Eudragit S-100 coated sodium alginate microspheres of naproxen sodium: Formulation, optimization and *in vitro* evaluation

ANUJ CHAWLA
POOJA SHARMA
PRAVIN PAWAR

Chitkara College of Pharmacy
Chandigarh-Patiala
National Highway, Rajpura-140401
Patiala, Punjab, India

The aim of the study was to prepare site specific drug delivery of naproxen sodium using sodium alginate and Eudragit S-100 as a mucoadhesive and pH-sensitive polymer, respectively. Core microspheres of alginate were prepared by a modified emulsification method followed by cross-linking with $CaCl_2$, which was further coated with the pH dependent polymer Eudragit S-100 (2.5 or 5 %) to prevent drug release in the upper gastrointestinal environment. Microspheres were characterized by FT-IR spectroscopy, X-ray diffraction, differential scanning calorimetry and evaluated by scanning electron microscopy, particle size analysis, drug loading efficiency, *in vitro* mucoadhesive time study and *in vitro* drug release study in different simulated gastric fluids. Stability studies of the optimized formulation were carried out for 6 months. SEM images revealed that the surface morphology was rough and smooth for core and coated microspheres, respectively. Core microspheres showed better mucoadhesion compared to coated microspheres when applied to the mucosal surface of freshly excised goat colon. The optimized batch of core microspheres and coated microspheres exhibited 98.42 ± 0.96 and 95.58 ± 0.74 % drug release, respectively. Drug release from all sodium alginate microsphere formulations followed Higuchi kinetics. Moreover, drug release from Eudragit S-100 coated microspheres followed the Korsmeyer-Peppas equation with a Fickian kinetics mechanism. Stability study suggested that the degradation rate constant of microspheres was minimal, indicating 2 years shelf life of the formulation.

Keywords: colon specific, Eudragit S-100, microspheres, naproxen sodium, sodium alginate

Various drug delivery approaches have been explored for successful delivery of drugs to the target site. However, the oral route of administration is considered to be the

---

* Correspondence; e-mail: pkpawar80@yahoo.com

most convenient and preferred route for a sustained as well as controlled drug delivery system (1).

Various drug delivery strategies have been employed to trigger the release of drugs to the large intestine; however, they do not reach the site of action in appropriate concentrations. Thus, to ensure an effective and safe therapy for the large bowel diseases, the colon specific drug delivery system is considered to be the preferable approach (2).

Targeting of drugs to the colon offers several potential therapeutic advantages, like increasing the systemic absorption of poorly absorbed drugs and effective treatment of the colonic diseases such as amoebiasis, ulcerative colitis, Crohn's disease, colorectal cancer, *etc*. This delivery system can be also used in certain conditions where drugs should be delivered after a lag time, like in chronopharmacotherapy of diseases showing Circadian rhythms in their pathophysiology (3). Several available approaches for targeting the drug selectively to the colon include pH sensitive polymers, time dependent dosage forms, use of carriers degraded by enzymes produced by colonic bacteria, prodrug based approaches, bioadhesive and osmotically controlled drug delivery systems (4). Colon specific drug delivery (CDDS) microspheres are advantageous owing to the fact that, in comparison with conventional dosage forms, CDDS microspheres provide a more consistent and reproducible transit through the gastrointestinal tract (GIT). Moreover, they also provide more uniform drug dispersion in the GI tract, which results in more homogeneous drug absorption. This helps in predicting gastric emptying, increases colon residence time, decreases local irritation (5).

The third most common deadly cancer in the world is colorectal cancer. Epidemiological investigations and clinical trials done in patients with hereditary colon cancer and familial adenomatous polyposis (FAP) highlight the importance of nonsteroidal anti-inflammatory drugs (NSAIDs), which act as COX inhibitors and hence, due to their mode of action similar to that of anticancer drugs, halt the development of colon cancerous cell growth (6).

Naproxen [(*S*)-2-(6-methoxynaphthalen-2-yl)propanoic acid], is a non selective COX inhibitor widely used as an analgesic in the treatment of rheumatoid arthritis and colitis. It can be used in FAP and in colon cancers due to its inhibitory action on COX-2 enzymes (7, 8). Moreover, because of the same mode of action, it shows synergistic action with that of anticancer drugs. Sodium alginate is a sodium salt of alginic acid and is a natural polysaccharide derived from brown seaweeds. It is composed of β-D-mannuronic acid and α-L-guluronic acid. It is biocompatible, biodegradable, non-toxic and shows mucoadhesive properties as well. Due to its mucoadhesion nature, its residence time in the colon can be increased, which subsequently results in maximum bioavailability (9). Eudragit belongs to another class of biocompatible polymers. Eudragit S-100 is a pH--sensitive anionic copolymer consisting of methacrylic acid and methacrylate in the ratio 1:2. It does not degrade below pH 7. Eudragit S-100 has been used to prevent drug release from microspheres in the small intestine (10).

Taking the above information into account, a study was designed for the preparation and characterization of naproxen sodium, as a non-selective COX inhibitor, incorporated in sodium alginate and Eudragit S-100 coated microspheres for the colon drug delivery system.

<div align="center">EXPERIMENTAL</div>

Materials

Naproxen sodium and Eudragit S-100 were procured as gift samples from Micro Labs Pvt. Ltd. (Bangalore, India) and Evonik Industries (Mumbai, India), respectively. Sodium alginate and Span 80 were received from Loba Chemie Pvt. Ltd. (Mumbai, India). All polymers and chemicals used were of analytical grade.

Methods

Preparation of Eudragit S-100 coated sodium alginate microspheres involved two steps, *i.e.*, step I and step II. In step I, sodium alginate microspheres were prepared and in step II, optimized formulations from step I were coated with Eudragit S-100 polymer to prevent the release of drug content in the stomach and small intestine. Procedures of step I and step II were as follows (11, 12).

