



Fabrication and characterization of chitosan-carbopol-71G polyelectrolyte complex-based mucoadhesive pellets of miconazole nitrate for vaginal candidiasis

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ABSTRACT

Various approaches have been used in the past to overcome the problems associated with the delivery of antifungal drugs, for effective treatment of vaginal candidiasis. In the present investigation, mucoadhesive polyelectrolyte complex pellets (MPECP) loaded with Miconazole nitrate (MN) were prepared using chitosan/carbopol-71G interpolymer complex (IPC) by Fluid Bed Process (FBP) method. IPC was prepared by precipitation method, followed by characterization with Fourier transform infrared spectroscopy (FT-IR) and Differential scanning calorimeter (DSC). The prepared MPECP were tested for physicochemical properties, *in vitro* drug release behavior, swelling studies and mucoadhesive strength. The possibility of chemical interaction between drug and excipients was ruled out in the prepared pellets as confirmed by FT-IR and DSC studies. SEM photomicrographs showed that pellets had rough surface and were spherical. The dissolution behavior of MN from MPECP into the simulated vaginal fluid (SVF) pH 4.2 was controlled and followed non-fickian release mechanism. Moreover, *in vitro* mucoadhesive study confirmed that complex MP3 showed pH independent controlled releases of MN without an initial burst release, with highest mucoadhesive property and satisfactory residence in vaginal cavity (8h) of rat. The adherence of swelled pellets (MP3), for over 8 hrs, to the vaginal mucous membrane was confirmed by *in vivo* X-ray studies. The results showed no significant change in the pellet properties and drug content. Thus, the formulated MPECP seems to be a potential candidate for controlled release of MN in vaginal pH with optimum swelling behavior and zero order release.

Keywords: Mucoadhesive polyelectrolyte complex pellets; Fluid Bed Process; Miconazole nitrate; *In vitro* mucoadhesive test; *In vivo* X-ray studies

INTRODUCTION

The objective of the present study was to develop mucoadhesive polyelectrolyte complex pellets (MPECP) loaded with Miconazole nitrate (MN) to achieve the therapeutic needs that are lacking in vaginal candidiasis treatment by minimizing toxic effect, enhancing efficacy and increasing patient compliance [1, 2]. The manuscript presents the critical issues of MN associate with vaginal candidiasis using MPECP. Over the last few decades a potential development in research towards the development of vaginal drug delivery for candidiasis was occurred. Now a day's extensive research was carried to deliver active drugs through vaginal cavity. Due to the vast permeation area, many advantages such as rich vascularization, avoidance of first pass metabolism and relatively low enzymatic activity are offered by the vaginal route. Active drugs can effectively be delivered both locally and systemically to

the mucosa [3]. Vagina is a fibro-muscular tube of roughly 10 cm in length, comprising of 3 distinct layers, (a) an outer adventitial layer, (b) a middle muscularis layer (c) an innermost mucosal layer [4]. Rugae and microridges of vagina on the epithelial cell surface permit the vagina to expand, easy to administer vaginal formulations; also increased surface area of the vagina thus offers more drug absorption [5]. Enzyme activity, pH, vaginal secretion and microflora affect formulation spreading, retention, absorption and drug release in vagina [6].

Recently, MPECP have gained much consideration in effective delivery of active drugs because of their potential applications [7]. Compared to other complexes, generally polyelectrolyte complexes were effective and efficient due to its potential in drug delivery [8]. Polyelectrolyte complexes are the association complexes formed due to electrostatic interaction between oppositely charged polycations and polyanions; thus possibly avoids the use of chemical cross linking agents, thereby reducing the toxicity and unwanted effects of the reagents [9-11].

Pellets are preferred in controlled release applications due to their soft tissue biocompatibility, drugs are dispersed easily in matrix [12]. Polymeric pellets offer greater flexibility during design and development, because it can be divided into different dose strengths without any processes changes and effectively deliver the therapeutic agent with different release profiles at same site or at different site [13]. Moreover, it is expected that due to their small size, they will distribute evenly, resulting in complete coverage of vaginal mucosa and long retention time [14].

Most of the research scientists targeted MN only on single drug delivery system. Only a few scientific papers revealed on diffusion studies and histological effects of MN. MN is an imidazole antifungal agent and acts by interfering with permeability of fungal cell membrane by killing the yeast fungus causing the infection. It has wide antifungal spectrum and also possesses some antibacterial activity. MN is incompletely absorbed from GI tract. MN is unlikely to cause any serious side-effects, although it can cause headache; mild vaginal burning, irritation, or itching; stomach cramps in some women [15].

Chitosan, [poly- β - (1, 4)-D-glucosamine] is a unique polysaccharide derived from chitin. Based on large number of studies published over the last few years, chitosan has been widely used for its mucoadhesive character, permeation enhancer and controlled release of drug [16]. Chitosan is a poly cationic polysaccharide that forms polyelectrolyte complex with oppositely charged carbopol. Chitosan complexes have already been used as biosensors, scaffolds in tissue engineering, for waste-water treatment and for drug delivery in different forms [17].

The present investigation aims to develop mucoadhesive polyelectrolyte complex pellets (MPECP) of MN using polyelectrolyte complex to maximize drug absorption, minimize the toxicity and to improve the patient compliance. The drug content uniformity, physical testing, *in vitro* drug release behavior and mechanism of drug release was also evaluated.

EXPERIMENTAL SECTION

Materials

Miconazole nitrate was received as gift sample from Micro Labs Ltd., Bangalore. Chitosan, Microcrystalline cellulose & Carbopol 71G were procured from Sigma Aldrich, Bangalore. All other chemicals and reagent used in this study were of analytical grade.

Preparation of Chitosan/Carbopol 71G polyelectrolyte complex

5% w/v of chitosan in 1% w/v acetic acid solution and 1% w/v of carbopol 71G aqueous solution were mixed. The prepared solutions were stirred for a period of 2 h at a speed of 4000 rpm with a mechanical stirrer. The resulting precipitate (carbopol 71G-chitosan IPC) was washed several times with distilled water and filtered under vacuum pump. The filtrate was dried in hot air oven and dried complex was ground with a grinder. The powder was passed through a 200 μ m sieve and used for further study [18].

Fourier transform infrared spectroscopic (FT- IR) analysis

The FT-IR spectra of chitosan, carbopol 71 G and IPC were analyzed using a FT-IR spectrophotometer (Shimadzu, Model 8400S, Japan). The pellets were prepared by pressing the sample with KBr.

Differential scanning calorimetry (DSC)

The thermal behavior of chitosan, carbopol 71 G and IPC were analysed using a differential scanning calorimeter (DSC Q2000) with a temperature range of 40–250 °C and a heating rate of 10 °C/min in a nitrogen atmosphere. The runs were made in triplicate.

Turbidity measurement of chitosan/carbopol 71G complex ratios

The chitosan/carbopol 71G ratio in the complex mixture was tested by monitoring the transmittance of a solution at a wavelength of 600 nm using a spectrophotometer (UV-1800, Shimadzu, Japan). An aqueous carbopol 71G solution (0.5, 1, 1.5, 2, 2.5, 3, 3.5, and 4 mM) and chitosan aqueous acetic acid solution (0.5, 1 and 2 mM) were used. The concentration was calculated by dividing the weight of chitosan and carbopol by the formula weight of each monomer unit. Each mixture was shaken vigorously. The mixtures were kept aside for 10 min before measuring the transmittance as a function of the various mixing ratios (chitosan/carbopol 71G).

Preparation of mucoadhesive pellets through FBP

The formulation batches were prepared using a fluid-bed processor [19]. The composition was made with 100 gm of powder containing variable amounts of excipients as shown in Table 1. The salts solutions were used as the binding-liquid with different concentrations (0.05, 0.1, 0.4, and 0.6 mol/L). Process conditions during granulation are, (a) batch size 100 gm, (b) inlet air volume 60 – 70 m³/h, (c) inlet air temperature 40 °C, (d) process time 60 min, (e) spray rate 25 gm/min, (f) purging interval time 20 sec, (g) spheronization time 20 min and (h) rotor rotation speed 700 rpm.

