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Modulation of drug release from nanocarriers loaded with a poorly water soluble drug (flurbiprofen) comprising natural waxes

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In this study, flurbiprofen (FLB) Solid Lipid Nanoparticles (SLN) composed from a mixture of beeswax and carnauba wax, Tween 80 and egg lecithin as emulsifiers have been prepared. FLB was incorporated as model lipophilic drug to assess the influence of matrix composition in the drug release profile. SLN were produced by microemulsion technique. *In vitro* studies were performed in Phosphate Buffered Saline (PBS). The FLB loaded SLN showed a mean particle size of 75 ± 4 nm, a polydispersity index $\sim 0.2 \pm 0.02$ and an entrapment efficiency (EE) of more than 95%. Suspensions were stable, with zeta potential values in the range of -15 to -17 mV. DSC thermograms and UV analysis indicated the stability of nanoparticles with negligible drug leakage. Nanoparticles with higher beeswax content in their core exhibited faster drug release than those containing more carnauba wax.

1. Introduction

Solid Lipid Nanoparticles (SLN) were developed at the beginning of the 1990 s as an alternative carrier system to the existing traditional carriers like emulsions, liposomes and polymeric nanoparticles (Müller and Lucks 1996). SLN made of solid lipids (lipids being solid at room and body temperatures) are submicron colloidal carriers (50–1000 nm) dispersed either in water or in an aqueous surfactant solution (Mühlen et al. 1997). By definition, the lipids can be highly purified triacylglycerols, complexes acylglycerol mixtures or waxes (Souto et al. 2007). Compared to other particulate carriers, SLN show advantages as drug delivery system, like good tolerability and biodegradability, physical stability, possibility of large-scale production, and efficient incorporation of lipophilic drugs in their lipid core (Mŭller and Keck 2004; Mühlen et al.1997).

SLNs are composed of physiological and compatible lipids with a high melting point as the solid core, which is coated by non-toxic amphiphilic surfactants as the outer shell (Mehnert and Mäder 2001). Studies have shown that the physiochemical characteristics and stability of drug-loaded SLNs depend on the properties of drug and ingredients (Lim and Kim 2002). Appropriate choice of lipids, surfactants, and their composition affect the particle size, long-term stability during storage, drug loading, and release behaviour (Kim et al. 2005).

Flurbiprofen (FP) [*(RS)*-2-(2-fluorobiphenyl-4-yl) propionic acid] was selected as a model drug. The potent nonsteroidal anti-inflammatory agent is commonly used for the treatment of acute and chronic rheumatoid arthritis. The usual dose of FP by the oral route is 50–100 mg, administered twice per day. Because of its short plasma half-life (2–4 h), it should be frequently administered. Indeed, FP is readily absorbed from the gastrointestinal tract, where a high concentration results in increased side effects (Martindale 1999; Doyle 2008).

The short half-life, poor solubility in water, low bioavailability, and side effects in the gastrointestinal tract make FP a promising candidate for formulation of a controlled-release dosage form (Xiaomei and Xing 2007; Loganathan 2010). To overcome the problems of FP administration, many studies have focused on preparing particular systems such as lipospheres (Jain et al. 2005), polymeric carriers, pellets containing FP, and formulations for dermal and topical use. Solid lipid particulate systems based on lipophilic materials like waxes or fats have also been developed (Manna et al. 2006; Vergote et al. 2001). Lipid-based drug delivery systems enhance the bioavailability of lipophilic drugs such as halofantrine and ontazolast by lymphatic transport of biosynthesized chylomicrons associated with the drugs. In this work, FP-loaded SLNs were prepared by a mixture of beeswax and carnauba wax as the lipid core and combination of egg lecithin and Tween 80 as emulsifier.

The effects of lipid proportion in the lipid mixture, surfactant concentration, and surfactant composition on the particle size, drug loading, thermal characteristics, and drug release behaviour of the resulting nano drug delivery systems were investigated.

