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Formulation Development and Characterization of Naproxen Sodium-Loaded Mucoadhesive Microspheres

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Abstract:
Development of new drug molecule is expensive and time consuming. Improving the safety efficacy ratio of old drugs has been attempted using different methods such as individualizing drug therapy and therapeutic drug monitoring. Delivering drug at controlled rate, slow delivery, and targeted delivery are other very attractive methods and have been pursued very vigorously. The present work was envisaged to reduce the dosing frequency and improve patient compliance by designing and evaluating sustained release mucoadhesive microspheres of Naproxen sodium for effective control of rheumatoid arthritis. Microspheres were prepared by Ionic gelation technique using sodium alginate, carbopol 974, and hydroxyl propyl methyl cellulose K15 M (HPMC) as a mucoadhesive polymers. Microspheres prepared were found discrete, spherical and free flowing. The microsphere exhibits good drug entrapment efficiency. Naproxen sodium release from these microspheres was slow and extended and dependent on the type of polymer used. The data obtained thus suggest that mucoadhesive microspheres can successfully design for sustained delivery of Naproxen sodium and to improve patient compliance.
Key words: Naproxen sodium, sodium alginate, carbopol 974, prolonged release & mucoadhesive microspheres.

INTRODUCTION

Nonsteroidal anti-inflammatory drugs are amongst the most commonly prescribed medications in the world. Almost all the nonsteroidal anti-inflammatory drugs available in the market have severe side effects. As awareness of the gastrointestinal adverse effects associated with nonsteroidal anti-inflammatory drugs increases, safety becomes a primary requisite in treatment. A trend in nonsteroidal anti-inflammatory drugs development has been to improve therapeutic efficacy and reduce the severity of gastrointestinal adverse effects through altering the dosage forms by modifying the release of the formulations to optimize drug delivery system. One such approach is using mucoadhesive polymeric microspheres as carriers of drugs[1]A trend in NSAID development has been to improve therapeutic efficacy and reduce the severity of upper GI side effects through altering dosage forms of NSAIDs by modifying release of the formulations to optimize drug delivery[2]. Mucoadhesive microspheres include microparticles and microcapsules (having a core of the drug) of 1—1000 µm in diameter and consisting either entirely of a mucoadhesive polymer or having an outer coating of it, respectively. Drugs are being administered since ancient times to treat various ailments. Gelanicals and powders were the initial dosage forms to deliver the drugs. Presently drugs are given as tablets, capsules, pills, suppositories, ointments, creams, liquid orals, and injectables. Even though these conventional dosage forms ensure the proper release of a drug, it is necessary to administer these types of dosage forms several times a day to achieve, as well as to maintain the drug concentration within the therapeutically effective range[3-4]. This results in significant fluctuations in the plasma drug concentration. An ideal drug delivery system should achieve a steady state blood or tissue level for an extended period of time that is therapeutically effective a nontoxic. This can be achieved by the use of new and better techniques known as novel drug delivery systems[5]. Many controlled-release dosage forms are designed to release the drug at a predetermined rate, thus maintaining relatively constant drug levels in the plasma for an extended period of time[6]. Several benefits may result from the use of such formulations. Reduction of frequency of dosing, lowered adverse effects, and improved patient compliance are considered the primary advantages of controlled-release dosage forms. One such formulation uses polymeric microspheres as carriers of drugs[7-8].

Naproxen sodium (NS) is chemically, (S)-6-methoxy-α-methyl-2-naphthaleneacetic acid, sodium salt (Fig.1). NS is a non-steroidal anti-inflammatory drug (NSAID) commonly used for the reduction of moderate to severe pain, fever, inflammation and stiffness. It works by inhibiting both the COX-1 and COX-2 enzymes[9].

