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European Journal of Pharmaceutics and Biopharmaceutics 58 (2004) 61–67
European Journal of Pharmaceutics and Biopharmaceutics
www.elsevier.com/locate/ejpb

Research paper

Dual coated erodible microcapsules for modified release of diclofenac sodium

S.S. Biju[a], S. Saisivam[b], N.S. Maria Gerald Rajan[b], P.R. Mishra[a,*]

[a]*Faculty of Pharmacy, Hamdard University, New Delhi, India*
[b]*K.M. College of Pharmacy, Madurai, Tamilnadu, India*

Received 26 June 2003; accepted in revised form 19 March 2004

Available online 19 May 2004

Abstract

Diclofenac sodium was formulated as novel enteric microcapsules for improved delivery to the intestine using the polymers cellulose acetate phthalate (CAP) and ethyl cellulose (EC). The enteric coating was given using an innovative technique combining the wet granulation and thermal change methods. The novel process was analysed for its capability to produce microcapsules of uniform size, good flowability, uniform drug loading and maximum entrapment efficacy and the absence of interaction between drug and process parameters as well as the polymers. In vitro release study was carried out in simulated gastric fluid (SGF) for first 2 h and simulated intestinal fluid (SIF) for next 6 h. The best formulation that contained cellulose acetate phthalate and ethyl cellulose in the concentration of 10:90 at 1:1.5 drug–polymer ratio (B3) was further evaluated using in vivo for its pharmacodynamic efficacy and ulcerogenicity. In addition to sustained and uniform release of drug, the formulation B3 showed better anti-inflammatory activity than the marketed formulation and retarded drug release in the gastric medium. The biological examination of incised stomach showed no histological alterations in term of mucous surface cells and glands.

Keywords: Diclofenac sodium; Microencapsulation; Enteric release; Anti-inflammatory activity; Microcapsules

1. Introduction

Diclofenac sodium is a new generation non-steroidal anti-inflammatory agent, which is widely used in the long-term therapy for chronic musculoskeletal pain and chronic inflammatory conditions like rheumatoid arthritis and osteoarthritis. Short biological half-life of 1–2 h necessitates multiple dosing for maintaining therapeutic effect throughout the day [1]. Albeit one among the best in long term therapy in management of arthritis, diclofenac sodium suffers from severe drawbacks like gastrointestinal disturbance, occult GI bleeding and peptic ulceration [2,3]. Ulcers that develop in areas of the GI tract exposed to acidic gastric juice are called peptic ulcer. The most common complication of peptic ulcer is bleeding, which can lead to anaemia if blood loss is serious. In the United States about 5–10% of the population develops peptic ulcer disease [4]. These adverse effects create a potential need for delayed release to intestine and bypass the stomach. Development of new drug molecule is expensive and time consuming. Improving safety, efficacy ratio of 'old' drugs has been attempted using different methods such as individualizing drug therapy, dose titration and therapeutic drug monitoring. Delivering drug at controlled rate, slow delivery, and in targeted fashion are other very attractive methods and have been pursued very vigorously.

In the present work, an innovative method was employed to prepare dual coated enteric microcapsules of diclofenac sodium. Enteric-coated products are designed to remain intact in the stomach and then to release the active substance in the upper intestine [5]. The enteric microcapsules were prepared by combining wet granulation and thermal change methods. Two polymers offered resistance for gastric erosion; enteric cellulose based polymer (CAP) and a water insoluble, hydrophobic polymer such as EC [6,7]. The drug was wet granulated using aqueous acacia mucilage with CAP and then coated with EC by thermal change method. The aim of this research work was to maintain constant blood levels for longer period of time, decreasing

* Corresponding author. Address: Faculty of Pharmacy, Hamdard University, New Delhi 110062, India. Tel.: +91-11-608-4685; fax: +91-11-608-8874.
E-mail address: mishrapr@hotmail.com (P.R. Mishra).

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doi:10.1016/j.ejpb.2004.03.021

GI side effects, and thus improved patient compliance. The release retardant property of two polymers in addition to preventing ulcerogenicity of drug also helps to sustain concentration of the drug for prolonged period.

