

Full Length Research Paper

Controlled release formulation of levocetirizine dihydrochloride by casein microparticles

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Accepted 5 March, 2013

The study aims to develop a microparticulate casein based delivery system by the controlled drug delivery approach using Levocetirizine dihydrochloride as the model drug. Fourier transform infrared spectroscopy (FTIR), X-ray and differential scanning calorimetry (DSC) studies substantially indicates the presence of molecularly dispersed drug within the particles with preserved stability during microencapsulation. *In vitro* release studies of levocetirizine dihydrochloride loaded microparticles were performed by simulating the condition of gastrointestinal tract, and showed the minimal drug leakage (less than 5%) at acidic pH (1.2) and significantly higher release at basic pH (7.4). The results were found to be critical in confirming the role of casein microparticles as potential candidate for the controlled and targeted release of levocetirizine dihydrochloride.

Key words: Extended release, casein, levocetirizine dihydrochloride, steric stabilization.

INTRODUCTION

Controlled drug delivery systems are found to be vital in rectifying some of the problems associated with conventional therapy and in enhancing the therapeutic efficacy of a given drug. To obtain maximum therapeutic efficacy, it becomes necessary to deliver the agent to the target tissue in the optimal amount, in the right period of time, to reduce toxicity and side effects. The usage of microspheres as drug carriers to deliver a therapeutic agent in sustained controlled fashion has been reported (Yanhong et al., 2012).

It is really fascinating to find the role of milk protein, casein, as a drug carrier mainly for the sustained delivery of various drugs (Willmott et al., 1992; Chen et al., 2006; Knepp et al., 1993; Yoav, 2010). Casein is an inexpensive, readily available, non-toxic and highly stable milk protein and as a natural food product, this generally

recognized as safe (GRAS) protein is biocompatible and biodegradable (Ana et al., 1999; Katz et al., 2009). Casein comprises about 94% protein and 6% low molecular weight compounds collectively called colloidal calcium phosphate. There are mainly four casein phosphoproteins, α S1-, α S2-, β -, and κ -casein, which exist approximately in proportions of 4:1:4:1 by weight, respectively in cow milk. Their molecular weights are between 19 and 25 kDa and average isoelectric point (pI) is between 4.6 and 4.8. All of the four caseins are amphiphilic and have ill-defined structures (Fox et al., 2003), with distinct hydrophobic and hydrophilic domains (Dalgeish, 1998). The usage of casein in various drug delivery studies has been well reported (Latha et al., 1994, 1995, 2000; Elzoghby et al., 2011; Arora et al., 2012).

Levocetirizine dihydrochloride, the active R enantiomer of cetirizine, is a selective H₁ histamine blocker that contains antihistaminic properties and is used to manage intermittent and persistent allergic rhinitis. It is zwitterionic and relatively polar, and thus, does not penetrate the blood brain readily. It is effective in relieving nasal symptoms, improving nasal air flow, reducing leukocyte infiltration, and diminishing the cytokine level, which results in evidence of the effectiveness of levocetirizine in seasonal allergic rhinitis (Ciprandi et al., 2004). Levocetirizine has several pharmacokinetic properties that are desirable for an antihistamine, providing a combination of both potency and safety (Cranswick et al., 2005; Molimard et al., 2004). Its clinical advantages are derived from its rapid and extensive absorption, limited distribution and its very low degree of metabolism (Ferrer et al., 2011). The incorporation of levocetirizine in an extended-release of oral dosage form would have many advantages such as aiding in enhancement of bioavailability and prolonged plasma level drug concentration (Prabakara et al., 2011; Patel et al., 2012).

MATERIALS AND METHODS

Levocetirizine was obtained as a gift sample from Praveen Laboratories Pvt Ltd, Gujarat and Casein (alkali soluble) was purchased from Himedia laboratories pvt. Ltd, Mumbai. All reagents used were of analytical grade.