2. Investigations, results and discussion

2.1. Effect of surfactant concentration on the particle size and drug loading

The particle size distribution of the nanoparticles presented in Fig. 1 shows that increasing the concentration of surfactant mixture from 0.5 to 1.5% (wt/vol) in a mixture with drug and lipid mixture resulted in reduced particle size of SLNs, as depicted in Table 1. Polydispersity index (PDI), zeta potential, and entrapment efficiency were not much affected by increasing surfactant concentration. But, as per Eq. (2), upon increasing surfactant concentration the amount of excipients increases, which results

Table 1: Effects of surfactant concentration on the characteristics of FP-loaded SLNs

Surfactant conc. % (wt/vol)	Z average (nm)	PDI*	Zeta potential (mV)	EE%	DL%
0.5	98 ± 6.54	0.28 ± 0.02	–15.50 ± 1.85	95.22 ± 0.8	11.20 ± 0.05
1.0	74 ± 3.44	0.32 ± 0.02	–16.10 ± 1.49	96.18 ± 0.2	10.12 ± 0.04
1.5	64 ± 5.22	0.22 ± 0.02	–14.50 ± 0.78	95.04 ± 0.5	9.80 ± 0.02

* polydispersity index

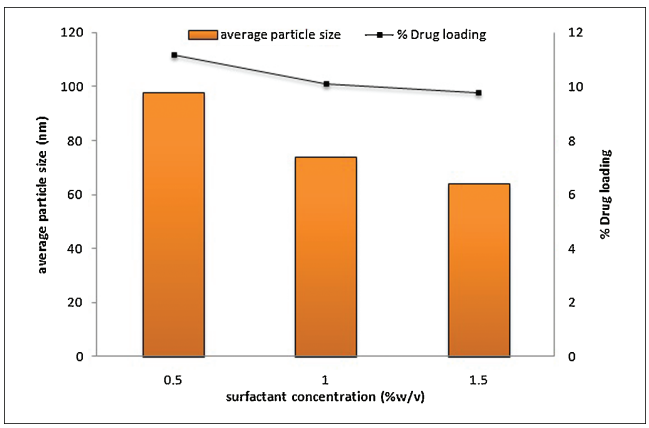


Fig. 1: Effect of surfactant concentration on the particle size (Z) and drug loading (DL %) of flurbiprofen loaded solid lipid nanoparticles (SLNs)

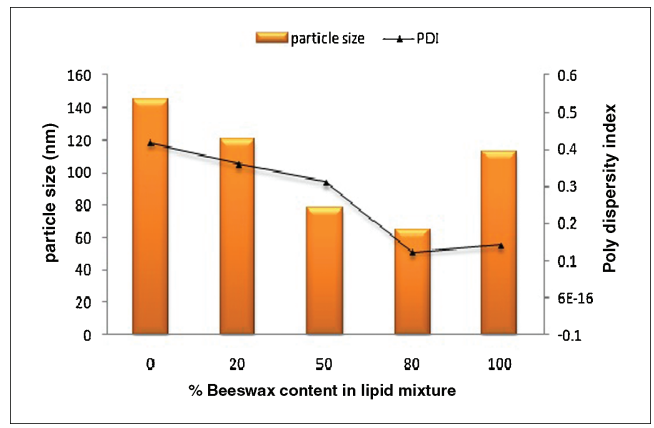


Fig. 2: Effect of beeswax content in lipid mixture on the size (Z) and polydispersity (PDI) of particles prepared using a 1% (wt/vol) surfactant mixture with 50% Tween 80 content

in reduced drug loading for constant amount of entrapped drug. Surfactant concentration was fixed at 1% (wt/vol) in subsequent experiments as average particle size from 1% (wt/vol) surfactant concentration is 82 nm, which is below 100 nm, as an acceptable particle size. It was found that particle size decreased by increasing surfactant content. As expected, the reduction of surface tension by increasing surfactant concentration facilitates the particle partition during homogenization (Mehnert and Mäder 2001) and contributes to reduction of particle size. Using a lipid mixture of beeswax and carnauba wax can decrease the size of SLNs as compared with pure lipids. These results indicate that lipid mixtures can be more suitable than pure lipids for production of SLNs.