Preparation of sodium alginate microspheres

An emulsification method was used for the preparation of alginate microspheres, followed by cross-linking with calcium chloride (5 %, *m/V*, in IPA). Core microspheres were prepared with different drug polymer ratios (NM1 to NM7), as shown in Table I. A weighed amount of sodium alginate was dissolved in warm distilled water and the drug was then dispersed in this aqueous solution. Subsequently, the dispersion was emulsified in light liquid paraffin containing Span 80 (2 %, *V/V*) with the help of a me-

Table I. Composition, particle size and drug loading efficiency of naproxen sodium microspheres[a]

Formulation code	Drug/polymer ratio	Particle size (μm)	Drug loading efficiency (%)
NM1	1 : 2	314.68	72.3 ± 1.07
NM2	1 : 3	317.09	75.9 ± 1.51[b]
NM3	1 : 4	426.32	76.6 ± 1.02[b]
NM4	1 : 5	444.08	78.1 ± 1.30[c]
NM5	1 : 6	450.41	87.6 ± 1.22[c]
NM6	1 : 7	478.65	74.9 ± 2.38
NM7	1 : 8	484.35	76.2 ± 1.35[b]
Coated formulations	core : coat ratio	–	–
NM8	1 : 2.5	454.26	92.4 ± 1.16[c]
NM9	1 : 5	459.28	86.8 ± 1.07[c]

[a] Data are expressed as mean ± SD (*n* = 3).
[b] Statistically significant difference at $p < 0.05$.
[c] Statistically significant difference at $p < 0.001$ from control (NM 1) as determined by one-way ANOVA, followed by Dunnett's test.

chanical stirrer (propeller type) (Remi Instrument Ltd, Mumbai, India) at 400 rpm for 1 h. A solution of calcium chloride (5 %, *m/V* in IPA) was added dropwise to the emulsion at a rate of 1 mL min^{-1} to harden the formed microspheres and stirring was continued for another 20 min to ensure efficient cross-linking. Microspheres were collected by filtration and washed three times with petroleum ether to remove the residual liquid paraffin. Microspheres were frozen for 10 h and then kept in vacuum desiccators for 12 h.

Preparation of Eudragit S-100 coated microspheres

The optimized batch (NM5) of sodium alginate microspheres was coated with two different concentrations of Eudragit S-100, as shown in Table I. Core microspheres were dispersed in an Eudragit S-100 solution (5 %, *m/V*) in acetone and isopropyl alcohol solution at room temperature, followed by emulsification in light liquid paraffin containing Span 80 (2 %, *V/V*) in a beaker with the help of a mechanical stirrer (propeller type) at 400 rpm. The system was agitated for 3 h at room temperature to allow solvent evaporation. Finally, encapsulated microspheres (NM8, NM9) were filtered and washed with petroleum ether to remove the traces of oil and dried in a vacuum desiccator for 24 h.

Characterization of naproxen sodium microspheres

FT-IR spectroscopy. – The FT-IR spectra of pure drug (naproxen sodium), sodium alginate, Eudragit S-100 and coated microsphere were recorded with an FTIR spectrophotometer (Mode spectrum RX 1, Perkin Elmer, England) using the potassium bromide disk method, in the range of 4000–400 cm^{-1}.

X-ray diffraction analysis (XRD). – XRD analysis investigates the effect of microencapsulation on the crystallinity of the drug as well as drug-loaded microspheres. X-ray diffractograms of naproxen sodium, sodium alginate, Eudragit S-100, physical mixture (naproxen sodium and sodium alginate), core microspheres, physical mixture (naproxen sodium, sodium alginate and Eudragit S-100) and coated microsphere were recorded with an X-ray diffractometer (XPERT-PRO, PAN analytical, The Netherlands) using the PRS measurement program using Ni-filtered, CuKα radiation with a voltage of 45 kV and a current of 40 mA. The instrument was operated at continuous scanning speed over 2θ range of 5 to 49°.

Differential scanning calorimetry (DSC). – Thermal analyses of naproxen sodium, sodium alginate, Eudragit S-100, mixture of drug and sodium alginate, core microspheres, physical mixture (naproxen sodium and sodium alginate and Eudragit S-100) and coated microspheres were performed using a differential scanning calorimeter (Shimadzu, DSC 60, Japan) to study the thermal behaviour of samples. All samples were heated in hermetically sealed aluminium pans at a constant scanning rate of 10 °C min^{-1} from 40 to 260 °C under air atmosphere (50 mL min^{-1}) by applying the minimum possible pressure. An empty aluminium pan was used as reference.

Surface morphology. – Shape and surface morphology of both core and coated microspheres were observed using scanning electron microscopy (JSM 6100 Jeol, Japan). Samples mounted on an aluminium stub were sputter coated with gold under reduced pressure and a thick gold coat was applied using a JFC 1100 (Japan) sputter coater. The

sample assembly was placed in the microscope and vacuum was applied. The microspheres were observed under SEM.

Particle size analysis. – The particle size distribution of core as well as coated microspheres was determined. Freeze-dried microspheres were dispersed in 20 mL isopropyl alcohol and sonicated for 5 min to bring about disaggregation of the microspheres. Microspheres were sized using a particle size analyser (Malvern Instruments, Mastersizer 2000, UK).

Drug loading and drug loading efficiency. – To determine the drug content in microspheres, an accurately weighed quantity of microspheres equivalent to 20 mg of the drug was crushed and dissolved in 100 mL phosphate buffer pH 7.4 in a volumetric flask and stirred for 12 h (13, 14). After stirring, the solution was filtered through Whatman filter paper, the filtrate was diluted using phosphate buffer pH 7.4 and absorbance was measured for the determination of unentrapped drug at 272 nm using a UV/Visible spectrophotometer (Systronics, Mumbai, India). Observations were taken in triplicate to calculate drug loading and drug loading efficiency.

