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Preparation and in vitro evaluation of Enteric Controlled release Pantoprazole loaded Microbeads using Natural Mucoadhesive Substance from *Dillenia indica* L.

Hemanta Kumar Sharma*, Siba Prasad Pradhan, Babita Sarangi

Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh-786004, Assam, India.

Corres. Author:hemantasharma123@yahoo.co.in
Tel.: 0373-2370254, Fax: 0373-2370323

Abstract: Pantoprazole is a proton pump inhibitor prodrug used in the treatment of gastric ulcers and gastroesophageal disease. Pantoprazole must be absorbed in the gastrointestinal tract and because it is unstable under acidic conditions, enteric delivery systems are required. The purpose of this study was to prepare Pantoprazole loaded microbeads by ionotropic gelation technique using sodium alginate and natural Mucoadhesive substance from the fruit of *Dillenia indica* followed by a coating with Eudragit L100-55. The microspheres have been characterized in terms of their morphology, particle size, encapsulation efficiency, swelling ratio, mucoadhesivity and ability of stabilizing Pantoprazole in acidic media. Different formulation variables like polymer-polymer ratio, drug-polymer ratio and coating concentration were considered. Almost spherical microbeads were obtained with sufficient swelling, Mucoadhesive property and acid resistance. Dissolution study was followed at phosphate buffer (pH 7.4) for 8 hr.
Key words: Pantoprazole sodium, Ionotropic gelation, Sodium alginate, Natural Mucoadhesive substance, Eudragit, Fickian diffusion.

Introduction and Experimental

Microsphere carrier systems made from the naturally occurring biodegradable polymers have attracted considerable attention for several years in sustained drug delivery. However, the success of these microspheres is limited owing to their short residence time at the site of absorption. This can be achieved by coupling bioadhesion characteristics to microspheres and developing bioadhesive microspheres. Bioadhesive microspheres have advantages such as efficient absorption and enhanced bioavailability of drugs owing to a high surface to volume ratio, a much more intimate contact with the mucus layer, and specific targeting of drugs to the absorption site [1].

Alginate is a naturally occurring biopolymer that is finding increasing applications in the biotechnology industry. Alginate has been used successfully for many years in the food and beverage industry as a thickening agent, a gelling agent and a colloidal stabilizer. Alginic acid is a linear copolymer of p-O-mannuronic acid and u-L-guluronic acid linked by (1 -4)-glycosidic bonds. Alginate gelation takes place when divalent cations (usually Ca^{+2}), interact ionically with blocks of guluronic acid residues, resulting in formation of three-dimensional network which is usually described by 'egg-box' model [2].

The natural mucoadhesive substance (NMS) used in the present work was obtained from the water soluble extract of the fruit pulp of *Dillenia indica* L. Dilleniaceae. According to Kirtikar and Basu [3], the aqueous extract of fruit of *D. indica* contains mostly pectous matter as the polysaccharides. The NMS has considerable swelling behavior in water and particularly in buffer 6.8. This may be considered as significant for its use in mucoadhesive drug delivery, particularly for controlled release.

Polymethacrylate copolymers are widely used as film-coating materials in oral pharmaceutical

formulations. Eudragit L 100-55 is insoluble below pH 5 and thus resistant to gastric fluid. By salt formation in the neutral to weakly alkaline medium of the intestinal fluid, the polymer dissolves step-wise at pH values above 5.5.

For this study, an acid-labile drug, Pantoprazole, was chosen to be microencapsulated by the ionotropic gelation technique using sodium alginate and NMS blend. Pantoprazole is a proton pump inhibitor, used in the treatment of digestive ulcers. It is a prodrug that degrades once protonated in acidic media. So, the drug protonation for activation must occur inside the gastric parietal cells, and the tetra cyclic form of Pantoprazole binds irreversibly to cystein residues of the proton pump (H+/K+ ATPase). In this way, Pantoprazole must be absorbed intact before activation and, because of this; it requires an enteric drug delivery system [4].

The proposed system is expected to provide several advantages: Firstly, gelation of the aqueous solution of alginate/NMS blend renders oral sustained drug delivery. Secondly, Eudragit-coating prevents the solvation of beads and acid labile drug leakage in the stomach, leading to intestinal drug release. Finally, the mucoadhesive property of NMS enhances adhesion on the mucosal surface with dissolution of the Eudragit-shell of beads in the intestine, resulting in the delivery of a drug across the mucous membrane for an extended period of time. So the aim of the present work was to prepare by ionotropic gelation technique and to characterize the controlled-release enteric microbeads containing Pantoprazole sodium.