2.2. Effect of lipid composition on the properties of SLNs

The effect of beeswax content in the lipid matrix on the properties of SLNs was studied by preparation of nanoparticles with various beeswax percentages in the lipid mixture while other ingredients were kept constant.

As shown in Fig. 2, variation of beeswax in the lipid mixture from 0 to 100% had a significant effect on the particle size and PDI. These results indicate that SLN prepared with mixed lipid matrix produced lower *z*-average sized particles when carnauba wax and Tween 80 were incorporated compared with SLN prepared with beeswax alone at polysorbate concentrations of 0 % (w/w) and 100 % (w/w), both particle size and PDI decreased upon increasing beeswax content in the lipid mixture. Zeta potential, entrapment efficiency and drug loading of nanoparticles did not change considerably upon increasing the beeswax content in the lipid mixture. As shown in Table 2, increase of beeswax percentages in the lipid mixture from 20% to 80%, and high entrapment efficiencies in the range of 96.4% to 96.9% were obtained. This was due to ease of particle disintegration and slower recrystallization in the mixed lipid as its melting point and crystallinity were lower than that of beeswax. These results suggest that FP favourably interacts with the different mixtures of beeswax and carnauba wax in the lipid core of SLNs.

Table 2: Effects of beeswax content (%) in lipid mixture on the entrapment efficiency (EE%) and drug loading (DL%) of SLNs

Beeswax (%) in 3% (wt/vol) lipid mixture	EE%	DL%
20	95.20 ± 0.5	10.12 ± 0.05
50	95.75 ± 0.6	10.65 ± 0.05
80	95.28 ± 0.2	10.35 ± 0.03

2.3. Effect of surfactant composition on the particle size

SLN prepared with mixed lipids and without polysorbate 80 showed high growth in particles up to 700 nm. Addition of a mobile surfactant is necessary for stabilization of the nanoparticles containing phospholipids.

For studying the effects of surfactant composition the amount of Tween 80 varied from 0 to 100%. The results are shown in Table 3 and Fig. 3. It is clearly shown that egg lecithin alone as emulsifier was not sufficient for stabilizing SLNs, and particle agglomerated leading to rise in an average particle size to 700 nm. These results may be due to the hydrophilic-lipophilic balance (HLB) value of surfactants required for stabilizing the lipid core. According to an earlier study, HLB values

Table 3: Effects of surfactant composition on the characteristics of SLNs

% T80 in surfactant mixture	Z average (nm)	PDI*	Zeta potential (mV)
0	700 ± 10.12	0.418 ± 0.02	–31.25 ± 1.3
40	103 ± 5.52	0.359 ± 0.04	–12.24 ± 1.11
50	80 ± 3.43	0.313 ± 0.05	–16.26 ± 0.68
60	65 ± 2.52	0.125 ± 0.03	–15.16 ± 0.44
100	262 ± 5.26	0.144 ± 0.02	–7.23 ± 0.90

* Polydispersity index

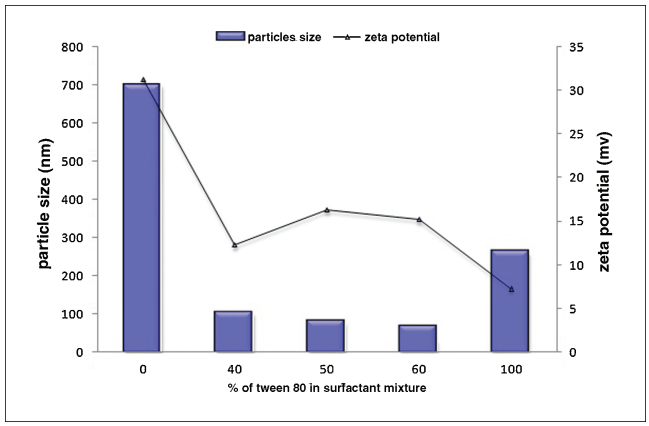


Fig. 3: Effect of Tween 80 concentration in surfactant mixture on the particle size and zeta potential of SLNs