In vitro *drug release study*

Core microspheres. – Microspheres equivalent to 2 mg of naproxen sodium were weighed accurately and suspended in 20 mL of phosphate buffer pH 7.4 containing 1 % (*m/V*) sodium dodecyl sulphate (SDS) to maintain the sink condition for the drug. The mixture was stirred at 37 °C using a magnetic stirrer at a stirring speed of 50 rpm for 3 h. At specified time intervals, samples were withdrawn (2 mL) and replaced with the same volume of fresh media. The withdrawn samples were centrifuged at 3000 rpm for 10 min and were then filtered and diluted with phosphate buffer pH 7.4. The drug content was measured by taking supernatant absorbance using a UV/Visible spectrophotometer (Systronics, Mumbai, India).

Coated microspheres. – Microspheres equivalent to 2 mg of naproxen sodium were weighed accurately and suspended in 20 mL of 0.01 mol L^{-1} HCl. The mixture was stirred on a magnetic stirrer at 37 °C at a stirring speed of 50 rpm for 2 h. Samples were withdrawn at specified intervals and an equivalent amount of fresh medium was added. Collected samples were centrifuged, filtered through a membrane filter (0.45 μm) and analysed for drug content using a UV/Visible spectrophotometer.

The coated microspheres equivalent to 2 mg of the drug were placed in 20 mL of phosphate buffer pH 6.8 containing 1 % (*m/V*) SDS (to maintain the sink condition) and stirred magnetically at 50 rpm. The pH of the medium was maintained at 6.8 for 2 h and then it was slowly increased to 7.4 by the addition of disodium hydrogen phosphate. Two millilitres of aliquots was withdrawn at predetermined intervals with replacement of the same volume of fresh medium. The samples were centrifuged, filtered and analysed for drug content at 272 nm using spectrophotometry.

Drug release kinetics. – The *in vitro* drug release patterns were fitted to various release kinetic models, zero order, first order, Higuchi model and Korsmeyer-Peppas power law equation.

Statistical analysis

The *in vitro* drug release profiles from the microspheres were compared by statistical analysis using one-way ANOVA, followed by Dunnett's test. A difference was considered statistically significant at a *p*-value less than 0.05.

In vitro *mucoadhesion study*

The *in vitro* mucoadhesion study of the microspheres was carried out using the *in vitro* wash-off test reported by Lehr *et al.* (15). Proximal portion of a freshly slaughtered goat's large intestine was cut to expose the mucosal surface and washed with distilled water and phosphate buffer pH 7.4. A 2×2 cm serosal side was attached *via* a thread onto a glass slide. Coated microspheres (5 mg) were spread over the exposed mucosal surface and rinsed with phosphate buffer pH 7.4. The assembly was then kept in a humidity chamber (Thermotech, India, Model TH-7004) at 37 °C/90 % RH for 30 min. In the above pretreatment, Eudragit S-100 coat got dissolved, exposing sodium alginate core microspheres. Mucoadhesiveness of the microspheres was measured by mounting the complete assembly onto a disintegration apparatus (EI Products, Panchkula, India) with the help of a clamp and a thread. The apparatus was operated in such a manner that the tissue was allowed to move in reciprocating motion at a frequency of 28–32 cycles per minute while immersed in phosphate buffer pH 7.4 contained in a 1000 mL beaker. The time taken by the tissue to completely wash off the microsphere was considered the mucoadhesion time.

Stability studies

Three different batches of all formulations were subjected to stability studies according to the International Conference of Harmonization (ICH) guidelines. Uncoated and coated microspheres were put ino hard gelatin capsules wrapped in aluminium foil laminated on the inside with polyethylene. The samples were kept at room temperature and under accelerated conditions in a stability chamber (Stability Oven, Nirmal Instruments, India). Real time stability studies were performed by periodical testing of the drug content at intervals of 0, 30, 90 and 180 days during 6 months.

RESULTS AND DISCUSSION

FT-IR spectra of naproxen sodium, sodium alginate, Eudragit S-100, and coated microspheres are depicted in Fig. 1. The drug sample showed characteristic functional group peaks at 1252 (acid C-O), 1583 (COO$^-$), 1631 (C-C aromatic), 2840 cm^{-1} (C-H aliphatic). Coated microspheres showed characteristic peaks at 1607 (sodium alginate), 3504 and 2953 (naproxen sodium), 1606 (sodium alginate), 1731 (Eudragit S-100), 1586 and 1244 cm^{-1} (naproxen sodium). Finally, the FT-IR study concluded that major characteristic peaks of naproxen sodium were found in entire coated microspheres, which confirms the presence of the drug in the polymer without any interaction.
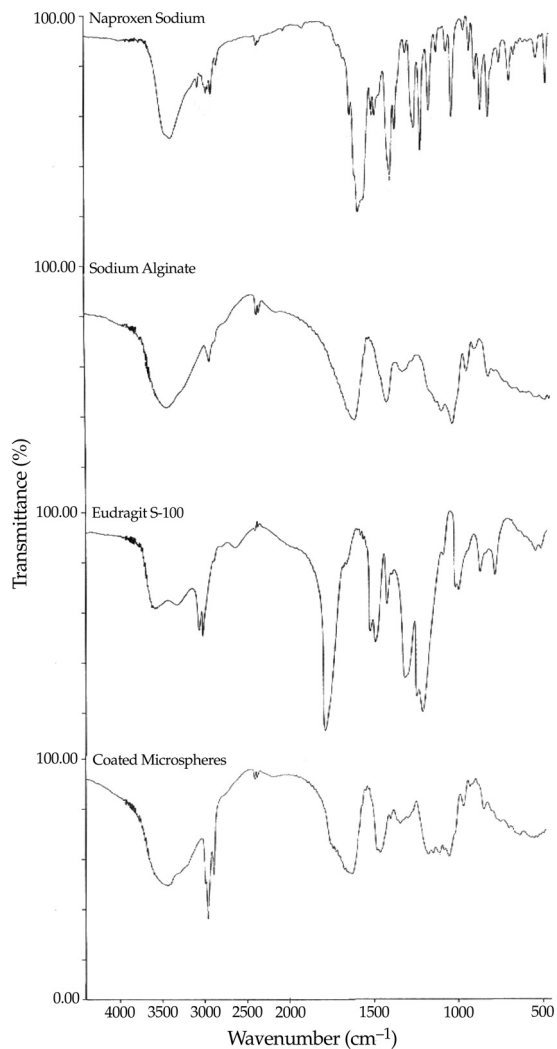
Fig. 1. Fourier transform infrared spectra of naproxen sodium, sodium alginate, Eudragit S-100, and coated microspheres (NM9).