CHARACTERIZATION OF PELLETS***Micromeritic properties***

The angle of repose (θ) was assessed to know the flowability of matrix pellets, by a fixed funnel method using the following equation,

$$\tan \theta = \frac{h}{r} \quad (1)$$

The friability test was performed on the pellets to ensure their mechanical strength and physical stability of the pellets. Pellets of known mass were placed in a Roche Friability tester (Electro lab Friability tester, EF -2, India) and subjected to impact testing at 25 RPM for 5 min. The pellets were passed through a sieve of mesh size 16, weight of pellets retained on the sieve was noted and the friability was calculated using the following equation,

$$\text{Friability (\%)} = \left[1 - \frac{\text{initial weight}}{\text{weight retained after 100 rotations}} \right] \times 100 \quad (2)$$

Scanning electron microscopy analysis (SEM)

SEM was conducted to analyze the morphological behavior of the prepared formulations. The samples were coated with a gold palladium alloy and mounted in a sample holder. The photomicrographs of the samples were recorded with different magnifications under a voltage of 20 kV.

X-ray diffraction analysis (XRD)

The crystallinity and structure of the pure MN and MN loaded pellets were recorded using X-ray diffraction (Phillips PW 1710, Tokyo, Japan) at a current of 30 mA with a voltage of 40 kV. The samples were scanned at a scan rate of 4 °/min.

Determination of drug content

100 mg of pellets were dispersed in 100 ml of methanol. The resulted solution was analyzed spectrophotometrically at 271 nm (Shimadzu-1601, Japan) after suitable dilution with simulated vaginal fluid pH 4.2. The experiments were repeated in triplicate [20].

Swelling Studies

The swelling index of the prepared pellets were determined by weighing 100mg of pellets and record their weights before placing them separately in weighed beaker. SVF pH 4.2 was added to each beaker and allowed to swell until a constant weight is attained in each medium. The pellets were removed and blotted with filter paper, and their changes in weight were measured. The formula for calculation of degree of swelling (α) is as follows given as [21],

$$\alpha = \frac{W_g - W_o}{W_o} \quad (3)$$

W_o = Initial weight of the product, W_g = Final Weight of the product

***In vitro* release studies in vaginal pH**

In vitro release studies were performed using USP XXI dissolution apparatus (make), type II, at 100 rpm and at a temperature maintained at 37 ± 0.5 °C. Accurately weighed quantities of drug loaded pellets were taken in 500 ml dissolution medium and drug release was studied in SVF pH 4.2 and pH 7. 10 ml of samples were withdrawn periodically using guarded sample collectors at regular intervals and immediately replaced with same volume of fresh medium. The withdrawn sample was filtered through a $0.45\mu\text{m}$ membrane filter and after appropriate dilution estimated for MN concentration spectrophotometrically at 271 nm. The experiments were performed in triplicate [22].

***Ex vivo* bioadhesion studies**

Bioadhesive properties of prepared pellets were evaluated by conducting *ex vivo* bioadhesion study employing modified wash-off test method. A freshly excised goat vagina was procured from a local butcher house (Mysore, India) within an hour of slaughter and kept in a cold isotonic saline (4 °C) and cleaned by washing. The goat vaginal tissue was cut-open and tissue was pasted on glass slide using cyanoacrylate glue with mucosal surface facing outside [23]. Drug loaded pellets were exposed to the dissolution medium (30 mins in both pH) before testing. About 20 mg pellets were adhered to intestinal mucosal tissue by applying light force with fingertip for 30 seconds. The glass slide was hung on to arm of USP tablet disintegrating machine which was suspended in 500 mL of simulated vaginal fluid pH 4.2 at 37 ± 0.5 °C and tissue specimen was given slow, regular up and down movement by operating the USP tablet disintegrating test apparatus. The number of pellets adhering to tissue was counted at regular intervals up to 8 h.

***In vivo* X-ray studies**

The animal experiments were evaluated and approved by the Institutional animal ethical committee of the JSS College of Pharmacy, JSS University, Mysore, India (Code: 108/2012). The study was conducted on a healthy female rabbit, weighing between 1 to 1.5 kg. The optimized formulation was selected in order to study *in vivo* performance of the preparation. Optimized formulation was modified by replacing equivalent amount of MN with X-ray grade barium sulfate. The prepared pellets were placed in the vaginal cavity of a healthy rabbit. During the study, the rabbit was not allowed to eat or drink. The rabbit was exposed to X-ray examinations and photographs were taken at 1st and 8th h after administration of the pellet.

Accelerated stability studies of pellets

Stability studies were performed at 40 ± 2 °C and $75 \pm 5\%$ relative humidity (RH) for up to 90 days (Thermolab, India) as per ICH guidelines. Various physicochemical parameters including physical appearance, percentage drug content and drug release profile were monitored periodically for 3 months.

RESULTS AND DISCUSSION

Characterization of the chitosan-carbopol 71 G complex

Figure 1 shows the FT-IR spectrum of chitosan, carbopol 71G and chitosan/carbopol 71G complexes in 2000-1000 cm^{-1} . Protonated chitosan and dissociated carbopol solutions were prepared by dissolving chitosan and carbopol in acetic acid solution and water, respectively. Electrostatic interaction between the COO^- group of carbopol and NH_3^+ group of chitosan in aqueous solution led to the formation of inter polymer complex. By dissolving chitosan and carbopol in acetic acid solution and water respectively, preparation of protonated chitosan and dissociated carbopol solutions were done. 2-aminoglucose units of chitosan and carbonyl group of carboxylic acid in carbopol@ 71G are the possible groups that are involved in the formation of the interpolymer complex. IR spectrum was used to confirm the absorption bands of various groups of interpolymer complex. The carbonyl group of carboxylic acid was assigned the peak at 1712 cm^{-1} in the IR spectrum of carbopol 71G. The amine group of the 2-aminoglucose unit and the carbonyl group of the 2-acetaminoglucose unit of chitosan showed absorption bands at 1575 and 1656 cm^{-1} [24]. Protonation of the amine group to a NH_3^+ group in IPC was indicated by IR spectrum of interpolymer complex, which showed a peak of 1575 cm^{-1} assigned to the amine band of chitosan was shifted to 1635 cm^{-1} . The

bands at 1560 and 1410 cm^{-1} were assigned to the symmetric and asymmetric stretching of the COO^- group; the NH_3^+ band was known to appear between 1600 and 1460 cm^{-1} . Besides, the peak of NH_3^+ groups in the complex between chitosan and poly (acrylic acid) was known to appear at 1518 cm^{-1} [25].