Materials

Pantoprazole sodium sesquihydrate was obtained from Sun pharma (Badodara). The polymers used were Eudragit L 100-55 (Rohm Pharma, Darmstadt, Germany) and Sodium alginate (Loba Chemi Pvt. Ltd. Mumbai). The fruit of *Dillenia indica* was procured from local market and was confirmed by local people. All other chemicals were of analytic grade.

Isolation of mucoadhesive substance

Extraction procedure followed in accordance to that followed for extraction of pectins [5].

Preparation of microbeads

The microbeads were prepared using blends of sodium alginate and NMS by micro-orifice ionic gelation method [6]. The sodium alginate and NMS gel were mixed in a ratio so as to have the 1.5:1, 1:1, and 0.5:1 ratio by weight of the NMS and sodium alginate by weight in the final blend (Table-1). The drug, Pantoprazole sodium, was added to this mixture and homogenized thoroughly with a magnetic stirrer to form a homogeneous dispersion. The resulting bubble free dispersion was added manually dropwise with a 5 ml syringe (18 gauge needle) into 100 ml of (8%w/v) calcium chloride ($CaCl_2$) solution stirred in a 250 ml beaker. The gelation time of 15 min was allowed to complete the curing reaction and produce spherical rigid microbeads. The beads so prepared were collected by decantation, washed with water and dried in hot air oven (NSW, India) at 60 ^{0}C for 2 hours.

Preparation of enteric-coated beads

The beads prepared were transferred into acetone solutions of Eudragit L 100-55 at various concentration of 7.5, 10 and 12.5%w/v, and coated for 15 min under stirring. The resulting coated beads were filtered and air dried. This coating process was repeated three times [7].

Characterization of Microbeads

Entrapment efficiency

The drug entrapment efficiency of beads was estimated by dispersing the beads in 100 ml of phosphate buffer at 7.4 by vigorous shaking on mechanical shaker (Remi Motors, Mumbai) for 12 hr. Then, the solution was filtered, and the Pantoprazole sodium content was assayed by a UV-spectrophotometer. The entrapment efficiency of microbeads was calculated using the following formula [8].

Entrapment efficiency =

$$\frac{\text{Estimateed percentage drug loading}}{\text{Theoretical percentage drug loading}} \times 100$$

Determination of Particles Size

The prepared (coated and uncoated) microbeads were mounted in light liquid paraffin, and the diameters of 50 particles were measured by means of an optical microscope equipped with a calibrated ocular micrometer. The mean diameter was then calculated [9].

Mean particle size = $\sum n.d / \sum n$

Swelling studies

The swelling studies of uncoated beads were performed in aqueous swelling media with pH 7.4 buffer at 37.5 □ 0.5 ^{0}C. The swelling ratio, S_{wt}, was calculated from the following expression [10]:

$S_{wt} = [(W_t - W_0)/ W_0] \square 100$

Where Wt and W_0 are weight of sample swollen at time t and weight of the original sample respectively.

Evaluation of mucoadhessive property

The mucoadhesive property of uncoated beads was evaluated by an *in vitro* adhesion testing method known as wash-off method [11]. Freshly excised pieces of chicken intestinal mucosa (2 □ 2cm) were mounted on to glass slides with cotton thread. About 50 microbeads were spread on to each prepared glass slide and immediately thereafter the slides were hung to USP tablet disintegration test (Tab. Machines, Mumbai). When the test apparatus was operated, the sample is subjected to slow up and down movement in the test fluid at 37 ^{0}C contained in a 1-liter vessel of the apparatus. At an interval of 30min up to 8 hours the machine is stopped and number of beads still adhering to mucosal surface was counted. The test was performed at intestinal (Phosphate buffer pH 7.4) condition.

Scanning electron microscopy

The surface morphologies of the uncoated microbeads, coated microbeads and microbeads collected after dissolution study were investigated by using HITACHI, S-3600 N, Scanning Electron Microscope at 15kv. Prior to examination samples were gold coated under vacuum (BIO RAD Microscience division, SC 502) to render them electrically conductive.

Differential scanning colorimetry

The DSC thermograms of the pure drug, drug-loaded microbeads, blank microbeads and NMS were obtained using the Perkin Elmer JADE DSC system, to identify any interaction between the components of formulation.

