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Infrared and Differential Scanning Calorimetric Studies on Solid Lipid Nanoparticles of Aceclofenac

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Solid lipid nanoparticles are particles produced by high pressure homogenization or other suitable technique having photon correlation spectroscopy diameters between 80-1000 nm. Aceclofenac is a NSAID which acts by inhibiting the secretion of tissue necrosis factor and interleukin-1. Stable solid lipid nanoparticles of this drug were prepared using different lipids namely cetyl alcohol, glyceryl palmitostearate and glyceryl behenate by Gasco microemulsion method. Batches were prepared as per Taguchi experimental design. In order to ascertain the status of drug and lipid inside solid lipid nanoparticles, infrared spectroscopic studies are required. Differential scanning calorimetric studies are done to check the amorphous/crystalline state of drug. Solid lipid nanoparticles were characterized on the basis of infrared, differential scanning calorimetric and photon correlation spectroscopy.

Key Words: Solid lipid nanoparticles, Aceclofenac, Taguchi experimental design, Infrared, Differential scanning calorimetry.

INTRODUCTION

In the recent years, it has become more evident that the development of new drugs alone is not sufficient to ensure progress in drug therapy. Exciting data obtained *in vitro* is very often followed by disappointing results *in vivo*. The *in vivo* fate of drug in no longer determined by the properties of the drug, but by the carrier system which should permit a controlled and localized release of the active drug according to the specific needs of the therapy[1].

The size of carrier depends on the desired route of administration and ranges from few nanometers (colloidal carriers) to several millimeters (implants). Solid lipid nanoparticles (SLN) have attracted increasing attention during recent years as alternative to colloidal systems, for controlled and targeted delivery[2]. Common ingredients used in the formulation of solid lipid nanoparticles are lipids (matrix materials), emulsifiers, co-emulsifiers, stealthing agents that improve long circulation time and targeting ability. Of late, work has been focused in the development of solid lipid nanoparticles as delivery system for anticancer drugs, peptides, genetic materials, cosmetics, *etc*.[3,4]

Aceclofenac (Fig. 1) is a non steroidal antiinflammatory drug which acts by inhibiting the secretion of tissue necrosis factor and interleukin-1.

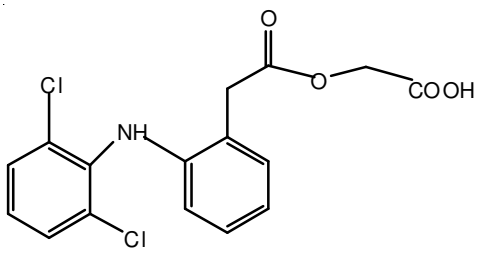

Fig. 1. Chemical structure of aceclofenac

In this study, an attempt has been made to prepare solid lipid nanoparticles of aceclofenac using lipids cetyl alcohol, glyceryl palmitostearate and glyceryl behenate. Solid lipid nanoparticles were characterized on the basis of IR, differential scanning calorimetry and photon correlation spectroscopy in order to ascertain the physical status of the drug (entrapped/free) and to find out the average size of prepared solid lipid nanoparticles.

EXPERIMENTAL

Glyceryl palmitostearate (Precirol ATO 5) and glyceryl behenate (Compritol ATO 888) were received as gift samples from Colorcon Asia Pvt. Ltd., India. Aceclofenac was received as gift sample from Arbro Pharmaceuticals, India. The surfactant,

poloxamer 188 (Lutrol F 68) was received *ex-gratia* from BASF, Germany. All other chemicals used were of analytical grade and procured from SD Fine Chemicals, India.

Preparation of solid lipid nanoparticles: Solid lipid nanoparticles were prepared by Gasco's method[14]. The lipid was heated to a temperature at least 5 °C above its melting point. 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 0.5 h. The pre-emulsion thus formed was homogenized at 3000 rpm for 0.5 h followed by ultrasonication (Sartorius LabsonicP Ultrasonicator, Germany) for 2 to 8 min. After ultrasonication, contents were diluted to 20 times with ice-cold water, where solid lipid nanoparticles were precipitated and were separated using membrane filters. Taguchi experimental design was used to reduce the number of experiments. The lipids used were cetyl alcohol, glyceryl palmitostearate and glyceryl behenate. The details of Taguchi design used are given in Table-1.

