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ORIGINAL ARTICLE

Preparation and characterization of spray-dried mucoadhesive microspheres of aceclofenac

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Abstract

Objective: Microencapsulation of the anti-inflammatory drug aceclofenac (ACE) was investigated as a means of controlling drug release and minimizing or eliminating local side effects. *Method*: Microspheres were prepared by a spray-drying technique using solutions of ACE and three polymers, namely, carbopol, chitosan, and polycarbophil, in different weight ratios. *Results*: The spray-dried mucoadhesive microspheres were characterized in terms of shape (scanning electron microscope), size (6.60–8.40 μm), production yield (34.10–55.62%), and encapsulation efficiency (58.14–90.57%). In vitro release studies were performed in phosphate buffer (pH 6.8) up to 10 hours. The spray-drying process of solutions of ACE with polymeric blends can give prolonged drug release. The in vitro release data were well fit into Higuchi and Korsmeyer–Peppas model and followed Fickian diffusion mechanism. In vivo data showed that the administration of ACE in polymeric microspheres prevented the gastric side effects. *Conclusion*: The formulations here described can be proposed for the oral administration of nonsteroidal anti-inflammatory drugs with minimal side effects on gastric mucosa.

Key words: *Aceclofenac; gastric side effects; mucoadhesive microspheres; spray-drying method*

Introduction

Spray-drying is a well-known technique in food industry and is used to dry solutions such as milk. Spray-drying, also, has been used in pharmaceutical industry since the early 1940s for drying heat-sensitive materials, increasing the solubility of poorly water-soluble drugs, masking the taste, enteric coating, improving the flow properties in tablet production, and coating of some drugs or drug microencapsulation[1]. In addition, it is a one-stage continuous process, easy to scale up, and only slightly dependent upon solubility of drug and polymer. The particle size of the microspheres prepared by the spray-drying method ranged from a micron to several tens of microns and had a relatively narrow distribution. Microspheres prepared by the spray-drying technique can be administered by oral, parenteral, or nasal routes. Recently, spray-dried microparticles have been demonstrated as controlled release carriers for water-soluble and water-insoluble drugs[2].

Aceclofenac (ACE), phenyl acetic acid derivative 2-[(2,6-dichlorophenyl)amino] phenyl acetoxy acetic acid, is a novel nonsteroidal anti-inflammatory drug (NSAID) indicated in the symptomatic treatment of pain and inflammation with a reduced side-effect profile especially regarding gastrointestinal (GI) complications[3,4]. Recommended dose is 200 mg daily in divided doses. The successful treatment of arthritis depends on the maintenance of effective drug concentration in the body for which a constant and uniform supply of drug is desired. Sustained release dosage forms deliver the drug at a slow release rate over an extended period of time and achieve this objective. The mean plasma elimination half-life of ACE is 4 hours[3]. To reduce the dosing

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frequency and adverse effects during prolonged treatment, it is necessary to formulate in long-acting dosage form. Different workers have attempted to prepare sustained release oral formulations of ACE like sustained release tablet, microparticulate system, and microemulsion[5–7]. Carbopol (CP), chitosan (CS), and polycarbophil (PL) are known to be nontoxic when taken orally and to protect the mucous membrane of the upper GI tract from the irritation of chemicals[8,9,10]. Since the property of swelling is susceptible to the environmental pH, the incorporation of acid-sensitive drugs into the microspheres protects them from the gastric juice[11]. Therefore, drug-loaded microspheres might provide these advantages for NSAIDs, such as ACE, which lead to gastric irritation. Several trials have been similarly undertaken to decrease the ulcerogenic activity of NSAIDs such as diclofenac, ibuprofen, and indomethacin[11–14]. The formulation of ACE as a controlled release dosage form seems to be an important approach to overcome the potential problems in the GI tract so as to achieve a reduction of the NSAID's adverse effects.

Various attempts have been made in order to prepare mucoadhesive microspheres by spray-drying technique using hydroxyl propyl methyl cellulose, CP, CS, hydroxyl propyl cellulose with excellent mucoadhesive properties[2,9,15]. The objective of this study was to prepare the ACE-loaded microspheres by the spray-drying method using CP, CS, and PL. Prepared microspheres were characterized for their surface morphology, swelling behavior, mucoadhesion, drug release profile, and in vivo GI side effects by using appropriate evaluative studies.

Materials and methods

Materials

ACE was obtained as a gift sample from Comed Chemicals Limited (Vadodara, India). Carbopol 974® PNF (CP) and Noveon AA-1 (PL) were obtained as a gift sample from Lubrizol Advanced Materials Inc. (Mumbai, India). CS, >85% deacetylation, was kindly contributed from the Central Institute of Fisheries Technology (Kochi, India). All other reagents and solvent used were of analytical grade and were used as obtained from the manufacturers.

Preparation of ACE-loaded mucoadhesive microspheres

Drug-loaded microspheres based on mucoadhesive polymers were prepared by spray-drying of dispersion using a SD-05 spray-drier (Lab Plant, Leeds, UK) with a standard 0.5 mm nozzle. The liquid was fed to the nozzle with peristaltic pump, atomized by the force of the compressed air, and blown together with a hot air to the chamber where the solvent in the droplets was evaporated. The dry product was then collected in a collection bottle. From preliminary experiments, the drying conditions were selected as follows: inlet air temperature of 168°C, outlet air temperature of 82°C, pump setting of 12 mL/min, and pressure bar at ~2 atm.

For the dispersion system, CP and PL were solubilized in deionized water at 0.75%, 0.80%, and 0.825% (w/v) concentration and CS was solubilized in 1% (v/v) aqueous acetic acid solution at 0.75%, 0.80%, and 0.825% (w/v) concentration. ACE was dissolved at 0.25%, 0.20%, and 0.165% (w/v) concentration in 10 mL of absolute ethanol, respectively. These drug solutions were then mixed with polymeric solution in order to achieve desired drug to polymer 1:3, 1:4, and 1:5 ratio. Each formulation was produced in triplicate and pooled prior to further studies.

