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Research article

FORMULATION DEVELOPMENT AND CHARACTERIZATION OF METRONIDAZOLE MICROENCAPSULATED BIOADHESIVE VAGINAL GEL

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ABSTRACT

The present study concerned with the development and characterization of Microencapsulated Bioadhesive Vaginal Gel (MBVG). Metronidazole encapsulated microcapsules were prepared by thermal change method using ethyl cellulose as rate controlling polymer in different ratios. The microcapsules were found to be discrete, spherical with free flowing properties and evaluated for particle size analysis, shape (scanning electron microscopy), flow properties, wall thickness, drug encapsulation efficiency, and *in vitro* release performance. The selected microcapsule formulation (MC_3, containing drug: polymer ratio 1:4) was incorporated in gels with a variety of bioadhesive polymers. The MBVGs were evaluated for pH, spreadability, extrudability, viscosity, vaginal irritation test, *in vitro* drug release, drug release kinetics, bioadhesion test, accelerated stability of selected gel formulation. *In vitro* drug release rate for selected MBVG (F5 gel, containing 1 % w/w of drug loaded microcapsules and 0.6 % w/w of carbopol 974) was found to sustain metronidazole over 36 h obeying zero order kinetic with a good bioadhesion quality. The results were compared statistically and found with satisfactory correlation. Thus in conclusion preparation protocol of MBVG studied may be adopted for a successful development of newer drug delivery system of other drugs for administration to vagina.

Keywords: Microencapsulated bioadhesive vaginal gel (MBVG), Microcapsules, Vaginal bio adhesion, Vaginal irritation.

INTRODUCTION

The vaginal route has been traditionally used for the conventional delivery of several locally acting drugs like antimicrobial agents[1]. However conventional vaginal delivery systems such as creams, foams, pessaries and jellies reside at the targeted site for relatively shorter retentivity because of the self cleaning action of the vaginal tract which limits effective drug levels for a shorter period and fluctuation in

drug dose level leads to increased dose frequency of the drug. This ultimately results into patient inconvenience and toxic conditions[2]. The use of prolong-release bioadhesive vaginal gel was thought to offer numerous benefits including prolong residence time of the dosage form at the site of absorption due to bioadhesion to the vaginal mucosa, prolong drug release, improved bioavailability and decreased side effect of drug and ultimately improved patient compliance. Metronidazole was used as a model drug in this study due to its bacteriostatic and bactericidal activity against gram negative bacteria and also effective against various vaginal infections[3]. Another important rationale of using metronidazole, is its unique, low molecular weight offering the greater permeation benefit through vaginal epithelial membrane. Ethyl cellulose was assumed to offer the control release behavior of drug due to its hydrophobic coating over metronidazole[4]. Bioadhesive polymer carbopol presumed to provide better vaginal bioadhesion.[5] Keeping in view of the above uniqueness, the present study was designed to develop a newer Microencapsulated Bioadhesive Vaginal Gel (MBVG) for prolong release of metronidazole to treat vaginal infections with increased patient convenience.

Experimental

Materials and methods

Metronidazole was received as a gift sample from Aristo Pharmaceutical Ltd., Kolkata (India). Ethyl cellulose (BDH, ethoxy content- 47.5% by weight, viscosity (ŋ), and 22 cps) was purchased from S.D. Fine Chem., Mumbai (India). All grades of Carbopol were received as gift sample from Corel Pharma Chem., Ahmedabad (India). All other chemicals and reagents used were of analytical grade and used as received.

Preparation of vaginal microcapsules Metronidazole

Vaginal microparticles were prepared by the solvent evaporation method. Accurately weighed quantity of polymer was dissolved in acetone, Metronidazole was dispersed slowly in polymer solution and this solution was added to heavy liquid paraffin with stirring (800 rpm). Microparticles were recovered by treating with petroleum ether. Then filtered, dried in a desicator. Microcapsules of various drug polymer ratios prepared accordingly for further evaluations. Metronidazole vaginal microcapsules were prepared by thermal change method. Accurately weighed quantity of ethyl cellulose and cyclohexane (50 ml) was heated in water bath. The temperature was gradually raised to 70°C over 20 min under

constant stirring (500 rpm). Metronidazole was dispersed slowly with maintaining temperature at 80°C for 30 min and it was cooled slowly under continuous stirring and temperature was dropped to 5°C in order to hardening of ethyl cellulose coated microcapsules. Then filtered, dried in a desiccator. Microcapsules of various drug polymer ratios prepared accordingly for further evaluations[6, 7].

