [9]. It is assumed that observed protective effects derive from the antioxidative and radical-scavenging which was shown in many in vitro experiments [110-112].

Consequently rutin has been widely used in treating diseases, its several pharmacological activities including antiallergic, antiinflammatory and vasoactive, antitumor, antibacterial, antiviral and antiprotozoal properties. It is also known that rutin has other pharmacologically actions such as hypolipidaemic, cytoprotective, antispasmodic and anticarcinogenic [9]. Rutin formulations can be found in the market as nutritional supplements for oral administration to be taken daily [109].

Table 3.1 Physicochemical properties of rutin [109, 113-115]

Rutin monograph	
CAS no.	250249-75-3
synonym	Quercetin-3-rutinoside; Quercetin- 3 6-O-(6- deoxy-alpha-L-mannopyranosyl)- beta-D-Glucopyranoside
molecular weight	610.518
formula	$C_{27}H_{30}O_{16}$
indication	antioxidant,
physical-state	yellow to greenish crystalline powder or needle
melting point	188.7 ^{o}C
solubility	one gram dissolves in about 8 liter water, about 200 ml boiling water, 7 ml boiling methanol. Soluble in pyridine, formamide, and alkaline solution; slightly soluble in alcohol, acetone, ethyl acetate. Practically insoluble in petroleum solvent.

3.1.2 Hesperidin

The flavonoid hesperidin is a flavanone glycoside (glucoside) being composed of the flavanone (a class of flavonoids) hesperitin and the disaccharide (**Fig. 3.2**) rutinose. Hesperidin is the predominant flavonoid in lemons and oranges. The peel and membraneous parts of these fruits have the highest hesperidin

concentrations. Therefore, orange juice containing pulp is richer in the flavonoid than that without pulp. Sweet oranges *(Citrus sinensis)* and tangelos are the richest dietary sources of hesperidin. Hesperidin is classified as a citrus bioflavonoid [116, 117].

Hesperidin, in combination with a flavone glycoside called diosmin, is used in Europe for the treatment of venous insufficiency and hemorrhoids. Hesperidin, rutin and other flavonoids are thought to reduce capillary permeability and to have anti-inflammatory action. They are collectively known as vitamin P and may have a contribution to the total antioxidant activity and act as detoxifiers. Vitamin P enhances the action of vitamin C. It is considered that it lowers down cholesterol levels if it taken with vitamin C. It is also known to have pharmacological actions e.g. antihistaminic and antiviral. These substances, however, are not vitamins per definition and thus are no longer referred to, except in older literature, as vitamin [116, 117].

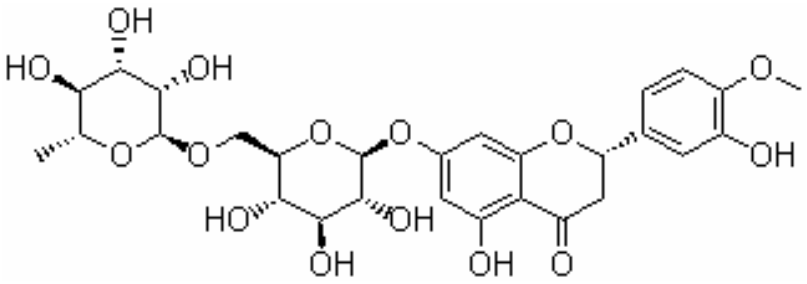


Fig. 3.2 Chemical structure of hesperidin

Table 3.2 Physicochemical properties of hesperidin [114, 116, 117]

Hesperidin monograph	
CAS no.	520-26-3
synonym	Hesperitin-7-rutinoside; (S)-7-[[6-O-(6-deoxy- alpha-L-mannopyranosyl)- beta-D-glucopyranosyl]oxy]- 2,3-dihydro-5-hydroxy- 2-(3-hydroxy-4-methoxyphenyl)-4H-1-Benzopyran-4-one; Cirantin
molecular weight	610.57
formula	$C_{28}H_{34}O_{15}$
indication	antioxidant
physical-state	white to yellow powder
melting point	257 - 262 ^{o}C
solubility	one gram dissolves in about 50 liter water. Soluble in formamide, DMF at 60^{o}C; slightly soluble in methanol, hot glacial acetic acid. Almost insoluble in acetone, benzene, chloroform. Freely soluble in alkalies and pyridine.

Hesperidin [(S)-7-[[6-O-(6-deoxy- alpha-L-mannopyranosyl)- beta-D-glucopyranosyl] oxy]- 2,3-dihydro-5-hydroxy- 2-(3-hydroxy-4-methoxyphenyl)-4H-1-Benzopyran-4-one] is a solid substance with low solubility in water. It is, however, much more soluble in water than its aglycone hesperetin. The molecular formula of hesperidin is $C_{28}H_{34}O_{15}$, and its molecular weight is 610.57 dalton. It is a white to yellow crystalline powder melting at approx. 260^{o}C.

3.1.3 Ibuprofen

Ibuprofen is a nonsteroidal anti-inflammatory drug (NSAID) and belongs to the group of propionic acid derivatives (**Fig. 3.3**). It inhibits the enzyme cyclooxygenase (prostaglandin synthesis) which catalyzes the transformation of unsaturated fatty acids to prostaglandins. One assumes that the inhibition of the prostaglandin synthesis is the cause for the analgesic, antipyretic, and anti-inflammatory action of the drug. It is used to treat inflammatory rheumatoid diseases and to relieve acute pain [118].

Table 3.3 Physicochemical properties of ibuprofen [22, 114, 118, 119]

Ibuprofen monograph	
CAS no.	15687-27-1
synonyms	(RS) 2-(4-isobutylphenyl)propionic Acid; Apsifen; Apsifen-F; Alpha-Methyl-4-(2-methylpropyl) benzeneacetic acid;
molecular weight	206.29
formula	$(CH_3)_2CHCH_2C_6H_4CH(CH_3)COOH$
indication	non steroid anti-inflammatory drug
physical-state	white crystalline powder
melting point	75 – 78 ^oC
pK_a	pK_{a1}: 4.4 pK_{a2}: 5.2
solubility	practically insoluble in water, readily soluble in most organic solvents

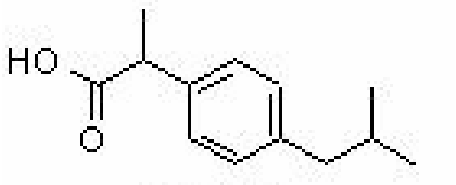


Fig. 3.3 Chemical structure of ibuprofen

In general, ibuprofen is suited for the treatment of pain, in particular for the treatment of pain being related to the musculoskeletal system, but also headaches, postoperative pain, dysmenorrheal, etc. Pain accompanying arthritis can be reduced and the mobility of the joints can be increased. In the case of chronic rheumatoid arthritis, ibuprofen not only reduces pain, but also joint swelling and morning stiffness. Ibuprofen has been used successfully for other rheumatoid diseases such as spondylitis ankylosans (Bechterew disease), juvenile rheumatoid arthritis, and acute gout seizures. However, nonsteroidal anti-inflammatory agents only have a symptomatic effect, but the development of the actual disease is not significantly influenced. There are even indications that a long-term treatment may have an unfavourable influence.

For soft tissue rheumatism (e.g. periarthropathy of the shoulder) NSAID can be beneficial; however, it has not been demonstrated whether they represent advantages in comparison with other methods of treatment [119].

3.1.4 Coenzyme Q10

Coenzyme Q10 (CoQ10), also known as Ubiquinone (**Fig. 3.4**) is naturally present in a variety of foods and it is also synthesised in the body. CoQ10 is a physiologically important compound acting as an electron shuttle in the mitochondrial respiratory chain and as a stabilizing agent in cellular membranes. Mainly it is found in the inner membrane of the mitochondrion membranes where it functions as cofactor in the electron transport chain. Apart from this CoQ10 serves as an important antioxidant in mitochondria and lipid membranes. Using conventional methods this compound was found to be very difficult to extract and to obtain in its purest form, implying a high cost per gram [120-122]. Because of its ability to transfer electrons it acts as an

antioxidant and thus it is not only used as dietary supplement but also as anti-aging active in cosmetic formulations [123, 124].

Table 3.4 Physicochemical properties of coenzyme Q10 [114, 123-125]

Coenzyme Q10 monograph	
CAS no.	303-98-0; 13448-14-1; 55870-43-4
synonyms	Ubidecarenone; Ubiquinone 10; Ubiquinone 50; Udekinon; (all-E)-2-(3,7,11,15,19,23,27,31,35,39-deca-methyl-2,6,10,14,18,22,26,30,34,38-tetracontadecaenyl) -5,6-dimethoxy-3-methyl-2,5-cyclohexadiene-1,4-dione
molecular weight	863.34
formula	$C_{59}H_{90}O_4$
indication	Antioxidant
physical-state	yellow to orange crystalline powder
melting point	48-50^{o}C
solubility	insoluble in water, soluble in fat oil

It has been reported that CoQ10 can also penetrate into the viable layers of the epidermis, where it reduces the level of oxidation measured by weak photon emission. Furthermore, also a reduction in wrinkle depth following CoQ10 application was also shown. CoQ10 was determined to be effective against UV A mediated oxidative stress in human keratinocytes in terms of thiol depletion, activation of specific phosphotyrosine kinesis and prevention of oxidative DNA damage. CoQ10 was also able to significantly suppress the expression of collagenase in human dermal fibroblasts following UV A irradiation. These results indicate that CoQ10 has the efficacy to prevent many of the detrimental effects of photo aging [126]. In addition, CoQ10 is sold as dietary supplement, also supporting skin function and condition. A

coenzyme antioxidant benefit to the brain is also studied for the treatment of neurodegenerative disease [125].

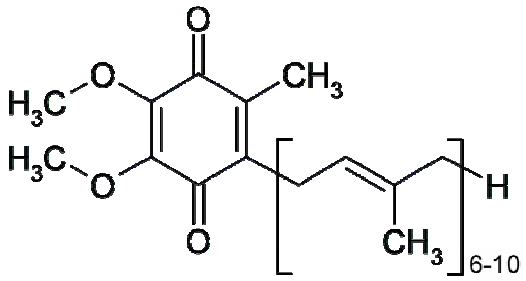


Fig. 3.4 Chemical structure of coenzyme Q10

3.1.5 Testosterone

Testosterone (17beta-Hydroxy-3-oxo-4-androstene, **Fig. 3.5**) is the major androgenic hormone. It is responsible for the growth and development of masculine characteristics. Testosterone is produced in the Leydig cells (interstitial cells) of the testes. It regulates gonadotropic secretion and Wolffian duct differentiation (formation of the epididymis, vas deferens, and seminal vesicle), and stimulates the growth of the skeletal muscles. It is also responsible for other male characteristics, e.g. spermatogenesis after its conversion to dihydrotestosterone by 5 alpha-reductase in the peripheral tissue. Testosterone is converted to dihydrotestosterone in the target tissues where it appears to mediate many of biological actions.

In addition, testosterone possesses protein anabolic properties, manifested by retention of nitrogen, calcium, phosphorus, and potassium. Furthermore it is important in maintaining muscle mass and bone tissue in the adult male. It is also converted by aromatization to estradiol in peripheral tissue. Testosterone can be used for treating male hypogonadism, cryptorchidism, frigidity, inoperable female breast cancer, oligospermia, to suppress lactation, and as an anabolic agent. It has been synthesized for replacement therapy, in the form of various esters having prolonged duration of effects. The esters are

administered by subcutaneous, intramuscular injection, buccally or intramuscularly include [127, 128].

Table 3.5 Physicochemical properties of testosterone [22, 114, 127, 128]

Testosterone monograph	
CAS no.	58-22-0
synonym	17beta-Hydroxy-androst-4-en-3-one
molecular weight	288.43
formula	$C_{19}H_{28}O_2$
indication	androgenic hormone
physical-state	white to off-white crystalline powder
melting point	155 ^{o}C
solubility	23.4 mg dissolves in 1 L water

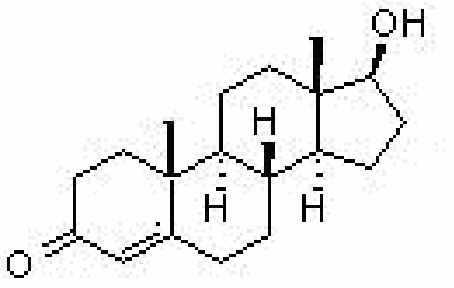


Fig. 3.5 Chemical structure of testosterone

3.1.6 Emulsifying agents

3.1.6.1 Tween 80

Polysorbat 80 (Polyoxyethylen(20)sorbitan monooleate), Tween 80 V Pharma was received from the company Uniqema (ICI Surfactants, Eversberg, Belgium). Its HLB value is 15. It is a non-ionic surfactant with a relatively low toxicity. For example for oral application, the product Combantrin® tablet and oral suspension (Pfizer Consumer Healthcare 2000) contains this surfactant. The **Fig. 3.6** represents the structure of the molecule.

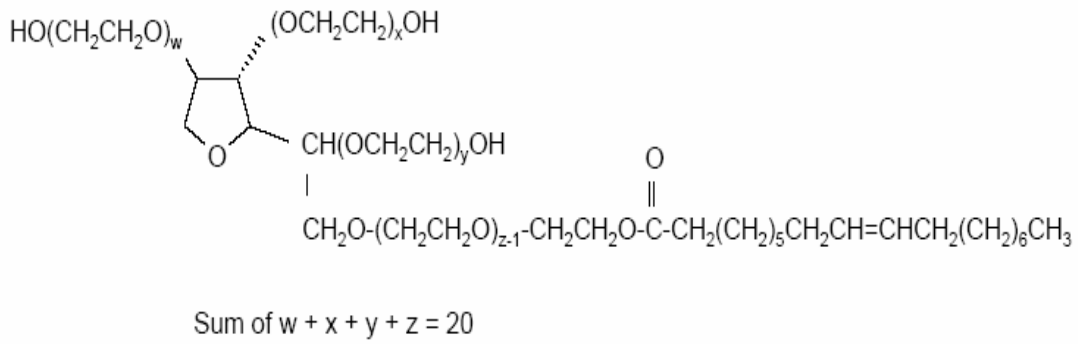


Fig. 3.6 Molecular structure of Tween 80

3.1.6.2 Poloxamer 188

Poloxamer 188 is a white or almost white waxy powder, microbeads or flakes with a melting point around 50°C. As all poloxamers, it is very soluble in water and alcohol [129]. Chemically, poloxamer is a synthetic block copolymer of α-Hydro-ω-hydroxypoly (oxyethylene) poly (oxypropylene) poly (oxythylene) with the general formula shown in **Fig. 3.7**.

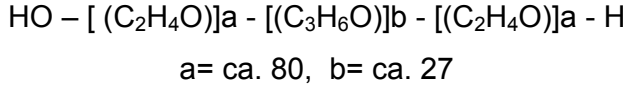


Fig. 3.7 Molecular structure of Poloxamer 188

It has an average molecular weight from 7680 to 9510. Poloxamer 188 has an HLB value of 29 [130]. Poloxamer 188 was ordered as Lutrol® F68 from BASF AG (Ludwigshafen, Germany).

3.1.6.3 Polyvinyl alcohol

Polyvinyl alcohol (PVA) is a water-soluble polymer. It is prepared by hydrolysis of a polyvinyl ester (polyvinyl acetate). It is used as a starting material for the preparation of other resins. It can be used as a component of elastomers used in the manufacture of sponges. This polymer is used in shielding agents that confer resistance to oils and greases upon paper and textiles, to make films resistant to attack by solvents or oxygen. It is used as a component of adhesives, emulsifiers, suspending and thickening agents. In pharmaceutical industry, polyvinyl alcohol is used as an ophthalmic lubricant and viscosity increasing agent. It thickens the natural film of tears in eyes [130]. Its functionality is as pharmaceutical excipient mainly as coating agent, non-ionic surfactant and viscosity-increasing agent.

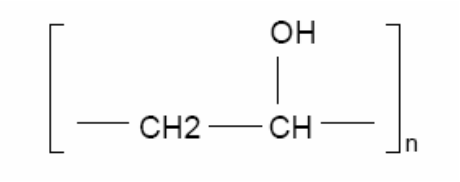


Fig. 3.8 Molecular structure of polyvinyl alcohol

Polyvinyl alcohol (PVA) is obtained by polymerisation of vinyl acetate followed by partial or complete hydrolysis in presence of catalytic amounts of alkali or mineral acids. Granules or powder are white to cream-coloured and odourless [129]. The PVA used in this work was purchased from Sigma Aldrich (Sigma Aldrich GmbH, Deisenhofen, Germany). The product is a polymer with a molecular weight of ~90.000. The chemical formula of the polymer can be seen in **Fig. 3.8**, where n is ~630 for the product used in the present work.

It is soluble in water, but due to partial hydrolization of the vinyl acetate polymer, it is not soluble in cold water. An effective dissolution of the polymer

requires the dispersion and continued mixing of the solid in cold or tepid water followed by sustained heating at 85-95°C until dissolved [130].

3.1.6.4 Purified soy lecithin

Lecithin is a non-synthetic emulsifying agent which can be totally biodegraded and metabolized, since it is part of biological membranes. A very important fact for the use of lecithin in oral administration is that lecithin vesicles are promising carriers in the oral delivery of drugs, considering their absorption enhancement effect and low toxicity [131]. This may reduce the risk of toxicity in oral formulations which are often intended for repetitive administration. Additionally, the surfactant possesses a GRAS status conferred by the FDA. The **Fig. 3.9** shows the chemical structure of the amphoteric molecule of phosphatidylcholine.

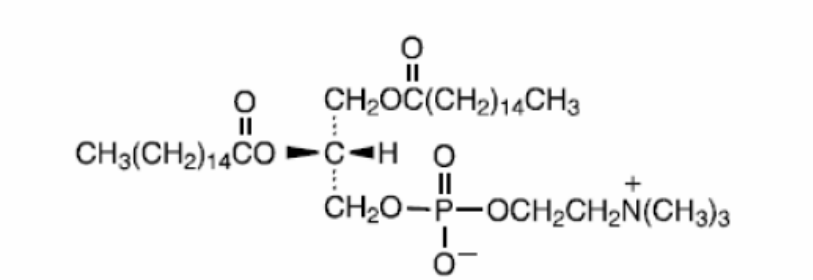


Fig. 3.9 Molecular structure of phosphatidylcholine

Purified soy phosphatidylcholine Phospholipon® 80 was a kind donation from the company Phospholipon GmbH (Cologne, Germany). Phospholipon® 80 is a yellowish to brown solid containing around 76 % of phosphatidylcholine, including small amounts of impurities such as phosphatidylethanolamine, sphingomyelin, and hydrogenated lysophosphatidylcholine.

3.1.6.5 Sodium dodecyl sulfate

Sodium dodecyl sulfate (SDS) has the chemical formula $C_{12}H_{25}NaO_4S$ or CH_3-(CH_2)11-O-SO_3-Na+ and its structure is presented in **Fig. 3.10**. SDS is a high production volume chemical (i.e., annual production and/or importation volumes above 1 million pounds in the United States). In solution, the sodium cation (Na+) dissociates from the anionic part of the compound (lauryl/

dodecyl sulfate), and this anionic compound is the active chemical. SDS is prepared by sulfation of lauryl alcohol and neutralisation with sodium carbonate. It is a surfactant which has its amphiphilic properties due to C_{12} chain (lipophilic) attached to a sulfate group (hydrophilic). This bi-functionality in one molecule provides the basic properties useful as surfactant [132].

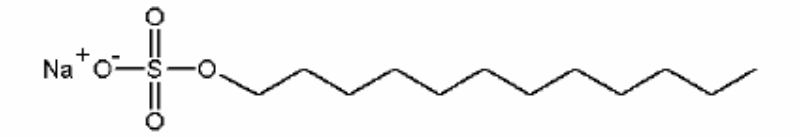


Fig. 3.10 Molecular structure of sodium dodecyl sulfate

SDS is an anionic surfactant lowering the surface tension of aqueous solutions. Surfactants can act as wetting agents by enhancing the spread of water over surfaces, and are a class of chemicals used for their detergent properties. One end of the molecule is charged and therefore has an affinity for water, and the other end is nonpolar and soluble in fats/oils. SDS has a faint odor of fatty substances. At room temperature it occurs as white or cream-colored crystals, flakes, or powder [133], or a clear to yellowish thick fluid. SDS is stable under ordinary conditions of use and storage but is incompatible with strong acids or strong oxidizing agents [134]. When heated to decomposition, SDS emits toxic fumes (sulfur oxides and sodium oxides) [135].

3.1.7.1 Polyvinyl pyrrolidone

Polyvinyl pyrrolidone (PVP) is a polymeric material and has a low reactivity. It reacts as a weak base. Although polyvinyl pyrrolidone has well known properties as emulsifying agent, it was used in this work as carrier polymer in the preparation of solid dispersions of buparvaquone. In the formulation of solid dispersions with poorly water soluble drugs, PVP prevents or delays the dissolved portion of the active substance from crystallizing out e.g. by forming soluble complexes with it. Increasing the amorphous component can lead in many cases to an enhancement of dissolution rate of lipophilic active

substances (hydrophilization effect) and can improve the physical stability of the formulations. The chemical structure of PVP can be seen in **Fig. 3.11**. It is a free flowing white or yellowish-white powder very soluble in water.

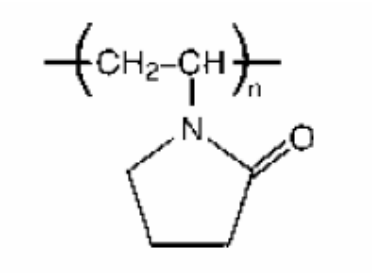


Fig. 3.11 Molecular structure of polyvinyl pyrrolidone

PVP K-30 was purchased from BASF AG (Ludwigshafen, Germany). The K value corresponds to the molecular weight of the product, which in this case is within the range 27.0 to 32.4 KDa, according to USP and Ph. Eur. specification.

3.1.7 Other materials

As tablet and capsule excipients, microcrystalline cellulose PH 101 and PH 102, AcDiSol, Explotab, magnesium stearate, talc were used. For effervescent granules, sodium bicarbonate, citric acid, tartaric acid, fumaric acids are used as excipients. The pellets produced consisted only of drug and lactose. As cryoprotectants, mannitol, trehalose, lactose and glucose were used. Others materials are usually used in the laboratory such as glycerol, water, ethanol etc.

3.2 Methods

3.2.1 Production of nanosuspensions by high pressure homogenization (HPH)

3.2.1.1 Operation principle of a piston-gap type high pressure homogenizer

Piston-gap homogenizers are based on a different homogenization principle than jet stream homogenizers. A liquid/dispersion is streaming through a narrow gap with a very high streaming velocity. A typical arrangement for this technology is to place the dispersion (emulsion or suspension) in a cylinder with a piston; a pressure is applied to the piston, pressing the suspension through a gap at the end of the cylinder. In the Micron LAB 40, the cylinder has a diameter of about 3.0 cm, and the narrow gap just has a width of only 25 µm; this narrowing by a factor of 120,000 leads to extremely high flow velocities up to 200-700 m/s depending on the pressure applied. The gap reduces in width with increasing pressure. For some homogenizer types, velocities up to 1000 m/s are given. According to the patent of DissoCubes®, the reason for disintegration of the particles is the cavitation occurring in this gap. According to the Hagen-Poiseulle equation, the flow rate in the system of different diameters is equal at each diameter.

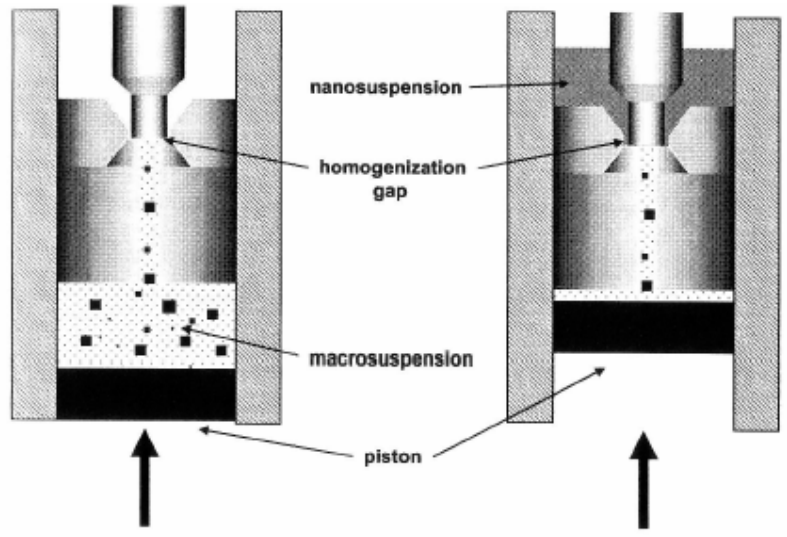


Fig. 3.12 Cross section of a piston gap – homogenizer
The macrosuspensions is forced through a very tiny homogenization gap in order to produce drug nanocrystals (with permission after [79])

According to the Bernoulli equation, the sum of static and dynamic pressure is constant at each diameter of the system [53, 57]:

$$P_s + \frac{1}{2}\rho V^2 = K \qquad \textit{equation 14}$$

where P_s, is the static pressure, $\frac{1}{2}\rho V^2$ is the dynamic pressure (P_{dyn}), ρ is density of the fluid, V its velocity and K is a constant.

In the gap, the dynamic pressure drastically increases (i.e., also streaming velocity), and simultaneously, the static pressure decreases and falls below the vapor pressure of the water at room temperature. This leads to the boiling of the water at room temperature, the formation of gas bubbles which implode when the liquid leaves the gap being under the normal pressure conditions again (cavitation). The formation of gas bubbles and their implosion cause shock waves, leading to the diminution of particles of a suspension. Nowadays, bubble formation and not the implosion is considered as the major reason for diminution [53, 57].

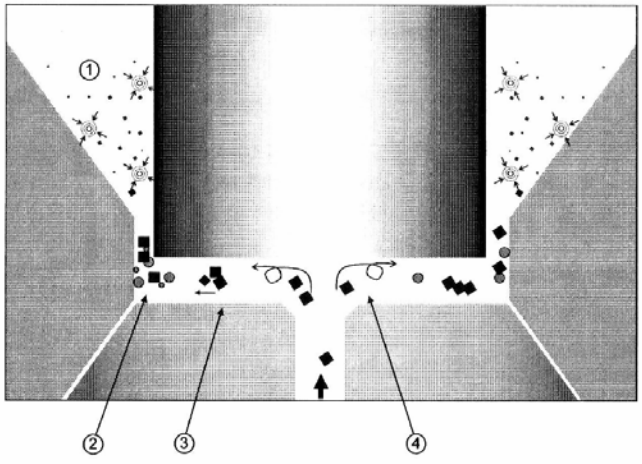


Fig. 3.13 Diminution principle during high pressure homogenization
(detailed illustration of a homogenization gap in cross-section)
1) implosion area (cavition); 2) boiling area; 3) shear forces;
4) turbulent flow (with permission after [79])

During a milling process, the particles/crystals break preferentially at weak points, i.e. imperfections. With decreasing particle size, the number of imperfections is getting less and less, that means the crystals remaining are

becoming more and more perfect. Thus, the force required to break the crystals increases with decreasing particle size. If the force (power density) in the homogeniser is equal to the interaction forces in the crystal, the particles can not further be diminuted, even when additional homogenization cycles are applied. That means the maximum dispersity at the given power density/homogenisation pressure is reached [6, 53, 57, 79, 80].

By definition the term "homogenization" includes reduction of particle or droplet size and simultaneous narrowing of the size distribution, that is, making the particle population not only smaller, but simultaneously more homogeneous in size [57].

3.2.1.2 Preparation of Nanosuspensions

A formulation screening was carried out to identify the most suitable formulation for further processing. Drugs were dispersed in water and stabilizers were added. Then a pre-suspension was prepared by stirring using an Ultra-Turrax T25 (Janke & Kunkel GmbH, Staufen, Germany) at 8000 rpm for 10 seconds. A Micron LAB 40 (APV Deutschland GmbH, Unna, Germany) was used for the HPH. The suspension was processed using 2 homogenization cycles of 150 bar and 2 homogenization cycles of 500 bars as pre-milling. The final nanosuspensions were produced applying 20 homogenization cycles at 1500 bar.

Dispersed drugs as microparticles were passed through a continuous Micron Lab 40 at room temperature applying the variable pressure profile described above. At certain cycles (normally each fifth homogenization cycle at 1500 bars), samples were withdrawn for particle size analyses. Profiles of the particle size reduction as function of cycle number were plotted to illustrate the homogenization effectiveness.

3.2.2 Ball Milling

As comparison, ibuprofen nanosuspensions was also produced by ball milling. The preparation steps are basically the same as performed when preparing nanosuspension by the high pressure homogenization. The dispersion medium is prepared, the drug is suspended in the dispersion medium using an Ultra Turax T25 (Janke & Kunkel, Staufen Germany) for 10 second at 8,000 rpm. The resulting pre-mix was then transferred into the chamber of a

planetary mono mill “pulverisette 6” (Fritsch GmbH, Idar Oberstein, Germany). The chamber was filled with 90g zircon oxide milling beads Yttrium stabilized, 1 mm in diameter. The bead/suspension ratio was 80:20. The mill was run for 1 hour; samples were taken at certain defined time points and subsequently characterized by particle size analysis.

3.2.3 Characterization of drug nanosuspensions: size distribution, crystal nature and zeta potential

3.2.3.1 Laser Diffractometry (LD)

LD has rapidly become a preferred standard method for particle sizing in the pharmaceutical industry. This is due to its short analysis time, robustness, high precision, reproducibility, wide measurement range and flexibility of operation using liquid, spray and dry dispersion attachment. Most LD instruments employ a standard He-Ne laser light source (632.8 nm wave length) and consist of an optical system for Fourier transform of the diffracted light onto a position-sensitive detector. The light scattering pattern from non-spherical particles is very complex, varying as a function of the scattering angle, particle size and shape, and complex refractive index which depends both on the light refraction (real component) and absorption (imaginary component). However, the forward (Fraunhofer) diffraction depends only on the particle size, an azimuthally averaged tangential (Feret’s) diameter. This diameter can be associated with the projected-equivalent diameter that is defined by the overall intensity of the diffracted light. The volume-equivalent diameter is not measured by the laser diffraction technique, although the volume particle size distribution (PSD) is derived from the Fraunhofer diffraction pattern using a system of linear equations incorporated into the algorithm of the instrument. Algorithms, based on rigorous Mie scattering theory should be ideally applied for particles below 25 μm, however this requires the knowledge of the complex refractive indices which are not available for most organic materials and are not directly measurable with the available instruments. Therefore, in practice, the dispersion errors with small particles and uncertainties introduced by the imaginary component of the refractive index may outweigh the inaccuracy associated with the simplified Fraunhofer theory.

For non-spherical particles, such as plates and needles oriented randomly, the volume diameter is typically overestimated because of the larger projection diameter for these shapes. Another error may originate from high concentration of particles analyzed/high obscuration of laser beam, leading to multiple light scattering and overestimation of particle fines (undersizing the particle size distribution). This can be easily avoided by keeping the obscuration, usually monitored during measurements, within a 0.1-0.3 interval. In some instruments, the LD method is combined with multiangle and multi wave length light scattering measurements, which enable expansion of the dynamic measuring range and essentially become the multiangle static laser light scattering (LLS) method [136].

The equivalent diameter measured by LD is not directly related to the particle volume or surface and therefore care should be taken when interpreting these data for any pharmaceutical application. For the quality control purposes it is sufficient to establish a reliable correlation between the LD data and, for example, particle size distribution measured by image analysis or aerodynamic measurements. In the instances where the particle size of nanocrystals is measured when incorporated in the final dosage form, different formulation ingredients may strongly affect the data. Examples include assessment of powder blending and tablet homogeneity, measurements of nebulizer sprays, phase separation and aggregation in a suspensions of metered dose inhalers and deaggregation in dry powder inhalers [136].

Particle size distribution of the nanosuspensions were examined by laser diffractometry (LD) (Coulter® LS 230, Beckman-Coulter Electronics, Krefeld, Germany) with applied PIDS. The LD data obtained were evaluated using the volume distribution diameters (d) 10%, 50%, 90% and 99%. For results based on the Mie theory, the real refractive (rf) and imaginary rf index was determined specifically each substance. The diameter values 10% to 99% indicate the percentage of particles possessing a diameter equal or lower than the given size value.

3.2.3.2 Photon Correlation Spectroscopy (PCS)

This method, also known as dynamic light scattering (DLS) or quasi-elastic light scattering, is primarily used to measure nanoparticulate systems such as e.g. emulsions, micelles, liposomes and nanosuspensions. When a laser beam is passed through liquid dispersion containing particles in Brownian motion, it experiences fluctuations in its intensity due to interferences of waves in light scattering. In the PCS instrument, measurements of this fluctuation of intensity at a given scattering angle are used to infer the particle size or the “hydrodynamic diameter” of the dispersed particles. The PCS instruments measure the fluctuations in the intensity of the scattered light with time in order to generate an exponentially decaying autocorrelation function. This function is then analyzed for characteristic decay times, to determine the diffusion coefficient unique to the scattering dispersed particles and, in conjunction with the Stokes-Einstein equation, the hydrodynamic radius. The primary advantage of the PCS method is that it provides an absolute measurement without the need of any further information about the composition and the optical properties of the particles in dispersion. The lower limit of the instrument depends on the laser power, the sensitivity of the detector and the signal-to-noise ratio and can be as low as 2 nm. Hence it can be used to measure the sizes of not only surfactant micelles and colloids but also macromolecules. The data obtained using the instrument is usually in two formats depending on the type of algorithms used for the inversion of the autocorrelation function. A Gaussian distribution is typically used to represent unimodal dispersions. A more complex analysis is required for multimodal (e.g., bimodal) particle size distributions. Introduction of multimodality can lead to a significant complication because the equations used to calculate the particle size become more ambiguous and less stable. The algorithms used provide information about the mean particle size, widths and peak modes of the particle size distributions. The intensity-based data, collected by the instrument, can be reduced to a volume-weighted particle size distribution (PSD) within certain limits of reliability. However, large particles (>3 μm) may completely distort the measurements and therefore a complementary analysis with LD or laser scattering instrument is recommended in order to corroborate the obtained results [136].

The particle size average of the nanosuspensions was analyzed by photon correlation spectroscopy (PCS) with a Zetasizer Nano ZS (Malvern Instruments, UK). PCS yields the mean particle size (z-average) and the polydispersity index (PI) which is a measure of the width of the size distribution. The z-ave and PI values were obtained by averaging of 10 measurements at an angle of 90° in 10 mm diameter cells at 25°C. Prior to the measurement, all samples were diluted with bi-distilled water to have a suitable scattering intensity.

3.2.3.3 Zeta potential

Zeta potential (ZP) shows the charge on the particle surface which indicates the physical stability of dispersed systems. The ZP was measured by determining the electrophoretic mobility using the Malvern Zetasizer Nano ZS. The measurements were performed in distilled water adjusted to a conductivity of 50 µS/cm with sodium chloride solution (0.9%w/v), to avoid changes in ZP values due to day-to-day variations occurring in the conductivity of the water. The ZP was calculated using the Helmholtz-Smoluchowski equation [137]. The measurements were repeated three times at 25°C at field strength of 20 V/cm. Under these measuring conditions the measured ZP is equivalent to Stern potential.

In addition, the ZP was also measured in the original media of the nanosuspensions. This was conducted to measure the ZP as a measure for the thickness of the diffuse layer and used to predict stability of electrically stabilized nanosuspensions.

3.2.3.4 Polarized light microscopy

Light microscopy was performed using an Orthophlan microscope (Leitz, Wetzlar, Germany). The employed magnification was 1000 fold (oil immersion) and each sample was investigated 3 times. The crystallinity was examined using polarized light.

3.2.3.5 Scanning Electron Microscopy (SEM)

The particle size, morphology of the drug powder and drug nanocrystals after high pressure homogenization were investigated by scanning electron microscopy. The materials were placed onto carbon plates and coated under an argon atmosphere with gold to a thickness of 5nm (SCD 040, Bal-Tec

GmbH, Witten, Germany). The samples were then observed with a scanning electron microscope (S-4000, Hitachi High Technologies Europe GmbH, Krefeld, Germany) using secondary electron imaging at 10 kV. These SEM studies were performed at the Zentraleinrichtung Elektronenmikroskopie (Technical University Berlin, Germany).

3.2.3.6 Kinetic solubility measurement

Solubility studies were performed with a shaker (Innova™ 4230, New Brunswick Scientific Edision, NJ-USA) at 25°C which allowed an accuracy in temperature of ±0.01°C. Excess drug was added in 40 ml capped vials, then sonicated in a water bath (Bandelin RK 514, Berlin, Germany) for 2 min. Vials were sealed to avoid changes due to evaporation and shaken for 1 week in a thermostated storage at 25 ± 0.01°C, shielded from light to prevent any degradation of the molecules. After the equilibrium was reached, suspensions were filtered through Sartorius® 0.1 µm filters (Sartorius AG, Goettingen Germany). An aliquot from each vial was withdrawn by 1ml glass syringe (Poulten & Graf GmbH, Germany) and assayed by HPLC to evaluate the amount of drug dissolved. The sample volume taken was not replaced by new solvent. Dilution was intentionally avoided, to prevent any possible interference with the chemical equilibrium, particularly considering the presence of colloidal particles. Experiments were carried out in triplicate, solubility data were averaged.

3.2.3.7 HPLC method to determine the content of the drugs

Drug concentrations were determined by high performance liquid chromatography (HPLC). A modified method has been developed for each substance. The chromatographic system consisted of a KromaSystem 2000 (Kontron Instruments GmbH, Neufahrn, Germany), a solvent delivery pump equipped with a 20 µl loop and a rheodyne sample injector. The analytical columns and mobil phases were specific for each drug. A diode array detector (DAD 540) was used as UV detector.

3.2.4 Preparation of dried nanocrystals

3.2.4.1 Spray drying process (SD)

In general, spray drying was employed to obtain nanocrystal powder. An aqueous nanosuspension was transferred into nanocrystals powder by a Mini Spray-dryer B-190 (Büchi Labortechnik AG, Switzerland). Mini Spray-dryer B-190 was set regarding to temperature inlet (110-100°C), outlet (74-76°C), air volume (600 L/h). Spray-dried nanocrystals were directly collected after the process. The nanocrystals were used for different purposes, e.g. as reference for crystalline state investigation.

In one specific case, testosterone nanosuspension was produced using the H42 technique. As starting material testosterone suspension was spray-dried by a Mini Spray-dryer B-190 (Büchi Labortechnik AG, Switzerland). In this process, the spray dryer was set to the conditions:

Temperature inlet: 72-76°C, outlet: 58-60°C, air Volume: 600 L/h, formulation: 10% (w/w) Testosterone, 0% or 1.11% or 10% of poloxamer 188 (w/w) and up to 100% ethanol.

3.2.4.2 Freeze-drying process (FD)

There are three stages in the complete freeze-drying process: Freezing, primary Drying, and secondary Drying [138]. The freezing process consists as first step of freezing the material. In a lab, this is often done by placing the material in a freeze-drying flask and rotating the flask in a bath, called a shell freezer, which is cooled by mechanical refrigeration, dry ice and methanol or liquid nitrogen. In this step, it is important to cool the material below its eutectic point, the lowest temperature at which the solid and liquid phase of the material can coexist. The freezing phase is the most critical in the whole freeze-drying process, because the product can be spoiled if badly done. Amorphous (glassy) materials do not have a eutectic point, but do have a critical point, below which the product must be maintained to prevent melt-back or collapse during primary and secondary drying.

a. Primary drying

During the primary drying phase the pressure is lowered (to the range of a few mbar and enough heat is supplied to the material for the water to sublimate. The amount of heat necessary can be calculated using the sublimating

molecules' latent heat. In this initial drying phase about 95% of the water in the material is sublimated. This phase may be slow because if too much heat is added the material's structure could be altered.

The vacuum speeds sublimation making it useful as a deliberate drying process. Furthermore, a cold condenser chamber and/or condenser plates provide a surface(s) for the water vapor to re-solidify on. This condenser prevents water vapor from reaching the vacuum pump, which could degrade the pump's performance. Condenser temperatures are typically below −50 °C (-58°F) [138].

b. Secondary drying

The secondary drying phase aims to sublimate the water molecules that are firmly bound by adsorption and cannot be removed during the freez-drying process. Only the more mobile water molecules were sublimated in the primary drying phase. This part of the freeze-drying process is governed by the material's adsorption isotherm. In this phase, the temperature is raised higher than in the primary drying phase, and can even be above 0°C, to break physicochemical interactions that exist between the water molecules and the frozen material. At the end of the operation, the final residual humidity in the product is around 1% to 4%, which is extremely low [138].

The nanosuspension was dried using lyophilization. In a 20 ml vial 2 ml of the nanosuspensions were frozen at -70°C. The frozen nanocrystals were freeze dried for 24 hours (Christ® Alpha I-5, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany).

3.2.5 Re-dispersability and phase separation

The re-dispersability of rutin nanosuspensions stored in glass bottles was determined by tilting the bottle up and down by hand until the sediment was dispersed in the aqueous phase uniformly. The number of times tilted was noted and rated as fast, medium and low. Phase separation was determined visually in all formulations during long-term storage.

3.2.6 Characterization of the drug nanocrystals

3.2.6.1 Differential Scanning Calorimetry (DSC)

DSC analysis was performed using a Mettler DSC 822e1200 (Mettler Toledo, Germany). The instrument was calibrated with indium (calibration standard,

purity 99.999%) for melting point and heat of fusion. Analysis was performed under a nitrogen purge (20 ml/min). Standard aluminum sample pans of 40 µl were used. About 2 mg drug nanocrystals were taken for analysis. A heating rate of 5^{o}C/min was employed. An empty pan was used as reference.

3.2.6.2 Powder x-ray diffraction (PXRD)

A powder X-ray diffractometer (wide angle scattering-WAXD, Philips, Amedo, the Netherlands) was used for diffraction studies. PXRD studies were performed on the samples by exposing them to CuK_{α} radiation (40 kV, 25 mA) and scanned from 0.06^{o} to 40^{o}, 2θ at a step size of 0.04^{o} and step time of 0.5 s. Samples used for PXRD analysis were same as those of DSC analysis.

3.2.6.3 In vitro dissolution behavior

The dissolution test was performed using a USP XXIV rotating paddle apparatus with a Pharmatest PTW SIII (Pharma Test, Hamburg, Germany) at 37 °C and a rotating speed of 100 rpm in 900 ml of water. Certain amounts of drugs were dispersed in the dissolution medium. At certain times, samples were withdrawn from the dissolution chamber. The samples were filtered through Sartorius® 0.1 µm filters (Sartorius AG, Goettingen Germany). An aliquot from each vial was withdrawn with a 1ml glass syringe (Poulten & Graf GmbH, Germany) and assayed by HPLC (HPLC, Kroma-System 2000, Kontron Instruments, Germany) to evaluate the amount of rutin dissolved. Again, dilution was intentionally avoided, to prevent any possible interference with the chemical equilibrium, particularly considering the presence of colloidal particles. That means the sample volume taken was not replaced by fresh dissolution medium.

3.2.6.4 HPLC method to determine the dissolved drugs

Applying the same method as determining drug content of the nanosuspensions, dissolved drug was also determined by HPLC. A modified method has been developed for each substance. The chromatographic system consisted of a KromaSystem 2000 (Kontron Instruments GmbH, Neufahrn, Germany), a solvent delivery pump equipped with a 20 µl loop and a rheodyne sample injector. The analytical columns and mobile phases were specific for each drug. A diode array detector (DAD 540) was used as UV detector.

3.2.7 High Performance Liquid Chromatography (HPLC)

3.2.7.1 Rutin

Rutin concentrations were determined by high performance liquid hromatography (HPLC). A modification method after Day *et al.* [139-141] has been developed. The chromatographic system consisted of a Kontron Instrument KromaSystem 2000, a solvent delivery pump equipped with a 20 µl loop and rheodyne sample injector. Eurospere C18 RS (25 cm x 4.6 mm lC) analytical column and buffer acetic (pH 4.8) : acetonitrile = 80 : 20 as solvent system were used. Diode array detector (DAD-Kontron Instrument HPLC 540) was used as UV detector and operated at 255 nm.

3.2.7.2 Hesperidin

Concentration of dissolved hesperidin in collected medium was determined by HPLC. The HPLC conditions were as following: column, Eurospher 100-5 C18, 250 x 4 mm (B6Y535); mobile phase: isocratic 5 mM ammoniumacetate/ acetonitril = 75 : 25 (v/v) and adjusted until pH of 4.45 with acetic acid; flow rate, 1.0 mL/min; UV/Vis detector; λ max. at 285nm, temperature; 40°C [142].

3.2.7.3 Ibuprofen

The amount of released ibuprofen in the collected medium was determined by high performance liquid chromatography (HPLC; Thermo Separation Product, USA). The HPLC conditions were as following: column, Spherisorb ODS 5 mm (diameter), 20 cm (length); mobile phase: methanol/Na_2HPO4 0.01 M (25%); flow rate, 1.0 mL/min; UV/Vis detector; λ max. at 220nm [143].

3.2.7.4 Co-enzyme Q10

The HPLC system consisted of Kontron Instrument KromaSystem 2000, a solvent delivery pump equipped with a 20 µl loop and rheodyne sample injector. As column, Bondapack® C18 RP, 10 mm, 125x4.6 mm was used. HPLC method and sample processing were modified as described by Kaikkonen, J. *et al* [144-146]. The mobile phase was Methano/Tetrahydrofuran (95:5 by volume). The flow rate was 1.2 ml/min and the column temperature was heated to 30 °C. UV detector was set at 275 nm.

3.2.8 Solid Dosage Form Development

3.2.8.1 Direct Compression of the tablet

The formulations of the rutin and hesperidin tablets are shown in **Table 3.6** and **Table 3.7**. Dried nanocrystals were admixed to the tablet excipients by a tumbler (Turbula, Willy A. Bachofen AG, Muttenz, Switzerland) and afterwards compressed by a single punch tablet machine (Korsch Pressen GmbH, Berlin, Germany) using direct compression. Direct compression was chosen to minimize agglomeration of the rutin nanocrystal during longer processing by granulation. The final product of the nanocrystal-loaded tablets can be seen in **Fig. 4.77** and **Fig. 4.78**.

Table 3.6 Rutin tablet formulations

Formulation	NC (mg)	MC (mg)	Excipients (mg)				
			AV	AC	Ex	Mg	T
A	50		42	5		2	1
B	50		42		5	2	1
C		50	42	5		2	1
Market		50					

NC = rutin nanocrystal, MC = rutin microcrystal, T = Talc, Mg = Mg stearic, AV = Avicel PH 101, AC = AcDiSol, Ex = Explotab, Market = marketed tablet.

Table 3.7 Hesperidin tablet formulations

Formulation	NC (mg)	MC (mg)	Excipients (mg)				
			AV	AC	Ex	Mg	T
X	50		594	35		14	7
Y	50		594		35	14	7
X		50	594	35		14	7
Market		50					

NC = hesperidin nanocrystal, MC = hesperidin microcrystal, T = Talc, Mg = Mg stearate, AV = Avicel PH 101, AC = AcDiSol, Ex = Explotab, Market = marketed tablet.

3.2.8.2 Extruding and Spheronizing of Pellets

The extrusion-spheronization process is a multiple step procedure, involving mixing, wet granulation, extrution, spheronization and drying (**Fig.3.14**). The first step was dry mixing of ibuprofen nanocrystal and lactose (mixing ratio 1:2). The water was poured to dry mass of them (final ratio ibuprofen : lactose : water = 1:2:1) and followed by wet granulation using a coating pan, which converted the ibuprofen and lactose into a plastic mass that can be easily extruded by a basket *extruder* (*Caleva Model 10*, Sturminster Newton, UK). The coating pan was rotated at 25 rpm within certain time until a plastic mass (pellet mass) formed.

The extruded strands were transferred into a *spheronizer* (*Caleva Model 120*, Sturminster Newton, UK) where, when upon contact with the rotating friction plate, they were instantaneously broken into short cylindrical rods and were pushed towards and up the stationary wall of the processing chamber by centrifugal force.

Due to gravity, the particles fall back to the friction plate and the cycle was repeated until the desired sphericity is achieved. Finally, they are dried either on trays or in a fluid bed-dryer prior to the further processing [50].

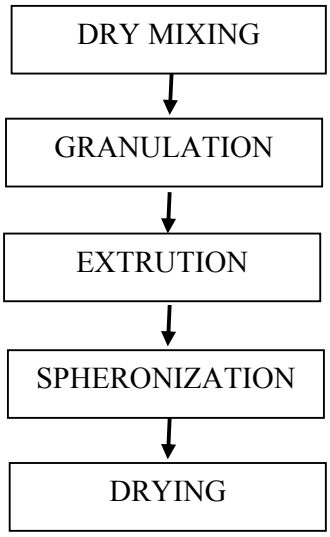


Fig. 3.14 Flow chart of extrusion-spheronization process after [50]

3.2.8.3 Fusion method of the effervescent granule

In the fusion method, the one molecule of water present in each molecule of citric acid acts as the binding agent for the powder mixture. Just before mixing the powder, the acid source were powdered and then mixed with the other powders (previously passed through a number 60 sieve) to ensure uniformity the mixture. The sieves and the mixing equipment should be made from stainless steel or other material resistant to the effect of the acids.

Table 3.8 The formulations of ibuprofen nanocrystal-loaded effervescent granules

Formulation	Composition	
Effervescent A	Nanocrystals	28%
	Mannitol	11 %
	Citric acid	26 %
	Sod Bicarbonat	35 %
Effervescent B	Nanocrystals	28%
	Mannitol	11 %
	Citric acid	22 %
	Sod Bicarbonat	33 %
	Fum. Acid	6 %
Effervescent C	Nanocrystals	28%
	Mannitol	10 %
	Citric acid	24 %
	Sod Bicarbonat	38 %
Effervescent D	Nanocrystals	28%
	Mannitol	16 %
	Citric acid	10 %
	Sod Bicarbonat	30 %
	Tartaric Acid	16 %

The mixing of the powder was performed as rapidly as is practical, preferably in an environment of low humidity to avoid the adsorption of moisture from the air leading for premature chemical reaction. After mixing the powder was

placed in a glass or a suitable dish in an oven previously heated to between 93^{o} and 104^{o}C. During the heating process, a spatula was used to turn over the powder. The heat causes the release of the crystallization water from the citric acid, which in turn dissolves a portion of the powder mixture, setting off the chemical reaction and consequently release of some carbon dioxide. This causes the softened mass of the powder to become somewhat spongy, and when of the proper consistency, it was removed from the oven and rubbed to a sieve to produce granules of the desired size. A no. 84 sieve was used to produce medium granules. When all of the mass had passed through the sieve, the granules were immediately dried at a temperature not exceeding 54^{o}C and immediately transferred to containers which are the promptly and tightly sealed [49].

