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Physical stability of nanosuspensions: Investigation of the role of stabilizers on Ostwald ripening

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ABSTRACT

The effect of stabilizer type (small molecule vs. polymeric) and the amount of micellar solubilized drug on Ostwald ripening of nanosuspensions was investigated. Indomethacin nanosuspensions were prepared with small molecule stabilizers (sodium lauryl sulfate (SLS) and Dowfax 2A1 (DF)) and a polymeric stabilizer (hydroxypropyl methyl cellulose (HPMC)). Two different drug:stabilizer ratios were used to evaluate the effect of micellar solubilized drug. The Ostwald ripening potential of nanosuspensions was evaluated by subjecting them to various stress conditions (temperature (15, 25, 35 and 45 °C), thermal cycling, and mechanical shaking) for three months. The mean particle size increased in all SLS and DF formulations stored under different stress conditions. No effect of micellar solubilized drug on the Ostwald ripening rate was observed. In the case of HPMC formulations only those stored at higher temperatures (35 or 45 °C) exhibited an increase in mean particle size. The increase in size in the HPMC formulation stored at 45 °C was attributed to dehydration of the HPMC chains and subsequent loss of protection of the nanoparticles. The cube of the mean particle diameter versus time plot was determined to be non-linear for all formulations exhibiting Ostwald ripening. Therefore, according to the Lifshitz, Slyozov and Wagner theory the process was not diffusion controlled. The most probable mechanism for Ostwald ripening was surface nucleation controlled.

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1. Introduction

Nanosuspensions are being widely employed for the delivery of water insoluble compounds. High dissolution rates of poorly water-soluble compounds can be achieved with these formulations due to their small particle size and high surface area (Patravale et al., 2004). The high dissolution rate aids in increasing the permeation of these compounds (Jia et al., 2002). Both increased dissolution and permeation greatly improve the oral bioavailability of water insoluble compounds and reduce the effects of fed and fasted states on the bioavailability (Rabinow, 2004). Improvement in oral bioavailability comes with the possible added advantage of dose reduction since less of the administered dose is wasted. Reduced dose not only benefits the patients in terms of reduced side effects and toxicity (Liversidge and Conzentino, 1995; Wu et al., 2004), but also leads to cost savings. In addition, nanosuspensions are very useful in toxicological studies of investigational compounds. The dose administered in such studies often exceeds their aqueous solubility and hence co-solvents are used to achieve solubilization. The use of harsh co-solvents can complicate toxicity studies and sometimes it becomes difficult to pinpoint the cause of toxicity (whether due to the solvents or the investigational compound). No harsh co-solvents are necessary when nanosuspensions are used and therefore these formulations are gaining popularity in early discovery research (Kesisoglou et al., 2007).

The small particle size of nanosuspensions, which is inherent to their success, is also responsible for their physical instability. Nanosuspensions consist of hydrophobic particles dispersed in a hydrophilic medium (usually water). The enormous surface area associated with the small size of these particles results in high interfacial tension, which in turn results in an increase in the free energy of the system. Accordingly, nanosuspensions are essentially thermodynamically unstable systems (Rabinow, 2004). To decrease their free energy nanoparticles tend to reduce interaction with water via flocculation, aggregation or crystal growth. However, these processes adversely affect the central characteristics of nanosuspensions (i.e., small size and high surface area) and consequently the benefits of the nanosuspension formulations, as discussed above, are lost. Stabilizers are added to reduce the free energy of the system by decreasing interfacial tension, and to

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prevent nanoparticle aggregation by electrostatic or steric stabilization. Stabilizers constitute an integral part of nanosuspensions and it is important to fully understand their role on physical stability of nanosuspensions (Verma et al., 2009c). Stabilizers can be surfactants, polymers or a mixture of both. Examples of some of the commonly used surfactants include Tween 80, sodium lauryl sulfate and poloxamer 188 (Jacobs et al., 2000). Polyvinylpyrrolidone (PVP), hydroxypropyl methyl cellulose (HPMC), hydroxypropyl cellulose (HPC), and polyvinyl alcohol (PVA) are examples of polymeric stabilizers (Kesisoglou et al., 2007; Patravale et al., 2004).

Ostwald ripening is the process in which larger particles grow at the expense of the smaller particles (Ostwald, 1901) due to the well known Kelvin effect (Hiemenz and Rajagopalan, 1997). There are two preconditions for Ostwald ripening: (i) the system should be polydisperse and (ii) the dispersed phase should have finite solubility in the dispersion medium. Both these conditions are frequently encountered in pharmaceutical nanosuspensions. In addition, most of the stabilizers used in the preparation of nanosuspensions also increase their solubility and hence may increase Ostwald ripening.