2. Materials and methods

2.1. Materials

Diclofenac sodium (IP) was a generous gift provided by Pharm Fabrikon, Madurai, India, the polymers used such as ethyl cellulose and cellulose acetate phthalate, sodium acetate, acacia and sulphuric acid were purchased from S.D. fine-Chemicals Ltd., Mumbai, India. Acetonitrile, benzene, acetone, cyclohexane, methanol and acetic acid were purchased from E.Merk, Mumbai, India.

2.2. Methods

2.2.1. Preparation of enteric microcapsules

Enteric microcapsules were prepared by using a simple but innovative process that was designed by combining wet granulation with thermal change method. Thermal dependent solubility of EC was advantageously clubbed with wet granulation of CAP to impart enteric release properties to the delivery system [8–11]. Diclofenac sodium was mixed with CAP; the resulting mixture was granulated with 30% w/w aqueous acacia mucilage and dried at 50 °C. Dried granules of uniform size (No. 30/40) were further encapsulated with EC by coacervation phase separation technique by thermal change method. The EC coating was given on drug-CAP core by using cyclohexane as solvent for EC and changing the temperature from 80 °C to room temperature with continuous stirring at 1000 rpm. Drug–polymer ratio was kept at two levels like 1:1 and 1:1.5 (Table 1). Although the amount of drug loaded in each batch was constant (1 g), the concentrations of CAP and EC were altered at each level in order to prepare ten batches of the enteric microcapsules with different polymer composition.

2.2.2. In vitro release studies

In vitro drug release studies were carried out for 8 h using the rotating basket method specified in USP XXI. The volume and ionic strength of the dissolution media were selected in order to simulate the gastrointestinal environment. Two phases of dissolution studies were carried out in 900 ml of simulated gastric fluid for first 2 h and simulated intestinal fluid for next 6 h. Samples were withdrawn and replaced with fresh media every hour. Samples were filtered using membrane filter of nylon type with pore size of 0.45 μm and analysed by HPLC. The in vitro release studies were carried out in triplicates.

2.2.3. HPLC for diclofenac sodium

A HPLC method with minor modifications [12,13] was followed. SGE ODS column was used with a pump operated at a flow rate of 1.5 ml/min and detection was done at 280 nm. Acetonitrile, acetic acid and sodium acetate was used to prepare the mobile phase. The volume was made up with distilled water so that the final ratio of acetonitrile to H_2O was 60:40 and the pH was 4.8. The sensitivity of the proposed method is 100 ng ml^{-1}. The between run reproducibility and accuracy of the method were examined on three different occasions. Over a range of 100–800 ng ml^{-1} the coefficient of variation was being in the range of 0.4–3% compared with predicted concentration of diclofenac which reflected the excellent accuracy of method with relative errors falling in the range of 0.2–2.5%.

2.2.4. Evaluation of enteric microcapsules

Surface morphology of enteric microcapsules was studied using a Hitachi Gold Scanning Electron Microscope

Table 1
Composition and physical characteristics of the enteric microcapsules

Batch code	Drug–polymer ratio	Composition			Average diameter (μm)	Entrapment efficacy[a] (%)	Angle of repose[a] (°)
		Drug (mg)	CAP (mg)	EC (mg)			
A1	1:1 (EC)	1000	–	1000	457 ± 2.1	94 ± 3.4	22
A2	1:1 (CAP)	1000	1000	–	498 ± 3.2	92 ± 2.9	22
A3	1:1 (10:90)	1000	100	900	447 ± 2.6	84 ± 2.8	23
A4	1:1 (30:70)	1000	300	700	431 ± 2.9	94 ± 3.2	22
A5	1:1 (50:50)	1000	500	500	422 ± 3.9	90 ± 2.6	23
B1	1:1.5 (EC)	1000	–	1500	493 ± 4.1	95 ± 1.9	24
B2	1:1.5 (CAP)	1000	1500	–	506 ± 3.3	92 ± 2.2	25
B3	1:1.5 (10:90)	1000	150	1350	490 ± 2.7	92 ± 3.3	24
B4	1:1.5 (30:70)	1000	450	1050	476 ± 3.1	90 ± 2.7	22
B5	1:1.5 (50:50)	1000	750	750	464 ± 4.0	85 ± 1.9	26

A1–A5 represent various formulations at 1:1 drug–polymer ratio and B1 to B5 represent various formulations at 1:1.5 level using CAP and EC at different proportion.

