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DEVELOPMENT OF PH-INDEPENDENT MUCOADHESIVE PELLETS FOR EFFECTIVE TREATMENT OF VAGINAL CANDIDIASIS

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ABSTRACT: Objective: The present investigation aims to develop pH-independent mucoadhesive pellets using the extrusion-spheronization procedure and the inter-polyelectrolyte complex (IPEC) of Chitosan-Carbopol 71G. Miconazole was used as a representative drug in this study. **Materials and Method:** It was found that the composition of the formulation impacts the extrusion of the prepared dough mass. The generation of pellets within a narrow size range was also studied as a function of spheronization speed and time. The precipitation method was utilized to create IPEC. Compatibility between the drug and the additives was confirmed through FT-IR and DSC testing. **Results:** The MP3 formulation demonstrated consistent swelling across a wide pH range, without any abrupt alterations in swelling at the pH study points of 4.5 or 7. Evidence from *in-vivo* X-ray scans indicated that the pellets remained intact in the vaginal canal for an approximate duration of 8 hours. Short-term stability studies of the formulation affirmed its consistency throughout the investigation. **Conclusion:** The study showed that a Chitosan-Carbopol 71G interpolymer complex can function as a pH-independent drug delivery system for the sustained release of miconazole nitrate. This makes it beneficial for treating candidiasis.

INTRODUCTION: Vaginal candidiasis, which is caused by species of the genus Candida, is the most common and consequential vaginal fungal disease [1, 2]. At least one case of vaginal candidiasis has been documented among women of childbearing age. It has been suggested by point-prevalence investigations that Candida species can be isolated from the vaginal tract of asymptomatic, healthy women of childbearing age. Candida species is the most prevalent causative agent of vaginal infections in Europe, but only the second most common in the United States.

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Candida overgrowth in the genital tract has been linked to psychological and emotional distress [3-5]. Vaginal candidiasis risk factors include pregnancy (30-40%), oral contraceptives with a high oestrogen content, chemotherapeutics, antibiotics, steroids, STI clinic attendance, and advanced age [6]. The increased production of reproductive hormones during pregnancy favours the genesis of infection. Epithelial tissues may be directly invaded by infection hyphae from candida through a variety of ways, including but not limited to those listed above.

During vaginal candidiasis, vagina in the usual pH range (pH 4.0-4.5), as contrast to mixed infections (bacterial, trichomonas), where pH rises to values more than 4.7. In the previous few decades, C. albicans infection rates have been rather high, ranging from 85% to 90%. Vulvo-vaginal itching, irritation, pain, burning, and dyspareunia [7-11] are

often reported symptoms of vaginal candidiasis. A vaginal examination may reveal erythema or a thick, curdy discharge [5, 12], as well as redness, swelling, fissures, or excoriations in the vulvar area. The absence of bacteria and a vaginal pH of 4.5 are both indicative of being in the premenopausal stage of life. When the pH of the vagina is over the normal range (which is between 5.0 and 6.5), it is likely that bacteria are to blame. A vaginal pH between 6.0 and 7.0, in the absence of bacterial infections, is highly indicative of menopause [13-16]. In light of this, the final formulation should show consistent swelling and release in both the low (pH 4.5) and high (pH 7) pH environments seen in the vaginal cavity.

Most pellets are made by the extrusion-speronization process, which is used extensively [17]. If the right excipients and fillers are utilised, pellets with the desired properties can be manufactured. Advantages of spherical pellets include their large surface area, superior flow characteristics, and uniform packing [18, 19]. Because of their uniform shape, pellets can be easily coated to add aesthetic value or to regulate the release of active ingredients. When it comes to excipients used in extrusion-speronization, microcrystalline cellulose (MCC) is the most popular choice. Formed spheres exhibit excellent properties [20] due to this effect. Because of the moisture trapped in the MCC microfibrils, speronization eventually improves the extrudates' plasticity [21], allowing for the formation of spherical pellets from short extrudates. Miconazole was employed as a model medicine in this work to treat vaginal candidiasis, and a pH-independent release pellet containing chitosan-Carbopol 71G IPEC (Inter polyelectrolyte complex) was used as a bio adhesive polymer.

MATERIALS AND METHODS: Sigma Aldrich was contacted for the purchase of chitosan and Carbopol 71G. Micro Labs Ltd. generously provided the miconazole (Bangalore, India). LobaChemie was the source for the microcrystalline cellulose (MCC), lactose anhydrous, and Avicel® PH 200. (Mumbai, India). In addition, only analytical reagent-grade chemicals and reagents were used in the present investigation.

Development of Chitosan-Carbopol 71G IPEC: There was a combination of mix 1mg of Carbopol 71G in 1ml of water, and 5mg of chitosan acetic acid in water. We filtered the Carbopol /chitosan complex precipitate using a vacuum to remove any impurities. After drying the filtrate in a hot air oven, the complex was crushed in a mortar and pestle. Powder was sieved using a 200 m screen for use in subsequent experiments.

Chitosan-Carbopol IPEC Percentage test of Suspended Solids: Using a spectrophotometer set to measure transmittance at a wavelength of 600 nm, we analysed the percentage of Carbopol 71G to chitosan in the structure (UV-1800, Shimadzu, Japan). 0.5, 1, 1.5, 2, 2.5, 3, 3.5, and 4 mM of Carbopol 71G aqueous solution and 0.5, 1, and 2 mM of chitosan acetic acid aqueous solution were utilised. Chitosan and Carbopol were weighed and then the formula weight of each monomer unit was used to determine the concentration. Each of the concoctions was given a good shake. After letting the combinations sit for 10 minutes, the transmittance was measured in relation to the chitosan/Carbopol 71G ratios.