Preparation of levocetirizine dihydrochloride loaded casein microparticles

A total of 10 ml of casein solution in 0.1 M NaOH was prepared and the calculated amount of levocetirizine dihydrochloride was dissolved in the 10 ml of casein solution. 4 ml of this solution was dropped into a 50 ml ethylcellulose solution. The ethyl cellulose solution was made by adding 1 g of ethylcellulose in 50 ml of chloroform. The resulting solution was homogenized for 30 min at 25,000 rpm. Crosslinking was done by adding glutaraldehyde at the concentration of 3.12% during the homogenization process. Thus, formed particles were stirred for 12 h. Then, the particles were separated by centrifugation at 6,000 rpm for 10 min and washed repeatedly with acetone to remove excess ethyl cellulose, chloroform and glutaraldehyde. The microparticles obtained were dried at room temperature (Jalil et al., 1990). Different concentrations of casein 10 and 15 % w/v were tried. The effect of ethyl cellulose concentration (0.5, 1.0, 2.0 and 3.0% w/v), as well as volume of casein (4 and 5 ml) on the microsphere formation, was investigated. Furthermore, the effect of stirring time on the properties of the prepared microspheres was studied.

Encapsulation efficiency

Encapsulation efficiency was calculated by weighing 50 mg of the loaded microparticles and dispersing them in 50 ml of phosphate buffer saline (pH 7.4). The sample was allowed to stir overnight at 50 rpm and the concentration of levocetirizine dihydrochloride was analyzed in the supernatant at 230 nm using ultra violet (UV)-Vis spectrophotometer (UV – 1700 Pharma spec - Shimadzu)

(Pradeep and Inderbir, 2010; Rao et al., 2007; Kim et al., 2005; Kim and Lee, 1992).

$$\text{Encapsulation efficiency(\%)} = \frac{\text{Actual Weight (W}_a\text{)}}{\text{Theoretical Weight (W}_t\text{)}} \times 100$$

Morphological characterization using scanning electron microscopy (SEM)

The drug loaded casein microparticles were analysed for its diameter and surface morphology using SEM (FEI Quanta FEG 200). The particles were sprinkled on adhesive aluminium stub and then surface coating was done with gold to a thickness of ~300 Å using a sputter coater.

Particle size distribution

Particle size distribution was performed using Mastersizer 2000 (Malvern India Pvt Ltd). The microparticles loaded with levocetirizine dihydrochloride were dispersed with poly (dimethylsiloxane). The refractive index for the powder samples was set to 1.52, and the poly (dimethylsiloxane) was 1.40.

X-ray powder diffraction studies

The X-ray diffractograms of the microparticles and the levocetirizine dihydrochloride loaded microparticles were obtained in a D8 Advance Model X-Ray Diffractometer (Bruker, Germany) using Ni filtered radiation ($\lambda = 15.4$ nm, 40 kV and 30 mA). The measurements were carried out using Poly (methyl methacrylate) (PMMA) sample holder and lynx eye detector.

Fourier transform infra red spectral analysis

The fourier transform infrared (FTIR) spectrum of the title compound was recorded in the range of 400 to 4000 cm^{-1} using KBr pellet with a FTIR spectrophotometer (Nicolet Avatar 330) at room temperature.

Differential scanning calorimetry

The DSC thermograms of placebo and levocetirizine dihydrochloride loaded casein microparticles were carried out using Netzsch DSC 204. The samples were heated from 50 to 230°C at a heating rate of 10°C at a heating rate of 10°C/min in an inert nitrogen atmosphere.

In-vitro release of levocetirizine dihydrochloride

Levocetirizine dihydrochloride loaded casein microparticles were subjected to *in vitro* release in the simulated gastric fluid pH 1.2 (SGF) [as per United States Pharmacopeia (USP)] and simulated intestinal fluid (phosphate buffer Saline (PBS)) pH 7.4 without enzymes. 50 mg of levocetirizine dihydrochloride loaded casein microparticles was taken in a dialysis cassette along with 0.5 ml of simulated intestinal fluid and immersed in the 50 ml of simulated intestinal fluid. The dissolution was done at 50 rpm at 37°C. Aliquots were collected at predetermined points and an equal

Table 1. Optimization of casein microparticles.