Encapsulation efficiency

To determine encapsulation efficiency, 100 mg of accurately weighed drug-loaded mucoadhesive microspheres were added to 100 mL of methanol. The resulting mixture was kept shaking on a mechanical shaker for 24 hours. Then, the solution was filtered and 1 mL of this solution was appropriately diluted with methanol and analyzed spectrophotometrically at 275 nm using a Shimazdu UV-700 (UV/VIS double beam spectrophotometer, Kyoto, Japan). The drug encapsulation efficiency was calculated using the following formula: (practical drug content/theoretical drug content) × 100.

Scanning electron microscope

A scanning electron microscope (ESEM TMP with EDAX, Philips, Holland) was used to characterize the surface topography of the microscope. The microspheres were placed on a metallic support with a thin adhesive tape and microspheres were coated with gold under vacuum. The surface was scanned and photographs were taken at 30 kV accelerating voltage for the drug-loaded microspheres.

Particle size

Prepared spray-dried mucoadhesive microspheres exhibited quick swelling in liquid medium and as a result, sizes could not be determined using laser diffraction in a particle size analyzer. Therefore, the particle size was determined by image analysis system (IAS). Microscopical imaging analysis technique for determination of particle size distribution was used[16]. Microsphere size and distribution were determined with an AXIOPALN microscope (Zeiss MPM400, Göttingen,

Germany), equipped with a computer-controlled IAS (Zeiss KS300).

Swelling study

The swelling ability of the microspheres in physiological media was determined[17]. Accurately weighted amounts of microspheres were immersed in a little excess of phosphate buffer (pH 6.8) and kept for 24 hours. The following formula was used for calculation of degree of swelling:

$$S_{sw} = \left(W_s - \frac{W_0}{W_s} \right) \times 100,$$

where S_{sw} = percentage swelling of microspheres, W_0 = initial weight of microspheres, and W_s = weight of microspheres after swelling.

Mucoadhesion

Mucoadhesion of different microspheres system was assessed using the method reported by Jain et al.[16] with little modification. A strip of rat intestinal mucosa was mounted on a glass slide and accurately weighed mucoadhesive microspheres in dispersion form was placed on the mucosa of the intestine. This glass slide was incubated for 15 minutes in a desiccator at 90% relative humidity to allow the polymer to interact with the membrane and finally placed in the cell that was attached to the outer assembly at an angle 45°C. Phosphate buffer (pH 6.8), previously warmed to 37 ± 0.5°C, was circulated to the cell over the microspheres and membrane at the rate of 1 mL/min. Washings were collected at different time intervals and microspheres were separated by centrifugation followed by drying at 50°C. The weight of microspheres washed out was taken and percentage mucoadhesion was calculated by the following formula:

$$\text{Percentage mucoadhesion} = W_0 - \frac{W_t}{W_0} \times 100,$$

where W_0 = weight of microspheres applied; W_t = weight of microspheres leached out.

Fourier transform infrared spectroscopy

The spectra were recorded for pure drug, drug-loaded microspheres and blank microspheres using Fourier transform infrared spectroscopy (FTIR; PerkinElmer, Spectrum GX, Chicago, IL, USA). Samples were prepared in KBr disks (2 mg sample in 200 mg KBr). The scanning range was 400–4000 cm^{-1} and the resolution was 2 cm^{-1}.

Differential scanning calorimetry

Differential scanning calorimetry (DSC) scans of drug, blank microspheres, and drug-loaded microspheres were performed using DSC-PYRIS-1 (PerkinElmer). The analysis was performed with a heating range of 50–300°C and a rate of 10°C/ min.

Drug release study

The drug release study was performed using USP XXIV basket apparatus (Electrolab, TDT-06T, Mumbai, India) at 37°C and at 50 rpm using 900 mL of phosphate buffer (pH 6.8) as a dissolution medium up to 10 hours[18]. Microspheres equivalent to 100 mg of ACE were used for the test. Five mL of sample solution was withdrawn at predetermined time intervals, filtered through a 0.45 mm membrane filter, diluted suitably, and analyzed spectrophotometrically at 275 nm. An equal amount of fresh dissolution medium was replaced immediately after withdrawal of the test sample. Percentage drug dissolved at different time intervals was calculated using the calibration curves constructed from the reference standards.

Release kinetics

In order to understand the mechanism and kinetics of drug release, the results of the in vitro drug release study were fitted with various kinetic equations. The kinetic models used are zero-order, first-order, Higuchi matrix, and Baker and Lonsdale models[19]. These are summarized below:

Zero-order release:

$$\frac{M_t}{M_\infty} = kt.$$

First-order release:

$$\ln\left(1 - \frac{M_t}{M_\infty}\right) = -kt.$$

The Higuchi square root of time model has been derived from Fick's first law of diffusion and is suited for the modeling of drug release from a homogeneous planar matrix, assuming that the matrix does not dissolve:

$$\left(\frac{M_t}{M_\infty}\right)^2 = kt.$$

The Baker and Lonsdale equation models drug release from diffusion rate-limiting matrixes of spherical shape:

$$\frac{3}{2}\left[1-\left(1-\frac{M_t}{M_\infty}\right)^{2/3}\right]-\frac{M_t}{M_\infty}=kt.$$

In order to define a model which will represent a better fit for the formulation, drug release data further analyzed by Peppas equation

$$\frac{M_t}{M_\infty}=kt^n,$$

where M_t is the amount of drug released at time t and M_∞ is the amount released at time ∞, the M_t/M_∞ is the fraction of drug released at time t, k is the kinetic constant, and n is the diffusional exponent, a measure of the primary mechanism of drug release. r^2 values were calculated for the linear curves obtained by regression analysis of the above plots.