The XRD study suggested that the X-ray diffractogram (Fig. 2) of naproxen sodium indicated the presence of crystalline material with sharp principle peaks at 13.50 and 17.45°, whereas both polymers, sodium alginate and Eudragit S-100, were found to be amorphous in nature. The X-ray diffractogram of core and coated microspheres of naproxen sodium showed an amorphous material devoid of any crystallinity due to the dilution effect of the amorphous polymers.
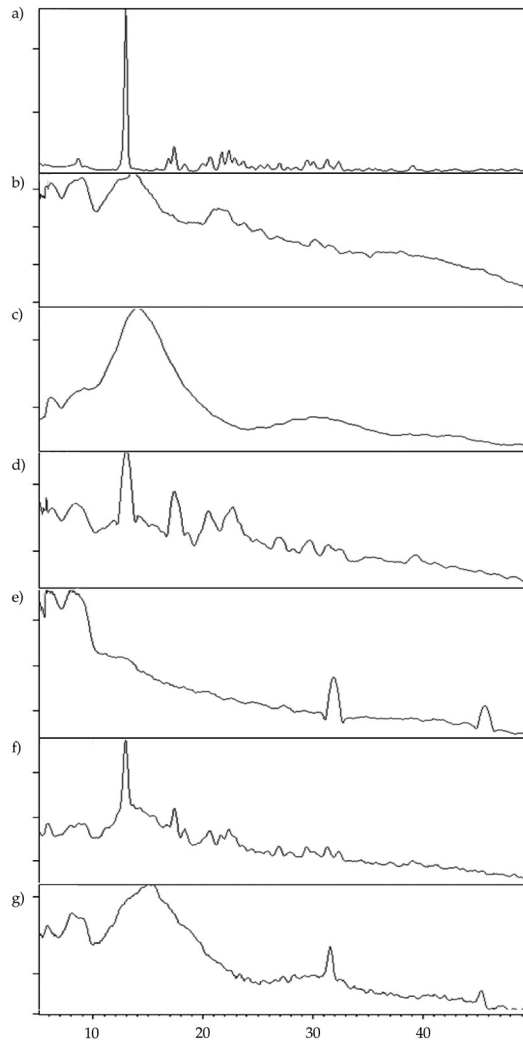
535

Fig. 2. X-ray diffraction spectra of: a) naproxen sodium, b) sodium alginate powder, c) Eudragit S-100, d) physical mixture of drug and sodium alginate, e) core microspheres, f) physical mixture (drug, sodium alginate and Eudragit S-100) and g) coated microsphere.

According to the differential scanning calorimetry thermograms (Fig. 3), naproxen sodium showed a sharp endothermic peak at 257.50 °C corresponding to its melting point in the crystalline form. DSC scans of sodium alginate under air depict a wide endothermic peak at 100.00 °C, which was attributed to water evaporation. A maximum exothermic peak was shown at 263.66 °C (16, 17). Eudragit S-100 exhibited two endothermic peaks at 82.51 and 230.01 °C. DSC scan of the uncoated microsphere of sodium

alginate showed a sharp endothermic peak around 252.00 °C and a broad exothermic peak at 119.00 °C. In the physical mixture, the presence of Eudragit S-100, interaction between Eudragit S-100 and sodium alginate (disappearance of sodium alginate exothermic peak at 283.00 °C) can be proposed. The endothermic peak of naproxen sodium at 257.00 shifted to 249.00 °C in the physical mixture with Eudragit S-100 and peak intensity was also reduced due to its low percentage in the physical mixture (2 %, *m/m*). However, thermograms of coated microspheres suggest that a depressed, broad endothermic peak at 93.00 °C could be a contribution of the dilution effect of amorphous polymer.
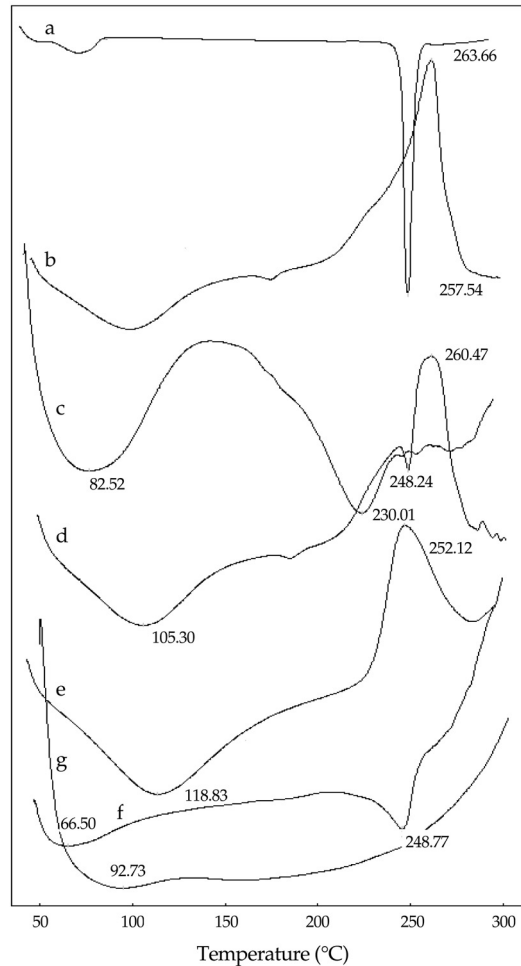