Formulation no.	Ethylcellulose concentration (%)	Casein concentration (%)	Casein volume (ml)
1	0.5	15.0	4.0
2	1.0	15.0	4.0
3	2.0	15.0	4.0
4	3.0	15.0	4.0
5	0.5	10.0	4.0
6	1.0	10.0	4.0
7	2.0	10.0	4.0
8	3.0	10.0	4.0
9	0.5	15.0	5.0
10	1.0	15.0	5.0
11	2.0	15.0	5.0
12	3.0	15.0	5.0
13	0.5	10.0	5.0
14	1.0	10.0	5.0
15	2.0	10.0	5.0
16	3.0	10.0	5.0

amount of buffer was replaced to maintain the volume. The amount of levocetirizine dihydrochloride was quantified by UV-Vis spectrophotometer at 230 nm. The release of levocetirizine dihydrochloride in simulated gastric fluid was also analyzed.

RESULTS AND DISCUSSION

Preparation and optimization of levocetirizine dihydrochloride loaded casein microparticles

The casein microparticles were produced by steric stabilization process by optimizing the concentration, volume of casein and ethylcellulose which is used as the stabilization agent as shown in Table 1. The result depicts that formulation No. 15 with 5 ml of 10% casein and 2% ethylcellulose yielded better particles. This shows that casein microparticles concentration and volume of casein solution was observed to have a strong influence on microsphere morphology. Ethyl cellulose used as a steric stabilization agent plays an important role in establishing emulsion between the aqueous (casein solution) and organic (chloroform) phases, hence influence the formation of particles. At decreased ethyl cellulose concentration, particles were formed as large clumps and at increased ethyl cellulose concentration, particles size decreased but particles were clumped and unstable.

Accordingly, the increased solubility of the drug in casein solution indicates strong hydrophobic interactions leading to association of the drug with the protein micelles (Bachar et al., 2012).

Particle size distribution and morphology

The mean diameter of the microparticles was found to be 50 microns (Figure 2) by the laser particle size analyzer. From the SEM report, the microparticles size was found to be less than 3 μm . Casein microparticles showed macropore surfaces with follicular structure appearance (Figure 1).

X-ray powder diffraction studies

X-ray diffraction (XRD) was implemented to investigate whether levocetirizine dihydrochloride retains its crystalline state in the microparticles obtained. Pure forms of the drug, polymer, placebo microparticles and drug loaded microparticles were scanned as shown in Figure 3. Several sharp and intense peaks can be identified clearly in the levocetirizine dihydrochloride diffractogram at 2θ values. However, the disappearance of levocetirizine dihydrochloride peaks in the drug loaded microparticles indicates that the drug is encapsulated in the polymer effectively.

FTIR spectral analysis

The FTIR spectra of placebo microparticles and levocetirizine dihydrochloride loaded casein microparticles are shown in Figure 4. The FTIR spectrum indicates that there is no modification or interaction of