In vivo studies

Pharmacology

The preclinical studies were carried out in Wistar rats. Male Wistar rats (200–250 g) were obtained from the Zydus Research Center (Ahmedabad, India). They were housed in polyethylene cages, four animals per cage, with free access to food (Pranav Agro Limited, Baroda, India) and water. The preclinical study protocol was approved by the Institutional Animal Ethical Committee (CPCSEA/IAEC/ARCP/07-08/05). Twenty-four hours before the experiment, the food was withdrawn, but free access to water was allowed. Free ACE (10 mg/kg), blank microspheres, and ACE-loaded microspheres equivalent to free ACE (10 mg/kg) were each suspended in 0.5% (w/v) NaCMC solution and given orally to each group ($n = 6$) by gavage. The animals were killed 7 hours after the oral administration and their stomachs were dissected. The existing hemorrhagic lesions were inspected visually. The specimens were then put into 10% formalin solution for microscopic examinations.

Histology

After removal of the stomach of rats, the tissues were placed in 10% buffered formaldehyde solution, fixed for 72 hours. For the purpose of histological study, tissues were dehydrated in ascending degrees of ethyl alcohol (70%, 80%, 90%, 96%, and 99%) and sequentially embedded in paraffin wax blocks according to the standard procedure, sectioned at 5 μm thickness. They were further deparaffinized with xylol, and histologic observations were performed after staining for functional stomach tissues by hematoxylin–eosin. The slides were examined using light microscope.

Results and discussion

The encapsulation method by spray-drying is simple and rapid, since spray-drying combines drying of feed solution and embedding of the drug into the polymeric network process into a one-step operation. Microspheres, fabricated from CS, CP, and PL were also shown to be mucoadhesive and 'retentive' in vitro under dynamic test conditions[20]. The aim of this study is to combine the potential advantages of mucoadhesion with those of controlled drug delivery.

Most spray-dried mucoadhesive microspheres were white fine powder except those containing CS, which were yellowish, while some microspheres of PL adhered into masses.

Characterization of microspheres

Yield, particle size, and encapsulation efficiency

The compositions of formulation, percentage yields of microspheres, particle size, and encapsulation efficiency obtained with different polymer/drug ratios and feed concentrations is listed in Table 1. Percentage yield is expressed as the weight percentage of the microspheres obtained with respect to the initial amount of drug and

Table 1. The composition, yield, particle size, and encapsulation efficiency of microsphere formulations.

	SDCP1[a]	SDCP2[a]	SDCP3[a]	SDCS1[b]	SDCS2[b]	SDCS3[b]	SDPL1[c]	SDPL2[c]	SDPL3[c]
Drug: polymer ratio	1:3	1:4	1:5	1:3	1:4	1:5	1:3	1:4	1:5
Yield (%)	46.87 ± 3.44	47.50 ± 5.66	43.60 ± 5.87	55.62 ± 4.54	44.50 ± 7.23	37.50 ± 3.46	43.50 ± 3.13	42.75 ± 4.67	34.10 ± 3.46
Mean particle size (μm)	6.60	7.22	7.89	6.74	7.55	8.40	6.88	7.33	6.94
Encapsulation efficiency	89.49	68.20	75.60	78.47	90.57	58.14	85.25	77.69	60.19

[a]Represents microspheres containing CP as polymer. [b]Represents microspheres containing CS as polymer. [c]Represents microspheres containing PL as polymer.

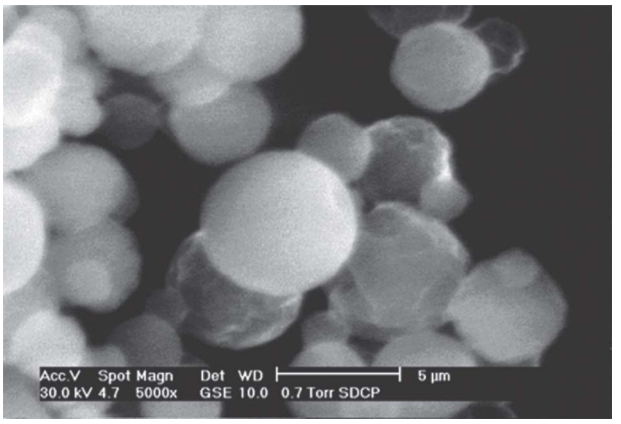
polymer used for the preparation. Encapsulation efficiencies calculated from actual drug contents were found to be high, lying between ~58% and 90%. Percentage yields were found to be between 34% and 55%. These low values can be explained both by the relatively low volumes of feed solution sprayed for the preparation of each batch of microspheres (~500 mL) and by the structure of the spray-drier apparatus that is not equipped with a trap to recover the smaller and lighter particles that are exhausted by the aspirator. Further, the loss of material during spray-drying process may be due to powder adhering to the cyclone walls[21]. The mean particle size of microspheres ranged from 6 to 8 µm, which indicated the narrow size distribution. It was also noted that increasing the drug to polymer ratio slightly increased the size of microspheres and the increased atomization nozzle flow reduced the particle size, which was consistent with previous finding[22].

Scanning electron microscopy

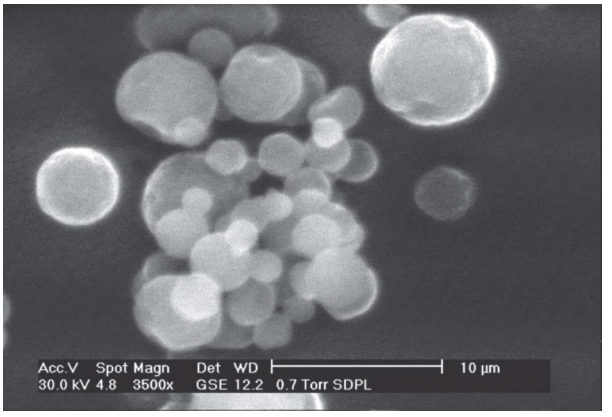
The polymeric composition of the microspheres does not influence their morphology, as shown in Figure 1, where SEM pictures of drug-loaded microspheres are reported. Blank microspheres have similar morphology (data not reported). It can be seen that spray-dried mucoadhesive microspheres are spherical with wrinkles on their surface due to shrinking of the microspheres following the formation of the solid crust at the surface of the droplets as a result of the solvent evaporation[23]. The increase in the concentration of polymeric solution leads to alteration of morphological characteristics such as rough surface of the microspheres and increased size of microspheres[24].