Preparation of Pellets through Extrusion/Spherization: For the preparation of spheroids, Extruder (EXT-65/037, R.R. Enterprises, Mumbai, India) and Spheronizer (SPH-150/010, R.R. Enterprises, Mumbai, India) were utilised. As a spherization booster, MCC was incorporated into the spheroid formulation. Here, several lots were made up of MCC-IPEC-drug with various weight-to-weight (w/w) proportions, such as 85%: 5%: 10%, 80%: 10%: 10%, 75%: 15%: 10%, 70%: 20%: 10%, 65%: 25%: 10% and 60%: 30%: 10%. Powder mixtures were made in 10-minute batches of 100 grammes by geometric mixing in a plastic bag. The aforementioned dry blend mixture was then granulated with demineralized water. An extruder of the cylinder roll type was used to squeeze the liquid out at a speed of 40 revolutions per minute. The opening diameter was 1 millimetre. A spheronizerwith a 150 mm in diameter and 2.5 mm thick cross-hatched rotor plate was used to spheronizer the resulting extrudates.

The spheres that resulted were dried in a hot air oven (Memmert 30, Germany) at 40 degrees Celsius for 8 hours. Extrudates from all the chosen MCC-IPEC-drug ratios were spheronizer at varying

rates (400, 600, 800, 1200, 1400, and 1600 rpm) to determine the optimal speronization speed. The optimal batch of spheroids was spheronizer at 1200 rpm for various times (5, 10, and 15 minutes) to determine the optimal speronization time.

Fourier Transform Infrared Radiation Measurements (FT-IR): Using an FT-IR spectrophotometer, we established the drug and excipient compatibility and polysaccharide modification (shimadzu 8400S). The mixture was compressed with potassium bromide at a ratio of 1:100 to create pellets. We did the tests three times to be sure.

Differential Scanning Calorimetry (DSC): The shimadzu DSC thermal analyser 60 was used for all of the dynamic DSC analyses. The instrument's precision was checked against a standard made of high-purity indium metal. Scans were performed in an environment of nitrogen at a heating rate of 10°C per minute. We did the tests three times to be sure.

Diffraction of X-rays by Powders: An X-ray diffraction study was conducted in powder form on a variety of polymers as well as their modified equivalents (Rigaku Corporation Miniflex II Desktop XRD Tokyo, Japan). The scan angles for the samples varied anywhere from 6 degrees to 40 degrees (2), with a resolution of 0.02 degrees and a measuring time of 10 seconds per degree.

Characterization of Spheroids:

Particle size Analysis: Malvern master sizer 2000 version 5.1 was used to determine the average particle size of the synthesised spheres (Malvern, UK). The pellets containing the active ingredient were diluted 1:20 with methanol and the concentration of the medication was determined at 37 degrees Celsius.

Angle of Repose: The fixed funnel method was used to determine the angle of repose of spheres and thus their flowability. Slowly, the spheres were poured through the funnel until a cone-shaped pile had formed at the end of the tool. We then calculated the pile's height (h) and its radius (r). Following this equation, we were able to determine the angle of repose (θ) for each sample (1).

Angle of repose (θ) = hr (1)

Percentage Yield: It is of the utmost importance to know whether or not the preparation process chosen for introducing a medicine into the polymers is successful. Raw materials, quantity of active component, Micro Crystalline Cellulose, and other process factors all impact product yield while making pellets.

The yield was calculated by weighing pellets containing the active ingredient as a percentage of the total drug and polymer weights utilised in the process. Yield percentage can be determined using the formula in equation (2)

Yield = Pellet weight / Medicine weight + 100 is the method for determining the yield of the product

How much polymers weigh multiplied by a hundred (2)

Effectiveness of the Drug loading and Encapsulation: A drug's loading has a significant impact on its release characteristics. Rapid drug release can be expected when drug loading is increased. The effectiveness of drug entrapment is measured as a percentage of the total amount of drug that was initially added that is now contained within the pellets.

Weighing 100 mg, pellets of the medication were added to a volumetric flask containing 100 ml of methanol. A 1 ml sample was taken from here and diluted to the correct concentration in volumetric flask of ten millilitres it takes another 1 ml of this solution to be adjusted to 10 ml.

Equation was used to derive the medication concentration (3).

Dosage = concentration from standard graph divided by 1000 (3)

If you take 100 mg of pellets and divide the amount of medicine by that amount, you get the encapsulation efficiency in percentage. The formula in equation is then used to determine the value (4).

Percentage Encapsulation Efficiency = ba × 100 (4)

Given that a represents the total amount of drug in theory and b represents the amount of drug actually trapped,

Scanning Electron Microscopic (SEM) Studies: Scanning electron microscopy (SEM) images were captured at room temperature with a JEOL-LV-5600 (USA) scanning electron microscope at the appropriate magnification. To verify the pellets' sphericity and examine their morphological properties, we looked at the images.

Swelling Studies: As a means of calculating the swelling index, 100mg of the produced mucoadhesive miconazole pellets were weighed and recorded before being divided across weighed beakers. Weighing in at a grand amount, the (W1). Each beaker contained four millilitres of pH 4.5 and 7 vaginal saline and was then placed in an incubator at 37 0.5 °C. Swelled pellets were weighed after 2, 4, 6, and 8 hours after meticulous removal of excess water (W2). The average W1 and W2 values were reported after the experiment was conducted three times. From this equation, we were able to calculate the swelling index (SI) (5).

$$SI=W2-W1W1 \times 100 \quad (5)$$

***In-vitro* Release Studies:** Two different 500 ml jars of pH 4.5 and pH 7 simulated vaginal fluid were used to test the medication release rates from mucoadhesive vaginal pellets. There was a constant 370.5 °C and 50 rpm in the room. Every hour, 10 mL of the solution was taken, filtered through a Millipore filter with a pore size of 0.45 m, and analysed using spectrophotometry set to detect wavelengths of 265 nm. Amounts of simulated vaginal fluid at pH 4.5 and pH 7 were added to the dissolving medium immediately following sample removal.