Table 1: Formulation chart of Pellets prepared through FBP

Ingredients	MP1 (%)	MP2 (%)	MP3 (%)	MP4 (%)
chitosan/ carbopol 71G mixture (1:1)	30	30	30	30
MCC	10	20	30	40
TCP	50	40	30	20
MN	10	10	10	10

Microcrystalline cellulose-MCC, Tricalcium phosphate – TCP, Miconazole nitrate - MN

Table 2: Micromeritic properties of prepared pellets

Formulation code	NaCl Concentration (mol/L)	Yield (%) Mean \pm SD*	Average Size(μm) Mean \pm SD*	Angle of repose (θ) Mean \pm SD*	Hardness (N) Mean \pm SD*	Friability (%) Mean \pm SD*
MP1	0.05	77.25 \pm 1.89	1298 \pm 0.89	23.35 \pm 1.26	8.12 \pm 1.56	1.53 \pm 0.78
MP2	0.1	82.14 \pm 1.24	1421 \pm 0.31	22.58 \pm 1.05	7.55 \pm 0.47	1.41 \pm 0.45
MP3	0.4	84.86 \pm 0.65	1523 \pm 0.56	21.47 \pm 1.32	6.52 \pm 0.73	1.43 \pm 0.82
MP4	0.6	88.47 \pm 1.79	1692 \pm 1.01	20.17 \pm 0.59	4.48 \pm 1.07	0.97 \pm 0.93

*Standard Deviation $n=3$

Table 3. Drug loading and encapsulation efficacy of pellets

Formulation	% Drug loading	% Encapsulation efficacy
MP1	15.56 \pm 0.63	93.50 \pm 0.75
MP2	17.97 \pm 0.75	96.69 \pm 0.83
MP3	18.61 \pm 0.86	98.18 \pm 0.46
MP4	17.21 \pm 0.93	95.92 \pm 0.87

*Standard deviation $n = 3$

Table 4A: *In vitro* dissolution data of formulations MPC, MPP and MP3 in SVF pH 4.2

Formulation Code	1 hour	2 hour	3 hour	Drug Release (%)				8 hour
				4 hour	5 hour	6 hour	7 hour	
MPC	24.55	51.62	79.72	91.82	---	---	---	---
	\pm 0.57	\pm 0.34	\pm 0.25	\pm 1.12				
MPP	48.53	67.18	84.88	93.86	96.78	---	---	---
	\pm 0.28	\pm 1.26	\pm 0.38	\pm 0.57	\pm 0.29			
MP3	13.41	26.36	38.36	54.27	64.95	81.27	92.75	96.37
	\pm 0.95	\pm 0.25	\pm 0.92	\pm 1.27	\pm 0.32	\pm 0.84	\pm 0.53	\pm 0.86

*Standard deviation $n = 3$

Table 4 B: *In vitro* dissolution data of formulations MPC, MPP and MP3 in SVF pH 7

Formulation Code	1 hour	2 hour	3 hour	Drug Release (%)				8 hour
				4 hour	5 hour	6 hour	7 hour	
MPC	76.35	89.42	94.72	---	---	---	---	---
	\pm 0.57	\pm 0.34	\pm 0.25					
MPP	34.37	51.41	62.28	73.37	85.43	98.78	---	---
	\pm 0.37	\pm 0.57	\pm 0.33	\pm 0.25	\pm 0.37	\pm 0.43		
MP3	20.32	32.41	45.23	53.28	65.42	78.43	85.75	98.37
	\pm 0.28	\pm 1.23	\pm 0.35	\pm 2.42	\pm 0.27	\pm 1.16	\pm 0.27	\pm 1.18

Figure 2 shows the DSC thermograms of chitosan, carbopol 71G, and the carbopol/chitosan inter polymer complex. At 95 $^{\circ}\text{C}$, appearance of a broad endothermic peak was noted, which can be attributed to bound water. An exothermic peak attributable to the decomposition of chitosan appeared at approximately 320 $^{\circ}\text{C}$ [26, 27]. Sequential decomposition of carbopol was observed approximately at 275 $^{\circ}\text{C}$ in the DSC thermogram of carbopol [28]. The broad endothermic peak of chitosan is ascribed to amine group; therefore the water absorption ability of chitosan depends on the account of its amine group. The smaller endothermic peaks in complex are due to bound water, which is due to the reduction in the amine group due to complexation. Hence, the water absorption capacity

of polymeric complex is higher than chitosan. Due to reduced water absorption capacity, results in slow disintegration of polymeric complex and the drug release will be extended.

Table 5A: Swelling index data of formulations MPC, MPP, MP3 in SVF pH 4.2

Formulation Code	Time in Hours							
	Swelling index Mean \pm S.D*							
	1 hour	2 hour	3 hour	4 hour	5 hour	6 hour	7 hour	8 hour
MPC	80 ± 0.47	105 ± 0.64	136 ± 0.35	170 ± 0.42	---	---	---	---
MPP	27 ± 0.71	58 ± 0.34	108 ± 0.48	172 ± 0.92	207 ± 0.87	---	---	---
MP3	95 ± 0.55	108 ± 0.72	141 ± 0.72	220 ± 0.82	249 ± 0.48	288 ± 0.58	334 ± 0.44	361 ± 0.74

*Standard deviation $n = 3$

Table 5B: Swelling index data of formulations MPC, MPP, MP3 in SVF pH 7

Formulation Code	Time in Hours							
	Swelling index Mean \pm S.D*							
	1 hour	2 hour	3 hour	4 hour	5 hour	6 hour	7 hour	8 hour
MPC	22 ± 0.45	38 ± 0.58	78 ± 0.64	---	---	---	---	---
MPP	39 ± 0.65	87 ± 0.74	142 ± 0.66	180 ± 0.87	298 ± 0.92	355 ± 0.75	---	---
MP3	97 ± 0.67	111 ± 0.83	123 ± 0.89	196 ± 0.78	240 ± 0.92	289 ± 0.97	330 ± 0.92	364 ± 0.85

*Standard deviation $n = 3$

Table 6: Stability studies data of MP3 formulation

Condition	Sampling interval (months)	Physical appearance	% Drug content (mean \pm S.D*)
25 \pm 2 $^{\circ}$ C/60 \pm 5% RH	0	No change	99.50 \pm 0.50
	3	No change	99.48 \pm 0.22
	6	No change	99.47 \pm 0.14
30 \pm 2 $^{\circ}$ C/65 \pm 5% RH	0	No change	99.50 \pm 0.50
	3	No change	99.49 \pm 0.12
	6	No change	99.48 \pm 0.10
40 \pm 2 $^{\circ}$ C/75 \pm 5% RH	0	No change	99.50 \pm 0.50
	3	No change	99.47 \pm 0.11
	6	No change	99.46 \pm 0.13

The X-ray diffraction pattern of chitosan powder showed two prominent diffraction peaks at 11 $^{\circ}$ (2 θ) and 20 $^{\circ}$ (2 θ) as shown in Figure 3. A shoulder peak and a minor peak appear at 22 $^{\circ}$ and 27 $^{\circ}$ respectively. Typical fingerprints of chitosan that were related to the hydrated and anhydrous crystals are the two prominent crystalline peaks at 11 $^{\circ}$ and 20 $^{\circ}$ respectively. The X-ray diffraction pattern of carbopol showed a prominent diffraction peak at 20 $^{\circ}$ (2 θ) and a minor peak at 35 $^{\circ}$ (2 θ); whereas that of the IPC showed a prominent diffraction peak at 22 $^{\circ}$. The formation of hydrogen bonding between amino groups and hydroxyl groups was hindered as the carbopol was introduced into chitosan, which disrupted the crystalline structure of chitosan and this can be evident from the disappearing of the typical peaks of chitosan after complexing.

Turbidity measurements of chitosan-carbopol 71G complex ratios

To determine the composition of the complex, the variation in transmittance as a function of chitosan to carbopol 71G (unit molar ratio) was measured, this is depicted in Figure 4. The aqueous acetic acid solution of chitosan and the aqueous solution of carbopol 71G were transparent, which did not relate to their concentration prior to mixing. No significant change was observed when the ratio of chitosan/carbopol was increased (1:1). Transmittance decreased as the ratio was altered from 1:1 to 1:4, which showed that a higher ratio does not signify change in transmittance. Due to the saturation of the electrostatic interaction sites of chitosan by carbopol, excess of chitosan did not react with carbopol. No significant change in transmittance was observed as the carbopol/chitosan ratio was changed from 1:1 to 1:4 (the amount of carbopol was fixed at 0.5 mM), confirming poor formation of inter polymer complex. The transmittance results clearly show that the complexation unit molar ratio of chitosan with carbopol

was 1:4. Hence, the chitosan and carbopol inter polymer complex with the mixing ratio 1:4 were used to characterize the complex and to study the release profile.