Morphological and topographical characterization

Microcapsules were observed and photographed with scanning electron microscopy (LEO, 435 VP, U.K.) and optical microscopy (OLYMPUS BX-50, Japan). Their diameters were determined with a pre-calibrated graduated eyepiece of the optical microscope. One hundred measurements were averaged for each microcapsule formulation prepared[8].

Wall thickness of microcapsules

Wall thicknesses of the microcapsules were determined by the method as suggested by Luu et al.[9], using equation, $h = r (1-P) d_1 / 3[Pd_2 + (1-P) d_1]$, Where, h is wall thickness; r is mean radius of microcapsules from optical microscopic observations; d_1 is density of the core material; d_2 is density of coat material; P is proportion of medicament in the microcapsules. All the test sample was examined for three times (n=3).

Flow properties

Flowability of microcapsules was investigated by determining angle of repose, bulk density, Carr's index and Hausner ratio[10]. The angle of repose was determined by fixed funnel method. The microcapsules were tapped using bulk density apparatus (Excel Enterprises, Kolkata) for 1000 taps in a cylinder and the change in volume were measured. Carr's index and Hausner ratio were calculated by the formula: Carr's index (%) = $(D_f - D_0) \times 100 / D_f$ and Hausner ratio = D_f / D_0,

Where, D_f is tapped density; D_0 is poured density. All the experimental units were studied in triplicate (n=3).

Drug content and drug encapsulation efficiency (DEE)

Accurately weighed microcapsules equivalent to 50 mg, were suspended in 10ml of diethyl ether to dissolve the polymer coat. The drug was extracted with 50 ml of simulated vaginal fluid (SVF, phosphate buffer I.P., pH 4.9) in separating funnel and analyzed by using UV-Visible spectrophotometer (UV-1700, Shimadzu, Japan) after suitable dilution at 320 nm. Drug encapsulation efficiency was calculated using the formula[11].

DEE (%) = (Practical drug content/Theoretical drug content) × 100, each sample was analyzed in triplicate (n=3).

***In vitro* drug release studies of microcapsule formulations**

In vitro drug release study was carried out in USP XXI paddle type dissolution test apparatus using SVF as dissolution medium (900 ml phosphate buffer I.P. pH 4.9, at 37±1 °C was adjusted to 100 rpm). An aliquot sample (5 ml) was withdrawn at an interval of 1 h with replacement of fresh medium and analyzed for metronidazole content by UV-Visible spectrophotometer at 320 nm[8]. The same method was adopted for each batch of microcapsules.

Release kinetic studies of microcapsule formulations

In order to study the exact mechanism of drug release from the Vaginal Microcapsules, drug release data was analyzed according to zero order, first order, Higuchi square root and Korsemeyer - Peppas equations. The criterion for selecting the most appropriate model was chosen on the basis of goodness of fit test[12, 13, 14].

***In vitro* polymer degradation**

In vitro degradation study of placebo microcapsules was carried out in the same *in vitro* SVF medium. Accurately weighed 100 mg of microcapsules in 150 ml of the SVF was shaken at 72 rpm and 37.0°C. At pre-set intervals, the vials were centrifuged at 5000 rpm for 20 min. After removing the upper clear solution; the microcapsules were dried under vacuum for 48 h. Then mass loss of the dried microcapsules was determined by digital microbalance[15].

Fourier transformed infrared spectroscopy (FT-IR)

IR spectroscopy was performed on Fourier transformed infrared spectrophotometer (840, Shimadzu, Japan). The pellets of drug and potassium bromide were prepared by compressing the powders at 20 psi for 10 min on KBr-press and the spectra were scanned in the wave number range of 4000-600 cm^{-1}.[8]

Scanning electron microscopy (SEM)

The SEM analysis was carried out using a scanning electron microscope (LEO, 435 VP, U.K.). Prior to examination, samples were mounted on an aluminium stub using a double sided adhesive tape and making it electrically conductive by coating with a thin layer of gold (approximately 20 nm) in vacuum. The scanning electron microscope was operated at an acceleration voltage of 05 kV and resolution of 4000[8].

Preparation of microencapsulated vaginal bioadhesive gels

Selected batches of metronidazole microcapsule were incorporated in gels by mechanical stirring method using various grades of bioadhesive polymer[5], such as carbopol 934, 940, 974 and 980 with other formulation additives. For all

batches, the microcapsules were mixed with prepared bioadhesive gels[16]. The prepared gels were packed in wide mouth plastic jars covered with screw capped plastic lid after covering the mouth with an aluminum foil and were kept in cool place for further study.

Estimation of metronidazole in vaginal gels

Accurately weighed gel (0.5 g) was suspended in 25 ml of SVF. It was filtered after constant stirring and analyzed by using same UV-Visible spectrophotometer after suitable dilution at 320 nm[17].