Ostwald ripening kinetics in disperse systems is governed by two basic processes: (i) diffusion of the solute molecules; and (ii) attachment or detachment (crystal growth and dissolution) to and from the particle surface. If crystal growth/dissolution at the particle surface is rapid then diffusion becomes the rate determining step (diffusion controlled growth). Whereas, if diffusion of the solute molecules is faster than their incorporation or removal to or from the solid particles then the coarsening of the system is governed by the mechanisms of crystal growth (including, surface energy and the presence of defects) (interface controlled growth). Depending upon the nature of the interface and crystal growth mechanism three different types of interface coarsening can be identified: (i) continuous growth, (ii) surface nucleation and (iii) spiral growth. The kinetics of diffusion controlled growth and two of the interface controlled growth (continuous growth and spiral growth) are given by:

$$d^n - d_0^n = k \times t$$

where d is the average diameter at time t, d_0 is the average initial diameter at $t = 0$ and k is the ripening rate. The exponent n is 3 for Lifshitz, Slyozov and Wagner (LSW) diffusion controlled processes (Lifshitz and Slyozov, 1961; Wagner, 1961) and continuous growth processes (Dehoff, 1984; Wagner, 1961) and 2 for spiral growth (Kahlweit, 1975; Ratke et al., 1995). The ripening rate is a given by:

$$k = \frac{64\, D C_\infty V_m \gamma}{9\, RT}$$

where D is the translational diffusion coefficient of the dissolved solute molecules, C_∞ is the bulk solubility of the dispersed phase, γ is the interfacial tension and V_m is the molar volume of the dispersed phase. R and T are the universal gas constant and the absolute temperature, respectively. The surface nucleation controlled growth of particles follows a logarithmic dependence on time (Cabane et al., 2005; Solomatov and Stevenson, 1993) and is given by

$$d - d_0 = k_1 \log\left(1 + \frac{t}{\tau}\right)$$

where τ and k_1 are constants with dimensions of length and time, respectively.

Although, the importance of Ostwald ripening on the physical stability of nanosuspensions has been underlined in a number of literature reports (Chaubal and Popescu, 2008; Eerdenberg et al., 2008; Jacobs et al., 2000; Lindfors et al., 2006; Moschwitzer et al., 2004; Pace et al., 1999; Verma et al., 2009a,b), detailed studies on the stability of nanosuspensions including the role of Ostwald ripening have not been reported. In this work Ostwald ripening of nanosuspensions has been investigated in detail with special emphasis on the stabilizer characteristics. The first aspect of this study deals with investigation of the effect of the micellar solubilized drug on Ostwald ripening. For this, indomethacin is used as a model drug and nanosuspensions were prepared at two different drug:stabilizer ratios (high and low) with two small molecule surfactants (sodium lauryl sulfate (SLS) and Dowfax 2A1 (DF)). Ostwald ripening of nanosuspensions was followed for three months under various stress conditions (elevated temperature, thermal cycling and mechanical shaking).

Both surfactants and polymers can be used as stabilizers in nanosuspension formulations. However, interfacial film characteristics differ significantly for these different types of stabilizers. Surfactants are usually small molecules; as a result their interfacial films are more dynamic as compared to the polymers which generally exhibit irreversible adsorption (Walstra, 1983). Adsorbed polymer layers are normally more robust and can prevent or slow down the attachment/detachment of drug molecules at the surface of dispersed particles and hence can affect Ostwald ripening. Moreover, polymers have been known to prevent crystal growth (Raghavan et al., 2001, 2003; Ziller and Rupprecht, 1990) in a number of cases. Therefore, the other aspect of this study deals with the effect of the characteristics of the interfacial layer on Ostwald ripening. For this, indomethacin nanosuspensions were prepared using a polymeric stabilizer; hydroxypropyl methylcellulose (HPMC). Ostwald ripening of these nanosuspensions was then compared to those prepared with small molecule surfactants (under similar stress conditions) to evaluate the role of the interfacial layer characteristics on Ostwald ripening.

2. Material and methods

2.1. Materials

Indomethacin USP, 1-(p-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetic acid, γ polymorph, was purchased from PCCA (Houston, TX). Methocel (hydroxypropyl methylcellulose) E5 Premium LV (HPMC E5) and Dowfax 2A1 (alkyldiphenyloxide disulfonate) (DF) were a generous gift from Dow Chemical Company (Midland, MI). Glycerin USP was purchased from PCCA (Houston, TX). Sodium lauryl sulfate (SLS) was purchased from Sigma–Aldrich (St. Louis, MO). Methanol HPLC grade was purchased from Fisher Scientific (Fair Lawn, NJ).

2.2. Preparation of nanosuspensions

The required amount of indomethacin was dispersed in 100 ml of the stabilizer solution using a mechanical stirrer to form a macro-suspension of the drug. The macro-suspension was homogenized at 10,000 rpm for 10 min using a PowerGen 700 D (Fisher Scientific) lab homogenizer to break up any lumps of the drug that may be present in the macro-suspension. Particle size reduction was carried out by processing this pre-conditioned macro-suspension through a microfluidizer model 110Y (Microfluidics, Newton, MA) at 18,000 psi for 70 min. The bulk temperature of the nanosuspension was maintained within $15 \pm 1\,^\circ$C during processing using a circulating water bath (Grant Ltd. 6, Grant Instruments, Cambridge, U.K.).