Swelling property

Swelling is an important characteristic as it affects mucoadhesion as well as drug release profiles of polymeric drug delivery systems. The adhesive strength of polymers on rat intestine correlates with their degree of hydration, which in turn is related to the expanded nature of the polymer network due to swelling. All obtained microspheres rapidly swelled in pH 6.8 phosphate buffer as shown in Figure 2. The swelling was ranked, CP>PL>CS microspheres. Increasing the amount of CP and CS in formulation increased the percentage of swelling. The high swelling property of CP and CS microspheres could be attributed to their ionized ability to uncoil the polymer into an extended structure[25]. Higher swelling of CP microspheres than CS microspheres was likely due to its higher molecular weight[26]. However, on increasing the amount of PL, the swelling capacity of the PL microspheres decreased considerably, which was not comparable to previous studies performed by using PL as polymer[27]. The water uptake in hydrogels depends upon the extent of hydrodynamic free volume and availability of hydrophilic functional groups for the water to establish hydrogen bonds[2].



(A)



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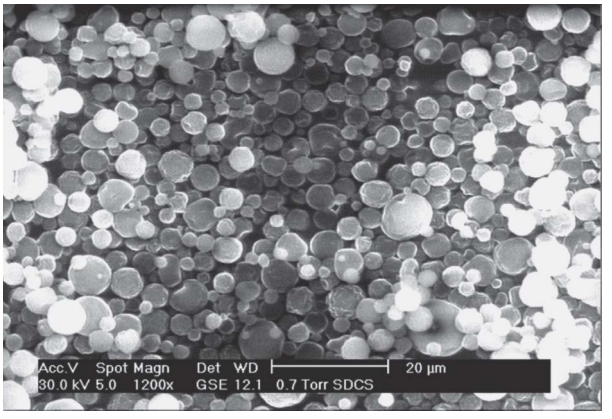

(C)

Figure 1. Scanning electron photomicrographs of drug-loaded microspheres. (A) Microspheres of CP, (B) microspheres of PL, (C) microspheres of CS.

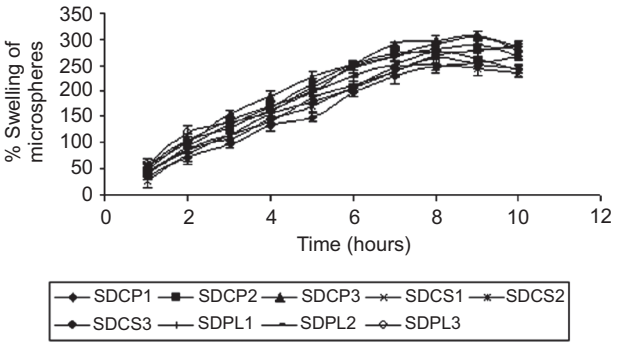

Figure 2. The profiles of percentage swelling with time of microspheres. Values are mean ± SD ($n = 3$).

Mucoadhesion property

Mucoadhesion is defined as an adhesion phenomenon occurring between a mucosal membrane covered with mucus and nonbiological materials consist of a two-step process. The first step is considered to be an interfacial phenomenon influenced by surface energy effects and spreading of both the mucus and mucoadhesive microspheres. The second step involves interdiffusion of polymer chains of both phases. This step requires hydration of the polymers and is influenced by molecular weight, molecular mobility and viscosity of adhesive, and swelling properties of both the mucus and microspheres[28].

The adhesion time of mucoadhesive microspheres (Figure 3) was ranked, CS>CP>PL microspheres. Poor mucoadhesion of PL microspheres may be due to its nonionic property, whereas excellent mucoadhesion of CS microspheres may be attributed to electrostatic attraction between CS and mucin[29]. This is the further evidence for the strong interaction between CS microspheres and mucous glycoprotein and/or mucosal surfaces. Not only can mucin be adsorbed on CS microspheres, but more importantly from the standpoint of bioadhesion, CS microspheres can be adsorbed onto mucosal tissue. The linear molecule of CS expressed sufficient chain flexibility for interpenetration and entanglement[30]. Although CP microspheres had negative charge in phosphate buffer (pH 6.8), causing negative charge repulsion with mucus, numerous hydrophilic functional groups such as carboxyl groups in CP molecules could form hydrogen bonds with mucous molecules, thus producing some adhesive force of this polymer[9,31]. In addition to type, amount, and molecular weight of polymer might have played a significant role on mucoadhesion. The comparatively low mucoadhesion for CP and PL may be attributed to their swelling characteristics[32]. Therefore, these polymers can hydrate more readily than CS which exhibited least swelling to form a nonadhesive mucilage and therefore be 'washed' away.

FTIR and DSC

The IR spectra of pure ACE, drug-loaded microsphere and blank microsphere are shown in the Figure 4. The peak at 3319 nm indicating the –NH stretching, two peaks at 1771 and 1717 nm for the –C=O stretching of –COO and –COOH group, respectively. The peaks at 1589, 1281, and 749 nm show as major peaks for drug. All the above peaks are present in drug-loaded microspheres that confirm the presence of drug in the polymer without any interaction.