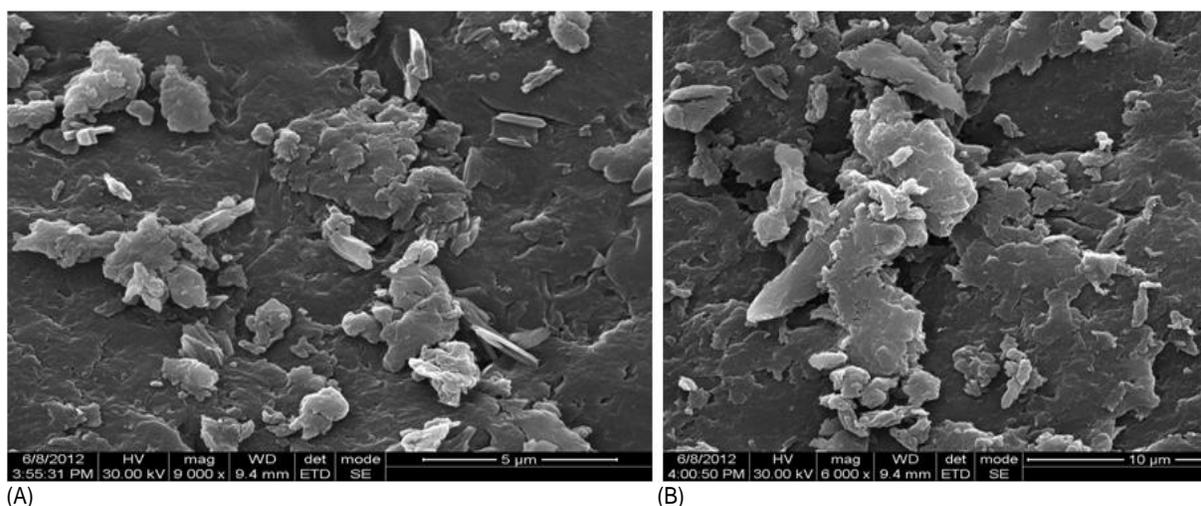
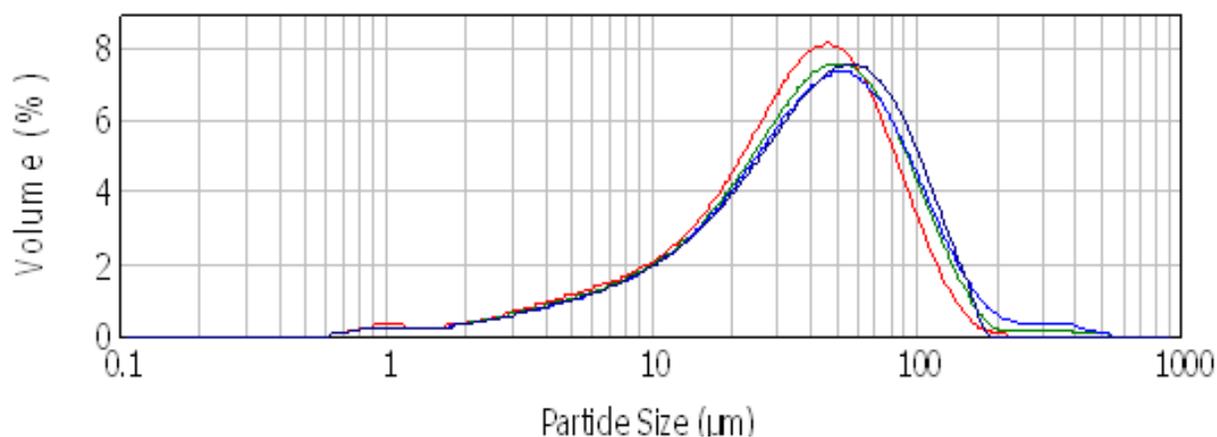


Figure 1. Scanning electron micrograph of casein microparticles.



- CaOX, Monday, March 19, 2012 10:23:01 PM
- CaOX - Average, Monday, March 19, 2012 10:22:31 PM
- CaOX, Monday, March 19, 2012 10:22:46 PM
- CaOX Monday, March 19, 2012 10:22:31 PM

Figure 2. Particle size distribution.

casein and levocetirizine dihydrochloride. The absence of the intense peaks (500 to 1700 cm^{-1}) corresponding to levocetirizine dihydrochloride assures the encapsulation of the drug within the protein matrix. The bands observed in the casein microparticles spectrum did not show any shift, suggesting that no new chemical bond was formed after preparing the formulation, and the results confirmed that the drug is physically encapsulated inside the polymer matrix.

Differential scanning calorimetry

The DSC thermogram showed endothermic peak of levocetirizine at 206.14°C , which corresponded to its melting point. The evaluation of the thermo gram obtained from DSC (Figure 5) shows only exothermic peaks at 95.3 and 96.8°C , respectively which revealed no interaction between the polymer and the drug in the film and the drug is encapsulated in the polymer matrix.