required for emulsifying beeswax and carnauba wax are 9 and 12, respectively, while the HLB value for egg lecithin is 8 so lecithin alone is not sufficient for stabilizing a 1:1 mixture of beeswax and carnauba wax (Myers 2006). The average particle size with 100% Tween 80 was found to be 250 nm, but combination of surfactants reduced the particle size as well as PDI. Tween 80 and egg lecithin had a significant effect on zeta potential. Combination of these surfactants changed the surface properties of the SLNs. Because an absolutely large negative or positive zeta potential is required for colloidal dispersion stability, the range of zeta potential obtained for SLNs with surfactant mixture was not high enough to provide a strong electrical field around the particles (Attama et al. 2008). Measuring the particle size of samples 30 days after preparation showed no remarkable changes in the average size of these samples (Fig. 4). This suggests that combination of steric and electrostatic stabilization can avoid nanoparticle aggregation.

In terms of controlled and sustained or prolonged drug delivery, SLN prepared with beeswax containing both egg lecithin and polysorbate 80 could be recommended because of the more crystalline nature compared with the mixed lipid matrix SLN. Mixed surfactants often reduce interfacial tension more than single-surfactant formulations and surfactant type can also affect the stability of particles during periods of storage (Olbrich and Müller 1999; Lim and Kim 2002). We used the combination of Tween 80 as a non-ionic surfactant, and egg lecithin as a lipidic and zwitterionic surfactant. The use of mixed surfactants resulted in the preparation of stable SLNs formulated with decreased particle size with respect to pure surfactant. Besides the electrostatic stabilization provided by egg lecithin, Tween 80

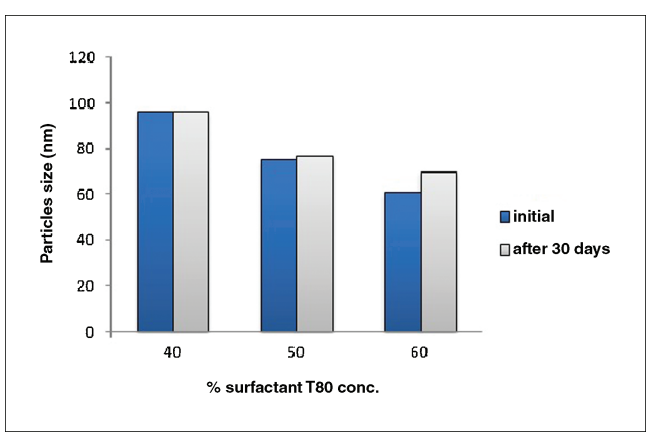


Fig. 4: Stability of flurbiprofen loaded SLNs prepared with different surfactant composition of 1:1 mixture of beeswax and carnauba wax, T80 = Tween 80

can also provide additional steric stabilization of particles (Kheradmandnia et al. 2010). Interpenetration of long polyethylene chains of Tween 80 limits freedom of the particles and prevents them from associating with one another, as previously shown (Lim and Kim 2002) and as reported above. However, when high drug incorporation is needed, SLN formulated with mixed lipid core containing lecithin and polysorbate 80 will be better as they were shown to possess low crystalline order which favours drug loading (Attama and Müller-Goymann 2007, 2008).

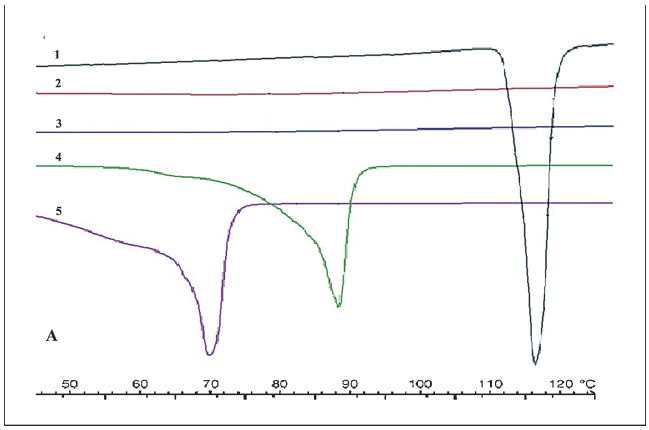
In subsequent stages of the study the amount of Tween 80 in the surfactant mixture was fixed at 50% because of the suitable size and zeta potential of particles obtained at this composition of surfactant mixture.