Drug content uniformity

Initially the formulations were tested for homogeneity by visual inspection. To further ensure the homogeneity of drug content in the formulation of the gel, six tubes were sampled from the different locations in the mixer and assayed for the drug content as stated above. Studies were performed in triplicate for all the formulations[18].

Determination of pH

The pHs of the microencapsulated carbopol gels were determined by digital pH meter (Model MK–VI, Kolkata, India). One gram of gel was dissolved in 25 ml of distilled water and the electrode was then dipped in to gel formulation for 30 min until constant reading obtained. And constant reading was noted. The measurements of pH of each formulation were replicated three times[19].

Determination of spreadability

Spreadability of the formulations was determined by an apparatus suggested by *Mutimer et al.*[20] Each formulation was replicated for three times.

Extrudability study

In conducting the test, a closed collapsible tube containing above 20 grams of gel was pressed firmly at the crimped end and a clamp was applied to prevent any rollback. The cap was removed and the microencapsulated gel was extrudes until the pressure was dissipated[20, 21].

Viscosity measurement

A Brookfield digital viscometer (Brookfield Engineering Laboratories, Model DV-II, Mumbai) with a suitable sample adaptor was used to measure the viscosities in cps of the micro-encapsulated gel prepared[19].

Vaginal irritation test

The study protocol (Regd. No. HPI / 07 / 60 / IAEC / 0013) was approved by the Institutional Animal Ethics Committee. Microencapsulated gels (0.5 g) were applied in to the vagina of the New Zeeland white rabbits. After 72 hours, the microencapsulated gel was removed and the following characteristics such as sensitization (allergic reaction),

photosensitization, edema and excess redness were observed in test animals and in control by visual inspection[23].

***In vitro* drug diffusion studies of microencapsulated vaginal gels**

In vitro drug release study was carried out in KC-Diffusion cell using SVF as diffusion medium. The processed cellophane membrane was used, simulating the vaginal i*n vivo* condition like vaginal epithelial barrier. The drug content in withdrawn sample was estimated by UV-Visible spectro-photometer at 320 nm[19]. The same method was adopted for each batch of microencapsulated gels.

Release Kinetic studies of micro-encapsulated vaginal gels

In order to study the exact mechanism of drug release from the microencapsulated gels, drug release data was analyzed according to zero order, first order, Higuchi square root and Korsemeyer-Peppas equations. The criterion for selecting the most appropriate model was chosen on the basis of goodness of fit test[12, 13, 14].

Vaginal bioadhesion measurements

The bio adhesion measurement was performed by using a modified balance method intact with mucosal membrane of goat vagina *in vitro*[25, 24].

Accelerated stability studies of microencapsulated vaginal gel

Stability studies were performed according to ICH guidelines[21]. The formulations were stored in hot air oven at 37 ± 2°, 45 ± 2° and 60 ± 2° for a period of 12 weeks. The samples were analyzed for drug content every two weeks by UV-Visible spectrophotometer at 320 nm. Stability study was also carried out by measuring the change in pH of gel at regular interval of time.

Statistical Analysis Statistical data analyses were performed using the ANOVA one way at 5 % level of significance $p < 0.05$[22].

Results and discussion

The obtained microcapsules were found to be none aggregated. The generalized microparticulation protocol depends on, choice of ingredient, successful preparation of microcapsules and optimization at every preparative steps. The formulation code and composition of vaginal microcapsules were presented in column 1 and 2 of Table 1.

Morphological and topographical characterization

That all microcapsules thus obtained, were opaque, discrete and spherical particles with smooth surfaces further confirmed by SEM study. The results of all particle size (mean diameter) were

given in column 4 of Table. 2. Particle size distribution of selected microcapsules and the mean particle size for all formulations. The mean diameter of the microcapsules was found to be increased with increase in proportion of coat material as expected.

Wall thickness

The wall thickness of the microcapsules was shown in column 3 of Table. 1. The wall thickness was found to be highest 3.888 ± 0.25 µm for MC_3 in comparison to others. The wall thickness of the microcapsules mainly built up with increase in polymer content.

Flow properties

The flow properties of the microcapsules were shown in column 4, 5 and 6 of Table 1. As usual, the flow properties increase with polymer ratio. Most of the formulations are having excellent (MC_1, MC_2 and MC_3) to good (MC_4 and MC_5) flow properties as represented in column 7 of Table 1.