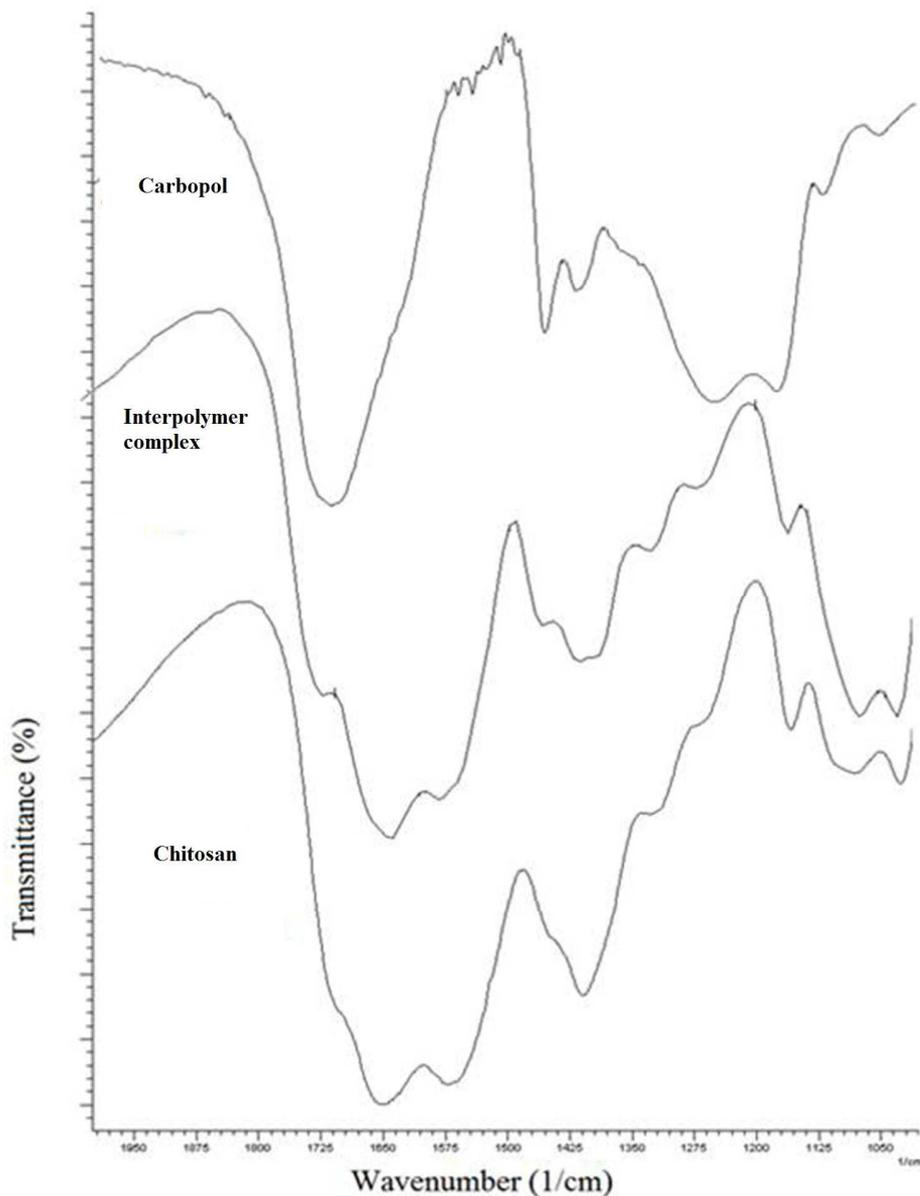


Figure 1: FT-IR spectrum of chitosan, carbopol 71G and chitosan/carbopol 71G complex

Formulation of mucoadhesive pellets through FBP

The production of pellets containing mucoadhesive polymer using extrusion spheronization method has been well reported in literature [29, 30]. Due to its swelling and gelling properties, the reduction of tack is necessary for a successful formulation, which otherwise cause's serious problems regarding formulation processes. According to the previous literature various agents such as sodium chloride, talc, glyceryl monostearate etc. were added as an anti-tack agent [31, 32].

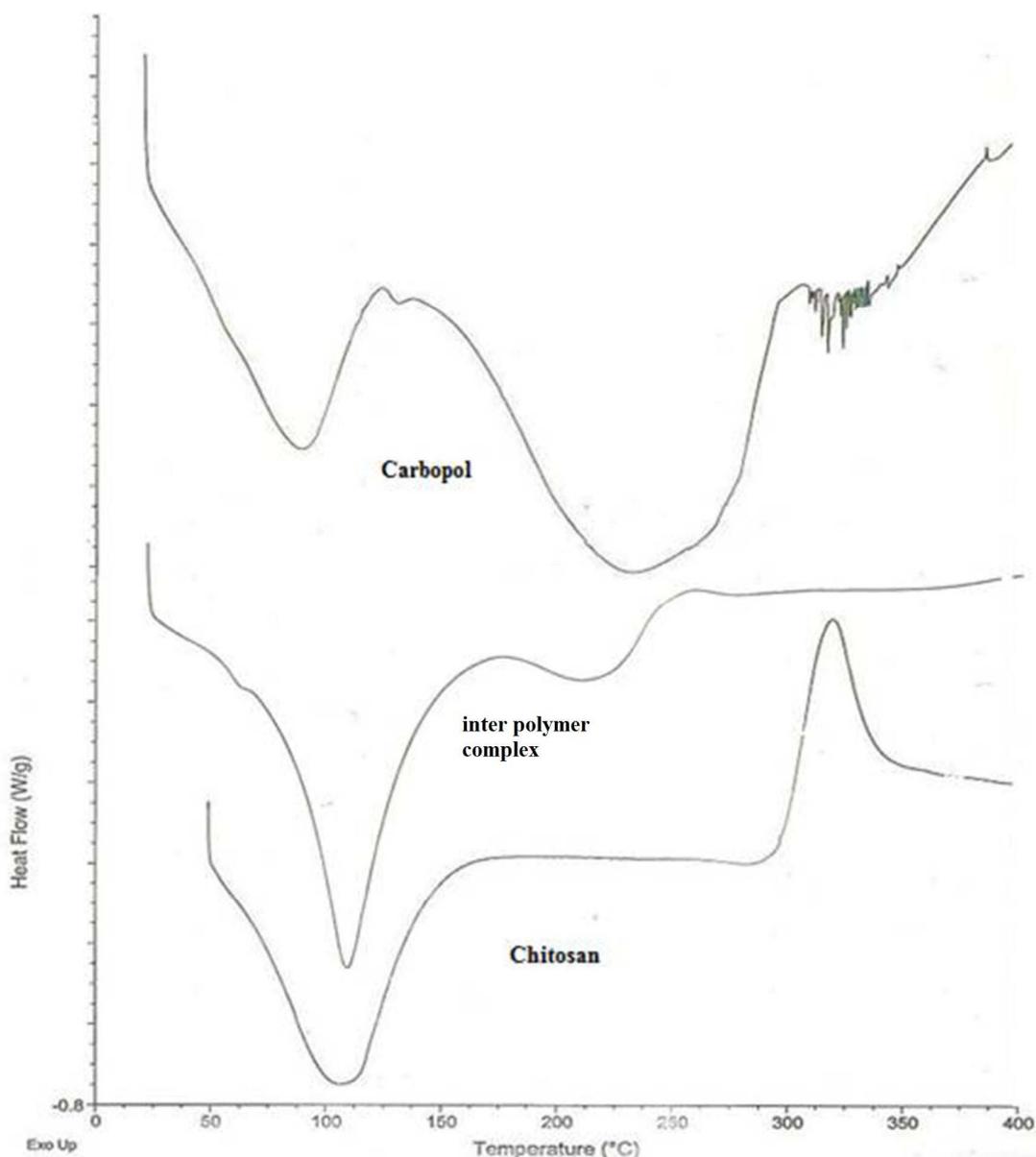


Figure 2: DSC thermogram of Chitosan, complex and Carbopol

Effect of salt solution in preparation of pellets through FBP

Different concentrations of NaCl salt solution (0.05, 0.1, 0.4, and 0.6 mol/L) were used as the binding-liquid; as the concentration of salt solution increased, better yield of pellets was observed. Further, pellet's hardness was decreased as the salt solution concentration increased which could be due to the salting-out effect of sodium chloride. Salt solution reduced the tack and decreased the gel formation, which results in reduced hardness of the pellets.