2.4. Thermal analysis

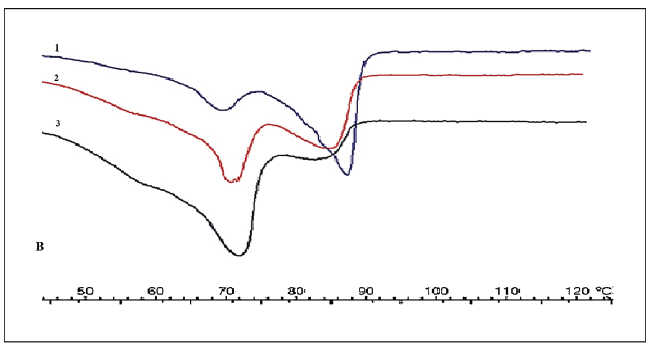
DSC was used to analyse the degree of crystallinity of the raw materials as well as for nanoparticles. The thermotropic phase behaviour of a lipid matrix system is highly affected by the presence of guest. Thermal analysis was performed initially and 30 days after sample preparation. Obtained results are shown in Fig. 5 for raw materials. The changes in heating peak, melting point and melting enthalpy in the thermograms of SLNs (Fig. 5, c) compared with raw materials (Fig. 5, a and b) can be assigned to the decreased particle size and large surface area i.e. colloidal dimensions. The lipid crystalline structure determines whether a drug will be expelled or incorporated in the nanoparticle i.e. a highly crystalline structure will lead to rapid expulsion of the drug molecules from the system on the other hand less ordered crystalline carrier gives spaces for incorporation of the drug. The high entrapment efficiency of FP in the SLNs (97%) would be due to a less ordered crystalline arrangement in the lipid carrier and increased crevices that allow incorporation of the drug molecules into the lipid matrix. Less ordered crystalline structure gives improved physical stability and less expulsion of drug from particles during its storage (Attama and Müller-Goymann 2008). FP entrapment efficiency at 0 and 30 days after preparation of the samples showed almost the same results (96% compared with 97%) by UV method. No peak of FP at 117 °C was detected in DSC thermograms of SLNs compared with thermograms of raw materials. The absence of a melting point has been observed for other drugs in previous studies. The thermograms of SLNs with different ratios of beeswax in lipid matrices (Fig. 5, c) indicate that crystallinity of the samples increased by decreasing beeswax content in the lipid mixture. This means that increasing proportions of carnauba wax in the lipid core of SLNs resulted in enhancement of melting enthalpy and lipid crystallization.

2.5. In vitro drug release

The release profile, presented in Fig. 6, shows an initial burst release during 1 h followed by a more sustained release for three samples. Small size of the nanoparticles (large surface area) and the fast rate of hydrolytic degradation are responsible for the initial burst release. Furthermore, slower drug release from nanoparticles with high carnauba wax content was obtained, which can be due to the increased hydrophilicity and crystallinity of SLNs (Attama and Müller-Goymann 2007). Faster drug release from nanoparticles with higher beeswax content can be attributed to a higher degradation rate, hydrophilicity, and less crystallinity of the beeswax structure. Two important mechanisms for drug release from SLNs are diffusion and erosion by hydrolytic degradation. SLNs degrade in the presence of water by acid-catalyzed ester hydrolysis reactions which are reversible (Hurrell and Cameron 2003). Degradation products are acidic, and the reaction also is autocatalytic. Increase of porosity in



(a)