The thermal behavior of microspheres prepared by spray-drying in comparison with thermograms of both pure ACE and blank microspheres is illustrated in Figure 5. It was used to determine the existence of possible interaction between the polymer and drug. The DSC thermogram of pure ACE shows sharp endothermic peak at 155°C, corresponding to its melting point. Also, ACE-loaded polymeric microspheres exhibit a single melting peak at 153°C due to presence of ACE in polymeric matrix. However, there was a slight decrease in

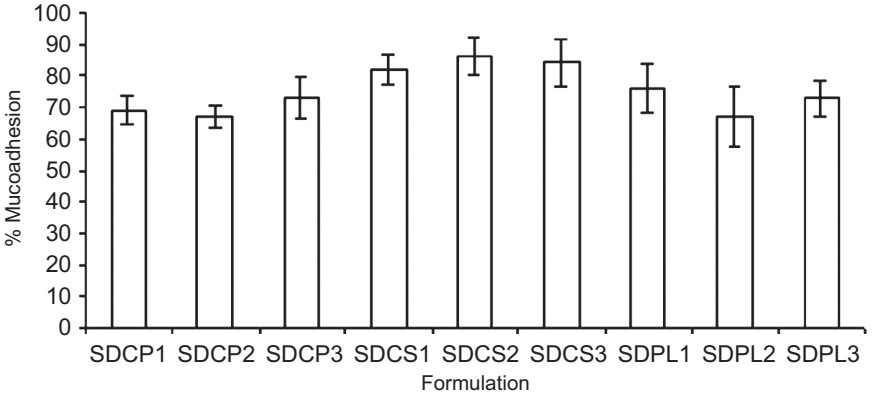

Figure 3. Percentage mucoadhesion of microspheres of different formulations. Values are mean ± SD ($n = 3$).

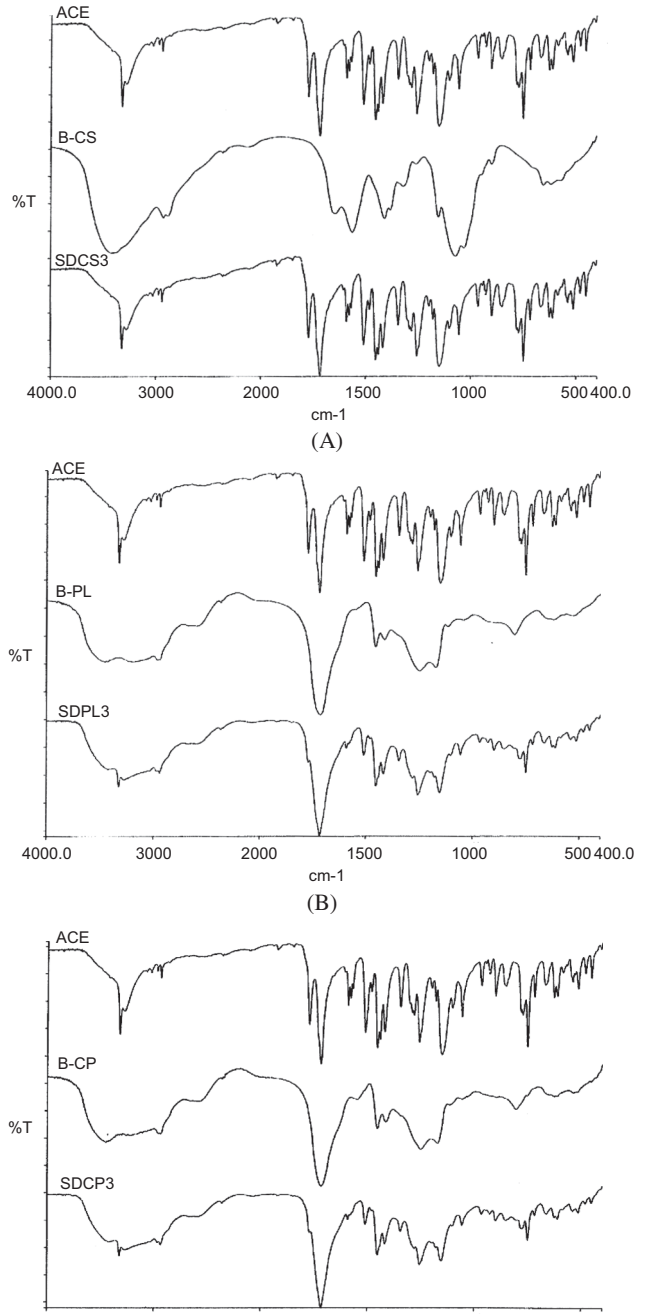

Figure 4. Comparative IR spectra of ACE, blank microsphere (B), and drug-loaded microspheres: (A) microspheres of CS, (B) microspheres of PL, (C) microspheres of CP.

the melting point of drug when prepared in the form of microspheres. It was also observed that there was a noticeable reduction in the enthalpy of the crystals in comparison with pure ACE. The evaluation of the thermograms obtained from DSC revealed no interaction between the polymer and the drug in the microspheres.

In vitro release study

Figure 6 shows the drug release profiles from various formulations of microspheres. The release of drug was prolonged when incorporated within mucoadhesive polymers. Drug release from microspheres is much slower and the cumulative drug release in 10 hours was decreased from 92.04% to 80.86% (CP microspheres), 91.02% to 88.30% (CS microspheres), and 93.04% to 89.01% (PL microspheres) on increasing polymer concentration from 3% to 5% and is attributed to the increase in the density of the polymer matrix and also an increase in the diffusional path length that the drug molecules have to traverse[23]. It was observed that the polymeric gel might have acted as a barrier to penetration of the medium, thereby suppressing the diffusion of ACE from the swollen polymeric matrix. It may be demonstrated that high swelling ability of ACE loaded microspheres, large contact surface between swollen microspheres and small lipophilic cores, as well as lower degree of ACE crystallinity due to spray-drying lead to similar release profiles for all the microspheres prepared by this method[33]. PL represented a greater mass in microspheres and because of swelling properties, relaxation of PL chains occurred and lipophilic drug could not form contact with solvent and did not dissolve. As a result the slow release of ACE was obtained[8]. The slow release of ACE from CP and CS microspheres prepared by the spray-drying method could be due to opposite nature of drug and polymer, hydrophilic nature of polymers, and hydrophobic nature of ACE; it would be possible to prepare drug-loaded microspheres with slow release characteristic by spray-dried method. Slow release of drug could also be attributed to the molecular dispersion of drug in the polymeric matrix (DSC and FTIR results).