2.3. Characterization of nanosuspensions

2.3.1. Particle size analysis

The particle size distribution of the nanosuspensions was determined via dynamic light scattering (DLS) using Submicron Particle Sizer Autodilute Model 370 (Nicomp Particle Sizing Systems, Santa

Barbara, CA) at 25 °C. Samples were diluted with 30% glycerin (including stabilizer to match the stabilizer concentration to that of the nanosuspensions) before measuring particle size. The glycerin solution was pre-saturated with indomethacin. Viscosities of the diluted samples were measured using a Brookefield viscometer (Model DV III, Stoughton, MA) and were incorporated in the particle size calculations. Three dilutions for each sample were prepared and their average intensity weighted particle size and standard deviations are reported. Sample run time was approximately 8 min.

2.3.2. Zeta potential measurement

The zeta potential values of the nanosuspensions were determined using a Zeta Plus (Brookhaven Instruments Corporation, Holtsville, NY). Samples were diluted with the respective original dispersion medium pre-saturated with indomethacin. All measurements were made in triplicate and the mean values and standard deviations are reported.

2.3.3. Light microscopy

Suspensions were diluted appropriately and observed using a Nikon Eclipse TE 200 microscope (400× magnification) equipped with a digital camera to determine the presence of any large crystals or aggregates, that may be generated during storage stability. Images were analysed with NIS-Elements BR 2.30 software.

2.3.4. Scanning electron microscopy

SEM of the initial formulations and formulations after storage were conducted to evaluate the bulk morphology of the particles initially and also to evaluate any changes after storage at different conditions. Nanoparticles were affixed onto a gold coated silicon chips by placing a 3 μl of the diluted nanosuspension on it and allowing the suspension to dry at room temperature. The images were obtained by a Ziess DSM-982 Gemini (Germany) FE-SEM at an accelerating voltage of 2 kV.

2.3.5. X-ray diffraction

One to two milliliters of the SLS or Dowfax based suspensions were filtered through 0.05 μm filters (polycarbonate) to separate the solids. In the case of HPMC formulations 1–2 ml of the nanosuspensions were centrifuged at 12,000 rpm (rcf: 9659 × *g*) using a Minispin centrifuge (Eppendorf, Westbury, NY) for 10 min to separate the solids. X-ray diffraction patterns were obtained using a X-ray diffractometer (Xcalibur, Varian Inc., Oxford Diffraction, Blacksburg, VA) using Cu-kα radiation (λ: 1.5418 Å) and an Onyx area detector. The powder was filled in the 0.5 mm thin walled glass capillaries and was exposed for 60 s to the X-ray. The detector distance was kept at 65 mm. Powder diffraction patterns were generated using CrysAlis^{Pro} software.

2.4. Physical stability of nanosuspensions

Physical stability of the nanosuspensions was evaluated under various stress conditions such as storage at elevated temperatures, thermal cycling and mechanical shaking. The details for the same are given below.

2.4.1. Effect of temperature

Prepared nanosuspensions were divided into four parts and kept at 15 °C, 25 °C, 35 °C and 45 °C for three months. The samples were withdrawn on days 3, 5, 7, 10 and 14, and 1, 2 and 3 months. The samples were characterized for particle size, zeta potential and physical form of the drug as described above.

2.4.2. Thermo-cycling

Two milliliters of suspensions were maintained in 10 ml vials and subjected to thermo-cycling to determine formulation robustness with respect to temperature variability. One cycle consisted of storing the formulation at 4 °C for 24 h followed by 40 °C for 24 h. Particle size distribution and zeta potential of the formulations were evaluated after four such cycles.

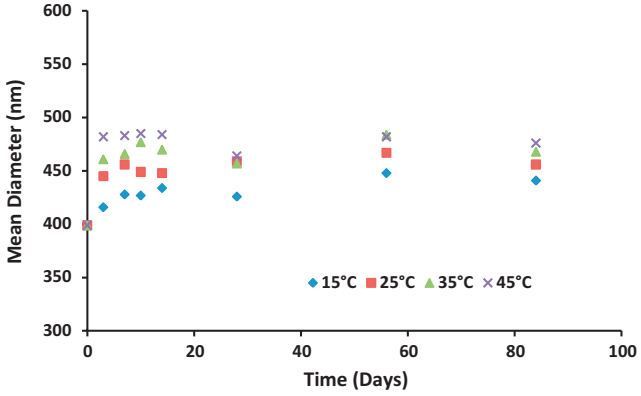

Fig. 1. Mean diameter of SLS 1 formulation as a function of temperature and time.