***Ex-vivo* Bio adhesion Studies:** In an *ex-vivo* bio adhesion research using a modified wash-off test method, the pellets were tested for their bio adhesive characteristics. Within an hour of slaughter, the reproductive organ, or obtained vagina, was bought at the marketplace, placed in cold isotonic saline (4°C), and washed. Cut vaginal tissue was glued on a glass slide with cyanoacrylate adhesive, mucosal side out, with the outside facing the lab. The drug-filled pellets were tested after being subjected to the dissolution media for 30 minutes at each pH level. Pellets weighing around 20 mg were adhered to intestinal mucosal tissue by pressing gently with the fingertip for 30 seconds. The tissue specimen was given slow, regular up and down movement by operating the USP pill dissolving test device, which involved suspending the glass slide on the arm of the machine and moving it up and down in 500 mL of SVF pH 4.5 at 37 0.5 C. Pellets stuck to tissue were counted at set intervals for up to 8 hours.

***In-vivo* X-ray Studies:** The research was conducted on a 1-1.5 kilogramme female rabbit in good health. For the purpose of investigating the preparation's efficacy *in-vivo*, an optimal formulation was chosen. Miconazole pellets' already-optimized composition was tweaked using 50 milligrams of barium sulphate for x-rays. A healthy rabbit had the pellet inserted into its vaginal canal. The rabbit was fasted during the duration of the experiment. At 1 and 8 hours after the pellet was given, the rabbit was subjected to X-ray and photographic tests.

Stability Studies: Optimal batches of the formed spheroids were blister packed with 100 mg of medication capsules and stored in the stability chamber at 25° C/60% RH, 40° C/75% RH, and room temperature. Physical appearance, friability, and drug content were assessed in samples taken at 15, 30, and 60 days.

RESULTS AND DISCUSSION:

Percentage of Turbidity to the Chitosan-Carbopol 71G Complex: For this reason, we investigated how the transmittance changed relative to the Carbopol 71G: chitosan molecular weight as shown in **Fig. 1**. Before being mixed together, the aqueous chitosan solution that had been treated with acetic acid and the Carbopol 71G solution both had a transparent appearance. With increasing concentrations of Carbopol 71G, there was no discernible change in the transmittance of the complex up to the point when the ratio of chitosan to Carbopol 71G was 1:1. However, the transmittance dropped when the ratio was increased from 1:1 to 1:4, which was a factor of four. When the ratio was increased further, the difference in transmittance was almost non-existent. It would appear that the electrostatic interaction sites of Carbopol (or chitosan) were saturated by that of chitosan (or Carbopol), which prevented the excess chitosan (or Carbopol) from reacting with the Carbopol (or chitosan) (or Carbopol). The ratio of chitosan to Carbopol 71G ranged from 4:1 to 1:1,

which caused the quantity of complex to be formed in a manner that was proportionate to the quantity of Carbopol (the concentration of Carbopol 71G was held constant at 0.5 mM). With a fixed chitosan concentration of when the chitosan to Carbopol ratio was changed from 1:1 to 1:4, the quantity of complex produced increased by 0.5 mM. Additional chitosan did not improve transparency past a chitosan: Carbopol ratio of 1:4. Evidence from permeability measurements shows that the chitosan to Carbopol complexation unit molar ratio was 1: 4. Using a chitosan: Carbopol complex with a 1:4 mixing ratio, we analysed the release profile and characterised the complex.