Fig. 3. DSC thermograms of: a) naproxen sodium, b) sodium alginate, c) Eudragit S100, d) physical mixture (drug and sodium alginate), e) core microspheres, f) physical mixture (drug, sodium alginate and Eudragit S-100) and g) coated microspheres.
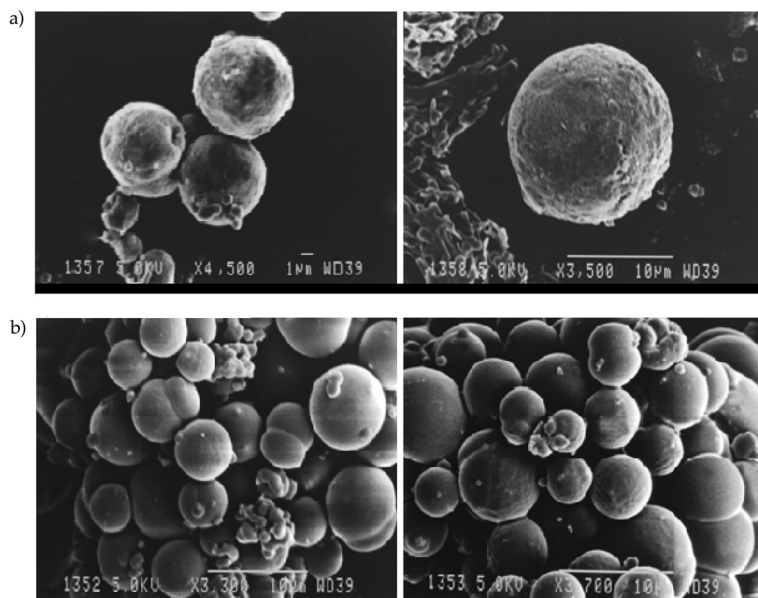
Fig. 4. SEM micrographs of a) core microspheres of formulation NM3 and b) Eudragit S-100 coated microspheres of batch NM8.

On the basis of scanning electron microscopy (Fig. 4) of core microspheres, it was concluded that the microspheres formed were spherical in shape for both core and coated microspheres due to surface attached crystals of the drug. In the case of core microspheres, they were discrete, having a rough surface with an increased number of pores causing rapid release of the drug in the medium. On the other hand, SEM images of coated naproxen sodium microspheres showed them to have a smooth surface and a smaller number of pores due to coating, which led to a decrease in the drug release rate from microspheres, as shown in Fig. 6.

The effect of polymer concentration on the particle size of microspheres was studied and is given in Table I. As the concentration of polymer increases in core microspheres, it leads to an increase in viscosity and thus to an increase in the emulsion droplet size, and finally to higher microsphere size. In the case of coated microspheres, particle size again increases with increased concentration (Eudragit S-100) and the coating thickness over the microspheres increases, which results in larger size.

The drug loading efficiency values for all formulations are given in Table I. The percentage drug entrapment was found to be in the range from 75.95 to 87.65 % for sodium alginate microspheres (core microspheres). The highest drug loading efficiency was found to be 87.6 % for NM5 at the drug/polymer ratio 1:6. ANOVA results reveal that a significant ($p < 0.01$, Table 1) effect on drug entrapment efficiency (%) of core microspheres was observed at varying polymer concentrations, except for formulation NM6. However, a significant effect ($p < 0.001$) on entrapment efficiency of coated microspheres (NM8 and NM9) in comparison with core microspheres (NM1) was also shown. The re-
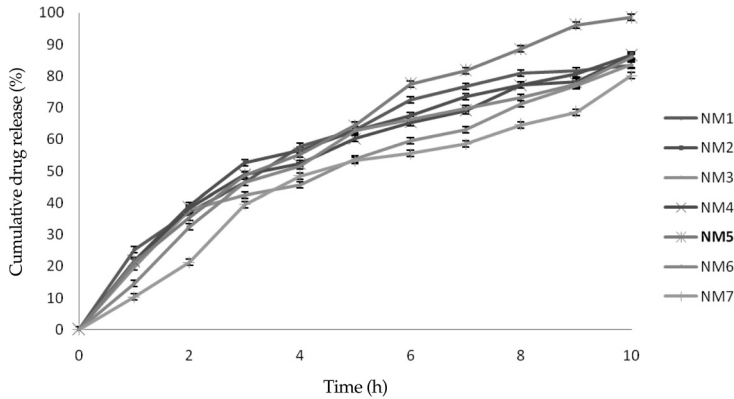
Fig. 5. *In vitro* release profile of sodium alginate core microspheres. Data are expressed as mean ± SD (*n* = 3).

sults revealed that as the concentration of polymer increased, the drug loading efficiency also increased, except for formulations NM6 and NM7. This decrease in drug loading efficiency effect may be due to the contribution of the saturation concentration effect of the polymer at a certain level, which resulted in reduced drug incorporating efficiency in the microspheres (18).

On the other hand, coated microspheres showed drug loading efficiency of 92.4 and 86.8 % for formulations NM8 and NM9, respectively. The drug loading efficiency of coated microspheres was higher compared to core microspheres, which may be attributed to the finding that due to the coating on microspheres, the integrity of core microspheres was retained, which caused less leaching of the drug from core microspheres and led to higher drug loading efficiency (12).
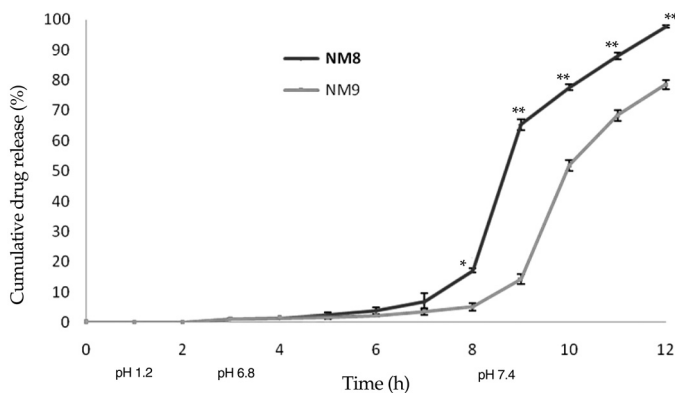

Fig. 6. *In vitro* release profile of Eudragit S-100 coated microspheres in different media. Data are expressed as mean ± SD (*n* = 3). * Statistically significant difference at $p < 0.001$. ** Statistically significant difference at $p < 0.0001$ from NM9 as determined by Student's *t*-test.