FTIR spectroscopy

The FTIR spectra of the drug (Pantoprazole sodium), NMS, sodium alginate, blank and drug-loaded microbeads, were obtained in KBr pellets using a Perkin-Elmer FT-IR spectrometer and at mid IR region (wavelength 25□ to 2.5□, wave-number from 400cm^{-1} to 4000cm^{-1}), in order to identify the possibility of any interaction between the drug and polymer materials.

In vitro drug release study

The release of Pantoprazole sodium from the microbeads was studied in phosphate buffer pH 7.4 as medium using USP XXVI dissolution test apparatus paddle type (Campbell Electronics, Mumbai) at 37± 0.2 ^{0} C with a rotating speed of 50 rpm [12]. A sample of microbeads equivalent to 40 mg of Pantoprazole sodium was used in each test. At preset time intervals 1ml aliquots were withdrawn and replaced by an equal volume of fresh dissolution medium. The samples were withdrawn through a membrane filter (0.45 □m) and were analyzed for Pantoprazole sodium content spectrophotometrically at 295nm using the UV-Visible Spectrophotometer (UV-1700(E), SHIMADZU).

Determination of the Gastro-Resistance of enteric coated beads

The gastro resistance of coated beads was determined by the method described by [4]. The samples (F2, F7, F8) were placed in dissolution bath containing 0.1N HCl at 37± 0.2 ^{0} C for 1 hour. During the acid step, no sample was collected for quantification because any amount of Pantoprazole released at this pH is quickly degraded. After the acid step, the HCl solution was replaced by phosphate buffer pH 7.4. Then, samples were collected at predetermined time intervals and analyzed spectrophotometrically at 295 nm.

Results and Discussion

Entrapment efficiency

The drug entrapment efficiency of different formulations (both coated and uncoated) has been summarized in the table-2. The Pantoprazole sodium being highly soluble in water is having tendency to diffuse out to the aqueous medium even though the sufficiently higher drug entrapment to the gel beads prepared with the NMS could be achieved that might be resulted due to hindered diffusion of the medicament through the gel barrier formed by the pectin of NMS. It was observed that, as the concentration of NMS increases, viscosity of resulting gel increases and there by increase in entrapment efficiency. An increase in drug load was also observed by increasing the concentration of drug. The decrease in entrapment efficiency in case of coated beads may be due to leakage of drug into coating solution during coating.

Particles Size analysis

The effect of different parameters on particle size of microbeads has been summarized in the table-2. Increase in gel concentration increases the mean particle size of the beads. This is due to the increase in viscosity, which in turn increase the droplet size during addition of the polymer solution to the cross-linking agent solution [13-14]. Particle size also increases by increasing the drug load.

Swelling studies

The swelling behavior of uncoated microbeads was determined gravimetrically. The result indicated that as

the amount of polymer (formulations F1, F2, F3) in microbeads was increased the swelling ratio also proportionately increased. The higher percentage of NMS in microbeads renders high swelling and gel formation. So the inclusion of NMS in alginate gel opens an option for the manufacture of cross linked matrix devices for gastrointestinal delivery (table-2).

Mucoadhesion testing

The adhesion of microspheres to the intestinal mucosa of goat was evaluated as the mean percent of microspheres remain adhered after a defined period of washing. Results indicated that the polymer to drug ratio had a significant effect on mucoadhesive property. The greater the concentration of the polymer associated with NMS–alginate matrix, greater will be the adhesion. An increase in drug load has no such effect on mucoadhessive property (Table-2).

Morphological and surface characteristics

The SEM photomicrographs of the microbeads prepared with different formulation composition and processing parameters before and after dissolution are shown below (Fig-2). The microphotographs of the prepared microbeads were almost spherical in shape and had a rough surface with shrinkage due to removal of water during drying process. Thus the rate of water removal from the micro beads exerts an influence on the morphology of final product. The enteric coated micro beads revealed smooth and almost discontinuous film on to the spherical surface of the beads. The formation of pores on the surface of the micro beads after dissolution indicates that the drug release from the beads is possibly by the diffusion. The decrease in size of beads after dissolution revealed that the release of drug was not only by diffusion but also by surface erosion of polymers from the microbeads. There was no sign of coating material on the surface of microbeads after dissolution in phosphate buffer (pH 7.4).