TABLE-1
COMBINATION OF VARIABLES OF TAGUCHI EXPERIMENTAL DESIGN

Batch	Type of lipid (CA or GP or GB)	Drug: lipid molar ratio	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

Characterization of prepared solid lipid nanoparticles

Infrared spectrometry: The prepared solid lipid nanoparticles, pure drug, pure lipids and drug-lipid physical mixture were subjected to IR analysis. A Shimadzu 8400 S FTIR spectrophotometer was used. Pellets were prepared using dried potassium bromide. Minimum possible pressure was used so as not to affect the integrity of prepared solid lipid nanoparticles.

Differential scanning calorimetric: For this purpose, cetyl alcohol, solid lipid nanoparticles of cetyl aclohol and drug, blank solid lipid nanoparticles of cetyl alcohol were used. Analysis was performed using a differential scanning calorimeter Differential scanning calorimetric Q 10V9.0 Universal DTA Instruments. The instru-ment was calibrated with indium for melting point and heat of fusion. A heating rate of 10 ºC/min in the range of 25-300 °C was performed under a nitrogen purge. Standard aluminium pans were used and an empty pan was used as reference. Triple runs were carried out on each sample to check reproducibility.

Photon correlation spectroscopy: This method is based on dynamic laser light scattering due to Brownian motion of particles in dispersion medium. Photon correlation spectroscopy measures the fluctuation of the intensity of scattered light, which is caused by particle movement. It is suitable for particles from 3 nm to 3 mm. It measures polydispersity index (P.I.). Polydispersity index for a monodisperse system is zero, whereas for polydisperse systems the polydisperity index should be < 0.5. A Malvern zetasizer was used to measure the zeta potential of prepared solid lipid nanoparticles. The samples are taken in a polystyrene cuvette which should be filled up to a minimum height of 10 mm. The instrument is first calibrated with standard polystyrene latex of 60 nm size. A sample is made to run 5 times, each run of 10 s duration. Zeta potential readings are normally an average of these five determinations.

RESULTS AND DISCUSSION

Stable solid lipid nanoparticles of aceclofenac have been produced by the stated method. Drug lipid molar concentration, concentration of surfactant, type of lipid and sonication time was found to influence the characteristics of prepared solid lipid nanoparticles. It was found that as the drug lipid molar concentration was raised, particles with smaller size were obtained irrespective of the nature of lipid. The surfactant poloxamer 188 gave best results when used at a concentration of 2.5 % w/v of dispersion.

An IR spectrum reveals the characteristic peaks of all functional groups present in a sample. IR spectrum of pure drug, aceclofenac, which is 2-[2-[2-(2,6-dichloroanilino) phenyl]acetyl]oxyacetic acid shows a secondary amine stretching peak at 3319 cm^{-1}, carbonyl peak at 1716 cm^{-1}, aryl chloride peak at 1055 cm^{-1}, C-H bending at 750 cm^{-1} and NH bending at 1508 cm^{-1}. However, in the IR spectrum of glyceryl palmitostearate solid lipid nanoparticles stretching and bending peaks of NH group and aryl chloride group are absent. It is evident that IR spectrum of solid lipid nanoparticles resembles that of lipid thus proving that the lipid forms the outer core and drug has been successfully incorporated inside. These facts are corroborated by the IR spectrum of physical mixture of glyceryl palmitostearate and aceclofenac which still contains peaks of aryl chloride at 1056 cm^{-1}, C-H bending at 749 cm^{-1} and NH stretching at 3319 cm^{-1} and NH bending at 1508 cm^{-1}. All these four peaks are common to aceclofenac and glyceryl palmitostearate-aceclofenac physical mixture but absent in drug loaded glyceryl palmitostearate solid lipid nanoparticles.

Differential scanning calorimetric aims at measuring heat of fusion. It is an important tool in the hands of a pharmaceutical scientist to establish the state of drug in a dosage form. When comparing differential scanning calorimetric thermograms of bulk lipids and corresponding solid lipid nanoparticles; differences in shape and positions of the signals are usually observed[6,7]. Differential scanning calorimetric thermogram of cetyl alcohol (Fig. 2) shows its latent heat of fusion as 29.21 J/g. Differential scanning calorimetric thermogram of blank solid lipid nanoparticles (Fig. 3) indicates higher heat flow. However, heat of fusion is greatly reduced as compared to lipid. Thermogram of drug loaded solid lipid nanoparticles of cetyl alcohol (Fig. 4) reveals that aceclofenac was present in an amorphous state. This is attributed to the absence of clear drug melting events in the differential scanning calorimetric thermogram.