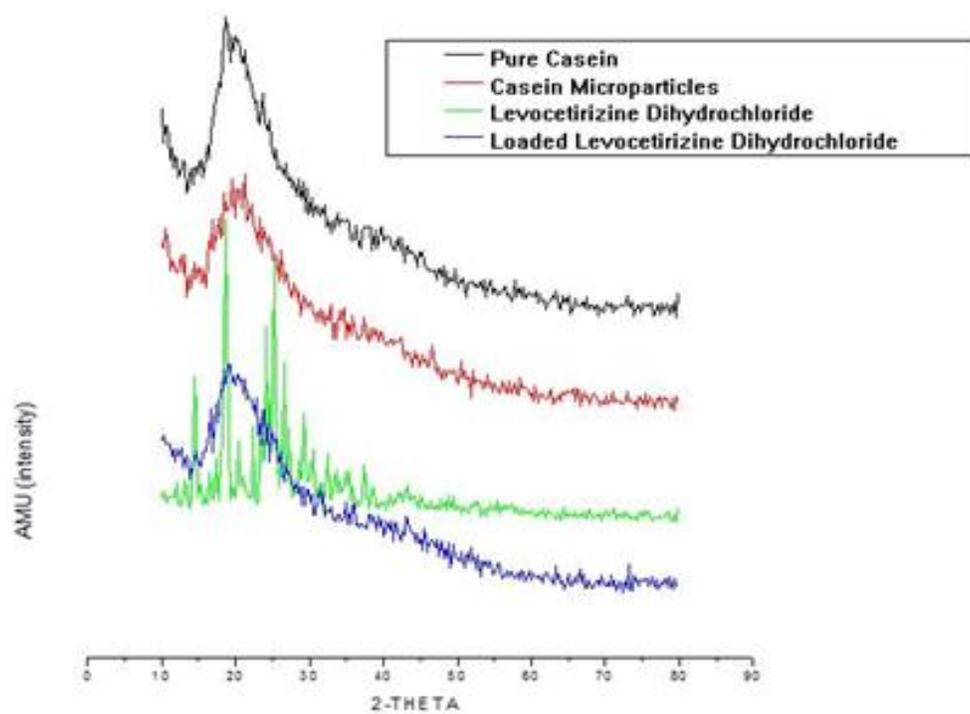


Figure 3. Combined XRD pattern having constant X-axis.

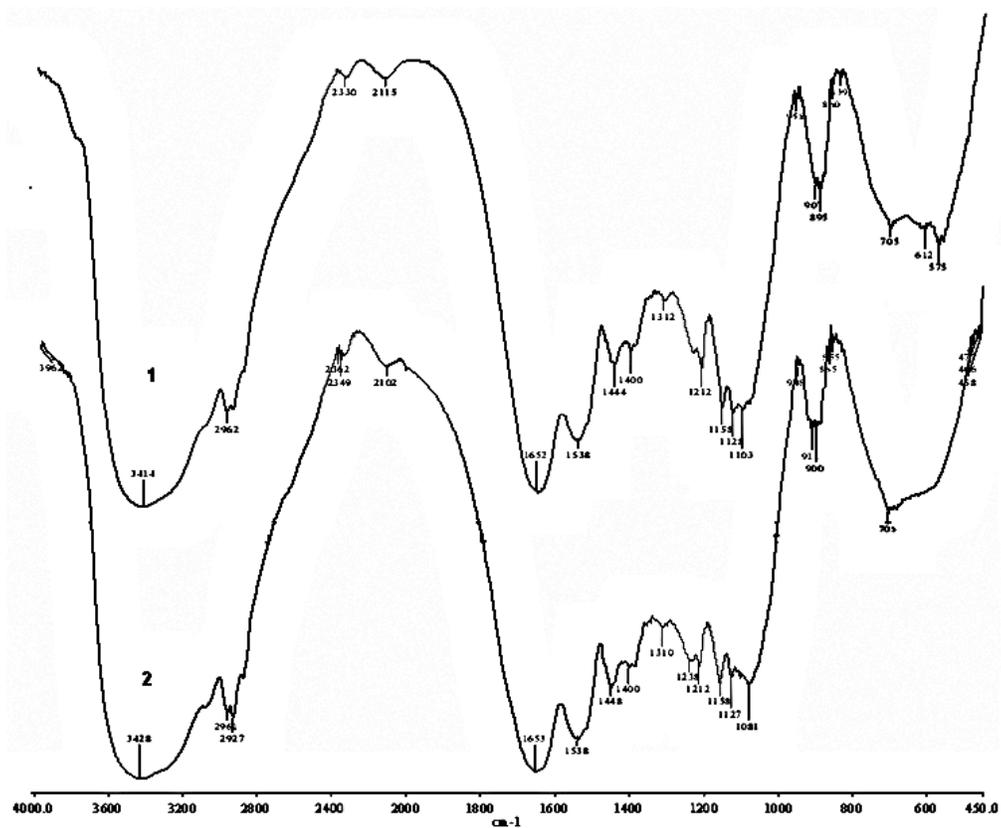


Figure 4. IR spectrum of placebo and levocetirizine dihydrochloride loaded casein microparticles.