Differential scanning calorimetry

The DSC thermogram of Pantoprazole sodium, blank microbeads and drug loaded microbeads are shown in Fig-3. A sharp endothermic peak at 148^0C was observed for pure Pantoprazole sodium corresponding to its melting point. There was no significant difference in the thermograms of blank and drug loaded microbeads suggesting that, presence of drug does not cause any change in thermal property of beads and the drug was homogenously dispersed through out the polymer matrix [15].

FTIR spectroscopy

The FTIR spectroscopy was used to identify the possibility of any interaction between the formulation components. As shown in Fig-4, there was no significant difference in the FTIR spectra of the drug and drug-loaded beads when compared to the spectra of individual components. The characteristic C = N and S = O stretching band of drug remained unchanged in case of the drug-loaded microbeads. Also the peaks corresponding to CF_2 stretching, C = C stretching in aromatic ring, C – O of -OCH_3 and C – H bending of CH_2, CH_3 were retained in the FTIR spectrum of the drug loaded microbeads reflecting the identity features of the Pantoprazole sodium. This finding indicates that possibly there was no chemical interaction between the drug and polymer backbone and the drug entrapment and modulation of release is due to the physical entanglement only.

In vitro drug release study

The drug-polymer ratio was found to affect the drug entrapment, particle size and ultimately the drug release characteristics of the prepared micro beads. At higher drug-polymer ratio the drug release from the microbeads was faster as compared to lower drug/polymer ratio (Fig 1.1). This may be due to the increase in the drug/polymer ratio with an increase in the amount of drug loaded in the polymer, suggesting that higher amount of drug is released per unit area of exposed surface of the polymer matrix.

A significant decrease in rate and extent of drug release was observed with the increase in polymer concentration in microbeads and is attributed to an increase in the density of polymer matrix and in the diffusion path length that the drug molecules have to traverse (Fig 1.2). The prolongation of the release rate from the hydrogel beads with increase of NMS concentration reflects the concomitant increases in gel strength which is a determining factor in this case since the release of drugs in polymer matrices are mainly through the diffusion of the drug through the pores of the polymer network which can be significantly reduced in size by increasing the polymer concentration.

The initial higher release from the uncoated beads (F2) reflects the lower diffusional resistance of these core beads compared with that of the coated beads caused by the absence of a barrier against drug diffusion (Fig 1.3). The results demonstrated that the enteric-coated beads provide a system of low permeability and a good barrier against drug diffusion under pH conditions, at which protection is required.

Determination of the Gastro-Resistance of enteric coated beads

As expected, the beads remain intact during the acid step because the degree of ionization of carboxylic acid groups in the Eudragit L 100-55

increased with pH. Eudragit L 100-55 is fully dissolved and rapidly releases the drug from the core beads, whereas, at pH 1.2, the one is almost intact and retard the release of the drug. The dissolution profile shows that as the polymer concentration in coating solution increases, loss of drug during the acid step decreases (Fig 1.4). In alkaline medium initially the enteric coating retard the release to some extent but as such enteric coating has no effect on drug release due to rapid dissolution of the coating layer in pH 7.4 medium [16]. The results demonstrated that the enteric-coated beads provide a system of low permeability and a good barrier against drug diffusion under low pH conditions, at which protection is required.

Conclusion

Pantoprazole sodium release from these enteric coated mucoadhesive micro beads was slow and extended over longer period of time and dependent over ratio of polymers. Results of the in vitro drug release indicated that the controlled drug release upto 10 hours were obtained from the so prepared carrier backbone. The acid resistance experiment carried out with microspheres showed that 84.8%, 73.8% and 90.68% of Pantoprazole remained stable for F2, F6, and F7 respectively, presenting the stability of drug protection. These studies demonstrated that Pantoprazole sodium can be encapsulated into microbeads having NMS and sodium alginate backbone by micro orifice ionic gelation technique having good batch to batch reproducibility with respect to particle size, entrapment efficiency and in vitro drug release profile of micro beads. Presence of enteric coating has a little effect on drug release but efficiently protect the acid labile drug from highly acidic environment of stomach. In conclusion the performed studies suggested that NMS may be a promising candidate for oral controlled drug delivery system because of its gel forming ability and sustaining the release of drug. Further clinical trial will confirm the result.

Table 1: Formulations with different formulation varibles.

