ORIGINAL PAPER

Glyceryl Behenate and Its Suitability for Production of Aceclofenac Solid Lipid Nanoparticles

Viney Chawla · Shubhini A. Saraf

Abstract The aim of this study was to explore the use of glyceryl behenate (GB) as a candidate lipid for the production of solid lipid nanoparticles (SLN) using the anti-inflammatory drug, aceclofenac, as an example. SLN were produced using the Gasco microemulsion method with three different lipids, namely GB, glyceryl palmitostearate (GP) and cetyl alcohol (CA). The prepared SLN were subjected to determination of entrapment, zeta potential, particle size, in vitro dissolution, entrapment efficiency and biodistribution. Stable SLN of GB having size 245 ± 5 nm were prepared with a polydispersity index of 0.470. The size range was higher with other lipids i.e., CA and GP. It was found that as the drug lipid molar concentration was raised, particles with a smaller size were obtained irrespective of the nature of the lipid. The surfactant poloxamer 188 gave best results when used at a concentration of 2.5% w/v of dispersion. The study recommends GB as a suitable candidate for the production of SLN.

Keywords Compritol · Glyceryl behenate · Solid lipid nanoparticles · Aceclofenac · Taguchi experimental design

Abbreviations
AC   Aceclofenac
CA   Cetyl alcohol
GB   Glyceryl behenate
GP   Glyceryl palmitostearate
SLN   Solid lipid nanoparticles
SR   Sustained release

V. Chawla
Faculty of Pharmacy, Northern India Engineering College, Lucknow 227105, Uttar Pradesh, India

S. A. Saraf (✉)
Faculty of Pharmacy, Babu Banarasi Das National Institute of Technology and Management, Lucknow 227105, Uttar Pradesh, India
e-mail: shubhini.saraf@gmail.com

Introduction

The science of dosage form design has always been haunted by the poor solubility of drugs. Among other approaches tried by scientists, colloidal systems like liposomes, polymeric nanoparticles etc., have shown promise but not many products have made it to the market. Solid lipid nanoparticles (SLN) are emerging as alternative carriers to colloidal systems, for controlled and targeted delivery. These are in submicron range (50–1,000 nm) and are made up of biocompatible and biodegradable material, capable of incorporating lipophilic and hydrophilic drugs [1–3]. SLN combine the advantages of different colloidal carriers, are physiologically acceptable and, like polymeric nanoparticles, controlled release of drug from lipid matrix can be anticipated [4, 5]. Aceclofenac (AC) is a non steroidal anti-inflammatory drug which acts by inhibiting the secretion of tissue necrosis factor and interleukin-1 [6]. Marketed sustained release preparations of aceclofenac include matrix tablets where accidental dose dumping may lead to higher concentrations at release sites leading to ulcers. Glyceryl dibehenate (European Pharmacopoeia) or Glyceryl behenate (GB) occurs as a fine powder with a melting range of 69–74 °C. Its saponification value lies between 145 and 165 mg KOH/gm. Glyceryl behenate (GB) is a lipid which contains 15–23% of monoglycerides, 40–60% of diesters and 21–35% of triesters content. The crystalline structure of GB is composed of very small amounts of unstable α

polymorphic form, characteristic of triacylglycerols, which disappears after thermal stress of bulk lipid. Mixing oils and drug molecules which are soluble in this lipid decrease its lattice organization and thus the lipid is suitable for production of lipid nanoparticles [7]. The aim of this study was to find out the suitability of GB, in preparation of SLN of anti-inflammatory drug, AC. The present study for the first time establishes the successful entrapment of drug AC in SLN through IR spectroscopy and also for the first time oral administration of AC-SLN in rabbits.

Materials and Methods

The drug, AC was given as a gift sample by Arbro Pharmaceuticals, India, and cetyl alcohol (CA) was procured from SD Fine Chemicals India. Glyceryl palmitostearate (GP) or Precirol and GB were received as gifts from Colorcon Asia Pvt. Ltd, India. Aceclo-SR® tablets were purchased from a local pharmacy. The surfactant, poloxamer 188 (Lutrol F 68) was received ex-gratia from BASF, Germany. The drug, surfactants and solid lipids were subjected to IR analysis for authentication. All the peaks observed were in accordance with the reference spectrum. An absorption maximum of the drug was determined using a Shimadzu Double beam UV 1700 spectrophotometer. This was in accordance with the reported absorption maxima of 275 nm. A standard curve of the drug was prepared in methanol at concentrations varying from 10 to 100 µg/ml. A concentration dependent increase in absorbance was observed with good correlation ($y = 0.0048x + 0.0016$, $R^2 = 0.9956$).