3.2.8.4 Capsule development (mixing and filling of capsules)

Formulations of the CoQ10 capsules can be seen in **Table 3.9.** CoQ10 was admixed to the capsule excipients by a tumbler (Turbula, Willy A. Bachofen, Basel, Switzerland). The mixed powder was filled into hard gelatin capsule no. 2 using a simple filling capsule equipment for lab scale. The final product of the capsules were collected and immediately transferred into dry plastic containers and tightly sealed.

Table 3.9 The formulations of coenzyme Q10 capsules

Formulation	NC (mg)	MC (mg)	Excipient (mg)		
			Lc	AV	Mg
K	30		15	190	1
L		30	15	190	1
Market		30			

NC = Coenzyme Q10 nanocrystal, MC = Coenzyme Q10 microcrystal,
Mg = magnesium stearate, AV = Avicel PH 102, Lc = lactose,
Market = marketed capsule.

Fig. 3.15 A simple capsule filler equipment for lab scale

3.2.9 Dissolution performance of the dosage forms

The dissolution test was performed using a USP XXIV rotating paddle apparatus with a Pharmatest PTW SIII (Pharma Test Apparatebau GmbH, Hainburg, Germany) at 37°C and a rotating speed of 100 rpm in 900 ml of water. Tablets/pellets/effervescent granules were placed in the dissolution chamber containing the dissolution media. At certain times, samples were drawn from each dissolution chamber. The samples were filtered through Sartorius® 0.1 μm filters (Sartorius AG, Goettingen Germany). An aliquot from each vial was withdrawn with a 1ml glass syringe (Poulten & Graf GmbH, Wertheim Germany) and assayed by HPLC to evaluate the amount of rutin dissolved. Again, dilution of the samples was intentionally avoided, to prevent any possible interference with the chemical equilibrium, particularly considering the presence of colloidal particles.

The dissolution test of the capsules was performed using a USP XXIV rotating paddle apparatus with a Pharmatest PTW SIII (Pharma Test, Hamburg, Germany) at 37°C and a rotating speed of 50 rpm in 900 ml of medium. Capsules were held to the bottom of the vessel using copper sinkers. Samples were analyzed for drug concentration using HPLC.

Chapter 4
RESULTS AND DISCUSSION

4. RESULTS AND DISCUSSIONS

4.1 Preparation of nanosuspensions

This thesis describes two methods for creation of nanosuspensions. First is the use of standard high pressure homogenization (HPH) and second is the development of nanosuspension by Nanopure® XP variant H42. The second method is a combination of spray drying and subsequent high pressure homogenization. As comparison, ibuprofen nanosuspension was also generated by ball milling using a planetary mono mill “pulverisette 6”

4.1.1 High pressure homogenization

Nanosuspension on a lab scale is typically produced by using a Micron LAB 40 in the discontinous version (APV Deutschland GmbH, Germany). The Micron LAB 40 can produce 20-40 ml in a batch. However, product capacity is low because only 20-40 ml can be produced in a batch. Therefore, a continuous Lab 40 can be employed yielding a batch size of 200–500 ml in its standard version but allowing also the production of a few liters in its modified version (use of larger product containers).

The aim of the research is to obtain a product of good quality. This means homogeneity of the nanosuspensions with a very small amount of microparticles, especially under 5 µm, to avoid capillary blocking for parenteral administration and to re-disperse easily into a medium after the drying process for oral administration. With high pressure homogenization it is possible to obtain a uniform product with a very low fraction of particles in the micrometer range, or even with all particles in the nanometer range. To avoid effects such as anaphylactic shock or other allergic reactions because of presence of surfactants, it was important to reduce the amount of stabilizer without losing a stable suspension. To achieve this screening of formulations was preformed with different types and concentrations of surfactants or polymers commonly used as stabilizer. The drug powder was dispersed in an aqueous surfactant solution using an Ultra Turrax (Janke und Kunkel GmbH, Staufen, Germany) for 10 seconds at 8,000 rpm.

4.1.1.1 Rutin

Four formulations of rutin nanosuspensions were prepared using four different stabilizers. The four formulations can be seen in the **Table 4.1**. Rutin microparticles were dispersed in the surfactant solution and passed through a continuous Micron Lab 40. Homogenisation was performed at room temperature by applying a variable pressure profile. In this process, the particle size reduction depends on the number of homogenization cycles. According to Grau *et al.* [147-149], two cycles were performed at 150 bar, two cycles at 500 bar as the pre-milling step and finally twenty subsequent cycles at 1500 bar as the milling step. Samples were withdrawn after pre-milling and after each fifth cycle of milling for particle size analysis. In this way, profiles of the particle size reduction could be clearly observed.

Table 4.1: Formulations of 10% rutin nanosuspensions

Subtances	**Formulation**			
	A	**B**	**C**	**D**
Rutin	10%	10%	10%	10%
Poloxamer 188	2%	-	-	-
SDS	-	0.2%	-	-
Tween 80	-	-	2%	-
PVA	-	-	-	2%
Water	88%	89.8%	88%	88%

PVA = polyvinyl alcohol, SDS = sodium dodecyl sulfate

The width of the homogenization gap is a function of the applied pressure, that means it will be decreased by increasing the pressure. In general, the particle size decreases with an increasing number of cycles and increasing homogenization pressure [147-151].

The width of the particle size distribution of the microsuspension can be reduced using pre-milling. **Fig. 4.1** (p. 96-97) in the particle size analyses section shows the effect of pre-milling after two cycles at 150 and 500 bar, compared to the particle size of the raw material before homogenization.

Applying a pressure of 1500 bar leads to a continuous particle size reduction with increasing number of cycles. Usually there is no further decrease after a certain number of cycles because the maximum dispersitivity at the given power density has been reached. After that product becomes yet more homogeneous in size, due to the disintegration of micrometer drug particles still present in the suspension [147-150].

4.1.1.2 Hesperidin

The physicochemical properties of hesperidin indicate that its crystalline phase has poor solubility in water and shows a slow dissolution rate from solid oral forms, restricting its uses in therapy. This property indicates that the dissolution of hesperidin may be the most critical factor or rate limiting step in its bioavailability than the passage through intestinal barrier. Various methods could be used to enhance its solubility including nanonization, and formation of amorphous coprecipitates [152] or preparation of inclusion complexes using material such as cyclodextrins [13, 153]. Nanonization such as high pressure homogenization (HPH) is also intensively used in pharmaceutical technology to reduce particle size and sometimes to produce amorphous drugs [56]. HPH could reduce the primary particle size to less than 1 µm, which is critical, to achieve the desired dissolution and solubility enhancement. Therefore, HPH was intensively performed to generate hesperidin nanosuspensions using various stabilizers.

Table 4.2 Formulations of 10% hesperidin nanosuspensions

Subtances	**Formulation**			
	E	**F**	**G**	**H**
Hesperidin	10%	10%	10%	10%
Poloxamer 188	2%	-	-	-
SDS	-	0.2%	-	-
Tween 80	-	-	2%	-
PVA	-	-	-	2%
Water	88%	89.8%	88%	88%

PVA = polyvinyl alcohol, SDS = sodium dodecyl sulfate

Four formulations of heperidin nanosuspensions were prepared using various different stabilizers **(Table 4.2)**. Using the same methods for producing rutin nanosuspensions, macrosuspensions of hesperidin were intensively homogenized using a Micron Lab 40. After pre-milling, hesperidin nanosuspensions were generated by milling process at 1500 bar with twenty homogenization cycles. At each fifth cycle, samples were drawn and the particle size was analyzed to plot profiles of particle size reduction. Particle size reduction will be described in the next section.

The pre-milling reduced effectively the width of the size distribution of the particles and applying the production pressure of 1500 bar led to a progressive decrease in size as the number of cycles increased. After a certain number of cycles, there is usually no further decrease of the particle size and only a more homogeneous product results but without smaller particles of the bulk population.

4.1.1.3 Ibuprofen

Interestingly it has found that physically ibuprofen is hard crystalline. Additionally, since it has a low melting point, the temperature should be intensively controlled during production [114]. Normal production of a nanosuspension using high pressure homogenization (HPH) requires five until twenty homogenization cycles. With respect to physical properties of ibuprofen, the ibuprofen nanosuspension produced using the LAB 40 required 40 homogenization cycles. Ten formulations were prepared with different stabilizers such as surfactants, polymers and the combination of surfactant and polymer. The hard crystalline ibuprofen needs an appropriate stabilizer to produce excellent ibuprofen nanosuspensions. Therefore, in the screening process, ten formulations were set up. All formulations can be seen in **Table 4.3**.

Table 4.3 Formulations of 1% ibuprofen nanosuspensions

Subtances	Formulation									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Ibuprofen	1%	1%	1%	1%	1%	1%	1%	1%	1%	1%
Poloxamer 188	1%	-	0.5%	1%	-	0.25%	1%	-	-	-
SDS	-	-	-	-	0.1%	-	0.25%	-	0.2%	0.2%
PVA	-	-	-	-	-	-	-	0.25%	-	0.2%
Phospholipon 80	-	-	-	1%	0.5%	0.5%	-	-	-	-
PVP	-	1%	0.5%	-	-	-	-	-	-	-
Water	98%	98%	98%	97%	98.4%	98.25%	97.75%	98.75%	98.8%	98.6%

PVA = polyvinyl alcohol, SDS = sodium dodecyl sulfate, PVP = polyvinyl pirolidon

Similar to other processes, ibuprofen microparticles were dispersed in the surfactant or stabilizer solution and passed through a discontinuous Micron Lab 40. After premilling at 150 bar and 500 bar, nanosuspensions were generated using forty homogenization cycles at 1500 bar. Important to note, homogenization was performed controlled at 5°C homogenize temperature and after each cycle, the temperature of the suspension was measured and kept to be below 10°C to avoid the melting of the ibuprofen. Again, samples were drawn after each fifth cycle for particle size analysis to plot a profile of the particle size reduction as function of cycle number.

For comparison, a nanosuspension from hard crystalline ibuprofen was also prepared by ball milling (BM). The particle size of this nanosuspension was compared to nanosuspensions produced by high pressure homogenization (HPH). Ibuprofen nanosuspensions consisting of ibuprofen, sodium dodecyl sulfate (SDS) 0.2% and water were investigated as to whether a sufficiently particle size of drug nanocrystals could be obtained by the BM process. A Planetary Mono Mill "pulverisette 6" was used for the ball milling. Dispersed microparticles of the hard crystalline ibuprofen were milled by BM for 60 minutes. Photon correlation spectroscopy (PCS) and laser diffraction (LD) were employed to determine the particle size. The size reduction was also plotted and can be seen in next section.

4.1.1.4 Coenzyme Q10

Coenzyme Q10 (CoQ10), a yellow crystalline powder with a melting point of about 50°C, is a lipid soluble vitamin and poorly soluble in water [123, 154]. Compared to other drugs crystalline CoQ10 is relative soft. Therefore CoQ10 nanosuspension could be produced with only 10 homogenization cycles. Since it has a low melting point, homogenization should be performed at low temperature. In this case a water bath was used and the temperature was set at 0°C during homogenization.

Two formulations of CoQ10 nanosuspensions were prepared using different stabilizers. The formulations were stabilized by Tween 80 and PVA (polyvinyl alcohol) as it can be seen in **Table 4.4**.

Table 4.4 Formulations of 10% Coenzyme Q10 nanosuspensions

Subtances	Formulation	
	I	J
Coenzyme Q10	10%	10%
Tween 80	2%	-
PVA	-	2%
Water	88%	88%

PVA= polyvinyl alcohol

The homogenization process was performed identical to the production of the rutin and hesperdin nanosuspensions. It should be noted that the melting point of coenzyme Q10 is very low. Therefore during homogenization, the temperature of the nanosuspensions was kept below 10°C.

To sum up: the number of cycles required for a nanosuspension depends on the desired size and uniformity of the product which depends of course on the envisaged route of administration (e.g. oral vs. parenteral, s.c. and i.m. vs. intravenous).

4.1.1.5 Homogenization method H42 for testosterone nanosuspensions

The newly developed nanonization technology Nanopure® XP variant H42 uses spray drying for production of nanocrystals in combination with subsequent HPH. It was found that the addition of surfactant (poloxamer 188) resulted in spray-dried drug powders with improved properties, such as improved diminution effectiveness during the HPH and flow ability of the powder [155]. Spray drying is well known for the production of micronized drug powders [156]. Depending on the drug, the spraying conditions and additives, micronized drug powders can be obtained in amorphous or crystalline state [157]. The powder morphology of spray-dried testosterone was found to be influenced by the content of surfactant (i.e. Poloxamer 188).

In addition, the use of a solvent for spray drying is no additional process step because after synthesis the drug has to go through a purification and re-crystallization process anyway, normally in volatile organic solvents. The last

step of this purification process is then changed from a re-crystallization in a non-solvent to a spray drying step. To simplify it, the last step of synthesis and purification is slightly modified to obtain an optimized material for the homogenization process.

The aim of the pre-treatment of material using spray drying before homogenization is to produce fragile material which can easily be broken up during the homogenization. In general, micronized drug particles tend to agglomerate; their flow properties are often poor. These problems could be circumvented by the addition of small amounts of surfactant to the drug solution before spray drying. Addition of surfactants (i.e. poloxamer 188) is also useful to avoid growth of the nuclei crystals during drying process. The surfactants can occupy voids among nuclei particles during agglomeration in the drying process. The particle size of the final product after spray drying is larger than for the raw material, but by the subsequent process of homogenization it is easily broken up into submicron materials [79, 158].

Three formulations were prepared as starting material of testosterone with different concentration of surfactant (i.e. Poloxamer 188) and 1 formulation of a “normal” nanosuspensions produced from micronized testosterone as the control. Hard crystalline testosterone needs a combination of surfactants to produce the starting material testosterone by spray drying. The screening process, employed 3 formulations of combination of surfactant and testosterone. All formulations are presented in **Table 4.5**.

Table 4.5 Composition of starting material formulations

Batch	Powder Composition (%, w/w)	
	Testosterone	Poloxamer 188
Testo-M[1]	100	0
Testo-SD I[2]	100	0
Testo-SD II[2]	90	10
Testo-SD III[2]	50	50

1) Old method (Dissocubes® technology, SkyePharma), micronized starting material
2) HPH new variant H42, SD = spray-dried testosterone

Spray-dried testosterone microparticles (starting material) were dispersed into 0.2% sodium dodecyl sulfate solution as stabilizer solution and passed through a continuous Micron Lab 40. Homogenisation was performed controlled at 5°C applying a variable pressure profile. Two cycles were performed at 150 bar, two cycles at 500 bar as a pre-milling step and finally twenty subsequent cycles at 1500 bar as the milling step. It should be noted, after each cycle at 1500 bar, temperature of the suspension was measured and kept to be below 10°C. Samples were drawn after each fifth cycle for particle size analysis to plot the profile of the particle size reduction. By comparison, testosterone nanosuspension was also produced using high pressure homogenization only (directly from micronized testosterone without spray drying).

4.2 Particle size and zeta potential analyses

Particle size analysis was performed by laser diffractometry and by photon correlation spectroscopy (PCS). Laser diffractometry yields a volume size distribution. As characterisation parameters the diameters 10, 50, 90 and 99% were used. For example diameter 99% means that 99% of the particles are below the given size value.

PCS yields the mean diameter of the bulk population (*z*-average, measuring range: 3 nm – 3 µm) and a polydispersity index (PI) as measure for the width of the size distribution. The PI ranges from zero (monodisperse particles) to 0.500 (broad distribution), values above 0.5 do not allow allocation of a logarithmic normal distribution to the PI.

4.2.1 Rutin

The particle size characterization data of the rutin nanosuspensions are shown in **Table 4.6**. The profiles of the particle size reduction as function of cycle number are shown in **Fig. 4.1** and **Fig. 4.2**. In general, the particle size decreases with an increasing number of cycles and increasing homogenization pressure. **Fig. 4.1** and **Fig. 4.2** show the effect of the pre-milling after two cycles at 150 and 500 bar in comparison to the particle size distribution of the raw material (microcrystals) before homogenization. The volume size distribution of the raw material revealed 10% (d10%) of the particles were below 0.8 µm, but 99% (d99%) of the particles were below 195

µm. It means the size difference between big particles and small particles of the raw material was very large. Pre-milling could reduce the width of size distribution of the particles as Grau *et al.* suggested. After pre-milling the particle size distribution of the suspension showed a d10% of 3 µm and a d99% of 11 µm. The pre-milling significantly improved the homogeneity of the suspension.

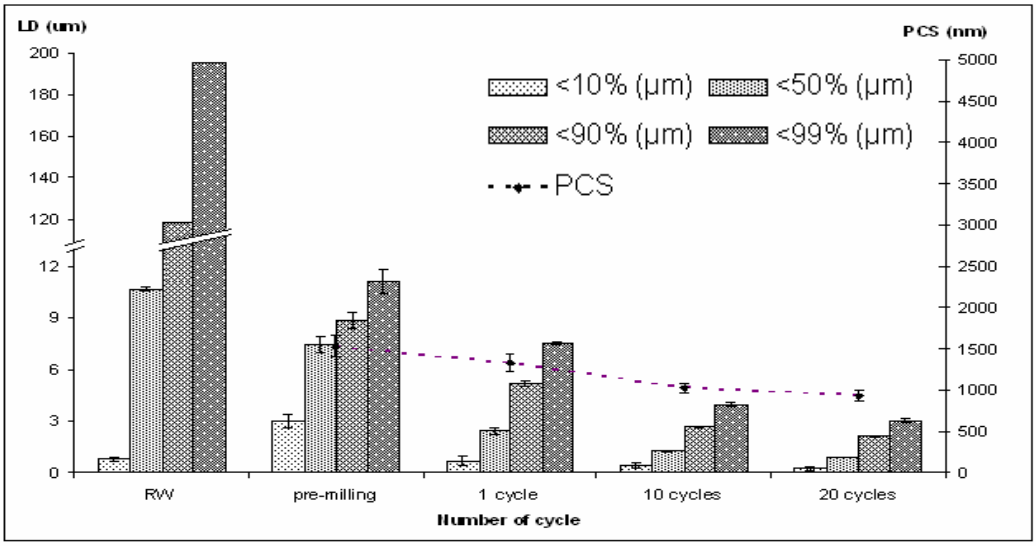
Table 4.6 Mean Particles size parameters of rutin nanosuspensions using 4 different stabilizers: (A) 2% of Poloxamer 188 (B) 0.2% of SDS (C) 2% of Tween 80 (D) 2% of PVA

Size parameters	Formulations			
	A	B	C	D
z - ave (nm)	648	727	912	547
PI	0.266	0.265	0.321	0.274
d50% (µm)	0.909	0.840	1.067	0.965
d90% (µm)	2.095	1.845	1.790	1.918
d99% (µm)	3.018	2.397	2.317	2.609

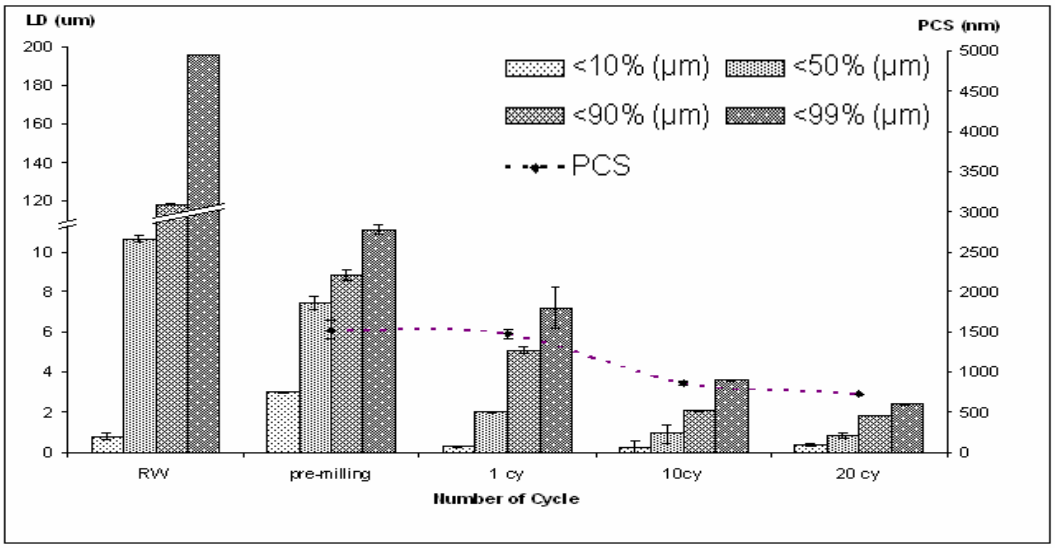
(S.D. <1%)

To obtain maximum physical stability (avoidance of Ostwald ripening) the nanosuspension should be as homogenous as possible, that means reduction of the number of micrometer particles. A low content of micrometer particles is also essential when the product should be injected intravenously. In particular, there should be a low content of particles larger than 5 µm to avoid capillary blockade [147]. **Table 4.6** shows the diameters 50–99% measured by laser diffractometry (LD). Typically, after twenty homogenization cycles the d99% of the particles was below 3 µm. It should be noted that the laser diffractometry data are a volume distribution and that the size range used for calculating the diameters is broader than for PCS. Therefore, in most cases the diameter d50% needs to be higher than the mean PCS diameter. Typically the volume size distribution d50% of the nanosuspensions by laser diffractometry (LD) were below 1 µm, except formulation C (**Table 4.6**). Formulations A, B and D

of the rutin nanosuspension had volume size distribution d50% by LD in the nanometer range.

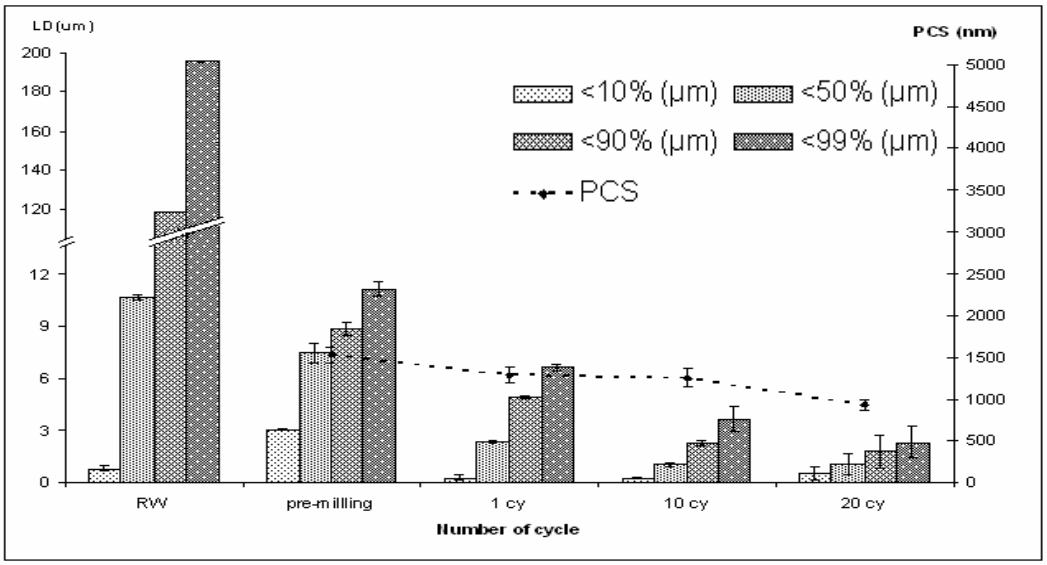


A

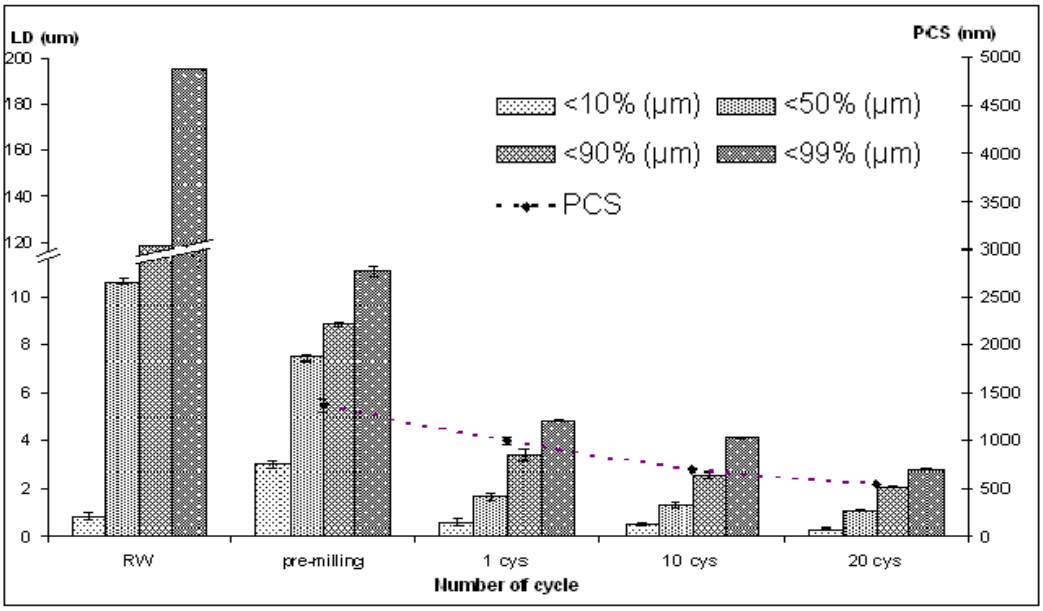


B

Fig. 4.1 Profiles of particle size reduction of rutin nanosuspensions of the formulations A, and B after pre-milling and after 20 homogenization cycles, for comparison the size of the raw material (RW) are given (PCS diameter (nm) and LD diameter d10% to d99% (µm))



C



D

Fig. 4.2 Profiles of particle size reduction of rutin nanosuspensions of the formulation C and D after pre-milling and after 20 homogenization cycles, for comparison the size of the raw material (RW) are given (PCS diameter (nm) and LD diameter d10% to d99% (µm))

According to Möschwitzer *et al.*, the typical particle size of a nanosuspension is around 200-1000 nm [159]. It is shown (**Table 4.6**, **Fig. 4.1** and **Fig. 4.2**) that mean particle sizes by PCS of all formulations were in the nanometer range. It means all formulations have fulfilled requirements of a nanosuspension. Particle sizes of the formulation A and D (stabilized by Poloxamer® 188 and PVA) were smaller than of formulation B. But both formulations were relatively instable. During storage time at room temperature the PCS particle sizes increased due to aggregation of the particles. The PCS particle sizes of formulation A increased to 1387 nm within 2 weeks, and formulation D to 984 nm within 2 months. In contrast, formulation B was relatively stable over a period of 12 months and therefore selected as the most suitable formulation for advanced development of an oral dosage form. The physical stability of the formulation B will be described in the next section.

Zeta potential analyses showed that the zeta potentials of the rutin nanosuspensions in water were higher than in the original dispersion media (**Table 4.7**). The zeta potential (ZP) in the original dispersion media was lower than in water because the ZP in water is equivalent to the Stern potential. However, the measured ZP in the original dispersion media is the ZP to predict the stability of the suspension [160]. Concerning formulation D, its zeta potential was relatively lower than the others. This formulation was stabilized by PVA with average molecule weight of ~90.000 (long chain polymer). The long chain polymer is adsorbed at the surface of the particle, leading to a shift of the plane of the shear in the diffuse layer. Therefore the measured zeta potential was lower [160].

Table 4.7 Zeta potensial analyses of 4 rutin nanosuspensions using 4 different stabilizers: (A) 2% of Poloxamer 188 (B) 0.2% of SDS (C) 2% 0f Tween 80 (D) 2% PVA in mV.

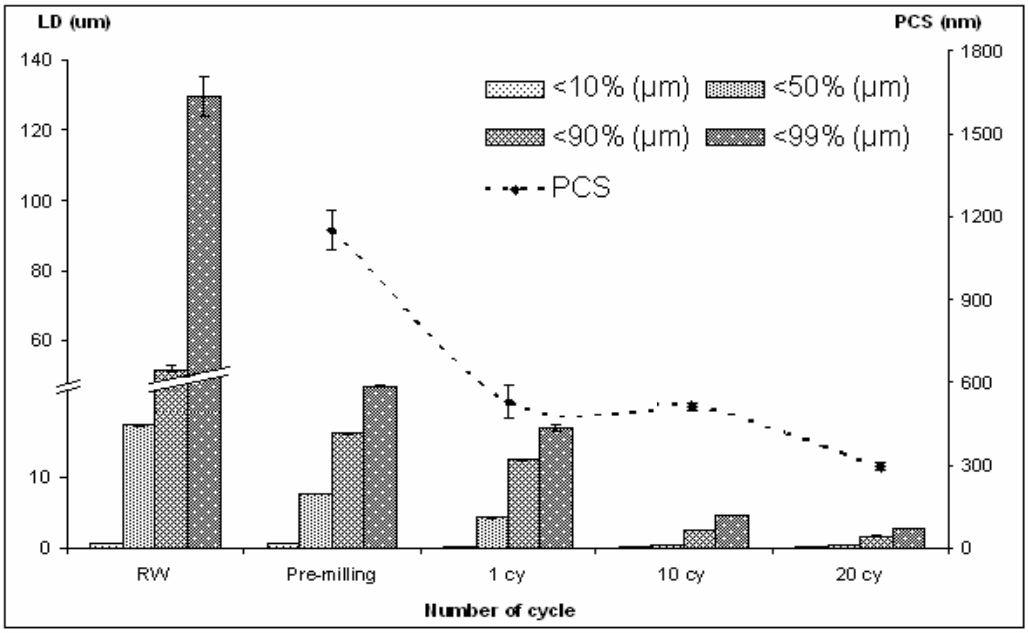
Medium	Formulations			
	A	**B**	**C**	**D**
water	-38.4	-35.3	-32.6	-26.6
Original medium	-24.7	-24.3	-22.6	-17.1

In general, ZP values of /30/ mV provide a good stability. The values in the dispersion media were below /30/ mV, around – 24 mV and lowest with -17 mV for the PVA–stabilized nanosuspension D. However, these values are still sufficient for stable suspensions in case electrostatic stabilization is combined with steric stabilization by polymers, e.g. PVA or poloxamer 188.

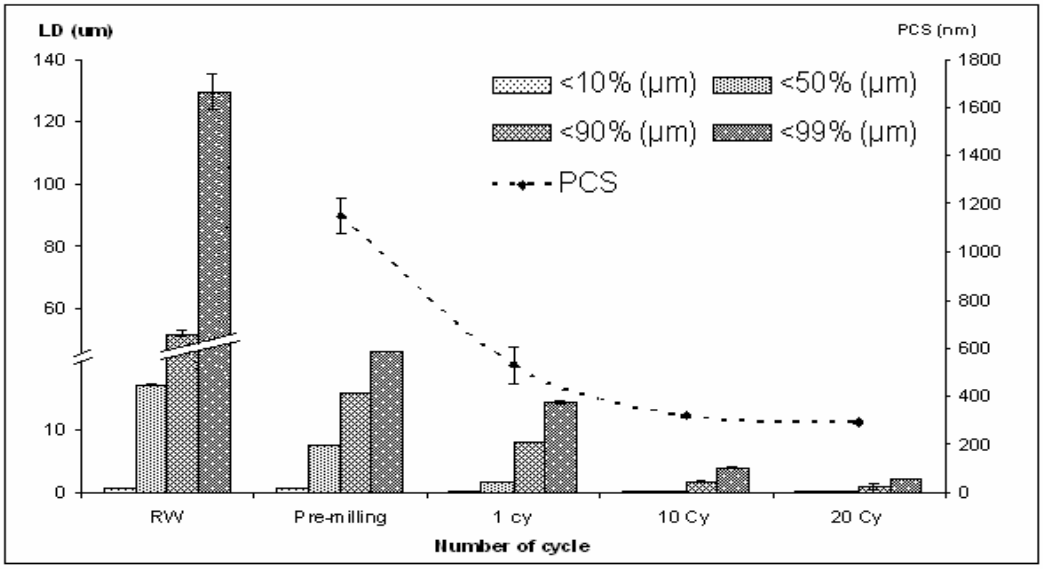
4.2.2 Hesperidin

Table 4.8 shows the particle size data of the hesperidin nanosuspensions stabilized by 4 different stabilizers. All profiles of the particle size reduction are shown in **Fig. 4.3** and **Fig. 4.4**. Similar to the rutin nanosuspensions, the particle size of the hesperidin nanosuspensions decreases with an increasing number of cycles and increasing homogenization pressure. The effectiveness of the pre-milling was clearly revealed by the reduced width of the particle size distribution of the particles. The width of the size distribution of the raw material was about 130 µm (d10% of 0.6 µm to d99% of 130 µm) and reduced by pre-milling to about 23 µm (d10% of 0.6 µm to d99% of 23 µm) after the pre milling process. A clear advantage was exhibited that pre-milling could increase the homogeneity of the macrosuspensions.

A homogenous nanosuspension is one of many advantages of the nanosuspensions produced by high pressure homogenization. This characteristic has been found to provide maximum physical stability (avoidance of Ostwald ripening). Therefore, the hesperidin nanosuspensions should be as homogeneous as possible, meaning a reduction of the number of micrometer particles. When the product should be injected intravenously, a low content of micrometer particles is essential for nanosuspensions for parenteral injections to avoid capillary blockage. **Table 4.8** shows the diameters 50–99% measured by laser diffractometry. Typically, after twenty homogenization cycles d99% of the volume size distribution of the particles is maximum 5 µm (a part from formulation G). According to the USP Pharmacopeial Forum and British Pharmacopoeia in 1980, the diameter of particles (globules in emulsion) should not exceed 5 µm for parenteral application [99]. According to the particle size, these hesperidin nanosuspensions can be officially used for parenteral administration.

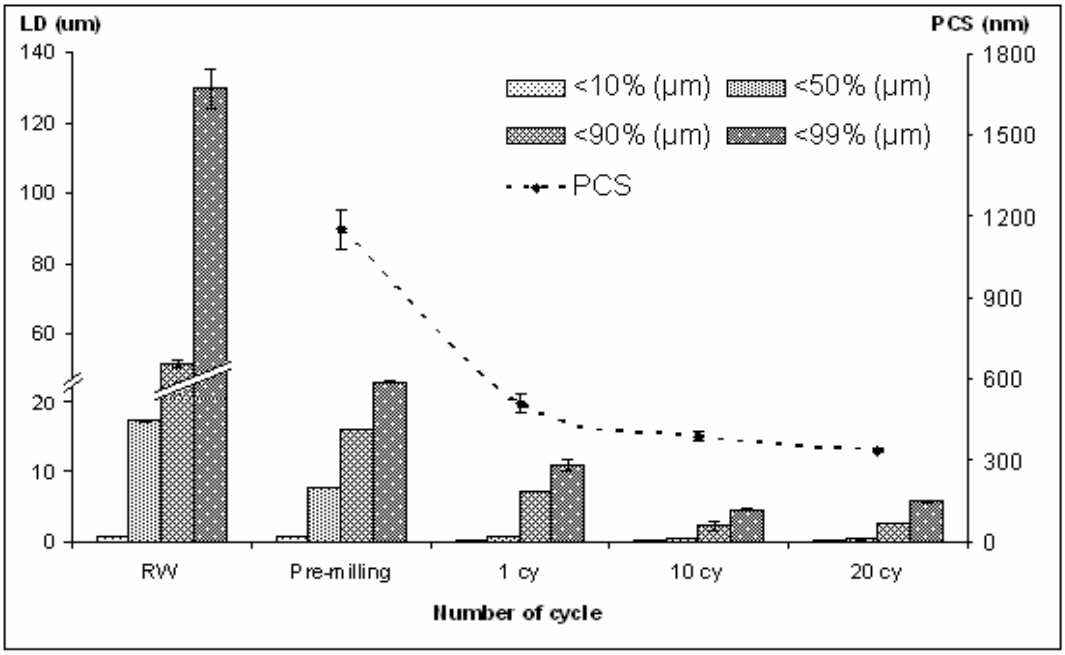


E

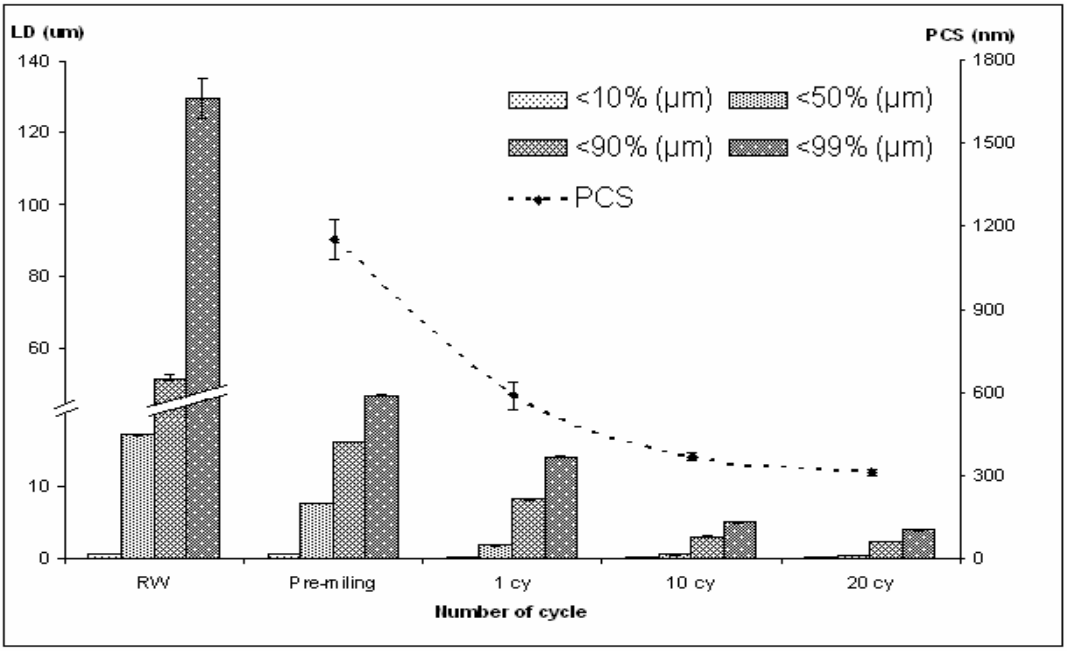


F

Fig. 4.3 Particle size reduction profiles of the hesperidin nanosuspensions of the formulation E and F after pre-milling and after 20 homogenization cycles, for comparison the size of the raw material (RW) are given (PCS diameter (nm) and LD diameter d10% to d99% (µm))



G



H

Fig. 4.4 Particle size reduction profiles of the hesperidin nanosuspensions of the formulation G and H after pre-milling and after 20 homogenization cycles, for comparison the size of the raw material (RW) are given (PCS diameter (nm) and LD diameter d10% to d99% (µm))

The LD diameter d50% of the nanosuspensions were below 1 µm (**Table 4.8**). Formulation E, F, G and H of the hesperidin nanosuspensions have volume size distribution d50% by LD in the nanometer range.

It is shown (**Fig. 4.3** and **Fig. 4.4**) that particle size averages by PCS of all of the formulations are in the nanometer range. This means formulations E, F, G and H have fulfilled the requirements of a nanosuspension. Particle size of the formulation F could reach d50% of 0.277 µm and d99% of 2.057 µm. This means formulation F resulted in smaller particle sizes compared to the other formulations.

Table 4.8 Mean particles size parameter of hesperidin nanosuspensions using 4 different stabilizers: (E) 2% of poloxamer 188 (F) 0.2% of SDS (G) 2% of Tween 80 (H) 2% of PVA.

Size parameters	Formulastions			
	E	F	G	H
Z ave (nm)	309	293	449	309
PI	0.434	0.307	0.308	0.404
d50% (µm)	0.344	0.277	0.343	0.378
d90% (µm)	1.632	0.831	2.415	2.172
d99% (µm)	2.728	2.057	5.627	3.942

(S.D. <1%)

Table 4.9 Zeta potensial analyses of 4 hesperidin nanosuspension using 4 different stabilizers: (E) 2% of poloxamer 188 (F) 0.2% of SDS (G) 2% of Tween 80 (H) 2% of PVA in mV

Medium	Formulations			
	E	F	G	H
water	-25.9	-36.4	-33.6	-9.9
Original medium	-19.6	-31.5	-19.2	-1.9

The zeta potential (ZP) was measured in two dispersion media. In comparison with the ZP in the original media, the ZP in water was higher (**Table 4.9**). This

is logic because as described before the ZP in water is equivalent to the Stern potential. However the measured ZP in the original media is the ZP at the plane of shear to predict the stability of the suspensions. As with the rutin nanosuspensions, the zeta potential of formulation H was relatively smaller than the others since this nanosuspension was stabilized by PVA (long chain polymer) which shifts the plane of shear in the diffuse layer.

4.2.3 Ibuprofen

After production, all formulations showed a different volume size distribution. A narrow size distribution is essential to prevent particle growth due to Ostwald ripening caused by different saturation solubilities in the vicinity of differently sized particles. In these ten formulations, only formulation 9 revealed a nanosuspension with mean particle size of 929 nm by PCS. The particle size reduction data is shown in **Table 4.10** and **Fig. 4.5**.

Formulations 4 and 7 could not generate a nanosuspension. Phospholipon 80 as stabilizer in formulation 4 could not disperse properly the ibuprofen particles in the aqueous solution. Phospholipon 80, a phospholipid, could not be used as single stabilizer for the nanosuspension. It works as stabilizer if combined with another stabilizer, for example with poloxamer 188 or SDS as can be seen in formulations 5 and 6. Formulation 7 used a combination of poloxamer 188 and SDS as stabilizer. This combination was not appropriate as stabilizer for ibuprofen nanosuspensions. During production, the particles agglomerated. The agglomerated nanosuspensions can be clearly observed by light microscopy (data not shown). Therefore single phospholipon 80 and the combination SDS and poloxamer 188 are not suitable as stabilizer for the ibuprofen nanosuspensions.

The next step of the investigation was to produce a higher concentration of ibuprofen nanosuspension. In this step, 10% nanosuspension was produced using a Micron Lab 40 and 0.2% SDS added as stabilizer. The particle size reduction profile of 10% ibuprofen nanosuspensions can be seen in **Fig. 4.6**. The volume size distribution of 10% ibuprofen nanosuspension was similar to 1% ibuprofen nanosuspensions. The particle size average by PCS of the 10% ibuprofen nanosuspension was 970 nm. Therefore 10% ibuprofen

nanosuspension stabilized by 0.2% SDS could be chosen as best formulation for producing a higher concentrated of the ibuprofen nanosuspension.

Table 4.10 Composition of ibuprofen formulation and particle size data

No	Formulation Composition	LD (µm)		PCS (nm)	
		D50%	D99%	Z-ave	S.D.
1	Ibuprofen 1% Poloxamer 188 1% Water q.s. 100%	2.021	5.335	1650	90
2	Ibuprofen 1% PVP 1% Water q.s. 100%	1.218	9.292	-	-
3	Ibuprofen 1% Poloxamer 188 0.5% PVP 0.5% Water q.s. 100%	1.691	7.362	1321	69
4	Ibuprofen 1% Phospholipon 80 1% Water q.s. 100%	-	-	-	-
5	Ibuprofen 1% Phospholipon 80 0.5% SDS 0.1% Water q.s. 100%	1.460	8.209	1394	129
6	Ibuprofen 1% Phospholipon 80 0.5% Poloxamer 188 0.25% Water q.s. 100%	1.701	4.553	1084	17
7	Ibuprofen 1% SDS 0.25% Poloxamer 188 1 % Water q.s. 100%	2.516	208.87	-	-
8	Ibuprofen 1% PVA 0.25% Water q.s. 100%	1.269 (40) 1.190 (60)	2.628 (40) 2.489 (60)	1061 (40) 1076 (60)	72 (40) 43 (60)
9	Ibuprofen 1% SDS 0.2% Water q.s. 100%	1.094	2.995	929	34.2
10	Ibuprofen 1% PVA 0.2% SDS 0.2% Water q.s. 100%	1.262 (60)	3.945 (60)	1050 (60)	72 (60)

Legend:
LD : Laser Difractometry
PCS : Photon Correlation Spectrophotometry
(40) : 40 cycles
(60) : 60 cycles

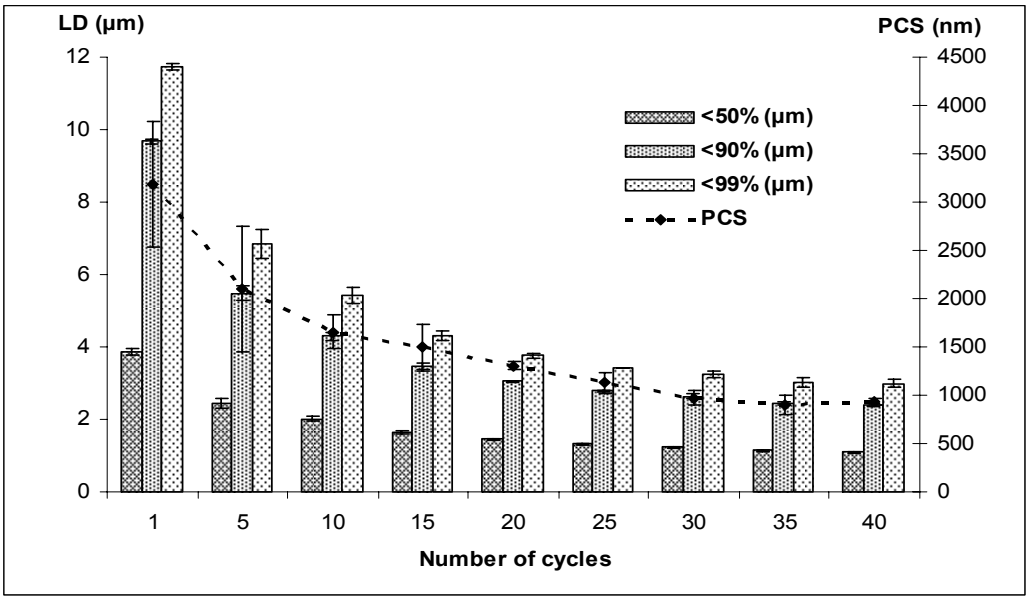


Fig. 4.5 Particle size reduction profile of 1% ibuprofen nanosuspensions formulation 9, stabilized by 0.2% SDS

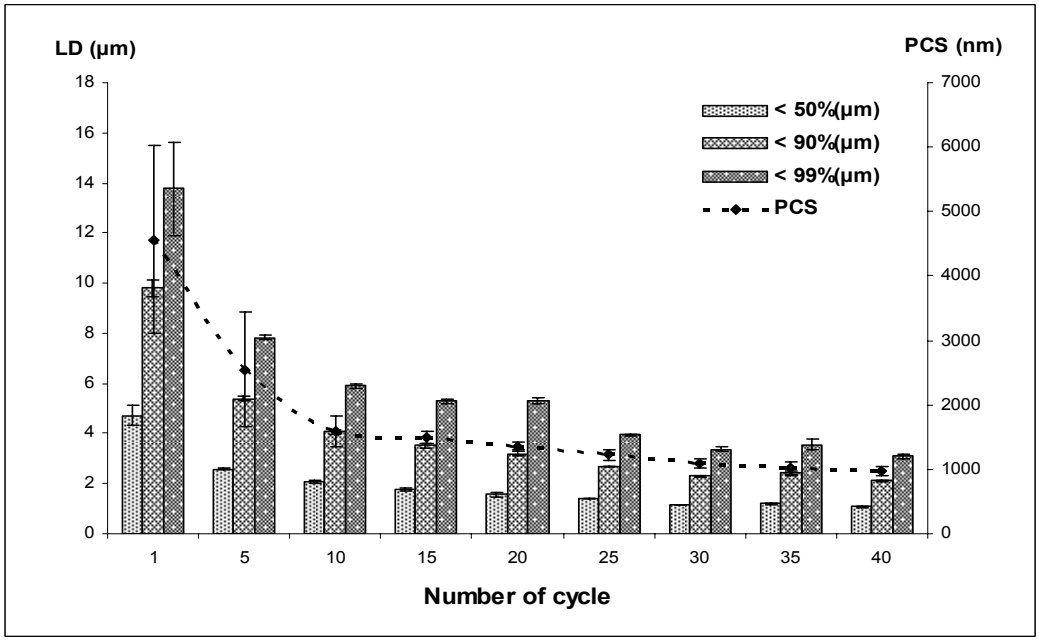


Fig. 4.6 Particle size reduction profile of 10% ibuprofen nanosuspension stabilized by 0.2% SDS

Zeta potential measurements were performed in distilled water with a conductivity adjusted to 50 µS/cm, field strength 20 V/cm. The zeta potential of formulation 9 was -68 mV. A zeta potential of at least approximately ± 30 mV is required for a stable dispersion. In this case, stability for ibuprofen nanosuspensions 9 was found to last at least a month (p. 131 - 132).

Particle size analyses of the nanosuspensions produced by ball miling (BM) were also performed. In contrast, BM leads only to ibuprofen microcrystals. The optimum particles size was achieved after 40 minutes milling time, the PCS size-average was 1270 nm with a PI of 0.571. The microscopic pictures showed that the suspension produced by BM formed microcrystals due to agglomeration of particles (*see in the morphology of particles section*). In contrast HPH could produce ibuprofen nanocrystals without agglomeration and being present generally as single crystals.