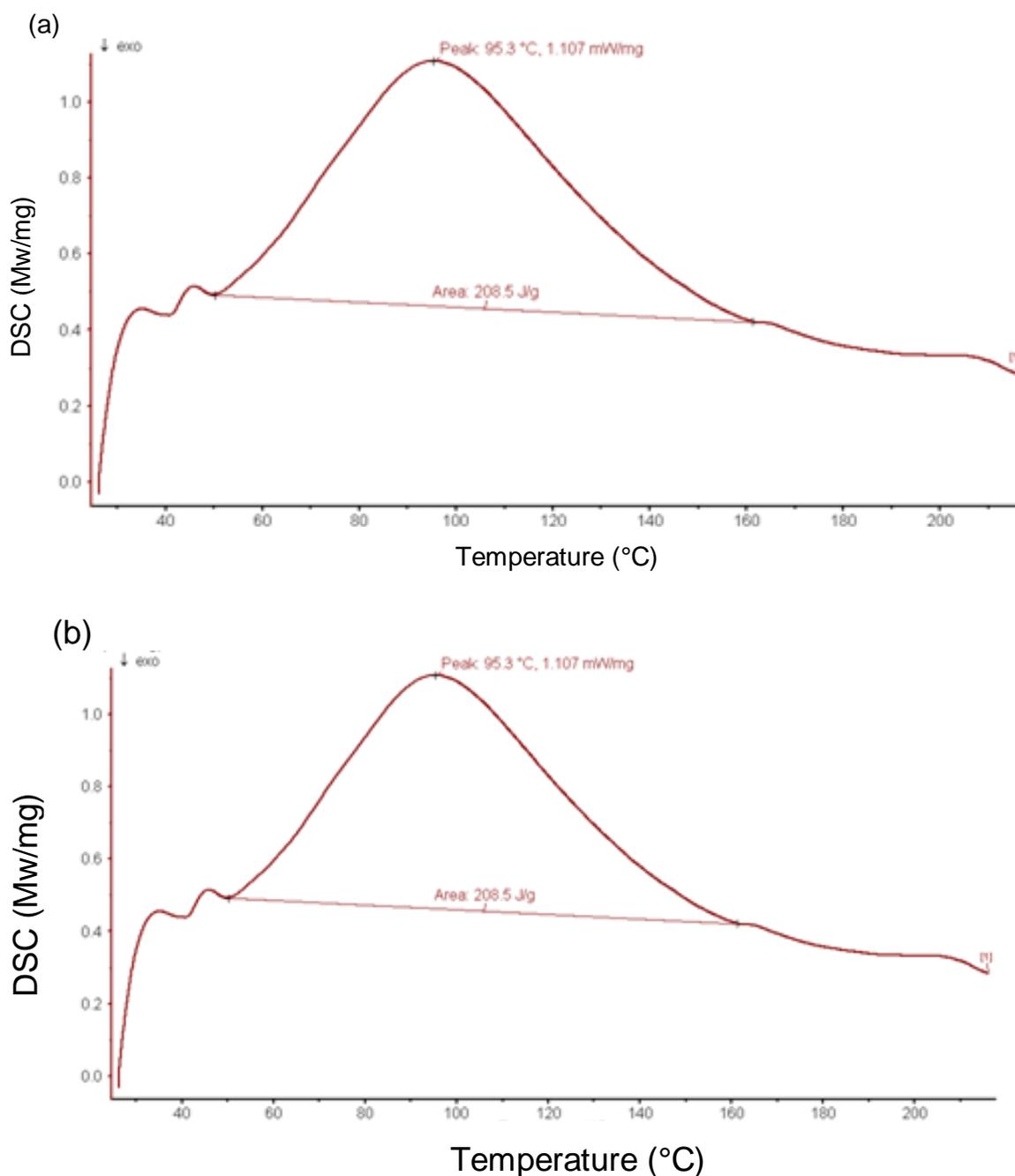


Figure 5. DSC thermograms.