Preparation of Solid Lipid Nanoparticles

SLN were prepared by Gasco's method [8]. The lipids used were CA, glyceryl palmitostearate, and GB. The lipid GB was heated to 80 °C (a temperature at least 5 °C above its melting point i.e., 74 °C; similarly CA was heated to 54 °C and GP was heated to 68 °C). Accurately weighed amount of drug was dispersed in the molten lipid. The aqueous phase (about 20 ml) containing surfactant poloxamer 188 was heated to the same temperature as that of the lipid phase. The lipid phase was then added to the aqueous phase and contents were stirred for 30 min. The pre-emulsion thus formed was homogenized at 3,000 rpm for 30 min (Remi Motors, India) followed by ultrasonication (Sartorius Labsonic^P Ultrasonicator, Germany) for 2–8 min. After ultrasonication, the contents were diluted to 20 times with ice-cold water, where SLN were precipitated and were separated using membrane filters. Taguchi experimental design was used to reduce the number of experiments [9]. The details of Taguchi design used are given in Table 1.

Characterization of Prepared SLN

The prepared SLN were characterized for various aspects.

IR Studies

An IR spectrum reveals the characteristic peaks of all functional groups present in a sample. In order to ascertain successful entrapment, the drug, lipid, their physical mixture and SLN were subjected to FTIR studies. An FTIR instrument from Shimadzu, model 8400, was used for this study. Pellets were prepared with anhydrous potassium bromide. The IR spectra of pure drug AC, GB, GB SLN, and a GB—AC physical mixture are shown in Figs. 1, 2, 3 and 4 respectively. The IR spectrum of pure drug, AC, which is 2-[2-[2-(2,6-dichloroanilino) phenyl]acetyl]oxyacetic acid shows a hydroxyl broad band at 3,276 cm^{-1}, a carbonyl peak at 1,716 cm^{-1}, an aryl chloride peak at 1,057 cm^{-1}, C–H bending at 750 cm^{-1} and NH stretching at 3,319 cm^{-1} [10, 11]. However, in the IR spectrum of GB SLN (Fig. 3) peaks of NH group and aryl chloride group are absent. It is evident that the IR spectrum of SLN resembles that of the lipid (Fig. 2) thus proving that the lipid forms the outer core

Batch	Independent variables			
Table 1 Combination of variables of Taguchi experimental design	Type of lipid	Drug:lipid	Conc. of surfactant (%)	Sonication time (min)
SLN 1	CA (1)	1:5 (1)	1.5 (1)	2 (1)
SLN 2	CA (1)	1:7 (2)	2.0 (2)	5 (2)
SLN 3	CA (1)	1:10 (3)	2.5 (3)	8 (3)
SLN 4	GP (2)	1:5 (1)	2.0 (2)	8 (3)
SLN 5	GP (2)	1:7 (2)	2.5 (3)	2 (1)
SLN 6	GP (2)	1:10 (3)	1.5 (1)	5 (2)
SLN 7	GB (3)	1:5 (1)	2.5 (3)	5 (2)
SLN 8	GB (3)	1:7 (2)	1.5 (1)	8 (3)
SLN 9	GB (3)	1:10 (3)	2.0 (2)	2 (1)

Figures in parentheses indicate the levels of different variables: *1* low, *2* medium, *3* high