Drug content and Encapsulation efficiency

Relatively high drug content and encapsulation efficiency were observed for each formulation presented in column 2 and 3 of Table 2. Although there is no significant difference among five different formulation in encapsulation efficiency, the DEE was found to be within the range of 70-80% and highest for MC_5 (lowest polymer content in comparison to others) perhaps. The increased encapsulation efficiency may be attributed to the hydrophobic nature of ethyl cellulose and metronidazole. The encapsulation efficiency was found to be increased with decrease in polymer content.

Table 1 : Composition, flow properties and wall thick ness of vaginal microcapsules.

Formulation code	Drug/ polymer ratio	Wall thickness (µm) (X ± S.D.)	Carr's index	Hausner's ratio	Angle of repose (°) (X ± S.D.)	Comment (U.S.P)
MC1	1:1	2.512 ± 0.21	08.600	1.093	24.8 ± 0.11	Excellent
MC2	1:2	3.567 ± 0.18	10.790	1.107	21.6 ± 0.09	Excellent
MC3	1:4	3.888 ± 0.25	07.525	1.081	20.0 ± 0.12	Excellent
MC4	2:1	1.848 ± 0.14	12.880	1.148	26.4 ± 0.14	Good
MC5	4:1	0.843 ± 0.27	13.630	1.158	27.9 ± 0.25	Good

Each value represents as mean ± standard deviation, n=3. Standard error mean < 0.156.

Table 2 : Physical properties and drug release data of Vaginal Microcapsules.

Formulation code	Drug content (mg) (X ± S.D.)	Encapsulation efficiency (%) (X ± S.D.)	Mean diameter (µm) (X ± S.D.)
MC1	19.03 ± 0.92	75.988 ± 0.91	45.950 ± 0.92
MC2	12.04 ± 0.56	69.601 ± 0.65	53.633 ± 1.05
MC3	07.53 ± 0.48	75.250 ± 0.84	68.830 ± 0.98
MC4	23.67 ± 0.83	69.048 ± 0.71	39.933 ± 1.11
MC5	33.98 ± 0.75	80.713 ± 0.95	24.016 ± 1.07
ANOVA			
F	19.716		
df	19		
p	1.27		

Each value represents as mean ± standard deviation. n=3. Standard error mean < 0.641.

***In vitro* drug release of prepared microcapsules**

The *in vitro* drug releases of acquired microcapsules were shown in column 2 of Table. 3 and Fig 1. In all the cases, the release rate was increased with decreased proportion of polymer. The microcapsule formulation MC_3 was found to release the drug only about 59.367 % even after 12hrs, thus concluded to have sustained release of drug for longer period of time when compared to other microcapsules formulations.

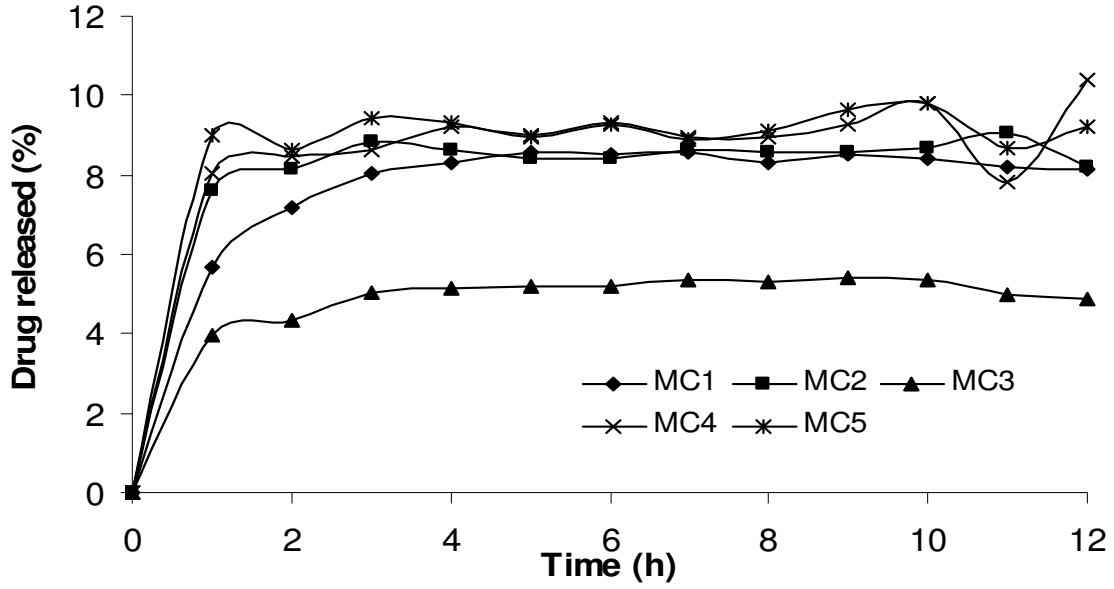

Fig. 1 : It Shows Dissolution profile of various vaginal microcapsule formulations. Each point represents as mean ± S.D., n=3.