The *in vitro* release study of naproxen sodium tablets in different dissolution media, *i.e.*, pH 1.2, phosphate buffer, pH 6.8 and phosphate buffer pH 7.4, revealed that drug release from the tablet was observed faster in pH 1.2, followed by pH 6.8 and pH 7.4. The naproxen sodium tablet showed total release within 7 h. However, the release from sodium alginate microspheres was slow and constant compared to naproxen sodium tablets. Optimized formulation of sodium alginate microspheres showed maximal drug release of 98.42 % in 10 h. It also resulted in lower release in pH 1.2 and pH 6.8 compared to the naproxen sodium tablet. Therefore, these findings suggest that sodium alginate showed complete drug release within 10 h, which is insufficient for targeting the drug to the colon. Hence, this indicates that there is a need for enteric coating, *i.e.*, Eudragit S-100 coating, on sodium alginate microspheres to target drug release to the colon.

The *in vitro* drug release profile of different core microsphere formulations containing different drug/sodium alginate ratios is shown in Fig. 5. With an increase in polymer concentration, the drug release rate of microspheres was found to decrease, which might be due to the formation of a rigid polymer matrix at higher polymer concentration in the microspheres. This result may be due to the increase in sodium alginate concentration leading to the formation of relatively strong walls of microspheres, and thereby retardation of drug release. Further, the increase in polymer chain entanglement and subsequent low dissolution of the polymer might be responsible for the retardation of drug release from the microspheres (19). Core microsphere formulation NM5 showed maximal drug release at pH 7.4 (98.42 %) compared to other core microsphere formulations. On the basis of drug release, NM5 (drug/polymer ratio 1:6) was selected for the preparation of Eudragit S-100 coated microspheres.

The *in vitro* release study (Fig. 6) of Eudragit S-100 coated microspheres revealed that drug release could be successfully retarded in an acidic environment, *i.e.*, pH 1.2. Drug release at pH 6.8 was found to be negligible due to Eudragit S-100 (as enteric copolymer methacrylic acid-methyl methacrylate) soluble at pH > 7. In release medium pH 7.4, the NM9 microsphere showed about 4 % drug release over a period of 2 h compared to 7 % drug release of NM8. The amount of coating in NM9 was larger, resulting in more time to dissolve and release the drug from the microsphere. Formulation NM8 showed a statistically significant ($p < 0.001$) difference in drug release characteristics compared to formulation NM9, as determined by Student's *t*-test. However, formulation NM8 having a lower concentration of Eudragit S-100 coating (2.5 %, *m/V*) showed higher drug release, *i.e.*, 95.6 % within a period of 5 h at pH 7.4, whereas formulation NM9 showed lower drug release (89.5 %) over 5 h at pH 7.4 due to thicker coating. It was observed in a previous study that the *in vitro* drug release of naproxen coated with Eudragit L-100 showed approximately 90 % drug release, which is lower compared to the present study (20).

The percentage drug release profiles obtained from *in vitro* release experiments were subjected to different kinetics models to find the drug release mechanism and kinetics. The zero order (cumulative amount of drug release *vs*. time), first order (log cumulative percentage of drug remaining *vs*. time) (21), and Higuchi kinetics (cumulative percentage of drug release *vs*. square root of time) (22) were used as the kinetics model. Also, the Korsmeyer-Peppas equation (log cumulative percentage of drug released *vs*. log time) (23) was used for the first 60 % of drug released from the microsphere. Regression coefficients (R^2) for all microsphere formulations using different kinetics equations are
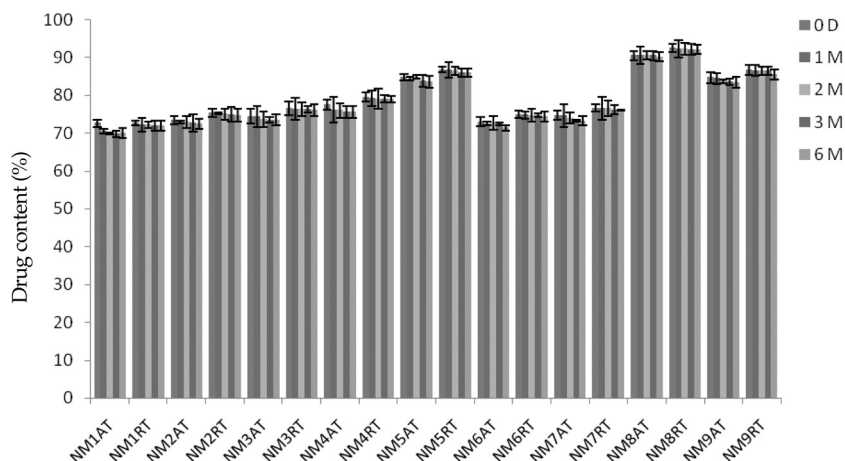
Fig. 7. Stability of naproxen sodium microspheres under accelerated conditions and at room temperature (mean ± SD, n = 3). AT – accelerated temperature (40 °C), RT – room temperature, D – days, M – months.

listed in Table II. The table data reveal that *in vitro* release from the core microsphere (sodium alginate microsphere) was better explained by the Higuchi equation, since the plots provide the highest linearity. For all sodium alginate microspheres, the n value as per Korsmeyer-Peppas model was found to be between 0.45 and 0.89, indicating anomalous release behaviour of the drug, *i.e.*, both diffusion and dissolution of the hydrated polymer matrix might be responsible for drug release from the microsphere (24, 25). Coated microspheres followed Fickian kinetics with the value n < 0.45 as per the Korsmeyer-Peppas model. This could be due to relaxation of the polymer matrix, followed by the diffusion matrix.

Determinations of *in vitro* mucoadhesion suggest that complete detachment of the microsphere from mucosal tissue took 90 min, indicating good mucoadhesive properties of sodium alginate. To provide better mucoadhesion, the polymer should show the presence of a strong hydrogen bonding group like –OH, –COOH and strong anionic charges spreading on the mucus (26).