Fig. 1 Infra red spectrum of
aceclofenac
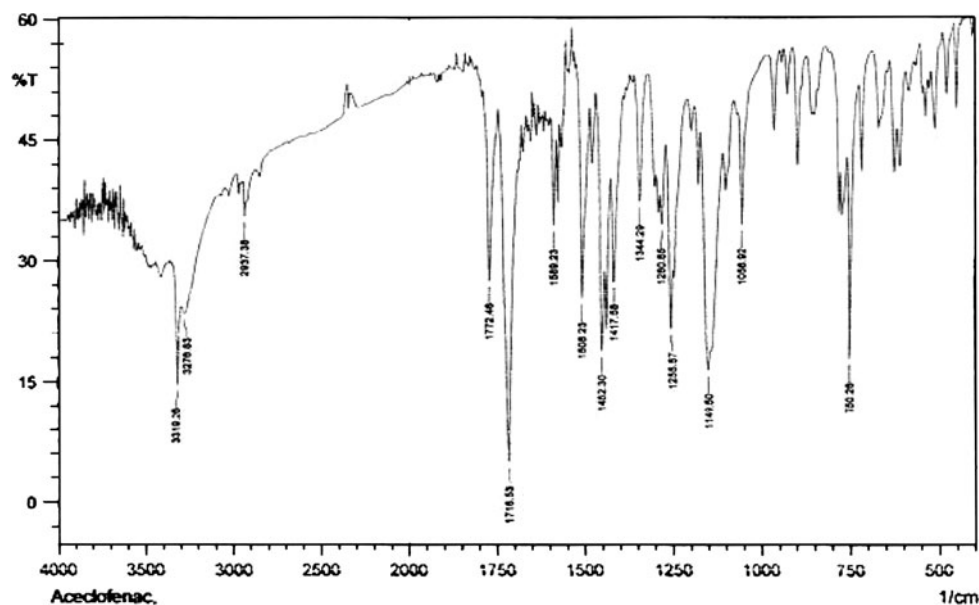
Fig. 2 Infra red spectrum of
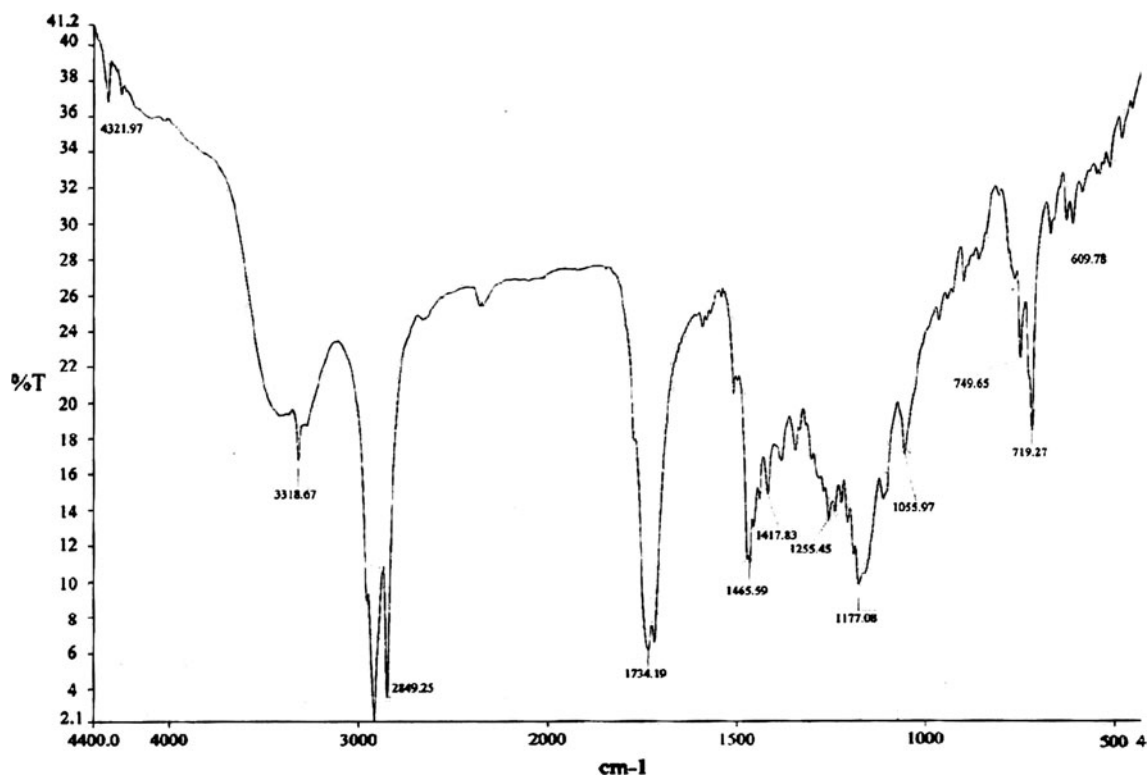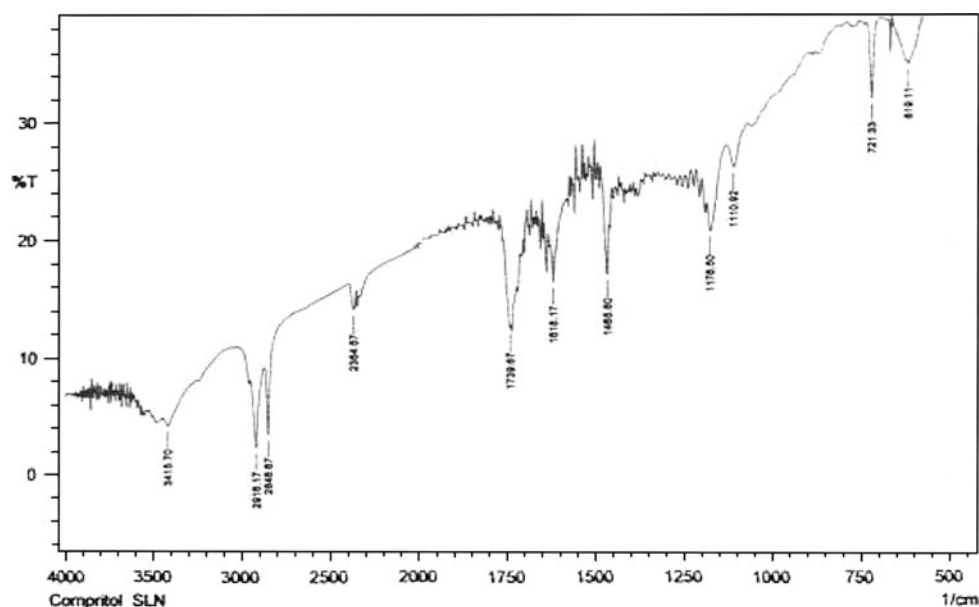lipid glyceryl behenate
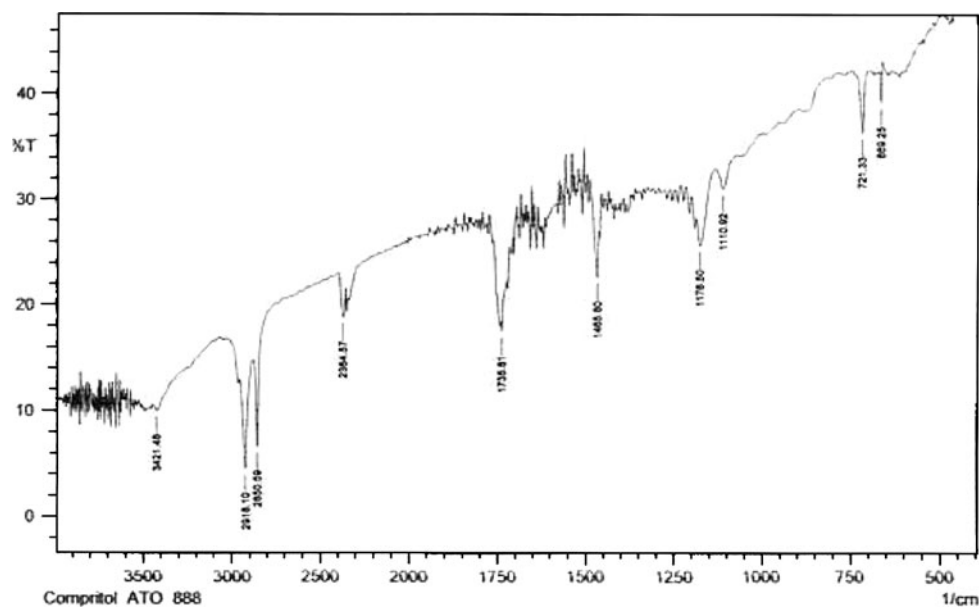
and the drug has been successfully incorporated inside. These facts are corroborated by the IR spectrum of physical mixture of GB and AC (Fig. 4) which still contains peaks of aryl chloride at 1,056 cm^{-1}, C–H bending at 750 cm^{-1} and NH stretching at 3,319 cm^{-1}. All these three peaks are common to AC and the GB–AC physical mixture but absent in drug loaded GB-SLN.