Drug release kinetics

The release rate was inversely proportional to wall thickness. The *in vitro* drug release of all the formulations (MC1-MC5) was found constant for each formulation and influenced by the polymer added. The *in vitro* drug release profile was presented in Table. 3, Fig. 1. To categorize the kinetics of drug release from microcapsule, release data was verified with different kinetic models. The column 3,4,5,6 of Table. 3 indicated that drug release from all formulations obeyed Higuchi kinetic equation, have diffusion controlled release rate which is depend on concentration of release regarding polymer with process variable. Column 7 of Table 3 showed that all the formulations released the drug by swelling followed by diffusion as per super Case II (n > 1) transport mechanism the release mechanism was not significantly influenced by formulation variables and was predominately swelling controlled, the drug is dispersed within a glassy polymer. Initially the polymer begin to swell in contact of water, .as the penetrant enters the glassy polymer , the glass transition temperature (T_g ,120-124ºC,)[29] of the polymer is lowered and become rubbery show diffusion allowing relaxation of macromolecular chains and drug diffuse out from the swollen rubbery area of polymer wall[30]. Statistical verification with one way ANOVA method attested the fact that the drug release data were found significant for F (20.252) at 5 % level of significance ($p < 0.05$).

Table 3 : Drug release and *in vitro* release kinetics data of Vaginal Microcapsule.

Formulation	Cumulative % Drug release (X ± S.D.) (12 h study)	Zero order equation	First order equation	Higuchi Square root eq.	Korsmeyer and Peppas equation	
		Regression co-efficient (r)			(n)	
MC 1	96.456 ±1.16	0.755	0.767	0.988	0.999	1.145
MC 2	101.731 ± 0.98	0.572	0.575	0.919	0.999	1.043
MC 3	59.367 ± 0.83	0.744	0.769	0.982	0.999	1.103
MC 4	104.551 ± 1.13	0.554	0.558	0.875	0.999	1.053
MC 5	100.752 ± 0.75	0.509	0.522	0.870	0.999	1.018
ANOVA						
F	57,95					
df	3					
p	6.12					

n- Diffusion exponent related to mechanism of drug release, according to Korsmeyer and Peppas equation, $\mathbf{m_t / m_\alpha = kt^n}$. Each value represents as mean ± standard deviation, n=3.Standard error mean < 0.756.

Infrared spectroscopy (IR)

The interaction between the drug and the carrier often leads to identifiable changes in the FT-IR profile of solid systems. FT-IR spectra at 45 scans and a resolution of 1 cm^{-1} were recorded in KBr pellets for pure drug (Fig. 2A), polymer (Ethyl cellulose) (Fig. 2B) and the selected (MC_3) microcapsule formulation (Fig. 2C) of 1:4 drug / polymer ratios, as represented in fig. 2. In FT-IR studies, the characteristic C-N stretching at around 1159 cm^{-1} was clearly distinguishable in the selected formulation (MC_3). Additionally characteristics peak of drug C=N stretching vibration at around 1487 cm^{-1}, N=O symmetrical and asymmetrical stretching at around 1369 cm-1 and 1535. cm^{-1} respectively, and characteristics peak of polymer C-O-C asymmetrical and symetrical stretching at around 1269 cm-1 and 1072 cm-1, cyclic alkanes C-H bending at around 1458 cm-1 and cyclic alkanes C-H stretching at around 3099 cm-1 were also observed unchanged in the formulation suggesting no drug polymer chemical interaction. The drug was therefore considered to have been encapsulated in unbound form in microcapsule formulation.