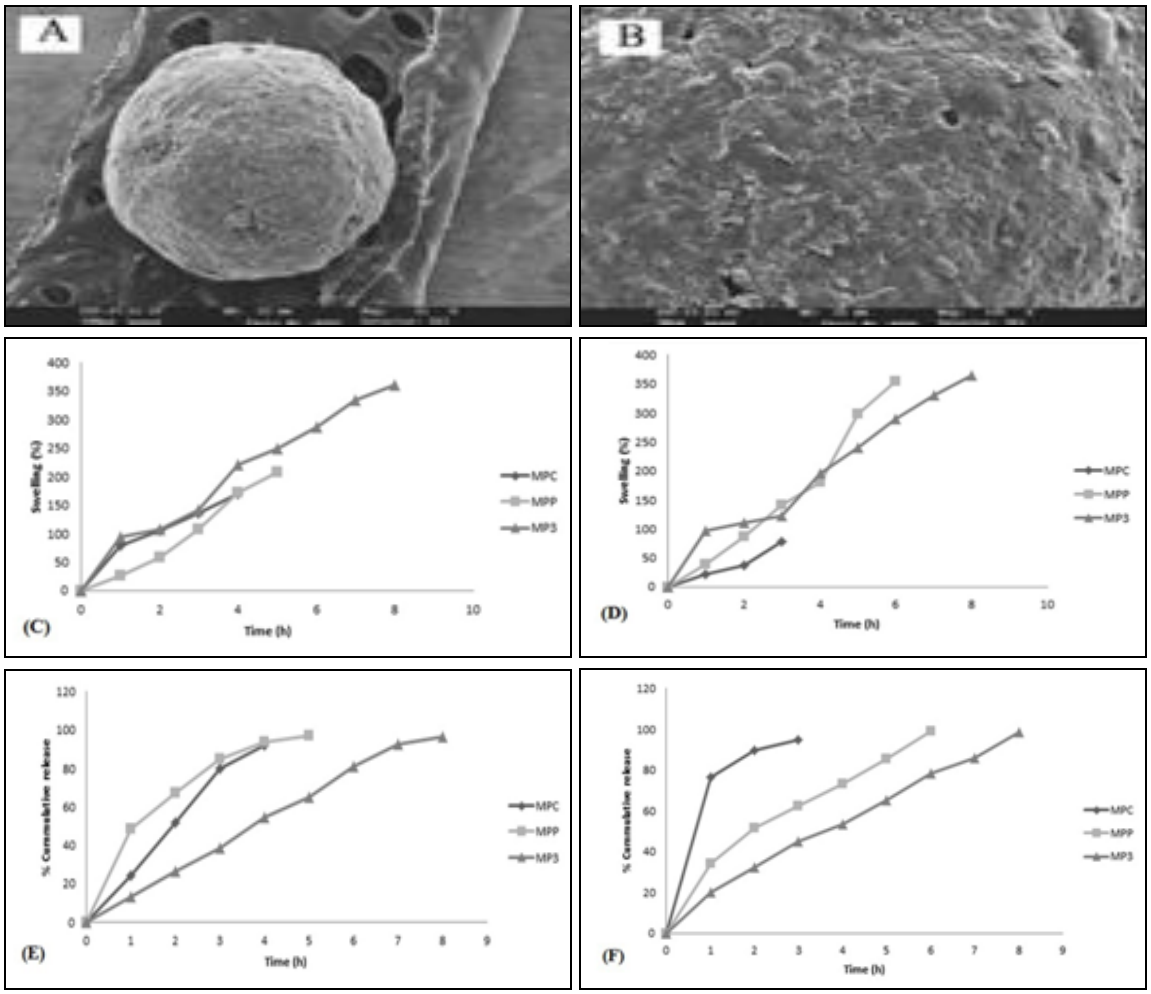

FIG. 1: TURBIDITY OF CHITOSAN-CARBOPOL 71G COMPLEX RATIOS

Optimization of Extrusion/ Spherization Process Parameters: The extrudates were generated at IPEC concentrations between 5% and 20%. As the IPEC content was increased over 20%, extrudates stopped forming.

When IPEC concentrations were raised above 20%, the dough mass became more elastic and difficult to extrude as shown in **Table 1**.

The diameter of the spheroids generated changed from wide to small when the IPEC percentage changed from 5% to 20%. Twenty spheroids with a small diameter range were obtained as shown in **Table 2**.

TABLE 1: RESULTS OF FORMATION OF EXTRUDATES

MCC-Drug-CMTKP	Extrudates
75-20-5	+
70-20-10	+
65-20-15	+
60-20-20	+
55-20-25	+
50-20-30	+
45-20-35	-
40-20-40	-
35-20-45	-

Researchers showed that the highest yields of rod- and dumbbell-shaped particles were achieved at the slowest spherization rates during the optimization

process. Even at greater rates, centrifugation prevented proper spherization, resulting in the production of rod- and dumbbell-shaped particles. A speed between 600 and 800 rpm produced pellets with a narrow size distribution.

TABLE 2: RESULTS OF OPTIMIZATION OF SPERONIZATION PROCESS

MCC-Drug-CMTKP	Speronization speed (rpm)	Spheroid description
75-20-5	500, 600, 800, 1000, 2000	Dumbbell shape, Spheroids with wide size range, Spheroids with wide size range, Dumbbell shape, Rod shape
70-20-10	500, 600, 800, 1000, 2000	Dumbbell shape, Dumbbell shape, Spheroids with wide size range, Dumbbell shape, Rod shape
65-20-15	500, 600, 800, 1000, 2000	Dumbbell, Spheroids with wide size range, Spheroids with wide size range, Dumbbell, Rod shape
60-20-20	500, 600, 800, 1000, 2000	Dumbbell shape, Spheroids with narrow size range, Spheroids with narrow size range, Dumbbell shape, Rod shape
55-20-25	500, 600, 800, 1000, 2000	Dumbbell shape, Dumbbell shape, Dumbbell shape, Rod shape, Rod shape
50-20-30	500, 600, 800, 1000, 2000	Dumbbell shape, Dumbbell shape, Rod shape, Rod shape, Rod shape

A major portion of the granulating mass was not converted into spheroids at shorter spherization times (i.e., less than 15 minutes), as shown in **Table 3**, for the optimization of spherization time, and at longer residence times, due to evaporation of water, the particles were dried and thus, size reduction was observed. Maximum production of spherical particles with limited size range was produced under the optimal conditions, namely MCC-IPEC-Drug ratio of 75:15:10, spherization period of 20 min at 800 RPM speed.

TABLE 3: OPTIMIZATION OF SPERONIZATION TIME

MCC-Drug-CMTKP	Spheronization speed (RPM)	Spheronization time (min)	Spheroid description
60-20-20	800	10	Not formed
		15	Not formed
		20	Formed
		25	Formed
		30	Formed

FT-IR, DSC, and XRD Characterization of the Chitosan-Carbopol 71G Complex:

Fourier Transform Infrared Spectroscopy (FT-IR): It is possible that the interpolymer complex is produced in aqueous solution due to electrostatic contact between the COO group of Carbopol and the NH3+ group of chitosan. Chitosan and Carbopol were dissolved in acetic acid and water, respectively, to make solutions of protonated chitosan and dissociated Carbopol, respectively. Those solutions were then used to make the interpolymer complex of chitosan and Carbopol.

The amine group of the 2-aminoglucose unit is responsible for the band at 1575 cm^{1}, and the carbonyl group of the 2-acetylaminoglucose unit is responsible for the band at 1656 cm1; the band at 1380 cm^{1} represents -CH2 bending, and the deacetylation degree of chitosan is 85%. The anti-symmetric stretching of the C-O-C bridge, which can be seen in its absorption bands at 1160 cm^{-1}, 1075 cm^{-1}, and 1040 cm^{-1}, characterises the saccharide structure of this substance (skeletal vibrations involving the C-O stretching). The O-H stretching (hydrogen-bonded), asymmetric CH2 stretching, and C-O stretching (hydrogen-bonded) are all responsible for the prominent band observed at 1715 cm^{1} in Carbopol 71G22, 23,24. Bands at 1246 and 1170 cm1 are attributed to C-O stretching 25; the faint band at 1417 cm1 is caused by the symmetric stretching of carboxylate anion (COO).