2.4.3. Mechanical shaking

To evaluate the effect of mechanical stress on the physical stability of nanosuspensions 1 ml of suspension was placed in 2 ml vials and maintained on a reciprocating shaker (Eberbach Corp., Ann Arbor, MI). Suspensions were shaken at a rate of 180 oscillations per min for 3 days at room temperature and the particle size distributions of the suspensions were determined.

3. Results and discussions

Nanosuspensions of indomethacin were prepared with three different stabilizers: sodium lauryl sulfate (SLS), Dowfax 2A1 (DF) and hydroxylpropyl methyl cellulose (HPMC E5). SLS and DF are small molecule ionic surfactants which stabilize the indomethacin nanosuspension via electrostatic stabilization. HPMC E5 is a large molecule non-ionic stabilizer and provides stability via steric stabilization. To study the effect of dispersed phase concentration on Ostwald ripening, nanosuspensions with two different concentrations of indomethacin were prepared. The details of the different nanosuspensions investigated are given in Table 1. The average particle size of nanosuspensions varied in the range of 330–430 nm. In general, the initial particle size of formulations with low drug:stabilizer ratio was lower than those with high drug:stabilizer ratio (Verma et al., 2009a). Detailed discussion of Ostwald ripening in the respective formulations is presented below.

3.1. Particle size: effect of temperature and duration of storage

3.1.1. SLS and DF formulations

Fig. 1 shows the mean particle diameter of the SLS 1 formulation as a function of storage temperature and time. Particle size increased initially from day 0 to day 3 for all the temperature conditions with the least increase at 15 °C and the highest increase at 45 °C. After day 3 no appreciable changes in particle size were observed in samples stored at 25 °C, 35 °C and 45 °C until the end of the study at three months. Samples stored at 15 °C increased in size until day 14 and after that there was no appreciable increase over the three month study period.

The variation in mean particle diameter of the SLS 2 formulation with respect to temperature and time is depicted in Fig. 2. Similar trends to those observed with the SLS 1 formulation were obtained. Significant increase in the mean diameter occurred between day 0 and day 3 for all storage conditions with the exception of the 15 °C

Table 1
Indomethacin nanosuspensions prepared with three different stabilizers.

Formulation	Stabilizer type and conc.	Indomethacin conc.	Drug:stabilizer ratio	Initial particle size (nm)[a]
SLS 1	SLS, 0.125% (w/v)	5% (w/v)	High (40)	399 ± 9
SLS 2		0.5% (w/v)	Low (4)	389 ± 8
DF 1	DF, 0.5% (w/v)	5% (w/v)	High (10)	361 ± 9
DF 2		0.5% (w/v)	Low (1)	332 ± 9
HPMC 1	HPMC, 0.5% (w/v)	5% (w/v)	High (10)	419 ± 19
HPMC 2		0.5% (w/v)	Low (1)	428 ± 13

[a] Mean ± std. dev.

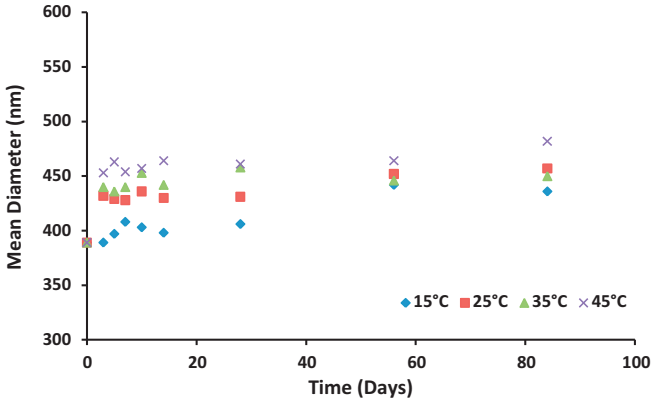

Fig. 2. Mean diameter of SLS 2 formulation as a function of temperature and time.

sample. The mean particle diameter of the sample stored at 15 °C increased thoroughout the study period of three months. The mean diameter did not change appreciably for all other storage conditions after day 3. Although, a slight increase in size was observed for the sample stored at 25 °C after 2 months.

The effect of storage temperature and time on the mean diameter of the DF1 formulation is shown in Fig. 3. Increase in mean particle size was observed for all storage conditions. The relative increase in mean particle size on day 3 compared to the initial mean particle size was highest for samples stored at 45 °C, followed by 35 °C and 25 °C. No significant increase in size was observed on day 3 for samples stored at 15 °C. After day 3, the mean particle size increased up to day 10 for the samples stored at 45 °C followed by a plateau. Similar trends were observed for samples stored at 35 °C, 25 °C and 15 °C, except that the samples stored at 25 °C and 15 °C exhibited a more gradual increase. Fig. 4 shows the evolution of particle size for the DF 2 formulation with time. A rapid increase in particle size followed by a decrease was observed under all storage conditions during the initial 14 days. After which particle size increased again, but at a much slower rate up to the end of the 3 month study period for samples maintained at 35 °C and 45 °C. In case of the 15 °C and 25 °C samples a plateau was reached around day 14.