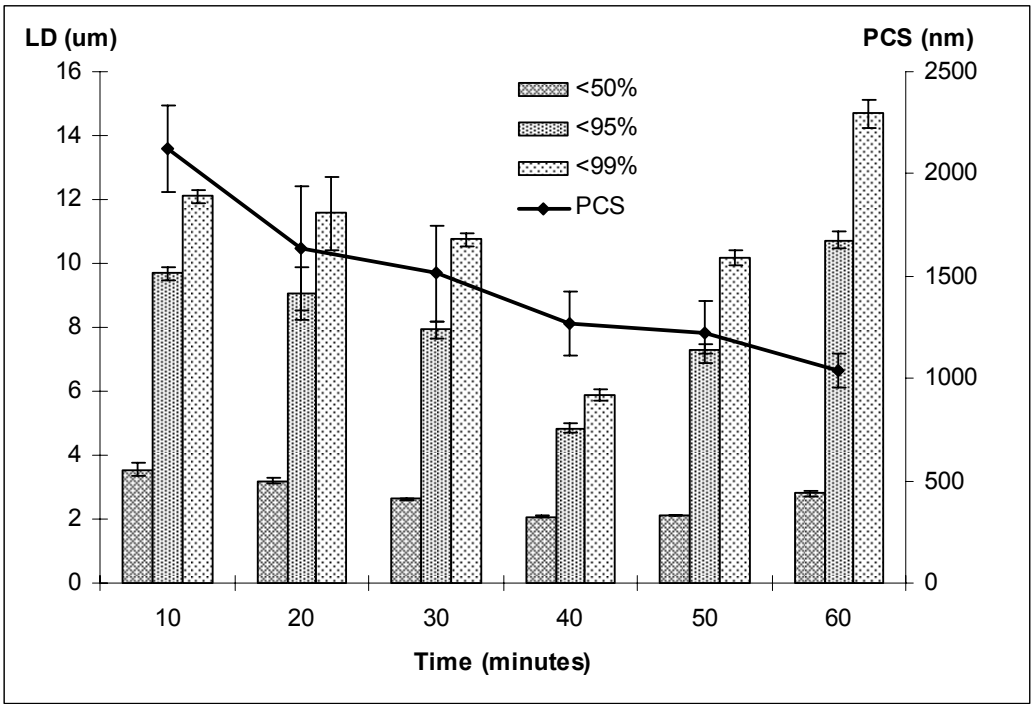


Fig. 4.7 Particle size reduction profile (LD and PCS) of ibuprofen nanosuspension produced by BM, composition: 1% of ibuprofen, 0.2% of SDS and ad water to 100%

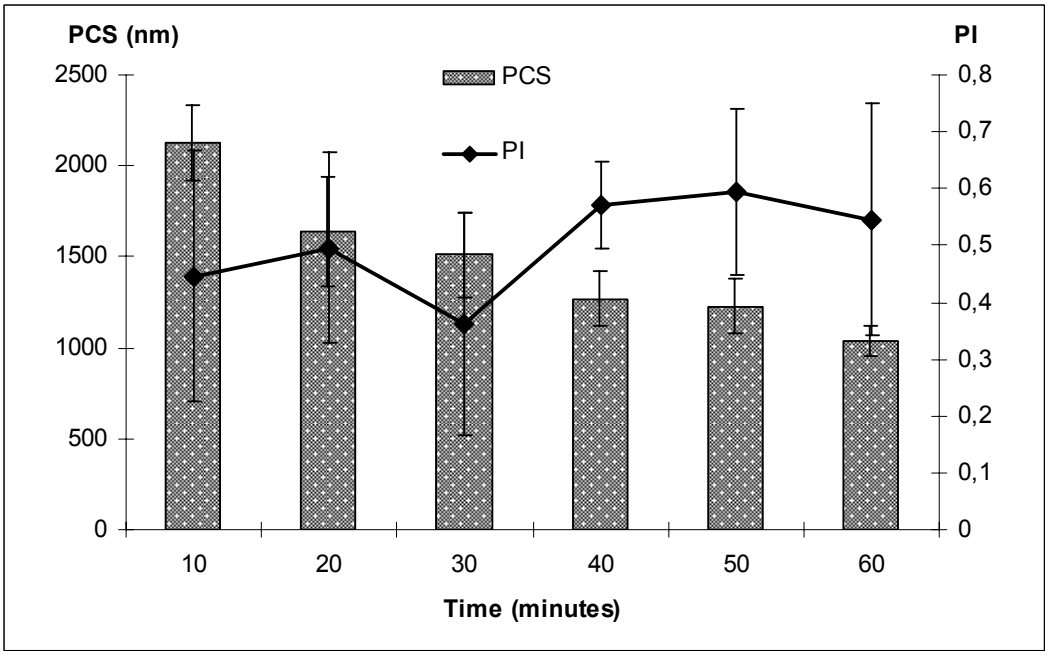


Fig. 4.8 Particle size reduction profile (PCS and PI) of ibuprofen nanosuspension produced by BM, PCS data taken from **Fig. 4.7**

The volume size distribution d50% of 2.066 µm and d99% of 5.893 µm have proven insufficient BM performance for this hard material. Longer milling times could not improve the results. Volume size distribution d50% and d99% by BM after 50 minutes was 2.115 µm and 10.160 µm and after 60 minutes was 2.809 µm and 14.685 µm. Another disadvantage of the BM method is that it can produce contaminants because of friction between the milling balls, which means the ball material can contaminate the product [57].

The performance of the nanonization technique depends strongly on the drug properties. In case of the hard crystalline ibuprofen, the high pressure homogenization (HPH) was superior to the ball milling (BM) [161].

4.2.4 Coenzyme Q10

High pressure homogenization (HPH) was very effective in reducing the particle size of coenzyme Q10. Nanonization by HPH was successful to obtain coenzyme Q10 nanosuspensions. After only 10 homogenization cycles, the particle size average of the nanosuspensions of formulation I and J by PCS was 221 nm and 219 nm respectively. Analysis by LD revealed that all of the particles were below 1 µm. It can be seen in **Fig. 4.9** and **Table 4.11,**

99% (d99%) of the particle size of the formulation I and J was below 0.679 µm and 0.443 µm respectively.

In addition, pre-milling was successful in significantly reducing the width of the size distribution of the raw material. This was shown clearly for formulation J. The volume size distribution of the raw material revealed 10% (d10%) of the particle size of the raw material was below 11 µm. The d99% of the particles was 276 µm. This means, size gap between big particles and small particles of the raw material was extremely large. After pre-milling this size gap among the particles could be reduced. The volume size distribution of the macorsuspension showing d10% and d99% was 0.5 µm and 6 µm, respectively. Pre-milling could increase the homogeneity of the macrosuspension.

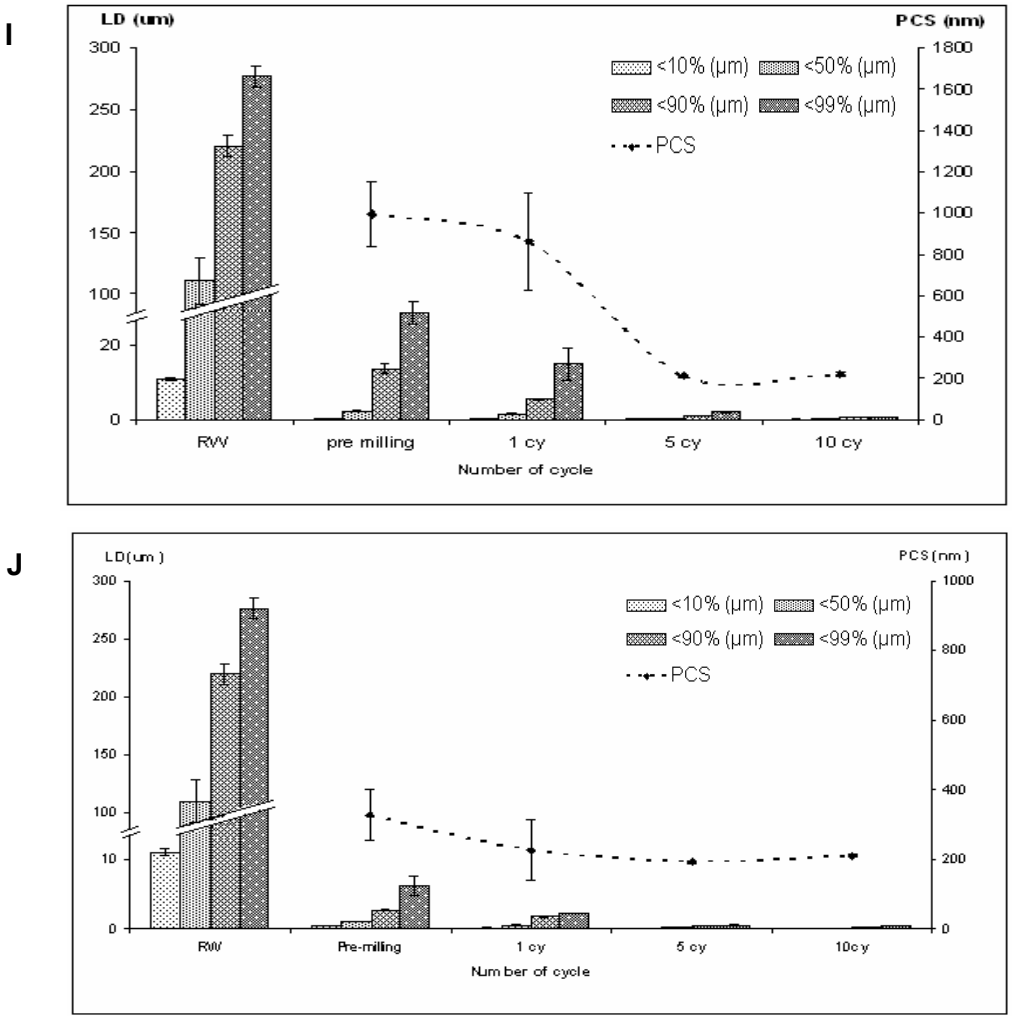


Fig. 4.9 Profile of particle size reduction of Coenzyme Q10 nanosuspension formulation I and J: (I) 2% of tween 80, (J) 2% of PVA

Table 4.11 Mean particles size parameter of CoQ10 nanosuspensions using 2 different stabilizers: (I) 2% of Tween 80, (J) 2% of PVA.

Size parameters	Formulations	
	I	J
z-ave (nm)	221	219
PI	0.250	0.136
d50% (µm)	0.363	0.214
d90% (µm)	0.538	0.310
d99% (µm)	0.679	0.443

(S.D. <1%)

4.2.5 Testosterone nanosuspensions by Nanopure® XP variant H42 method

Interestingly it was found that the micronization of testosterone by spray drying distinctly improves the performance of the high pressure homogenization process. **Table 4.12** shows the compositions and size characteristics of the investigated nanosuspensions after 20 homogenization cylces. However, the addition of poloxamer 188 during the spray drying process has an important impact on the achievable particle size of the drug nanocrystals. In general, the testosterone/poloxamer 188 co-processed powder resulted in nanosuspensions with a distinctly reduced particle size in comparison to suspension produced from the micronized drug material (Testo-M).

The mean particle size all of the H42 nanosuspensions was almost half or less compared to nanosuspension Testo-M after 20 homogenization cycles. Although the number of homogenization cycles was increased to 40 cycles, the average size of Testo-M nanosuspensions was still larger compared to H42 nanosuspensions. The polydispersity index was reduced for all nanosuspensions prepared by Nanopure®XP variant H42. The drug content seems to have almost no influence on the diminution effectiveness. The

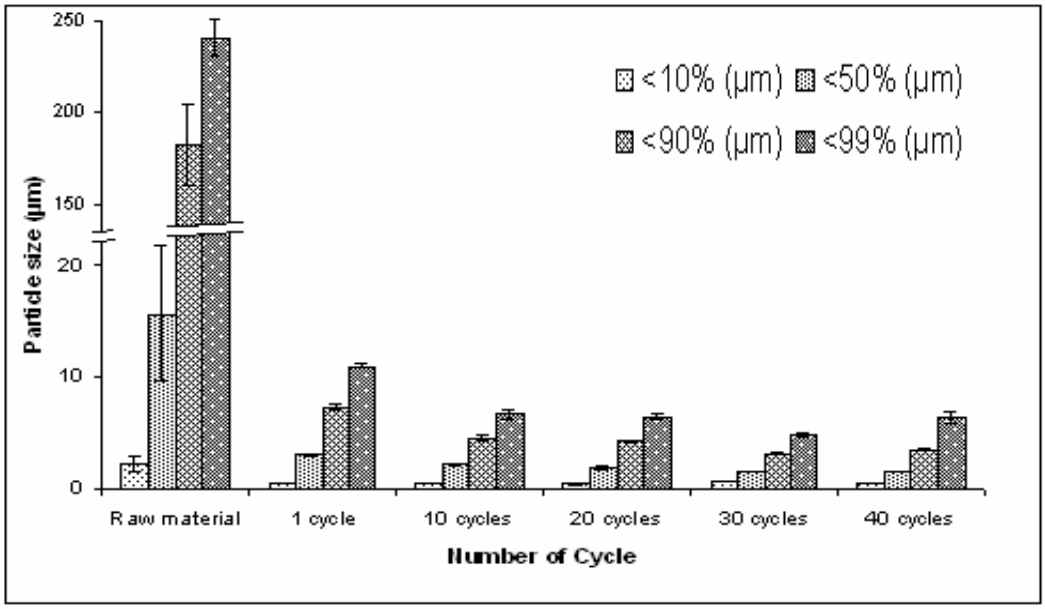
particles sizes achieved after 20 homogenization cycles for the nanosuspensions with 1% drug content were comparable. The results are in agreement with previous research by Möschwitzer *et al.* [158].

Table 4.12 Minimal achievable particle size as a function of the homogenization method (LD and PCS results)

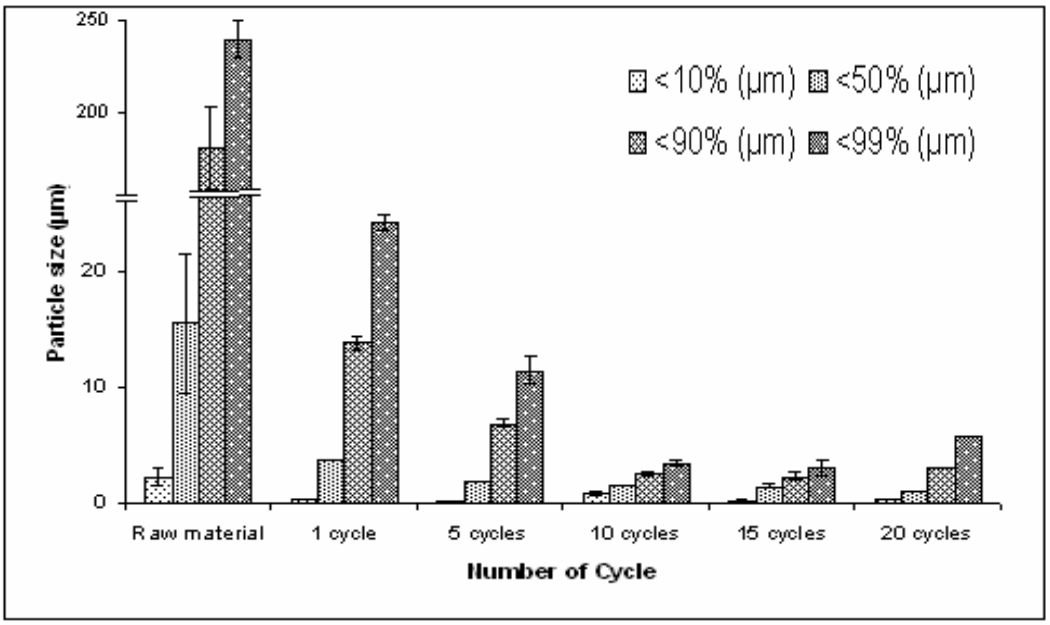
Formulation	Starting materials	Laser diffractometry (µm)			PCS (nm)	
		d 50%	d 95%	d 99%	z-ave	PI
Testo-M[1] (20)	Micronized	1.882	4.179	6.405	1336	0.402
(40)		1.501	3.446	6.345	1100	0.239
Testo-SD I[2]	Spray-drying	1.002	3.037	5.713	643	0.069
Testo-SD II[2]	Spray-drying	1.013	2.589	5.618	780	0.042
Testo-SD III[2]	Spray-drying	1.014	2.010	2.913	645	0.066

1) Standard method (Dissocubes® technology, SkyePharma), micronized starting material, in brackets are homogenization cycles
2) HPH new variant H42, SD = spray-dried testosterone
Composition testosterone : poloxamer 188 are 100 : 0 (I), 90 : 10 (II), 50 : 50 (III)

Fig. 4.10 and **Fig. 4.11** present the particle sizes reduction of testosterone nanosuspension produced with the conventional homogenization and H42 nanosuspensions as a function of homogenization cycles at 1500 bar. Due to the modification of the starting material, the diminution effectiveness could be significantly improved by H42. Considering the result of cycle number 40 for the micronized starting material and the result after the first 20 homogenization cycle for the spray-dried starting material; Testo-SD 1:0, 9:1, and 1:1, the advantages of the new process are clear. As a result of the starting material modification by spray drying - especially in conjunction with the addition of poloxamer - the particle size reduction requires much less energy input. In addition, the minimal achievable particle size is distinctly reduced to half or less. Particularily important is also the reduction of the polydispersity. Polydispersity index (PI) values for all nanosuspensions can be seen in **Table 4.12**.

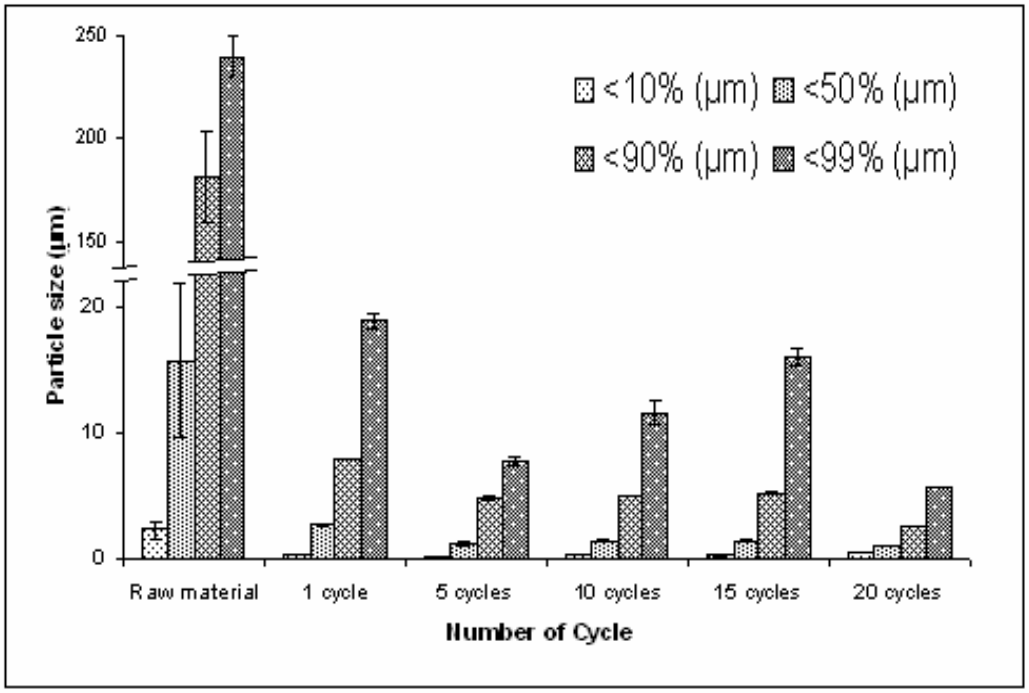


Testo-M

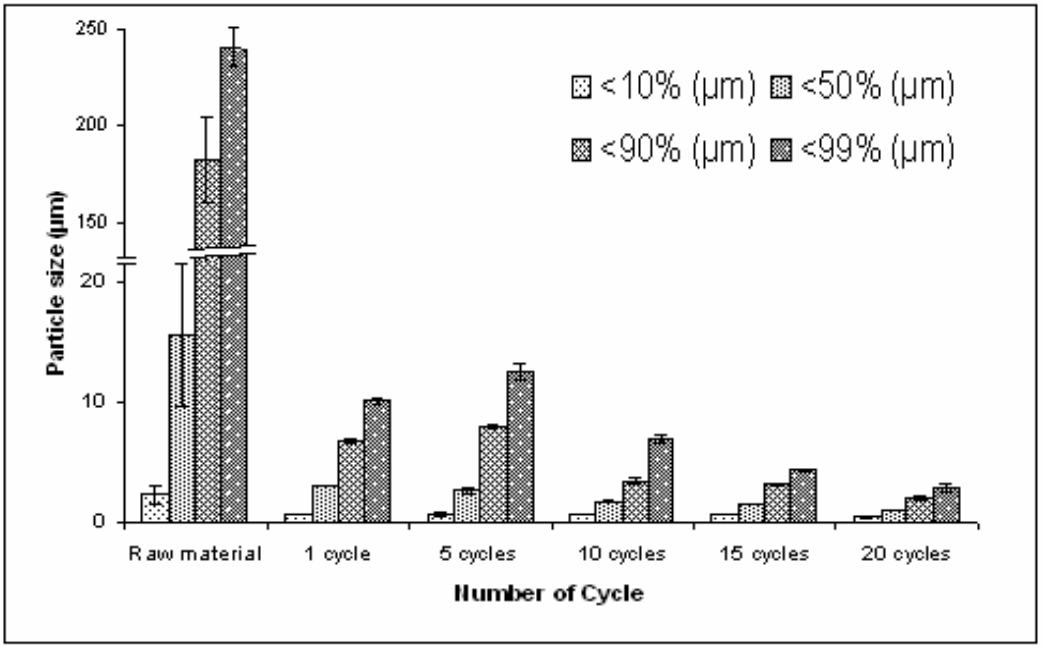


Testo-SD I

Fig. 4.10 Profiles of particle size reduction of testosterone suspension produced by standard HPH using 40 homogenization cycles (Testo-M) and testosterone nansuspension produced by H42 technology using 20 homogenization cycles (Testo-SD I), for comparison the size of the raw material (RW) are given (LD diameter d10% to d99% (µm))



Testo-SD II



Testo-SD III

Fig. 4.11 Profiles of particle size reduction of testosterone nansuspension produced by H42 technology using 20 homogenization cycles (Testo-SD II, Testo-SD III), for comparison the size of the raw material (RW) are given (LD diameter d10% to d99% (µm))

The use of the modified starting material led to homogeneously dispersed nanosuspensions without significant microparticle contamination. In contrast to the results of a previously published study dealing with co-processing of drug and poloxamer by spray drying alone [158], increasing the poloxamer content did not positively affect the particle size reduction.

Yin *et al.* have reported producing nanoparticles by spray drying only [162]. In contrast to Yin *et al.*, the Nanopure®XP variant H42 process involves spray drying and subsequent high pressure homogenization to obtain homogeneously dispersed nanosuspensions.

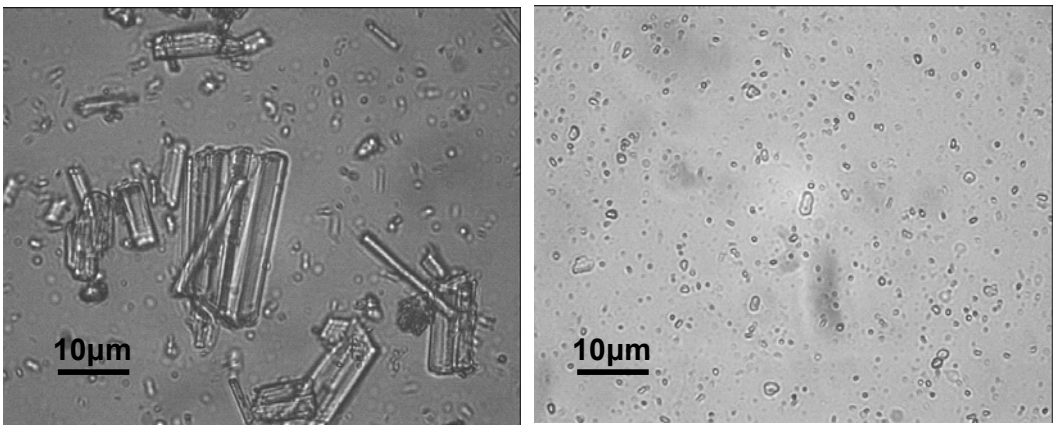
4.3 Morphology of the particles

The purpose of the study was to conduct a visual investigation of crystals in nanosuspensions. The images of the nanosuspensions either from light microscopy or scanning electron microscopy (SEM) can be intensively studied. Information about crystallinity and agglomerated particles could be provided from images of the nanosuspension by polarized light microscopy or scanning electron microscopy (SEM). In addition, particle size reduction can be also observated by these methods.

4.3.1 Polarized Light Microscopy

4.3.1.1 Rutin

The images in **Fig 4.12** show raw material of the rutin (a) and the nanosuspension (b) after homogenization (HPH) with 1000 times magnification. These pictures provide information about the particle size reduction effectiveness of the nanosuspensions by HPH. After high pressure homogenization, the large crystals of rutin could be transformed to fine particles of rutin nanosuspensions. The images also show homogeneous nanosuspensions. The particle distribution of the nanosuspensions was very narrow and the difference in size between large particles and small ones was very small. This data was in agreement with the low polydiversity index (PI) value (below 0.500).



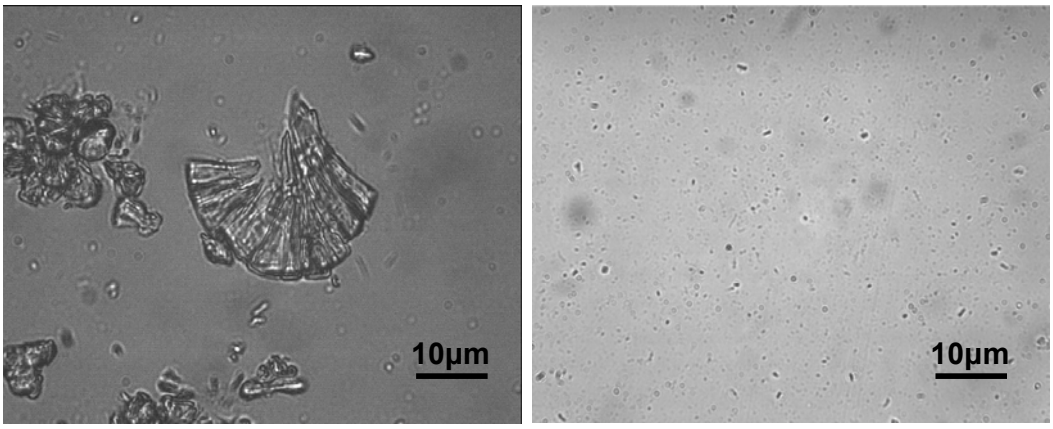
(a) (b)

Fig. 4.12 Light microscopic pictures of rutin: (a) raw materials (b) nanosuspensions B (stabilized by SDS) directly after producted by HPH using 20 homogenization cycles (magnified 1000 times)

Light microscopic pictures show effectiveness of high pressure homogenization (HPH). HPH could distinctly reduce the particle size of the drugs from the micrometer range to nanometer range. Particle size averages of the rutin nanosuspensions by PCS were found to be distinctly reduced to 500-700 nm. The fineness of the rutin nanocrystals could be seen by the light microscopy (**Fig. 4.12**). The most important information was that the particles of the rutin nanosuspension were dispersed mostly as single particles. It is hard to find agglomerated nanocrystals.

4.3.1.2 Hesperidin

Similar to the rutin nanosuspensions, the images of the hesperidin nanosuspensions can be clearly seen under a light microscope at 1000 times magnification (**Fig. 4.13)**. Again, HPH was effective in reducing the particle size of hesperidin. The big crystals of the raw material (a) could be successfully reduced to fine crystals of the hesperidin nanosuspension (b) with particle size in the nanometer range.



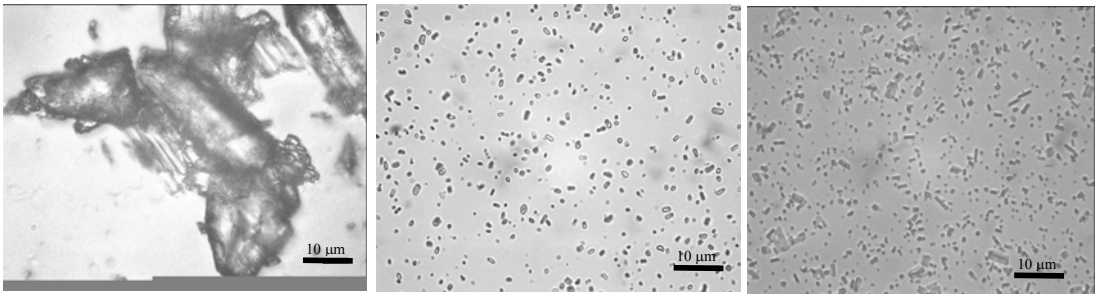
(a) (b)

Figure 4.13 Light microscopic pictures of (a) raw material and (b) heperidin nanosuspension H (stabilized by PVA) directly after produced by HPH using 20 homogenization cycles (magnified 1000 times)

The fine crystals of the hesperidin nanosuspensions were mostly dispersed as single particle. Moreover, there were no aggregated/ agglomerated particles indicating the absence of instability.

4.3.1.3 Ibuprofen

An image of the hard crystalline ibuprofen raw material is presented in **Fig. 4.14**(a). The very hard crystals of ibuprofen raw material (a) were successfully homogenized using the HPH technique. The fine crystalline ibuprofen nano-suspensions were homogenously distributed as single particles (b).



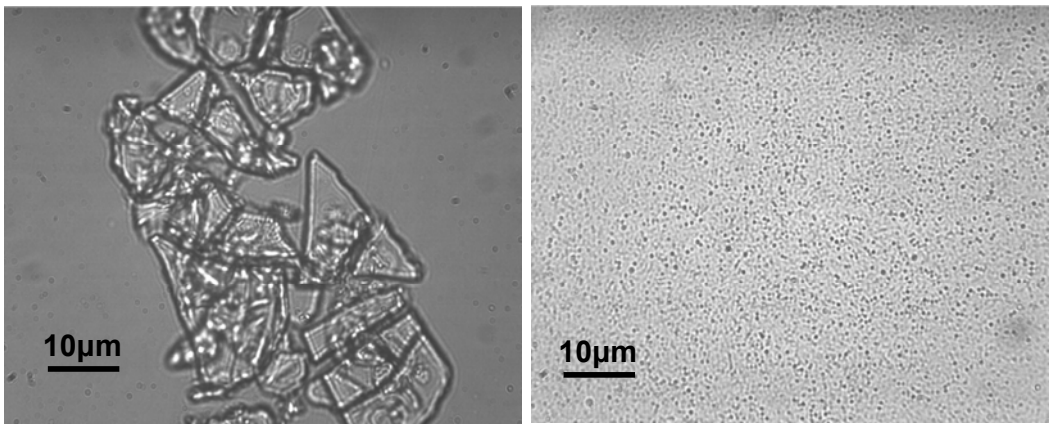
(a) (b) (c)

Fig. 4.14 Light microscopic pictures of ibuprofen: (a) raw materials (b) ibuprofen nanosuspension 9 (stabilized by SDS) after produced by HPH using 40 homogenization cycles at 5°C and (c) after producted by BM within 40 minutes at 5°C (magnified 1000 times)

Furthermore, no agglomerated/aggregated particles were found and the resulting nanosuspensions tended to be stable. The very low PI value was also in agreement with the homogenous nanosuspensions.

In contrast, the ibuprofen nanosuspensions produced by BM was clearly agglomerated and also particle size distribution was visibly found to be extremely broad (**Fig. 4.14**, C). This means that the ball milling is ineffective for generating an ibuprofen nanosuspension.

4.3.1.4 Coenzyme Q10

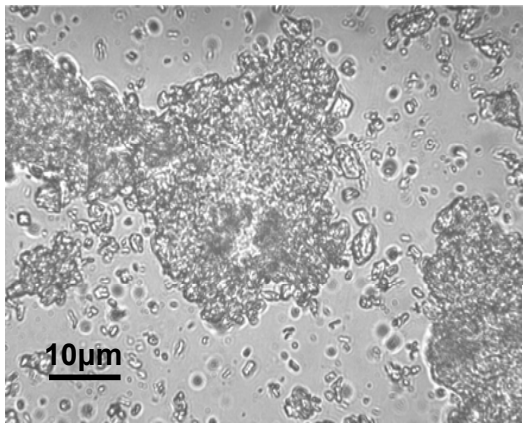


(a) (b)

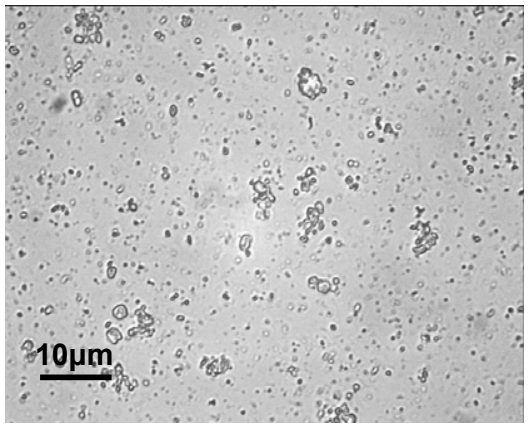
Fig. 4. 15 Light microscopic pictures of coenzyme Q10: (a) raw materials (b) coenzyme Q10 nanosuspension J (stabilized by PVA) after producted by HPH using 20 homogenization cycles at 10^{o}C (magnified 1000 times)

In general, the effectiveness of the high pressure homogenization (HPH) can be confirmed by light microscopy. This means that particles in the nanometer range could be generated by HPH even the original material consists of hard crystalline microparticles. In the particular case of coenzyme Q10 nanosuspensions, the coenzyme Q10 particle size was clearly reduced from microparticles to nanoparticles. Mean particles size of the CoQ10 nanosuspensions by PCS was about 200 nm. The fineness of the nanosuspensions rendered them almost invisible to light microscopy (**Fig. 4.15**). In addition, aggregated/agglomerated particles were hardly found for sure and almost all particles were distributed as single particles. The fineness of the nanosuspensions leads to better physicochemical properties such as increased surface area and consequently improved dissolution behavior.

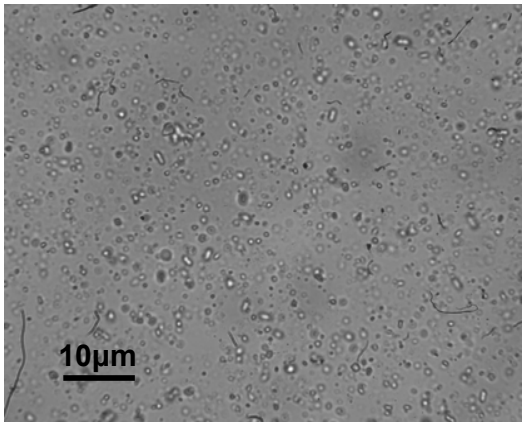
4.3.1.5 Testosterone



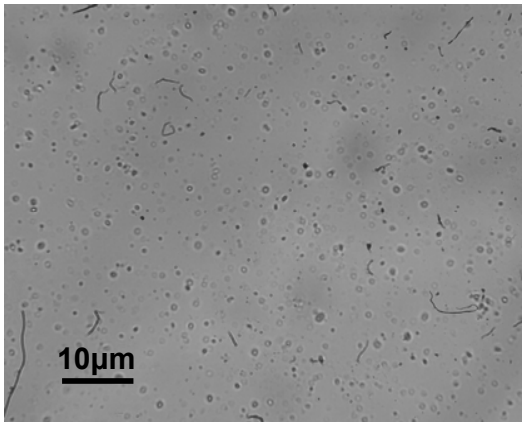
Raw Material



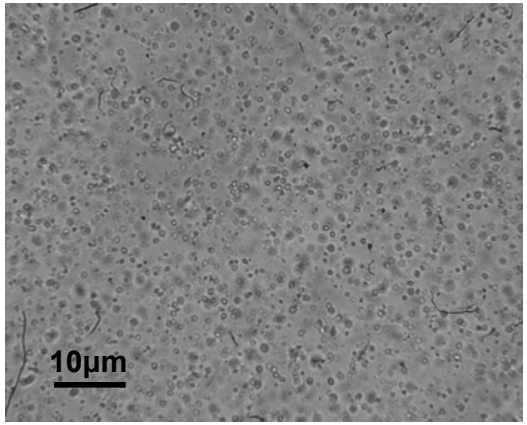
Testo-M



Testo-SD I



Testo-SD II



Testo-SD III

Fig. 4.16 Light microscopic pictures of testosterone raw materials, after produced by standard HPH using 40 homogenization cycles (Testo-M, magnified 1000 times) and after produced by H42 technology using 20 homogenization cycles (Testo-SD I, II, III, magnified 1000 times)

The effectiveness of the Nanopure variant H42 method is clearly visible in light microscopy. This method distinctly reduced testosterone particle size from the micrometer range (raw material) to submicron particulates (nanometer range) such as Testo-SD I, II, and III (**Fig. 4.16**). The particle sizes of testosterone nanosuspensions were effectively transformed from micrometer to nanometer. Using PCS, particle size average of the nanosuspensions was distinctly reduced to about 600 nm. In addition, aggregated/agglomerated particles were not found and almost all of the particles were definitely distributed as single particles [155]. In contrast, the standard HPH was ineffective in producing a testosterone nanosuspension. The resulting suspension was contaminated by numerous large particles. The image of the large crystals is easily visible in **Fig. 4.16** (Testo-M).

4.3.2 Scanning Electron Microscopy (SEM)

4.3.2.1 Rutin

Scanning electron microscopy was employed to determine the nanocrystal shape and size after high pressure homogenization. **Fig. 4.17** shows the rutin raw material. Reduction of the rutin particle size is clearly visible in **Fig. 4.18.**

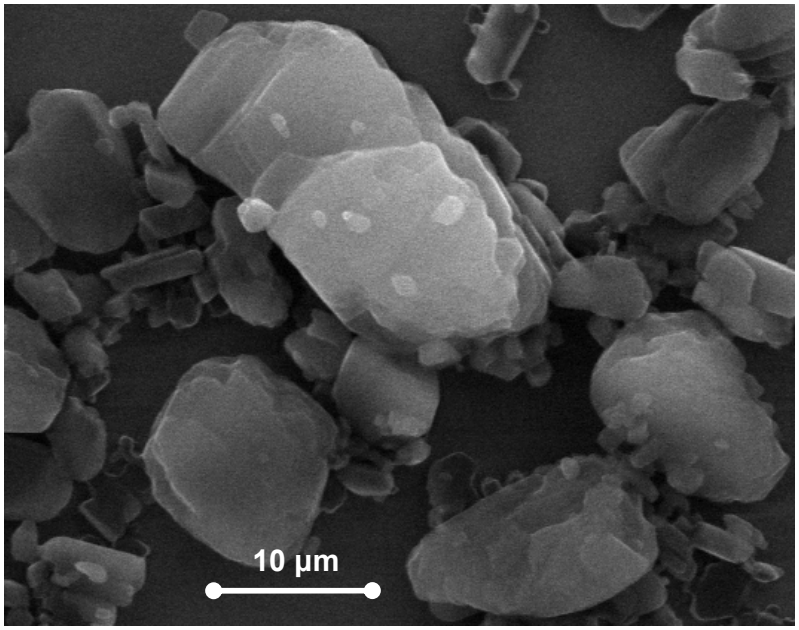


Fig. 4.17 Electron microscopic image of rutin raw material.

This picture shows very fine crystals in the rutin nanosuspension compared to large crystals of the raw material (**Fig. 4.17**). Some of the raw material crystals were bigger than 10 µm. In contrast, almost all particles in the rutin nanosuspensions (**Fig. 4.18**) were below 1 µm. The electron microscopic photograph of the rutin nanosuspension also shows that the nanocrystals possessed a cuboid shape. This shape could be preferentially formed by the crystallization at two opposite sides of the cubes.

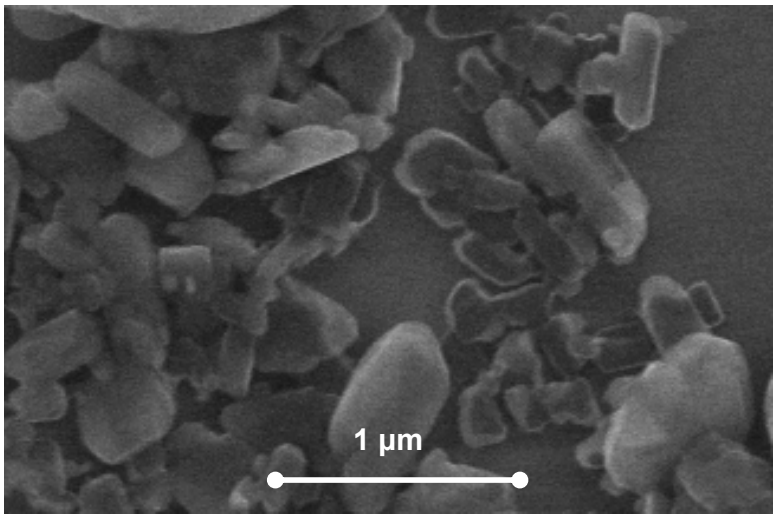


Fig. 4.18 Electron microscopic image of rutin nanosuspension B (stabilized by SDS) after produced by HPH using 20 homogenization cycles

The cubic shape of nanosuspensions lead to the selection of the trade name DissoCubes (Skye Pharma PLC) [56, 57]. The homogeneity of the size is an essential prerequisite for long-term stability, which means avoiding Ostwald ripening (**Fig. 4.19**) and crystal growth as mentioned before.

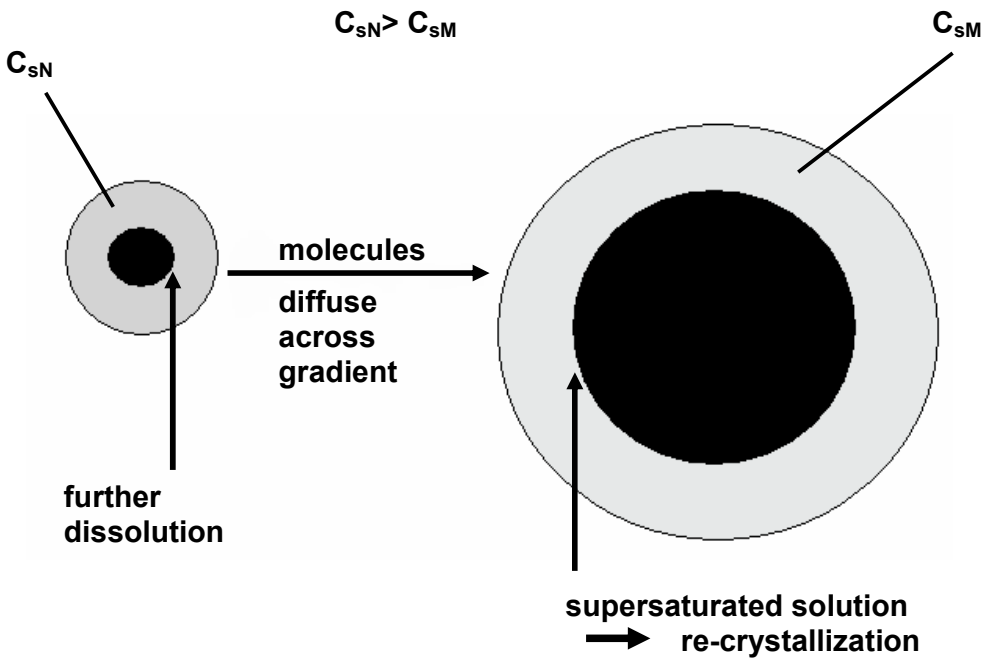


Fig. 4.19 Principle of Ostwald ripening and crystals growth (after [57])

High pressure homogenization can be used to produce rutin nanosuspensions. The degree of particles fineness in the nanosuspension was found to increase with production pressure and number of cycles. A high quality of rutin nanosuspension, in this case homogeneous nanocrystals with a low fraction of microcrystals, was obtained using the optimized formulations. According to the Ostwald-Freundlich and the Kelvin-Gibbs equations, the saturation solubility of the nanosuspensions can be distinctly increased compared to microsuspensions. The increase in saturation solubility, dissolution rate and intrinsic dissolution rate will accelerate drug dissolution after i.v. administration and also enhance absorption from the GIT after oral administration - especially when considering the adhesiveness of nanoparticles to the gut wall. Increased absorption in the GIT will consequently increase the oral bioavailability of drugs [75].

4.3.2.2 Hesperidin

In addition to particle size and shape of the rutin nanosuspension, electron microscopy was also employed to investigate the particle size and shape of the hesperidin nanosuspensions. The result is shown in **Fig. 4.20** and **4.21**.

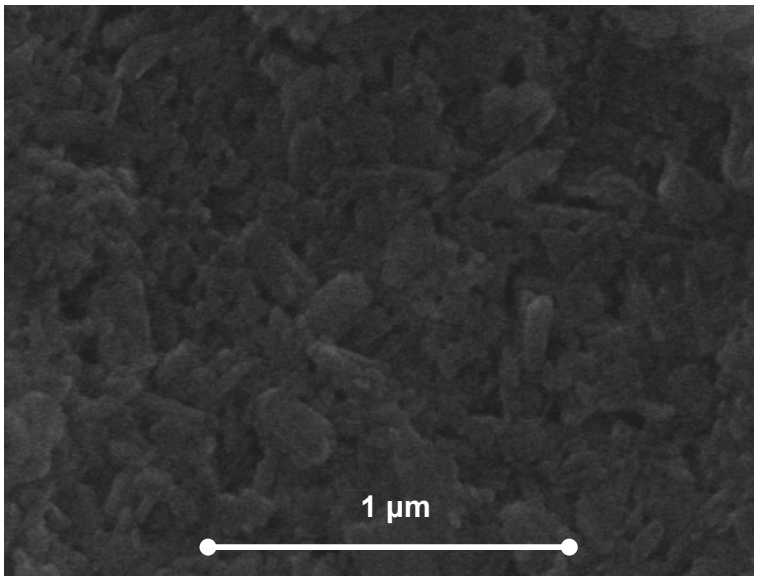


Fig. 4.20 Electron microscopic image of hesperidin nanosuspension H (stabilized by PVA) after produced by HPH using 20 homogenization cycles

Mean particle size of the hesperidin nanosuspensions by PCS was about 300 nm smaller than for rutin nanosuspensions, that means around 700 nm. The electron microscopic pictures of the hesperidin nanosuspensions show the fineness of the hesperidin nanosuspension particles. This picture not only shows the size of the nanocrystals (particle about 300 nm) but also homogeneity of the hesperidin nanosuspensions. As revealed in **Fig 4.20** and **4.21**, the particles of the hesperidin nanosuspensions were almost distributed uniformly. In addition, the electron microscopic photographs of the hesperidin nanosuspensions also show that the nanocrystals are of cuboid shape. Again this shape could be preferentially formed by the crystallization at two opposite sides of the cubes.

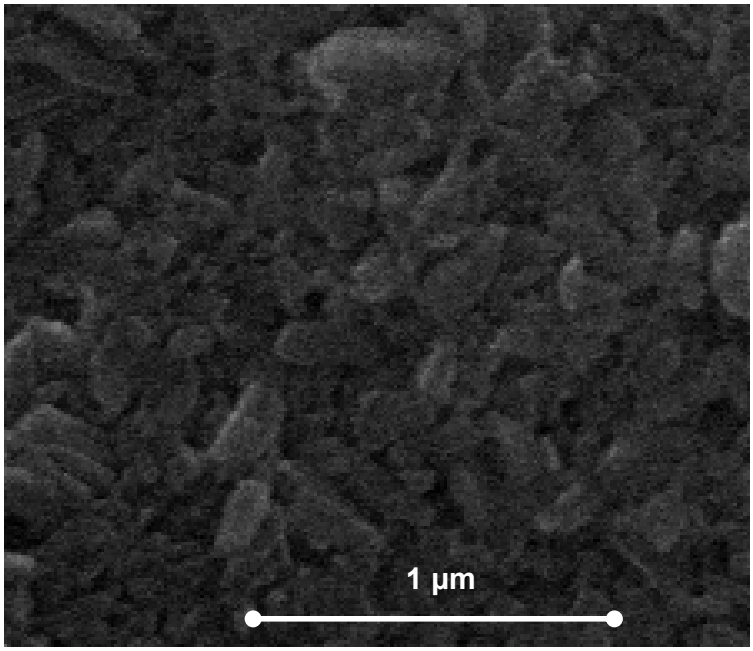


Fig. 4.21 Electron microscopic image of hesperidin nanosuspension G (stabilized by Tween 80) after produced by HPH using 20 homogenization cycles

Unlike the rutin nanosuspensions, the particle shape of the hesperidin nanosuspesions was clearly finer and smoother. However, the polydisperse index (PI) value of the hesperidin nanosuspensions was below 0.500. The polydisperse index data are in agreement with the homogeneous of hesperidin nanosuspensions, which can clearly be seen in the electron microscopic pictures.

4.4 Evaluation of physical stability

4.4.1 Rutin

Two formulations of the rutin nanosuspensions (B and C) have shown sufficient physical stability for long-term storage at room temperature. Increases in size were almost indetectable over a monitoring period of 1 year.