Sodium alginate is in the first-generation mucoadhesive polymer group, which contains higher hydrogen bond forming polymers (27). The presence of a large number of hydroxyl groups, which form a complex of hydrogen bonds with the hydroxyl group present in mucin, will result in mucoadhesion. However, it has been already reported that the time required to completely wash off the NSAID agents like valdecoxib containing sodium alginate microspheres is 95 min compared to similarly prepared microspheres of naproxen sodium containing alginate (90 min).

All the formulations under storage were analysed for drug content and no significant differences in drug content were observed. All the formulations showed less than 90 % drug content, except formulation NM8 which showed 92.18 % drug content under both accelerated and room temperature storage conditions (Fig. 7). The degrada-

Table II. Comparison of different dissolution kinetics models

Formulation code	Zero-order	First-order	Higuchi	Korsmeyer-Peppas	
	R^2	R^2	R^2	R^2	N
NM 1	0.892	0.986	0.990	0.979	0.50
NM 2	0.896	0.986	0.990	0.976	0.71
NM 3	0.950	0.884	0.988	0.978	0.56
NM 4	0.916	0.976	0.993	0.970	0.67
NM 5	0.951	0.956	0.987	0.984	0.54
NM 6	0.919	0.977	0.980	0.975	0.89
NM 7	0.934	0.957	0.959	0.951	0.92
NM 8	0.949	0.930	0.949	0.958	0.36
NM 9	0.954	0.955	0.956	0.953	0.39

tion rate constants (k_{cal}) and shelf life (t_{90}) of NM8 at room temperature were 2.46 day^{-1} and 795.96 days, respectively, which indicates that formulation NM8 has a shelf life of more than 2 years.

CONCLUSIONS

Data obtained from this study demonstrate that sodium alginate microspheres containing naproxen sodium were successfully prepared, followed by coating with the pH--sensitive polymer Eudragit S-100. The study also concluded that naproxen sodium tablets showed total release in 7 h in comparison with sodium alginate microspheres (core microspheres) that showed complete drug release within 10 h. This suggests that both naproxen sodium tables and sodium alginate microspheres were unable to target drug release into the colonic region. Hence, this indicates that there is a need for enteric coating, *i.e.*, Eudragit S100 coating, on sodium alginate microspheres to target drug release to the colon. Coated microspheres showed a longer residence time in the colon after removing the Eudragit S100 coating due to better mucoadhesion properties of sodium alginate. The *in vitro* release profile revealed that microspheres retard drug release in the upper part of GIT due to the pH-sensitive polymer coating. Hence, Eudragit S-100 showed promising drug delivery to the colon.

REFERENCES

1. P. R. Veerareddy and R. P. Manthri, Formulation and evaluation of compression coated piroxicam tablets for colon specific drug delivery, *Acta Pharm. Sci.* **52** (2010) 281–294.

2. G. V. Mooter, Colon drug delivery, *Exp. Opin. Drug Deliv.* **3** (2006) 111–125; DOI: 10.1517/17425 247.3.1.111.

3. K. V. V. Kumar, T. Sivakumar and T. T. Mani, Colon targeting drug delivery system: A review on recent approaches, *Int. J. Pharm. Biomed. Sci.* **2** (2011) 11–19.

4. M. K. Chourasia and S. K. Jain, Pharmaceutical approaches to colon targeted drug delivery systems, *J. Pharm. Pharm. Sci.* **6** (2003) 33–66.

5. S. Jose, M. T. Prema A. J. Chacko, A. C. Thomas and E. B. Souto, Colon specific chitosan microsphere for chronotherapy of chronic stable angina, *Colloids Surf. B Biointerfaces* **83** (2011) 277–283; DOI: 10.1016/j.colsurfb.2010.11.033.

6. P. Ricchi, R. Zarrilli, A. Palma and A. Acquaviva, Nonsteroidal anti-inflammatory drugs in colorectal cancer: from prevention to therapy, *Br. J. Cancer* **88** (2003) 803–807; DOI: 10.1038/sj.bjc. 6600829

7. M. J. Thun, J. Henley and C. Patrono, Nonsteroidal anti-inflammatory drugs as anticancer agents: mechanistic, pharmacologic, and clinical issues, *J. Natl. Cancer Inst.* **94** (2002) 252–266; DOI: 10.1093/jnci/94.4.252.

8. H. Berkel, R. F. Holcombe, M. Middlebrooks and K. Kannan, Nonsteroidal antiinflammatory drugs and colorectal cancer, *Epidemiol. Rev.* **18** (1996) 205–217.

9. M. George and T. E. Abraham, Polyionic hydrocolloids for the intestinal delivery of protein drugs: alginate and chitosan-a review, *J. Control. Release* **114** (2006) 1–14; DOI: org/10.1016/j. jconrel.2006.04.017.

10. W. M Obeidat and J. C Price, Preparation and evaluation of Eudragit S 100 microspheres as pH-sensitive release preparations for piroxicam and theophylline using the emulsion-solvent evaporation method, *J. Microencapsul.* **23** (2006) 195–202; DOI: 10.1080/02652040500435337.

11. N. K. Thakral, A. R. Ray, D. B. Shalom, A. H. Eriksson and D. K. Majumdar, The quest for targeted delivery in colon cancer: mucoadhesive valdecoxib microspheres, *Int. J. Nanomed.* **6** (2011) 1057–1068; DOI: 10.2147/IJN.S19561.

12. Z. Rahman, K. Kohli, R. K. Khar, M. Ali, N. A. Charoo and A. A. A. Shamsher, Characterization of 5-fluorouracil microsphere for colonic delivery, *AAPS PharmSciTech.* **7** (2006) E1-9; DOI: 10. 1208/pt070247.

13. K. Ganguly, T. M. Aminabhavi and A. R. Kulkarni, Colon targeting of 5-fluorouracil using polyethylene glycol cross-linked chitosan microspheres enteric coated with cellulose acetate phthalate, *Ind. Eng. Chem. Res.* **50** (2011) 11797–11807; DOI: 10.1021/ie201623d.