The batches (formulation code: SDCP1, SDCS2, SDPL1) demonstrated a satisfactory encapsulation, mucoadhesion, and drug release property from among all the formulated microspheres and were chosen for in vivo trials. In Figure 6, the release of ACE from the said batches at the end of 4th and 9th hours was found to be ~50% and 85%, respectively.

Release kinetics

The mechanism responsible for the release of ACE from the polymeric microspheres under consideration may be due to diffusion phenomena, due to degradation effects, or due to a combination of both processes. To examine the drug release kinetics and mechanism, the release data were fitted to models representing zero-order, first-order, and Higuchi's square root of time, Korsmeyer–Peppas, and Baker and Lonsdale models.

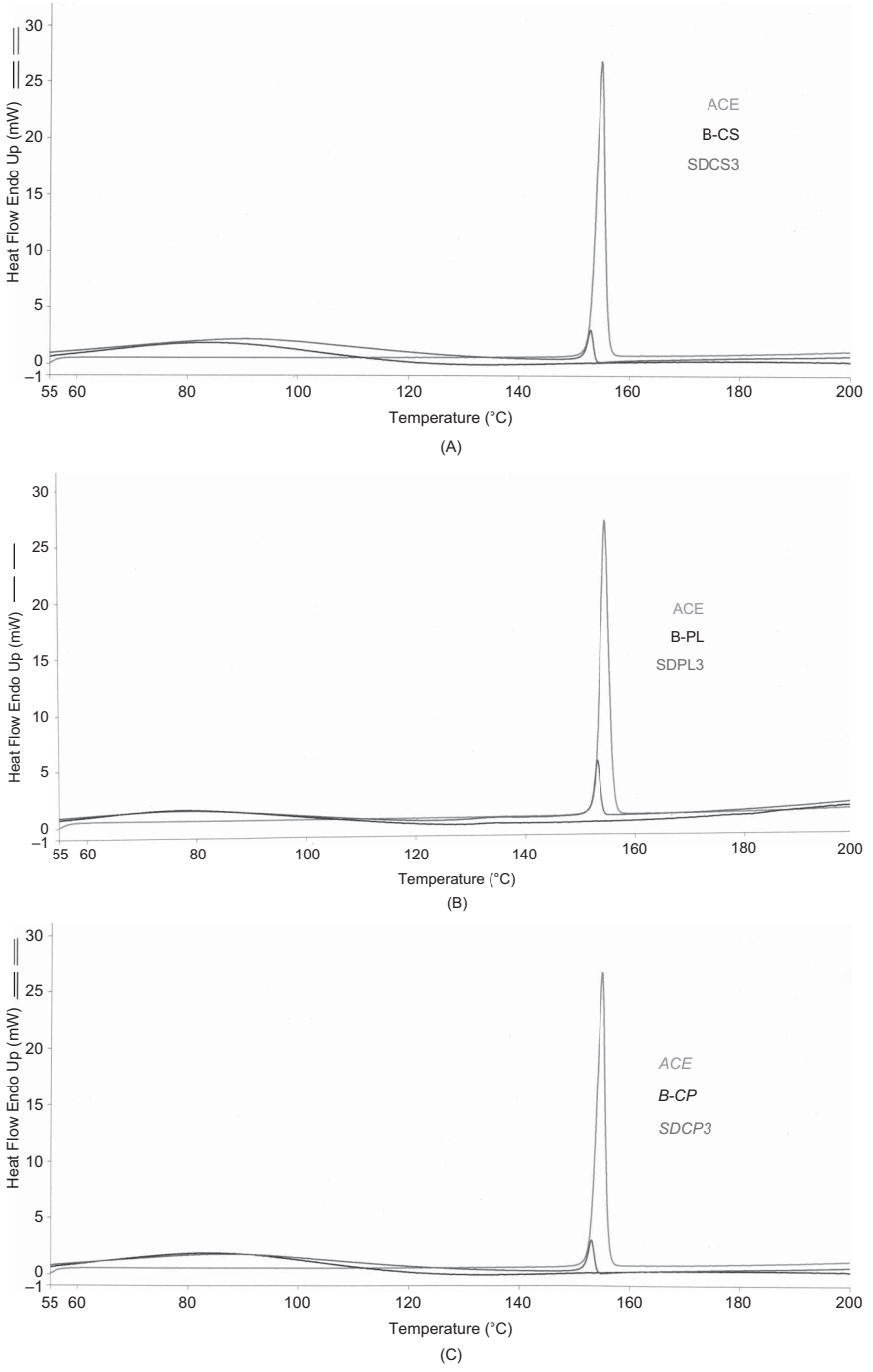

Figure 5. Comparative DSC spectra of ACE, blank microsphere (b), and drug-loaded microspheres: (a) microspheres of CS, (b) microspheres of PL, (c) microspheres of CP.