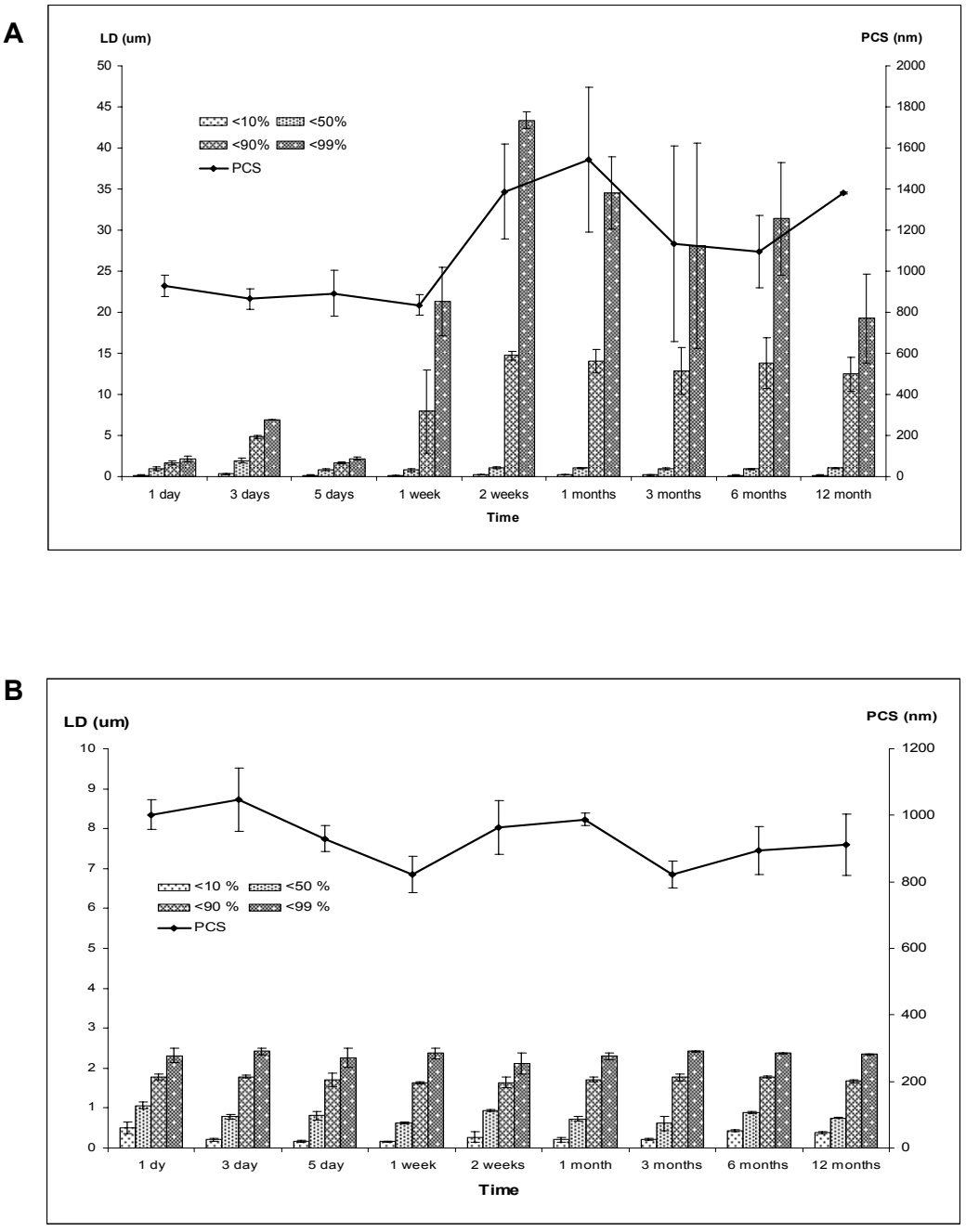
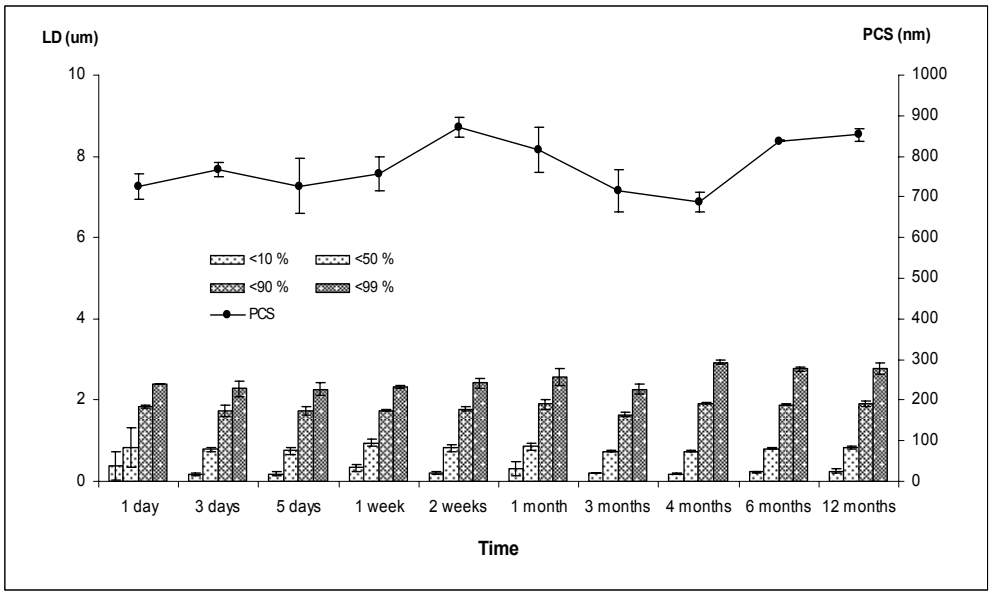


Fig. 4. 22 Physical stability profiles of rutin nanosuspensions with Poloxamer 188 (A) and SDS (B) as stabilizer over 12 months

C



D

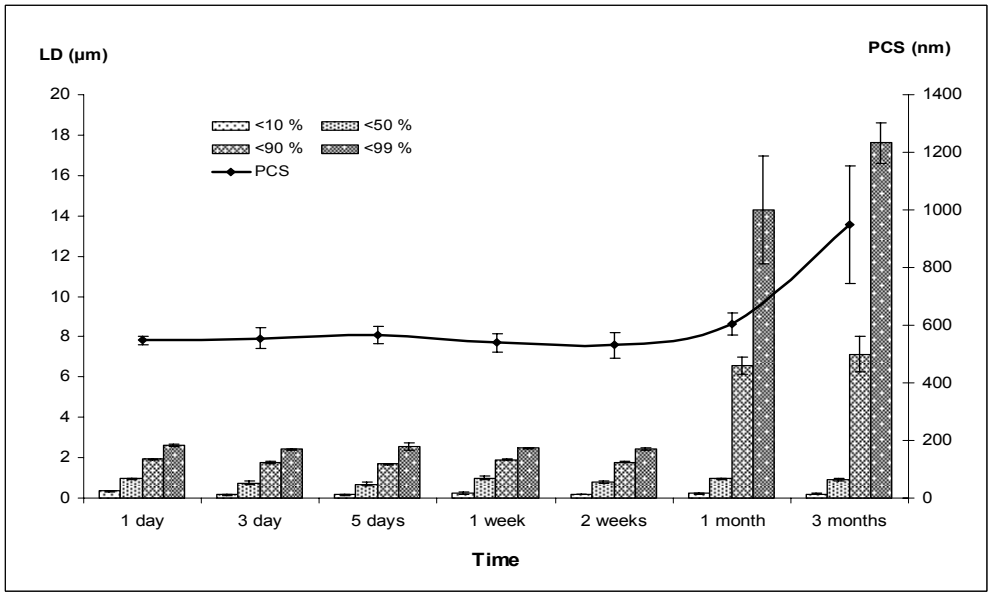


Fig. 4. 23 Physical stability profiles of rutin nanosuspensions with Poloxamer Tween 80 (C), and PVA (D) as stabilizer over 12 months

Fig. 4.22 and **Fig 4.23** show the results of the long term stability test. Formulations stabilized by SDS and Tween 80 were found to be more suitable

to achieve a stable nanosuspension (formulation B and C) compared to formulation A and D stabilized by poloxamer 188 and PVA respectively. Over the 12 months, 99% of the particles underwent only a slight increase in diameter. Possible reasons for this include crystal growth or alternatively particle aggregation. However, light microscopic pictures proved the absence of the large crystals (**Fig. 4.24)**. An increase in particle size can reasonably be caused by aggregation of particles. The pictures also show that increased particle size was hardly detectable over a monitoring period of 1 year. Meaning that the rutin nanocrystals were stable for over 1 year. In addition, these pictures show tiny crystals and the rutin nanocrystals were almost completely distributed as single crystals.

The increased particle size of the formulations A and D was not caused by crystal growth but by a tendency of agglomeration/aggregation of the particles. The size increases monitored during this long term-storage of course indicates that these nanosuspensions will not go to a next step of an investigation. Meanwhile the stable rutin nanosuspensions (formulation B and C) were intensively used for the next processing steps. Formulation B was selected for further research since this formulation using SDS as stabilizer appeared better suitable for an oral dosage form compared to Tween 80. For oral administration Tween 80 has a bitter taste. Therefore it is not convenient for patients to take an orally rutin in an oral dosage form, such as tablet, capsule or especially suspension. In addition, the rutin nanosuspenions will be lyophilized using freeze dryer to get rutin powder nanoscrystals. During lyophilization, Tween 80 can cause a tendency to form agglomerated/ aggregated particles. The rutin nanosuspensions stabilized by Tween 80 is more suitable when added to cream or gel for topical dosage form.

Moreover, the particle size of stable rutin nanosuspension (formulation B) was microscopically analyzed (**Fig. 4.24**). This nanosuspension showed a PCS size average of 727 nm and a PI of 0.265 at day 0. The LD volume size distribution showed that 50% (d50%) of the particles were smaller than 0.840 µm. The nanosuspensions was stable and after 12 months the mean particle size of the nanosuspensions by PCS was 854 nm and the d50% diameter

0.836 µm (**Fig. 4.22 and 4.23**). Data from the particle size analysis could be confirmed by light microscopic pictures (**Fig. 4.24**).

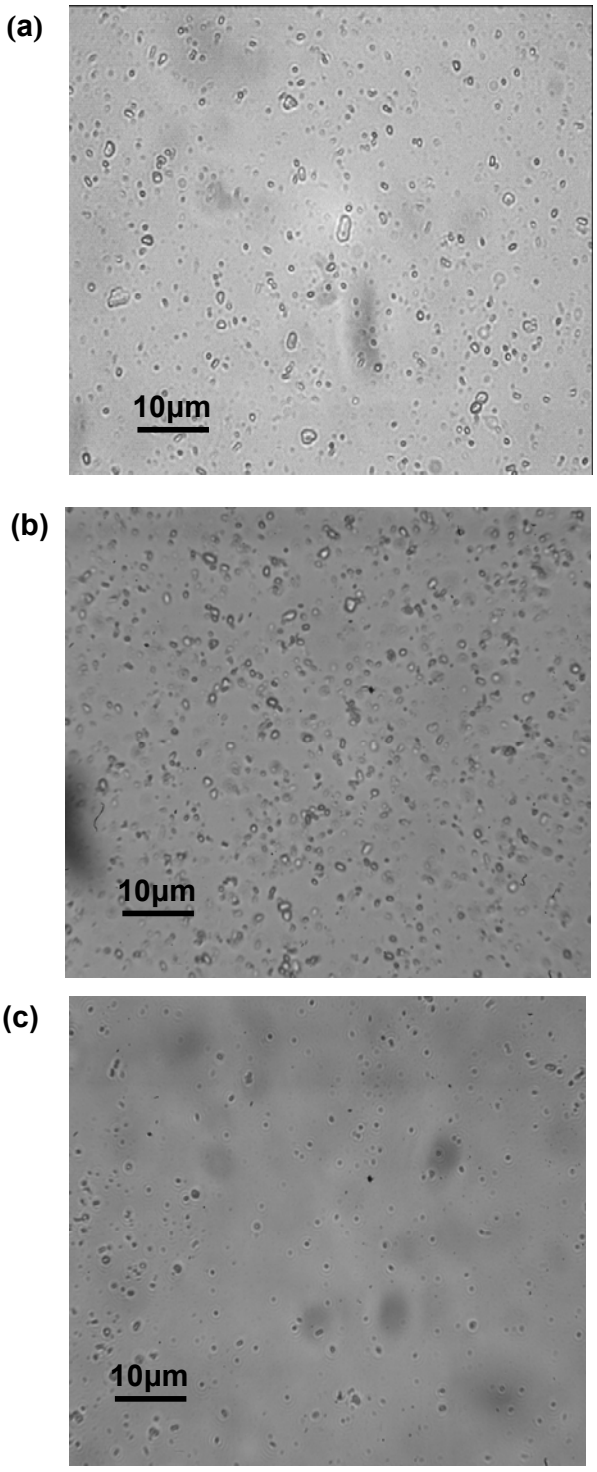


Fig 4.24 Light microscopic pictures of rutin nanosuspension stabilized by SDS: (a) after production (b) 3 months (c) 12 months storage at room temperature (magnified 1000 times)

4.4.2 Hesperidin

Physically stability of all formulations of the hesperidin nanosuspensions was also evaluated over a period of 1 year. At certain times, particle sizes of samples were measured to evaluate potential particle size growth.

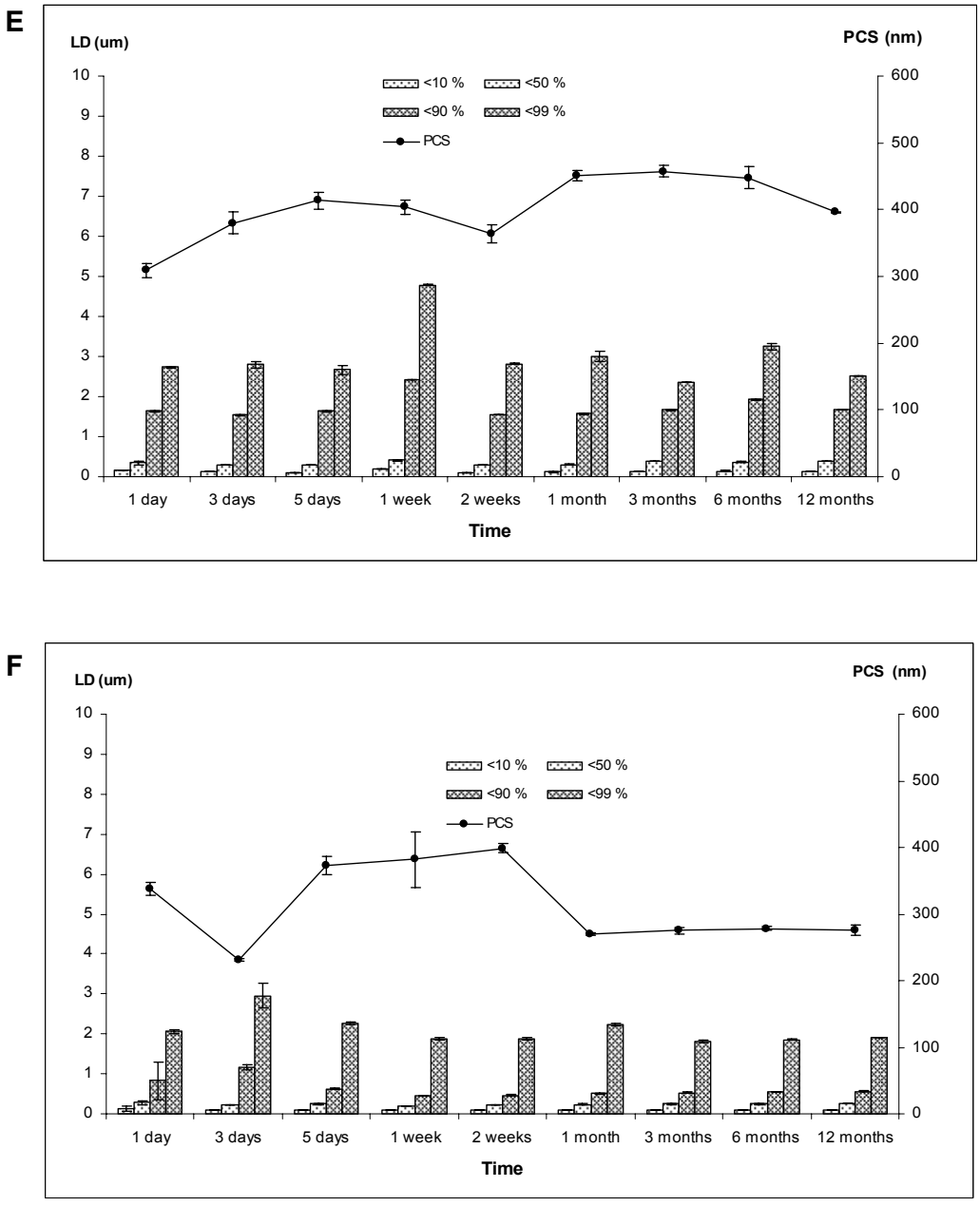


Fig. 4.25 Physical stability profiles of hesperidin nanosuspensions stabilized by Poloxamer 188 (E) and SDS (F)

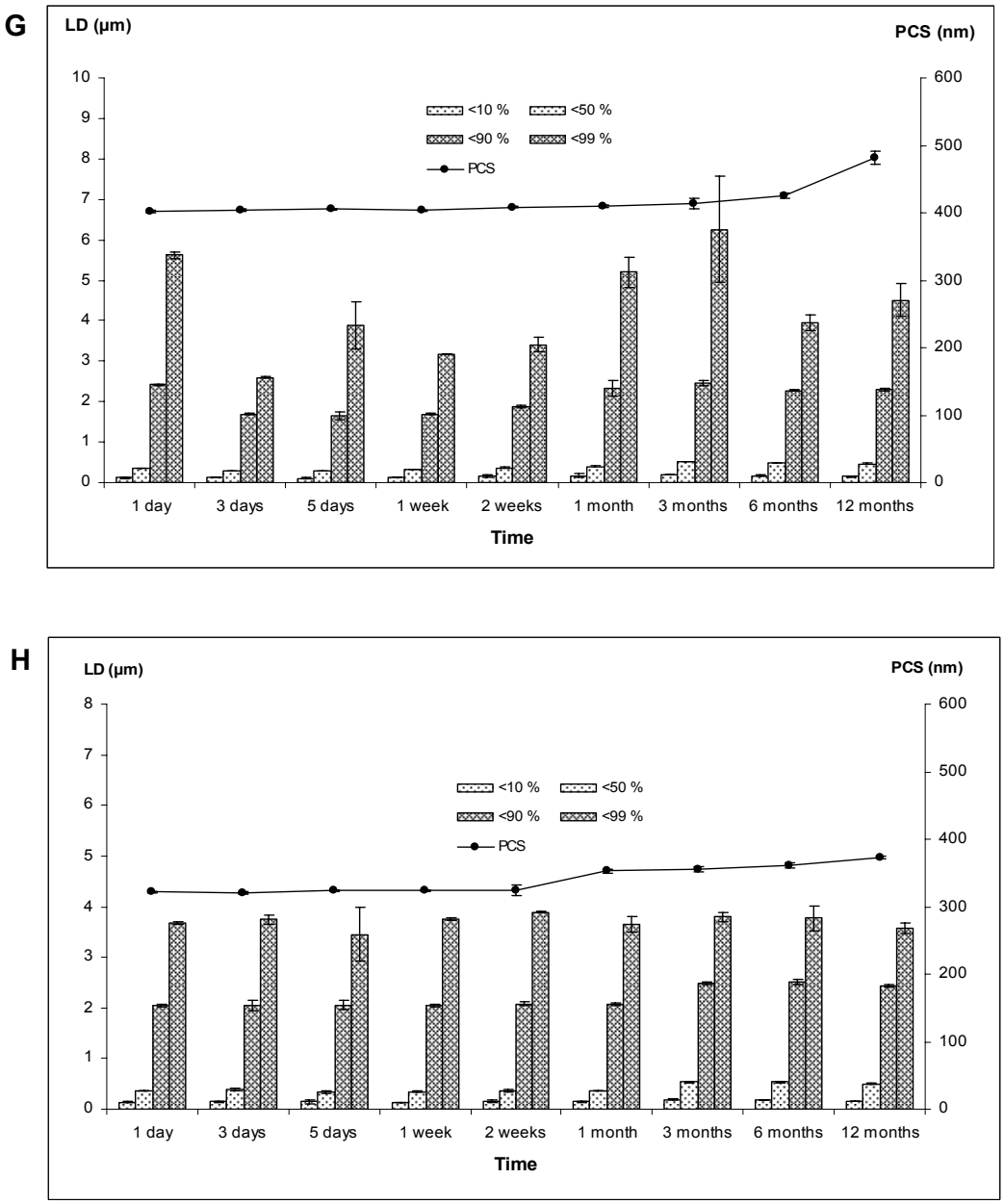


Fig. 4.26 Physical stability profiles of hesperidin nanosuspensions stabilized by Tween 80 (G) and PVA (H

All of the hesperidin nanosuspensions were relatively stable, but the hesperidin nanosuspensions stabilized by polyvinyl alcohol (PVA) was the best one. With a starting PCS particle size average of 309 nm, this nanosuspensions only very slight increased in particle size to 373 nm within 1 year. LD particle size distribution of this nanosuspensions was little different

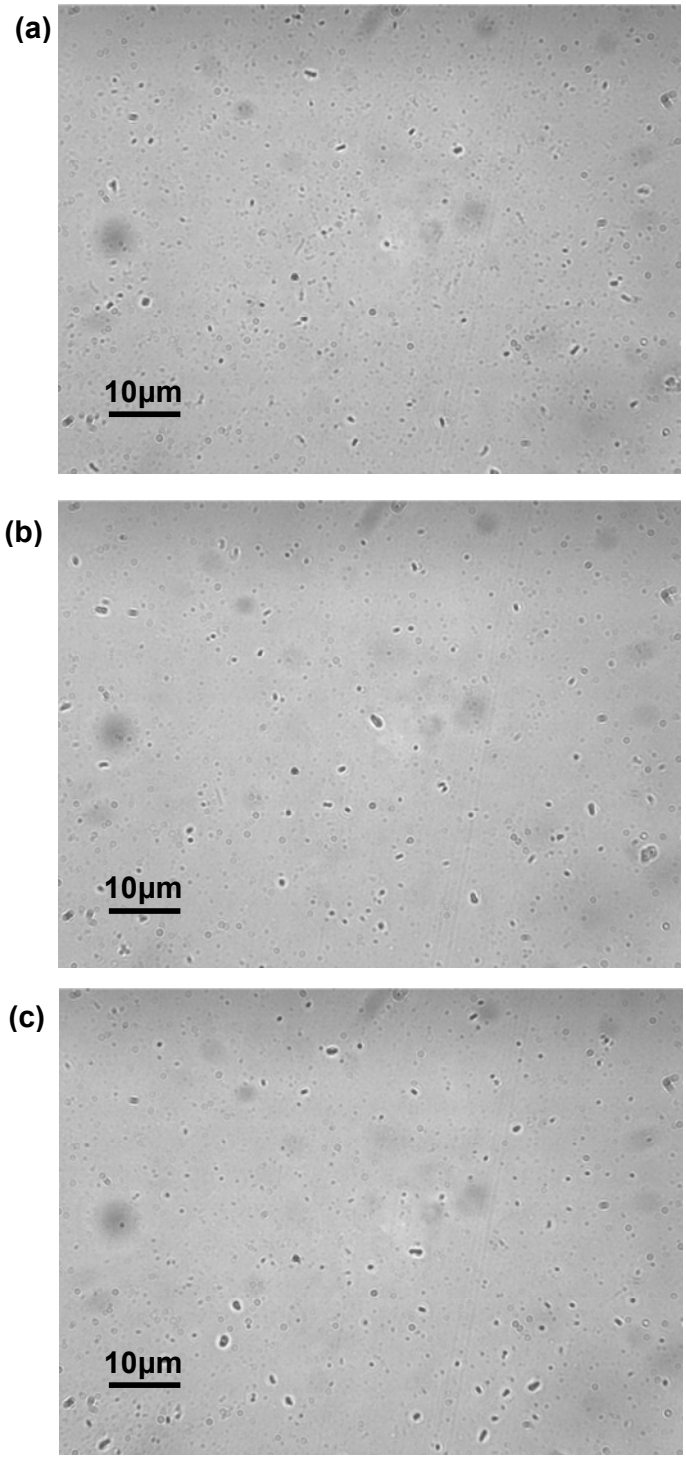


Fig. 4.27 Light microscopic pictures of the hesdperidin nanosuspensions stabilized by PVA: (a) after production (b) 3 months (c) 12 months storage in the fridge (magnified 1000 times)

from that on production day (day 0). D50% was below 0.5 µm on production day, and after 1 year the particles size distribution had little changed. According to the PI (polydispersity index) value of the hesperidin nanosuspensions below 0.5, the hesperidin nanosuspenions was relatively homogeneous. The PI value is in agreement with the stability of the nanosuspenions.

Fig. 4.27 shows light microscopic pictures of the hesperidin nanosuspension stabilized by PVA on production day, after 3 months and 12 months storage time. These pictures prove that almost all of the hesperidin nanoparticles remined as single particles. Hardly any were found to be agglomerated or aggregated. No particle growth was observed as a result of an Oswald ripening effect. The particles of the hesperidin nanosuspensions were relatively small and singular. This indicates that PVA is a very good stabilizer for hesperidin nanosuspensions. In addition, PVA as polymer can also inhibit crystalline growth of hesperidin nanosuspensions and prevent the Ostwald ripening effect [163-165].

4.4.3 Ibuprofen

Physical stability of the ibuprofen nanosuspensions was intensively evaluated over 1 month period. In this case, formulation 9 of the ibuprofen nanosuspension was chosen to study the physically stability because the particle size of this formulation was in the nanometer range. **Fig. 4.28** shows the particle size distribution of the ibuprofen nanosuspensions over 30 days. According to this data, the ibuprofen nanosuspension could retain the particle size distribution in the nanometer range within 14 days. After that the particle size began to increase and within 30 days the particle size of the ibuprofen nanosuspensions had grown rapidly to big particles. The particle growth was due to agglomerated or aggregated particles and also due to the Ostwald ripening phenomenon. The particle size of the ibuprofen nanosuspension after production was in the upper of the nanometer range. For this reason it was not easy to keep the ibuprofen nanosuspenion in the nanometer range due to contamination by micorparticles in the nanosuspension. The microparticles promote the Ostwald ripening phenomenon and can cause particles growth to big particles.

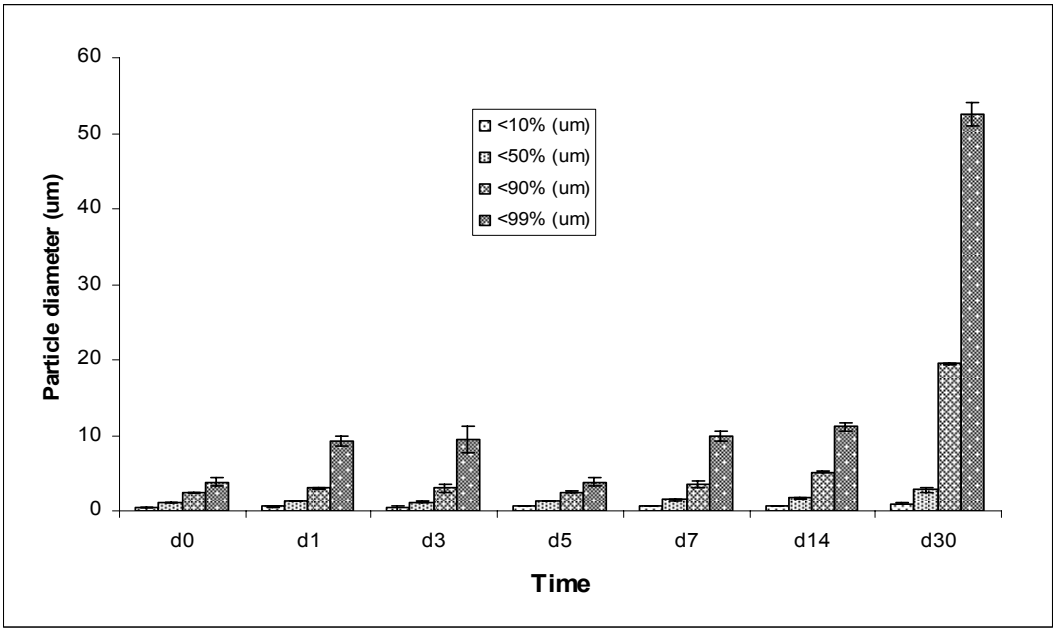


Fig. 4.28 Physical stability profile of the ibuprofen nanosuspensions stabilized by SDS within 30 days

The next step in characterization was investigation of the nanosuspension by light microscopy. **Fig. 4.29** shows that after production, the ibuprofen nanosuspensions were clearly distributed as fine, single particles (a). Over 14 days, the fine particles of ibuprofen grew into larger particles. These pictures clearly depict the growth of the ibuprofen nanocrystals into big particles due to Ostwald ripening phenomenon (**Fig 4.29 b**).

Within 30 days the ibuprofen particles underwent a pronounced changed to large single crystals but also agglomeratedcrystals. Light microscopic pictures were well in agreement with LD data. According to these pictures, agglomeration and Ostwald ripening phenomena explained the instability of the ibuprofen nanosuspensions within 30 days (**Fig 4.29 c**).

To circumvent the instability of ibuprofen nanosuspensions, the nanosuspensions drying process should be directly performed after production. Dried ibuprofen nanocrystals were more stable than ibuprofen nanosuspensions. The investigation could be taken further by incorporating dried ibuprofen nanocrystals into solid state dosage forms, i.e. pellets, tablets, capsules and effervescent tablets.

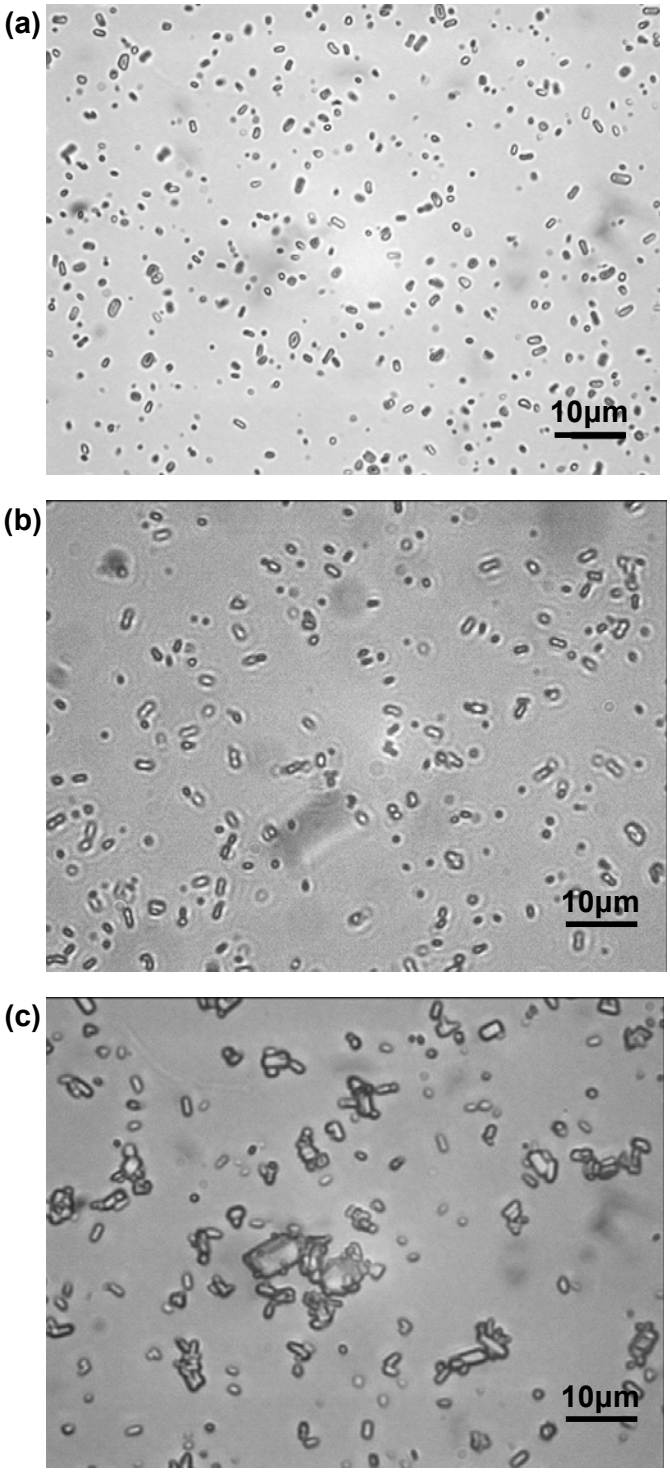


Fig. 4.29 Light microscopic pictures of ibuprofen nanosuspension stabilized by SDS: (a) after production (b) 14 day (c) 30 day storage in RT (magnified 1000 times)

4.4.4 Coenzyme Q10

Both formulations of coenzyme Q10 (Tween 80 and PVA) were evaluated for physical stability at room temperature over a 6 month period. In general, both formulations of the CoQ10 nanosuspensions were stable over period of 6 months and did not show any significant growth in particles. However, particle distribution in formulation I (stabilized by Tween 80) fluctuated as shown at the third month. At this time, 99% (d99) of particle distribution was almost 1 µm higher than others and decreased again after 6 months of the storage time to about 0.6 µm. A definite explanation for this cannot be given. It should be noted that PCS data did not increase at 3 month, they were within their normal range. In contrast, formulation J was more stable and d99% was always below 0.6 µm within 6 months.

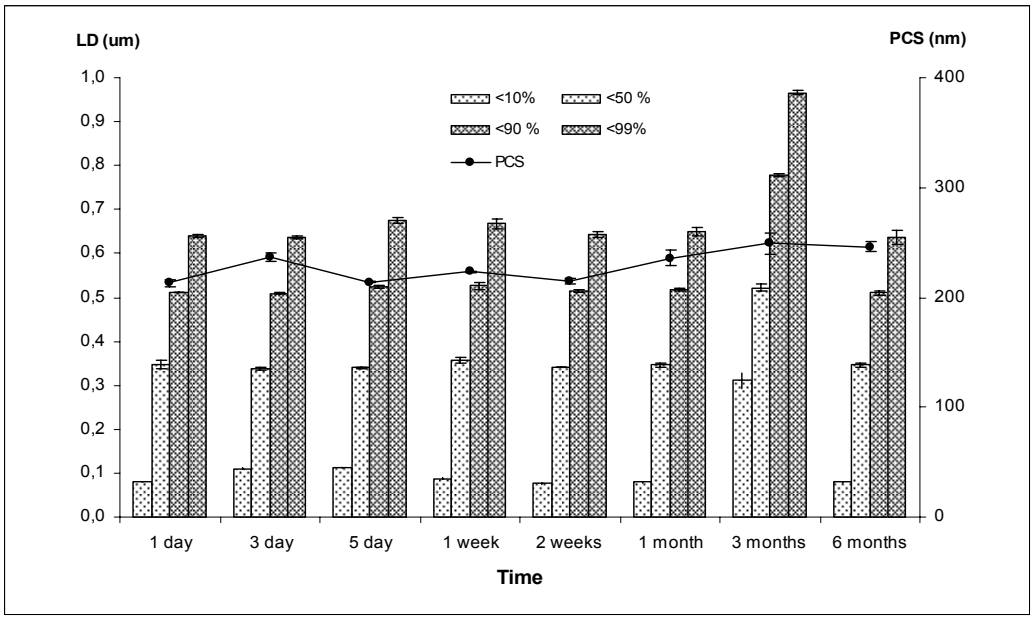


Fig. 4.30 Physical stability of the CoQ 10 nanosuspensions stabilized by Tween 80 over a 6 month period

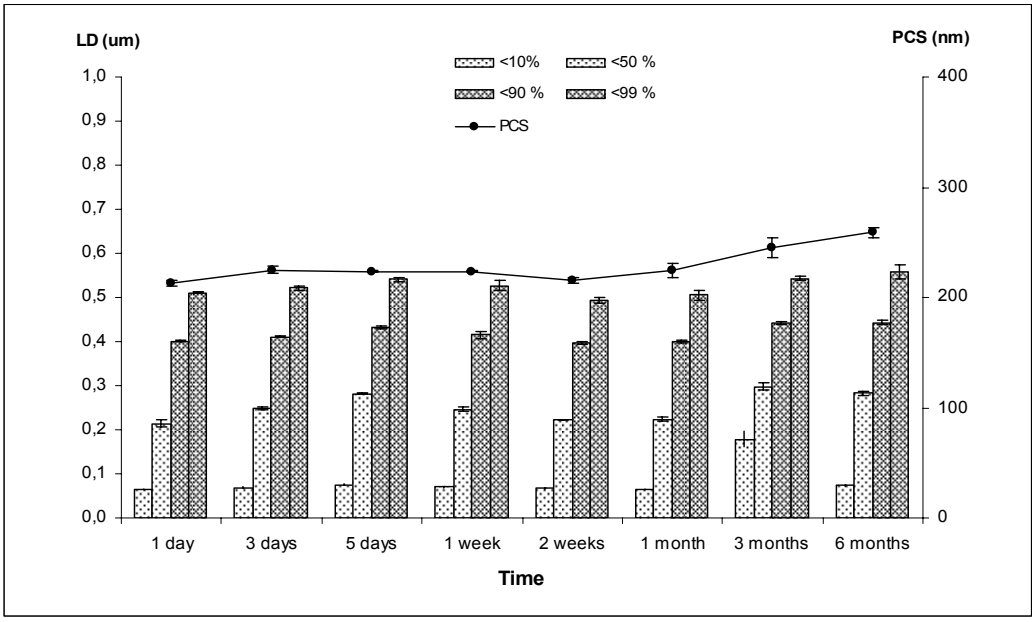


Fig. 4.31 Physical stability of the CoQ 10 nanosuspensions stabilized by PVA over a 6 month period

The mean particle size of nanosuspensions J by PCS was to 259 nm and the d50% diameter to 0.283 µm after 6 months (**Fig. 4.31**)and like the particle size average and distribution after production (the particles size average at 219 nm and d50% at 0.214 µm). Therefore, formulation J was selected for the next step of the investigation.

4.4.5 Testosterone

Reduction of particle size increases the surface area of the particles which is associated with an increased free energy. Very fine particles tend to decrease this energy as result of agglomeration caused by the van der Waals force or of crystal growth caused by Ostwald ripening (crystalline growth). On the other hand, very fine particles increase saturation solubility according to the Kelvin-Gibbs equation and Ostwald-Freundlich equation [57, 74, 75].

Table 4.13 Particle size analyses by Laser Difractometry and Photon Correlation Spectrophotometry (PCS) of testosterone nanosuspensions, with composition testosterone : poloxamer 188 are 100 : 0 (Testo-SD I), 90 : 10 (Testo-SD II), 50 : 50 (Testo-SD III)

Formulation	Laser Difractometry (µm)				PCS (nm)		P I	
	0 day		1 year		0 day	1 year	0 day	1 year
	d50	d99	d50	d99	Z -ave	Z -ave	mean	mean
Testo-SDI	1.002	5.713	0.791	2.528	643	632	0.069	0.090
Testo-SD II	1.013	5.618	0.767	2.510	779	793	0.042	0.314
Testo-SD III	1.014	2.913	0.708	2.583	645	616	0.066	0.219

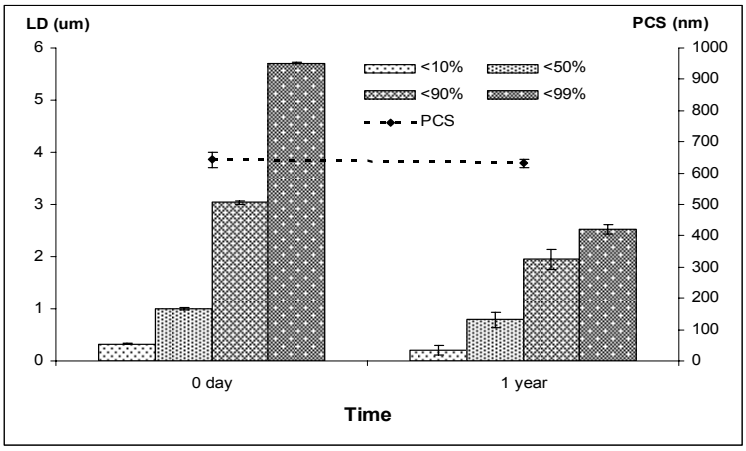


Fig. 4.32 Physical stability of Testosterone nanosuspensions (Testo-SD I) over 1 year

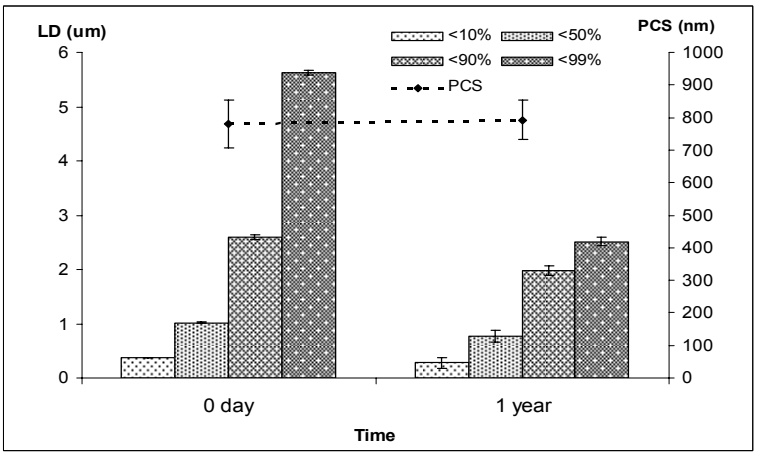


Fig. 4.33 Physical stability of Testosterone nanosuspensions (Testo SD II) over 1 year

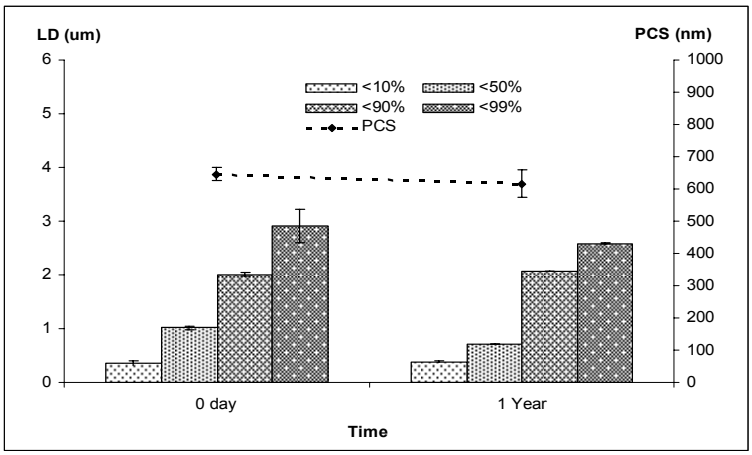


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Fig. 4.34 Physical stability of Testosterone nanosuspensions (Testo-SD III) over 1 year

Surprisingly, a decrease in the LD particle size data of testosterone nanosuspensions was observed after 1 year. The PCS diameter remained unchanged. This phenomenon is not attributable to dissolving testosterone in agreement with the Kelvin-Gibbs and Ostwald-Freundlich equations. Poloxamer 188 added as stabilizer could be a third acting component in the system e.g. promoting de-aggregation due to its sterical stabilizing ability and increasing saturation solubility of drugs [166, 167].

4.5 Evaluation of chemical stability

4.5.1 Rutin

The nanosuspensions produced via high pressure homogenization not only could provide fine crystals, but also protect the drugs from chemically degradation. Stabilizers such as surfactants and long chain polymers have an important role not only in overcoming agglomeration of particles, but also in protecting drugs from chemical degradation. The molecules of the surface stabilizer are able to shield the chemical compound. In other words, the crystalline structure in a nanoparticulate sized formulation results in improved drug stability [168, 169].

The rutin nanosuspensions have shown an excellent chemical stability. Stored at room temperature, the rutin nanosuspensions showed no significant chemical degradation and were stable over the 12 month monitoring period

Tabel 4.14 Chemical stability of rutin nanosuspensions stabilized by Poloxamer 188 (A) and SDS (B), Tween 80 (C) and PVA (D)

Formulation	Stability over time (months)			
	0	**3**	**6**	**12**
A	100%	103.64%	99.76%	96.60%
B	100%	103.05%	97.86%	99.09%
C	100%	102.03%	97.57%	100.40%
D	100%	104.18%	99.78%	96.03%

(**Table 4.14** and **Fig. 4.35**). It could therefore be proven that chemical stabilization of drugs could be achieved by formulating nanosuspensions stabilized by different surfactants or long chain polymers.

In an investigation of chemical degradation of a rutin aqueous solution due to light exposure, Nadja Buchner *et al.* [170] explained that during a thermal treatment of a rutin, degradation of this substance was detected. In addition, the amount of decay decreased when pH was adjusted to 5, indicating a higher stability of flavonoids in an acidic medium. A comparison between the degradation of the model substances in the presence and absence of oxygen illustrated that the reaction rate was affected. The presence of oxygen accelerated the decay of rutin. Hence it is suggested that the cause for the decay are so-called reactive oxygen species (ROS). The results for the oxidation products of rutin in an aqueous system underline the theory of oxidation by ROS. After hydrogen donation took place, the quinones formed reacted with either methanol or water, or both, leading to diverse products [170].

The nanosuspension technique is suitable for production of particulate drug formulations in order to protect drugs and especially chemical labile drugs from degradation, e.g. as also previously shown for paclitaxel [168]. The possibility of processing drugs at decreased temperature, e.g. 0^{o}C, opens the possibility for producing a nanosuspensions and further minimalizing chemical degradation of drugs during production. The rutin nanosuspensions are easily to produce; the systems can be easily transferred from the lab scale to the

large production scale. It is possible to produce highly concentrated chemically stable nanosuspensions, and to protect the rutin from degradation.

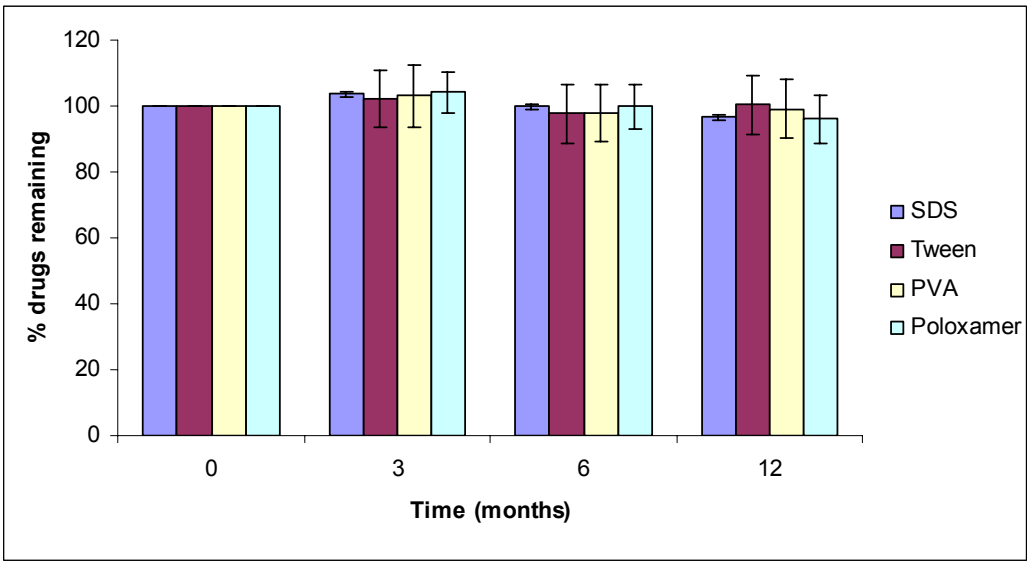


Fig. 4.35 Chemical stability of rutin nanosuspensions monitored over a 12 month period

4.5.2 Hesperidin

Similar to rutin, the chemical stability of hesperidin nanosuspensions was also evaluated over a period of 12 months. Considering that hesperdin originates from plant material (Citrus cultivation) [116], it is subjected to the inherent chemical instability of substances from natural sources. Hesperidin lacks stability when exposed to light or aqueous medium and stored at room temperature. Therefore, to prevent chemical instability it should be placed in a fridge and protected from direct light exposure.

Table 4.15 and **Fig. 4.36** show the chemical stability of hesperidin nanosuspensions over 12 months at 4^{o}C (placed in a fridge). This proves that the nanosuspensions could protect againt chemical degradation. All four investigated nanosuspensions showed excellent protection from chemical degradation over a 12 month period.

Table 4.15 Chemical stability of hesperidin nanosuspensions stabilized by Poloxamer 188 (E) and SDS (F), Tween 80 (G) and PVA (H)

Formulation	Stability over time (months)			
	0	**3**	**6**	**12**
E	100%	97.87%	101.13%	101.00%
F	100%	101.85%	100.01%	98.14%
G	100%	94.30%	102.94%	102.76%
H	100%	99.37%	100.59%	100.05%

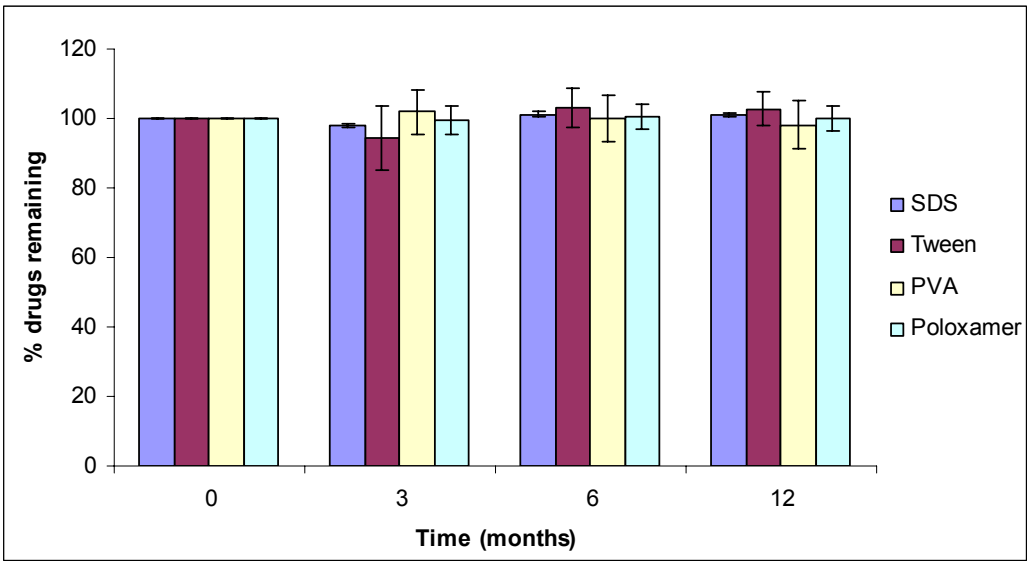


Fig. 4.36 Chemical stability study of hesperidin nanosuspensions monitored over a 12 month period

As can be seen in **Fig. 4.36**, hesperidin remained at 100% over 12 months. This result is in agreement with Liversidge and Troester [168, 169].