Particle Size, Shape, and Zeta Potential

SLN were further characterized on the basis of scanning electron microscopy. This technique uses electrons transmitted from the specimen surface to determine the overall shape and morphology, i.e., both particle size as well as distribution. SEM has a high resolution and sample preparation is easy. The SEM micrograph of GB SLN is shown in Fig. 5. The measurement of zeta potential allows prediction of the storage stability of colloidal dispersions. At higher zeta potential, particle aggregation is less likely to occur due to electrical repulsion. Size and zeta potential were measured on a Malvern Zetasizer using double distilled water as the dispersant. The samples were taken in a polystyrene cuvette and filled up to a minimum height of 10 mm. The instrument was first calibrated with standard polystyrene latex of 60 nm size. A sample was made to run for 5 times, each run with 10 s duration. Zeta potential readings are normally the

Fig. 3 Infra red spectrum of aceclofenac loaded glyceryl behenate SLN

Fig. 4 Infra red spectrum of aceclofenac–glyceryl behenate physical mixture

average of these five determinations. The results of particle size determination, polydispersity and zeta potential are shown in Table 2. Particle size showed a distinct correlation with drug:lipid molar ratio. The drug:lipid molar ratio of 1:10 yields particles with smallest size in each of the lipids. The smallest particles were produced by GB (245 ± 5 nm). Moreover, these SLN have a favorable polydispersity index