[a] $n = 3$.

after coating the microcapsules with gold vapors [14]. The microcapsules were coated uniformly with gold after fixing the samples in individual stubs. Morphological evaluation was carried out at different magnifications. The average particle size was calculated using Malvern particle size analyzer using water as a refractive medium. Measuring the repose angle using funnel method tested flowability of enteric capsules [15,16]. Drug content was confirmed by analysing the drug content in each batch after dissolving the microcapsules in acetone and then extracting the drug from the organic layer using water. Drug content in aqueous layer was determined using HPLC. Drug entrapment efficacy of the process was calculated using the drug content data. Amount of drug entrapped in the microcapsules in each batch was compared with the amount of drug, which was intended to be loaded in order to get the entrapment efficacy of this newly, designed process.

Drug integrity was checked by carrying out the thin layer chromatographic studies and IR spectral analysis for the enteric microcapsules of best in vitro formulation B3, and the results were compared with that of pure drug. Thin layer chromatographic analysis was carried out using benzene:methanol:acetone in the ratio of 7:2:3 as mobile phase and sulphuric acid in methanol as detector in the form of spraying solution [17]. These studies were carried out in order to authenticate the non-interaction of the process parameters with molecular integrity of the drug.

In vitro release studies were conducted by exposing enteric microcapsules to SGF for first 2 h and to SIF for next 6 h and analysed for metamorphosis of structural integrity, which was checked by taking microphotographs.

2.2.5. Pharmacodynamic activities

Gastrointestinal compatibility and pharmacodynamic efficacy of the enteric microcapsules were evaluated by carrying out anti-inflammatory and ulcerogenicity studies, respectively, using animal models.

Animals. Male Wistar rats (BW 150–200 g) were used which were housed in standard plastic cages and given standard laboratory diet and water ad libitum.

2.2.5.1. Carrageenan-induced paw oedema. The rats were divided into four groups containing six rats each. While one group served as control the remaining three groups were administered orally with pure drug suspension, marketed formulation and formulation B3 (containing CAP and EC in the ratio of 10:90 at 1:1.5 drug–polymer ratio) that was selected based on the in vitro release parameters. The first group served as control (received 0.75% CMC; 5 ml/kg). Second group was administered diclofenac sodium (5 mg/kg) as a standard drug. Group 3 was given the marketed formulation and the fourth group was administered the dual coated microcapsules. Oedema was produced by the method described by Winter et al. [18]. The paw volume was measured plethysmographically at 0, 1, 2, 4, 6 and 8 h, after the injection of carrageenan. Drug pretreatment was given 1 h before the injection of carrageenan. Mean increase in paw volume was measured and percentage inhibition was calculated.

2.2.5.2. Ulcerogenicity studies. After 24 h, the animals were sacrificed and the stomach was excised. The stomach was then incised and then examined for ulcer development under stereomicroscope.

2.2.5.2.1. Preparation of tissue and staining. Dehydrating. The water content of the tissue was replaced using increasing concentration of ethanol.

80% alcohol, 1 h; 95% alcohol, 1 h; 100% alcohol, 1 h.

Clearing. The reagent used for clearing must be miscible with dehydrant and paraffin. When dehydrant was removed, the tissue clears and becomes translucent, signifying the completion of process. Chloroform was used as clearing agent.

Impregnation. Complete removal of clearing agent by substitution is done by paraffin wax as it penetrates the tissues. Impregnation was done with 3-paraffin bath for 3 h. Paraffin with melting point of 56–58 °C was used. Precautions were taken so that heating above 58 °C was not done, which might shrink and harden the tissues. The tissue was then cast into blocks of paraffin wax.

Sections of the tissue block 3–5 μm in thickness were cut with the help of rotary microtome.

The section ribbons were made to float on water and then placed on glass slides to remove wrinkles, slightly warmed and dried.

Hydration. The sections were hydrated with xylene for 2 min and 70% alcohol for 2 min. They were rinsed with distilled water.

Staining. Sections were stained with 1% haematoxylin, rinsed in distilled water and then eosin 1% in 90% alcohol was added in 1 min and slides were dried.