Physicochemical properties of MPECP

MPECP containing various ratios of chitosan, carbopol 71G and IPC loaded with MN were formulated and their technological properties were evaluated. The average size, angle of repose (θ), hardness and friability of the obtained MPECP are presented in Table 2. The values of angle of repose was in the range of 20.17 to 23.35, indicating that the values were within the limits & prepared pellets had reasonably good flow potential. The size (1298 to 1692 μm) & % yield (77.25 to 88.47 %) of the pellets was found to be increased, as the MCC ratio was

increased. However, increased ratio of MCC showed decreased friability & hardness of the prepared pellets. From the obtained data MP3 formulation was selected for further studies.

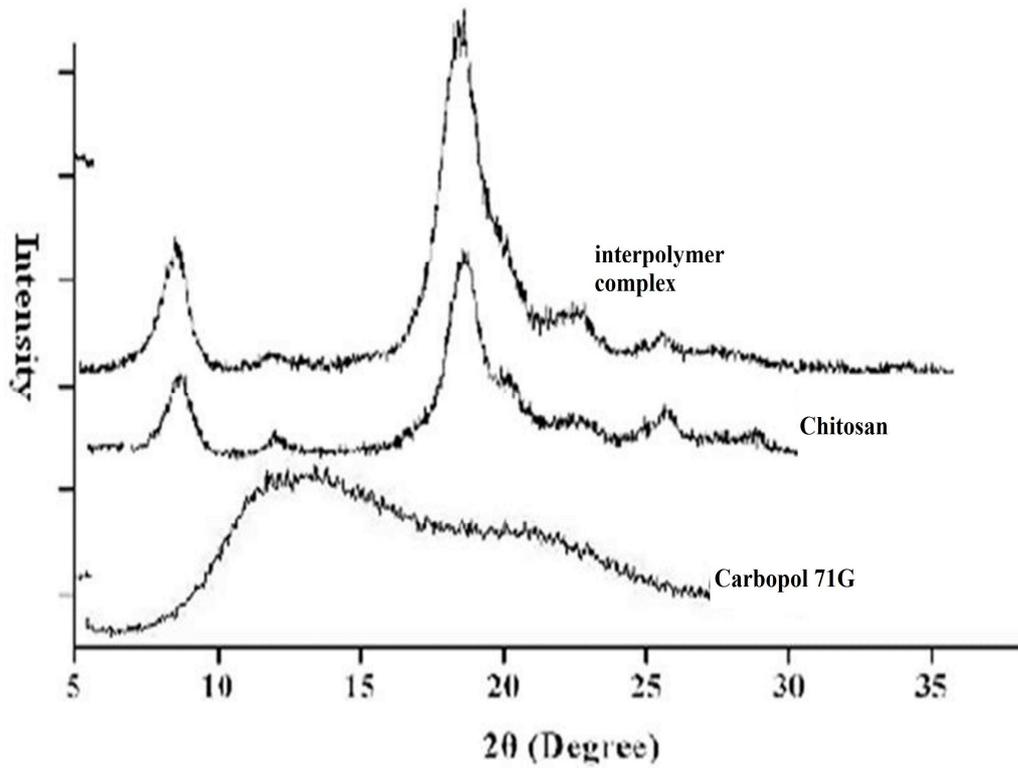


Figure 3: X-ray diffraction spectra of chitosan, complexMP3 and carbopol

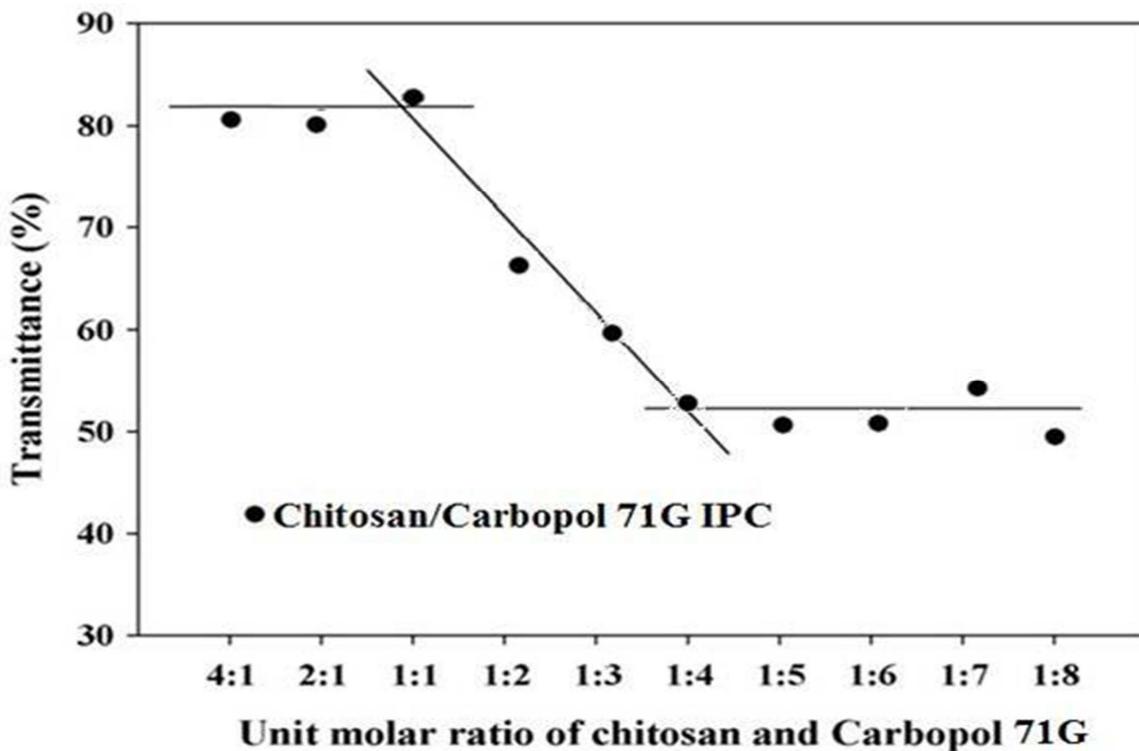


Figure 4: Transmittance of solutions of chitosan and carbopol 71G in different ratios