The average particle diameter increased in SLS and DF nanosuspensions for all storage conditions, as depicted in Figs. 1–4 and Table 2. Among all the storage conditions investigated, the relative change in particle size on day 3 compared to that on the initial time point was the highest for samples maintained at 45 °C. This can be explained on the basis of the increased molecular solubility of indomethacin in the stabilizer solutions at elevated temperatures. As a result, a larger percentage of smaller particles are dissolved at higher temperature which shifts the mean particle diameter of the samples stored at 45 °C to a relatively large diameter as compared to those at 15 °C. The above explanation is also consistent with the observation that the overall increase in size in the three month study period was approximately 10% and approximately 20% for samples stored at 15 and 45 °C, respectively.

The highest increase in size was observed in the SLS 2 formulation stored at 45 °C which exhibited a maximum size of approximately 125% of the original size in three months. However, a closer look at the kinetics of increase in particle size of the SLS formulations stored at 45 °C (Figs. 1 and 2) clearly shows that most of the particle growth was attained by day 3, with no significant increase after that. Similar trends can be observed for both SLS formulations at other storage conditions (25 °C and 35 °C), however a gradual increase in size was observed at 15 °C. Although, temperature is inversely related to the Ostwald ripening rate as per the LSW theory, temperature also influences solubility, interfacial tension and diffusion coefficient. In the present case it is plausible that the lower saturation solubility of indomethacin at 15 °C as compared to higher temperatures may be responsible for the gradual increase in size at this storage condition.

Kinetics of increase in particle size in DF formulations follows a similar pattern to that of the SLS formulations with a few exceptions. The rate of Ostwald ripening is greatest for the period

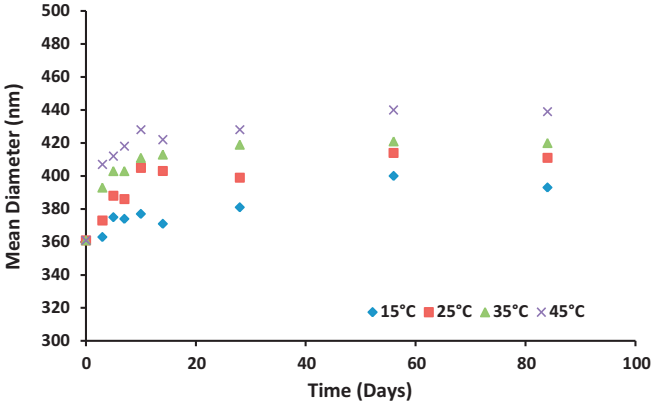

Fig. 3. Mean diameter of DF 1 formulation as a function of temperature and time.

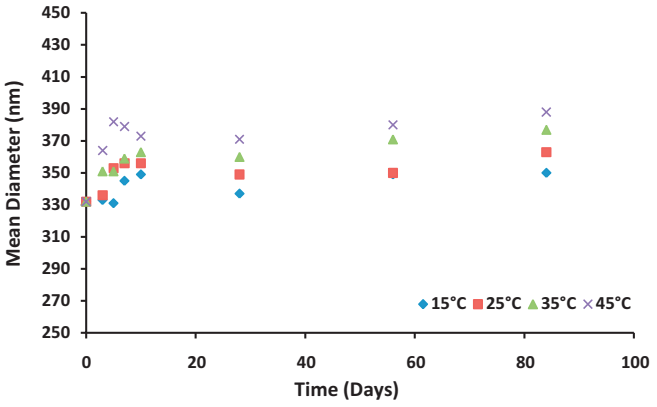

Fig. 4. Mean diameter of DF 2 formulation as a function of temperature and time.

Table 2
Relative particle size after storage at different temperatures.

Formulation	Drug:stabilizer ratio	Initial particle size (nm)	Relative size (%) after 3 months of storage[a]			
		Mean ± std. dev.	15 °C	25 °C	35 °C	45 °C
SLS 1	High	399 ± 9	110 ± 2	114 ± 1	117 ± 6	119 ± 4
SLS 2	Low	389 ± 8	112 ± 5	118 ± 2	116 ± 3	124 ± 4
DF 1	High	361 ± 9	109 ± 3	114 ± 3	116 ± 4	122 ± 2
DF 2	Low	332 ± 9	105 ± 1	109 ± 2	114 ± 2	117 ± 4
HPMC 1	High	419 ± 19	100 ± 2	99 ± 2	110 ± 3	210 ± 1
HPMC 2	Low	428 ± 13	94 ± 3	93 ± 4	96 ± 2	108 ± 4

[a] Mean ± std. dev.

between day 0 and day 3 for samples stored at 45 °C and 35 °C. However, these particles continued to grow until day 10, though at a reduced rate. DLS formulations stored at 25 °C exhibited an increase in size until day 10 compared to the SLS formulations which did not show appreciable increase in size after day 3. Analogous to the SLS formulations, the mean particle size of the DF formulations stored at 15 °C increased gradually with time until day 10. The longer time taken to reach a plateau for DF formulations stored at 25, 35 and 45 °C (compared to the SLS formulations) may be ascribed to the superior characteristics of the DF interfacial film which prevented Ostwald ripening of these nanosuspensions.