(PI) of 0.470 (<0.5). SEM picture of GB SLN (Fig. 5) reveals that particles are essentially spherical in shape. The zeta potential of CA SLN was found to vary between −8.17 and −15.7 mV, that of GP SLN between −3.04 and −11.20 mV whereas for GB SLN the limit was between −11.0 and −16.4 mV (Table 3). The higher magnitude of zeta potential indicates the stability of formulation.
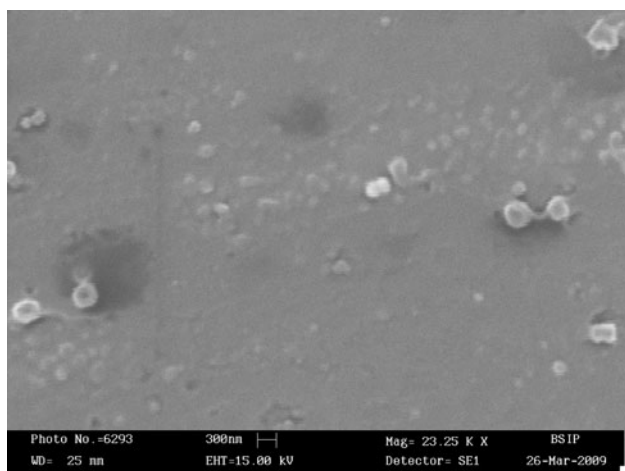
Fig. 5 Scanning electron micrograph of glyceryl behenate SLN

Measurement of Entrapment Efficiency
and In Vitro Dissolution Studies

Entrapment efficiency was determined by measuring the concentration of the free drug in the dispersion medium. The following formula was used:

$$EE \% = \frac{\text{weight of drug in system} - \text{weight of drug in aqueous phase} \times 100}{\text{weight of drug in system}}$$

The entrapment efficiency of different batches is shown in Table 2 and depicted graphically in Fig. 6. Entrapment efficiency for different batches varied between 68 and 90%.

Solid lipid nanoparticles prepared from the three lipids were subjected to dissolution studies ($n = 3$). They were carried out by using phosphate buffer, pH 7.4 and a temperature of 37 °C, in a Franz diffusion apparatus. Samples were taken at regular intervals i.e., 0, 15, 30, 60, 120, 180, 240, 300, 360, 480, 720, and 1,440 min. An equal volume was replaced with phosphate buffer. Samples were suitably diluted and filtered through Whatman filter paper. Absorbance was measured at 275 nm using a Shimadzu Double beam UV 1700 spectrophotometer and the amount of drug was calculated. About 55% of the drug was released in 24 h from GP SLN whereas in the case of CA it was 69%. However, in the case of GB SLN, only 36% of the drug was released in the same period. It may be concluded that as the melting point of the lipid increases the release of insoluble AC in the dissolution medium decreases (Fig. 7). A marketed sustained release tablet of aceclofenac

Table 2 Zeta potential (mV), mean particle size (nm), polydispersity index (PI) and entrapment efficiency (%) of different batches ($n = 3$)

Batch	Zeta potential (mV)	Particle size (nm)	Polydispersity	Entrapment efficiency (%)
SLN 1	-8.17 ± 0.21	969 ± 16	0.969	68 ± 1.2
SLN 2	-8.86 ± 0.27	642 ± 9	0.616	71 ± 2.0
SLN 3	-15.7 ± 0.66	432 ± 7	0.549	74 ± 2.3
SLN 4	-3.04 ± 0.30	774 ± 10	0.726	76 ± 2.8
SLN 5	-11.20 ± 0.58	806 ± 11	0.660	75 ± 2.5
SLN 6	-7.19 ± 0.39	321 ± 6	0.597	80 ± 3.1
SLN 7	-11.00 ± 0.60	662 ± 8	1.000	84 ± 4.0
SLN 8	-11.5 ± 0.49	761 ± 9	0.865	88 ± 4.1
SLN 9	-16.40 ± 1.22	245 ± 5	0.470	90 ± 3.6

**Table 3** Stability studies of prepared batches

Batch	Lipid	Initial drug content	Final drug content (4 °C)	Final drug content (RT)	Final drug content (40 °C)
SLN 1	Cetyl alcohol	13.00	12.69[*]	12.50[#]	12.56[$]
SLN 2	Cetyl alcohol	15.60	15.00[*]	15.21[#]	15.40[$]
SLN 3	Cetyl alcohol	16.20	15.86[*]	15.90[#]	16.00[$]
SLN 4	Precirol	1.61	1.48[*]	1.56[#]	1.50[$]
SLN 5	Precirol	2.24	2.10[*]	2.05[#]	1.89[$]
SLN 6	Precirol	15.71	15.20[*]	14.66[#]	15.52[$]
SLN 7	Compritol	16.54	16.05[*]	16.23[#]	15.90[$]
SLN 8	Compritol	4.46	4.22[*]	4.31[#]	4.10[$]
SLN 9	Compritol	22.60	22.33[*]	21.95[#]	22.30[$]