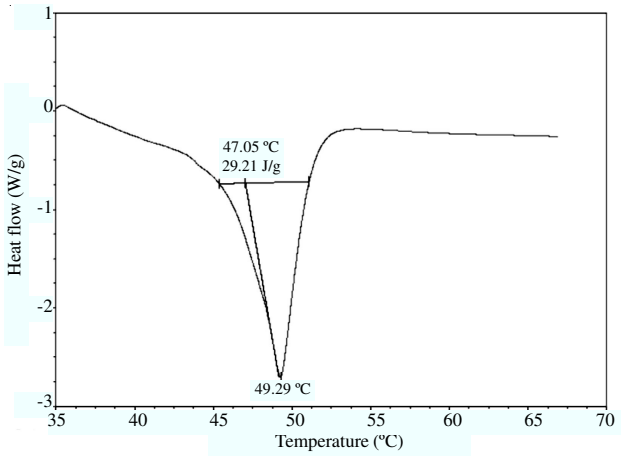

Fig. 2. DSC thermogram of cetyl alcohol

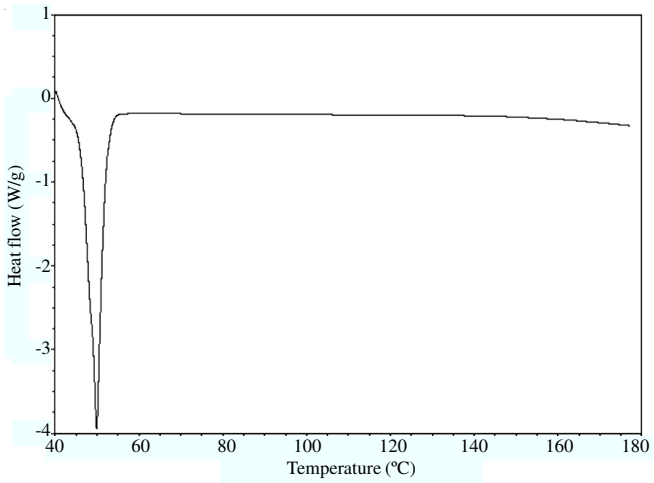

Fig. 3. DSC thermogram of blank solid lipid nanoparticles

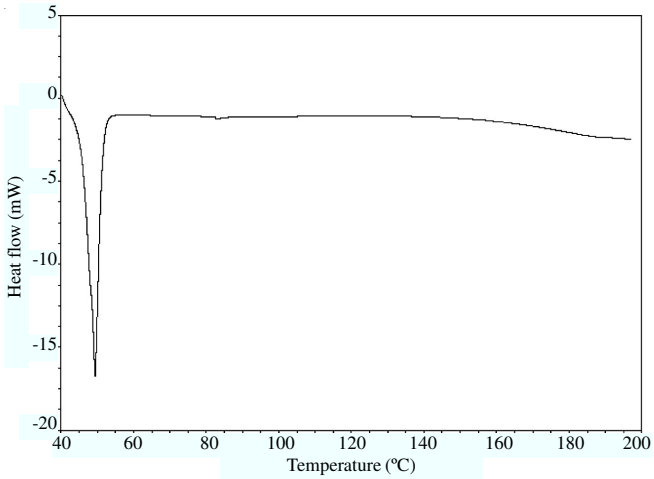

Fig. 4. DSC thermogram of drug loaded cetyl alcohol solid lipid nanoparticles

Zeta potential studies: Dynamic light scattering is the measurement of Brownian motion and relating it to particle size. Zeta potential is a measure of electrical repulsion between particles and therefore indicates stability of the formulation. The results of particle size, zeta potential and polydispersity of prepared solid lipid nanoparticles are shown in Table-2.

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	-15.70±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	-11.50±0.49	761±9	0.865	88±4.1
SLN 9	-16.40±1.22	245±5	0.470	90±3.6

These results indicate that drug:lipid molar concentration of 1:10 is desirable for smaller size of particles. The magnitude of zeta potential is least in case of glyceryl palmitostearate solid lipid nanoparticles. The smallest particles have been produced by GB (245 ± 5 nm). Moreover these solid lipid nanoparticles have a favourable polydispersity of 0.47 (< 0.5). The higher magnitude of zeta potential of this batch indicates stable formulation.

Conclusion

The study has for the first time proved the successful entrapment of drug aceclofenac in different lipids when formulated as solid lipid nanoparticles through IR spectroscopy. Optimized formulation has been prepared and needs to be characterized further.

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