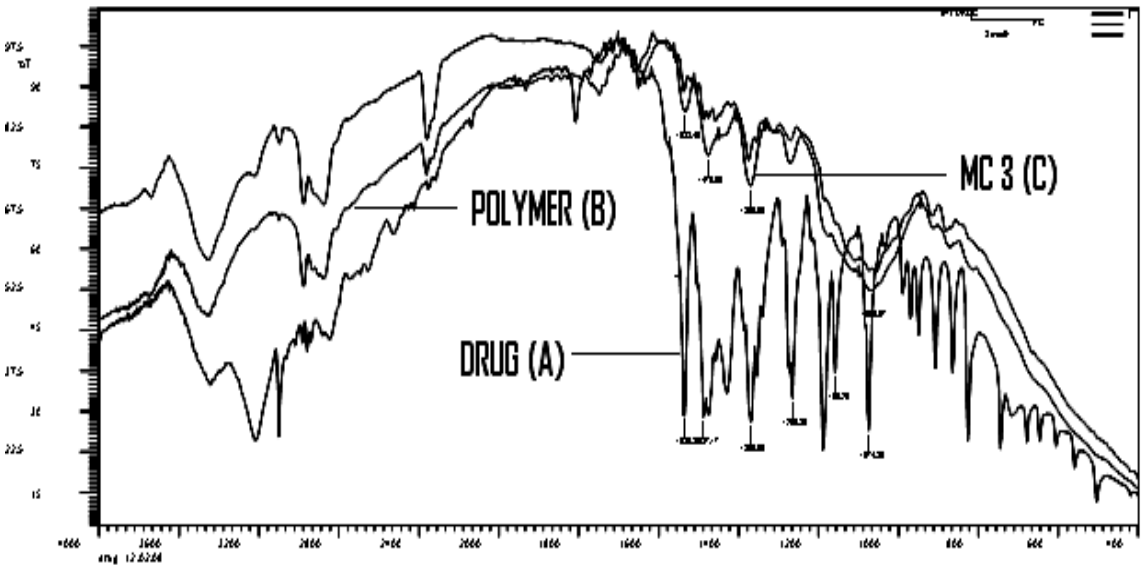

Fig. 2 : It Shows Entire FT-IR spectra and analysis region (In inset) of pure drug (A), ethyl cellulose (B), vaginal microcapsule formulation (C).

Scanning electron microscopy (SEM)

The morphology of the ethyl cellulose metronidazole systems prepared by thermal change method was investigated by SEM analysis (Fig. 3). Microcapsules appear as small spherical particle with smooth surfaces of homogenous morphology and no aggregation was seen.

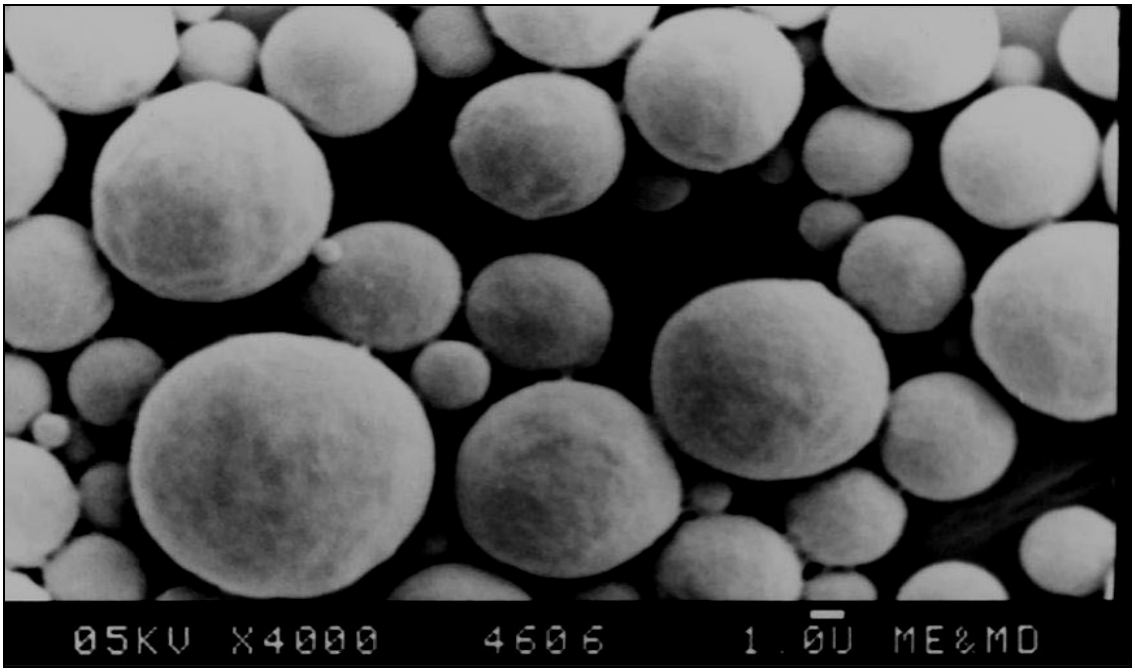

Fig. 3 : It shows scanning electron microscopy photograph of vaginal microcapsule at 05KV × 4000.

Preparation of microencapsulated gel

Selected batches of prepared vaginal microcapsules were then incorporated in gels prepared by mechanical stirring with various grades of bioadhesive[5] polymer, such as carbopol 934 (ŋ 37200 cps), 940(ŋ 49000 cps), 974 (ŋ 35850 cps) and 980(ŋ 47200 cps) and other formulation additives. The experimental design of the formulated gels was expressed in Table. 4.