The release data were seen to fit all models well ($R^2 > 0.90$). The release rates were calculated from the slope of the appropriate plots. Batches SDCP1, SDCP2, SDCS1, SDCS2, SDPL1 showed higher correlation with Higuchi plot than zero-order, first-order, and Baker and Lonsdale models that indicates diffusion controlled mechanism. Batches SDCP3, SDCS3, SDPL3 showed higher correlation with zero-order equation than Higuchi, first-order, and Baker and Lonsdale models. It was observed from zero-order model that drug dissolution from microspheres that do not disaggregate and release the drug slowly[34]. To find out release mechanism, the in vitro release data were applied in Korsmeyer–Peppas equation. The release exponent n was determined and given in Table 2. Batches with higher correlation to Higuchi model showed ($n < 0.5$) Fickian

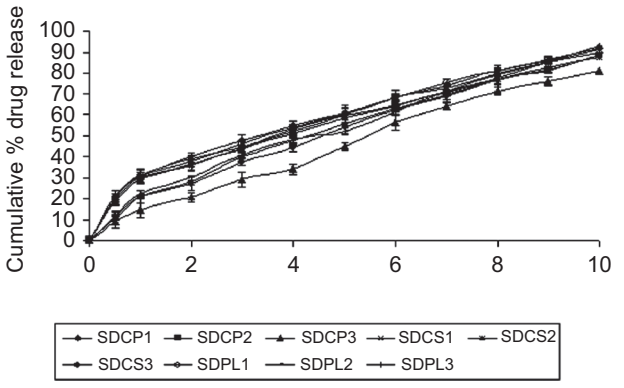

Figure 6. Cumulative % drug release profile of different microspheres. Values are mean ± SD ($n = 3$).

diffusion and batches with higher correlation to zero order showed ($n > 0.5$) anomalous (non-Fickian) diffusion; this suggested that other events occurred during drug diffusion process such as matrix swelling and dissolution that might contribute to the overall release mechanism[35].

In vivo studies

In vivo studies were carried out with free ACE (10 mg/kg), blank polymeric microspheres, ACE (10 mg/kg)-loaded polymeric microspheres (SDCP1, SDCS2, SDPL1), and as a control 0.5% (w/v) NaCMC solution. Figures 7–10 show photographs of rat stomach mucosa after administration of free ACE (10 mg/kg) and ACE-loaded microspheres containing an equivalent amount of drug.

Table 2. Various parameters of the model equations on the in vitro release kinetics.

	Higuchi model		Zero order		First order		Baker and Lonsdale model		Korsmeyer–Peppas model	
Batch	r^2	K_h	R^2	K_0	r^2	K_1	r^2	K_{bl}	R^2	n
SDCP1	0.9943	28.01	0.9870	7.02	0.9468	–0.092	0.9433	0.028	0.9947	0.472
SDCP2	0.9939	27.39	0.9843	6.86	0.9687	–0.079	0.9546	0.024	0.9927	0.494
SDCP3	0.9688	30.93	0.9919	7.88	0.9736	–0.071	0.9314	0.020	0.9874	0.753
SDCS1	0.9901	28.03	0.9878	7.05	0.9531	–0.089	0.9450	0.028	0.9884	0.481
SDCS2	0.9920	27.23	0.9869	6.83	0.9752	–0.078	0.9592	0.024	0.9898	0.487
SDCS3	0.9882	31.32	0.9914	7.09	0.9627	–0.085	0.9345	0.025	0.9936	0.662
SDPL1	0.9929	28.77	0.9876	7.22	0.9571	–0.094	0.9505	0.029	0.9916	0.491
SDPL2	0.9862	31.36	0.9888	7.90	0.9577	–0.090	0.9305	0.027	0.9925	0.640
SDPL3	0.9894	32.74	0.9917	8.25	0.9130	–0.099	0.8960	0.029	0.9942	0.692

K, release rate constant; r^2, coefficient of determination; n, release exponent.

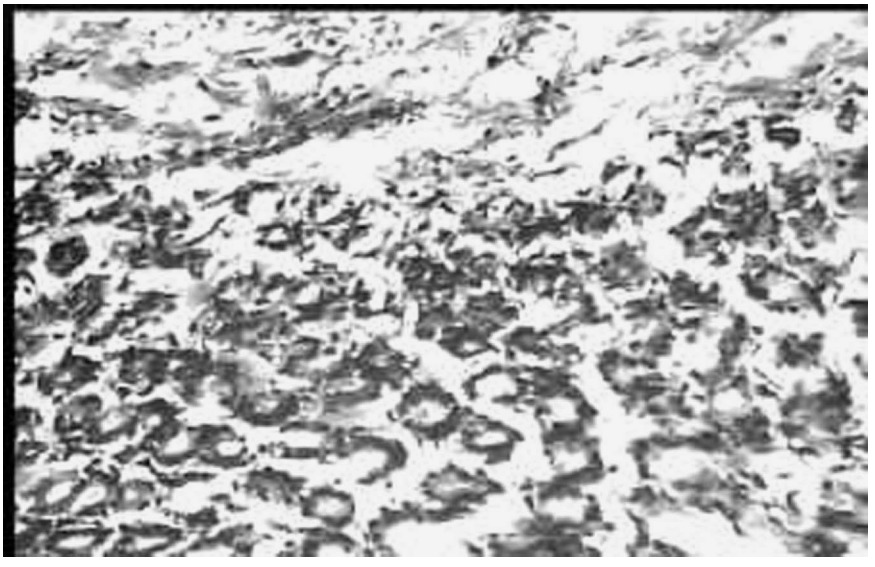

Figure 7. Stomach from the free ACE in NaCMC solution group. One of the eroded areas was observed. The surface epithelium was exfoliated, whereas gastric glands were partially intact.