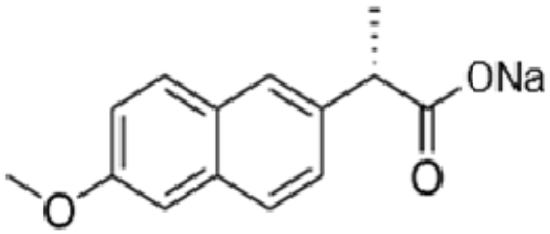

Figure 1: Naproxen sodium

Table 1. Composition of Naproxen sodium-loaded various microsphere formulations

F. Code	D/P Ratio	Drug (gm)	Na.alginate(g)	HPMC K15 (g)	Carbopol 974 (g)	Ca.Chloride (30 %w/v)
NS1	1:1	1	0.5	0.5	-	30
NS2	1:1.5	1	0.75	0.75	-	30
NS3	1:2	1	1	1	-	30
NS4	1:1	1	0.5	-	0.5	30
NS5	1:1.5	1	0.75	-	0.75	30
NS6	1:2	1	1	-	1	30

MATERIALS AND METHODS

Instruments used: An Elico model SL 244 (India) UV Visible double beam spectrophotometer, India; Magnetic stirrer (2MLH), Remi instrument, India; Scanning Electron Microscope (JEOL, JSM – 6100 SEM, Japan).

Reagents used: The following chemicals and solvents were used: Naproxen Sodium (a gift sample from Dr. Reddy's Laboratories, Hyderabad), Sodium alginate, Hydroxypropyl methyl cellulose (HMPC) and carbopol 974P (Loba Chemical Pvt. Ltd., Mumbai), calcium chloride, petroleum ether, light liquid paraffin, and heavy liquid paraffin (S.D Fine chemicals, India). All the solvents and chemicals used were of analytical grade satisfying pharmacopoeial standards.

Methods

Preparation of Microspheres

Preparation of mucoadhesive microspheres with drug and different polymer ratios were prepared by Ionic gelation technique[10-12]. Weighed quantity of sodium alginate and polymer (Carbopol 974, HPMC KI5) as per the ratios given in table no.1, the polymers were dissolved seperately in purified water (30 ml) to form a homogenous polymer solution. The drug Naproxen sodium was added to the polymer solution and mixed homogenously to get a smooth viscous homogenous dispersion. The resulting dispersion was then added in a thin stream to about 500 ml of liquid paraffin containing 400 ml of light liquid paraffin and 100 ml of heavy liquid paraffin in a 1000 ml beaker, stirring with 600 rpm for 5 mins to emulsify. Thereafter, 30ml of calcium chloride (10 %w/v) solution was added slowly while stirring for solvent emulsion method that resulted in spherical microspheres. The microspheres were separated and washed with chloroform followed by a stream of water. These were dried at 45˚C for 8 hrs & kept in a dessicator.

Assay

Naproxen Sodium was estimated by ultraviolet visible spectrophotometric method (Elico model SL 244, India). Aqueous solutions of Naproxen sodium were prepared with phosphate buffer (pH 7.4) and absorbance was measured on UV/Vis spectrophotometer at 332 nm (The United States Pharmacopoeia 2003). The method was validated for linearity, accuracy and precision. The method obeys Beer's Law in the concentration range of 10-50 µg/ml.

CHARACTERIZATION OF MICROSPHERES

Various evaluation tests[13-18] were performed for the prepared formulations as below,

Production yield (w/w)

The dried microspheres were weighed and their percentage yield (w/w) was determined by using following formula.

$$\%\ yield = \frac{Amount\ of\ dried\ microspheres\ recovered}{Amount\ of\ drug + Amount\ of\ polymer}$$

Particle size analysis

Particle size analysis is performed by using ordinary microscope. The microspheres were mounted on a slide using glycerine and observed, the average size is determined by using eye piece micrometer which is previously calibrated with a stage micrometer. Then the obtained value is multiplied with calibration factor know the

average particle size. Mean particle size of Naproxen sodium microspheres is given in the table no.2.

Flow properties of microspheres

Angle of repose

Weighed quantity of microspheres was passed through a funnel fixed on a stand at a specific height upon graph paper. A static heap of powder with only gravity acting upon it was tending to form a conical mound. The height of the heap (h) and radius (r) of lower part of cone were measured and reported in table no.2. The angle of repose was calculated using formula:

$$\tan \theta = h/r$$

Therefore,

$$\theta = \tan^{-1} h/r$$

Where,
θ = angle of repose,
h = height of cone and
r = radius of cone base

Shape and surface morphology

From the dissolution profiles, the best formulation (NS5) was selected for further evaluation. A scanning electron microscope (JEOL, JSM – 6100 SEM, Japan) was used to examine the shape and surface morphology of the microsphere. Best formulation (NS5) microspheres were mounted on an adhesive stub and then coated with gold palladium under vacuum using an ion coater. The coated specimen was then examined under the microscope at 15 kV and photographed and reported in fig no.2.