Table 4. Experimental design of Microencapsulated Bioadhesive Vaginal Gels.

Formul-ation	Microencapsulated Bioadhesive Vaginal Gels compositions Amount taken in percentage (w/w)					
	Micro-capsules	Carbopol	Triethanol-amine	Alcohol	Propylene glycol	Distilled Water
F1	1	0.6	0.5	20	10	q.s.
F2	1	0.8	0.6	20	10	q.s.
F3	1	0.6	0.5	20	10	q.s.
F4	1	0.8	0.6	20	10	q.s.
F5	1	0.6	0.5	20	10	q.s.
F6	1	0.8	0.6	20	10	q.s.
F7	1	0.6	0.5	20	10	q.s.
F8	1	0.8	0.6	20	10	q.s.

F1, F2: Carbopol 934, F3, F4: Carbopol 940, F5, F6: Carbopol 974 and F7, F8: Carbopol 980. q.s. quantity sufficient.

Drug content and uniformity

The column 2 and 3 of Table. 5 showed the drug content and homogeneity of microencapsulated gel formulations. The drug contents of the prepared microencapsulated gels were found to be in the range of 53.433 - 94.188 % indicating the applications of the present method for the preparation of novel semi-solid MBVG system with high drug content uniformity.

pH measurement

The pH of gels as showed in column 4 of Table. 5 were found to be within the range of 6.8 to 7.8 which is within the limit of semisolid specifications. The almost neutral pH reflected, the gel will be non irritant to vagina. This was further confirmed by vaginal irritation study in rabbit.

Spreadability and extrudability

The spreadability plays an important role in patient compliance and helps in uniform application of gel to the skin. A good gel takes less time to spread and will have high spreadability. The spreadabilty of formulated gels was decreased as the concentration of polymer increased. The extrusion of gel from tube is important during application and for the patient compliance. Extrudability of gel formulations with low polymer content was found satisfactory Fig. 8. Dissolution profile of various Microencapsulated Bioadhesive Vaginal Gels.

Each point represents as mean ± S.D., n=3.

while the high polymer content, good extrudability was observed. From the data of spreadability and extrudability as given in column 2 and 3 of Table. 6, among all the formulations, formulation F5 having good spreadability and extrudability and selected.

Table 5. Physical properties of microencapsulated bioadhesive vaginal gels.

Formulation	Drug content (%) (X ± S.D.)	Drug content uniformity	pH (X ± S.D.)
F1	78.72 ± 0.030	**	7.5 ± 0.011
F2	81.93 ± 0.042	***	7.4 ± 0.024
F3	53.33 ± 0.055	**	7.3 ± 0.016
F4	79.74 ± 0.021	***	7.1 ± 0.025
F5	94.52 ± 0.043	***	6.8 ± 0.027
F6	78.67 ± 0.051	**	7.1 ± 0.033
F7	72.98 ± 0.029	**	7.2 ± 0.025
F8	76.40 ± 0.054	*	7.3 ± 0.015

Each value represents as mean ± standard deviation, n=3. Standard error mean < 0.317. * (good), ** (very good), ***(excellent).

Viscosity

Viscosity is an important parameter for characterizing the gels as it affects the spreadability, extrudability and release of drug. The column 4 of Table. 6 showed the data of viscosity. The viscosity of gels was increased with the increase in carbopol content which may be due to the increase in formation of three dimensional cross linking structure of gel, as expected.

Vaginal irritation study

The result of vaginal irritation study was shown in column 5 of Table. 6. All formulations were found to be non irritant to vagina of New Zeeland white rabbits.

Table 6 : Rheological properties and vaginal irritation data of microencapsulated bioadhesive vaginal Gels

Formulation	Spreadability (g.cm/sec) (X ± S.D.)	Extrudability	Viscosity (cps) (X $\times 10^4$)	Irritation
F1	046.87 ± 0.098	*	2.015	-
F2	028.85 ± 0.181	**	4.175	-
F3	075.02 ± 0.134	**	1.742	-
F4	057.69 ± 0.174	**	2.397	-
F5	150.01 ± 0.324	***	1.802	-
F6	166.67 ± 0.112	**	1.645	-
F7	187.51 ± 0.315	***	1.555	-
F8	125.12 ± 0.114	**	2.702	-

Each value represents as mean ± standard deviation, n=3. Standard error mean < 0.187.
* (good), ** (very good), *** (excellent) and - (no irritation).