On comparison of Figs. 1 and 2 (SLS 1 and SLS 2) along with the % increase in mean particle size (Table 2) it is evident that the rate of Ostwald ripening was similar in both formulations. Interestingly, in the case of the DF nanosuspensions, the DF 2 formulation consistently exhibited a slightly lower percentage increase in size when compared to the DF 1 formulation at all temperatures studied suggesting a lower Ostwald ripening rate (Figs. 3 and 4, Table 2). The SLS 1 and DF 1 formulations had a high drug:stabilizer ratio (surfactant concentration is one tenth of indomethacin) whereas SLS 2 and DF 2 formulations had a low drug:stabilizer ratio (surfactant concentration is equal to that of indomethacin) (Table 1). Despite having higher concentration of surfactants in the SLS 2 and DF 2 formulations (and hence a greater number of micelles) the rate of Ostwald ripening was similar to that of the SLS 1 and DF 1 formulations, respectively. Thus, it would appear that micellar solubilized indomethacin did not contribute to the Ostwald ripening (Kabalnov et al., 1990). The slower ripening kinetics in the DF nanosuspensions with relatively higher concentration of surfactants can be attributed to the superior interfacial film properties reducing the rates of attachment and detachment of the indomethacin molecules at the nanoparticle surface.

3.1.2. HPMC formulations

Samples of HPMC 1 and HPMC 2 formulations stored at 15 °C, 25 °C and 35 °C did not show any increase in mean particle size. The HPMC 1 sample stored at 45 °C exhibited a steep increase in mean particle diameter up to day 14 followed by a slower rate of ripening until the end of the three month study period (Fig. 5). The mean particle diameter almost doubled compared to the initial mean diameter (Table 2). In contrast to the HPMC 1 sample, the HPMC 2 sample stored at 45 °C, exhibited a gradual increase in mean particle diameter. The steep increase in particle diameter oh the HPMC 1 formulation can be attributed to desolvation of the HPMC molecules at 45 °C and subsequent loss in steric stabilization of the nanoparticles. HPMCs are known to exhibit temperature dependent solubility. The chains of the HPMC molecules dehydrate at higher temperature leading to phase separation and gelation (Sarkar and Walker, 1995). The temperature at which phase separation occurs is known as the lower critical solution temperature (LCST). LCST of HPMC E5 is approximately 55 °C at 10% (w/w) in water (O'Connor and Gehrke, 1997). It is plausible that in samples stored at 45 °C partial dehydration of the HPMC molecules led to

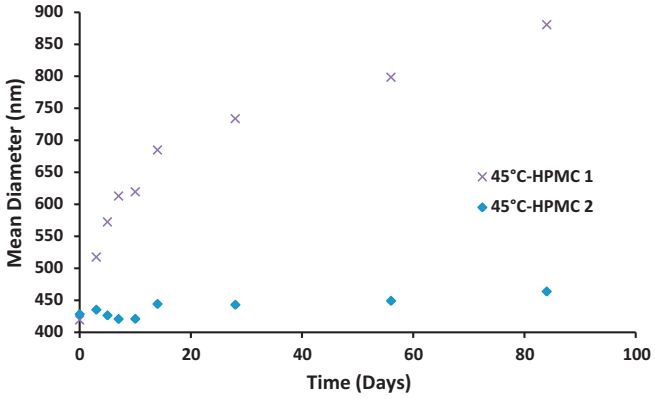

Fig. 5. Mean diameter of HPMC formulations at 45 °C as a function of time.

changes in the arrangement of the HPMC molecules in the interfacial film adsorbed on the nanoparticles resulting in reduced surface coverage of the nanoparticles. The reduced surface coverage would in turn result in an increase in particle size due to Ostwald ripening. Reduced surface coverage also explains why increase in particle size was much greater in the HPMC 1 formulation compared to the HPMC 2 formulation. The HPMC 1 formulation has a higher drug:stabilizer ratio (10:1) compared to the HPMC 2 formulation where the drug:stabilizer ratio is 1:1. A higher concentration of excess HPMC is available in the HPMC 2 formulation, compared to the HPMC 1 formulation. Although, dehydration of the HPMC molecules occurred in both formulations, it can be speculated that the excess HPMC present in the HPMC 2 formulation was able to fill the gaps in the surface coverage due to HPMC dehydration for samples stored at 45 °C. However, in the case of the HPMC 1 formulation there is insufficient excess HPMC present to achieve adequate surface coverage following HPMC dehydration.