Interaction between drug and polymer

Infrared spectra for the formulations are recorded using FTIR spectrophotometer by potassium bromide disc method. The absorption maxima in spectrum obtained with the substance being examined corresponds on position and relative intensity to those in the IR spectra of Naproxen sodium. The IR spectra of Naproxen sodium raw material and various physical mixture compositions are shown in figure no. 3,4,5,6,7, & 8.

Encapsulation efficiency

Accurately weighed amount (50 mg) of the microsphere formulations were dispersed in 50 ml of phosphate buffer pH 7.4. The sample was ultrasonicated for three consecutive periods of 5 min each, with a resting period of 5 min each. It was left to equilibrate for 24 hr at room temperature, and the suspension was then centrifuged at 3000 rpm for 15 min. The supernatant was diluted appropriately with phosphate buffer pH 7.4 and analyzed spectrophotometrically at 332 nm using ultraviolet visible spectrophotometric method (Elico model SL 244, India).

The encapsulation efficiency of microspheres was determined by the formula and results are shown in the table no.2.

$$E = Qp / Q_t \times 100$$

Where,
E = percentage of encapsulation of microspheres
Qp = quantity of drug encapsulated in microspheres
Q_t = quantity of the drug added for encapsulation

The drug encapsulation results are shown in table no. 2.

Drug loading

The drug loading in microspheres was estimated using the formula

$$L = Qm / Wm \times 100$$

Where,
L = percentage of loading of microspheres
Wm = weight of the microspheres
Qm = quantity of drug present in Wm of microspheres

The drug loading results are shown in the table no.2.

Swelling studies of microspheres

Swelling index was determined by measuring the extent of swelling of microspheres in phosphate buffer and results are shown in the table no.2. To ensure complete equilibrium, exactly weighed 100 mg of microspheres were allowed to swell in simulated intestinal fluid pH 7.4 for 24 h. The excess surface adhered liquid drops were removed by blotting and swollen microspheres were weighed by using microbalance. The degree of swelling was then calculated by the following formula,

$$\textbf{Degree of swelling} = M_o - M_t / M_t \times 100$$

Where,
M_t = Initial weight of microspheres,
M_o = Weight of microspheres at equilibrium swelling in the media.

***In-vitro* drug release studies**

The *in-vitro* dissolution studies were performed by using 0.1 N HCl and 7.4 pH buffer solution as a medium. In vitro drug release studies were carried out using US Pharmacopoeia paddle type-II dissolution apparatus at 37 ± 0.5°C with constant stirring rate of 50 rpm. 100 mg equivalent of Naproxen sodium microspheres were weighed and placed in a rotating basket, 750 ml of 0.1 N Hcl is taken as medium and temperature was maintained at 37 ± 0.5°C and fluid was agitated properly. For every 1 hr time interval the fluid from the medium is withdrawn and replaced with the same amount

of fluid for maintaining proper sink conditions. The samples were filtered and about 3 ml of the filtrate was taken and diluted to 10 ml with 0.1 N Hcl. These were analysed spectrophotometrically at 332 nm. After 2 hrs 250 ml of 7.4 pH phosphate buffer was added to make the pH to 7.4. The above procedure was repeated for every 1 hr and process was continued for 12 hrs. All dissolution studies were carried out and results are shown in figure 9.

Stability study

The formulation NS5 was stored in a stability chamber at 40 ± 2^{O}c temperature and 75% ± 5% relative humidity. Samples were analysed at 0 day, 15^{th} day, 30^{th} day for drug loading and drug encapsulation. The results are shown in table no.3.