4.6 Lyophilization/ Freeze-drying

Lyophilization is the process of freeze-drying a composition to remove excess water. The process involves sublimation of the frozen water, usually under reduced pressure conditions [138, 171]. Freeze-drying works by freezing the material and then reducing the surrounding pressure and adding enough heat

to allow the frozen water in the material to sublimate directly from the solid phase to gas.

4.6.1 Rutin

Rutin nanosuspension should be converted into powder. Lyophilization was performed to dry rutin aqueous nanosuspension into powder (nanocrystals). As discussed before, formulation B was chosen for further investigation because it showed highest physical stability. After 24 hours, lyophilized rutin nanocrystals were collected from the freeze dryer. Lyophilized rutin nanocrystals were further investigated with respect to redispersability, crystalline state, morphology, saturation solubility and dissolution velocity. In many processes of lyophilization, cryoprotectant is usually added to the nanosuspension to protect nanoparticles or nanosuspension from freeze damage (damage due to ice formation) and to reduce particle size growth during lyophilization [171]. But in this study added cryoprotectant did not minimize as avoid nanocrystals aggregation/agglomeration. Surprisingly, it was found that without cryoprotectant, lyophilized rutin nanocrystals can be re-dispersed properly in water. **Fig. 4.37** shows rutin aqueous nanosuspensions and lyophilized rutin nanocrystals.

Fig. 4.37 Rutin nanosuspensions after producing (left) and lyophilized rutin nanocrystal powder (right)

4.6.2 Hesperidin

Freeze-drying was also employed as a method to generate dried hesperidin nanocrystals. This method yielded a high yield of final dried hesperidin product. Compared to other drying processes, freeze-drying provides almost 100 percent dry content, meaning that the effectiveness of the freeze-drying is higher than other drying processes, e.g. spray drying, fluid bed drying, evaporation etc. Therefore this process is frequently used in many industries. The characterization of lyophilized hesperidin was intensively evaluated with respect to re-dispersability, crystalline state, morphology, saturation solubility and dissolution velocity. Like with rutin, cryoprotectant was also not added, since the hesperidin nanocrystals themselves – identical to rutin - already had good re-dispersion properties. Detailed results are presented in the re-dispersability section.

4.6.3 Ibuprofen

Dried ibuprofen nanocrystals could be generated by means of freeze-drying. A 10% ibuprofen nanosuspension was frozen in the freezer at -70^{o}C. Frozen ibuprofen nanosuspension was directly placed in the freeze dryer chamber and lyophilized within 48 hours. The freeze-dryer could produce high percentages of the final dried ibuprofen. Lyophilization was set to 0.03 mbar and to hold temperature below -20^{o}C over a period of 48 hours. The dried ibuprofen was further investigated with respect to crystalinity. In the next chapter, the crystalline state of lyophilized ibuprofen will be explained in detail. In this case, cryoprotectant was not added, since dried ibuprofen was directly incorporated into effervescent formulations. The characteristics of the nanocrystals in pellets and effervescent granules were studied in relation to morphology, crystalline state and re-dispersion from effervescent granules.

4.6.4 Coenzyme Q10

Coenzyme Q10 is intended to be incorporated into a solid dosage form, such as tablets or capsules for oral administration. Therefore coenzyme Q10 nanosuspensions should be converted to coenzyme Q10 nanocrystals powder. Lyophilisation (freeze-drying) was employed as with the previous drugs, with a 10% coenzyme nanosupension frozen in a freezer and then the

frozen nanosuspension lyophilized in a freeze-dryer at 0.03 mbar and temperature set at -20^{o}C within 48 hours.

In many cases of lyophilization, cryoprotectant is added to nanosuspensions to protect nanoparticles or nanosuspensions from damage (damage due to formation) and reduces particle size growth of nanoparticles or liposome during lyophilization [171-173]. Therefore in this study cryoprotectants were added to the nanosuspensions before the freezing process. Lactose, manitol, threhalose and glucose were used as cryoprotectants.

4.7 Spray drying

Spray driers convert a liquid, solution or suspension into powder in a one-step process. Spray driers consist of the following components: the feed delivery system, an atomizer, a heated air supply, drying chamber(s), solid-gas separators and product collection systems [174]. The nanosuspensions is pumped through an atomizer device that produces fine droplets in the main drying chamber. Particles may tend to agglomerate during production and processing, thereby undesirably altering the effective size of the particles to the detriment of the performance and/or reproducibility of the particle formulation. Under such circumstances, the particles made may simply be larger than desired for a particular application. Therefore, after they are produced, particles may require additional processing for size reduction and/or deagglomeration [175].

The final product produced (nanocrystals powder) by spray drying in this research was multi component, i.e. containing drug(s) and excipient (a small amount of stabilizer with/without cryoprotectant). In the former cases, changes in crystalinity observed in spray drying may have resulted from the formation of a more disordered form of a normally crystalline drug on drying. The crystal lattice is generally considered to be a highly ordered structure repeating itself in three dimensions. In practice, crystal lattice imperfections such as point defects (e.g. vacancies, impurity defects), line defects (e.g. edge and screw dislocations) and plane defects (e.g. grain boundaries and crystal surfaces) are present. Rapid drying processes such as spray drying are likely to increase the level of such defects, and these in turn will be reflected in the thermal properties of the material. More striking changes which may be

observed include polymorphic changes, solvate formation and in some cases the production of drug in an amorphous or glassy form. When multi component solutions or slurries are spray-dried, the above changes are possible for each of the solid constituents in the final dried product. In addition, there is the possibility of solid solution and complex formations. These changes may not be evident on visual examination. Thermal degradation of products can be overcome by using lower operating temperatures and larger chamber sizes for increased residence times [174].
Thermal analytical methods (microscopy, X-ray diffraction methods and DSC) were performed to identify the changes in the observed nanocrystals.

4.7.1 Rutin

As alternative to obtain rutin nanocrystals powder for comparison with lyophilized nanocrystals, a spray dryer was used to obtain rutin nanocrystals. A 10% rutin aqueous nanosuspensions was dried using a mini Büchi spray dryer to convert rutin aqueous nanosuspension to dried rutin nanocrystals (spray-dried rutin nanocrystals). The physicochemical properties of the spray-dried rutin nanocrystals were investigated with respect to kinetic solubility, crystalline state and morphology. The yield of the production of rutin nanocrystals on a laboratory scale was not so high. The spray dryer could convert only 50%-60% of the rutin nanocrytals to the final product and the rest of the material was lost during drying.

4.7.2 Hesperidin

A spray drying process was also employed to obtain dried hesperidin nanocrystals. Although this method was only moderately effective (low yield), the spray-dried nanocrystals had better flow characteristics compared to lyophilized nanocrystals [155, 158]. Therefore, spray-dried hesperidin nanocrystals were chosen for further investigation. As usual, the spray-dried nanocrystals were evaluated with respect to physicochemical properties e.g. redispersability, crystalline state, morphology, saturation solubility and dissolution velocity.

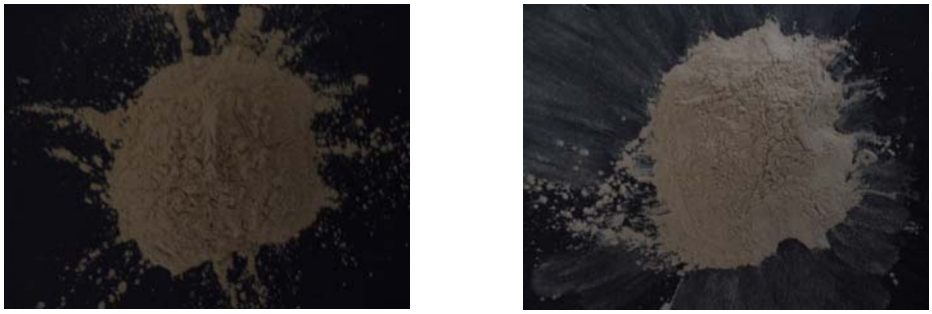


Fig. 4.38 Hesperidin raw material of (left) and spray-dried hesperidin nanocrystals (right)

4.7.3 Ibuprofen

A spray dryer was also employed to produce dried ibuprofen nanocrystals. A 10% ibuprofen nanosuspension was dried directly after production. Spray drying conditions can be seen in the chapter method on methodology. On the lab scale, a Mini Spray-dryer B-190 was used to obtain dry powder Ibuprofen nanocrystals. In this process inlet and outlet temperature was set at 75^{o}C. This temperature condition was adjusted to air volume (600 L/h) and aspirator.

Further investigation was carried out with respect to morphology particle, re-dispersion and crystalline state. The results of all these investigations are presented in the next chapter.

4.8 Re-dispersability

The aqueous nanosuspensions can be post processed for dispersability as dry powder for solid dosage forms such as tablets, capsules, pellets, effervescents or lyophilized material for injectable products. These dried powders from nanocrystals are designed to re-disperse into nanometer-sized particles when placed in water or an alternate water-based environment. Ideally the same size and size distribution should be obtained as before drying. The ability of the dried nanocrystals to re-disperse into non aggregated/non agglomerated nanopartuculate dispersion is critical to the development of a solid dosage form that maintains the benefits of this enabling drug delivery technology.

4.8.1 Cryoprotectant

Cryoprotectants (cryoprotective agents or compounds) are agents that protect chemical compounds, cells, or tissues from the deleterious effects of freezing that may accompany lyophilization. In the case of nanoparticles, cryoprotectants protect from agglomeration caused by the lyophilization process. Cryoprotectants include carbohydrates such as the saccharide sucrose, sugar alcohols such as lactose, mannitol, and surface active agents. Preferred cryoprotectants are carbohydrates (saccharide, disaccharide or oligosaccharide). Cryoprotectants are present in the nanoparticles of the present invention in an amount sufficient to allow the nanoparticles to be lyophilized, i.e. 0.5% to 90%, preferably 1-50%, and most preferably in an amount of about 2% to about 25%, based on the total weight of the nanosuspensions [171].

4.8.1.1 Coenzyme Q10

Cryoprotectants were added in this research only to the coenzyme Q10 nanosuspensions. Lactose, mannitol and trehalose were employed and added to the 10% coenzyme Q10 nanosuspension before lyophilization.

Table 4.16 presents the amount of cryoprotectants added to the coenzyme Q10 nanosuspensions. Cryoprotectants were added in a range 2.5% to 10% of total weight of the coenzyme Q10 nanosuspensions.

Table 4.16 Addition of cryoprotectants to 10% coenzyme Q10 nanosuspensions (stabilized by 2% of Tween 80 and 2% of PVA) before lyophilization

Cryoprotectant	Amount (percentage related to total nanosuspension)	
	Stabilized by Tween 80	Stabilized by PVA
Lactose	-	2.5%
	5%	5%
	10%	10%
Mannitol	5%	2.5%
	10%	5%
	-	10%
Trehalose	5%	-
	10%	-

4.8.2 Particle size analyses of re-dispersed dried nanocrystals

The aim of the transform of an aqueous nanosuspension to dried nanocrystals is to process them to solid dosage forms. The choice of drying technology is a critical obtaining an optimal final dried nanocrystal product. Dried powders of nanocrystals are designed to re-disperse into nanometer-sized particles. Therefore, this study must focus on whether the dried powder is properly formulated and able to be re-dispersed completely prior to formulating it as tablets, capsules, pellets and effervescent tablets (solid dosage forms).

4.8.2.1 Rutin

Lyophilized rutin nanocrystals stabilized with 0.2% of SDS could be redispersed completely into aqueous dispersion. Upon the addition of water the lyophilized rutin nanocrystals could be easily re-dispersed without aggregates or agglomerates. **Fig. 4.39** shows that the particle size distribution of the re-dispersed rutin nanocrystals is not so much different to the original aqueous rutin nanosuspension. The d50% of lyophilized rutin nanocrystals was similar to the d50% of the rutin nanosuspension after production.

The particle size (PCS) of the original rutin nanosuspension was 727 nm with a polydispersity index (PI) of 0.265. The PCS size average and polydispersity index (PI) of re-dispersed rutin were of 721 nm and of 0.288 (**Fig. 4.40**). The data are identical to the original rutin nanosuspension. These results confirm that the transform process of the rutin aqueous nanosuspensions to lyophilized rutin nanocrystals (dry powder) using a freeze dryer definitely did not or just little influenced the particle size (e.g. growth by agglomeration).

Usually, in many processes of the lyophilization, cryoprotectant is added to the nanosuspension to protect nanoparticles or nanosuspensions from freezing damage (damage due to ice formation) and to minimize the particle size growth during lyophilization. In this study it was found that added cryoprotectant did not minimize nanocrystal growth efficiently. Surprisingly, it was found that lyophilized rutin nanocrystals without any cryoprotectant can be re-dispersed in water properly.

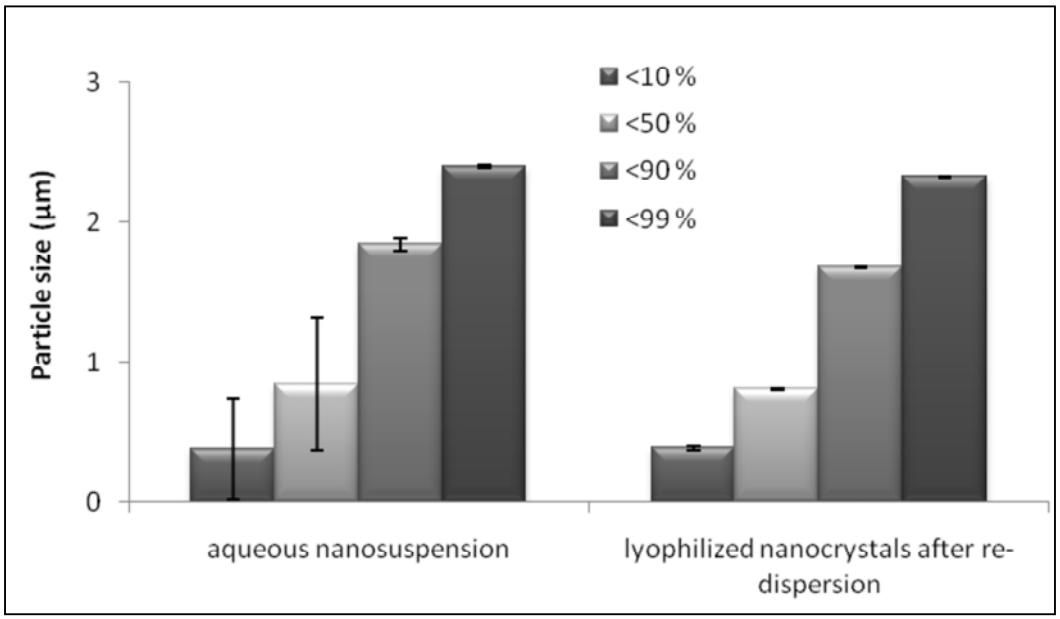


Fig. 4.39 Particles size diameters by LD of rutin aqueous nanosuspensions and re-dispersed rutin nanocrystals

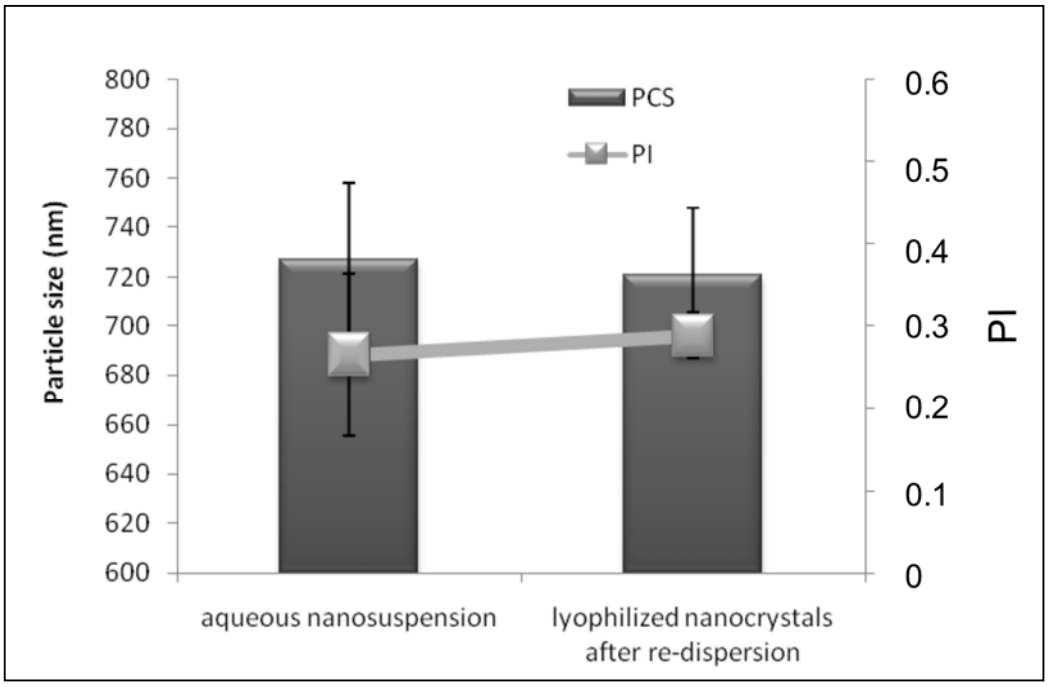
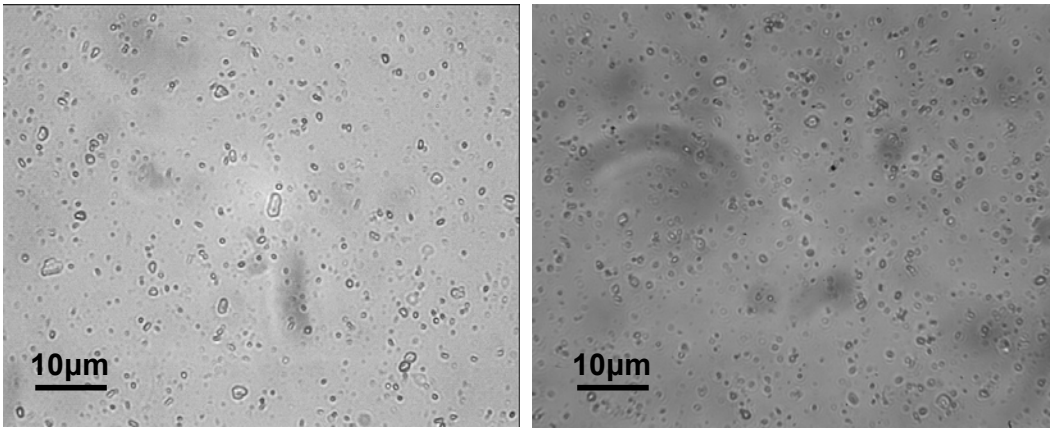


Fig. 4.40 Particles size average by PCS of rutin aqueous nanosuspensions before lyophilization and re-dispersed rutin nanocrystals (formulation from **Fig. 4.39**)



a b

Fig. 4.41 Light microscopic pictures of rutin nanosuspensions after production (a) and re-dispersed rutin nanocrystals (b), (formulation from **Fig. 4.39**)

Light microscopy pictures of the rutin nanosuspensions and re-dispersed rutin nanocrystals were visibly similar. No particle size difference could be observed between both pictures of the rutin nanosuspensions (**Fig. 4.36**). These pictures reveal that the particles of the lyophilized rutin nanocrystals were distributed homogenously as single particles. This is well in agreement with the low polydispersity index (PI) value. It could hardly be found any agglomerated or aggregated rutin nanocrystal after re-dispersion. In this study, lyophilized rutin was further investigated and incorporated into tablets. In addition spray-dried rutin was employed as control in crystalline state evaluations.

4.8.2.2 Hesperidin

Both dried forms of hesperidin nanocrystals (spray-dried and lyophilized) were almost completely redispersable in water. Even without the addition of cryoprotectants, the lyophilized and spray-dried hesperidin could easily be redispersed. The particle size average by PCS differed little from the original hesperidin nanosuspensions. For example, lyophilized hesperidin nanocrystals stabilized by poloxamer 188 had an average the PCS size and polydispersity index (PI) of 396 nm and 0.555 after redispersion. The d50% of hesperidin nanocrystals was 0.370 µm. This particles size analysis was

distinctly similar to the original nanosuspensions with the PCS size average of 309 nm (**Fig. 4.42**).

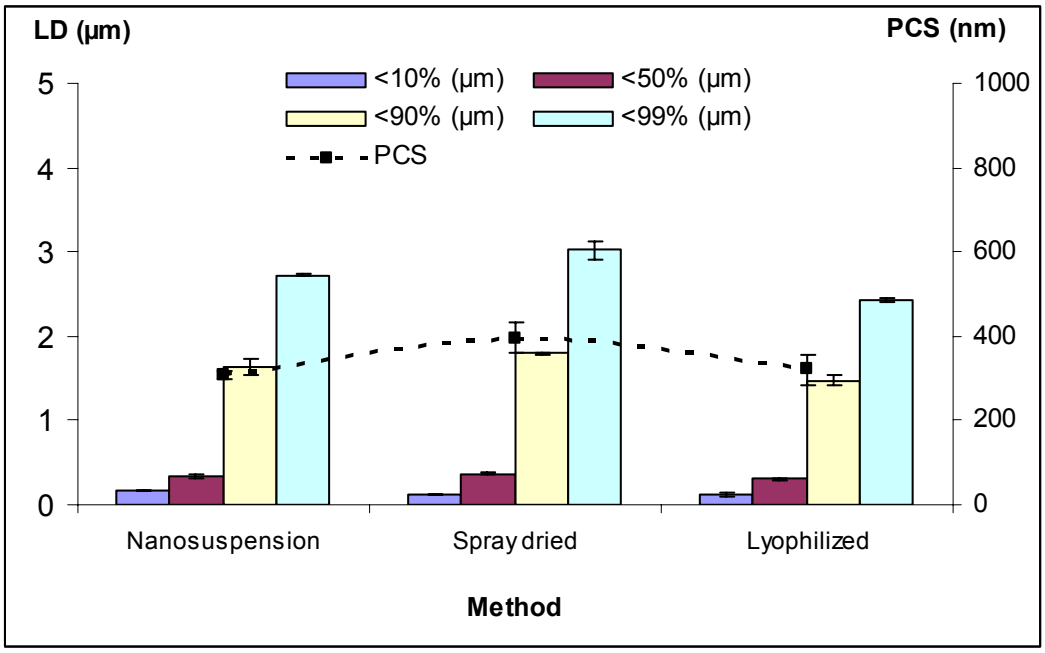


Fig. 4.42 PCS and LD particles size of 10% hesperidin nanosuspensions stabilized by 2% of poloxamer 188 before drying and redispersion of spray-dried and lyophilized hesperidin nanocrystals

Fig. 4.42 compares the particle size of the hesperidin nanosuspensions, and of redispersed spray-dried and lyophilized hesperidin nanocrystals. According to this result, the dried hesperidin nanocrystals is a promising powder for incorporation into solid dosage forms. Therefore in the next step of this research, hesperidin nanocrystal powder was incorporated into tablets. The dissolution velocity of the tablets was then evaluated and compared to marketed tablets.

The particle size average by PCS revealed little difference between the different formulations of spray-dried hesperidin nanocrystals. The physical properties of spray-dried hesperidin were better than for the lyophilized product. Flow ability of spray-dried nanocrystals was distinctly improved. This result is in agreement with Möschwitzer *et al.* who claims that spray drying can improve the flow properties of powder [155, 158].

Moreover, spray-dried hesperidin nanocrystals stabilized by PVA had the smallest average particle size after redispersion. The average particle size of this nanocrystal was 315 nm, practically identical to the original nanosuspension (particle size average of 309 nm) and showing little growth. Therefore this hesperidin nanocrystal formulation was preferable for incorporating into a solid dosage form for oral administration. The particles size difference is clearly visible in **Fig. 4.43**.

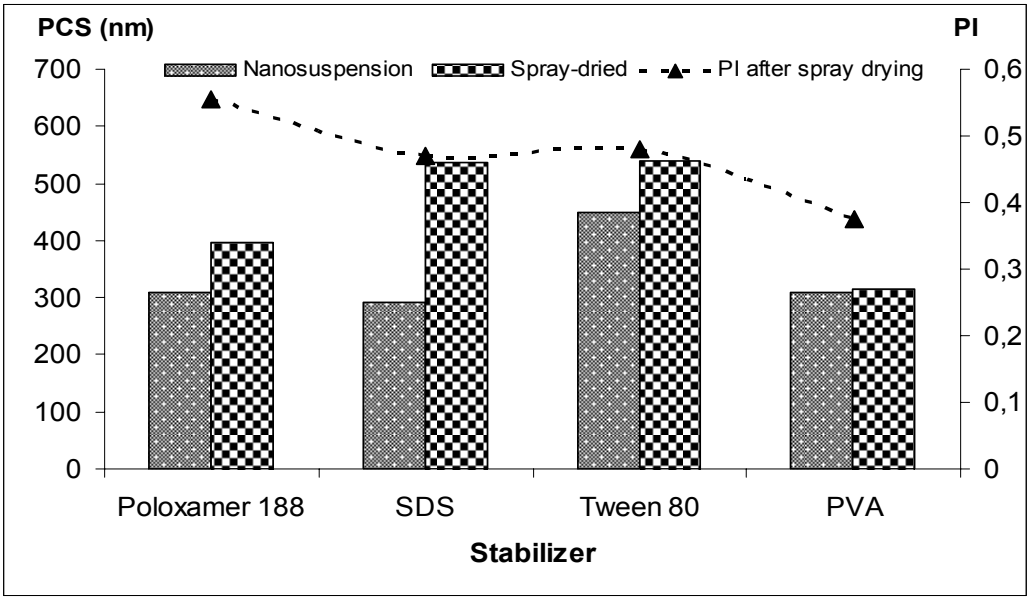


Fig. 4.43 Mean particle size by PCS of spray-dried hesperidin nanocrystals stabilized by poloxamer 188, SDS, Tween 80 and PVA after redispersion in water

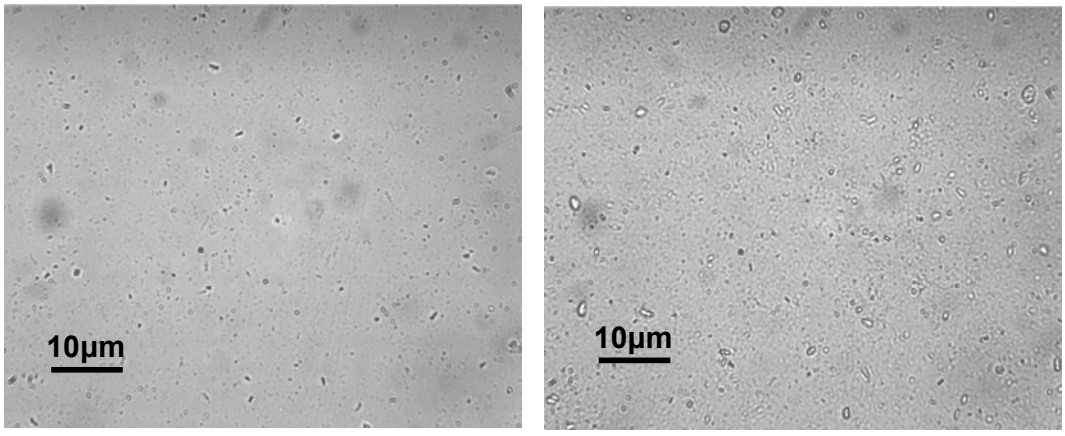


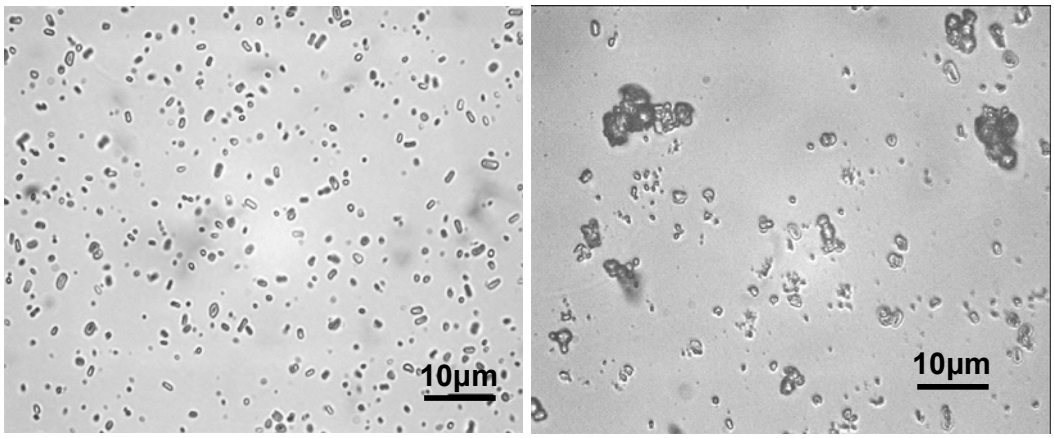
Fig. 4.44 Light microscopic pictures of hesperidin nanosuspensions stabilized by PVA after production (a) and re-dispersed spray-dried hesperidin nanocrystals (b)

In addition observation of morphology of the nanocrystals in the PVA-stabilized nanosuspension was performed using light microscopy **Fig. 4.44**. These pictures show that all particles were almost distributed as single crystals and clearly homogeneous. No agglomerated or aggregated particles were visible. This result is confirmed by the PI value. The low PI (below of 0.5) indicates a relative narrow distribution of the particles.

4.8.2.3 Ibuprofen

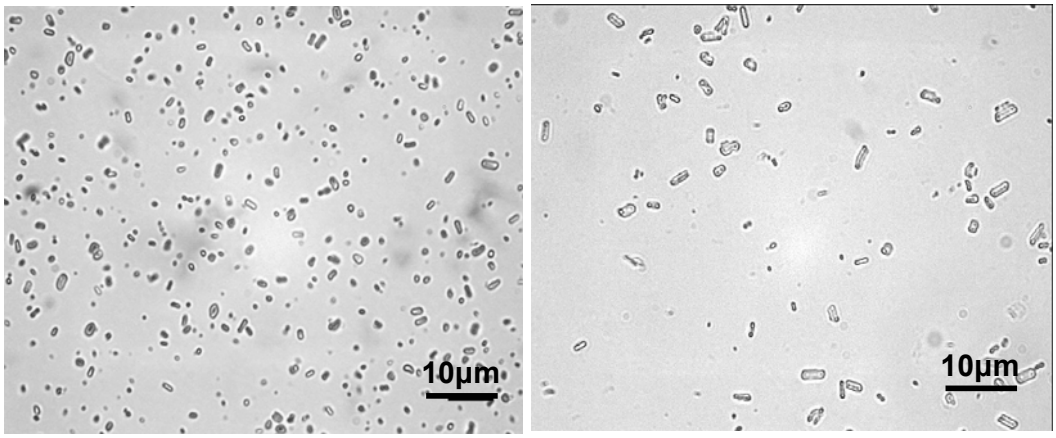
Particle size analyses of homogenized ibuprofen nanosuspensions showed that the aggregation or ibuprofen nanosuspensions was in the upper nanometer range. Therefore this nanosuspensions was slightly unstable. Drying directly after production is one method for inhibiting the aggregation or growth of ibuprofen nanocrystals [176]. **Fig. 4.45** shows a microscopic picture of re-dispersed spray-dried ibuprofen nanocrystals compared to homogenized ibuprofen nanosuspensions. In general, spray-dried nanocrystals had a larger particle size compared to ibuprofen nanosuspensions. Despite the larger particle size, the ibuprofen nanocrystals were clearly homogeneously distributed. Hardly any agglomerated or aggregated particles were found.

No difference was found between re-dispersed spray-dried ibuprofen nanocrystals and lyophilized ibuprofen. Both nanocrystals were bigger compared to those of the original nanosuspensions. Particle size distribution of the lyophilized ibuprofen nanocrystals, while exceeding the nanocrystals range, was relatively narrow with homogeneous distribution (**Fig. 4.45** and **Fig. 4.46**). Lyophilized ibuprofen nanocrystals could be admixed with other excipients to produce effervescent granule. In next section, the particle size distribution of the ibuprofen nanocrystals was analyzed after re-dispersion from effervescent granule in water.



a b

Fig. 4.45 Light microscopic pictures of ibuprofen nanosuspension after production (a) and re-dispersed ibuprofen nanocrystals after spray drying (b)



a b

Fig. 4.46 Light microscopic pictures of ibuprofen nanosuspensions after production (a) and re-dispersed ibuprofen nanocrystals after lyophilization (b)

In the other study, ibuprofen nanosuspensions was directly incorporated into lactose to produce ibuprofen pellets. Production of pellets and effervescent granules will be explained in detail in next section.

4.8.2.4 Coenzyme Q10

Cryoprotectant effectiveness was investigated for various concentrations and different type of cryoprotectors. **Fig. 4.47** and **Fig. 4.48** illustrate the effectiveness of cryoprotectants in inhibiting particle growth in CoQ10

nanosuspensions during lyophililzation by showing the particle size of lyophilized CoQ10 after re-dispersion into water. The best type and concentration of cryoprotectant was obtained with addition of 5% lactose to formulation J. With 5% lactose, particle growth was prevented in lyophilized CoQ10 nanocrystals, enabling proper re-dispersion into aqueous dispersion. The PCS size average and polydispersity index (PI) of the re-dispersed CoQ10 was 263 nm and 0.305. The particle size average of the original CoQ10 nanosuspensions was 219 nm with a polydipersity index (PI) of 0.136. The d95% was 0.593 µm, similar to d95% of CoQ10 nanosuspensions after production (d95% of 0.441). Therefore, the particle size analyses of the re-dispersed CoQ10 nanocrystals differed little from CoQ10 aqueous nanosuspension (**Fig. 4.47**). In contrast, the addition of mannitol as a cryoprotectant was not effective in preventing particle growth. The most effective concentration of mannitol as cryoprotectant was 5% of the total nanosuspension. LD distribution of particles in this re-dispersed indicated 99% of particles being below 12 µm with mean particle size by PCS of 632 nm (**Fig. 4.48**).

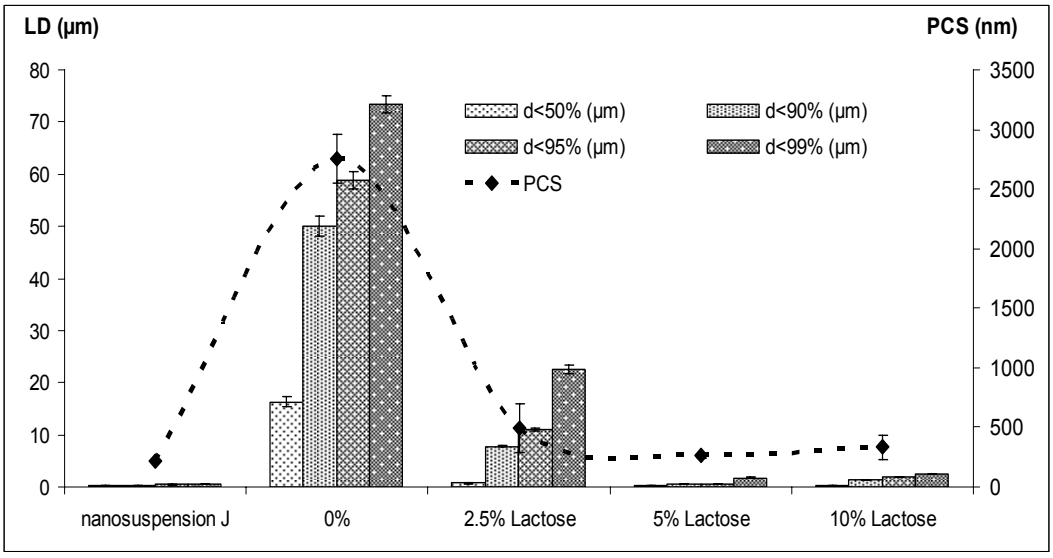


Fig. 4.47 LD particle sizes of re-dispersed CoQ10 nanocrystals (formulation J) with addition of various concentrations of lactose as cryoprotectans

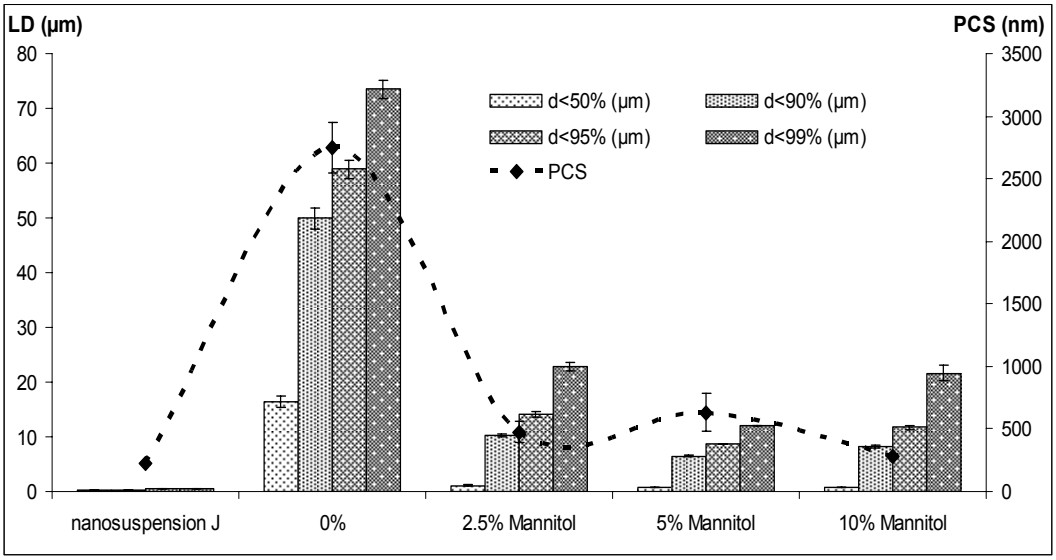


Fig. 4.48 LD particle sizes of re-dispersed CoQ10 nanocrystals (formulation J) with addition of various concentrations of mannitol as cryoprotectans

The addition of various cryoprotectants to formulation I before lyophilization failed to inhibit particle growth. **Fig. 4.49** shows that re-dispersion of lyophilized nanocrystals I yielded sizes in the micrometer range (micron particulate). It was not so much different to the particle size of the raw material. The addition of 10% lactose into formulation I produced the optimum formulation of re-dispersed nanocrystals with a d50% of 4.14 µm.

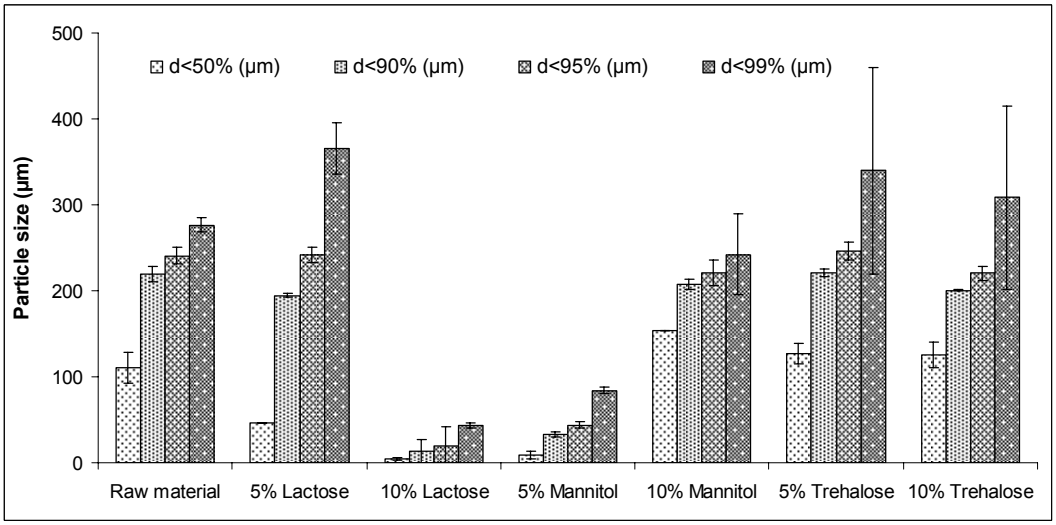


Fig. 4.49 LD particle sizes of re-dispersed CoQ10 nanocrystals (formulation I) with addition of various types and concentrations of cryoprotectans

Nanosuspension I was stabilized by Tween 80 which is a liquid surfactant and cannot be dried after lyophilization. The final lyophilized products were sticky and moist, preventing re-dispersion into water as finer particles. Instead, they were re-dispersed as agglomerated /aggregated particles. This confirms that Tween 80 is not a suitable stabilizer for nanosuspensions converted to dry powder by lyophilization.

Summarized results for re-dispersion of lyophilized CoQ10 nanocrystals are presented in **Fig 4.50** and **Table 4.17**. They show the particle LD and PCS data of the raw material, CoQ10 nanosuspension J and re-dispersed CoQ10 nanocrystals of formulation J with addition of 5% lactose. Particle size reduction by HPH and addition of 5% lactose were distinctly effective in maintaining the nanocrystals powder in submicron particulate form.

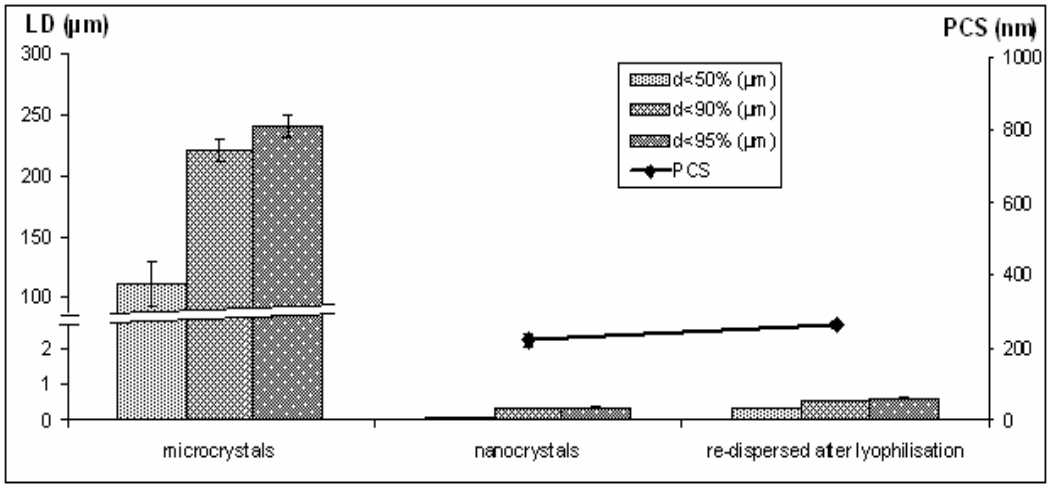


Fig. 4.50 LD particle sizes of microcrystals (raw material), nanocrystals (formulation J) and after re-dispersion of lyophilized CoQ10 nanocrystals with addition 5% lactose as cryoprotector

The addition of 5% lactose into formulation J and the use of freeze dryer technology for transformation of CoQ 10 aqueous nanosuspensions to lyophilized CoQ 10 nanocrystals (dry powder) had little or no influence on particle size distribution of the CoQ 10 nanocrystals. This result is in agreement with Liversidge *et al.* and Han *et al.,* that cryoprotectors can retain the particle size of the nanocrystals during lyophilization [171, 177, 178]. Lyophilized CoQ 10 nanocrystals can be re-dispersed into nanometer-sized particles without agglomerated nanocrystals.

Table 4.17 Particle size analyses (by LD and PCS) of microcrystals (raw material, nanocrystals (formulation J) and after re-dispersion of lyophilized CoQ10 nanocrystals with addition 5% lactose as cryoprotector

Samples	Particles size analyses							
	LD measurement (µm)				PCS (nm)			
	d50%	s.d.	d95%	s.d.	Z-ave	s.d.	PI	s.d.
Microcrystals	111	18.6	230	8.2	-	-	-	-
Nanocrystals	0.214	0.009	0.441	0.003	219	7	0.136	0.052
Re-dispersed lyophilized nanocrystals	0.322	0.004	0.593	0.081	263	17	0.302	0.055

Fig. 4.51 Coenzyme Q10 nanosuspensions (formulation J) with a particle size of 219 nm (left), lyophilized CoQ10 nanocrystal powder (middle) and re-dispersed lyophilized CoQ10 nanocrystals (addition of 5% lactose) with a particle size of 263 nm (right).

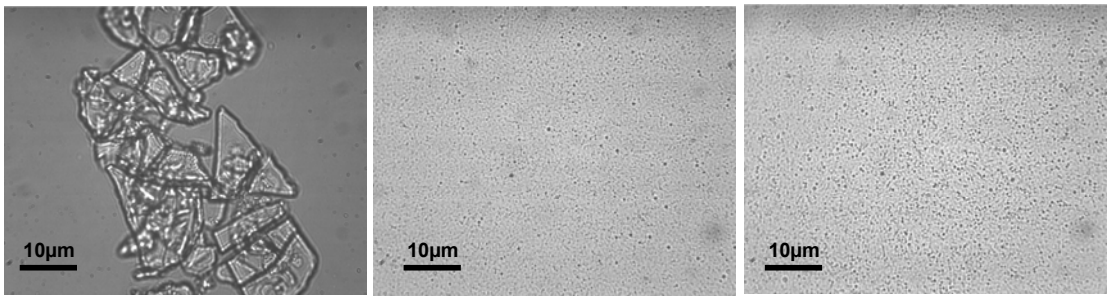


Fig. 4.52 Microcrystals (raw material) of coenzyme Q10 with a particle size of 110 µm (left, magnification 1000x) was transferred into coenzyme Q10 nanosuspension J (middle, magnification 1000x) and re-dispersed coenzyme Q10 nanocrystals after addition of 5% lactose (right, magnification 1000x)

Light microscopic pictures of CoQ 10 nanosuspension and re-dispersed CoQ10 nanocrystals were visibly similar. Difference in particle size between CoQ10 nanosuspensions and re-dispersed CoQ 10 nanocrystals could hardly be found (**Fig. 4.52**). These pictures reveal that the particles of the lyophilized CoQ 10 nanocrystals were distributed almost entirely as single particles and clearly homogeneous with low polydipersity index (PI) value. Hardly any agglomerated or aggregated CoQ10 nanocrystal could be found after redispersion.

4.9 Crystalline state evaluation

Various techniques are employed to investigate the crystalline state of substances such as drugs and excipients. Two of these techniques are DSC and the powder x-ray diffraction method. Both methods used together can yield enough information to confirm the crystalline state of a sample. Therefore in this research, both methods were employed to investigate the crystallinity of the dried nanocrystals.

4.9.1 DSC

DSC is part of a group of techniques called thermal analysis (TA). Thermal Analysis is based upon the detection of changes in the heat content (enthalpy) or the specific heat of a sample at a certain temperature. As thermal energy is supplied to the sample, its enthalpy increases and its temperature rises by an amount determined for a given energy input by the specific heat of the sample. The specific heat of a material changes slowly with temperature in a particular physical state, but alters discontinuously at a change of state. As well as increasing the sample temperature, the supply of thermal energy may induce physical or chemical processes in the sample, e.g. melting or decomposition, accompanied by a change in enthalpy, the latent heat of fusion, heat of reaction etc. Such enthalpy changes may be detected by thermal analysis and related to the processes occurring in the sample [179].

4.9.1.1 Rutin

Taha et al. [113] confirm that the raw material of rutin as a crystal contains water ($3 \cdot H_20$), and becomes anhydrous at 110°C and 10 mm Hg. However, the anhydrous form browns at 125°C, melts at 188,7°C, becomes plastic at 195-197°C and decomposes with effervescence at 214-215°C [114].

Thermal analysis of the rutin nanocrystal powder was performed and compared to the raw material and to the data in the literature. The rutin nanocrystal powders investigated were obtained by lyophilization and spray drying. The temperature of fusion of lyophilized rutin as well as spray-dried rutin was investigated by DSC. To study crystalline state alteration, the melting points of lyophilized and spray-dried rutin were compared to the raw material. The crystalline state of drugs can be transformed after physical treatment such as homogenization [56], grinding [157, 180], or environment condition altering (e.g. heat or humidity) [181], by increasing the amorphous fraction or by transformation into another polymorph.

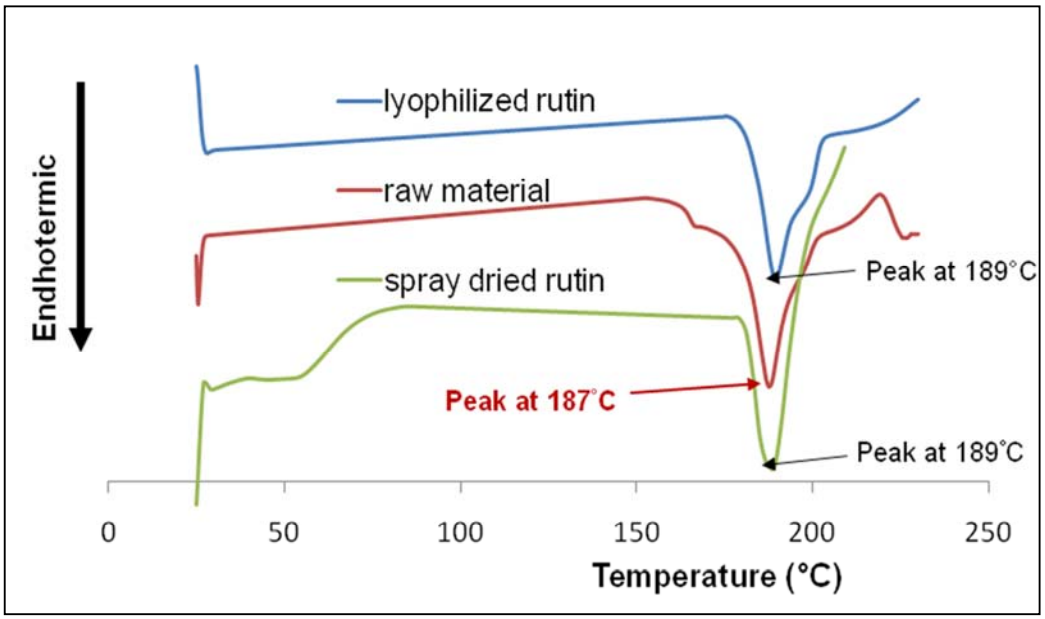


Fig. 4.53 DSC thermogram of lyophilized, and spray-dried rutin nanocrystals and raw material

The results of thermal analysis by DSC can be seen in **Fig. 4.53**. The diagram reveals a similarity in the melting temperatures. According to these results, neither lyophilized rutin nor spray-dried rutin nanocrystals modified the

crystallinity of the drug after the physical treatment of high pressure homogenization (HPH) and the subsequent drying process (lyophilization or spray drying). As it is shown in the DSC thermograms (**Fig. 4.53**), the melting points of rutin (raw material and both nanocrystal powders) are similar. The only difference observed was a slight shift in melting temperature (from 187^{o}C to 189^{o}C). This modification was attributed to the presence of SDS as stabilizer. In addition, the width of the dried nanocrystals was slightly reduced compared to the raw material.