Formula -tion code	Pantoprazole sodium (%)	Sodium alginate (%)	NMS (%)	Stirring speed (rpm)	Cross Linking Agent, %w/v ($CaCl_2$)	Curing time (min)	Coating concent-ration, %w/v
F1	2	2.5	1.25	200	8	15	10
F2	2	2.5	2.5	200	8	15	10
F3	2	2.5	3.75	200	8	15	10
F4	1	2.5	2.5	200	8	15	10
F5	2.5	2.5	2.5	200	8	15	10
F6	2	2.5	2.5	200	8	15	7.5
F7	2	2.5	2.5	200	8	15	12.5

Table 2: Particle size, Entrapment efficiency, % Swelling and % Mucoadhesion of differenent formulations.

Formulation code	Mean particle size (µm) (Mean±SD)		Mean Drug Entrapment Efficiency (%) ± SD		Swelling (%)	Mucoadhesion (%)
	uncoated	coated	uncoated	coated		
F1	934.61±15.23	992.41±11.67	39.28±1.58	37.95±1.13	440	69
F2	973.45±12.46	1045.36±13.24	51.51±1.34	50.32±1.18	470	71
F3	1013.22±16.52	1078.37±13.84	58.37±1.83	57.11±1.49	520	79
F4	912.79±14.69	980.21±12.95	28.57±2.12	27.1±1.58	465	70
F5	980.74±16.33	1039.97±14.79	55.78±2.48	53.8±1.75	473	72
F6	980.15±18.94	1053.58±15.76	50.79±2.32	48.74±2.2	468	66
F7	979.31±13.75	1068.85±14.89	49.18±1.41	48.1±1.14	477	74

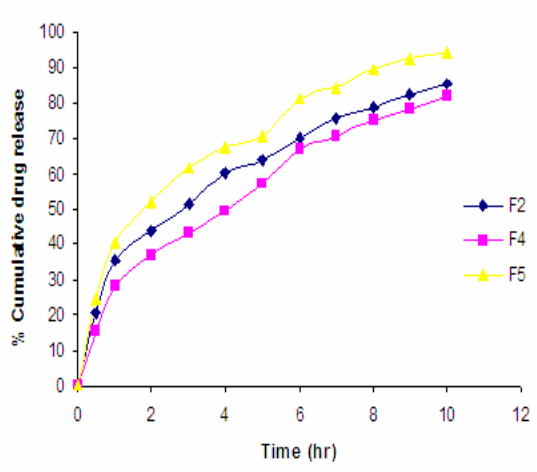


Fig 1.1:Effect of drug-polymer ratio on drug release.

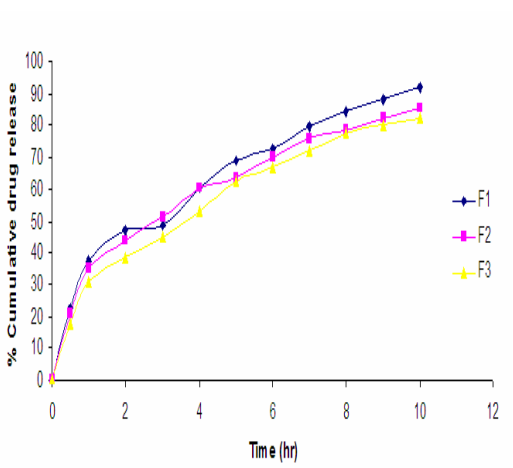


Fig 1.2: Effect of polymer polymer ratio on drug release.

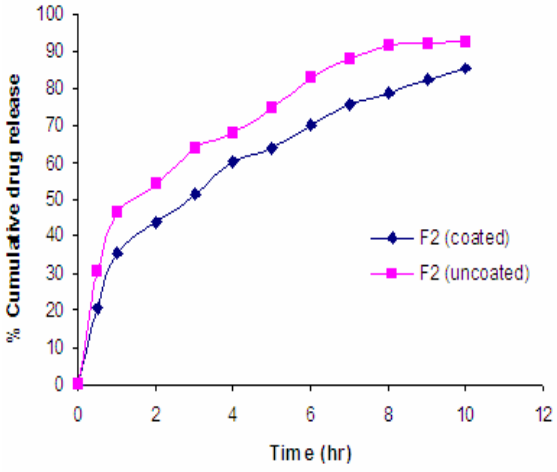


Fig 1.3: Effect of coating on drug release.