All values are in percentages

[*] $P = 0.9238$, [#] $P = 0.9092$, [$] $P = 0.9302$
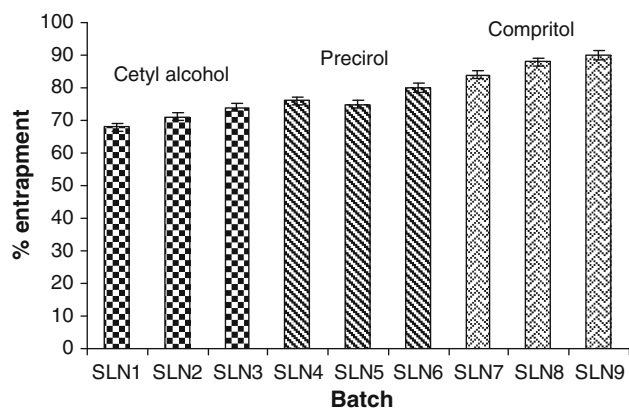
Fig. 6 Entrapment efficiency of SLN prepared from three lipids ($n = 3$)
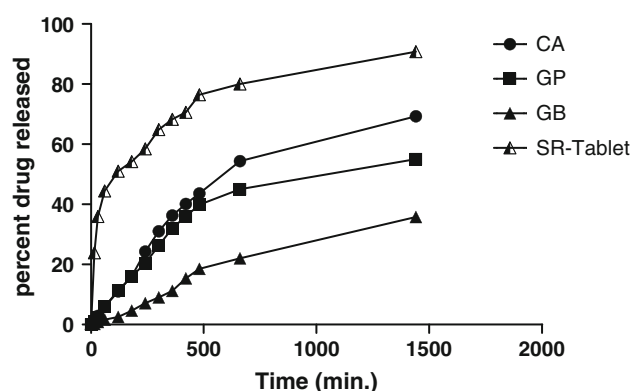
Fig. 7 Comparison of dissolution curves of SLN prepared from three lipids and a marketed sustained release tablet ($n = 3$)

(Aceclo-SR®) was also subjected to dissolution studies as a control and it showed a 90% release in the same period. However, such tablets are prone to the problem of dose dumping in vivo.

Stability Studies

Batches 1–9 were subjected to 6-month long stability studies. The batches were kept under refrigerated conditions, at room temperature and in a stability chamber at 40 ± 2 °C/$75 \pm 5\%$ RH conditions. After 6 months, batches were analyzed for changes in physical characteristics and for the drug content. A weighed quantity of stored SLN was digested in methanol and filtered through a Whatman filter paper. The filtrate was suitably diluted and the absorbance was measured at 275 nm and the concentration of drug was calculated. The results of stability studies indicate that there is no significant difference in the amount of drug when the prepared SLN were stored in three different conditions for 6 months. All the values of the experimental results (Table 3) are expressed as

means $\pm$ SEM. The values were analyzed by ANOVA (Dunnett's test) for possible significant differences between various conditions. $P < 0.05$ was considered statistically significant. All batches were physically and chemically stable.