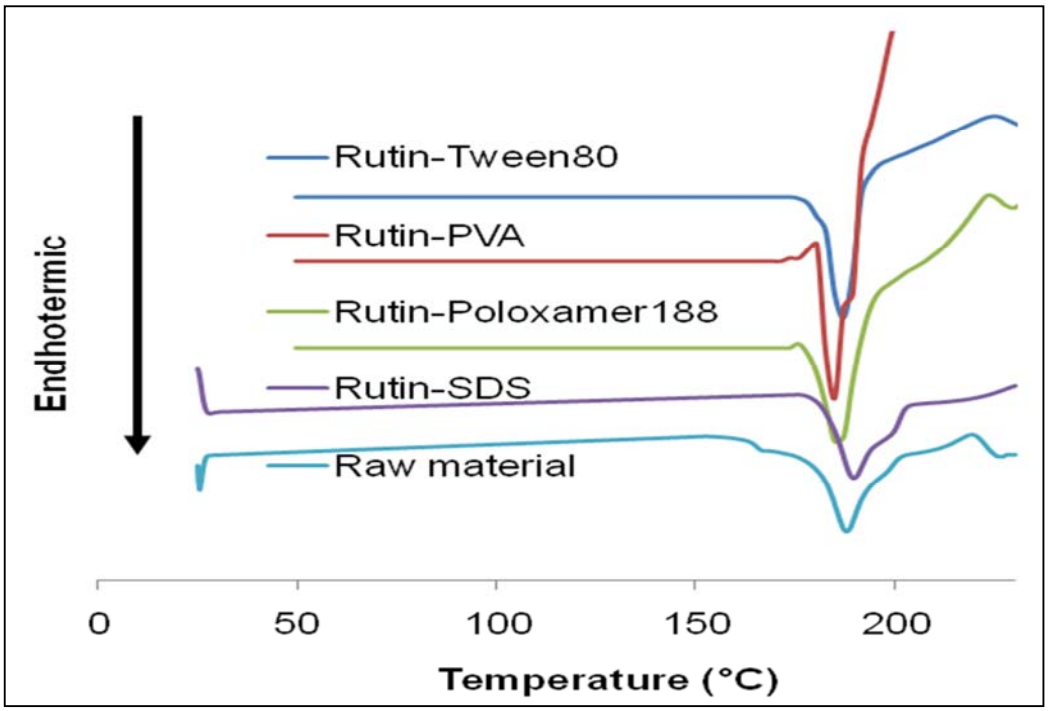


Fig. 4.54 Influence of stabilizer on fusion temperature and crystallinity of lyophilized rutin nanocrystals

Moreover, thermal analysis was performed to study the influence of the stabilizer on the crystalline state of the rutin nanocrystals. Lyophilized rutin nanocrystals stabilized by different stabilizers (SDS, PVA, Poloxamer 188, Tween 80) were investigated with respect to melting temperature and crystallinity by DSC. The results of the thermal analyses are shown in **Fig. 4.54**. In these thermograms, the melting points of the rutin nanocrystals are not changed significantly. The fusion temperatures of those rutin nanocrystals were in the range from 185^{o}C to 189^{o}C. The lowest melting point was 185^{o}C

revealed by rutin nanocrystals stabilized by PVA and the maximum was 189°C for the SDS-stabilized nanocrystals, respectively. In conclusion, the presence of stabilizers has minor influence on the melting temperature and has not altered the crystallinity of the drug.

According to those data, neither stabilizer nor applied physical treatment, such as HPH and drying, did affect the crystalline state of the nanocrystals such as to be fully or partial amorphous. In fact, all lyophilized rutin nanocrystals were still in the similar crystalline state as the original, unprocessed raw material.

4.9.1.2 Hesperidin

Some literature mentions the melting point and the crystalinity of hesperidin. Pure hesperidin exists as long hair-like needles, tan or pale yellow in colour, with melting point ranging from 258° to 262°C (softening at 250°C). The molecular formula for this compound is $C_{18}H_{34}O_{15}$ and molecular weight is 610.57 daltons [114].

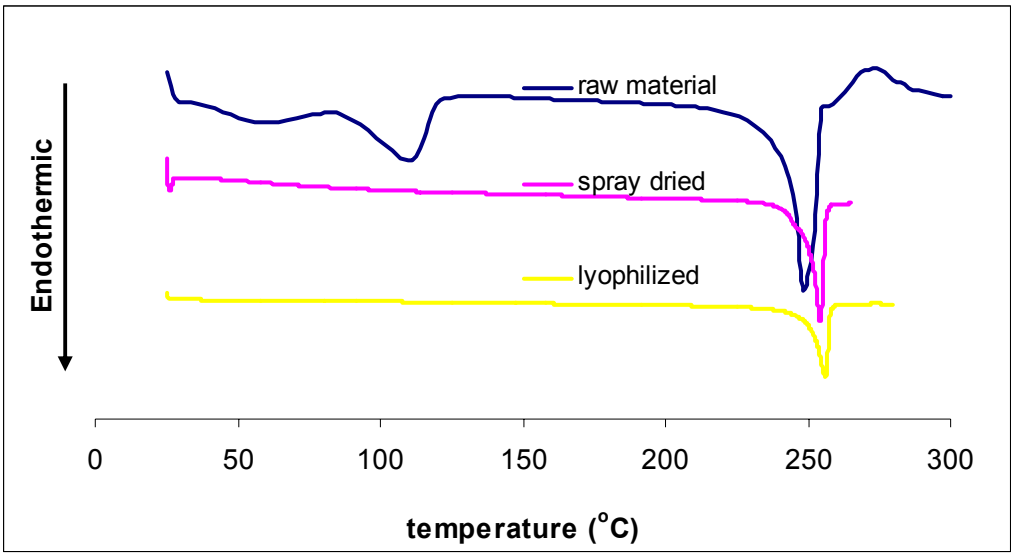


Fig. 4.55 DSC thermogram of lyophilized and spray-dried hesperidin nanocrystals (formulation E with 2% of poloxamer 188 as stabilizer) and raw material

As expected, the crystalline state of hesperidin was unaffected by the drying process. Exposure to energy during homogenization and the subsequent drying process did not alter crystallinity. According to the DSC thermogram in **Fig. 4.55,** fusion temperature peaks for the raw material, spray-dried and lyophilized hesperidin nanocrystals were similar and showed no significant

change. The peaks of fusion temperature indicate that dried hesperidin nanocrystals were in the same crystalline state with raw material. Again, the apparent modification in the hesperidin nanocrystals was only a minor shift of the drug melting points. Differences in the drying process produce an alteration of these melting points, but there was no modification of crystallinity to an amorphous state.

The presence of different stabilizers in the hesperidin nanocrystal formulations also had no influence on the crystalline state. **Fig. 4.56** shows fusion temperatures of the dried hesperidin nanocrystals with various stabilizers. According to these thermograms, the melting points of the nanocrystals did not change significantly. The melting points occurred in the range of 252°C to 254°C. The presence of stabilizer could explain the slight shift in those melting points.

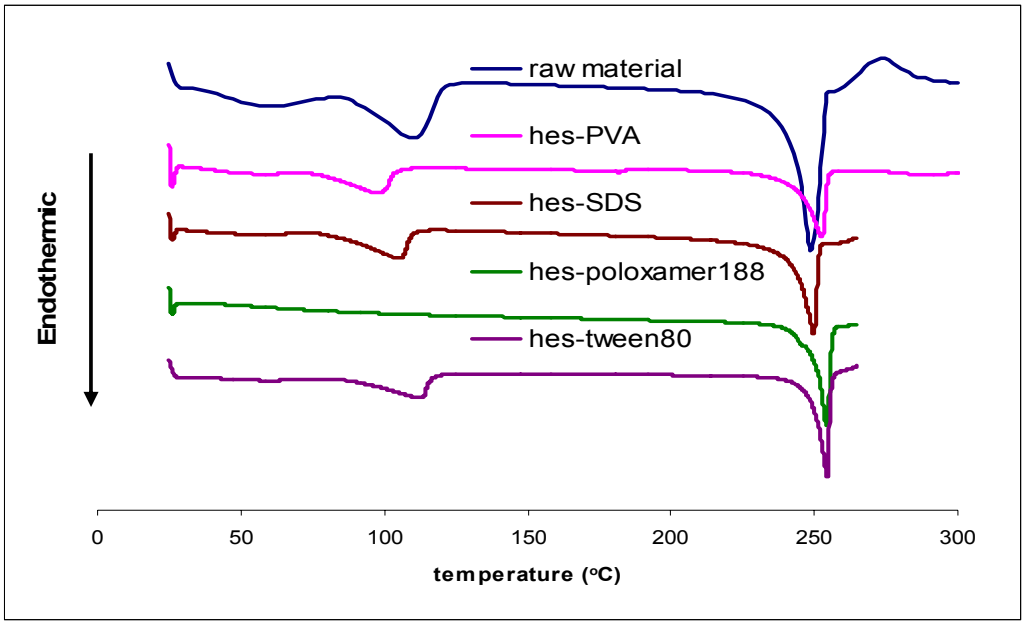


Fig. 4.56 Influence of stabilizers on fusion temperature and crystallinity of spray-dried hesperidin nanocrystals

Water was also evaporated from dried nanocrystals and from hydrated form of hesperidin during heating process, as clearly shown by the peaks at around 100°C in **Fig. 4.55** and **Fig. 4.56**. However, all peaks in the thermogram indicate the same crystallinity for the hesperidin nanocrystals as for the raw material. This means that the dried hesperidin nanocrystals were not converted to an amorphous state and remained in crystalline form.

4.9.1.3 Ibuprofen

Thermal analyses of lyophilized and spray-dried ibuprofen nanocrystals were performed. According to the DSC data, the spray drying and lyophilization have almost no influence on the crystallinity of the ibuprofen nanocrystals. The melting point of all nanocrystals was similar to that of the raw material (between 74°C to 76°C). Similar to rutin and hesperidin, the slight difference in melting point might be explained by the presence of stabilizer (**Fig. 4.57** and **Fig. 4.58**) [182].

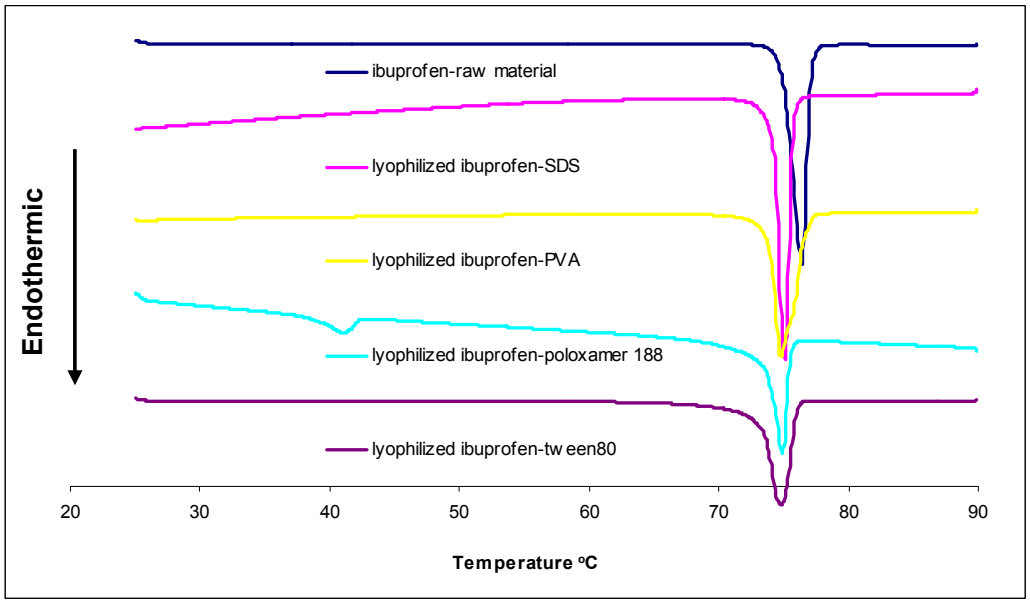


Fig. 4.57 Influence of stabilizers on fusion temperature and crystallinity of lyophilized ibuprofen nanocrystals

Due to the presence of poloxamer 188 in the nanosusupensions, temperature peaked at around 40°C in either lyophilized or spray-dried, indicating that poloxamer 188 melted at around 40°C. In contrast, other stabilizers did not reveal a peak because of their very low concentration such as only 0.2% for SDS or liquid state at room temperature, such as Tween 80 [182].

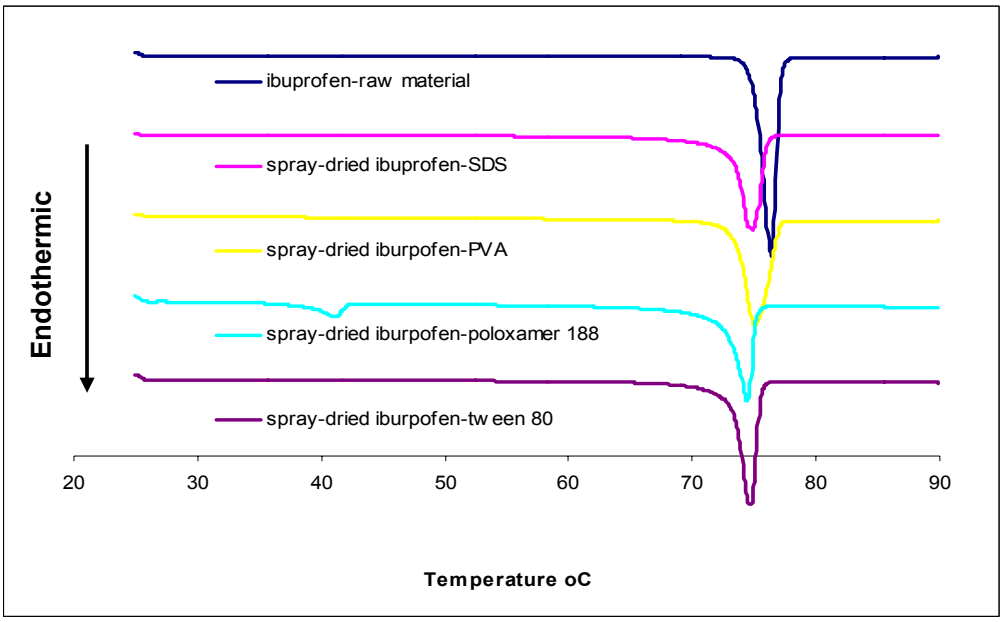


Fig. 4.58 Influence of stabilizers on fusion temperature and crystallinity of spray-dried ibuprofen nanocrystals

4.9.1.4 Coenzyme Q10

Coenzyme Q10 is an orange-yellow crystalline powder with a melting point of about 50°C [120, 123]. Thermal analysis of the CoQ10 powder was studied and compared to data in the literature. Dried CoQ10 nanocrystals were obtained by lyophilization. Temperature fusion of lyophilized CoQ 10 was investigated by DSC. The melting point of lyophilized CoQ10 nanocrystals was compared to that of the raw material to study the crystalline state.

Fig. 4.59 shows result of thermal analysis by DSC of both formulations. This graph reveals a minor shift in melting point, for formulation I in comparison to the melting point of the raw material. The difference observed was a slight shift in fusion temperature from 50°C to 48°C, attributable to the presence of Tween 80 as stabilizer. Moreover, the DSC thermograph of formulation I again showed a high endothermic peak in fusion temperature. It assumed that little or no diminishing occurred in the crystalline intensity of lyophilized formulation I.

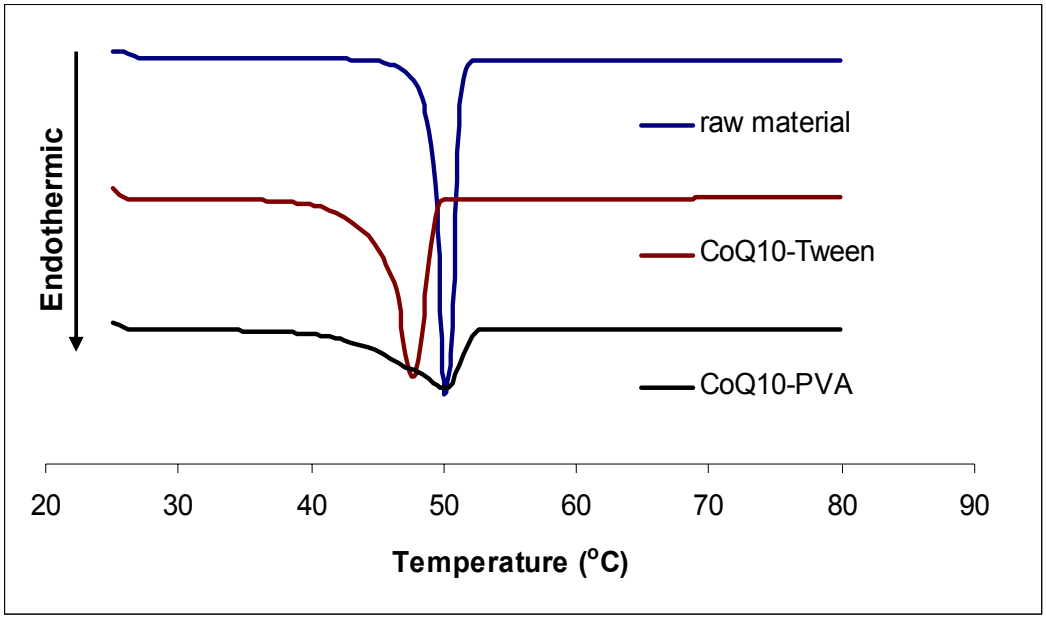


Fig. 4.59 DSC thermogram of the Coenzyme Q10 nanocrystals (stabilized by Tween 80 or PVA) and the raw materials

In addition, DSC thermograms (**Fig. 4.59**) revealed similar fusion temperature (about 50°C) of lyophilized formulation J and the raw material. However, the endothermic peak of the lyophilized CoQ10 nanocrystals was wider and lower than the raw material peak. These modifications were attributed to the presence of an imperfect crystallinity. According to Müller *et al.* [56] high pressure homogenization (HPH) can induce the change to an increased amorphous fraction. However, no amorphous corona was found, indicating transformation of the substance to the complete amorphous state or at least a major amorphous fraction.

According to this, neither stabilizer nor applied physical treatment such as HPH and drying process to the CoQ 10 brought about an alteration in the

crystalline state of the CoQ 10 to a completely amorphous state. Lyophilized CoQ 10 nanocrystals underwent a minor shift in their melting points and showed only slightly decreased crystalline intensity. However, no amorphous halo could be detected [183].

4.9.1.5 Testosterone

Testosterone nanosuspensions were produced by the Nanopure® XP variant H42. The 3 different starting materials of the testosterone production obtained after spray drying and tetstosterone produced by standar HPH (Testo-M) were investigated by DSC to study the crystalline state. This showed that the spray drying had almost no influence on the crystalline state of the starting materials and only a slight influence on the melting point of the spray-dried testosterone (**Fig. 4.60**).

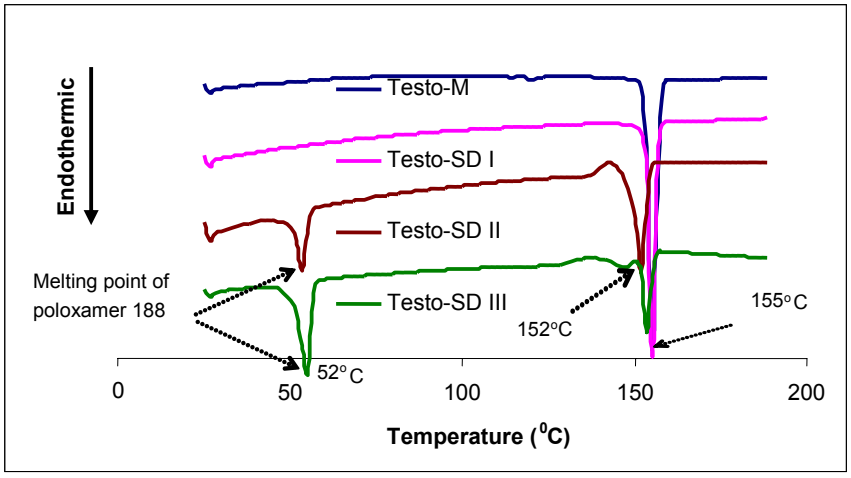


Fig. 4.60 DSC curves of various testosterone starting materials (composition of formulations is given in **Table 4.5**, page 93)

As shown in the DSC thermograms in **Fig. 4.60**, all Testosterone spray-dried starting material (SD-I to SD-III) and the micronized material (Testo-M) showed similar behaviour. Only a slight shift in the melting point (155°C to 152°C) was observed. These modifications were also attributed to the presence of poloxamer 188. It was also found that the addition of surfactant (poloxamer 188) resulted in drug powders with improved properties, such as

improved powder flowability and improved diminution effectiveness during the HPH [155].

4.9.2 PXRD

X-ray diffraction is used to study the atomic and molecular structure of crystalline substances such as drugs and excipients. The sample, a single crystal or powder, is exposed to x-rays at various angles; the diffraction patterns produced are then compared with reference standards for identification. X-rays diffraction patterns (diffractograms) can be used to confirm the crystalline nature of a sample. Therefore, this information is used to verify whether the substances are amorphous, partially amorphous crystalline or fully crystalline [184] as well as the polymorphic form being present.

4.9.2.1 Rutin

To confirm the crystalline state of the dried rutin nanocrystals, x-ray diffraction was performed with all of the lyophilized rutin nanocrystals stabilized with the various stabilizers. X-ray diffraction patterns were visualized in diffractograms. All XRD patterns can be seen in **Fig. 4.61**, proving the crystallinity of lyophilized rutin nanocrystals with the different stabilizers. The diffractograms reveal no different peaks for all rutin nanocrystals. All the peaks were confirmed as finger print of rutin. In addition, the diffractogram showed a similarity of the peak intensities for all rutin formulations.

According to these results, all nanocrystals were still in the same crystalline state as the raw material. Applying energy by being homogenized and subjecting them to the drying processes did not transform rutin nanocrystals to be fully or partially amorphous. Likewise the different stabilizers did not influence the crystallinity of the rutin nanocrystals. In general, more crystalline substances are physically more stable compared to amorphous forms. Therefore, lyophilized rutin nanocrystals will be physico-chemically stable during the storage time. In addition, better physicochemical properties such as the observed enhanced solubility and dissolution velocity can be attributed to the particle size reduction and not to alterations in crystalline state.

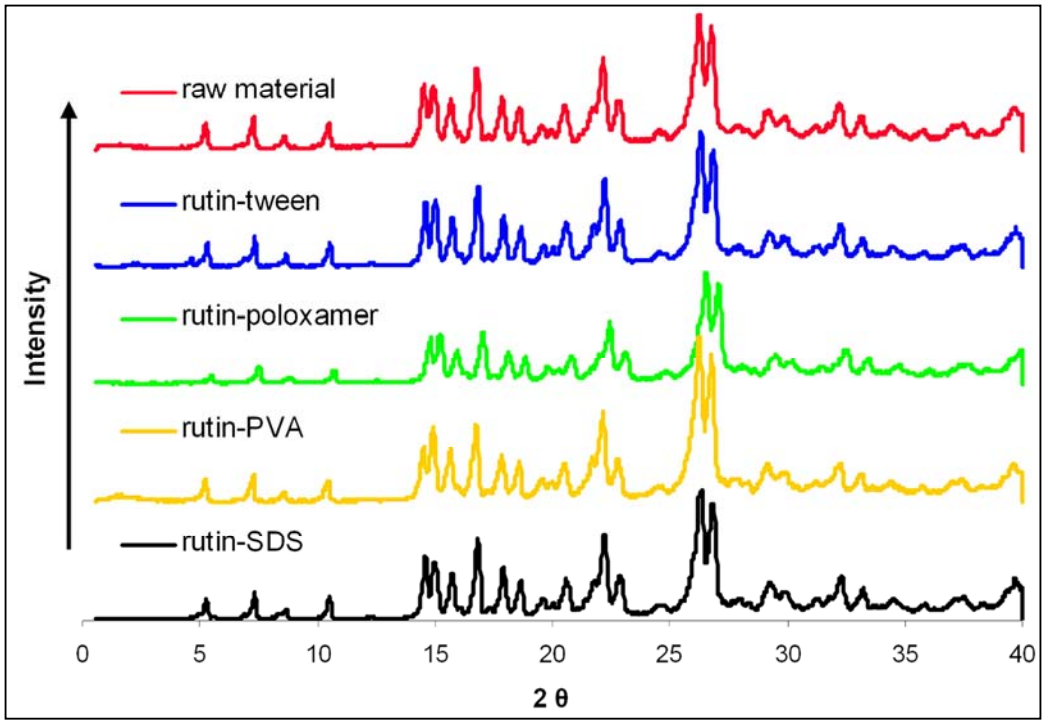


Fig. 4.61 X-ray diffractogram pattern of all lyophilized rutin nanocrystals and raw materials

4.9.2.2 Hesperidin

Similar to rutin nanocrystals, dried hesperidin nanocrystals were investigated by powder x-ray diffractions. In this study, the peaks for each hesperidin in the difractogram pattern were compared which each other and with a diffractogram of the raw material as reference. In this case the hesperidin raw material was proven already as being in a crystalline state. In addition, peak intensities were also studied to investigate crystallinity of the hesperidin nanocrystals. **Figure 4.62** reveals that all of the peaks were at the same position as in the raw material without new peaks. These results confirm that there was no decreased crystallinity in the hesperidin nanocrystals that might indicate alteration to amorphous state.

Dried hesperidin nanocrystals have the same finger prints as the raw material. In addition, the intensity of the peaks was about the same. Again, this study found that applying energy during homogenization and drying process did not transform the crystalline state and dried hesperidin nanocrystals were still in the same crystal form. No process led to an amorphous state.

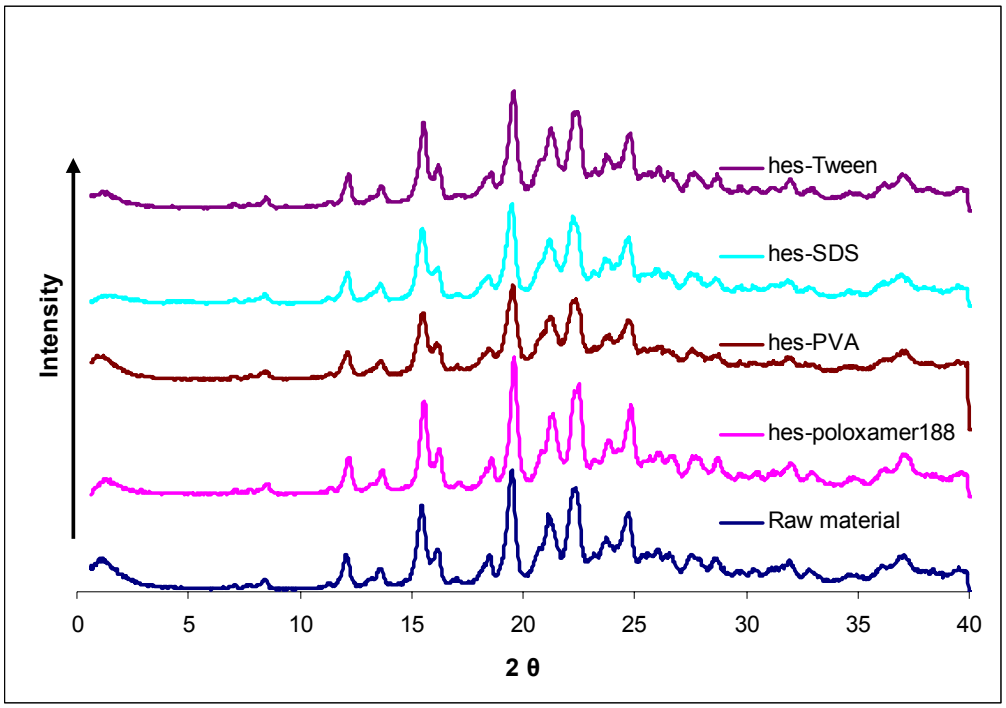


Fig. 4.62 X-ray diffractogram pattern of all lyophilized hesperidin nanocrystals and raw material

4.9.2.3 Ibuprofen

Like with the other drugs, the crystalline state of the ibuprofen nanocrystals was evaluated by x-ray diffraction. The diffractogram patterns obtained by PXRD confirmed that the HPH process did not alter the crystalline state. All diffractogram patterns of the ibuprofen nanocrystal powders (lyophilized or spray-dried) were similar to the raw material. The peaks around 6, 16, 20 and 22 degrees 2θ are characteristic finger prints for crystalline ibuprofen. With the SDS used as a stabilizer in the spray-dried nanosuspension, peaks were exhibited around 2, 5 and 7 degrees 2θ of the nanocrystals. Those peaks are in agreement with difractogram of physical mixing ibuprofen-SDS indicating these peaks belong to SDS and no polymorph is present. By comparison, the diffractogram pattern of the physical mixtures ibuprofen-SDS was similar to that of the ibuprofen nanocrystals stabilized by SDS (**Fig. 4.63**).

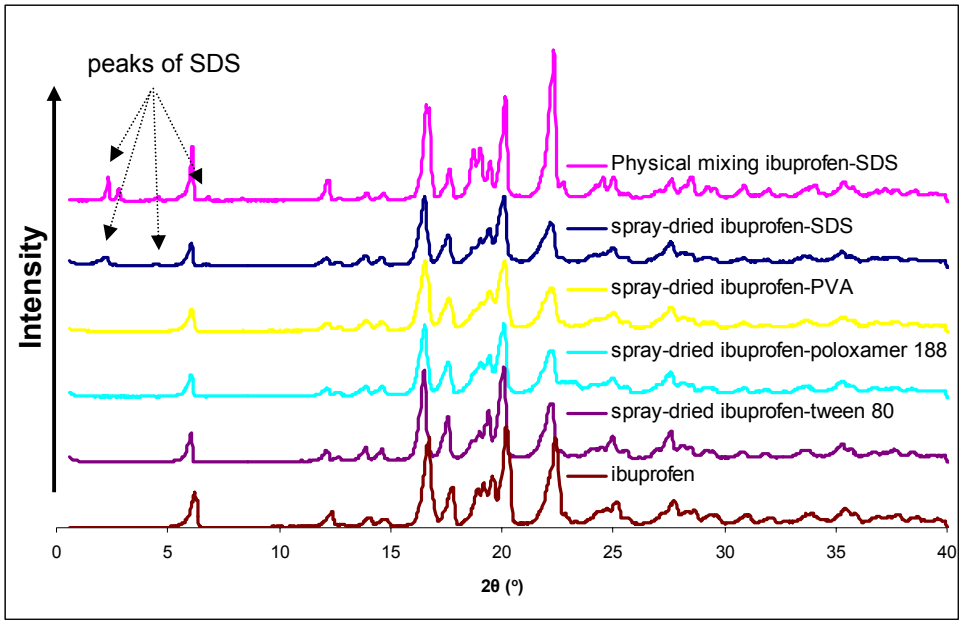


Fig. 4.63 X-ray diffractogram patterns for all spray-dried ibuprofen nanocrystals and raw material and physical mixture of ibuprofen with SDS (mixing ratio 1 : 0.2)

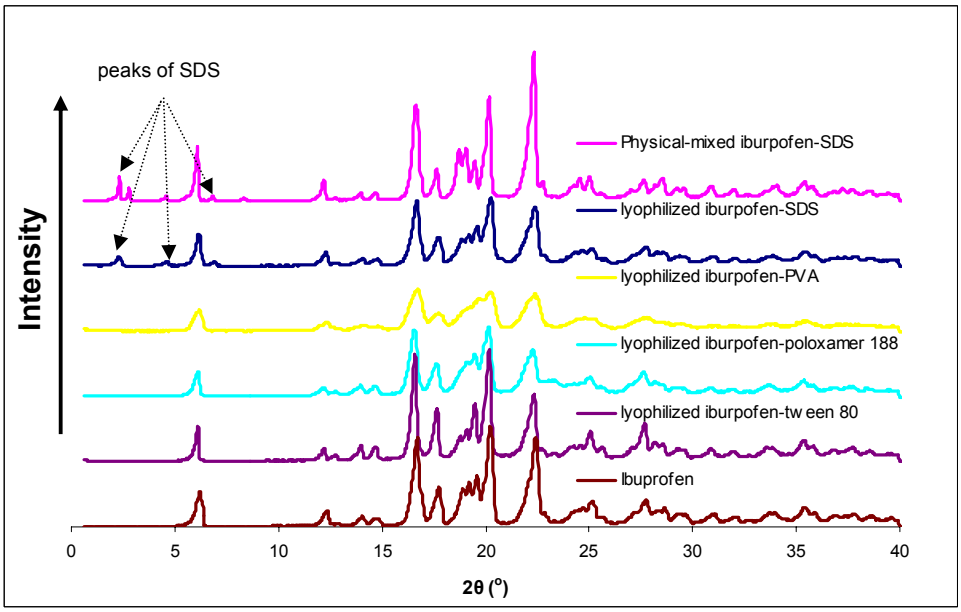


Fig. 4.64 X-ray diffractogram patterns for all lyophilized ibuprofen nanocrystals and raw material and physical mixture of ibuprofen with SDS (mixing ratio 1 : 0.2)

The peak location and peak intensities of the ibuprofen nanocrystals are comparable within each other and to the raw material, for both spray-dried

and lyophilized nanosuspensions (**Fig. 4.63 and 4.64**). They did not significantly reveal decreased intensity [182].

4.9.2.4 Coenzyme Q10

X-ray diffraction was performed also to confirm the crystalline state of dried CoQ10 nanocrystals (both formulations). The diffractograms in **Fig. 4.65** shows the X-ray diffraction patterns. This patterns show the crystalline intensity of both lyophilized CoQ10 nanocrystals with different stabilizers compared to the raw material. The diffractograms reveal no differences in peak location between the CoQ10 nanocrytals and the raw material. All peaks were confirmed as finger prints of CoQ10. The diffractograms showed only a reduction in the intensity of the peaks. This could indicate that the crystalline intensity of the CoQ10 nanocrystals was a little lower compared to the raw material. The reduced peak intensities can also be due to fraction of the crystals in the sample, because no amorphous halo could be detected, that would have otherwise indicated completely amorphous material [183].

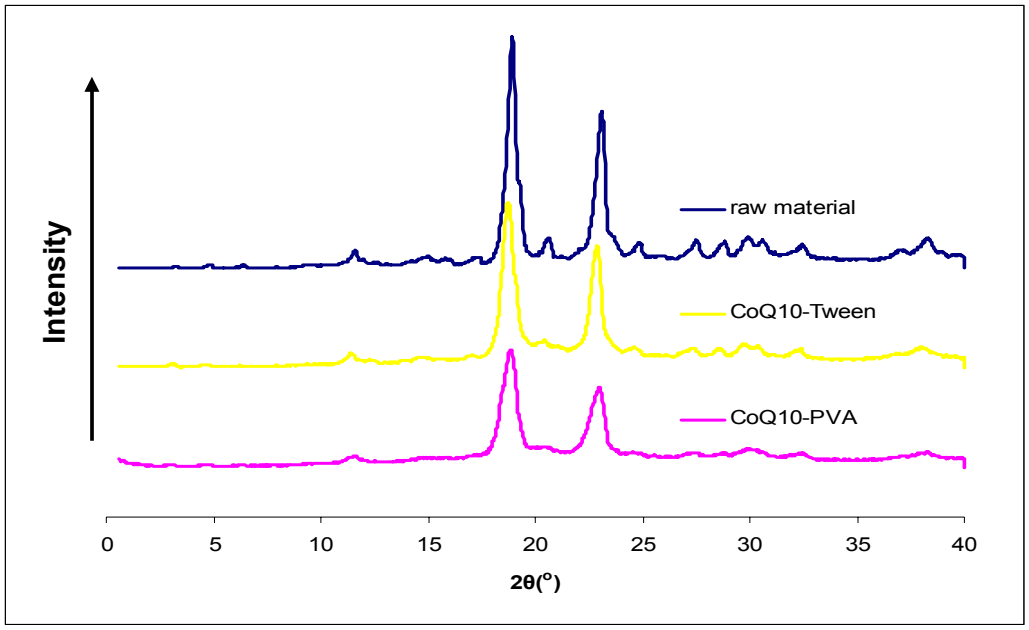


Fig. 4.65 X-ray diffractograms of the Coenzyme Q10 nanocrystals (stabilized by Tween 80 and PVA) and the raw materials

According to these results, both nanocrystals were still in the same crystalline state as the raw material. Applying energy through homogenization and drying processes did not transform CoQ 10 nanocrystals into completely or partially

amorphous form and brought about only a minor diminution of crystalline peak intensity. One of the advantages of the crystalline forms is greater stability compared to partially or completely amorphous forms. In addition, the change in physicochemical properties, such as enhanced solubility and dissolution velocity are attributable to particle size reduction and not to change in crystallinity alteration to completely amorphous form.

In summary, the crystallinity study of the drug nanocrystals confirms that all nanocrystals remained in a crystalline form. Applying energy during homogenization and the drying process did not alter or influence the crystallinity of the drug nanocrystals and the nanocrystals were not transformed to completely or partially amorphous form. Slightly reduction of crystallinity was only evident in lyophilized CoQ10. However, no amorphous halo of lyophilized CoQ10 could be detected which would have otherwise indicated amorphous material. Crystalline drug nanocrystals provide better physicochemical properties such as physical and chemical stability, as discussed in the physicochemical properties section.

4.10 Saturation Solubility

Due to poor solubility in water, many drugs show inadequate bioavailability after oral administration. This poor solubility also imposes other restraints on further pharmaceutical use. As mentioned in the introduction, there are many approaches which can be used to increase the solubility of poorly soluble drugs in water. In recent years, increasing attention has been paid to alter the physical, chemical and biological properties of drugs, especially to increase solubility and thus improving bioavailability. Particle size reduction such as the production of nanocrystals, is one of the most promising pharmaceutical technology approaches in improving the physicochemical properties of substances. It is generally acknowledged that drug nanocrystals offer a variety of physicochemical advantages over large-sized drugs, including the possibility for increased solubility, bioavailability and physical dispersion stability. Buckton *et al.* assume that enhancement of solubility is valid only for sparingly soluble particles of less than 1 μm in size [74, 75]. As reported before, in general the particle size average of re-dispersed drug nanocrystals

was below 1 μm. Therefore, rutin drug nanocrystals can in theory overcome the bioavailability problem associated with low aqueous solubility.

The fundamental basis for the observation that size influences solubility is found in the Kelvin equation, which gives the increase in pressure for a curved surface by comparing pressure in a small bubble (of radius *r)* with that in an infinitely large one (reference). It is argued that the Kelvin equation is equally applicable to the solid-liquid interface. Replacing the pressure terms with solubilities (C_s), as activity coefficients are effectively set equal to unity in the dilute solutions considered here, produces the so-called Gibss-Kelvin relation. The Gibbs-Kelvin relation is used as a model for crystallisation, whereby homogeneous nucleation will occur when a stable nucleus is formed; a stable nucleus being one which has exceeded a certain size and thus is described as having a lower solubility (and consequent longer existence). It is also considered a method by which solid-liquid interfacial energies can be determined from changes in solubility as a function of size [185]. Finally, the Kelvin-Gibss equation can be presented in the following forms:

$$\log \frac{C_r}{C_\sim} = \frac{2\gamma V}{2.303rRT} \qquad \textit{equation 15}$$

or

$$\ln \frac{S_r}{S_\infty} = \frac{2\gamma M}{\rho r R T} \qquad \textit{equation 16}$$

where C_r/S_r and $C_\sim/S_\infty$ are the solubility of a spherical particle of radius *r* and the solubility of a noncurved solute surface ($r\rightarrow\infty$), respectively; *V* is the molar volume of the solute; M is molarities of the solute; *ρ* is density; γ is the solid–liquid interfacial tension; *R* is the universal gas constant; *T* is the absolute temperature; and *r* is the particle radius.

As mentioned in chapter II, this equation form (equation 15) is presented in many pharmaceutical text books such as Physical pharmacy and Bentley's Textbook of Pharmaceutics. The increase in saturation solubility can be explained also by the Ostwald–Freundlich equation (equation 16). The Ostwald–Freundlich equation described the dependency of the saturation

solubility in comparing the solubilities of two particles with different diameters [60, 74, 186, 187].

4.10.1 Rutin nanocrystals

It has to be differentiated between kinetic and thermodynamic solubility. The thermodynamic solubility is the concentration in the solute in equilibrium with a normally sized powder, this condition being physically stable. The kinetic solubility, e.g. achieved by size reduction or from amorphous powders, is higher than the thermodynamic solubility but physically not long–term stable. With regard to the thermodynamic solubility, it is a supersaturated solution with higher energy, transforming to a lower energy level and lower concentration by precipitation of crystals, finally reducing the concentration of the kinetic solubility. The kinetic saturation solubility of rutin nanocrystals was investigated over 1 week. The results of this testing showed that the solubility of the rutin nanocrystals in water was increased at 25^{o}C. The kinetic solubility was with 133.3 ± 3.8 µg/ml higher than the one of the raw material (**Fig. 4.66**). The equilibrium of its solubility was achieved on fifth to seventh day. Saturation solubility of the raw material was 124.4 ± 1.8 µg/ml. According to the crystalline state investigation, the solubility enhancement of the rutin nanocrystals is not due to presence of the rutin amorphous form. It is due to the particle size reduction to the submicron range. This result is in agreement with the Kelvin-Gibbs equation and Ostwald–Freundlich equation [60, 74, 186, 187].

The increase in solubility is only around 10%, and not as high as reported for other compounds (e.g. increase by factor 2-10). For coenzyme Q_{10} nanocrystals an increase by a factor about 500 was observed. However the important point is, that despite the low increase in kinetic solubility, there was a clear advantage of the nanocrystals in dissolution velocity (cf. below).

It is remarkable that the kinetic solubility remained for seven days, without decreasing to the thermodynamic solubility of the raw material. This is favorable for oral administration. Some nanocrystals show a disappointing low bioavailability because they dissolve in the gut, but immediately re-crystallization to large microcrystals occurs. Thus, the dissolved concentration and subsequently the bioavailability decrease.

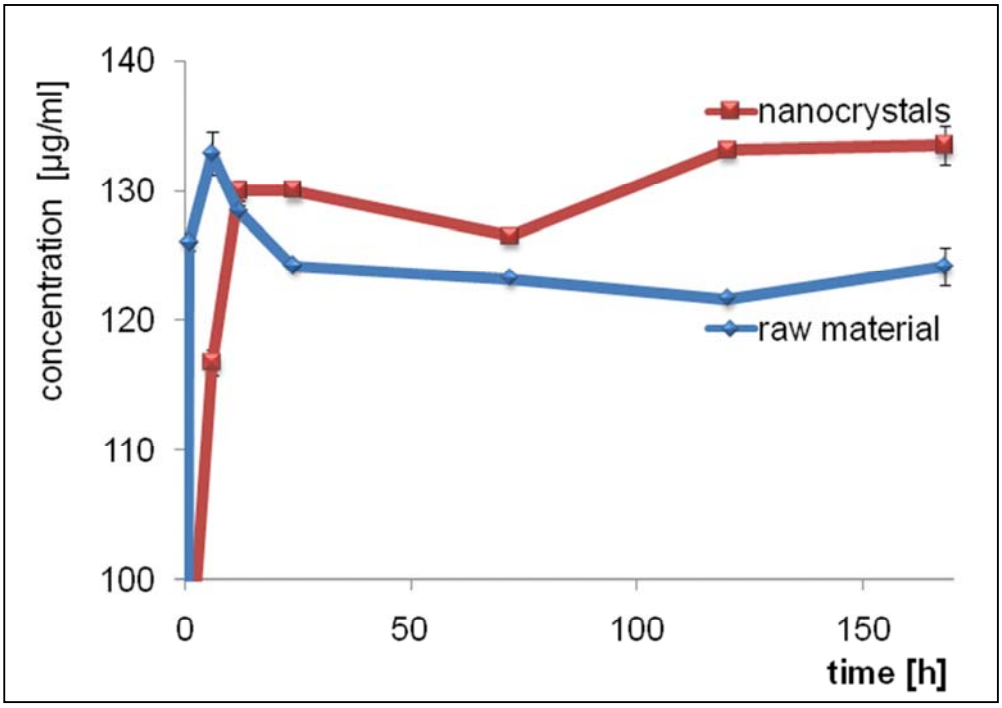


Fig. 4.66 Saturation solubility of the rutin nanocrystals (stabilized by SDS) and raw materials in water at 25°C

Surprisingly, during the first hours a similar high dissolved concentration was obtained for the raw material. Then the concentration decreased. The explanation for this is simple. The raw material was very fine, more than 10% of the particles were in the nanometer range (d10% = 0.8 µm). The powder was added in access for the solubility study. And the nanocrystals in the raw material dissolved immediately, giving logically the same kinetic solubility as nanocrystals from the nanosuspension. However, due to the presence of the micrometer crystals (act as crystallization cores) the concentration immediately decreased to the thermodynamic solubility by re-crystallization (growth of microcrystals being present). This effect nicely demonstrates why it is important to have no or little microcrystals in a nanosuspension. The microcrystals de-stabilize the kinetically supersaturated solutions. If microcrystals are not or only limited present – as in the nanocrystals formulation - the kinetic solubility remains unchanged, the system shows higher stability.

A slight solubility enhancement was observed in the other media (buffer solution having a pH of 6.8 and at pH 1.2). However, statistically ($P<0.05$) the kinetic solubility of both rutin nanocrystals and rutin raw material was not significantly different. For example, the saturation solubility of the raw material was of 72.8 ± 2.0 µg/ml in buffer solution having a pH of 1.2. It is not statistically different to the saturation solubility of rutin nanocrystals with 74.3 ±1.5 µg/ml in same dispersion medium. This is again attributed to the nanocrystals present in the micrometer sized powder. Adding the micrometer powder in access to the dissolution medium provides a sufficient amount of nanocrystalline material to achieve the kinetic solubility of nanocrystals. Obviously, in the buffers this kinetic solubility was stable during the observation period – despite the presence of microcrystals.

4.10.2 Hesperidin nanocrystals

Theoretically, according to Kelvin-Gibbs and Ostwald-Freundlich equations, the reduction of the drug particle size to the nanometer range can improve the solubility of that drug [60, 74, 186, 187]. As with rutin, solubility of the hesperidin nanocrystals was evaluated in three different media comprising water, buffer having a pH of 1.2 and 6.8 within 7 days. As predicted before, in all of those media, the solubility of the hesperedin was significantly pronounced.

In water, hesperidin solubility was improved about 5 fold. Saturation solubility of dried hesperidin nanocrystals in water was 87.2 ± 0.8 µg/ml higher. This was distinctly higher than the saturation solubility of hesperidin microcrystals (19.8 ± 0.4 µg/ml). In the literature it is mentioned that 1 g hesperidin dissolves in 50L water [114, 116]. Accordingly the solubility of hesperidin in water is 20 µg/ml, which differs little from the obtained result (**Fig. 4.67**).

In addition, saturation solubility was improved not only in water, but also in buffer. In buffer at pH 1.2 the solubility of nanocrystals was 24.0 ± 2.5 µg/ml or 2 times higher than the solubility of the raw material in the same medium (**Fig. 4.68**). In buffer at pH 6.8, the solubility increased more than 5 fold. Saturation solubility of hesperidin nanocrystals in buffer of pH 6.8 was with 62.0 ± 2.5 µg/ml higher compared to hesperidin microcrystals (12.2 ± 2.2 µg/ml) (**Fig. 4.69**).