The amine band of chitosan, originally assigned to 1595 cm1, changed to 1640 cm^{1} in the IR spectrum of the complex, showing protonation of the amine group to an NH3+ group. The COO group's symmetric and asymmetric stretching are responsible for the bands observed at 1550 and 1408 cm^{1}. It was also established that the NH3+ peak occurred at a wavelength of 1460 cm1 (between 27 1600 cm1 and 1460 cm^{1}). As an added bonus, it was previously established that the NH3+

group peak in the chitosan/poly (acrylic acid) complex appeared at 28 1520 cm^{1}. Inferring that COO and NH3+ peaks overlap, we get a broad peak around 1550 cm^{1}. Based on these findings, we can hypothesise that an electrostatic contact between the COO group of Carbopol and the NH3 + group of chitosan was responsible for the formation of the chitosan-Carbopol complex. As shown in **Fig. 2(B)** and **Fig. 2(C)** the FTIR spectra of chitosan, Carbopol, and complex in the range of 2000 to 1000 cm^{-1} and 1800 to 1400 cm^{-1}, respectively.

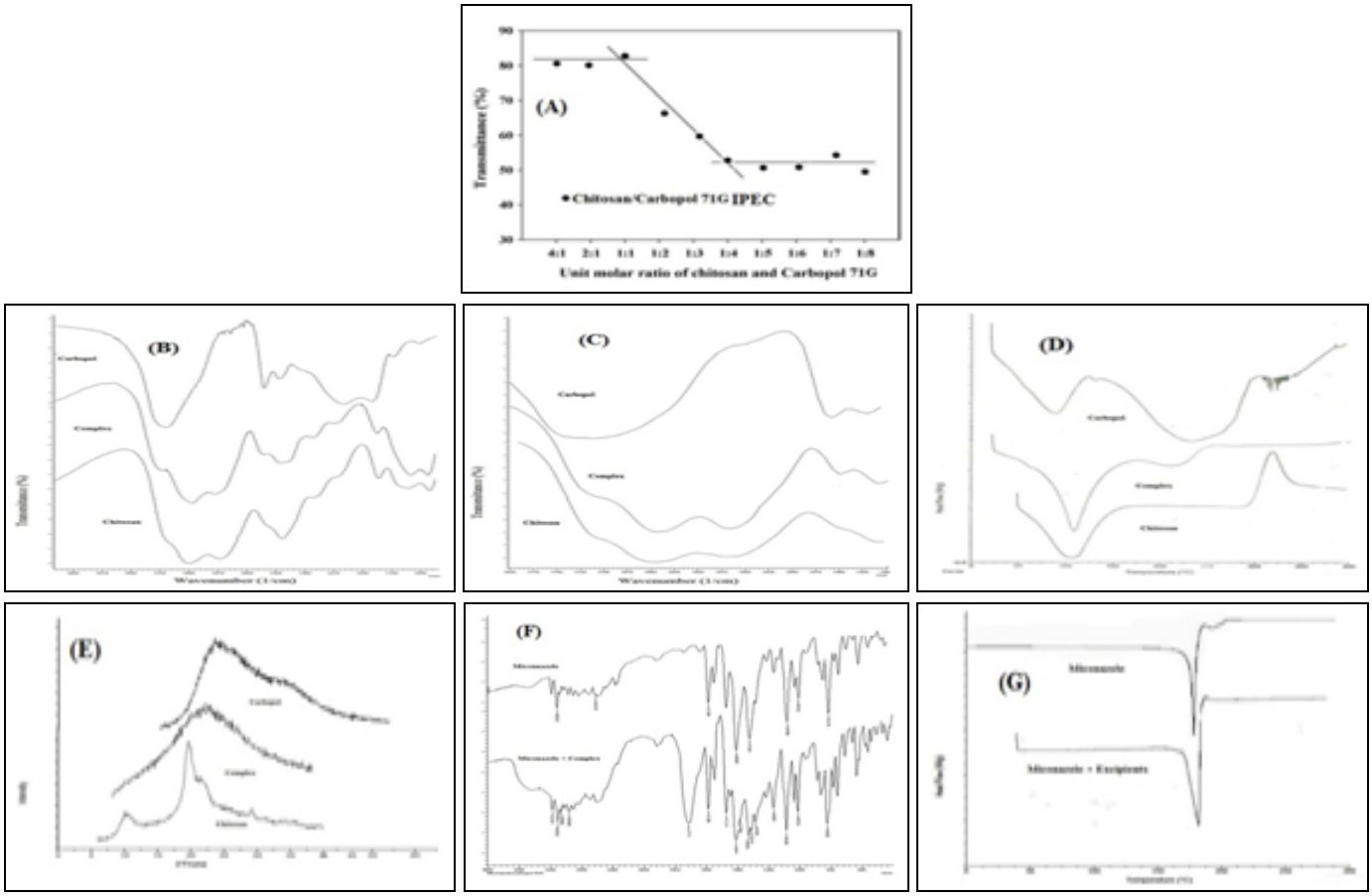

FIG. 2: XRD AND FTIR OF CHITOSAN-CARBOPOL 71G COMPLEX

Differential Scanning Calorimetry (DSC): Around temperatures above 100 degrees Celsius, chitosan's endothermic peak appears, while its exothermic peak occurs at 320 degrees Celsius. The peak around 320 °C is likely due to the initial step of chitosan degradation 29, and the peak at 100 °C was attributed to the evaporation of absorbed water. Chitosan has a distinctive peak at 320 degrees Celsius. To begin the pyrolysis of chitosan, the glycosidic linkages randomly rupture, and then the resulting fragments are further broken down into acetic acid, butyric acid, and lesser fatty acids 30.

The glass transition temperature of Carbopol, as seen in the DSC thermogram, is around 135 °C, possibly as a result of the breaking of hydrogen bonds between carboxylic acid groups. Carbopol 71G melted and disintegrated sequentially at around 280 °C, as was reported in experiments 31, 32. Two endothermic peaks may be seen in the thermogram's intricate pattern. At temperatures around 100 °C, bound water causes the first endothermic peak. When compared to chitosan, the endothermic peak of the complex caused by bound water was less pronounced. Complexation of chitosan with Carbopol may reduce the amine group, which in turn increases the water-absorbing ability of chitosan. As a result, the complex could have a poorer water-absorption capacity than chitosan.