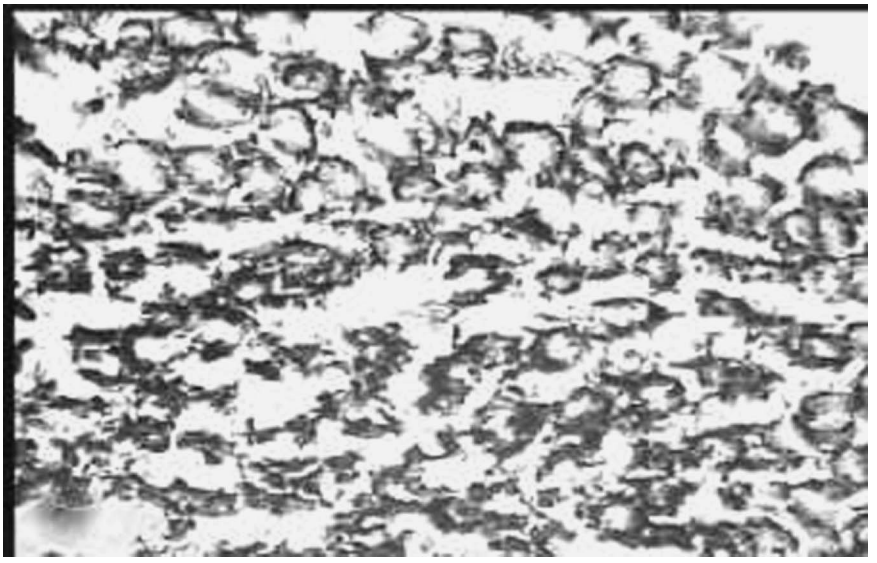

Figure 8. Stomach from the ACE-loaded CP microspheres in NaCMC solution group. One of the eroded areas was observed. The surface epithelium and gastric glands were intact.

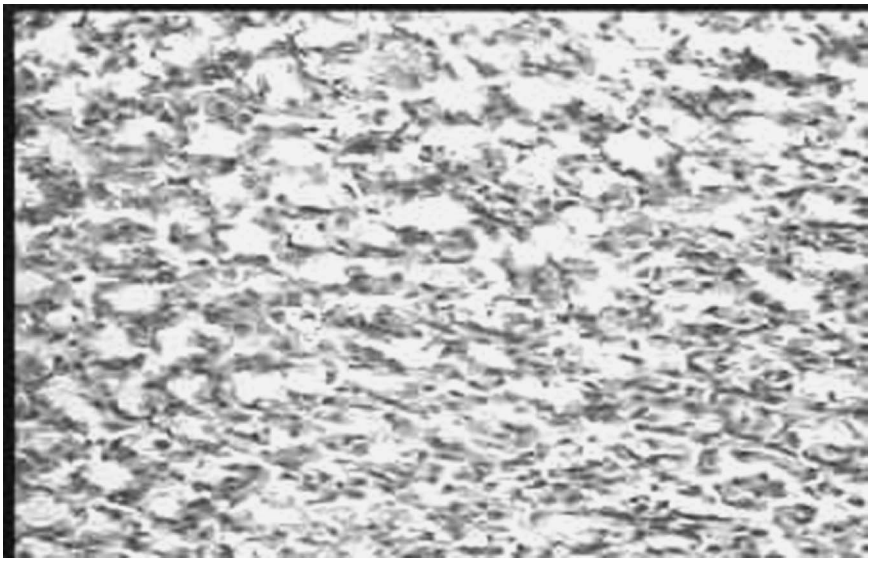

Figure 9. Stomach from the ACE-loaded CS microspheres in NaCMC solution group. No eroded area was observed. The surface epithelium and gastric glands were intact.

In the microscopical examination of the stomach of the group receiving free ACE in NaCMC solution, small erosive areas <1 mm in diameter were observed in some regions. Histologically, there were a few eroded areas in the gastric mucosa. In these eroded areas, only the surface epithelium was found to be exfoliated. Few of the gastric glands were damaged (Figure 7).

The mucosa of stomach is composed of surface epithelium, lamina propria, and muscularis mucosae. The lamina propria of the stomach is occupied by closely packed gastric glands. In the control NaCMC solution, blank microspheres and ACE-loaded polymeric microsphere groups; mild ulcer but no hemorrhage was observed macroscopically. Microscopically the structures of the gastric mucosa were normal. Neither erosion nor hemorrhage was observed in the gastric mucosa (Figures 8–10). According to these findings, it was concluded that ACE used in this dosage may lead to superficial mild mucosal erosion in the gastric mucosa. The erosive effect of ACE disappeared when it was entrapped into polymeric matrix and mucoadhesive polymers did not lead to any damage in gastric mucosa. Several trials have been similarly

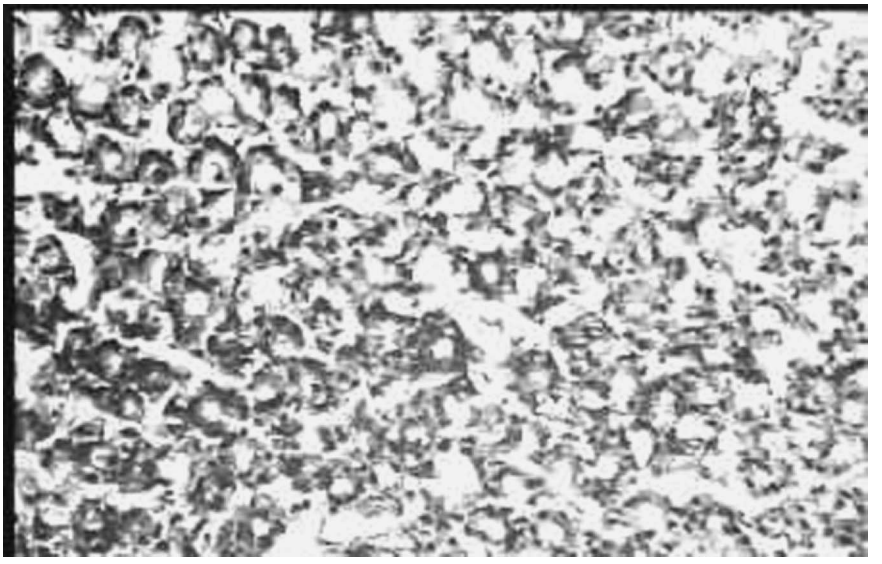

Figure 10. Stomach from the ACE-loaded PL microspheres in NaCMC solution group. The surface epithelium and gastric glands were intact.

undertaken to decrease the ulcerogenic activity of NSAIDs such as diclofenac and ibuprofen[12,13].

Conclusion

It may be concluded that mucoadhesive microspheres of ACE prepared using CS by spray-drying seems to be the promising formulation, providing controlled delivery of ACE with minimal gastric side effects.

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