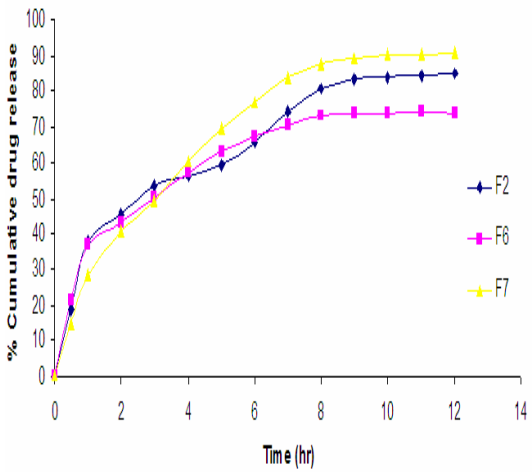
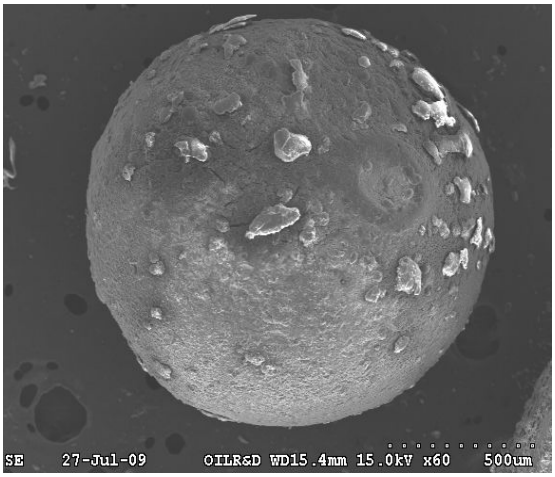
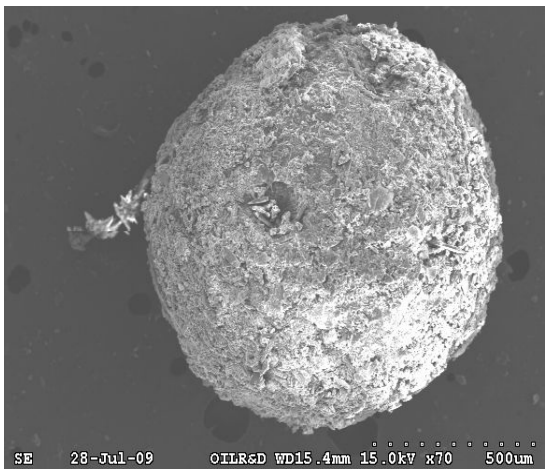


Fig 1.4: Effect of coating conc. on drug release



(a)



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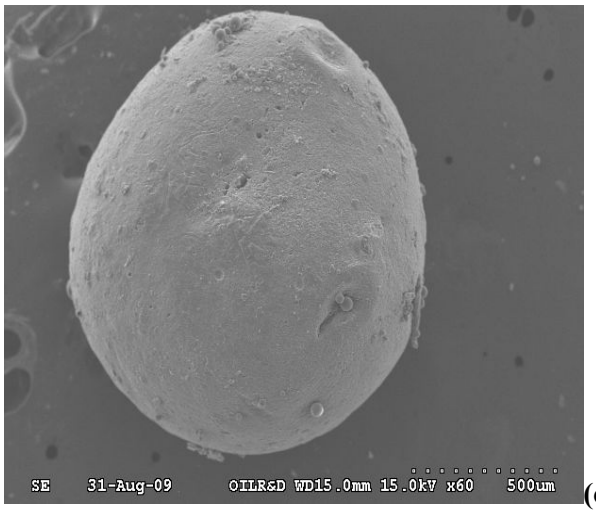


(c)

Fig 2: Scanning electron microscopic figures of (a) Microbead before dissolution (b) Microbead after dissolution and (c) Enteric coated microbead