Biodistribution Studies

The optimized formulation was subjected to biodistribution studies using an animal Photoimager. SLN tagged with dye (Cy 5.5 Bis NHS ester) were administered orally to male Wistar rats. The animal imager counts the photons emitted by animals. Images were taken at regular intervals. In the second part of study, a dispersion of SLN in distilled water was radiolabelled with Technetium-99 and administered through oral gavage to white New Zealand rabbits with a dose of 20 mg/kg body weight. An equivalent dose of AC was given orally in soluble form as a control. A gamma camera was used to measure the radioactivity 3 h after oral administration. Before performing these experiments, ethical clearance was obtained from the Institutional Animal Ethics Committee (IAEC) duly approved by CPCSEA (Reg. no. NIEC-IAEC/Clear/12/2009). The image, taken 4 h after oral administration to rats (Fig. 8), shows the distribution to central organs. The photons emitted by animals in the near IR region (676–690 cm^{-1}) were counted and put together as a color signal. Some of the dye is seen on the tail of animal suggesting excretion through urine. Region (a) indicates least concentration of dye thereby the least concentration of the drug; region (b) indicates medium concentration of dye whereas region (c) indicates the organs with maximum concentration of drug, i.e., intestines and liver. Studies in rabbits indicate that the radioisotope-labeled drug reaches the last parts of intestine 3 h after oral administration in the case of a solution,
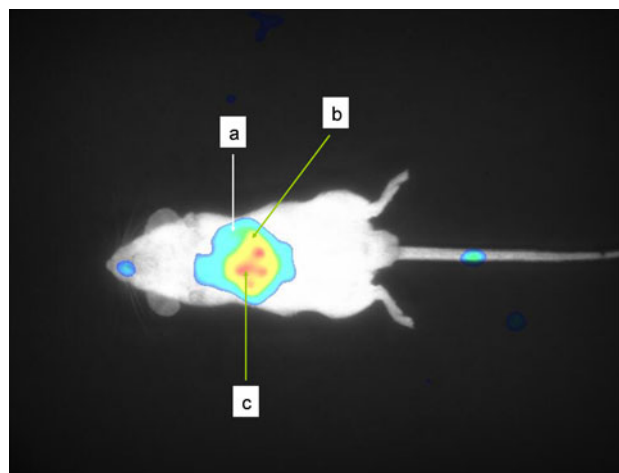
Fig. 8 Photograph taken by animal imager 4 h post oral administration in rats
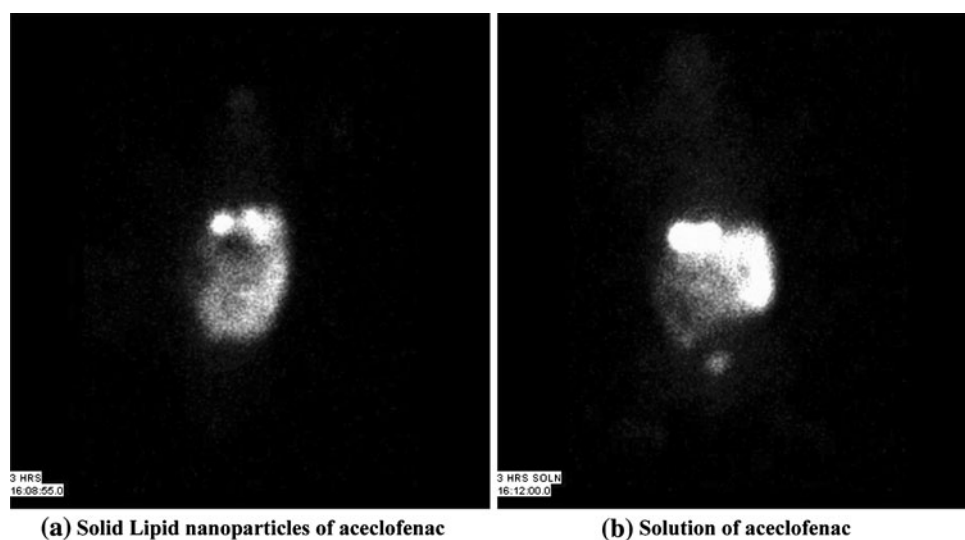
whereas in the case of SLN it was still present in the stomach and small intestine (Fig. 9).