A complex matrix may undergo a gradual breakdown and a prolonged drug release due to its lower water absorption capacity. Cleavage of electrostatic connections between the two oppositely charged polymers likely accounts for the

second endothermic peak at 210 °C, which is not seen for the pure molecules 33. As shown in **Fig. 2(D)** displays a differential scanning calorimetry (DSC) thermogram of Chitosan, a complex, and Carbopol (D).

X-ray Diffraction (XRD): As shown in **Fig. 2(E)** depicts the X-ray diffraction pattern of chitosan powder, which reveals two distinct diffraction peaks at 11° (2) and 20° (2). Two peaks, one a shoulder peak at 22 degrees and the other a minor peak at 27 degrees, may be seen. Crystalline peaks at 11° and 20°, characteristic of chitosan, are linked to the hydrated and anhydrous crystals, respectively 34, 35. Carbopol's X-ray diffraction pattern exhibited a dominant diffraction peak at 20° (2) and a secondary peak at 35° (2). Contrarily, a 22° diffraction peak was clearly seen in the X-ray diffraction pattern of the complex. After complexation, none of chitosan's characteristic peaks were present, indicating that the presence of Carbopol in chitosan disturbed the crystalline structure of chitosan and prevented hydrogen bonding between amino groups and hydroxyl groups 36.

Drug and Excipient Compatible Studies:

Fourier Transform Infrared Spectroscopy: FT-IR spectra were used to examine the relative drug-polymer compatibility. Peak locations in FT-IR spectra of pure Miconazole nitrate and Miconazole nitrate with excipients were compared. Miconazole nitrate was detected in all spectrums of medication and excipients with no disappearance or shift in peak position, confirming their compatibility. Therefore, it is possible to deduce that medications can be used with the selected excipients without causing instability in the formulation. For the spectra, as shown in **Fig. 2(F)**.

Differential Scanning Calorimetry (DSC): Miconazole nitrate and its 1:1 polymer combination were subjected to differential scanning calorimetry, and the resulting thermograms are as shown in **Fig. 2(G)**. Thermograms showed that miconazole nitrate's melting point (182 degrees Celsius) was not altered by the addition of excipients. Therefore, it can be established that pellet-making polymers do not react with miconazole nitrate.

Evaluation of Pellets: As shown in **Table 4** that a higher ratio of microcrystalline cellulose in a powder mixture results in even more round pellets. Furthermore, the amount of microcrystalline cellulose used enhanced the yield significantly. More round pellets were produced when the percentage of microcrystalline cellulose was raised from 70% to 850%. It follows that pellet quality improved with increasing amounts of microcrystalline cellulose in the powder blend.

TABLE 4: RESULTS OF EVALUATION OF PELLETS

Formulation code	% yield*	Averagesize*(µm)	Angle of repose* (θ)	Hardness* (N)	Friability*(%)
MP1	79.23±0.89	1635±0.56	37.35±1.26	9.89±0.78	1.53±0.78
MP2	82.14±1.24	1489±1.45	33.58±1.05	7.55±0.47	1.41±0.45
MP3	84.86±0.65	1245±1.23	32.47±1.32	6.11±0.45	1.43±0.82
MP4	88.47±1.79	1012±0.98	27.17±0.59	5.54±1.23	0.97±0.93

Drug Loading and Encapsulation Efficiency: Drug loading in the various formulations was calculated to be between 15.56 and 18.61%. Encapsulation success rates ranged from 93.50% to 98.188%. The data collected as shown in **Table 5**. The purpose of the drug content test is to determine if the drug is spread out evenly throughout the formulation. Growing the polymer concentration improves drug loading and entrapment efficiency. The results indicate that the pellets contain an appropriate amount of medication and that any discrepancies are within tolerance levels.

TABLE 5: DRUG LOADING AND ENCAPSULATION EFFICIENCY OF PELLETS

S. no.	Formulation	% Drug loading*	% Encapsulation efficiency*
1	MP1	15.56±0.63	93.50±0.75
2	MP2	17.97±0.75	96.69±0.83
3	MP3	17.21±0.93	95.92±0.87
4	MP4	18.61±0.86	98.18±0.46

Scanning Electron Microscopy (SEM): Due to its low cost, small sample size requirements, and user-friendliness, scanning electron microscopy (SEM) is a popular choice for assessing drug delivery

systems. The coating films' surface morphology, texture, and porosity were characterised through scanning electron microscopy. Images obtained using scanning electron microscopy are as shown in **Fig. 3(A),** and in **Fig. 3(B)**, the surface topography of the pellets, with their ideal, spherical shape, is displayed. SEM images of pellets indicate that the medicine is evenly distributed within the pellet, with no visible drug particles on the pellet's surface.