The section was then covered with glycerin jelly and cover slip was placed carefully on section, taking care no air bubble could enter.

The slides were observed under the stereomicroscope and photographs taken subsequently.

3. Results and discussion

In the present investigation we have evaluated enteric microcapsules for improved intestinal uptake of diclofenac sodium bypassing the stomach. These microcapsules were prepared by employing wet granulation and thermal change methods simultaneously. The method of preparation of these dual coated microcapsules is based on the simple idea that the mixture of CAP and drug is first converted into uniform granules (to act as core) employing wet granulation method and subsequently EC was coated using thermal change method which results in the formulation of dual coated microcapsules. The technique involves the principle of solvent evaporation. The composition of formulations

were varied and optimized for dual coated microcapsules based on degree of enteric coating.

The introduction of dual coating of EC and CAP in microcapsules allows both sustained release and prevention of enteric release owing to their versatile properties. Surface characteristics of these microcapsules revealed that these microcapsules are almost spherical and devoid of cracks. This method of preparing enteric-coated microcapsules is more advantageously compared to coating on gelatin capsules, which generally suffer from insufficient adhesion between the shell and the coating [19].

Results of measurements of the angle of repose of the microcapsules are summarized in Table 1. The values in the range of 22–26° for all batches revealed that they have good flow properties [15], which is a desired parameter for further processing. The entrapment efficiency of all batches (A1 to B4) except B5 (85%) were above 90% (irrespective of interpolymer ratio and drug–polymer ratio changes) potentially indicates the process efficacy.

Thin layer chromatography rules out the possibility of any drug–polymer interaction and changes in molecular integrity of the drug. Since there was no significant change in Rf values and additionally no secondary spot was observed. This analysis confirmed the absence of any interaction between drug and polymer.

The dissolution profile conducted both in simulated gastric and intestinal fluid has been shown in Fig. 1. The drug release was minimum over 2 h in simulated gastric fluid from the formulations A2, A3, A4, A5, B2, B3, B4 and B5 containing CAP. However, the drug release was dependent on degree of CAP concentration employed. The formulations A1 and B1 containing only EC failed to sustain the drug release and released 25.2 and 23.4%, respectively, in gastric medium. Similarly the batches A2 and B2 formulated, with CAP alone using wet granulation method, showed burst effect and released 54 and 50.4%, respectively, after 1 h in simulated intestinal fluid. However, they did not release the drug in simulated gastric fluid. The batches A3, A4, A5, B4 and B5 containing both CAP and EC not only reduced gastric release but also released 90, 87.75, 87.75, 81 and 82.8% of the drug, respectively, in the simulated intestinal fluid. The formulation B3 released 85.5% of the drug in simulated intestinal fluid in addition to completely retarding the drug release in the gastric medium.

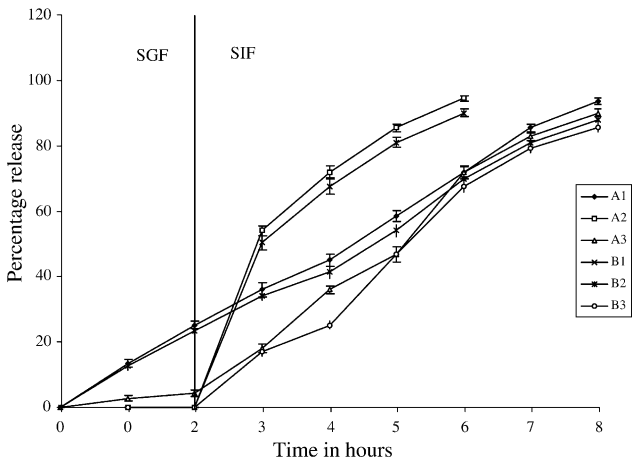

Fig. 1. Comparative in vitro release profile of formulations using simulated gastric fluid for first 2 h and simulated intestinal fluid for next 6 h.

This may be due to clubbing of EC and CAP where surface coating by EC probably prevents the permeation of hydrolytic enzymes, which are predominantly in the small intestine. CAP prevents gastric release which demonstrates that the granulation with CAP provides a system of low permeability at the acidic pH, where protection is required [6].