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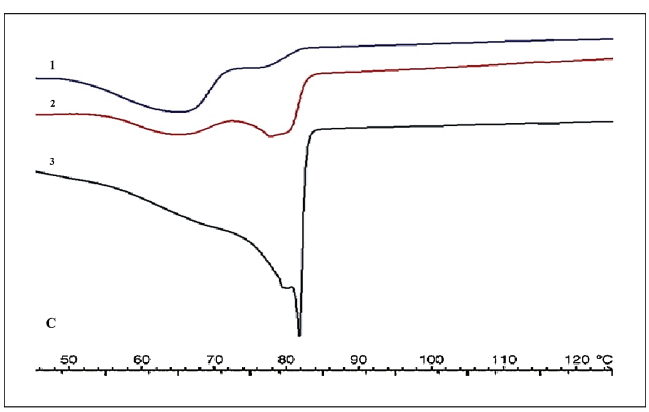

(c)

Fig. 5: Differential scanning calorimetry thermograms of the ingredients and of lipid nanoparticles. (a) Raw materials: 1. Flurbiprofen, 2. lecithin, 3. Tween 80, 4. carnauba wax, 5. beeswax, (b) lipid mixtures (ratio of beeswax to carnauba wax):1. 20:80, 2.50:50, 3.80:20, (c) Solid lipid nanoparticles (SLNs) prepared with different beeswax content of lipid mixture: SLNs with 80% beeswax (1), SLNs with 50% beeswax (2), SLNs with 20% beeswax (3)

lipid matrix and the mass loss steadily affects the drug release profiles. Drug release was expected to be slower from more lipophilic matrices than hydrophilic ones (Özyazıcı et al. 2006). Carnauba wax is a more lipoidal matrix and also contains 5% of resins that resist water to penetrate into the pores of the lipid structure. Therefore, the release from nanoparticles containing more carnauba wax is slow. Higher numbers of hydroxyl groups and free fatty acids in the beeswax structure increase the degradation rate and allow the water to penetrate easily into the pore of the matrix, leading to greater FP release with respect to particles containing more carnauba wax.

2.6. Conclusion

In the present study solid lipid nanoparticle containing Flurbiprofen were prepared by microemulsion technique using

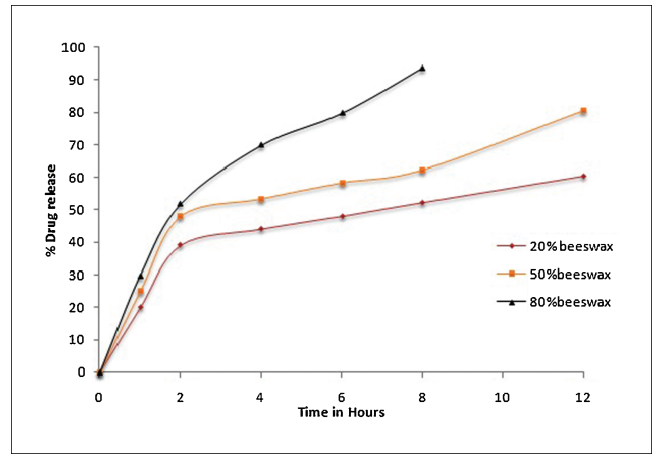

Fig. 6: Release profiles of flurbiprofen from SLNs prepared with different beeswax content in the lipid mixture

beeswax, carnauba wax, Tween 80 and egg lecithin as emulsifiers. The effect of surfactant content and composition and lipid composition on the properties of FP loaded SLNs prepared from beeswax and carnauba wax was studied. Prepared SLN had good average particle size, stability with acceptable PDI and high entrapment efficiency indicating a good compatibility between FP and the waxy core of SLNs. DSC thermograms showed that by increasing carnauba wax content in the lipid mixture, crystallinity of nanoparticles increased as a result of the chemical composition of the wax. Nanoparticles with more beeswax content in their core exhibited faster drug release as compared with those containing more carnauba wax in their structure.

3. Experimental

3.1. Drug and chemicals

Carnauba wax, Beeswax, egg lecithin, and FP were obtained as a gift sample from Sun Pharma (Mumbai, India). Tween 80 was purchased from Merck chemicals (India). Phosphate buffered saline (PBS), Acetonitrile, and all other solvents used were of analytical grade.