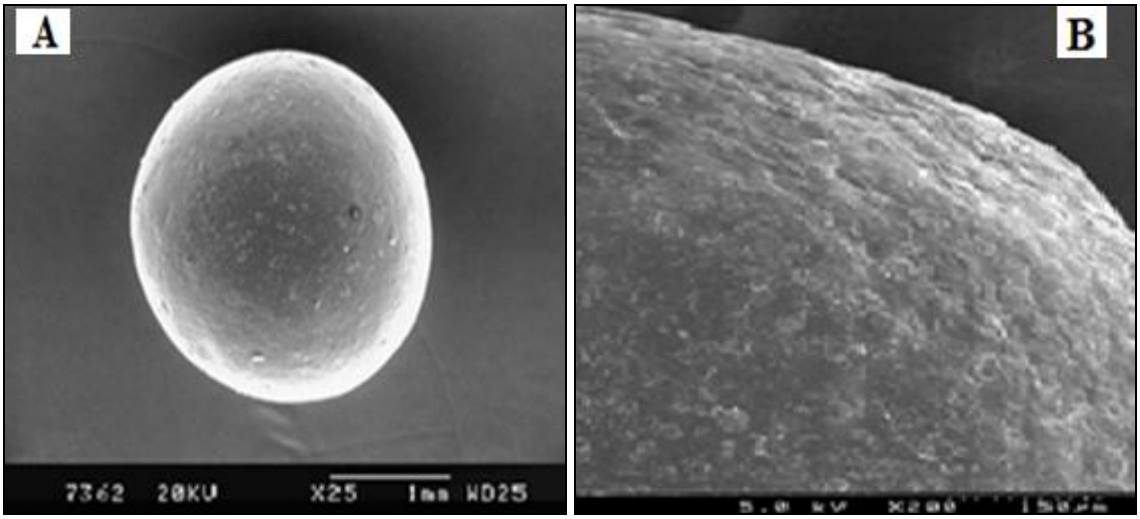

FIG. 3: SCANNING ELECTRON MICROSCOPY OF PELLETS

Swelling Studies: Before evaluating mucoadhesion, it is crucial to consider swelling, a property that impacts mucoadhesion and the drug release profiles of polymeric drug delivery systems 37, 38. The swelling data was reported as an index of swelling**. Fig. 1(C)** and **1(D)** display the swelling index profiles of the different formulations in SVF at pH 4.5 and at pH 7.

The dose form was created for the vaginal cavity; a vaginal pH of 4.5 is consistent with a pre-menopause and the absence of bacterial infections. When the pH of the vagina is over the normal range (which is between 5.0 and 6.5), it is likely that bacteria are to blame. A vaginal pH between 6.0 and 7.0, in the absence of bacterial infections, is highly indicative of menopause. Thus, the swelling tests were conducted at pH 4.5 and pH 7. Due to water entering through metastable pores in the pellets, swelling happens quickly at first. Hysteresis of the swelling that follows swelling due to diffusion processes describes this mechanism. Diffusion may be the most crucial factor controlling the rate of drug release from the system, provided that an intact hydrated layer is established over the study period. There may be a swelling-controlled mechanism for drug release from hydrophilic matrices. The swelling index was found to be the lowest for formulations that contained just chitosan in both the simulated vaginal fluid (pH 4.5) and the vaginal fluid (pH 7). (MPC). Because MPC formulation may form the gel layer around the pellet at acidic pH, the swelling index is greater in simulated vaginal fluid with a pH of 4.5 compared to fluid with a pH 7 value.

To the best of our knowledge, MPP formulations consisting just of Carbopol are capable of expanding for up to 8 hours. The swelling quality of Carbopol is directly influenced by the dissociation of the carboxylic group at neutral and basic pH levels. Therefore, a formulation that contains Carbopol expands more in phosphate at P^H 7 than it does in pH 4.5 simulated vaginal fluid. This is because of the higher pH. The swelling ratios of pellet formulations in environments of varying pH are affected by factors such as the free volume of the expanded polymer matrix, the relaxation of the polymer chain, and the presence of ionisable functional groups such as -COOH that can form hydrogen bonds with the liquid medium. At a pH of 4.5, Carbopol -only multi-purpose formulations (MPPs) experience less swelling than they do at a pH of 7. Because the pKa of any polymer containing carboxylic acid is about 4.5, and because the carboxyl groups of Carbopol have a tendency to dissolve at a pH greater than 4.5, the

osmotic pressure inside the hydrogels gradually increases at pH 4.5. This results in slow swelling. Because of this, the pH of the oedema progressively increased to 4.5 over the course of time. However, an enhanced for greater dissociation of carboxylic acid was detected in pH 7 in comparison to pH 4.5, which resulted in a larger swelling. This was the case because of the elevated pH. The IPEC-containing formulation showed a slow but steady swelling trend. The swelling index of complex-containing formulations is about the same at 4.5 and 7 pH. These results suggest that a complex is present, as the degree of swelling is slow, uniform, and pH-independent.

***In-vitro* Dissolution Studies:** The pH 4.5 and pH 7 simulated vaginal fluids were used in the USP class II dissolving device for the dissolution investigations. After 8 hours, the pellets were analysed to see how they released their contents. **Fig. 1(E)** shows dissolution profiles at pH 4.5, while **Fig. 1(F)** shows them at pH 7.

Dissolution of the drug from the chitosan pellets (MPC) took 4 hours longer in the SVF at pH 4.5 than at pH 7. This could be because chitosan forms a gel at low pH, slowing the release of the medication from the pellet. The dissolution rate of drugs in MPP formulations containing Carbopol was affected by the pH of the dissolution medium. At a pH of 4.5, Carbopol pellets showed an initial burst action, and the full medication was released within 5 hours. Rapid drug release 39 may be caused by the fact that the carboxylate group of Carbopol does not dissolve at 4.5 pH, leading in a less viscous gel layer surrounding the pellet. When complex-containing formulations were exposed to pH 4.5 vaginal saline, they underwent a swelling-driven phase transition from a glassy state to a rubbery state, where molecules diffused rapidly. Miconazole release kinetics in these systems were found to be proportional to the gel formation around the dosage form. Drug release rates were shown to be affected by both the diffusion of water and the thickness of the gel layer. Miconazole was liberated when the complex hydrates and this layer slowly disintegrated. In a pH 7 test, the chitosan pellets (MPC) had the fastest drug dissolution rate of all the formulations. Most of the drug from an MPC formulation was absorbed within 3 hours. Possible reasons for this include chitosan's neutral pH breakdown and its failure to create a gel layer around the pellet. The solubility rate of the medication in an MPP formulation incorporating Carbopol was affected by the pH of the dissolving medium. At a pH of 7, the carboxylic acid groups in Carbopolunderwent maximum ionisation, leading to maximum swelling, which reduced the number and size of micro viscosity zones. The quick development of the gel creates a barrier that slows the miconazole's release for up to 6 hours.