The in vitro release profile was subjected to graphical treatment using Hixon–Crowell's equation, which further confirm the order and mechanism of drug release (Table 2). The extent of influence of polymer concentration and combinations were studied based on comparative analysis of t_{50} value of different formulations. The t_{50} value was found to be maximum (6.56 h) for B3 formulation containing highest concentration of EC at the drug–polymer ratio of 1:1.5. The presence of CAP, in addition to EC at this ratio in B3 formulation has remarkable capacity to retard gastric release and to sustain the enteric release. Higher content of EC complied with presence of CAP makes the formulation B3 perfectly suitable, since it sustains the enteric release to an expected range and simultaneously shows uniform release in simulated intestinal fluid. Therefore formulation B3 was selected for further in vivo performance.

Fig. 2 represents the control structural texture of dual coated microcapsules. The changes in integrity of these

Table 2
In vitro release kinetic data of enteric microcapsules

Batch code	First order plot (a)	Erosion lot (b)	Release rate (min^{-1})	Dissolution rate constant (K) (Hixson–Crowell equation)	t_{50} (h)
A1	0.9428	0.9738	0.3472	0.0770	4.26
A2	0.9885	0.9848	0.7028	0.1326	2.46
A3	0.9832	0.9730	0.4313	0.7992	5.20
A4	0.9758	0.9497	0.4292	0.0775	4.37
A5	0.9669	0.9326	0.4424	0.0782	4.26
B1	0.9669	0.9326	0.4424	0.0782	4.26
B2	0.9959	0.9841	0.5338	0.1160	2.58
B3	0.9895	0.9750	0.3726	0.0740	6.56
B4	0.9750	0.9501	0.3515	0.0684	5.29
B5	0.9640	0.9306	0.3859	0.0712	4.45

(a) Correlation coefficient of log of cumulative % drug remaining versus time curve. (b) Erosion equation in which $(1 - M_t/M_o)^{1/3}$ is plotted against time (M_t, % drug remaining after time t; M_o, original content of drug ≅ 100).

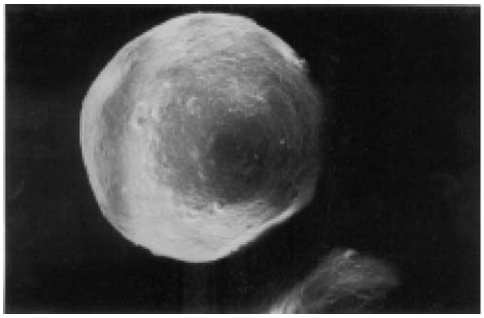

Fig. 2. Structural texture of dual coated microcapsules (magnification, 60 × 2).

Fig. 4. Dual coated microcapsule after 2 h in SIF. The microcapsule developed pores on the surface due to entry of dissolution fluid (60 × 2).

microcapsules were studied under scanning electron microscopy using both SGF and SIF. Degradation pattern of these microcapsules revealed that mechanism of release is erosion. When these microcapsules were kept in SGF for 2 h, the structural integrity of the microcapsules was almost maintained (Fig. 3) which may be ascribed to prevention of gastric release. But when these microcapsules were kept in SIF for 2 h, there was a development of pores (Fig. 4) on the surface of microcapsules due to entry of dissolution fluid, which indicates sustained release of the drug which is in conformity with in vitro release profile. After 6 h, the integrity was almost abolished (Fig. 5). During stay in the SIF, the microcapsules progressively developed pores and tortuous pathways, which may be the reason for the uniform release of the drug.

The pharmacodynamic studies were conducted using carrageenan induced paw oedema method. The carrageenan treated animals showed significant increase in volume compared to the control group ($P < 0.001$) as evident from the pharmacodynamic data (Table 3). There was no observed effect after 1 h when the formulation B3 was administered compared to the marketed formulation (43.5%) and pure drug (48.3%). After 2 h the anti-inflammatory activity (42.6%) was observed in case of our formulation but it was less prominent compared to the marketed formulation (70.5%). Overall the anti-inflammatory activity of marketed formulation and pure drug was maintained only for 2 h, which was found to be subsided by 4 h. But in case of our formulation the anti-inflammatory activity was 77.6% at the end of the 6 h, which was maintained almost up to 8 h (70.5%) significantly. These data clearly shows sustained and uniform release of drug from our optimized formulation.