To determine the mechanism of Ostwald ripening, the cube of the particle size was plotted against time. A representative plot of the cube of the mean diameter versus time is shown in Fig. 6 for the HPMC1 formulation, which exhibited continuous increase

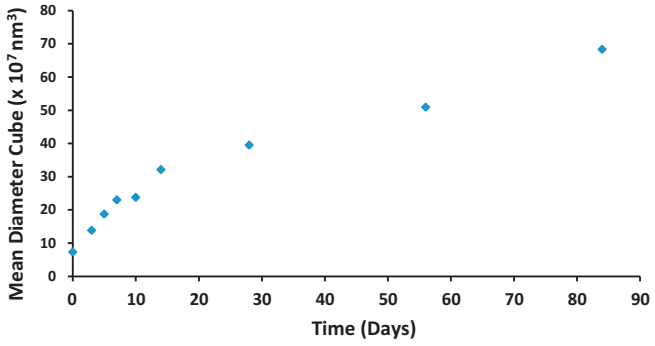

Fig. 6. Mean diameter cube of HPMC 1 formulation at 45 °C as a function of time.

Table 3
Effect of thermal cycling on relative particle size of various formulations.

Formulation	Drug:stabilizer ratio	Initial particle size (nm)	Relative size (%) after 4 cycles of thermal cycling[a]
		Mean ± std. dev.	
SLS 1	High	399 ± 9	114 ± 1
SLS 2	Low	389 ± 8	114 ± 4
DF 1	High	361 ± 9	108 ± 4
DF 2	Low	332 ± 9	105 ± 4
HPMC 1	High	419 ± 19	98 ± 5
HPMC 2	Low	428 ± 13	97 ± 2

[a] Mean ± std. dev.

in the mean diameter for the duration of the study. A non linear increase in the cube of the particle diameter was observed with time. The growth process was linear up to day 14 after which the ripening process slowed down significantly. Similar, observations were made for the SLS and DF formulations except that the deviation from linearity was much earlier when compared to HPMC 1 formulation. The higher the effect of the stabilizer on the solubility of the indomethacin the earlier the deviation from linearity was observed.

The non-linearity in the ripening process indicates that the ripening process cannot be described by diffusion controlled or spiral growth mechanisms. A plot of the square of the mean diameter versus time (not shown) was also non-linear. The most probable reasons for these deviations can be the presence of the stabilizer film at the interface and the non-negligible volume fraction of the dispersed solid particles in the nanosuspensions. Both of these factors have been assumed to be insignificant in the derivation of the above rate laws. A plot of the log time versus the mean diameter of the HPMC 1 formulation from day 3 to the end of the study at three months (Fig. 7) yields a straight line suggesting surface nucleation to be the most likely mechanism for ripening of the indomethacin nanosuspensions.

3.2. Zeta potential

The zeta potential of all formulations was measured in their respective original dispersion media. SLS and DF are anionic surfactants and, as expected, formulations prepared with these stabilizers exhibited high negative zeta potentials in the range from −84 mV to −90 MV. HPMC being a steric stabilizer resulted in nanosuspensions with low zeta potential values ranging from −17 mV to −20 mV. No significant difference was observed in the high or low drug stabilizer ratio formulations for each of the stabilizers indicating that in both cases the stabilizer concentration was sufficient to cover the surface of the nanoparticles. No change in zeta potential was observed for any of the formulations after storage at different temperatures or after thermal cycling.

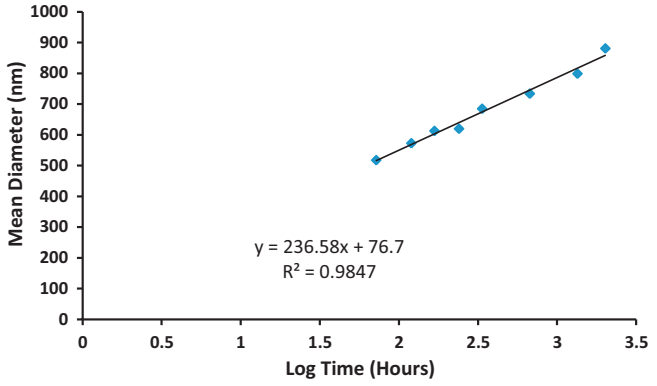

Fig. 7. Mean diameter of HPMC formulations at 45 °C plotted against log time.