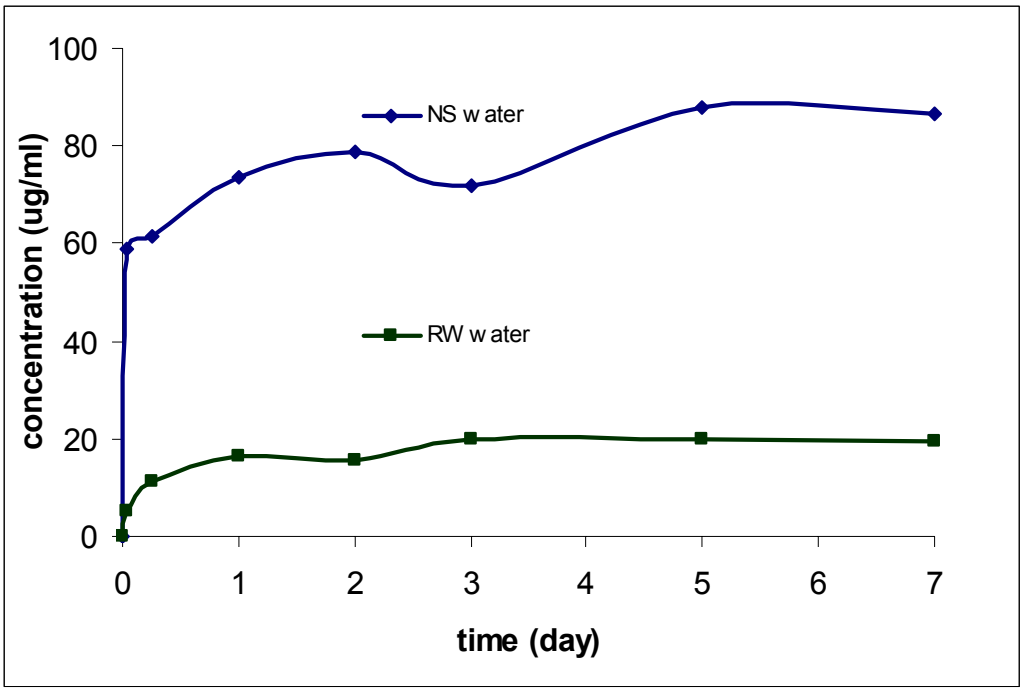


Fig. 4.67 Saturation solubility of heperidin nanocrystals and raw material in water at 25°C

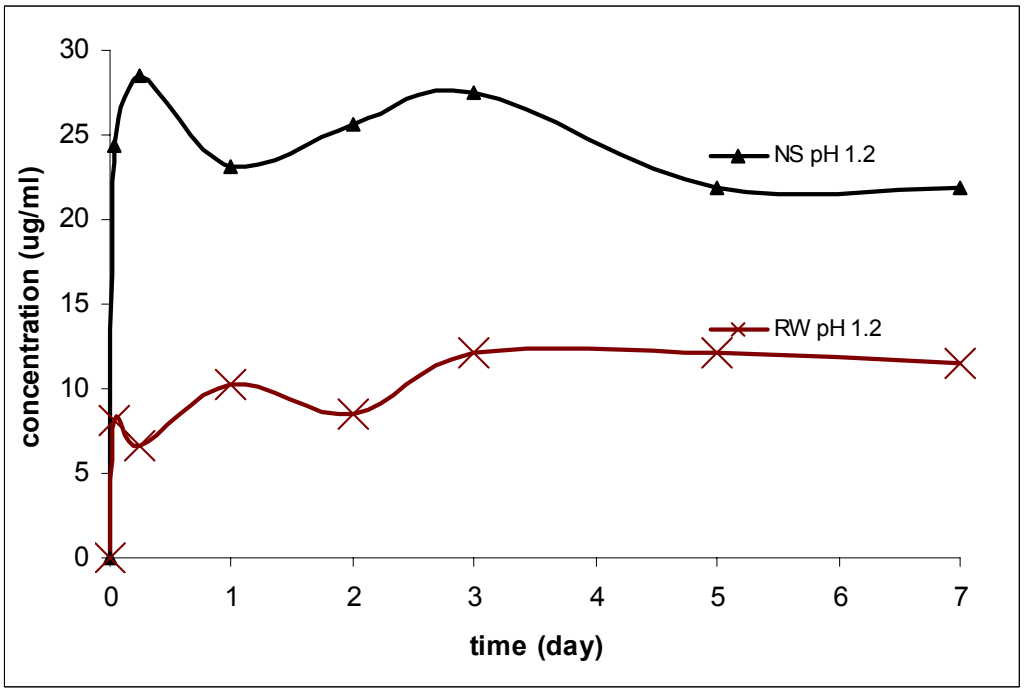


Fig. 4.68 Saturation solubility of heperidin nanocrystals and raw material in buffer having a pH of 1.2 at 25°C

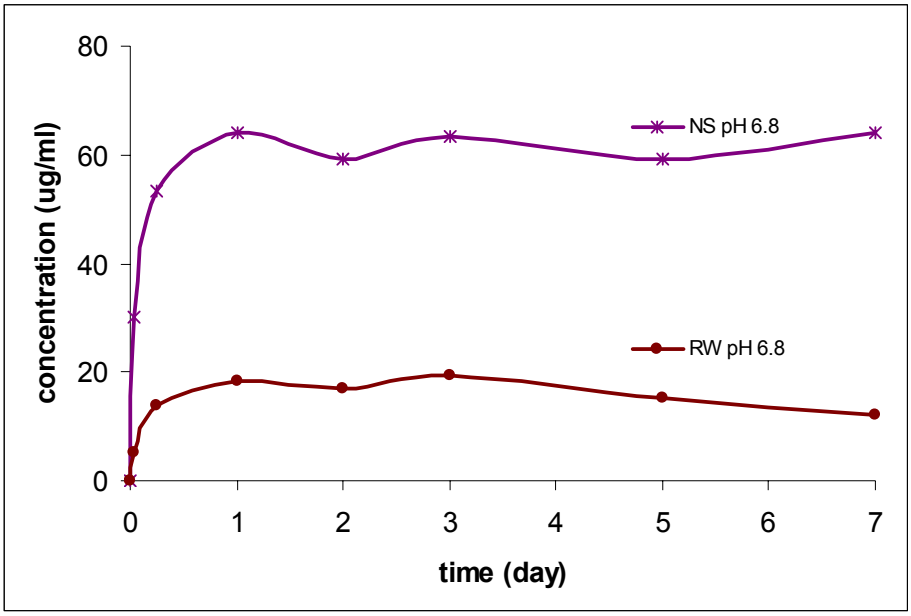


Fig. 4.69 Saturation solubility of heperidin nanocrystals and raw material in buffer having a pH of 6.8 at 25°C

Due to the presence of fine nanocrystals and potential a very small fraction of amorphous substance, the saturation solubility data at early stages (before equilibrium reached) clearly fluctuated. This was clearly evident especially in the kinetic solubility study in the buffer having a pH of 1.2. Afterwards, solubility decreased again. At equilibrium, the solubility was relativly steady at a certain concentration. In general, saturation solubility of the nanocrystals was distinctly higher than for microparticles.

4.10.3 Ibuprofen nanocrystals

Solubility of ibuprofen nanocrystals was also evaluated in three different media. As shown in **Fig. 4.70,** the saturation solubility of ibuprofen nanocrystals increased slightly but not to a significant extent. At steady state (after 3 days) ibuprofen nanocrystals showed a similar saturation solubility to ibuprofen microcrystals.

Similar to the saturation solubility in water, saturation solubility of ibuprofen nanocrystals in buffer at pH 1.2 was also evaluated. In buffer at pH 1.2, solubility was slightly enhanced at day 3 (at steady state conditions) compared to microcrystals. Unlike the saturation solubility in water, the saturation solubility of ibuprofen nanocrystals was initially lower than that of microcrystals. However after 3 days, saturation solubility increased for the

nanocrystals and was slightly higher than for microcrystal suspension. Again, saturation solubility at the early stages (before equilibrium reached) of the study clearly fluctuated due to presence of fine nanocrystals and potentially amorphous fractions of dried ibuprofen as mentioned before.

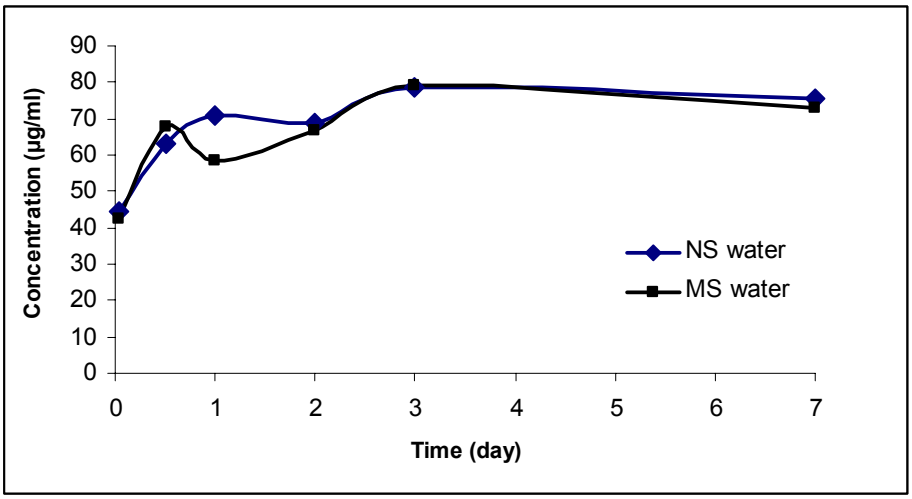


Fig. 4.70 Saturation solubility of ibuprofen nanocrystals and raw materials in water at 25°C

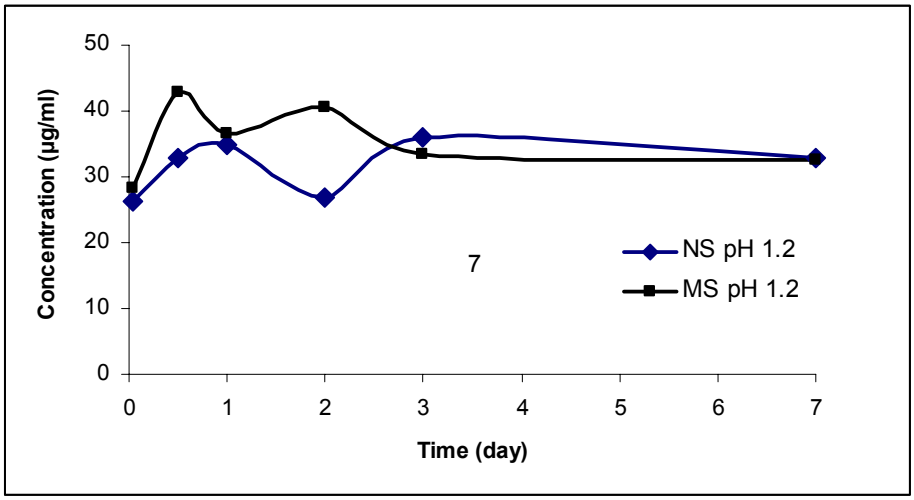


Fig. 4.71 Saturation solubility of ibuprofen nanocrystals and raw materials in buffer having a pH of 1.2 at 25°C

Chemically structure of ibuprofen is weak acid. Therefore, in a basic solution it will form salts and the solubility will be increased. Solubility of the ibuprofen nanocrystals was compared with ibuprofen microcrystals in buffer solution

having a pH of 6.8. In this solution, both ibuprofens will form the salt of ibuprofen and solubility will be increased.

The particle size reduction influenced the saturation solubility of ibuprofen in buffer at pH 6.8. **Fig. 4.72** shows the enhanced solubility of ibuprofen nanocrystals in comparison to ibuprofen microcrystals. In this medium, the saturation solubility of ibuprofen nanocrystals was 1824±8.4 µg/ml, only slightly higher than the solubility of ibuprofen microcrystals. In the same condition solubility of ibuprofen microcrystals was only 1751±6 µg/ml. The difference in saturation solubility between ibuprofen nanocrystals and microcrystals was assumed by the difference in particle size of both ibuprofens. This result indicates that superiority of drug nanocrystals compared to drug microcrystals points to better physicochemical properties in the drug nanocrystals.

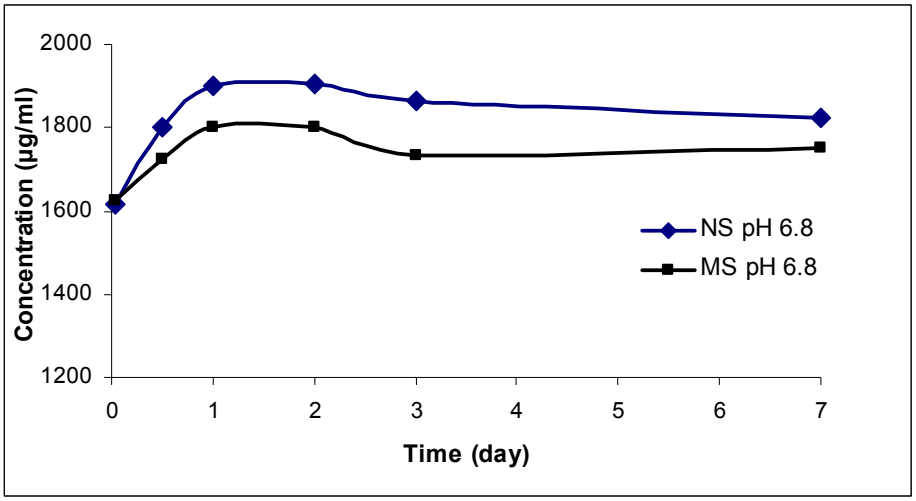


Fig. 4.72 Saturation solubility of ibuprofen nanocrystals and raw materials in buffer having a pH of 6.8 at 25°C

In general it can be concluded that in case of ibuprofen there was not solubility enhancement (water, at buffer pH 1.2) or very little (at buffer pH 6.8). This is well in agreement with the theory, because ibuprofen is not a poorly soluble drug, with solubility up to almost 2000 µg/ml. According to Buckton, a pronounced increase can only be found for sparingly soluble drugs [74].

4.10.3 Coenzyme Q10 nanocrystals

Investigation of the kinetic saturation solubility of CoQ10 nanocrystals was performed over a period of 5 days. Results of this testing showed that solubility of CoQ10 nanocrystals in water distinctly increased at 25^{o}C. Equilibrium of kinetic solubility was achieved on third and fifth day. The kinetic solubility of lyophilized CoQ10 nanocrystals in water was distinctly higher and determined to be 15.6 µg/ml (**Fig. 4.73**). Limit of detection (LOD) was 0.035 µg/ml, meaning that solubility was increased at least by a factor of 446. Kinetic solubility at early stages (before equilibrium reached) was distinctly found to be around 20 µg/ml and decreased again until reached equilibrium. This phenomenon could be caused by the presence of amorphous fractions of CoQ10 nanocrystals, as shown by DSC thermogram and x-ray diffractogram. After oversaturation, the amorphous fraction of the nanocrystals was re-crystallized and transformed to the crystalline form. Afterwards, equilibrium was reached. Therefore, solubility was decreased and at equilibrium the solubility will be constant. However, solubility at equilibrium was distinctly higher than for the CoQ10 microcrystals, this means CoQ10 microcrystals were not soluble in water (solubility was below the detection limit of HPLC).

Kinetic solubility enhancement was not observed in other media (buffer solutions having a pH of 6.8 and pH of 1.2). In both media, CoQ10 did not dissolve or solubility was below the detection limit of HPLC. According to the crystalline state investigation, solubility enhancement of the CoQ10 nanocrystals had little relation to the presence of increasing amorphous fractions. Instead, it is mostly explained by reduction in particle size to its submicron particulate character.

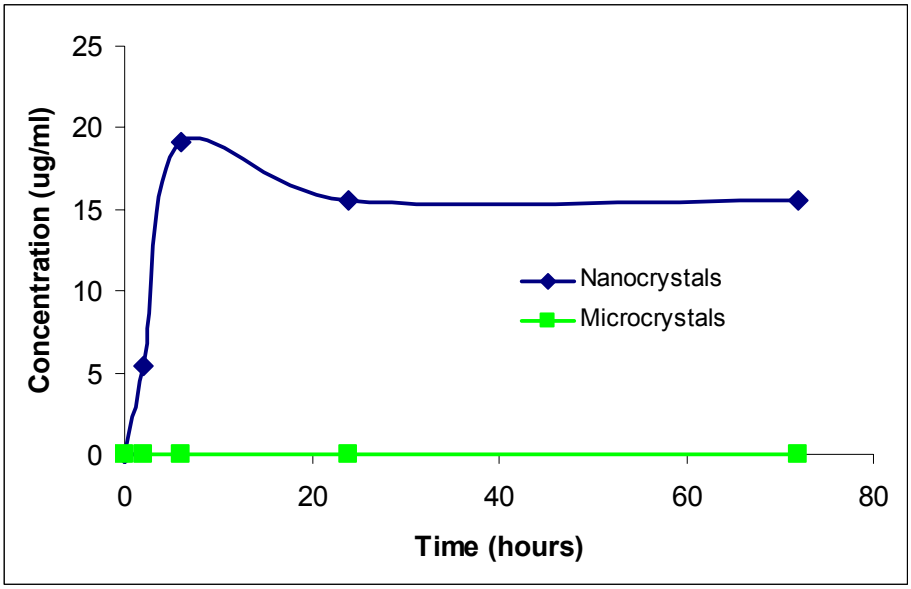


Fig. 4.73 Kinetic solubility of Coenzyme Q10 nanocrystals (stabilized by PVA) and raw material in water at 25°C

4.11 Dissolution velocity of drug nanocrystals

The most important feature of nanocrystals is the increase in the dissolution velocity of the compound, of course caused by the increase in saturation solubility. The law of Noyes-Whitney describes the dissolution velocity dc/dt [34]:

$$\frac{dc}{dt} = \frac{D \cdot A}{h}(c_s - c_x) \qquad \textit{equation 17}$$

D is the diffusion coefficient, A the surface area, c_s is the saturation solubility, c_x the bulk concentration, and *h* is the so-called "diffusional distance" over which the concentration gradient occurs. It is obvious that an increase in the surface area consequently increases the dissolution velocity, e.g. exploited in micronized and nanosized products [60, 187]. In addition, drug nanoparticles are characterized by an increase in saturation solubility c_s. According to Noyes-Whitney, the increase in c_s - in addition to the enlarged surface area - further increases the dissolution velocity.

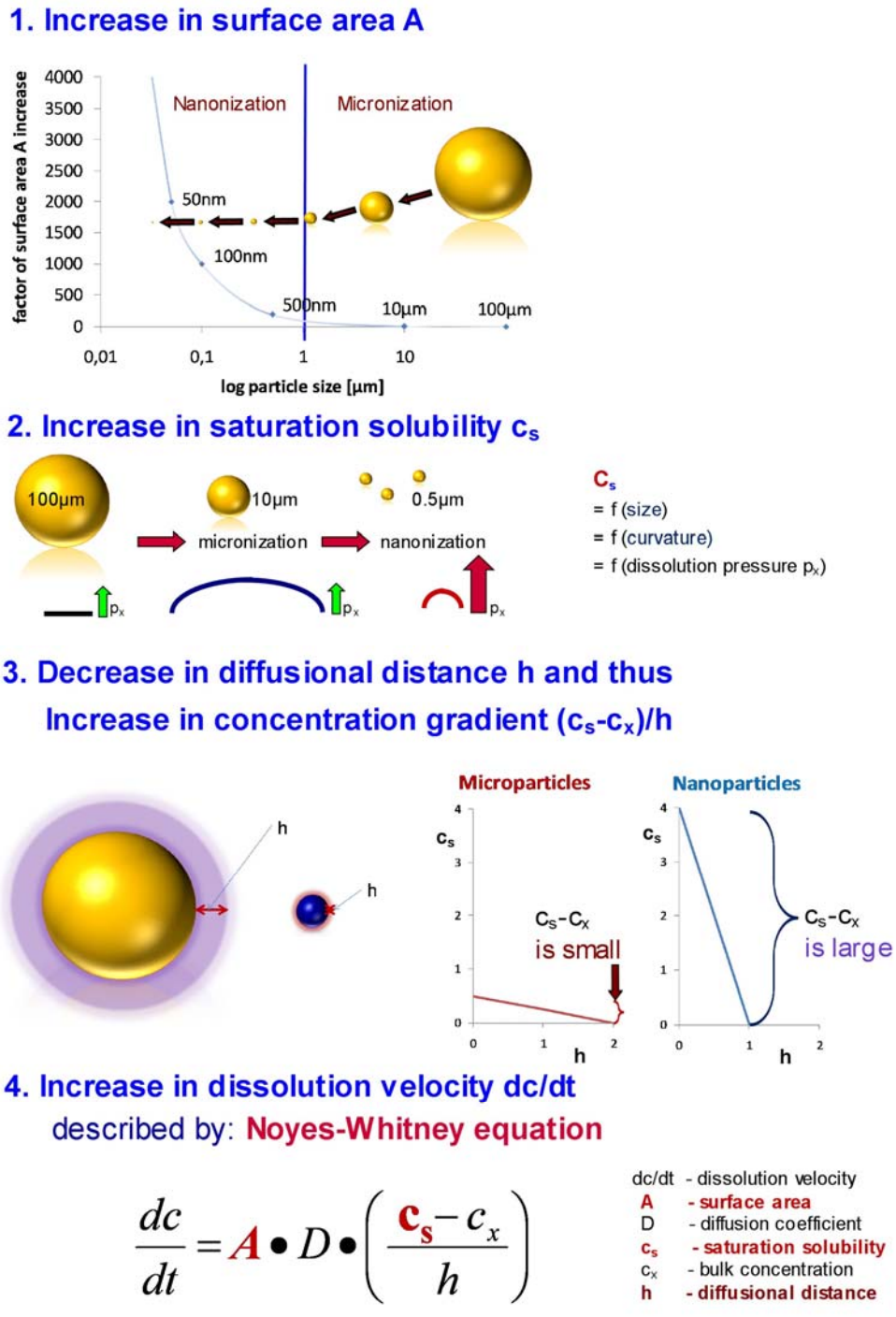


Fig. 4.74 Transfer of microcrystals to nanocrystals leads to an increase in surface area (upper). Increase in saturation solubility c_s, decrease in diffusional distance h and increase in the concentration gradient c_s-c_x/h. Both effects increase the dissolution velocity dc/dt (with permission after [188])

The final saturation solubility achieved is, of course, compound-specific based on the differences in compound specific dissolution pressures. The dissolution velocity is reversely proportional to the diffusional distance *h*, which means that reducing *h* leads to a further increase in dissolution velocity. According to the Prandtl equation [70], the diffusional distance *h* decreases for very small particles. The simultaneous increase in saturation solubility c_s and decrease in *h* leads to an increased concentration gradient $(c_s - c_x)/h$ (**Fig. 4.74**), thus enhancing the dissolution velocity in addition to the surface effect [56, 57, 60, 70, 75].

4.11.1 Rutin nanocrystals

Dissolution studies of the lyophilized rutin nanocrystals clearly showed this advantageous phenomenon. Dissolution velocities of the dried rutin nanocrystals were distinctly superior compared to the raw material (despite having a large fraction of nanosized crystals present in the raw material). Lyophilized rutin nanocrystals were almost completely dissolved within 15 minutes in water, buffer at pH 1.2 and pH 6.8. In contrast to this, the raw material (rutin microcrystals) in water, buffer of pH 1.2 and of pH 6.8 only dissolved to 65%, 78% and 81% within 15 minutes, respectively (**Fig. 4.75**, upper and lower). A large percentage of the dissolved raw material is attributed to the nanosized fractions resent in the raw material. In case of the class II drugs of the biopharmaceutics classification system (BCS), the increase in dissolution velocity is most important because it is the rate limiting step for oral absorption. Therefore, despite the fact that the kinetic solubility is only limited increased for the rutin nanocrystals, the roughly doubled dissolution velocity is the key point for oral absorption enhancement.

The increased dissolution velocity of the lyophilized rutin nanocrystals is in agreement with the theory of dissolution by Noyes-Whitney. These improved physicochemical properties can lead to the next step of investigation of the lyophilized rutin nanocrystals. The nanocrystals can be incorporated into solid dosage forms such as tablets, pellets or capsules. Based on these physicochemical studies, lyophilized rutin nanocrystals provide excellent physicochemical properties such as increased dissolution velocity, enhanced kinetic solubility in water and improved physical stability. Therefore, oral

dosage forms of dried rutin nanocrystals should lead to an improved oral bioavailability.

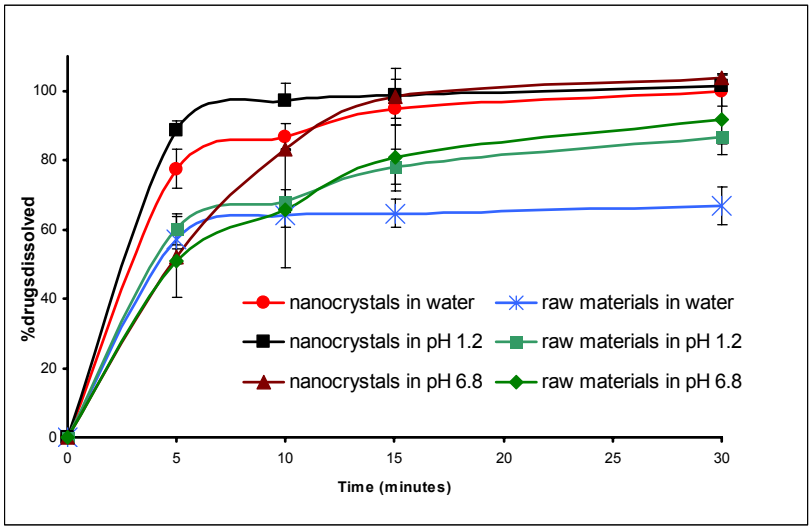


Fig. 4.75 Dissolution profile of rutin nanocrystals and raw materials in water and buffers pH 1.2 and 6.8

4.11.2 Hesperidin nanocrystals

Similar to rutin nanocrystals, the dissolution velocity of hesperidin nanocrystals was subjected to intensive evaluation in three different media. As expected, dissolution of hesperidin nanocrystals was distinctly superior to the raw material (microcrystals). Only in 15 minutes, all hesperidin nanocrystals were dissolved completely in water, buffer having a pH of 1.2 and 6.8. In contrast, hesperidin microcrystals were only to 37%, 34% and 36% dissolved in water, buffer having a pH of 1.2 and 6.8 respectively (**Fig. 4.76**). This result shows the superiority of hesperidin nanocrystals in dissolution behavior and is in agreement with the Noyes– Whitney equation.

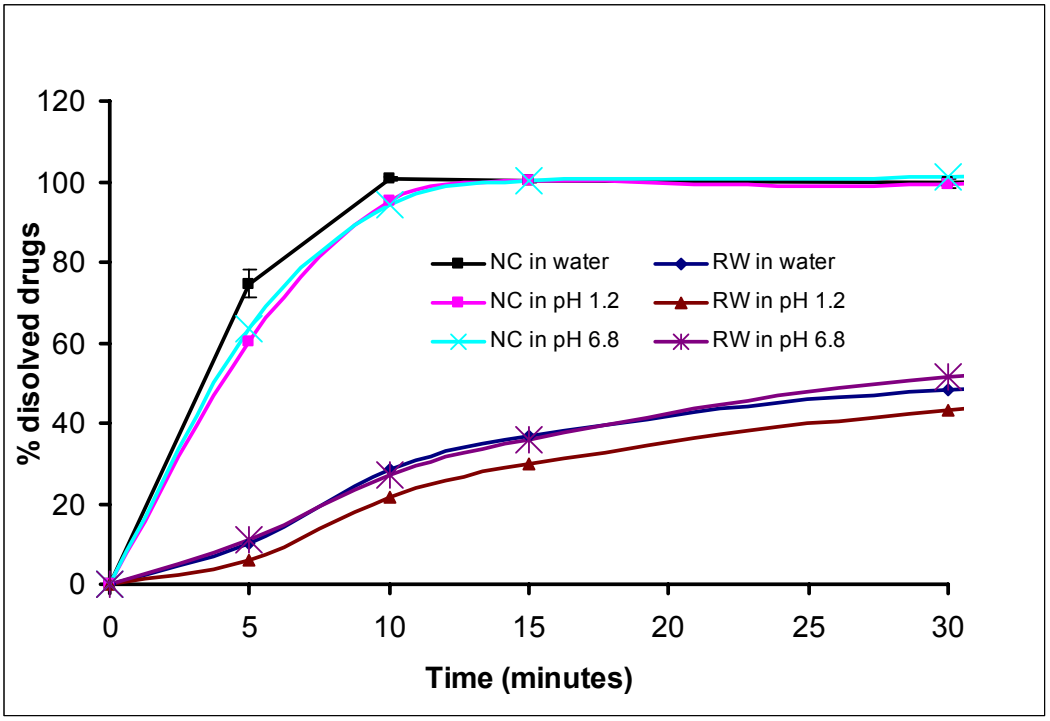


Fig. 4.76 Dissolution profile of hesperidin nanocrystals and raw materials in water and in buffer pH 1.2 and 6.8

According to this result, hesperidin nanocrystals are suitable for incorporation into solid dosage form, such as tablets, capsules, pellets etc. The final product of a solid dosage form containing hesperidin nanocrystals will be evaluated with respect to dissolution testing and compared to marketed dosage forms.

4.12 Preparation of solid dosage forms

Nanosizing refers to the reduction of drug particle size down to the submicron range. While reduction of particle size has been employed in pharmaceutical industry for several decades, recent advances in milling technology and our understanding of such colloidal systems have enabled the production of drug particles of 50–200 nm size in a reproducible manner. The sub-micron particles are stabilized with surfactants or polymers in nanosuspensions which can be further processed into standard dosage forms suitable for oral administration. These nanosuspensions offer increased dissolution rates for drug compounds and complement other technologies used to enhance the bioavailability of insoluble compounds (BCS Class II and IV), such as

solubility enhancers (i.e. surfactants), liquid-filled capsules or solid dispersions of drugs in their amorphous state.

4.12.1 Tablet

Based on the Noyes-Whitney principle, reduction in the particle size will increase the dissolution rate due to increased effective particle surface area. This size-dependency comes only into effect for particles of a size below approximately 1 µm (submicron particulate) — a phenomenon observed in tabletting which leads to an increase of the dissolution rate of such fine drug particles [75].

4.12.1.1 Formulation

a. Rutin

Formulations of the rutin tablets can be seen in **Table 3.6**. Rutin was admixed to the tablet excipients (**Table. 3.6**) by a tumbler .The tablets were prepared using direct compression by a single punch tablet machine.

Tablets were prepared by direct compression to avoid agglomeration of the rutin nanocrystal during producition. Avicel PH 101, AcDiSol, Explotab are excipients usually used in direct compression. These excipients have good characteristics for direct compression such as good compressibility and excellent flow ability. Therefore, these excipients were chosen as filler, binder and tablet disintegrates. As lubricant, antiadherent and glidant, magnesium stearate and talc were used.

Table 3.6 Rutin tablet formulations

Formulation	NC (mg)	MC (mg)	Excipients (mg)				
			AV	AC	Ex	Mg	T
A	50		42	5		2	1
B	50		42		5	2	1
C		50	42	5		2	1
Market		50					

NC = rutin nanocrystal, MC = rutin microcrystal, T = Talc, Mg = Mg stearic, AV = Avicel PH 101, AC = AcDiSol, Ex = Explotab, Market = marketed tablet.

b. Hesperidin

On the market, no oral hesperidin tablet is found in single dose form. Hesperidin is normally combined with other drugs such as in Daflon®500 tablets, which contain diosmin. Daflon®500 tablet contain of 50 mg hesperidin and 450 mg diosmin [189, 190]. In this study, only the dissolved hesperidin from the tablet was evaluated. Therefore, in formulations prepared from nanocrystals, diosmin was not included. In addition, diosmin could not be provided from industry sources. For weight adjustment to 700 mg, tablet filler was added such as microcrystals cellulose (Avicel PH 101) to substitute for diosmin. The complete hespridin tablet formulations are presented in **Table 3.7**. Microcrystal cellulose (Avicel PH 101) was used as tablet filler due to good compressibility properties. AcDiSol and Explotab were used as dry disintegrant. Mg stearate and Talc were incorporated as lubricant, glidant and antiadherent.

Table 3.7 Hesperidin tablet formulations

Formulation	NC (mg)	MC (mg)	Excipients (mg)				
			AV	AC	Ex	Mg	T
X	50		594	35		14	7
Y	50		594		35	14	7
Z		50	594	35		14	7
Market		50					

NC = hesperidin nanocrystal, MC = hesperidin microcrystal, T = Talc, Mg = Mg stearate, AV = Avicel PH 101, AC = AcDiSol, Ex = Explotab, Market = marketed tablet, .

The subsequent process was similar to that of the rutin tablet production. Dried hesperidin nanocrystals were admixed gently to the other tablet excipients. A tumbler was used to mix hesperidin nanocrystals with the excipients. The tablet mass was finally compressed using a single punch tablet machine. Similar to rutin tablet productions, hesperidin nanocrystal–loaded tablets were produced using direct compression.

4.12.1.2 Direct Compression

a. Rutin

Direct compression is known as the simplest method for tablet production. This method requires only a short time to obtain a final product. However not all drugs can be produced using direct compression method. To use this method, drugs must have good compressibility drugs and as a rule, crystalline drugs are used in direct compression. Therefore dried rutin nanocrystals are a suitable nanocrystals powder for this method. For direct compression it is useful to use excipients with good flowability and good compressibility. Avicel PH 101, Acdisol, Explotab are suitable for production of direct compression tablet [191-193]. This method consists of a 2-step process: mixing and compressing. Simply put, the tabletting process is admixing of rutin nanocrystals with the tablet excipients (**Table. 3.6**) by a tumbler and followed by compression by a single punch tablet machine. Finally, the 100 mg rutin tablets were produced in simple form and the tablets are shown in **Fig. 4.77**.

Fig. 4.77 Rutin nanocrystal-loaded tablets (left) and marketed tablets (right)

b. Hesperidin

The direct compression method was also employed to produce hepseridin nanocrystal-loaded tablets. This method is evidently more effective in avoiding particle agglomerates and aggregates in tabletting. Avicel PH 101, AcDiSol, Explotab are good excipients for direct compression tablets. As mentioned before, they offer suitable properties such as good flow ability and compressibility for the direct compression method. AcDiSol and Explotab are

superdisintegrants to designed improved dissolution behaviour and are normally used for the direct compression method [194].

To ensure successful tablet production using direct compression, spray-dried hesperidin nanocrystals were selected for the reason that spray-dried hesperidin nanocrystals offer better properties for the direct compression compared to lyophilized nanocrystals.

Fig. 4.78 Hesperidin nanocrystal-loaded tablets (left) and marketed tablets (right)

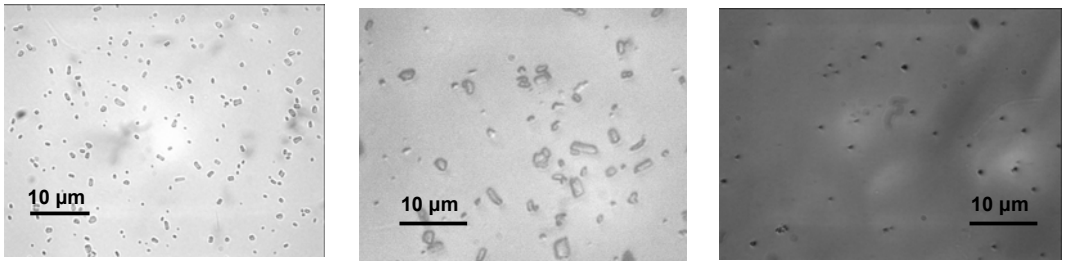
Hesperidin nanocrystals were admixed to other tablet excipients (**Table. 3.7**) in a tumbler. The process continues with compressing in a single punch tablet machine. Finally, the hesperidin nanocrystals-loaded tablets were produced in simple form. Hesperidin microcrystals tablets were also produced using the same method. The same procedure was employed, with hesperidin nanocrystals substituted for hesperidin microcrystals in the tablets. The final hesperidin nanocrystal-loaded tablets can be seen in **Fig. 4.78**.

4.12.2 Ibuprofen nanocrystal-loaded pellets

Ibuprofen nanocrystals were also incorporated into a pellet formulation. In this formulation, ibuprofen nanosuspension was directly admixed with lactose to form the pellet mass using a mixing pan. In the next step of the process, an extruder and a spheronization were used to generate pellets. **Fig. 4.79** shows ibuprofen nanocrystal-loaded pellets.

Fig. 4.79 Ibuprofen nanocrystal-loaded pellets

Considering the drying process, ibuprofen nanocrystals were agglomerated after spray drying as revealed in **Fig 4.80**. For this reason, liquid aqueous nanosuspension was directly admixed to lactose to avoid agglomeration of particles. This technique was effective as it enabled the nanosuspension to remain properly re-dispersed as fine particles in water, be incorporated non-aggregated into the pellets and finally be released as fine nanocrystals after dissolution of the pellets, as illustrated in **Fig. 4.80** (c) [176].



a b c

Fig. 4.80 Light microscopic pictures of ibuprofen nanosuspension (a), nanocrystals after spray-dryer (b), redispersion nanocrystals from pellets (c)

4.12.3 Ibuprofen nanocrystal-loaded effervescent granules

Effervescent granule is one of the solid dosage forms in which nanocrystals can be incorporated. Theoretically, effervescent formulations consist of an acid source and a gas source. In this research, simple effervescent

formulations were chosen for ease of preparation in a laboratory. There are many methods for producing effervescent granule, in this study the fusion method was employed to produce the final product. The composition of the effervescent granule formulations can be seen in **Table 3.8**.

Table 3.8 Ibuprofen nanocrystal-loaded effervescent granule formulations

Formulation	Composition	
Effervescent A	Nanocrystals	28%
	Mannitol	11 %
	Citric acid	26 %
	Sod Bicarbonat	35 %
Effervescent B	Nanocrystals	28%
	Mannitol	11 %
	Citric acid	22 %
	Sod Bicarbonat	33 %
	Fum. Acid	6 %
Effervescent C	Nanocrystals	28%
	Mannitol	10 %
	Citric acid	24 %
	Sod Bicarbonat	38 %
Effervescent D	Nanocrystals	28%
	Mannitol	16 %
	Citric acid	10 %
	Sod Bicarbonat	30 %
	Tartaric Acid	16 %

Fig. 4.81 Ibuprofen nanocrystal-loaded effervescent granule of formulation C

Light microscopic pictures of ibuprofen nanocrystals after redispersion revealed no agglomeration/aggregation among particles. The particle size of the nanocrystals was visibly larger than for the original nanosuspension (Fig. **4.81** and **4.82**).

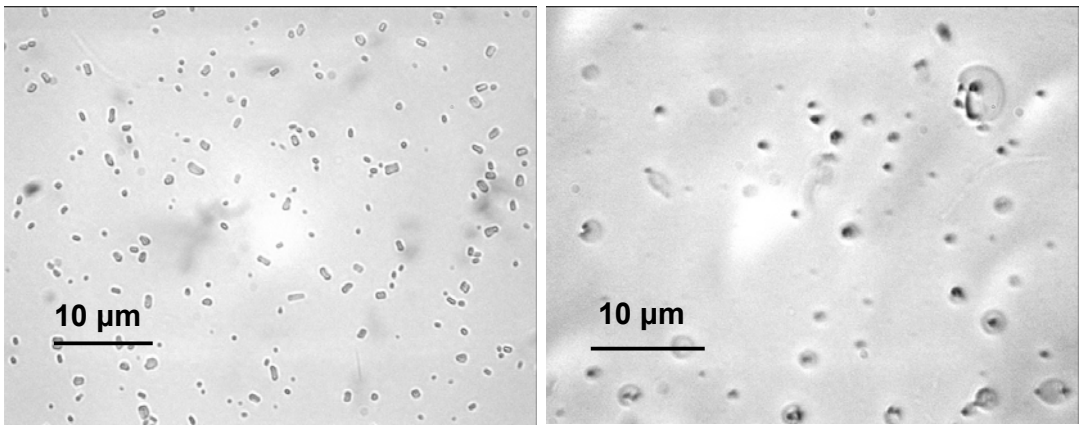


Fig. 4.82 Light microscopic pictures of nanosuspension (formulation 9) (a), re-dispersion of nanocrystals from effervescent granule (formulation C) (b)

4.12.3.1 Re-dispersed ibuprofen from pellet and effervescent formulations

Redispersion of ibuprofen nanocrystals from pellets and effervescent granules was also observed microscopically and the particle size distribution of the redispersed ibuprofen nanocrystals was anlayzed. **Fig. 4.83** shows the redisperion of all ibuprofen nanocrystals-loaded effervescents. Formulation C was found to be the best formulation, with ibuprofen nanocrystals homogenously distributed in the water medium.

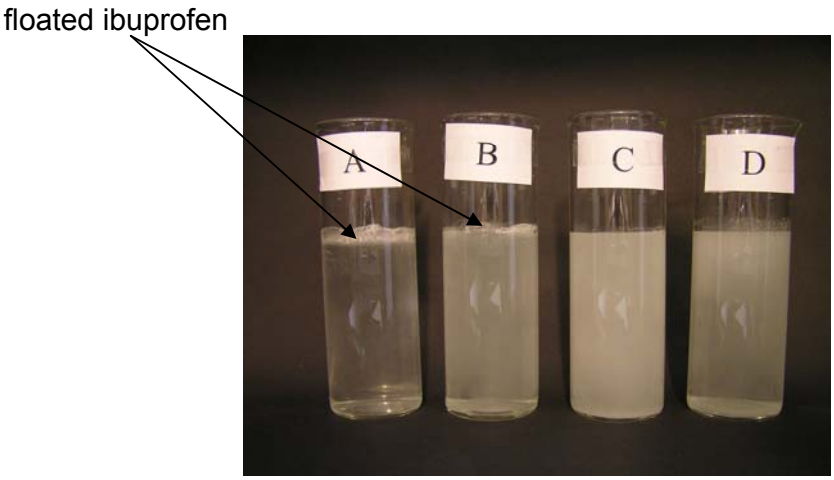


Fig. 4.83 Re-dispersion of ibuprofen effervescent formulations, best formulation is C

In the other 3 formulations ibuprofen nanocrystals were not homogeneously distributed, instead they agglomerated, were floating at the surface of the dispersion media. In formulation A in particular, ibuprofen nanocrystals were clearly agglomerated and floated at the surface. Formulation C was significantly fastest in redispersion of nanocrystals as shownin the **Table 4.18.** Therefore formulation C was selected for subsequent investigation and dissolution performance of this formulation was also studied.

Table 4.18 Dispersion times for ibuprofen effervescent granule

Formulation	Time of re-dispersion (seconds)
Effervescent A	60
Effervescent B	60
Effervescent C	20
Effervescent D	40

The volume size distribution after redispersion showed that 50% (LD 50%) of the ibuprofen nanocrystals had a diameter smaller than 1.983 μm for the

effervescent formulation C and 1.763 µm for the pellet formulation (**Fig. 4.84**). Same re-dispersed ibuprofen nanocrystals grew into big crystals. According to microscopic pictures, the nanocrystals were distributed as single particles with very few agglomerated or aggregated particles (**4.80** and **4.82**). That means it needs to be considered that LD provides a volume distribution emphasizing the few large particles by volume.

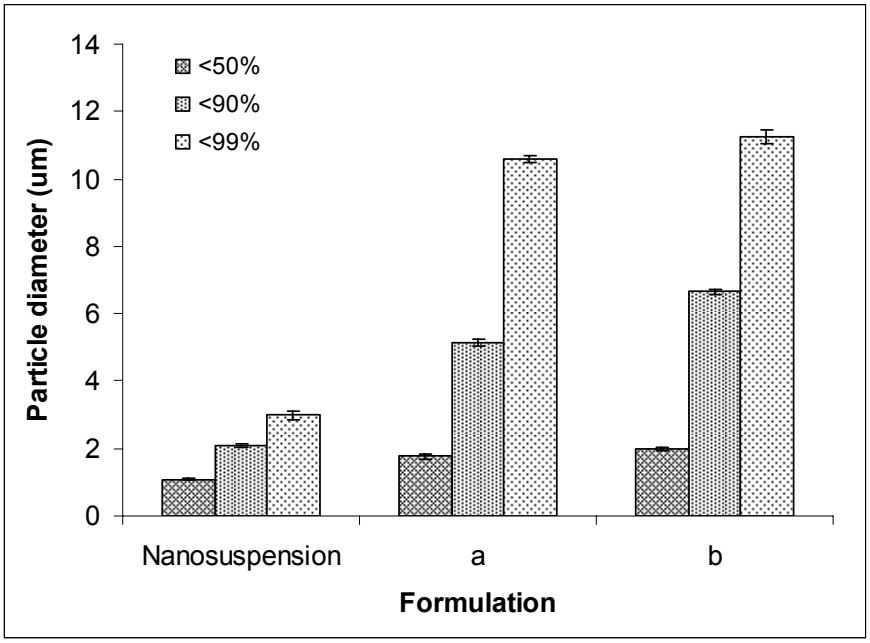


Fig. 4.84 LD particle sizes of ibuprofen nanosuspension and re-dispersed ibuprofen from pellet formulation (a) and effervescent formulation C (b)

4.12.4 Coenzyme Q10 Capsule

The formulations of CoQ10 capsules can be seen in **Table 3.9**. CoQ10 nanocrystals were admixed to the capsule excipients in a tumbler. On a lab scale, the capsules were prepared using a simple capsule filling equipment. The mixed powder was gently poured into capsule no. 2 using the capsule filling equipment. Avicel PH 102, lactose, magnesium stearate are commonly used excipients for capsule fillers. Avicel 102 has good characteristics for a capsule filler, such as excellent flow ability in comparison to Avicel 101. Therefore Avicel PH 102 was mostly chosen as the capsule filler [195]. These capsules were furthermore evaluated with respect to their dissolution behavior.

Table 3.9 Coenzyme Q10 capsule formulations

Formulation	NC (mg)	MC (mg)	Excipient (mg)		
			Lc	AV	Mg
K	30		15	190	1
L		30	15	190	1
Market		30			

NC=Coenzyme Q10 nanocrystal, MC=Coenzyme Q10 microcrystal,
Mg=Mg stearic, AV=Avicel PH 102, Lc=lactose, Market= marketed capsule.

Fig. 4.85 Coenzyme Q10 nanocrystal-loaded capsules (left) and marketed capsules (right)

4.13 Dissolution study of solid dosage forms

Dissolution test of drug tablets was evaluated using a USP XXIV rotating paddle apparatus. This method is known as method 2 in USP XXIV [46].

4.13.1 Rutin

Three formulations of rutin were prepared in this study. Formulation A and B contained rutin nanocrystals and formulation C contained rutin microcrystals. Detailed compositions of the formulations are given in **Table 3.6** in page 186. Released rutin from all of the formulations was evaluated in three different dissolution media.

4.13.1.1 In water

Dissolution velocity of rutin from the rutin nanocrystal-loaded tablets (nanocrystal tablet) was faster compared to the rutin microcrystal-loaded tablet (microcrystal tablet). Within 5 minutes, almost 80% of the rutin was dissolved into water from the nanocrystal tablet (formulation B). This was

definitely better than the microcrystal tablet, which dissolved only about 40% of the rutin under the same conditions. Moreover, within 30 minutes rutin was dissolved completely in water from the nanocrystal tablets (formulation A and B) (**Fig. 4.86**). In contrast, only 71% of the rutin from the microcrystal tablets was dissolved in water after 30 minutes.

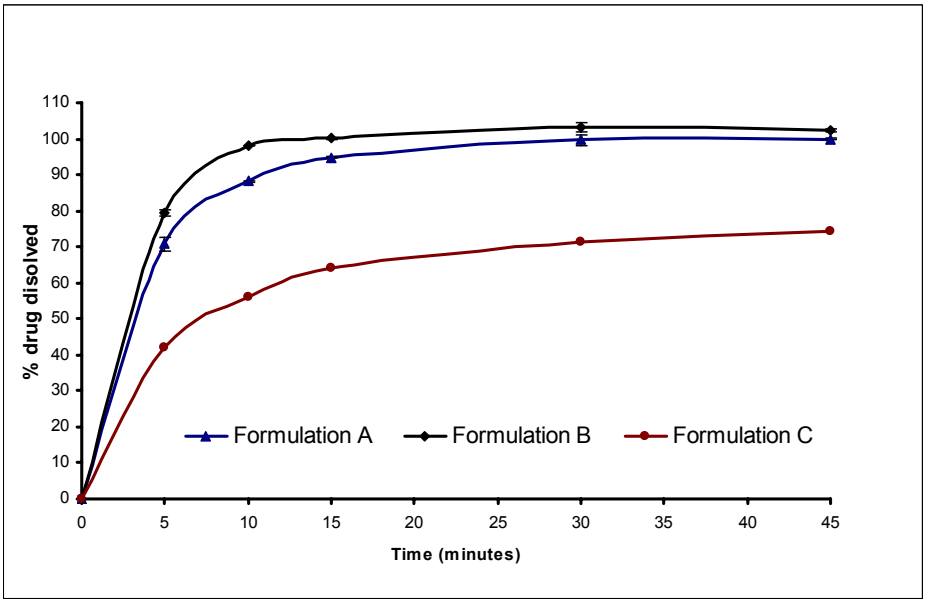


Fig. 4.86 Percentage of dissolved rutin from nanocrystal formulations A and B versus microcrystal tablet C in water (detailed compositions of the formulations are given in **Table 3.6** in page 186)

4.13.1.2 In buffer at pH 1.2

In buffer at pH 1.2, the dissolution velocities of the nanocrystal tablets were also superior when compared to the microcrystal tablets (formulation C). Formulations A and B dissolved 68% and 67% of rutin within 30 minutes, respectively, whereas only 59% of rutin was dissolved from the microcrystal tablets (**Fig. 4.87**).

4.13.1.3 In buffer at pH 6.8

In buffer having a pH of 6.8 formulations A and B are distinctly faster in releasing rutin compared to formulation C. The difference was similar large as in water. Within 5 minutes 95% of rutin could be dissolved from the nanocrystal tablet (formulation A) and only within 10 minutes all of the rutin was dissolved completely. Compared to formulation C, the dissolved rutin from formulation B was definitively increased. Only 50% of rutin could be

dissolved from formulation C within 5 minutes. In a period of 10 minutes only 68% of drug content was dissolved (**Fig. 4.88**).