The biological examination of incised stomach shows that on administration of the formulation B3, no histological alterations in term of mucous surface cells and glands were seen (Fig. 6). On the contrary, when the marketed formulation was administered, the stomach wall showed dilated and congested blood vessels in the submucosa and a mild inflammatory cell infiltration along the base of the gastric glands (Figs. 7 and 8). These changes indicate a mild irritation of the stomach lining in the rats exposed to this drug. These studies clearly indicate that our formulation did not produce any ulcerogenic effect [20], which is supported by SEM studies, where the structural integrity of the sphere was completely organized.

In conclusion, formulation B3 (containing cellulose acetate phthalate and ethyl cellulose in the ratio of 10:90 at 1:1.5 drug–polymer ratio) achieved the targets of

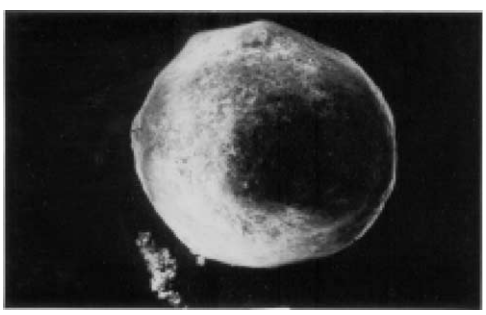

Fig. 3. Dual coated microcapsules after 2 h in SGF. The structural integrity was maintained which may be ascribed to prevention of gastric releases (60 × 2).

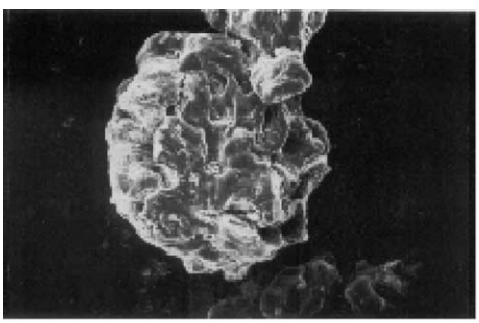

Fig. 5. Dual coated microcapsule after 6 h in SIF. Integrity of the microcapsule was abolished. The capsules developed pores and tortuous pathways, which may be the reason for the uniform release of the drug (60 × 2).

Table 3
Effect of intestinal specific microcapsules of diclofenac sodium on carrageenan-induced paw oedema

Group ($n = 6$)	Oedema volume (ml)					
	Dose	1 h	2 h	4 h	6 h	8 h
Control (1% CMC)	5 ml	0.62 ± 0.076	0.68 ± 0.088	0.74 ± 0.056	0.68 ± 0.022	0.67 ± 0.085
Marketed	Equivalent to 5 mg/kg	0.35 ± 0.062* (43.5)	0.20 ± 0.035* (70.5)	0.51 ± 0.024* (31.9)	0.54 ± 0.050* (20.5)	0.59 ± 0.010* (11.9)
Formulation (optimized formulation B3)	Equivalent to 5 mg/kg	0.62 ± 0.065^{NS} (0.0)	0.39 ± 0.076* (42.6)	0.28 ± 0.011* (62.1)	0.15 ± 0.012* (77.6)	0.20 ± 0.012* (70.5)
Pure (diclofenac sodium)	5 mg/kg	0.32 ± 0.052* (48.3)	0.20 ± 0.081* (70.5)	0.42 ± 0.010* (38.23)	0.50 ± 0.016* (26.4)	0.58 ± 0.092* (12.1)
ANOVA		$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$

ANOVA followed by Dunnet t-test. Each value is the mean ± SEM of six rats (figures in parentheses indicate the % anti-inflammatory activity). *$P < 0.001$; compared to control. NS, statistically not significant. Formulation, the optimized formulation B3 at drug–polymer ratio 1:1.5 using polymers CAP and EC in the ratio of 10:90.