14. S. K. Umadevi, R. Thiruganesha, S. Suresha and K. B. Reddya, Formulation and evaluation of chitosan microspheres of aceclofenac for colon-targeted drug delivery, *Biopharm. Drug Dispos.* **31** (2010) 407–427; DOI: 10.1002/bdd.722.

15. C. M. Lehr, J. M. Bowstra, J. J. Tukker and H. E. Junginger, Intestinal transit of bioadhesive microspheres in an in situ loop in the rat, *J. Control. Release* **13** (1990) 51–62; DOI: org/10.1016/ 0168-3659(90)90074-4.

16. J. P. Soarse, J. E. Santos, J. O. Chierice and E. T. G. Cavalheiro, Thermal behavior of alginic acid and its sodium salt, *Ecl. Quím. Sao Paulo* **29** (2004) 53–56.

17. M. S. Crcarevska, M. G. Dodov and K. Goracinova, Chitosan coated Ca–alginate microparticles loaded with budesonide for delivery to the inflamed colonic mucosa, *Eur. J. Pharm. Biopharm.* **68** (2008) 565–578; DOI: org/10.1016/j.ejpb.2007.06.007.

18. A. Vaidya, A. Jain, P. Khare, R. K. Agrawal and S. K. Jain, Metronidazole loaded pectin microspheres for colon targeting, *J. Pharm. Sci.* **98** (2009) 4229–4236; DOI: 10.1002/jps.21742.

19. P. Kumar and I. Singh, Formulation and characterization of tramadol loaded IPN microgels of alginate and gelatin: optimization using response surface methodology, *Acta Pharm.* **60** (2010) 295–310; DOI: 10.2478/v10007-010-0021-z.

20. M. Maghsoodi, Physicomechanical properties of naproxen-loaded microparticles prepared from eudragit L100, *AAPS PharmSciTech.* **10** (2009) 120–128; DOI: 10.1208/s12249-009-9186-5.

21. J. G. Wagner, Interpretation of percent dissolved-time plots derived from *in vitro* testing of conventional tablets and capsules, *J. Pharm. Sci.* **58** (1969) 1253–1257; DOI: 10.1002/jps.2600581021.

22. T. Higuchi, Mechanism of sustained-action medication; Theoretical analysis of rate of release of solid drugs dispersed in solid matrices, *J. Pharm. Sci.* **52** (1963) 1145–1149; DOI: 10.1002/jps.2600521210.

23. P. Costa and J. M. S. Lobo, Modeling and comparison of dissolution profiles, *Eur. J. Pharm. Biopharm.* **13** (2001) 123–133.

24. N. A. Peppas, Analysis of fickian and non-fickian drug release from polymers, *Pharm. Acta Helv.* **60** (1985) 110–111.

25. H. R. Chueh, H. Zia and C. P. Rhodes, Optimization of sotalol floating and bioadhesive extended release tablet formulations, *Drug Dev. Ind. Pharm.* **21** (1995) 1725–1747; DOI: 10.3109/03639049509069261.

26. N. A. Peppas and P. A. Burim, Surface, interfacial and molecular aspects of polymer bioadhesion on soft tissues, *J. Control. Release* **2** (1985) 257–275; DOI: 10.1016/0168-3659(85)90050-1.

27. J. D. Smart, I. W Kellaway and H. E. C. Worthington, An in-vitro investigation of mucosa-adhesive materials for use in controlled drug delivery, *J. Pharm. Pharmacol.* **36** (1984) 295–299; DOI: 10.1111/j.2042-7158.1984.tb04377.

S A Ž E T A K

Alginatne mikrosfere naproksen natrija obložene Eudragitom S-100: Priprava, optimizacija i *in vitro* vrednovanje

ANUJ CHAWLA, POOJA SHARMA i PRAVIN PAWAR

Cilj istraživanja bila je ciljana isporuka naproksen natrija koristeći natrijev alginat i Eudragit S-100 kao mukoadhezivne, odnosno pH-osjetljive polimere. Jezgra mikrosfera od alginata pripravljena je modificiranom metodom emulgiranja te umrežavanjem pomoću otopine CaCl$_2$. Sljedeći korak u pripravi mikrosfera bilo je oblaganje s pH osjetljivim polimerom Eudragit S-100 (2,5 ili 5 %) čime je spriječeno oslobađanje lijeka u gornjem dijelu gastrointestinalnog trakta. Mikrosfere su okarakterizirane FT-IR spektroskopijom, difrakcijom rendgenskih zraka, diferencijalnom pretražnom kalorimetrijom i pretražnom elektronskom mikroskopijom. Nadalje, analizirana je veličina čestica, količina uklopljenog lijeka, mukoadhezivna svojstva *in vitro* te oslobađanje lijeka *in vitro* u različitim simuliranim gastričnim fluidima. Testovi stabilnosti optimiziranih pripravaka praćeni su tijekom 6 mjeseci prema smjernicama ICH. SEM snimke otkrile su da je površina jezgre mikročestica neravna, dok je površina obloženih mikrosfera glatka. Jezgre mikrosfera imale su jače izražena mukoadhezivna svojstva nego obložene mikrosfere na testovima prove-

denim na svježe izrezanim dijelovima debelog crijeva koze. Iz optimiziranih neobloženih i obloženih mikrosfera oslobađa se 98.42 ± 0.96, odnosno 95.58 ± 0.74 % lijeka. Oslobađanje lijeka iz svih formulacija slijedilo je kinetiku po Higuchiju. Oslobađanje iz obloženih mikrosfera slijedilo je Korsmeyer-Peppasovu jednadžbu i kinetiku po Ficku. Studije stabilnosti pokazale su minimalnu razgradnju, te prihvatljivu stabilnost tijekom dvogodišnjeg skladištenja.

Keywords: specifična isporuka u kolonu, Eudragit S-100, mikrosfere, naproksen natrij, natrijev alginat

Chitkara College of Pharmacy, Chandigarh-Patiala National Highway, Rajpura-140401, Patiala, Punjab India