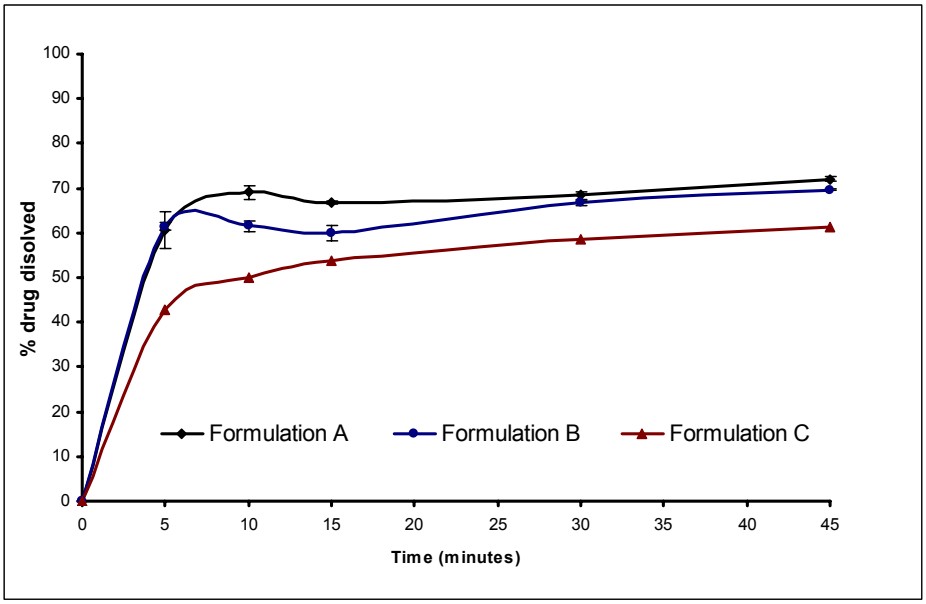


Fig. 4.87 Percentage of dissolved rutin from nanocrystal formulations A and B versus microcrystal tablet C in buffer at pH 1.2 (detailed compositions of the formulations are given in **Table 3.6** in page 186)

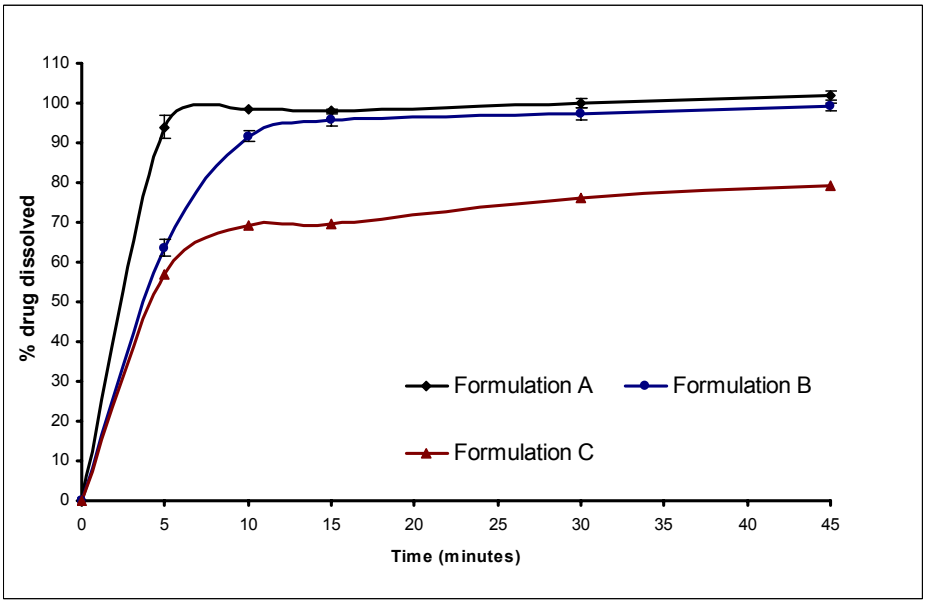


Fig. 4.88 Percentage of dissolved rutin from nanocrystal formulations A and B versus microcrystal tablet C in buffer at pH 6.8 (detailed compositions of the formulations are given in **Table 3.6** in page 186)

4.13.2 Hesperidin

Given the drug contents in the tablets, the dissolution test was not performed in sink conditions (drugs amount in tablets above 30% of saturation solubility). Therefore, at certain time the percentage of dissolved drugs will be constant or change little because the saturation solubility in the dissolution media is going to be approached.

4.13.2.1 In water

Three formulations of hesperidin tablets were prepared successfully. The dissolution behavior of these tablets was evaluated and compared with each other. In water medium, Formulation X could release hesperidin faster than the other formulations. Formulation X contained hesperidin nanocrystals with AcDiSol used as dry disintegrant. Compared to the microcrystal tablets (formulation Z), the nanocrystals tablet showed superior dissolution velocity. Within 30 minutes, nanocrystal tablets of formulation X dissolved 24% of their hesperidin content. In contrast, microcrystal tablets let to the dissolution of only 16% of hesperidin over the same time.

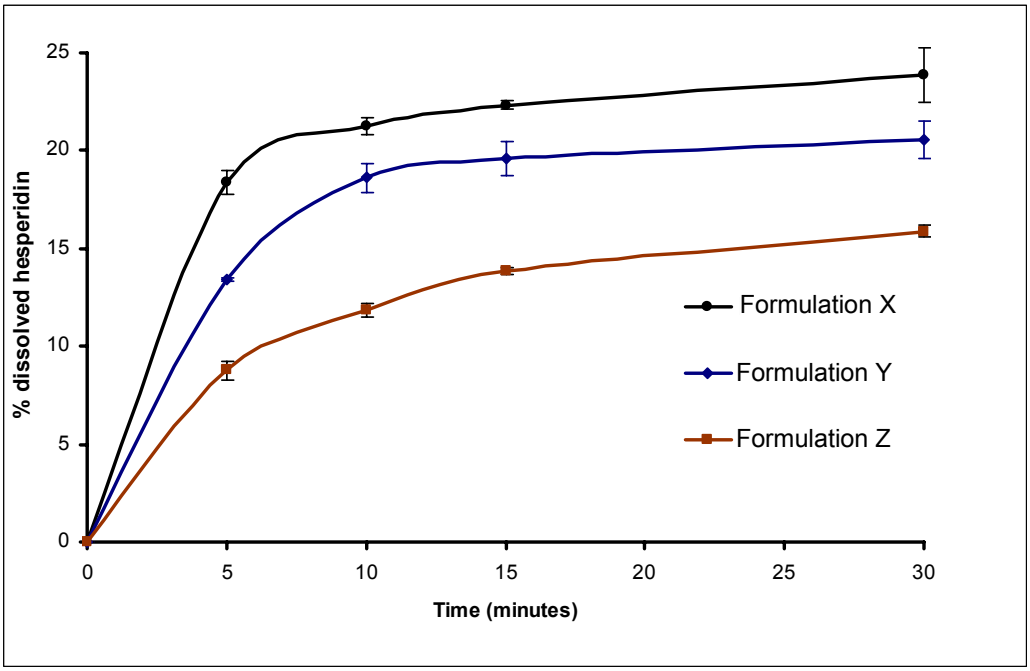


Fig. 4.89 Percentage dissolved hesperidin in water from nanocrystal formulations X and Y versus microcrystal formulation Z (detailed compositions of the formulations are given in **Table 3.7** in page 187)

The dissolution profiles of the three hesperidin tablets are presented in **Fig. 4.89**. This shows the superiority of hesperidin nanocrystal-loaded tablets (formulation X and Y), which were fastest in dissolution of hesperidin.

4.13.2.2 In buffer at pH 1.2

Similar to rutin tablets, hesperidin dissolution from hesperidin tablets was evaluated in buffer solution having a pH of 1.2. Hesperidin was dissolved faster from nanocrystals tablet compared to microcrystals tablet, resembling the dissolution behavior in water. Within 5 minutes, the nanocrystal tablets (formulation X) could dissolve almost 25% of drugs. By comparison only 7% of the hesperidin in the microcrystal tablets (formulation Z) dissolved in the same time. Dissolution of hesperidin from nanocrystal tablets distinctly increased over time. In a period of 30 minutes, almost 30% of hesperidin was dissolved from the nanocrystal tablets. This was twice as fast as the dissolution of hesperidin from microcrystal tablets (**Fig. 4.90**).

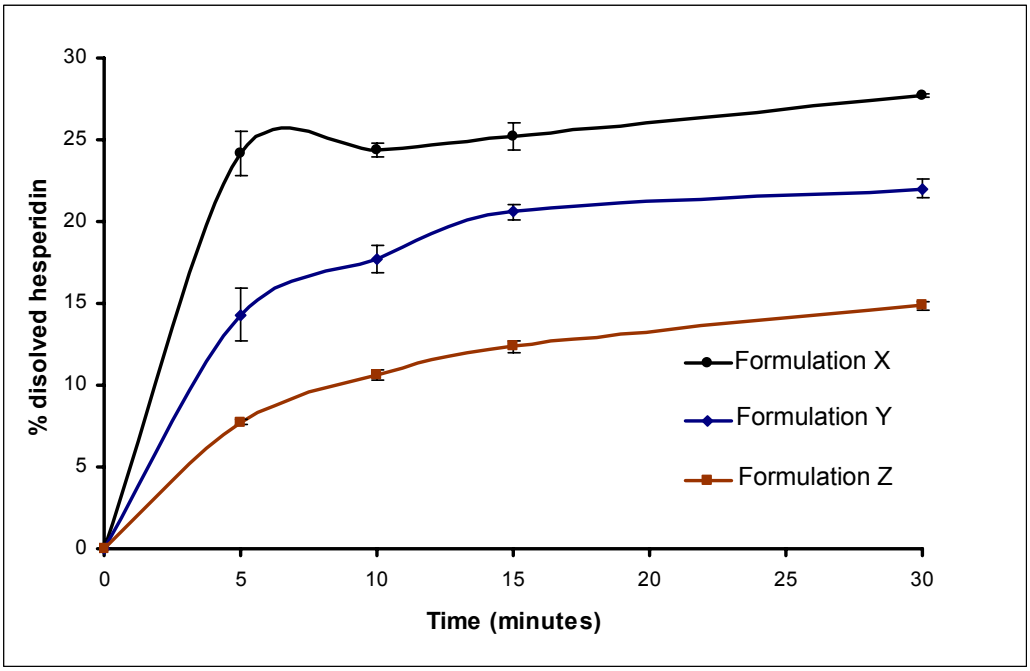


Fig. 4.90 Percentage dissolved hesperidin in buffer at pH 1.2 from nanocrystal formulations X and Y versus microcrystal formulation Z (detailed compositions of the formulations are given in **Table 3.7** in page 187)

4.13.2.3 In buffer at pH 6.8

Nanocrystal tablets were again superior in dissolving hesperidin in buffer at pH 6.8. In this media, 26% of hesperidin was dissolved from the nanocrystals tablet (formulation X) within 15 minutes. In contrast, microcrystal tablets dissolved only 14 % of the drug under the same conditions. This means that nanocrystal tablets could dissolve drugs at double the rate the microcrystal tablets over a 15 minute period (**Fig. 4.91**).

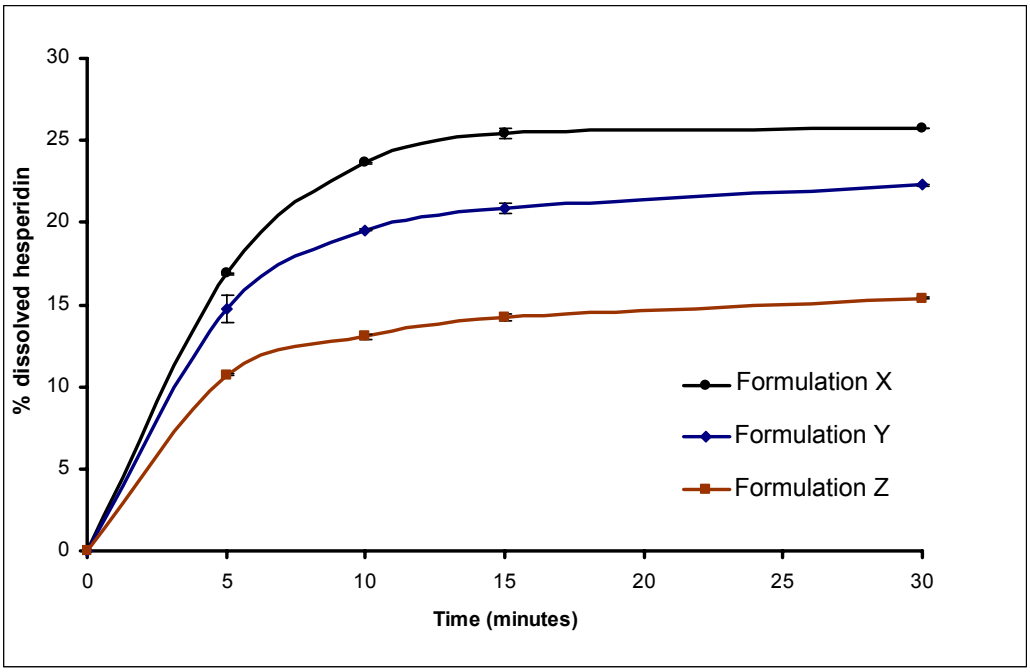


Fig. 4.91 Percentage dissolved hesperidin in buffer at pH 6.8 from nanocrystal formulations X and Y versus microcrystal formulation Z (detailed compositions of the formulations are given in **Table 3.7** in page 187)

4.13.3 Ibuprofen

Ibuprofen nanocrystals could completely dissolve in water in a period of 10 minutes. This result was superior to the dissolution velocity of micronized raw material (drug dissolved 62% within 10 minutes). Under the same condition, ibuprofen nanocrystals were also dissolved completely from the effervescent formulation. Within 15 minutes 100% of the ibuprofen nanocrystals could be dissolved from the pellet formulation (**Fig. 4.92**). This was a little slower than the dissolution velocity of the effervescent formulation. However, dissolution

of the effervescent formulation was superior even compared to the dissolution of the raw material. Accordingly, incorporation of ibuprofen nanocrystals into pellet and effervescent formulations does not delay the release of ibuprofen from these dosage forms (**Fig. 4.92 and 4.93**).

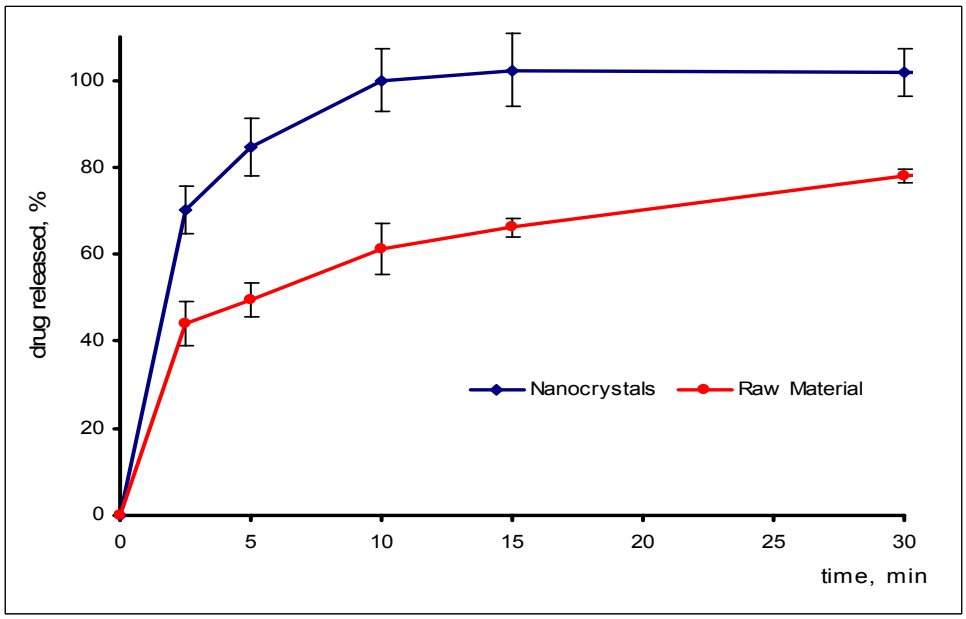


Fig. 4.92 Dissolution velocity profile of raw material (mean size d50%: 56 µm, LD) and ibuprofen nanocrystals (mean size: 929 nm, PCS) in water

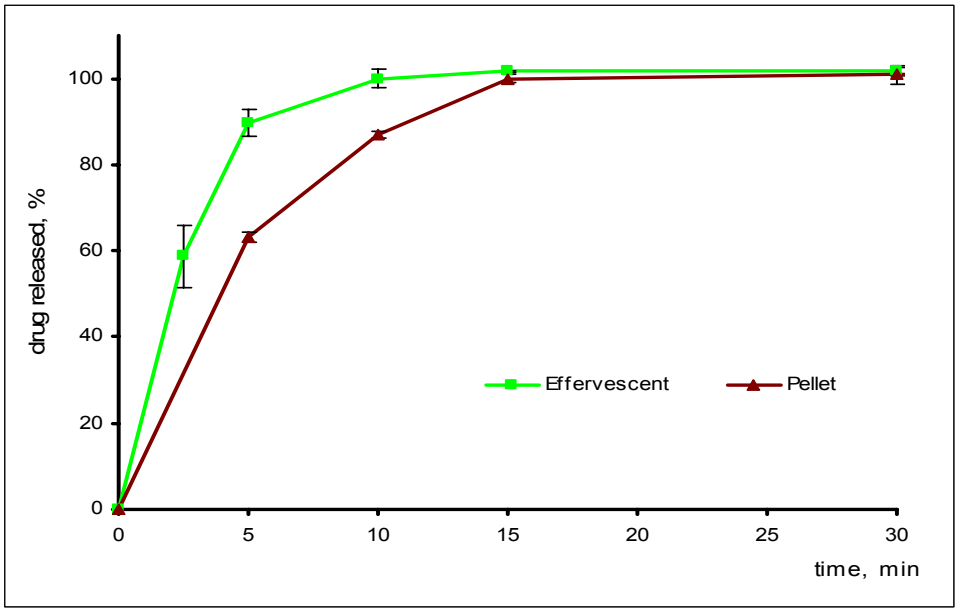


Fig. 4.93 Dissolution velocity profile of ibuprofen nanocrystals from effervescent formulation C and pellet formulations in water (detailed compositions of the effervescent formulations are given in **Table 3.8** in page 191)

4.13.4 Coenzyme Q10

In vitro dissolution testing of coenzyme Q10 (CoQ10) capsules was performed in water and buffer at pH 1.2, 37°C. Like with hesperidin, the dissolution test was not performed in sink conditions. Therefore at a certain time, release of CoQ10 will be constant or further increase little because the saturation solubility is going to be approached.

Dissolution velocity of CoQ10 from the CoQ10 nanocrystal-loaded capsules (nanocrystal capsules) was distinctly improved and superior to that of mycrocrystals capsules and marketed capsules.

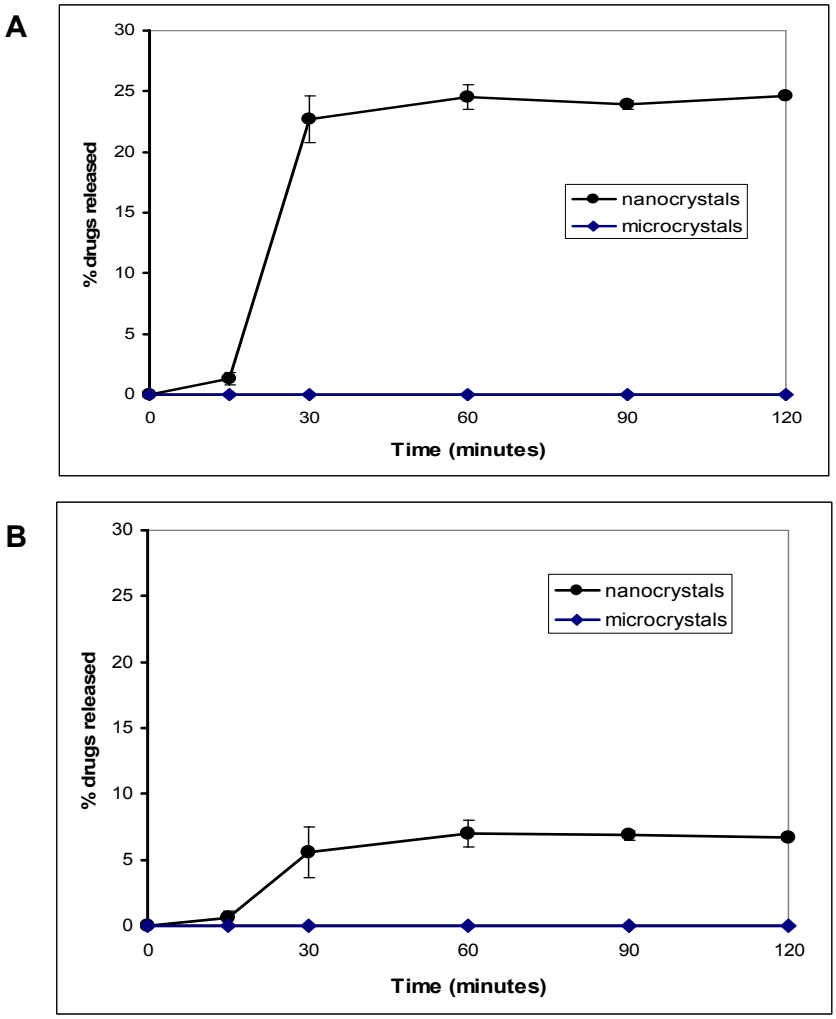


Fig. 4.94 Percentage of dissolved coenzyme Q10 from nanocrystal-loaded capsules (formulation K) compared to microcrystal-loaded capsules (formulation L) in water (A) and buffer at pH 1.2 (B); mean size of nanocrystals: 263 nm, PCS; d50% of microcrystals: 111 µm, LD (detailed compositions of the formulations are given in **Table 3.9** in page 195)

Within 60 minutes, about 25% of CoQ10 was dissolved from the nanocrystal capsules (Formulation K) in water and 7% in buffer having a pH of 1.2 (**Fig.4.94**). In contrast, no CoQ10 was dissolved from the microcrystal capsules (Formulation L) in water and in buffer having a pH of 1.2 even after 120 minutes (below the detection limit of HPLC).

4.14 Comparison of nanocrystal-loaded tablets or capsules and marketed tablets

4.14.1 Rutin

In all of three dissolution media, the nanocrystal-loaded tablets (formulation A and B) were superior compared to the marketed tablet. Within 5 minutes almost 80% of rutin was dissolved from formulation B in water. It was definitely better compared to the marketed tablet which dissolved only about 30% of rutin in the same conditions. In addition, within 30 minutes 100% rutin was dissolved from the nanocrystal-loaded tablets (formulation A and B) in water and buffer of pH 6.8. In the same time, 68% and 67% of rutin were dissolved also from nanocrystals tablets (formulation A and B) in buffers having a pH of 1.2. In contrast, after 30 minutes the marketed tablet dissolved only 55%, 51% and 62% of rutin in water, and buffers having a pH of 1.2 and 6.8, respectively.

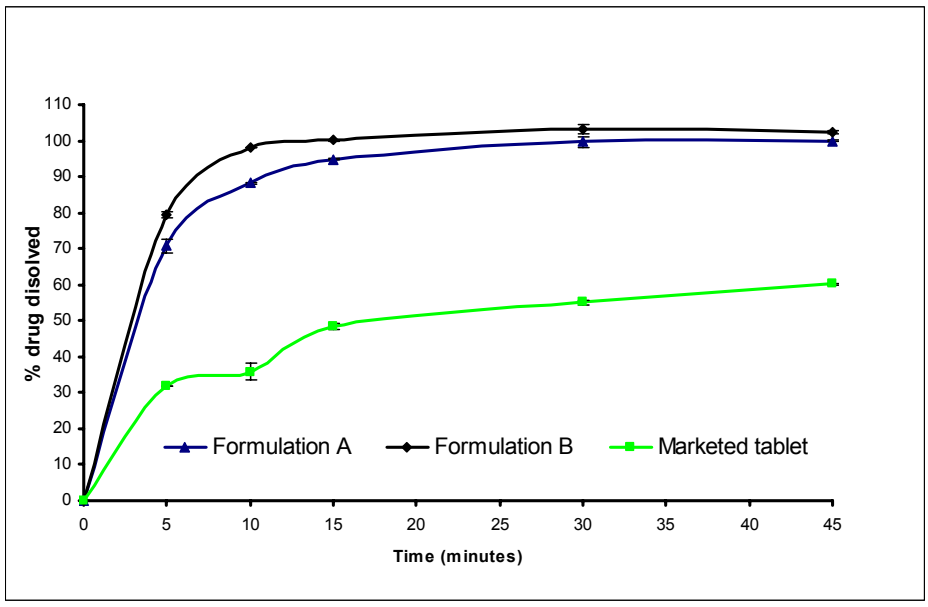


Fig. 4.95 Percentage of dissolved rutin from nanocrystal tablet formulations A and B versus marketed tablet in water (detailed compositions of the formulations are given in **Table 3.6** in page 186)

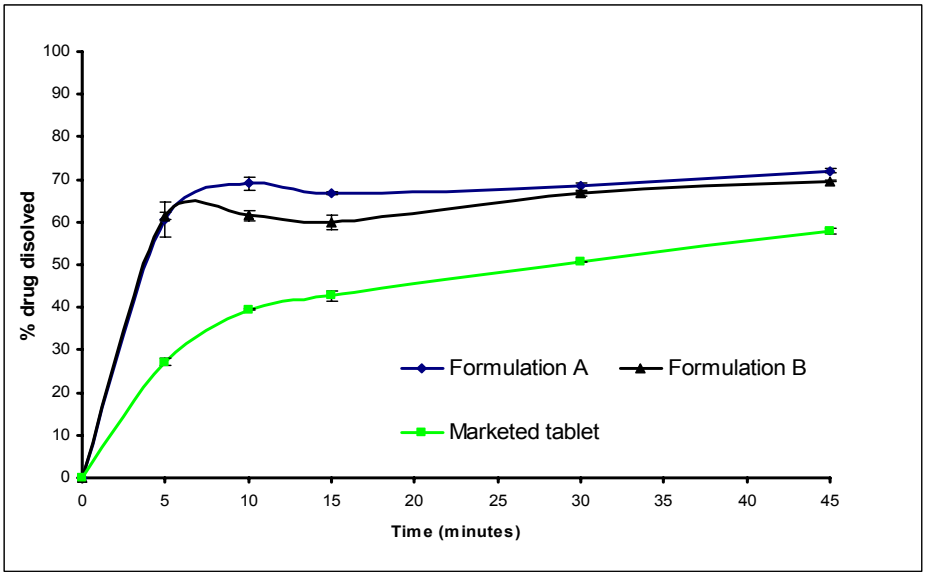


Fig. 4.96 Percentage of dissolved rutin from nanocrystal tablet formulations A and B versus marketed tablet in buffer at pH 1.2 (detailed compositions of the formulations are given in **Table 3.6** in page 186)

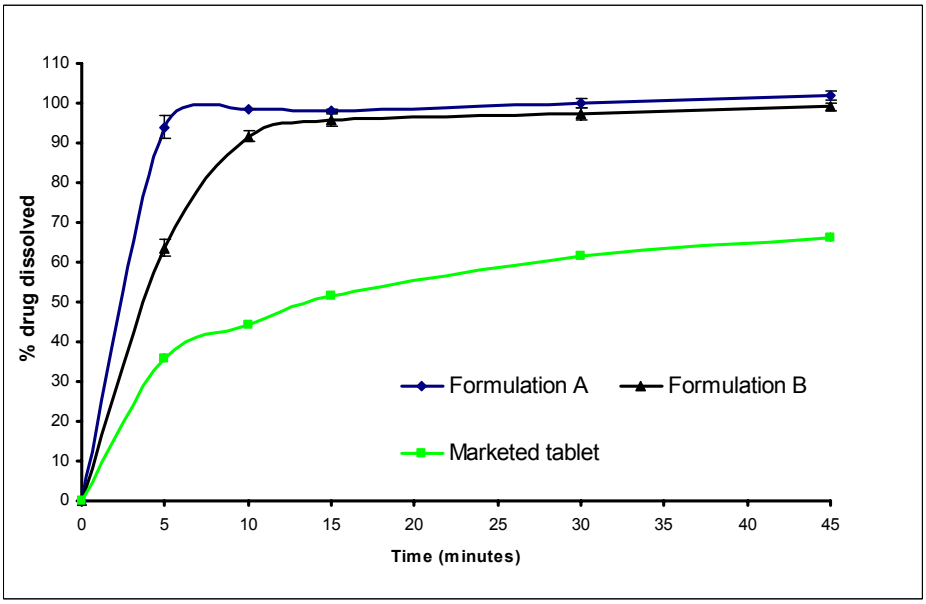


Fig. 4.97 Percentage dissolved rutin from nanocrystal tablet formulation A and B versus marketed tablet in buffer of pH 6.8 (detailed compositions of the formulations are given in **Table 3.6** in page 186)

The dissolution velocity profiles of rutin from the different tablet formulations investigated can be seen in **Fig. 4.95, 4.96** and **4.97**.

4.14.2 Hesperidin

Hesperidin nanocrystal-loaded tablets (Formulations X and Y) were also compared to the marketed hesperidin tablets. **Figs. 4.98, 4.99** and **4.100** show dissolved hesperidin from the formulations X, Y and the marketed tablets. The nanocrystal tablets were distinctly superior in all dissolution media. In water, the nanocrystal tablets (formulation X) dissolved about 22% of the drug within 15 minutes. Within the same time, the marketed tablets dissolved only 1% of their hesperidin. The nanocrystal tablets (Formulation X) thus dissolved hesperidin 22 times faster than marketed tablets in the first 15 minutes. In other media, the dissolution behavior was also distinctly improved. After 15 minutes, the nanocrystal tablets (formulation X) dissolved 25% and 26% of the drugs in buffer having a pH of 1.2 and 6.8. Meanwhile in the same time, only 3% and 1% of hesperidin was dissolved from the marketed tablets in buffer having a pH of 1.2 and 6.8. This suggests that hesperidin nanocrystal-loadeds tablets have a high potential for oral administration and can improve the bioavailability of drugs in cases in which dissolution performance restricts bioavailability of drugs in the body.

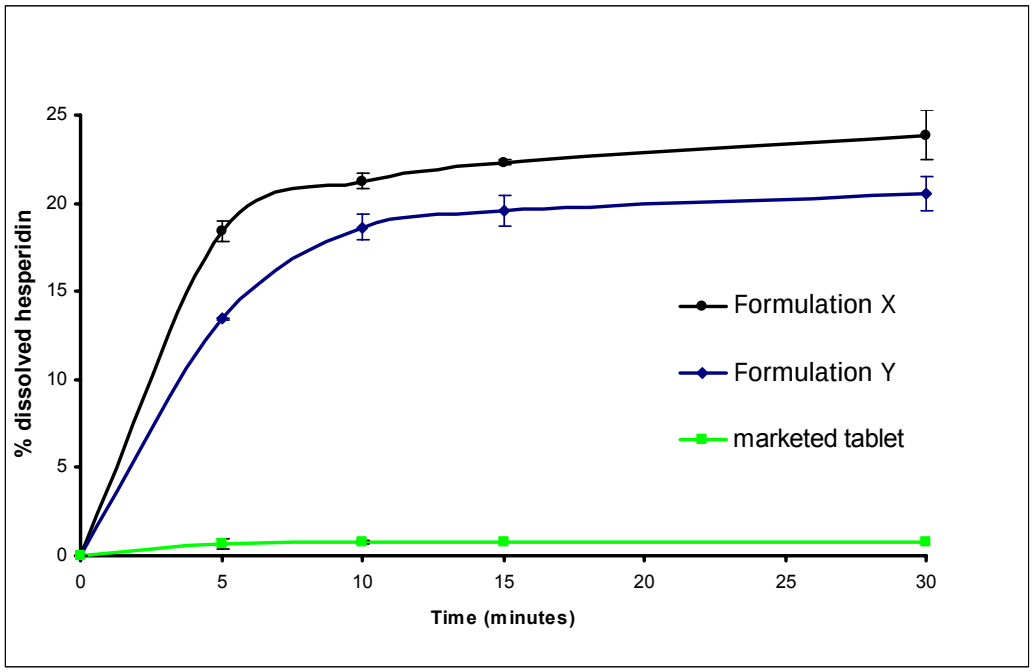


Fig. 4.98 Percentage of dissolved hesperidin from nanocrystal tablet X and Y versus marketed tablet in water (detailed compositions of the formulations are given in **Table 3.7** in page 187)

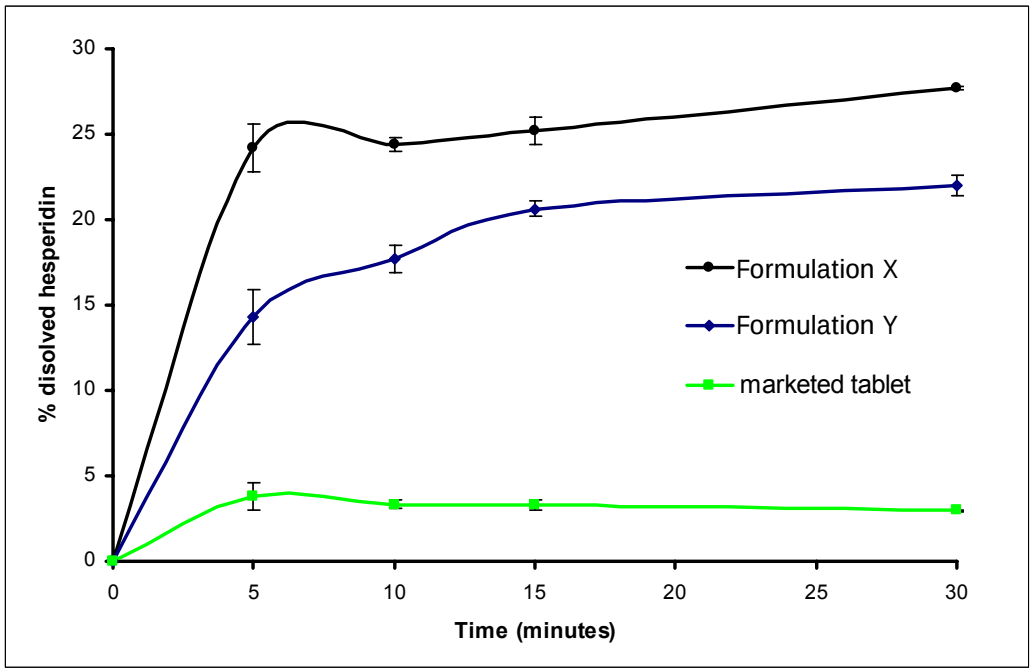


Fig. 4.99 Percentage dissolved hesperidin from nanocrystal tablet X and Y versus marketed tablet in buffer having a pH of 1.2 (detailed compositions of the formulations are given in **Table 3.7** in page 187)

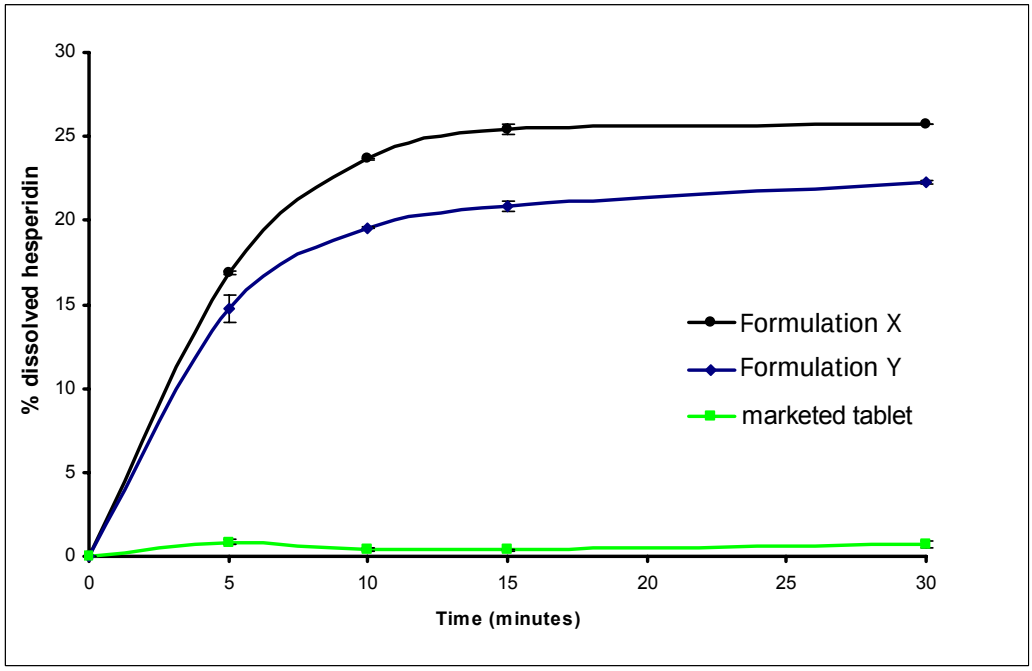


Fig. 4.100 Percentage of dissolved hesperidin from nanocrystal tablet X and Y versus marketed tablet in buffer having a pH of 6.8 (detailed compositions of the formulations are given in **Table 3.7** in page 187)

Dissolution performance:

Compared to microcrystal tablets and marketed tablets, the nanocrystal tablets definitively showed higher levels of percentage dissolved rutin and hesperidin. In all three dissolution media, nanocrystal tablets dissolved rutin and hesperidin at distinctly faster rates compared to microcrystals and marketed tablets. Therefore, nanocrystal tablets have superior characteristics to microcrystals and marketed tablets, indicating a major opportunity to enhance the bioavailability of drugs by nanosuspensions for oral administration, in cases when dissolution is a rate limiting factor in bioavailability in the body.

4.14.3 Coenzyme Q10

Neither microcrystal capsules nor marketed capsules dissolved CoQ10 into the dissolution media. After 120 minutes, no CoQ10 was dissolved from marketed capsules in water and in buffer having a pH of 1.2 (below the detection limit of HPLC). In the kinetic solubility test of coenzyme CoQ10 microcrystals, the CoQ10 microcrystals were found to be insoluble in water (solubility below the HPLC detection limit).

Due to the poor aqueous solubility of CoQ10 microcrystals, microcrystal-loaded capsules and marketed capsules were unable to release CoQ10. In contrast, CoQ10 nanocrystals showed an increased kinetic solubility. Therefore, the dissolve of CoQ10 from these capsules was distinctly improved (**Fig. 4.101**).

Bhandari *et al.* have evaluated the improved *in-vitro* dissolution of CoQ10 at 37°C in distilled water. The result shows that dissolution of CoQ10 was considerably enhanced by solid dispersion [123]. Other studies of CoQ10 nanoparticles have been published by Nehilla *et al.*, and Siekmann *et al*. However, their results did not specifically describe improved dissolution of CoQ10 nanoparticles [196, 197], instead mentioning only prolonged release of CoQ10 from nanoparticles until 2 months [196]. Dissolution velocity of nanocrystal-loaded capsules was comparable with solid dispersion. Among factors for consideration, nanocrystals are simpler to manufacture and require

less material. Nanotechnology is therefore more effective in increasing solubility and dissolution velocity. Moreover this technology offers cost saving.

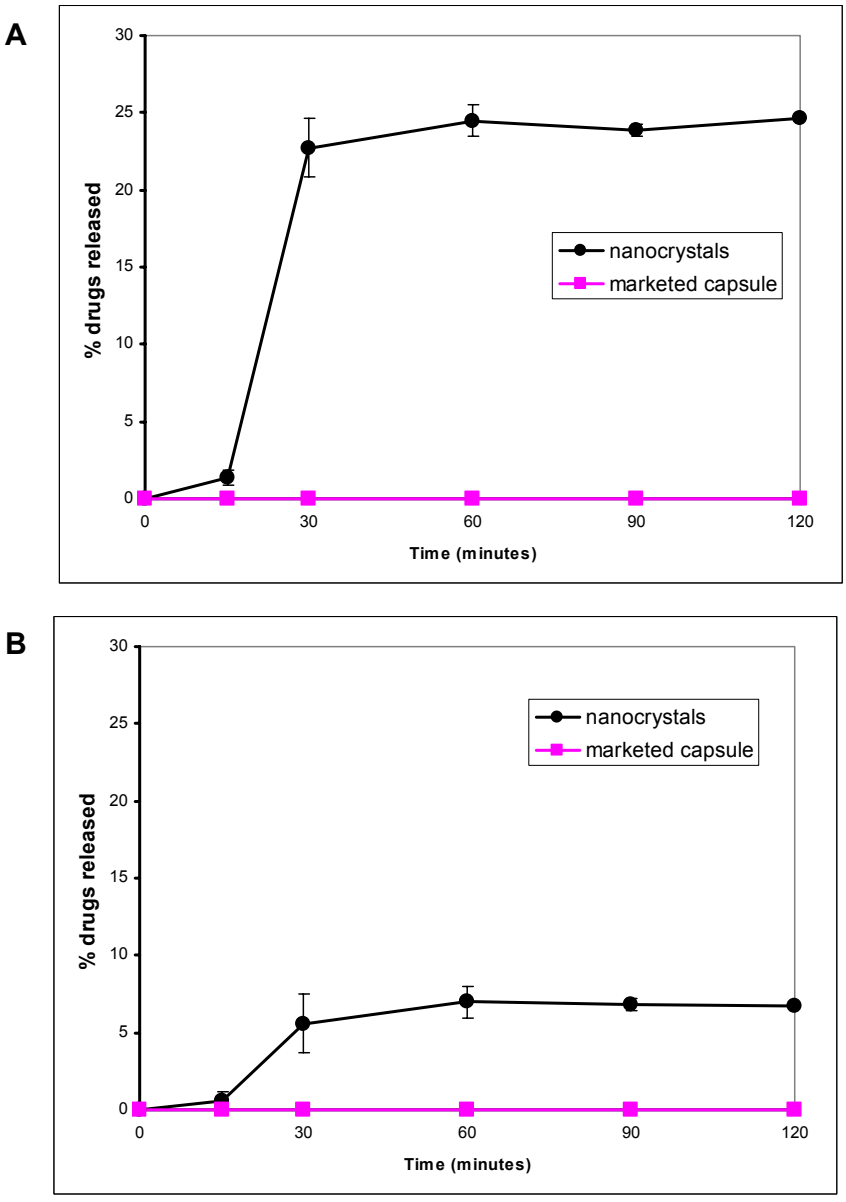


Fig. 4.101 Percentage of dissolved coenzyme Q10 from nanocrystals-loaded capsule in comparison with marketed capsule in water (A) and in buffer of pH 1.2 (B) (detailed compositions of the formulations are given in **Table 3.9** in page 195)

The more rapid release of CoQ10 from the nanocrystal-loaded capsules is thought to be beneficial since, once being administered orally, the fast dissolved CoQ10 may be passively partitioned into the gastrointestinal tract

tissues in a shorter time (because of the favorable concentration gradient) resulting into a rapid onset of action and improved bioavailability. Therefore, CoQ10 nanocrystal-loaded capsules offer promise for a dosage form with superior physicochemical properties that overcomes problem of low bioavailability in the human body.

4.15 Perspectives of drug nanocrystals for oral application

Recently, the particle size reduction effectiveness of drug substances-loaded tablets on oral bioavailability has been intensively investigated. It has been proven that particle size reduction leads to improved oral bioavailability in the body. Takano *et al.* have specified that particle size reduction leads to improved dissolution rate and bioavailability. In addition, the rate-limiting steps of oral absorption were simulated. An increase in the dissolution rate and administered dose showed a shift from dissolution rate-limited to solubility-limited absorption. In the study in dogs, the particle size reduction of the drugs improved the oral absorption [198]. Such studies provide a powerful tool to predict dose linearity and will aid in the development of formulating poorly soluble drugs (Biopharmaceutical Classification System (BCS) class II drugs).

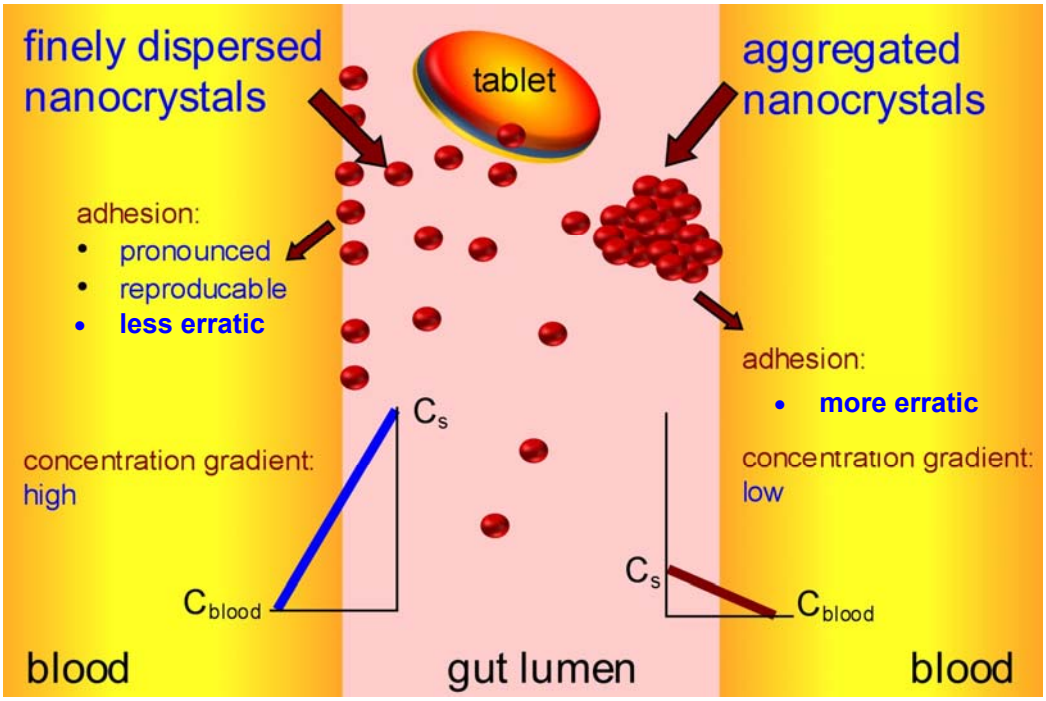


Fig. 4.102 Mechanism of action: finely dispersed nanocrystals versus aggregated nanocrystals (similar to micrometer crystals)

According to Hintz *et al.*, a computer method has been developed to describe the theoretical dissolution rate of a polydisperse powder under non-sink conditions based on its weight percent particle size distribution. It is shown that finer particles in the size distribution showed an improved dissolution behavior. Moreover the particle size distributions were used to simulate their effect on the amount of drug absorbed orally [199]. Based on those results, it has been suggested a promising rutin tablet dosage form for oral administration. It leads to superior physicochemical properties and should overcome the in vivo absorption problem of the poorly soluble rutin as class II BCS drug. **Fig. 4.102** summarizes the effects on bioavailability enhancement in the gut. Important is that the nanocrystals are released from the tablet or capsule as fine nanocrystals. It could be shown that a slight aggregation does not yet impair the dissolution velocity [200], but pronounced aggregation will decrease the dissolution velocity strongly [201].

4.16 General conclusions

High pressure homogenization (HPH) can be employed to produce aqueous drug nanosuspensions that are stable up to 12 months. The zeta potential values are about -30 mv or higher, i.e. in the range for a long-term stable suspension. Aqueous nanosuspension can be converted to dry drug nanocrystals by a lyophilization or spray drying process with/without any addition of cryoprotectant.

Dried drug nanocrystals offer superior physicochemical properties. The very fine particles of the dried nanocrystals re-disperse completely in water. This characteristic is crtitical in improving the kinetic solubility and the dissolution behavior of drugs, especially in tablet dosage forms. Dried drug nanocrystals in tablets and capsules showed significantly improved saturation solubility and particularly dissolution velocity in comparison to microcrystals (raw material). Rutin and hesperidin nanocrystal-loaded tablets can be produced using direct compression and coenzyme Q10 nanocrystal-loaded capsules can be made by means of simple capsules filling equipment. Dissolution velocity of drug nanocrystals from solid dosage forms was superior compared to microcrystal-loaded solid dosage forms and the marketed solid dosage forms (tablet or capsules). Improved dissolution behavior in drug nanocrystal-loaded solid

dosage forms should lead to better bioavailability of poorly soluble drugs in the body.

Chapter 5
SUMMARY/ZUSAMMENFASSUNG

5. SUMMARY/ZUSAMMENFASSUNG

5.1 Summary

Nanosuspensions were formulated by homogenization (HPH) to overcome problems caused by poor aqueous solubility. The physicochemical properties of the drug nanosuspensions were evaluated.

Kinetic solubility was significantly improved. For example Coenzyme Q10 (CoQ10) microcrystals are insoluble in water. However, the saturation solubility of CoQ10 nanocrystals in water considerably higher, determined being at 15.6 µg/ml.

Drying of nanosuspensions was completed by spray drying and freeze-drying. Agglomeration frequently occurs after drying of all nanosized products. Dried nanocrystals could be completely re-dispersed into water with or without cryoprotectant.

The crystalline state evaluation showed that the HPH process did not produce amorphous forms. As shown in DSC thermograms and XRD diffractograms, all patterns were similar to those for the original drugs.

Increase in surface area enhances the dissolution rate. From the Noyes-Whitney equation, the increased surface area and saturation solubility due to the decreased radius result in increased dissolution velocity. This phenomenon was clearly demonstrated by the rutin, hesperidin and coenzyme Q10 nanocrystals.

Dissolution velocity of nanocrystal-loaded solid dosage forms was evaluated. Drug nanocrystals were released from the nanocrystal tablets at a faster rate compared to microcrystal tablets or marketed tablets.

5.2 Zusammenfassung

Arzneistoffe wurden mittels Hochdruckhomogenisation (HPH) als Nanosuspension formuliert. Die physikalisch-chemischen Eigenschaften der Nanosuspensionen wurden bestimmt.

Die kinetische Löslichkeitserhöhung des Nanosuspensions ist gegenüber entsprechenden Mikrokristallen signifikant besser. Zum Beispiel CoQ10 Mikrokristalle sind in Wasser unlöslich. Die Sättigungslöslichkeit der lyophilisierten Coenzym Q10-Nanokristalle in Wasser erhöhte sich beträchtlich und betrug 15.6 µg/ml.

Das Wasser der Nanosuspensionen wurde durch Sprühtrocknung oder Gefriertrocknung entfernt. Agglomeration trat häufig nach dem Trocknen aller Nanosuspension auf. Getrocknete Nanokristalle konnten in Wasser mit oder ohne Kryoprotektor vollständig redispergiert werden.

Die DSC-Thermogramme und die XRD Diffraktogramme des homogenisierten Arzneistoffes waren ähnlich mit den von Ausgangsarzneistoffe bestätigten, dass der Hochdruck-homogenisationprozeß den kristallinen Zustand des Arzneistoffes nicht veranderte wurde.

Die Zunahme der Oberfläche kann verwendet werden, um die Auflösungsrate zu erhöhen. Von der Noyes-Whitney Gleichung ist bekannt, dass die vergrößerte Oberfläche und die Sättigungslöslichkeit wegen des verminderten Radius zu größerer Auflösungsgeschwindigkeit führen. Dieses Phänomen wurde durch Nanokristalle von Rutin, Hesperedin und Coenzym Q10 bestätigt.

Die Freisetzunggeschwindigkeit von Nanokristall-feste Arzneistoffformen waren schneller, als die von Mikrokristalltabletten bzw. als die handelsüblicher Tabletten oder Kapseln.

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