RESULTS AND DISCUSSION

Naproxen sodium-loaded mucoadhesive microspheres were prepared by ionic gelation technique. Naproxen sodium, a hydrophilic drug, can partition out into the aqueous processing phase during the preparation of microspheres by external gelation method. The infrared spectrum studies revealed that there is no interaction between the drug and polymers (shown in figure no. 3,4,5,6,7,&8) and in order to improve the indication of safety, efficacy of the product. Depending on the processing conditions as much as 80 - 90% of the drug can partition out into the external aqueous processing medium. In this study attempt was made to encapsulate Naproxen sodium with sufficiently high incorporation efficiency. An external oil phase (liquid paraffin) was used as the harvesting medium with the expectation that for Naproxen sodium it would be non-favourable to diffuse out of the microspheres before they form as rigid and discrete particles.

Naproxen sodium-loaded mucoadhesive microspheres composed of alginate in combination with HPMC K15M and carbopol 974 were prepared by the ionic gelation technique. The microspheres were found to be discrete, spherical, free flowing and of the monolithic matrix type. The microspheres were uniform in size with a particle size range of 129.7-395.9 µm. The particle size ranges are shown in Table 2. The size of the microspheres was in increasing trend with increasing with the polymer concentrations. This may be due to the increase in viscosity, which in turn increases in droplet size during addition of the polymer dispersion to the harvesting medium. The SEM photomicrographs (Figure 2) indicated that the microspheres were spherical in shape having particle size of 200 mm and the drug remained dispersed in the polymer matrix at amorphous state. SEM photomicrographs in Figure 2 reveal that the mean microspheres size as observed by optical microscope is significantly higher than that observed under scanning electron microscope. It might be explained by the fact that the incompletely dried microspheres (remaining at swollen state) were observed under optical microscope, whereas the microsphere particles were fully dried when SEM study was performed.

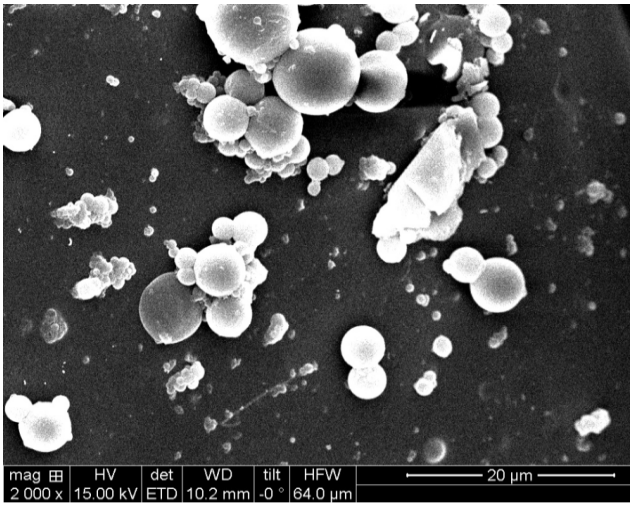

Figure 2. SEM photomicrograph of best formulation (NS5)

Table 2. Physical characteristics of mucoadhesive microsphere of Naproxen sodium.

Code	Particle size µm	% Yield	% drug loading	Encapsulation efficiency (%)	Angle of repose	Degree of swelling
NS1	129.7±2.850	64.05±1.125	33.63±0.689	69.91±0.646	26◦ 51′±0.254	0.693
NS2	141.9±2.261	63.08±1.128	34.81±0.606	69.66±0.798	27◦ 11′±0.490	0.734
NS3	134.8±1.135	65.35±0.488	31.91±0.588	72.82±0.696	26◦ 96′±0.515	0.679
NS4	170.4±1.033	64.72±0.623	35.07±1.045	63.89±0.640	37◦ 56′±0.570	0.597
NS5	199.7±2.348	67.99±1.351	39.06±0.990	76.27±0.862	39◦ 97′±0.715	0.898
NS6	186.6±0.723	70.08±0.960	36.01±1.022	68.79±0.554	29◦ 63′±0.954	0.617