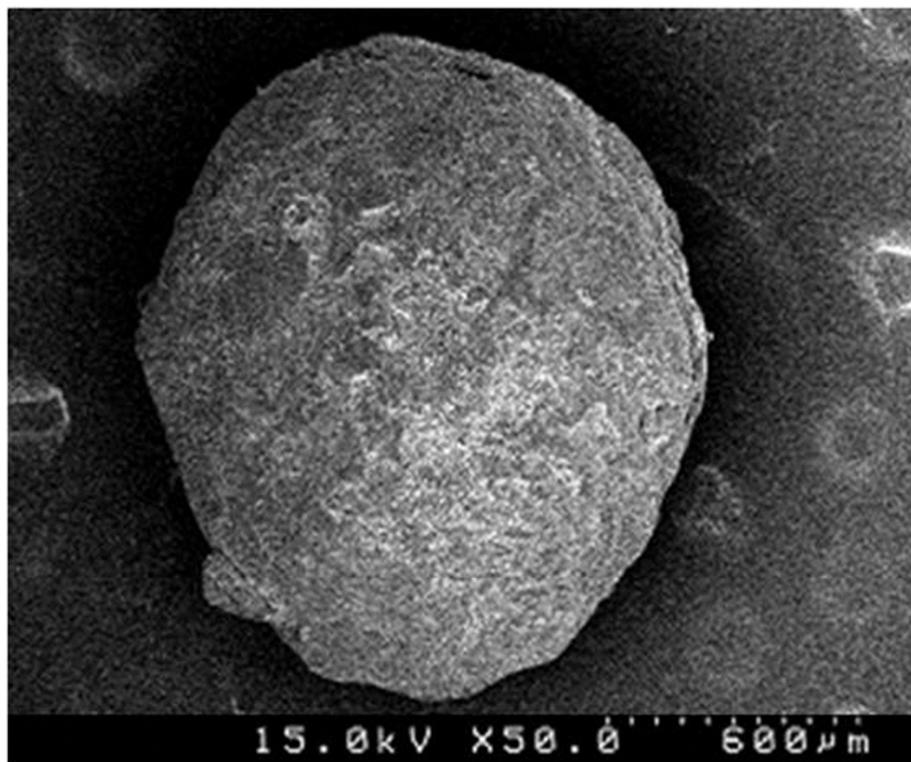


Figure 5: Scanning electron microscopy of pellet

Figure 5 showed the Scanning electron micrographs of the prepared MPECP. It was observed that the MPECP were spherical in shape with outer rough surface and surface dents. The dents on the outer surface of the pellets were developed during drying. SEM photo micrographs displayed the absence of drug particles on the pellet surface showing uniform distribution of the drug in the pellet. As the concentration of MCC (40% w/w) was increased in powder blend, resulted in the production of spherical, solid, discrete, free flowing pellets. Figure 6 and Figure 7 showed the FT-IR and DSC spectrum of MN and complex MP3 respectively. The results revealed that there was no incompatibility between drug and polymer.

Drug loading and encapsulation efficacy

The formulations were found to have the percentage drug loading in the range of 15.56 to 18.61; 93.50 to 98.18% was found to be the encapsulation efficiency percentage and the so obtained results are shown in Table 3. As the polymer concentration increases, the entrapment efficiency and the drug loading increase. It can thus be inferred that the pellets have proper distribution of drugs and the deviation is within the acceptable limits.

***In vitro* dissolution studies in vaginal pH**

The dissolution studies were conducted in USP type II dissolution apparatus using simulated vaginal fluid pH 4.2 and pH 7 as the dissolution medium (Figure 8). Release pattern of all the formulated pellets were tested for a period of 8 h, and are shown in Table 4A and 4B. Chitosan pellets (MPC) showed a slower drug dissolution rate in SVF pH 4.2 when compared to pH 7. 90 % of MN was released in 4h, which could have resulted because chitosan forms gel at low pH and this in turn slows down the pellet's drug release rate.

pH of the dissolution medium influences the rate of drug dissolution in the case of MPP formulations. The carbopol pellets showed an initial burst effect in the pH 4.2 and within 5 h, entire drug was released. Rapid drug release might have resulted due to the less viscous gel layer around the pellet as the carboxylate group of carbopol might not dissociate at pH 4.2. The complex containing formulations encountered swelling-driven phase transition from a glassy state to a rubbery state upon contact with simulated vaginal fluid (pH 4.2), the molecules diffused rapidly. Here, the rate of MN release is influenced by thickness of gel layer and the water transport rate. As the complex hydrates, the gel layer dissolves slowly releasing MN.

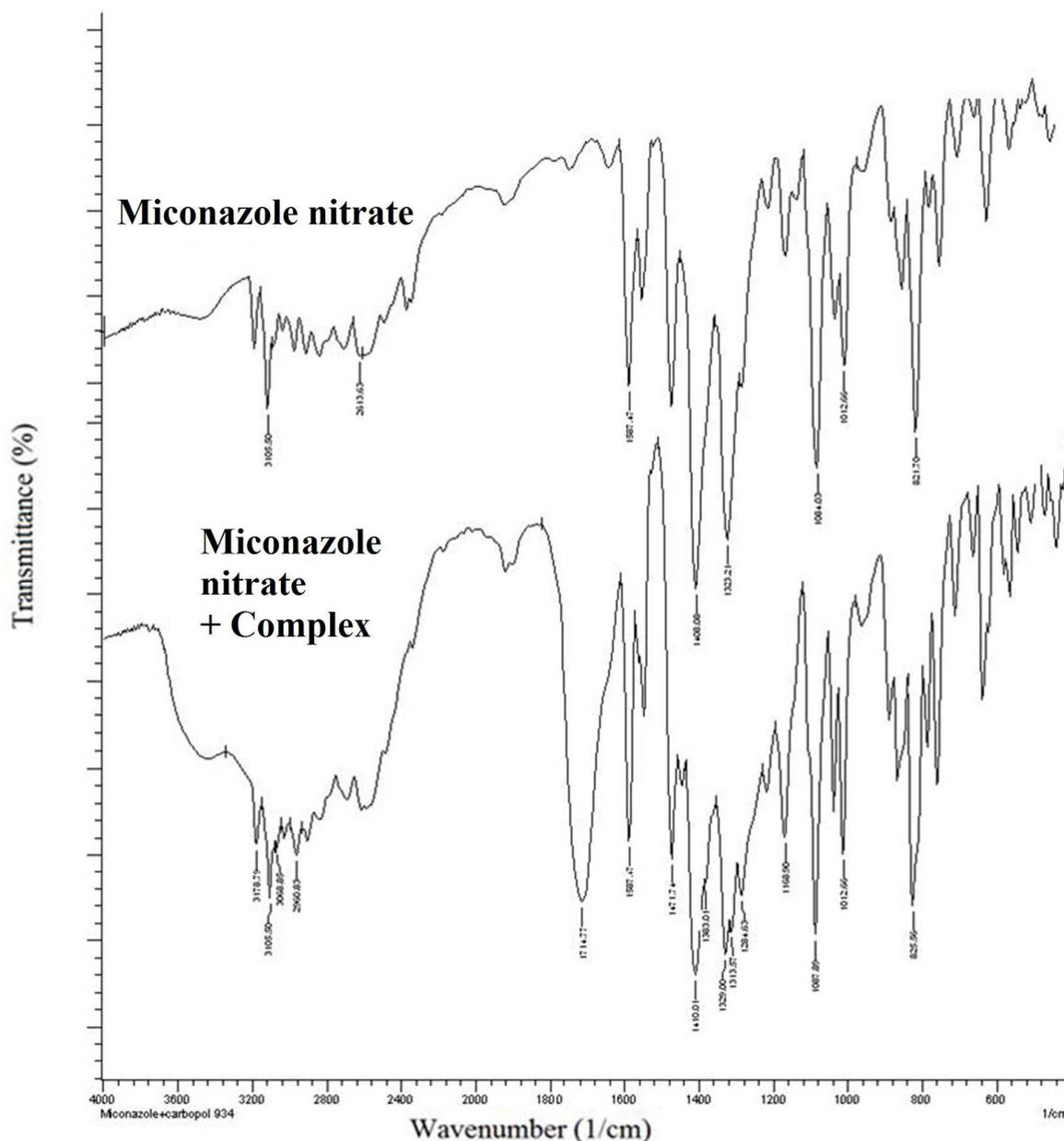


Figure 6: FT- IR Spectra of MN and Complex MP3

When compared with other formulations tested in pH 7, chitosan pellets (MPC) showed faster rate of drug dissolution whereas it showed 90% of drug release in 3 h. The reason for this could be their inability to form gel layer around the pellet and the characteristic of chitosan easy disintegration at neutral pH. pH of the dissolution medium influenced the drug dissolution rate in case of MPP formulation containing carboxypol. Fewer and smaller regions of micro viscosity were resulted when the carboxylic acid groups underwent ionization, which in turn caused maximum swelling in pH 7. Drug release is prolonged till 6 h due to the rapid formation of gel that acts as a barricade for MN release.