3.2. Preparation of SLNs

A microemulsion technique was used to prepare the SLNs (Ugazio et al. 2002). The specified quantities of mixture of beeswax, carnauba wax, egg lecithin, and drug were melted on a water bath at 90–92 °C. Tween 80 (surfactant, HLB 15.0) was added to purified water while at 90 °C and mixed for 2 min at 2000 rpm. Surfactant water mixture was added to the molten lipid mixture. The obtained emulsion was dispersed using a rotor-stator (Ultra-Turrax, Germany) at 25,000 rpm for the period of 5 min. Resulting nanoemulsion was dispersed in cool water at 2 °C with a volume ratio of 1:10 at 3000 rpm.
The formulation of various batches is as shown in Table 4.

3.3. Characterization of SLN

3.3.1. Particle size and zeta potential

Measurement of the average size, polydispersity, and zeta potential of the SLNs were determined by photon correlation spectroscopy (Malvern Zeta sizer, Nano Z-S; Malvern Instruments, Worcestershire, United Kingdom). Measurements were carried with an angle of 90 degrees at 25 °C. To determine average diameter and zeta potential, SLN dispersion samples were diluted with water.

3.3.2. Entrapment efficiency and drug loading

Entrapment efficiency and drug loading were determined by measuring concentration of free drug in the aqueous phase using the UV method. Free drug (unloaded) in suspension for UV analysis was separated from nanoparticles with ultra filtration method (Müller and Keck 2004; Mühlen et al. 1997). Entrapment efficiency (EE %) and drug loading (DL %) were estimated using the total added drug, drug in precipitate (total drug added – free drug)

Table 4: Experimental conditions for preparation of solid lipid nanoparticles

Number	Beeswax (%) in 3% (w/v) lipid mixture	% FP* added (w/v.)	% Surfactant amount (w/v.)	% T80 ** in surfactant mixture
1	50	0.5	0.5	50
2	50	0.5	1.5	50
3	50	0.5	1	50
4	50	0.5	1	0
5	50	0.5	1	40
6	50	0.5	1	60
7	50	0.5	1	100
8	0	0.5	1	50
9	20	0.5	1	50
10	80	0.5	1	50
11	100	0.5	1	50

* Flurbiprofen, ** polysorbate 80

and added excipients (lipid + surfactant mixtures), according to the following equations:

$$\text{EE}\,\% = \frac{\text{drug in precipitate}}{\text{total drug added}} \times 100 \quad (1)$$

$$\text{DL}\,\% = \frac{\text{drug in precipitate}}{\text{drug in precipitate + added excipients}} \times 100 \quad (2)$$

3.3.3. Differential scanning calorimetry (DSC)

DSC thermograms of the starting synthesis materials and SLNs samples were obtained on a Mettler instrument DSC-822e (Mettler Toledo, India) 30 days after sample preparation. For measurements, 6 mg of freeze-dried sample were placed on an aluminium pan and the thermal behaviour determined in the range of 5–125 °C at a heating rate of 5 °C per minute.

3.3.4. Drug release studies

For *in vitro* release studies, 35 mg of freeze-dried samples was redispersed in 1 ml PBS (0.1 M) and was charged into a cellulose acetate dialysis bag. A dialysis bag was inserted in a glass receptacle that contained 30 ml of PBS. The resulting dissolution medium was continuously stirred with a small magnetic stirring bar at 37 ± 0.5 °C and 1000 rpm to maintain homogeneity. At specified intervals, 1 ml of the dissolution medium was withdrawn and 1 ml of fresh PBS was added to the receptacle. Drug concentration was determined by an UV method. After filtration and appropriate dilution, the sample solution was analyzed at 247 nm for flurbiprofen by a UV spectrophotometer (Shimadzu 1700, Japan). The amounts of drug present in the samples were calculated with the help of appropriate calibration curves constructed from reference standards. Drug dissolved at specified time periods was plotted as percent release versus time (hours) curve.

References

Attama AA, Müller-Goymann CC (2007) Characterization of solid lipid nanoparticles with mixed lipid core for controlled drug delivery applications. Eur J Pharm Biopharm; 67:48–57.

Attama AA, Müller-Goymann CC (2008). Effect of beeswax modification on the lipid matrix and solid lipid nanoparticle crystallinity. Colloids Surf A, 315:189–195.