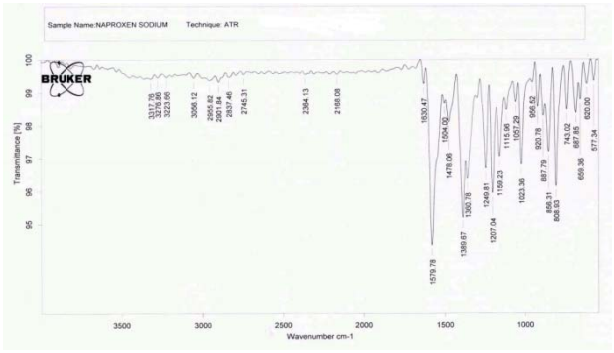

Figure 3: Infrared spectrum of Naproxen sodium

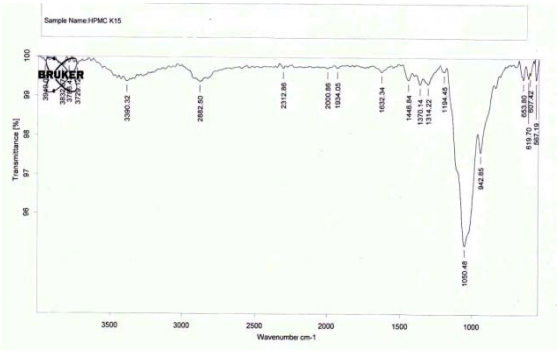

Figure 4: Infrared spectrum of HPMC K15

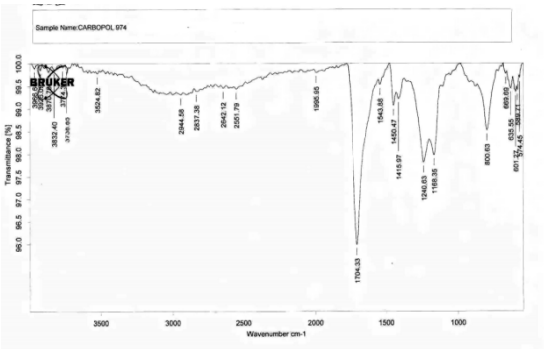

Figure 5: Infrared spectrum of carbopol 974

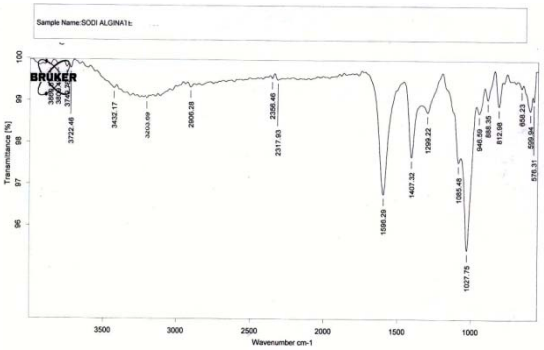

Figure 6: Infrared spectrum of Sodium alginate

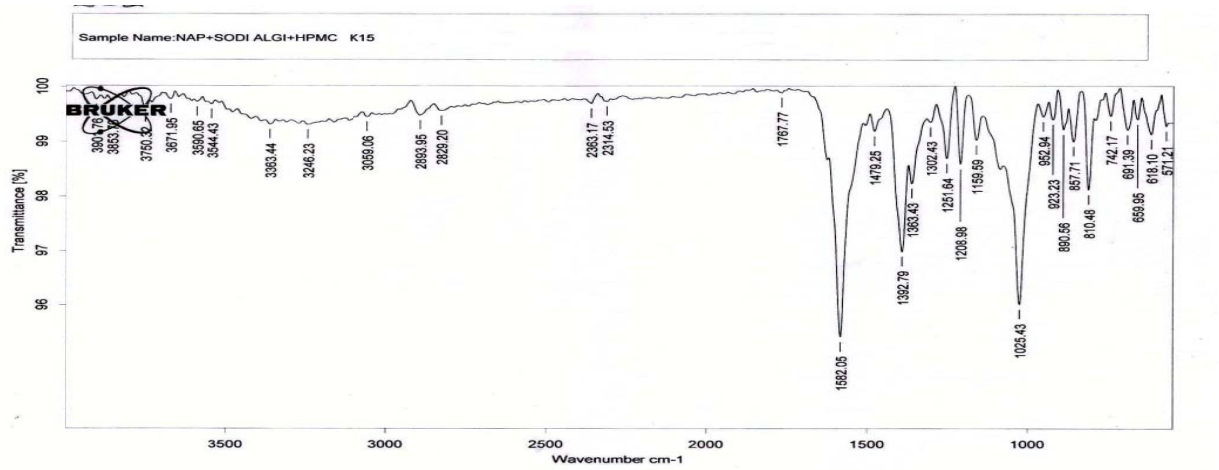

Figure 7: infrared spectrum of Naproxen sodium+sodium alginate+HPMC K15

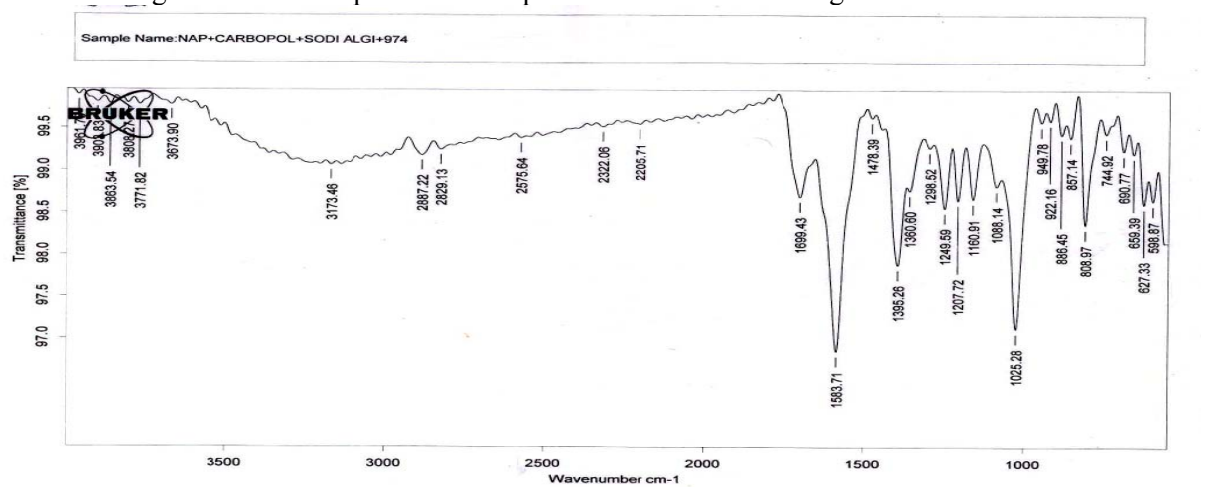

Figure 8: infrared spectrum of Naproxen sodium+sodium alginate+carbopol 974

The effects of alginate concentrations and polymer compositions on the drug incorporation efficiency of microspheres are shown in Table 2. The highest incorporation efficiency (76.27) and loading efficiency (39.06) were achieved with 1% w/v sodium alginate in combination with 1.5% w/v carbopol. The higher incorporation and loading efficiency were observed in the NS5 formulation. The drug loading efficiency greatly improved when alginate was blended with carbopol at 1.5% level.

The invitro drug release studies were carried out in the simulated gastric fluid (0.1 N HCl) and simulated intestinal fluid (phosphate buffer, pH 7.4). The microspheres were prepared by ionic gelation technique using $CaCl_2$ as cross linking agent. The rate of drug release of HPMC K15 and carbopol 974 loaded microspheres were provided better drug release effect but the rate of sustainability was less in the case of HPMC K15 loaded microsphere and shown in figure no. 9. The microspheres cross-linked with Ca^{2+} showed delay in disintegration and consequently a slow release of drug was obtained. Our results are in good agreement with the report of Das and Senapati who used the alginate microspheres containing furosemide prepared by the ionic external gelation technique using $BaCl_2$. Sodium alginate at in combination with 1%, 1.5% & 2% w/v of HPMC K15M and carbopol 974 P were utilized for the preparation of microspheres. The drug release behaviors are shown in figure 9 and 10. It was observed that the formulation NS5 shows better sustain release when compared to the other formulations. It can be attributed to an increase in the densities of the polymer matrix resulting in larger microspheres and this in turn increase the diffusion path length, which the drug molecules have to be traverse. It was observed that alginate containing carbopol 934 microspheres had swollen more in phosphate buffer of pH 7.4 than in 0.1 N HCl. Therefore, the release would depend on diffusion of Naproxen sodium through the insoluble matrix of alginate polymer in 0.1 N HCl and a sustained drug release behavior was observed.