Discussion

Drug lipid molar concentrations, concentration of surfactant, type of lipid and sonication time were found to influence the characteristics of prepared SLN. The three lipids were chosen because of increasing complexity in their structure. CA is a straight chain alcohol, GP is a triacylglycerol, with fewer imperfections, and GB is a triacylglycerol but with more imperfections. An increase in the amount of lipid with respect to the amount of drug led to better encapsulation and smaller particle size. This may be due to more lipid being available to form an outer core. However, when the drug:lipid ratio was raised to 1:12 and 1:15 during preliminary studies, polydispersity and aggregation behavior increased. Surfactant at concentrations below 1.5% w/v was not effective and at concentrations above 2.5% exhibited frothing problems. Sonication times below 2 min were ineffective whereas above 8 min there were no visible signs of improvement. The fact that lipid forms an outer core has been established in this study. The IR spectrum of drug, AC (Fig. 1) is different from the IR spectrum of GB SLN (Fig. 3). However, the IR spectrum of GB SLN is very similar to plain lipid (GB) (Fig. 2), thus, proving that the lipid forms an outer core whereas the drug is entrapped inside. The presence of characteristic functional group peaks belonging to AC, for example peaks of aryl chloride, C–H bending and N–H stretching in IR spectrum of AC–GB physical mixture (Fig. 4) further add weight to this hypothesis. The entrapment efficiency of straight chain alcohol, CA is least since there are no spaces to accommodate the drugs because of a relatively perfect crystalline lattice. However, in the case of GP which is an equal mixture of palmitic and stearic acids, there are some anomalies in the crystalline lattice, leading to better entrapment efficiency than CA. In the case of GB, which is a mixture of mono, di, and triglycerides containing fatty acids of different chain lengths, there are many imperfections and therefore maximum entrapment efficiencies are observed (Fig. 6). The higher concentration of surfactant poloxamer 188 can stabilize a larger surface area; therefore better results were obtained when the surfactant was utilized at 2.5% w/v of the dispersion medium. The measurement of the zeta potential allows prediction about the storage stability of colloidal dispersions. At higher zeta potential, particle aggregation is less likely to occur, due to electrical repulsion. Among the prepared SLN, GB SLN had the highest magnitude of zeta potential (Table 2) indicating that SLN made from GB are more stable. There appears to be no burst release from the prepared SLN. The release is expected to occur through swelling of the matrix and diffusion of the drug from the matrix into the dissolution medium. Some component of the erosion of the matrix is also involved because as the melting point of lipid increases the release of insoluble AC in the dissolution medium decreases (Fig. 7). Such a release pattern can also be expected in vivo since it has been established that the longer the fatty acid chains in the glycerides, the slower the enzymatic degradation [12]. The PI for a monodisperse system is zero, whereas for polydisperse systems the PI should be <0.5. The optimized batch (SLN9) has a favorable PI (Table 2). Biodistribution studies in rats (Fig. 8) indicate that the drug is distributed primarily in the intestines and liver after 4 h and a minor quantity had been eliminated through urine. The facts are further corroborated by the gamma scintigraphy studies in rabbits (Fig. 9), where it is clearly seen that radioisotope-labeled drug was

Fig. 9 Photograph taken by a gamma camera 3 h post oral administration to rabbits

(a) Solid Lipid nanoparticles of aceclofenac

(b) Solution of aceclofenac

reaching the last parts of the intestine 3 h after oral administration in the case of a solution, whereas in the case of SLN it was still present in the stomach and small intestine. This confirms a sustained release from prepared SLN, which is in agreement with the findings of in vitro release studies. If the release would not have been sustained, radioactivity would have been seen similar to solution dosage form.

Conclusion

This study establishes that GB is a promising lipid for the formulation of SLN. It has successfully been used in the preparation of stable SLN of aceclofenac. GB solid lipid nanoparticles have shown excellent characterization parameters with regard to particle size, PI, zeta potential and in vitro cum in vivo release profiles.

References

1. Manjunath K, Reddy SJ, Venkateshwarlu V (2005) Solid lipid nanoparticles as drug delivery systems. Methods Find Exp Clin Pharmacol 27:1–20

2. Utreja S, Jain NK (2001) Solid lipid nanoparticles. In: Jain NK (ed) Advances in controlled and novel drug delivery. CBS Publishers, New Delhi, pp 408–425

3. Mader K (2006) Solid lipid nanoparticles as drug carriers. In: Trochilin VP (ed) Nanoparticulates as drug carriers. Imperial College Press, London, pp 187–212

4. Müller RH, Mäder K, Gohla S (2000) Solid lipid nanoparticles (SLN) for controlled drug delivery—a review of the state of the art. Eur J Pharm Biopharm 50:161–177

5. Mehnert W, Mäder K (2001) Solid lipid nanoparticles production, characterization and applications. Adv Drug Deliv Rev 47:165–196

6. Akimoto H, Yamazaki R, Hashimoto S, Sato T, Ito A (2000) 4'-Hydroxy aceclofenac suppresses the interleukin-1 induced production of promatrix metalloproteinases and release of sulfated-glycosaminoglycans from rabbit articular chondrocytes. Eur J Pharmacol 401:429–436

7. Souto EB, Mehnert W, Muller RH (2006) Polymorphic behaviour of compritol® ATO 888 as bulk lipid and as SLN and NLC. J Microencapsul 23:417–433

8. Gasco MR (1993) Method for producing solid lipid microspheres having a narrow size distribution. US Patent 5,250,236

9. Roy RK (2001) Design of experiments using the Taguchi approach, 1st edn. Wiley, New York

10. Silverstein RM, Webster FX (2004) Spectrometric identification of organic compounds. Wiley, New York, pp 79–109

11. Pavia DL, Lampman GM, Kriz GS, Vyvyan JR (2007) Introduction to spectroscopy. 1st Indian ed. Brooks/Cole CENGAGE Learning, New Delhi, pp 26–107

12. Jose GR, Josephine D, Rios SA (2003) Controlled release pharmaceutical composition. US Patent 6,596,308