The complex containing MP3 formulation encountered swelling-driven phase transition from a glassy state to a rubbery state upon contact with pH 4.2, the molecules diffused rapidly. Here, the rate of drug release is influenced

by the formation of gel layer around the pellet. The water transport rate and the gel layer thickness controlled the rate of drug release. As the complex hydrates, the gel layer dissolves slowly releasing drug.

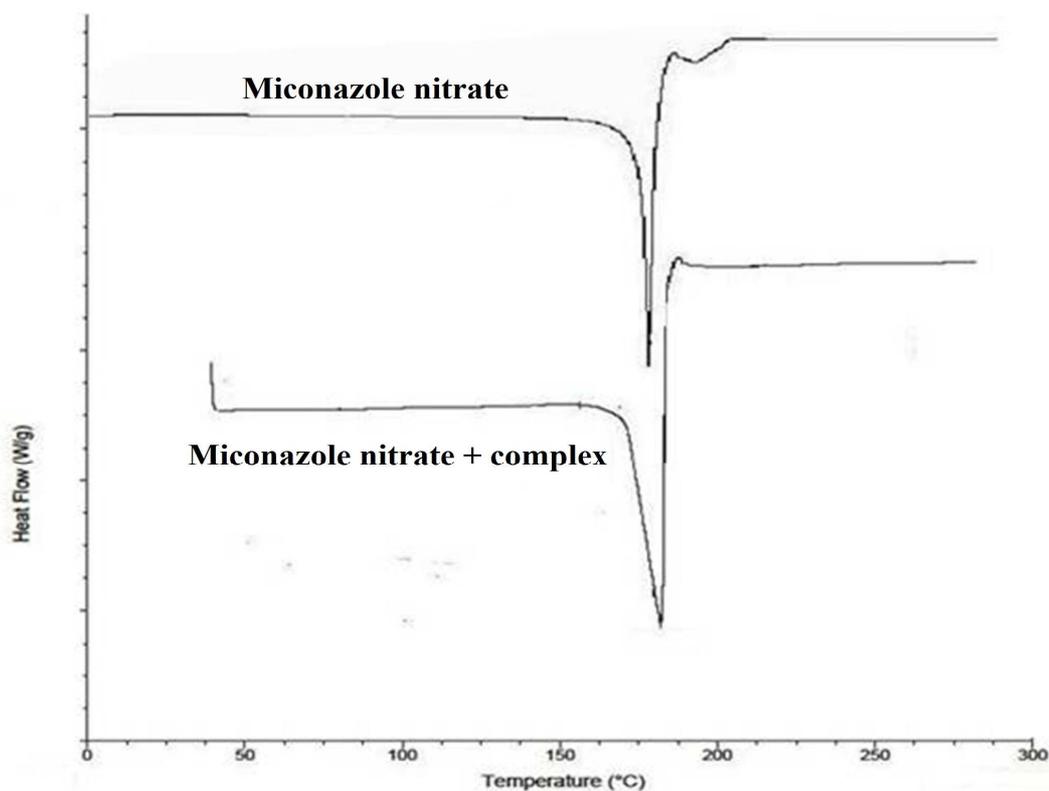


Figure 7: DSC thermogram of MN and Complex MP3

Initial burst effect was produced by the disintegration property of chitosan and its inability to form gel layer in neutral pH and exhibition of the pH independent drug release due to the presence of complex in pellets was indicated from the obtained results. To know the similarity of dissolution profiles of MP3 formulation between simulated vaginal fluid pH 4.2 and simulated vaginal fluid pH7, the similarity factor (f_2) was calculated and it was found to be 88.15. Since, the f_2 values were higher than 50 (as per USFDA guidelines), these results confirmed that the drug release profiles were almost similar for MP3 formulation for both the vaginal pH. Release data were fitted into release models using PCP Disso V2.01 dissolution software to determine the drug release mechanism. To know the release mechanisms, parameters like 'n' the time exponent and 'R' the regression co-efficient were determined. Results Model fitting of formulation MP3 in both pH was found to be following zero order kinetics with regression co-efficient value of 0.9902 and 0.9953 for 4.2 and 7 pH respectively. The n (0.8142) value in peppas is between 0.5 to 1 indicating non-fickian diffusion as the release mechanism.

Swelling studies

Swelling is an important characteristic as it affects mucoadhesion as well as drug release profiles of the polymeric drug delivery systems. It is also a parameter that has to be studied before considering mucoadhesion; the swelling results were expressed in terms of swelling index. The swelling index data of the individual formulations in simulated vaginal fluid (SVF) pH 4.2 and SVF pH 7 are shown in Table 5A and 5B, respectively.

With the condition of pre menopause and absence of bacterial pathogens, the vaginal pH is consistent with 4.2; and the pH of 6.0 to 7.0 is strongly suggestive of menopause in the absence of bacterial pathogens. The swelling studies were therefore carried out in pH 4.2 and pH 7. Due to the entry of water via metastable pores in the pellets, the

swelling occurs rapidly in the initial stages. This mechanism is known as hysteresis of the swelling that is followed by swelling as a result of diffusion processes. An intact hydrated layer established over the period of study, diffusion may be the most important factor controlling the rate of drug release from the system diffusion. By swelling-controlled mechanism, drug release could occur from hydrophilic matrix.

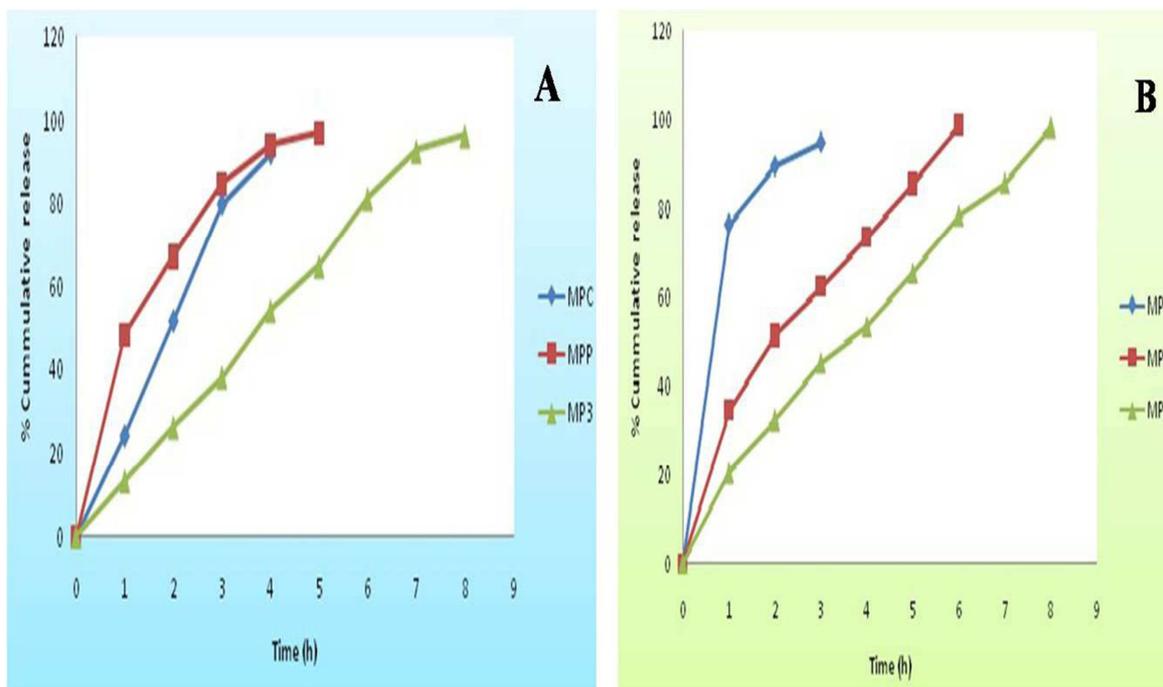


Figure 8: In vitro Dissolution studies of formulations MPC, MPP and MP3 in SVF (A) pH 4.2 and (B) pH 7