In contrast, swelling and erosion of the microspheres prepared from alginate polymer was observed in phosphate buffer of pH 7.4. Slow erosion of calcium cross-linked alginate microspheres could occur through slight degradation of alginate backbone into smaller fragments. In addition, the exchange of Ca^{2+} ions in the microspheres with Na^{+} ions of the phosphate buffer causes the sustained erosion of the microspheres, which greatly increase the drug release rate in phosphate buffer of pH 7.4. As described in table no. 3, there was no significant change in drug-loading, encapsulation of microspheres, stored at 40 °C and 75% RH, after 30 days of study.

Table 3. Results of accelerated stability data of best formulation (NS5)

No. of days	Temp(°C)	Relative humidity %	% drug loading	% drug encapsulation
0	40	75	39.06±0.428	76.27±0.363
15	40	75	38.29±0.380	75.58±0.394
30	40	75	38.02±0.392	75.31±0.351

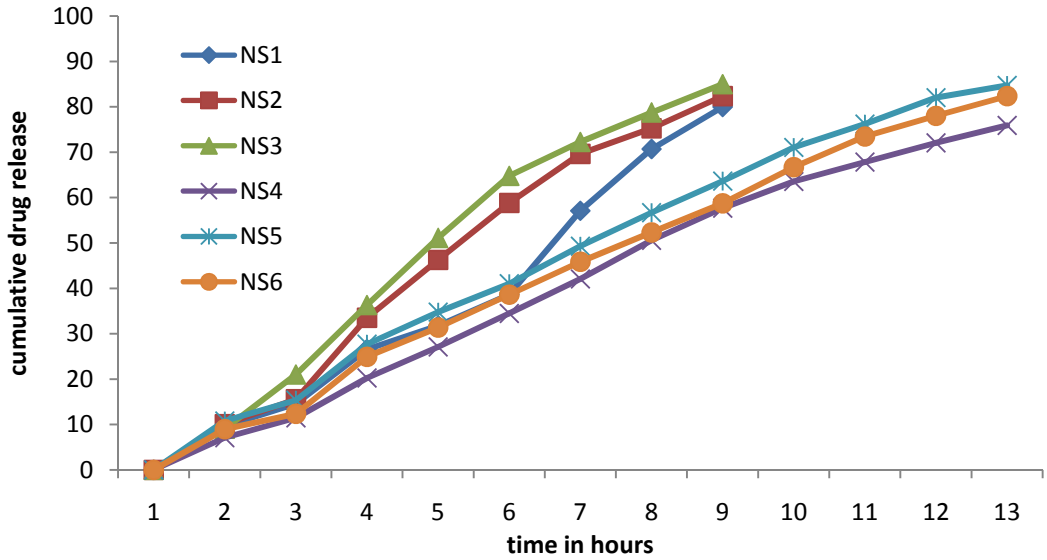

Figure 9: *Invitro* release profile of Naproxen sodium (HPMC K15 & Carbopol 974)

CONCLUSION

In the present study effort have been made to prepare and characterize the mucoadhesive microspheres of Naproxen sodium and the results obtained in the study have been summarized. The polymer concentration is a major factor affecting the release strength of the prepared microspheres. Naproxen sodium-loaded mucoadhesive microspheres were successfully prepared by ionic gelation technique with a maximum encapsulation efficiency of 76.27. The microspheres were spherical in shape and the drug remained dispersed in the polymer matrix at amorphous state. The carbopol 974 loaded Naproxen sodium release rate was followed non-fickian type, controlled by swelling and relaxation of polymer chain. There was no significant change in loading efficiency and encapsulation efficiency of drug-loaded microspheres, stored at different storage conditions after 30 days of study.

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