Doyle RM (ed), (2008) Nursing drug handbook. 28th ed. Philadelphia: Wolters Kluwer Health; 2008; 382–383.

Hurrell S, Cameron RE (2003) The effect of buffer concentration, pH and buffer ions on the degradation and drug release from polyglycolide. Polym Int 52:358–366.

Jain SK, Chourasia MK, Masuriha R, Soni V, Jain A, Jain NK, Gupta Y (2005) Solid lipid nanoparticles bearing flurbiprofen for transdermal delivery. Drug Deliv 12: 207–215.

Kheradmandnia S, Vasheghani-Farahani E, Atyabi M, Nosrati M (2010) Preparation and characterization of ketoprofen-loaded solid lipid nanoparticles made from beeswax and carnauba wax. Nanomedicine: NBM 6: 753–759.

Kim BD, Na K, Choi HK (2005) Preparation and characterization of solid lipid nanoparticles (SLN) made of cacao butter and curdlan. Eur J Pharm Sci 24: 199–205.

Lim SJ, Kim CK (2002) Formulation parameters determining the physiochemical characteristics of solid lipid nanoparticles loaded with all-trans retinoic acid. Int J Pharm 243: 135–146.

Loganathan V, Sivaprasada Reddy, M.V. (2010) formulation development and evaluation of Flurbiprofen lipospheres. Int J Advancem Sci Arts 1: 90–96.

Manna L, Banchero M, Sola D, Ferri A, Ronchetti S, Sicardi S (2006) Impregnation of PVP microparticles with ketoprofen in the presence of supercritical CO2. J Supercrit Fluids 42: 378–384.

Martindale (1999) The Complete Drug Reference. 32nd edition. United Kingdom: Pharmaceutical Press pp. 61–62.

Mehnert W, Mäder K (2001) Solid lipid nanoparticles production, characterization and applications. Adv Drug Deliv Rev47: 165–196.

Mühlen AZ, Schwarz C, Mehnert W (1997) Solid lipid nanoparticles (SLN) for controlled drug delivery—drug release and release mechanism. Eur J Pharm Biopharm 45: 149–155.

Muller RH, Keck CM (2004) Challenges and solutions for the delivery of biotech drugs—a review of drug nanocrystal technology and lipid nanoparticles. J Biotechnol 113: 151–170.

Müller RH, Lucks JS (1996) Azneistoffträger aus festen Lipidteilchen feste Lipid Nanosphären (SLN) European Patent 0605497; Germany.

Myers D (2006) Surfactant science and technology. 3rd ed. Hoboken: NJ: Wiley, p. 380.

Olbrich C, Müller RH (1999) Enzymatic degradation of SLN—effect of surfactant and surfactant mixtures. Int J Pharm 180: 31–39.

Özyazıcı M, Gökçe EH, Ertan G (2006) Release and diffusional modeling of metronidazole lipid matrices. Eur J Pharm Biopharm 63: 331–339.

Souto EB, Müller RH (2007) Lipid nanoparticles (SLN and NLC) for drug delivery. In: Domb AJ, Tabata Y, Kumar MNVR, et al. (ed) Nanoparticles for pharmaceutical applications. American Scientific Publishers, pp. 103–122.

Ugazio E, Cavalli R, Gasco MR (2002) Incorporation of cyclosporin A in solid lipid nanoparticles (SLN). Int J Pharm 241: 341–344.

Vergote GJ, Vervate C, Van Driessche I, Hoste S, De Smedt S, Demeester J, Jain RA, Ruddy S, Remon JP (2001) An oral controlled release matrix pellet formulation containing nanocrystalline ketoprofen. Int J Pharm 219: 81–87.

Xiaomei WJY, Xing T (2007) *In vitro* release and pharmacokinetics of Flurbiprofen sustained-release capsules containing coated pellets. Asian J Pharm Sci 2: 77–84.

Yang S, Zhu J, Lu Y, Liang B, Yang C (1999) Body distribution of camptothecin solid lipid nanoparticles after oral administration. Pharm Res 16: 751–757.