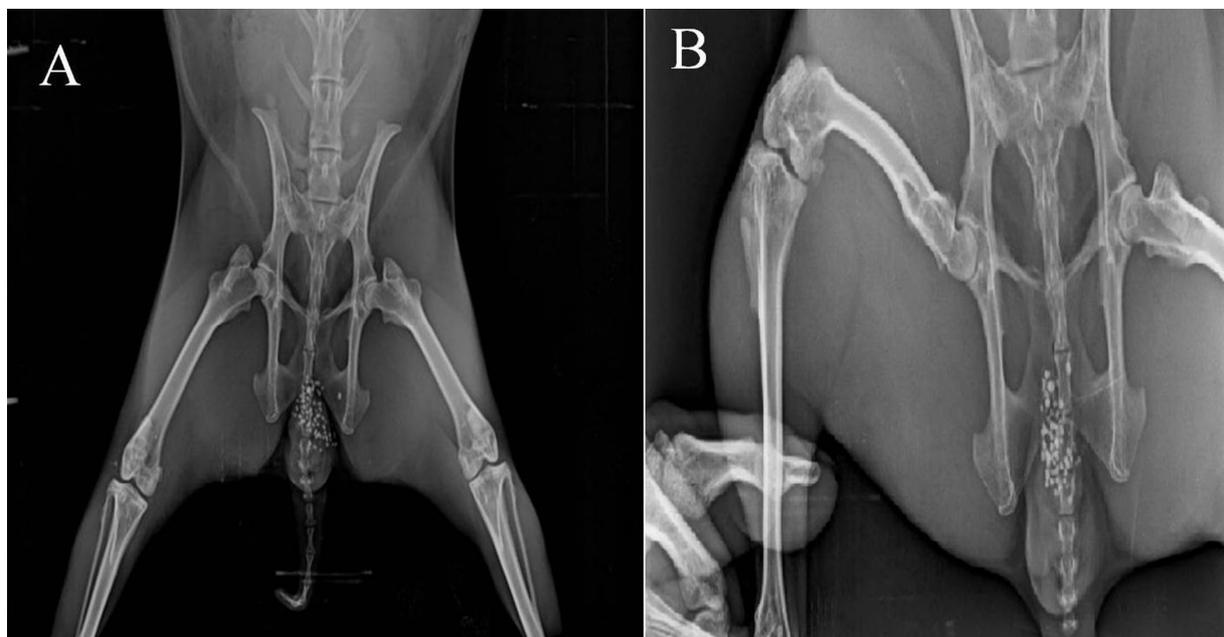


Figure 9: X-ray radiographic images of vaginal cavity at (A) 1 h and (B) 8 h after ingestion of BaSO₄-loaded optimized MP3 pellets in rabbit

Least swelling index was exhibited by the formulations containing chitosan (MPC) alone in both SVF pH 4.2 and simulated vaginal fluid pH 7. In acidic pH, MPC can form gel layer around the pellet and therefore, simulated vaginal fluid's pH is more (4.2); whereas formulations containing carbopol (MPP) showed swelling up to 8 h. Dissociation of carboxylic group occurring in neutral pH and basic pH is directly responsible for the swelling property of carbopol. Therefore, swelling index of carbopol containing formulations is more in phosphate pH (pH 7) when compared to that of the simulated vaginal fluid (pH 4.2). Swelling ratios of the pellet formulations at various pH environments depend upon the available free volume of the expanded polymer matrix, polymer chain relaxation and availability of ionizable functional groups such as $-\text{COOH}$ able to form hydrogen bonds with dissolution medium. Less swelling in pH 4.2 than in pH 7 was showed by the formulation (MPP) containing carbopol only. Swelling gradually increases in pH 4.2 because, pKa of any polymer containing carboxylic acid is about 4.2 and at a pH > 4.2 , the carboxyl groups of carbopol tend to dissociate, thereby increasing the osmotic pressure. Gradual increase in swelling and almost similar swelling index in both pH 4.2 and 7 were shown by the formulation containing complex. It can thus be inferred that slow uniform pH independent swelling degree can be exhibited by the presence of complex.

Ex vivo bioadhesion studies

Increased availability of adhesive sites of natural polymer with mucin might have caused the enhanced mucoadhesive strength, which in turn leads to the increased bond strength. Polymer swelling permits a mechanical entanglement by exposing the bioadhesive sites for hydrogen bonding and/or electrostatic interaction between the polymer and the mucous network and also building of secondary bonds favoring chemical and mechanical interactions. 98% of the mucoadhesive pellets strongly adhered to the vaginal mucosa and the adhered pellets could be retained for 8 h; this was confirmed by the *ex vivo* mucoadhesive tests. This shows that the pellets have the ability to remain localize on vaginal mucosa.

In vivo X-ray studies

The project proposal has been cleared and approved by Institutional animal ethical committee, J.S.S. College of pharmacy, Mysore (Code: 108/2012). Albino rabbits were used to evaluate the mucoadhesion and retention property; the X-ray photographic images are shown in Figure 9 A and 9B. The rabbit was administered with the optimized formulation of MP3 that was developed using barium sulfate and radiograms were used to monitor the duration of pellet in the vaginal cavity. Apparently, the pictures showed that the pellets remained intact, adhered to the vaginal mucous membrane for 8 h and showed swelling.

Kinetic Analysis

Based on the drug release behavior it was observed that the prepared mucoadhesive pellets followed zero order release for MN. MP3 formulation exhibits the controlled release profile with zero order release ($R= 0.9984$) in vaginal pH 4.2, which might be due to the rapid swelling and erosion.

Stability studies

Stability studies of pellets formulation MP3 was carried out to determine the physical stability of the formulation. The stability studies were carried out at 25 ± 2 °C and 60 ± 5 % RH, 30 ± 2 °C and 65 ± 5 % RH and 40 ± 2 °C and 75 ± 5 % RH for 6 months. The drug content in the formulation was evaluated. The observation of conditions is shown in Table 6. There was no significant change in the pellet properties and drug content.

CONCLUSION

Modest and reproducible methods were used to prepare the mucoadhesive complex pellets. The complexation unit molar ratio of chitosan with carbopol was shown to be 1:4 of the transmittance results. Cross-linking between chitosan and carbopol was confirmed by the FT-IR and DSC studies; the crystal lattice change was confirmed by XRD studies. In order to establish the compatibility between the drug and the polymers by FT-IR and DSC studies, pre-formulation studies were carried out and the results showed that the drug and the polymers were compatible. Using MCC, tri-calcium phosphate, chitosan-carbopol complex and drug, through FBP, the mucoadhesive pellets were prepared successfully. The obtained pellets were known to be spherical and had good flow property; this was confirmed from the results of the micromeritic properties. As the polymer concentration increased, drug loading and entrapment efficiency simultaneously increased. It could be concluded from the results that drug in the pellets were properly distributed and the deviation was within the acceptable limits. The surface topography of the pellets showed rough surface could be observed with optimal, spherical shape through the obtained scanning electron

micrographs. Uniform distribution of the drug in the pellet was seen as the SEM photographs revealed the absence of drug particles on the surface of pellets. It was observed that the formulation containing complex exhibited gradual increase in swelling from the swelling studies; almost similar swelling index in both pH4.2 and pH 7 was exhibited by the formulations containing complex. The results indicated that a slow uniform pH independent swelling degree was shown in the presence of complex. Presence of complex in pellets displayed pH independent drug release; this was indicated by the *in vitro* studies. 98% of the mucoadhesive pellets adhered strongly to the vaginal mucosa, which could be retained there for 8 h and this was confirmed by the *in vitro* mucoadhesive tests. It was also evident from the pictures of *in vivo* X-ray studies that the pellets remained intact, showed swelling and adhered to the vaginal mucous for about 8 h. Stability studies for MP3 formulation illustrated no significant change in the pellet properties and drug content.

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