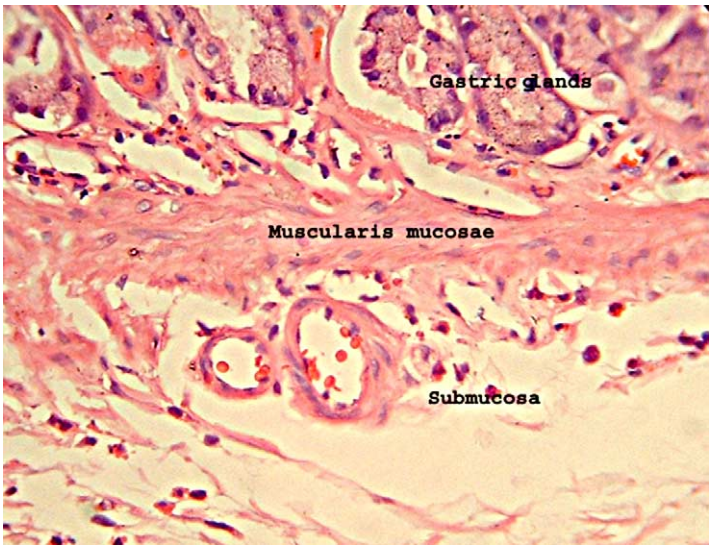

Fig. 6. Section from stomach wall of animal treated with formulation (B3) showing normal submucosal blood vessels and absence of inflammatory cells in lamina propria of mucosa and in the submucous layer (H&E stain, 400 ×).

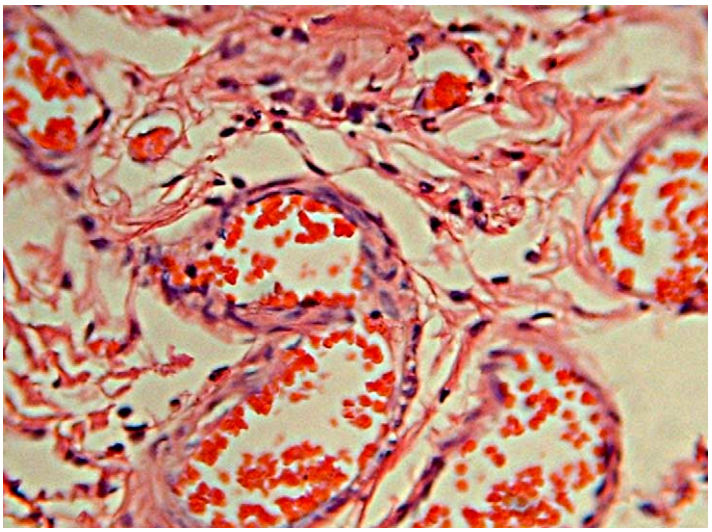

Fig. 7. Section of stomach wall from animal treated with marketed formulation showing dilated and congested blood vessels in submucosa (H&E stain, 400 ×).

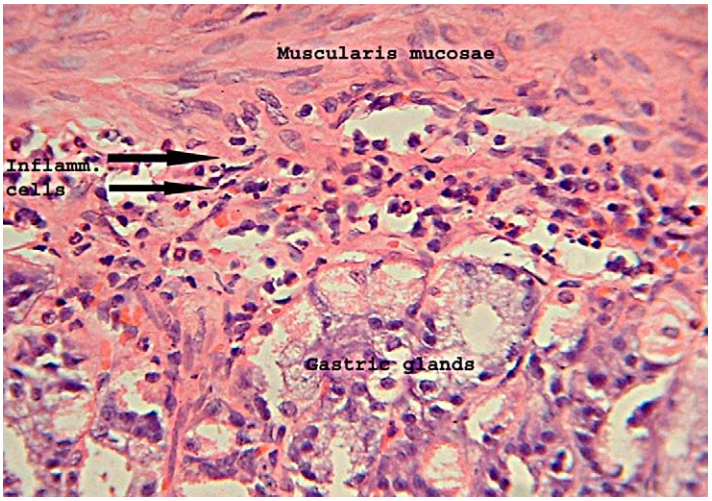

Fig. 8. Another area of the section of stomach wall from animal treated with marketed formulation showing collection of inflammatory cells along the base of the muscularis mucosae (H&E stain, 400 ×).

the present study such as uniform enteric release, improved anti-inflammatory effect and prevention of gastric ulcer and thus improves the patient compliance. These enteric microcapsules prevent gastric release in a substantial way and therefore the technique of dual coating for the preparation of enteric microcapsules is in offing.

Acknowledgements

We thank Dr Ashok Mukherjee for his help in carrying out the histopathology experiments.