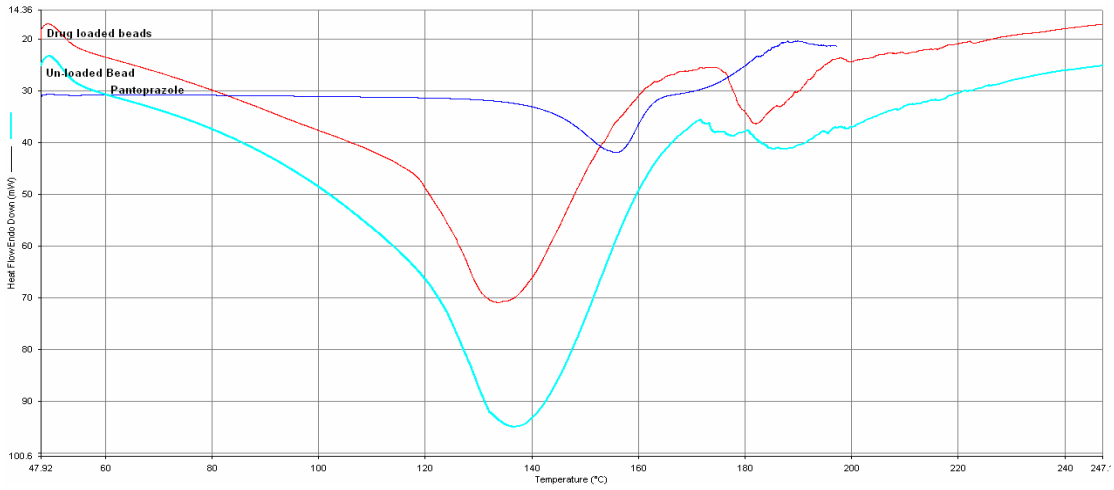
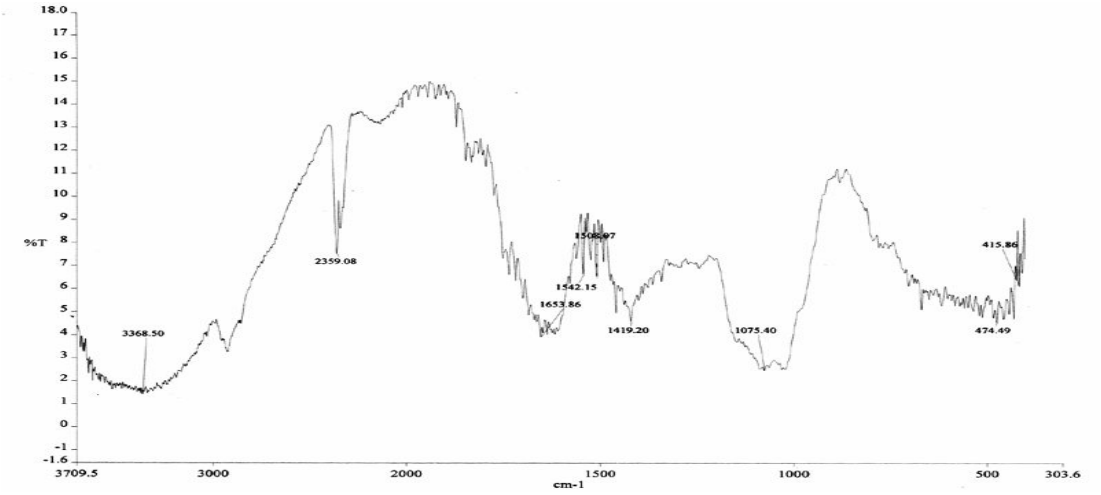


Fig 3: Comparative DSC thermogram of Pantoprazole sodium, beads without drug and drug loaded microbeads.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1419.20
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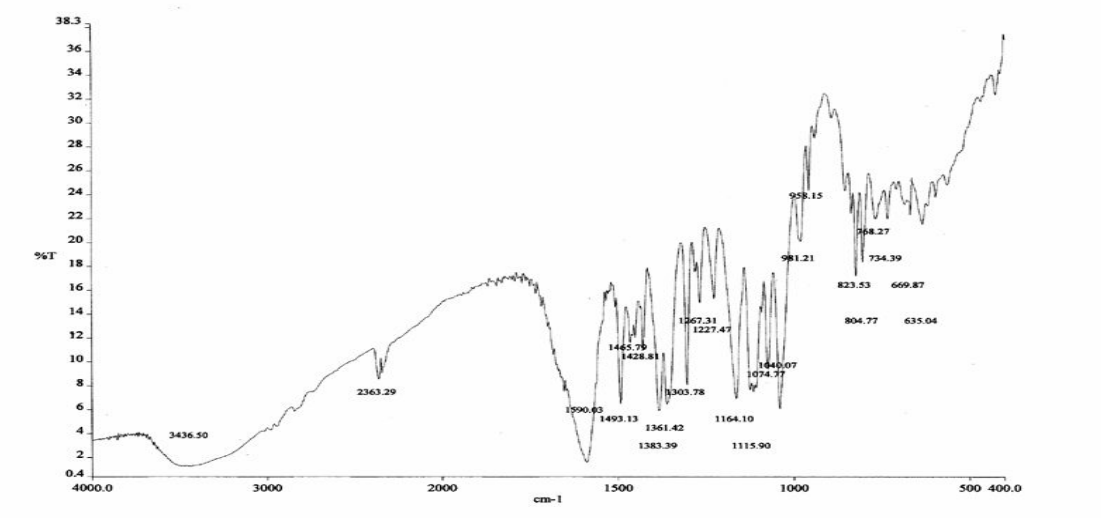
474.49
415.86