3.3. Thermal cycling

Nanosuspensions consist of extremely small drug particles in equilibrium with a saturated solution of the drug. Variations in temperature can alter the solubility of the nanoparticles to a great extent and thus exposure of nanosuspensions to different temperatures can lead to crystal growth and increase in particle size. Increase in particle size can promote settling which may further lead to agglomeration and destabilization of the nanosuspensions (Pace et al., 1999). Temperature fluctuations can also affect the characteristics of the adsorbed stabilizer on to the nanoparticles. Both the extent and the geometry of the adsorbed stabilizer can vary leading to destabilization of the nanosuspensions. Table 3 shows the effect of thermal cycling on the prepared nanosuspensions. Increase in particle size was observed in the formulations made with SLS and DF, while the HPMC based formulations were able to maintain their size. This can be explained on the basis of differences in stabilizer characteristics. HPMC is a polymeric stabilizer and polymer adsorption is known to be irreversible when compared to the more dynamic adsorption of small molecule stabilizers (SLS and DF) (Verma et al., 2009a; Walstra, 1983). No effect of drug:stabilizer ratio was observed in SLS and HPMC based formulations. Increase in particle size was similar in both formulations (SLS 1 vs. SLS 2 and HPMC 1 vs. HPMC 2) for each of the stabilizer solutions. A relatively greater increase in size was observed in the DF nanosuspension with higher drug:stabilizer ratios (DF 1) as compared to that with lower drug stabilizer ratios (DF 2).

Another interesting observation is that in the case of the SLS formulations, the particle size increased to 114% of the original size as compared to the DF formulation where the increase was only up to 105–108%. SLS has a Krafft point of approximately 9 °C (Broze, 1997). Below this temperature, increase in the concentration of SLS leads to precipitation rather than micelle formation. Therefore, storage at 4 °C during the thermal cycling not only resulted in increase in supersaturation due to decreased molecular solubility of indomethacin but also due to decrease in its micellar solubility as a result of precipitation of SLS which resulted in a greater increase in the size in SLS formulations. No such precipitation was observed in the case of the DF formulations. Another contributing factor could be the superior characteristics of the DF interfacial film interfering with the Ostwald ripening of the nanoparticles.

3.4. Mechanical stress

Nanosuspensions were subjected to mechanical stress to gauge their stability against aggregation. The relative particle size of the formulations obtained after three days of shaking at room temperature is shown in Table 4. No effect of mechanical stress was observed in all formulations investigated. Although, relative par-

Table 4
Effect of mechanical stress on relative particle size of various formulations.

Formulation	Drug:stabilizer ratio	Initial particle size (nm)	Relative to initial size (%)[a]	
		Mean ± std. dev.	Storage at 25 °C for 3 days	Shaking at 25 °C for 3 days
SLS 1	High	399 ± 9	111 ± 2	114 ± 2
SLS 2	Low	389 ± 8	111 ± 4	116 ± 1
DF 1	High	361 ± 9	103 ± 5	107 ± 2
DF 2	Low	332 ± 9	101 ± 1	101 ± 3
HPMC 1	High	419 ± 19	98 ± 3	97 ± 3
HPMC 2	Low	428 ± 13	100 ± 2	100 ± 2

[a] Mean ± std. dev.

ticle size increased in SLS and DF formulations, the increase was similar to the samples stored at 25 °C without shaking, signifying that the increase was not due to mechanical shaking but was a result of the storage temperature.

Evaluation of changes in particle size due to the physical instability of the nanosuspensions is a difficult task. In this study DLS was used to follow the growth of the nanoparticles in the prepared suspensions subjected to various stress conditions. It was assumed that the increase in the particle size occurred due to Ostwald ripening. Flocculation and aggregation either alone or in combination with Ostwald ripening can also contribute to the increase in size, as determined by DLS. However the absence of these processes was confirmed by light microscopy and electron microscopy data and therefore the particle size data is discussed in the context of Ostwald ripening. Other factors that may complicate the data analysis include drug degradation and polymorphism. No significant degradation of the indomethacin was observed in any nanosuspension formulation (data not shown). Generation or conversion of the starting material (indomethacin, γ-polymorph) to a new physical form (i.e., α-indomethacin and amorphous form) during processing or at storage may affect the Ostwald ripening of the drug nanosuspension due to known differences in the water solubility of the various polymorphs of the drug. However, no change in the physical form of the drug was observed in X-ray diffraction studies even following three months storage at 45 °C.

4. Conclusions

The physical stability of indomethacin nanosuspensions was investigated under different stress conditions. Increase in the particle size observed was attributed to Ostwald ripening. Increase in the amount of the micellar solubilized drug did not have a significant effect on the ripening rate. In fact, relatively lower rates of particle size increase were observed in DF suspensions with higher concentrations of stabilizer. This was attributed to interference of the stabilizer layer at the interface with both dissolution and growth at the nanoparticle interface. With the exception of the HPMC 1 formulation, the particle size in all other formulations increased by less than 25% of the original size under the most stressful condition (45 °C for three months). Excessive increase in particle size in the HPMC 1 formulation was attributed to dehydration of the HPMC molecules resulting in loss of protection of the suspended nanoparticles. All formulations exhibited a non-linear increase in size with time and this could not be described by the LSW diffusion controlled theory of Ostwald ripening. The probable reasons for the deviations are the presence of the stabilizer layer which interfered with the growth and dissolution of the nanoparticles and the high volume fraction of solid particulates in the nanosuspension systems. The growth kinetics of the HPMC 1 formulation can be described more closely by the surface nucleation mechanism rather than by the diffusion controlled mechanism of Ostwald ripening.

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