Loading efficiency and *in vitro* release

Levocetirizine dihydrochloride loaded casein microparticles possess a high percentage of encapsulation efficiency which was found to be 76.5%. *In vitro* release studies were performed in simulated intestinal fluid (SIF) and simulated gastric fluid (SGF) in a pH-responsive drug delivery system. In a pH sensitive drug delivery system in

which the drug is ionically linked, the release of the drug is controlled firstly by the rate of cleavage of electrostatic bonds and secondly by diffusion. The cumulative percentage drug release profile (Figure 6) reveals that the amount of drug releases in SGF is $2.33 \pm 0.13\%$, which is a significantly negligible fraction, and the drug was found to be released in a controlled manner in SIF ($58.14 \pm 3.11\%$) over the period of eight hours.

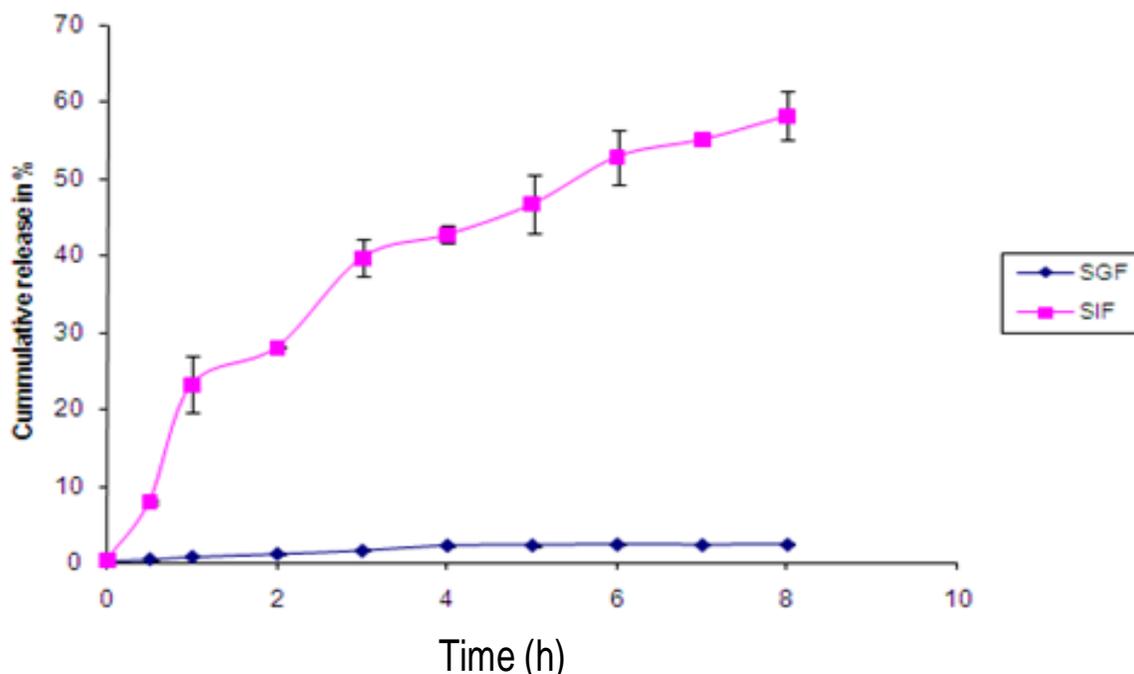


Figure 6. *In vitro* release profile of levocetirizine dihydrochloride.

Conclusion

The main theme of this research is focused on the preparation of casein microparticles which can be loaded with a range of drugs and the effects of processing conditions on particle size, drug loading and release. Casein microparticles were prepared by steric stabilization process. The parameters such as ethyl cellulose concentration, casein concentration and volume of casein solution were optimized. Ethyl cellulose concentration of 2% was found to be effective in obtaining spherical, uniform and evenly distributed microparticles. SEM reports that the microparticles size was found to be less than 3 μm . Casein microparticles showed macropore surfaces with follicular structure appearance. The bands observed in the casein microparticles spectrum suggest that drug is physically dispersed in the polymer. The DSC studies were also performed to investigate the casein-levocetirizine electrostatic interaction which indicates the absence of electrostatic interaction and it proves that the drug is encapsulated within the casein matrix. Release profile suggests that the drug be released till 8 hours in a controlled fashion.

ACKNOWLEDGEMENTS

The authors would like to gratefully acknowledge the financial and technical support of VIT University. Thanks are due to SAIF, IIT-Madras for providing the SEM and

Thermal analysis data.

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