(a)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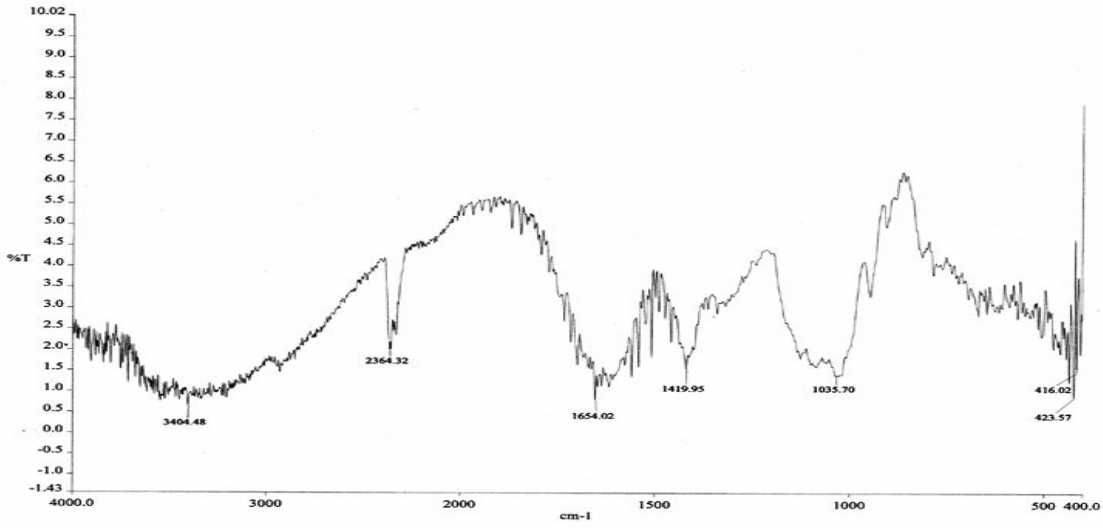
(b)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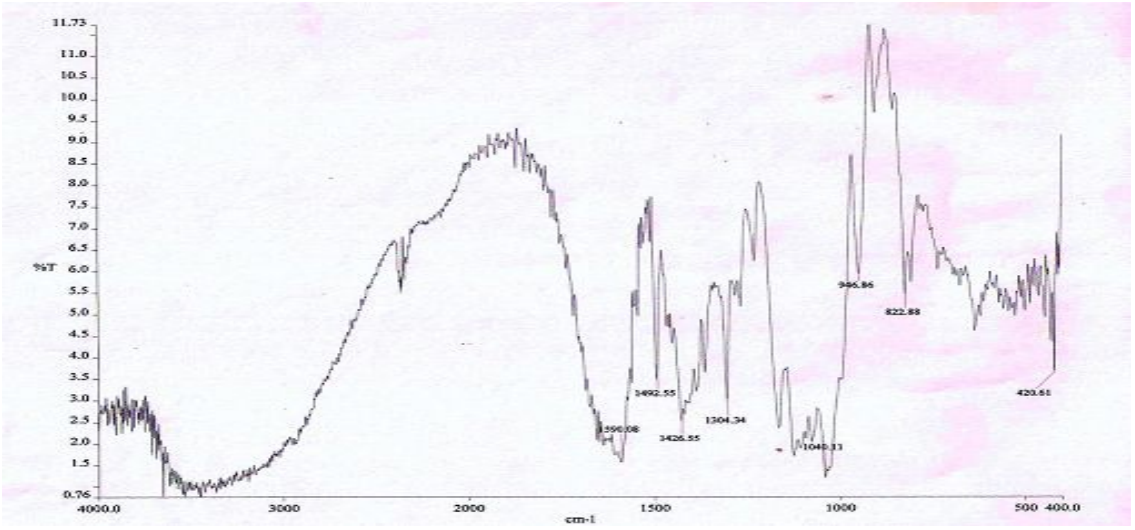
(c)



(d)

Fig 4: FTIR spectrum of (a) pure NMS, (b) Pantoprazole sodium, (c) Beads without drug (d) Dug loaded beads.

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