At pH 4.5, the complex in MP3 formulation changed phase from glassy to rubbery, allowing the drug to diffuse rapidly. In both setups, drug release kinetics were linked to the rate of gel formation around the pellet. Drug release rates were shown to be affected by both the diffusion of water and the thickness of the gel layer. This layer of protection for the medication steadily dissolved as the compound hydrated.

The initial explosion effect was brought on by chitosan's disintegration feature and its inability to form a gel layer in neutral pH. Studies of drug release found that pellets containing the compound showed pH-independent drug release. The similarity factor (f2) was determined to be 88 between the dissolution patterns of MP3 formulation in simulated vaginal fluid at pH 4.5 and enhanced vaginal fluid at pH 7. These findings verified that the medication release patterns for the MP3 formulation were nearly the same for both vaginal pH values since the f2 values was greater than 50 (in accordance with USFDA requirements).

***Ex-vivo* Bio adhesion Studies:** The increased availability of adhesive sites of natural polymer with mucus may account for the augmented mucoadhesive strength. Swelling the polymer exposes its bio adhesive sites, which can then form hydrogen bonds and/or electrostatic contacts with the mucosal network, resulting in a mechanical entanglement as well as the formation of secondary bonds that promote chemical and mechanical interactions. 95% of mucoadhesive pellets showed strong adhesion to the vaginal mucosa and could be held for 8 h in *in-vitro* experiments.

***In-vivo* X-ray Studies:** The animal experiment project was cleared and approved by Institutional Animal Ethical Committee, the IAEC Code

1082012. The X-ray images of an albino rabbit used to test the mucoadhesion and retention property are as shown in **Fig. 4(A)** and **4(B)**. Rabbits were given an MP3 version of the barium sulphate-optimized formulation. Radiographs were used to track the time that the pellet remained in the uterine cavity. Pictures indicated that the pellets swelled, stayed together, and stuck to the vaginal mucous membrane for more than 8 hours.

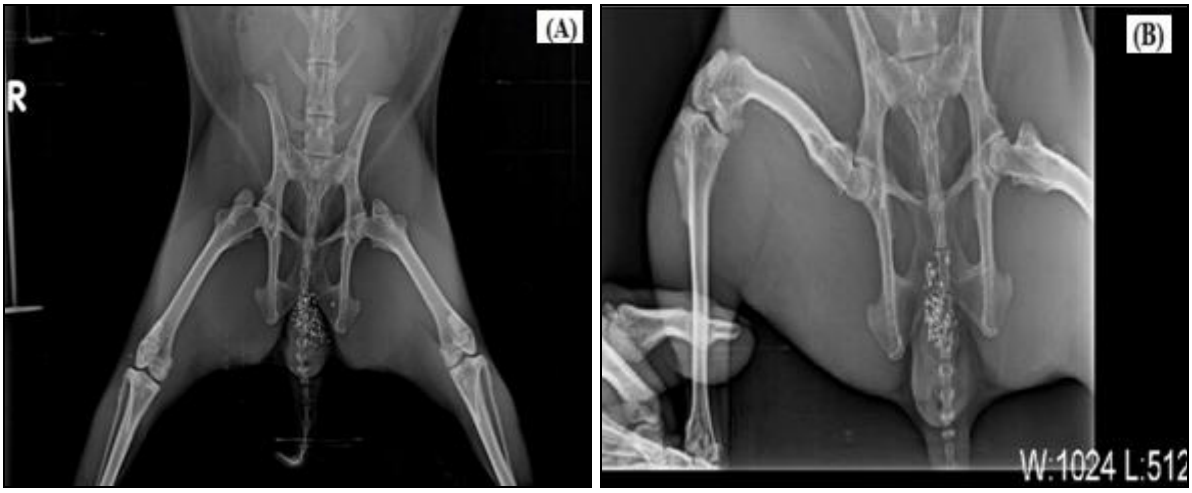

FIG. 4: X-RAY IMAGES OF AN ALBINO RABBIT MUCOADHESION AND RETENTION PROPERTY

Mathematical Model Fitting: The release data were fitted into release models using PCP Disso V2.01 dissolving software to determine the drug release mechanism and release rate. To learn about the release processes, values for parameters such as the time exponent n and the regression coefficient R were calculated. Results The MP3 formulation was shown to follow zero-order kinetics after model fitting at both 4.5 and 7 pH, with regression coefficient values of 0.9902 and 0.9953. The release mechanism in Peppas is non-fickian diffusion, as indicated by the n value (0.8142), which is between 0.5 and 1.

Stability Studies: In order to ascertain the physical stability of the pellet's formulation MP3, stability investigations were conducted. Stability tests were conducted for a total of 6 months at 25 2 °C and 60% RH, 30 2 °C and 65% RH, and 40 2 °C and 75% RH.

A measurement of the active pharmaceutical ingredient concentration was made. The results of the investigation are displayed as shown in **Table 6**. The pellets retained all of their original characteristics and pharmacological content.

TABLE 6: STABILITY STUDIES DATA OF MP3 FORMULATION

Stability condition	Sampling interval (months)	Physical appearance	% Drug content*
25°±2°C/60±5% RH	0	No change	99.50±0.50
	3	No change	98.00±0.22
	6	No change	97.81±0.14
30°±2°C/65±5% RH	0	No change	99.50±0.50
	3	No change	99.12±0.10
	6	No change	97.80±0.14
40°±2°C/75±5% RH	0	No change	99.50±0.50
	3	No change	98.40±0.60
	6	No change	98.20±0.72

CONCLUSION: It was shown that a chitosan-Carbopol 71G interpolymer complex can serve as a pH-independent drug delivery system for the sustained release of miconazole nitrate, making it useful for the treatment of candidiasis. Carbopol 71G's pH-dependent release profile is attenuated by the IPEC, while the drug's release follows a pH-independent pattern without a burst effect at the outset and approaches zero-order kinetics. Excellent swelling, bio adhesion, and pH-independent drug release are all characteristics of the MP3 formulation.

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