References

[1] P.A. Todd, E.M. Sorkic, Diclofenac sodium a reappraisal of its pharmacodynamic and pharmacokinetic purpose and therapeutic efficacy, Drugs 35 (1988) 244–285.
[2] H.P. Armstrong, A.L. Blower, Nonsteroidal anti-inflammatory drugs and life threatening complications of peptic ulceration, Gut 28 (1987) 527–532.
[3] S. Levi, R.A. Goodland, C.Y. Lee, G. Stamp, M.J. Walport, N.A. Wright, H.J.F. Hodgson, Inhibitory effect of non-steroidal anti-inflammatory drugs on mucosal cell proliferation associated with gastric ulcer healing, Lancet 336 (1990) 840–843.
[4] G.J. Tortora, S.R. Grabowski, Principles of Anatomy and Physiology, Eighth ed., 1996, pp. 799–800.
[5] E.A. Hosny, G.M. el Mahrouk, M.W. Gouda, Formulation and in vitro and in vivo availability of diclofenac sodium enteric-coated beads, Drug Dev. Ind. Pharm. 24 (1998) 661–663.
[6] J.I. Perez-Martinez, E. Morillo, C. Maqueda, J.M. Gines, Ethyl cellulose polymer microspheres for controlled release of norfluazon, Pest. Manag. Sci. 57 (2001) 688–694.
[7] G.F. Palmieri, S. Michelini, P. Di Martino, S. Martelli, Polymers with pH-dependant solubility: possibility of use in the formulation of gastroresistant and controlled release matrix tablets, Drug Dev. Ind. Pharm. 26 (2000) 837–845.
[8] J. Singh, D.H. Robinson, Controlled release captopril microcapsules: effect of ethyl cellulose viscosity grade on the in vitro dissolution from microcapsules and tableted microcapsules, J. Microencapsul. 7 (1990) 67–76.
[9] C. Sajeev, G. Vinay, R. Archna, R.N. Saha, Oral controlled release formulation of diclofenac sodium by microencapsulation with ethyl cellulose, J. Microencapsul. 19 (2002) 753–760.
[10] J. Liu, S.Y. Chan, P.C. Ho, Polymer-coated microparticles for the sustained release of nitrofurantoin, J. Pharm. Pharmacol. 54 (2002) 1205–1212.
[11] S.Y. Lin, Y. Kawashima, Drug release from tablets containing cellulose acetate phthalate as an additive or enteric-coating material, Pharm. Res. 4 (1987) 70–74.
[12] J. Klimes, J. Sochor, P. Dolezal, J. Korner, HPLC evaluation of diclofenac in transdermal therapeutic preparations, Int. J. Pharm. 217 (1) (2001) 153–160.
[13] D.R. Krishna, J. Surya Kumar, HPLC determination of diclofenac sodium in human plasma, Ind. J. Pharm. Sci. 53 (1991) 212–213.
[14] A.H. El-Kamal, M.S. Sorkar, S.S. Al Gamal, U.F. Naggar, Preparation and evaluation of ketoprofen floating oral delivery system, Int. J. Pharm. 220 (2001) 13–21.
[15] Martindale, The Extrapharmacopoeia, 28th ed., The Pharmaceutical Press, London, 1982, p. 1071.
[16] R.E. Notary, Biopharmaceutics and Pharmacokinetics. An Introduction, Fourth ed., Marcel Dekkar, New York, 1987, p.145.
[17] P.D. Sethi, D. Charegaonkar, Identification of Drugs in Pharmaceutical Formulations by Thin Layer Chromatography, Second ed., 1999, pp. 116–117.
[18] C.A. Winter, E.A. Risley, G.W. Nuss, Carrageenan induced oedema in hind paw of the rats—an assay for anti-inflammatory drugs, Proc. Soc. Biol. Med. 111 (1962) 544–547.
[19] E.T. Cole, R.A. Scott, A.L. Connor, T. Beckert, D. Cade, Enteric coated HPMC capsules designed to achieve intestinal targeting, Int. J. Pharm. 231 (2002) 84.
[20] R.S. Cotran, V. Kumar, T. Collins, Robbins Pathologic Basis of Disease, Sixth ed., 1999, p. 796.