***In vitro* drug diffusion studies and release kinetics**

The release mechanism was not significantly influenced by formulation variables and was predominately diffusion controlled. The release rate was inversely proportional to wall thickness. The *in vitro* drug release of all the formulations (F1-F8) was found constant for each formulation and influenced by the polymer added. The *in vitro* drug release profile was presented in column 3 of Table. 7, Fig. 4 and Fig.10 indicated release from microcapsule retarded by incorporating in gel network. To categorize the kinetics of drug release from microencapsulated gel, release data was verified with different kinetic models. The column 4, 5, 6, 7 of Table. 7 indicated that drug release from all formulations obeyed Higuchi kinetic equation except formulation F1, F4 and F5 which obeyed Korsemeyer and Peppas kinetics. The column 8 of Table 7 showed that all the formulations

released the drug by diffusion following Fickian (n<0.5) transport mechanism except the formulation F2, F3 and F6 which follow non-Fickian (n>0.5) transport mechanism. Statistical verification with one way ANOVA method attested the fact that the drug release data were found significant for F (20.252) at 5 % level of significance ($p < 0.05$).

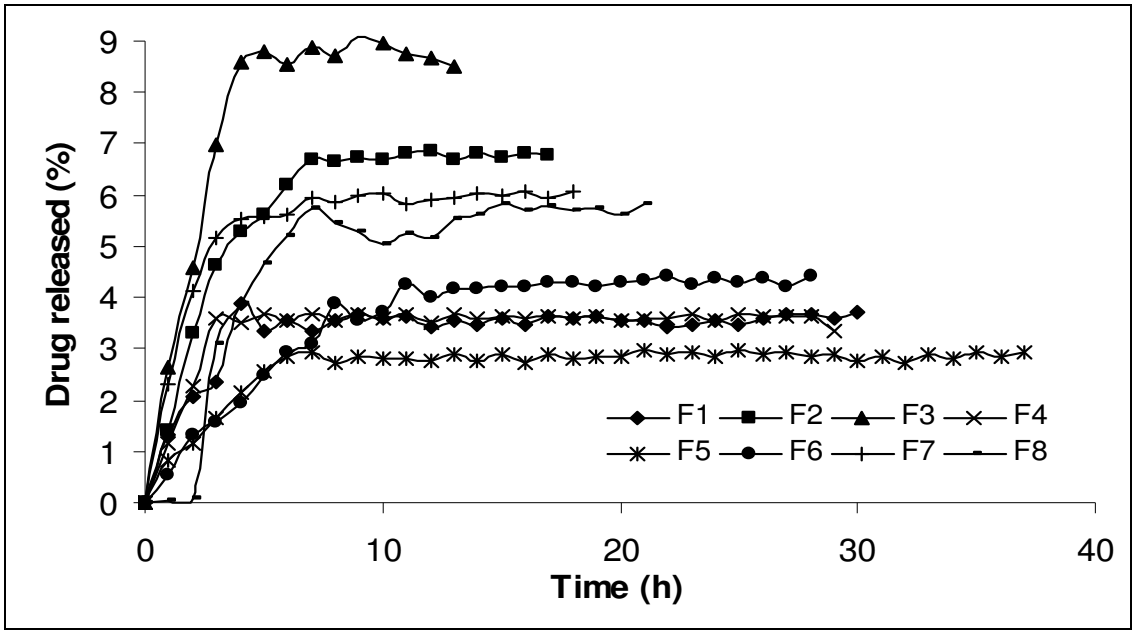

Fig. 4 : It shown dissolution profile of various microencapsulated bioadhesive vaginal gels. Each point represents as mean ± S.D., n=3.

Table 7 : Vaginal bioadhesive strength, drug release and *in vitro* release kinetics data of microencapsulated bioadhesive vaginal gels.

Formulation	Vaginal Bioadhesive Strength (Kg) (X ± S.D.)	Cumulative % Drug release (X ± S.D.) (12 h study)	Zero order equation	First order equation	Higuchi Square root eq.	Korsmeyer and Peppas equation	
			Regression co-efficient				
				(r)		(n)	
F1	0.069 ± 0.011	37.985 ± 1.12	0.560	0.716	0.858	0.883	0.403
F2	0.100 ± 0.010	66.823 ± 1.31	0.784	0.750	0.930	0.911	0.608
F3	0.080 ± 0.015	93.168 ± 0.98	0.751	0.702	0.889	0.848	0.624
F4	0.120 ± 0.013	39.460 ± 1.25	0.486	0.656	0.824	0.846	0.401
F5	0.210 ± 0.014	28.097 ± 0.88	0.581	0.797	0.925	0.942	0.456
F6	0.190 ± 0.016	33.271 ± 1.09	0.819	0.876	0.979	0.971	0.682
F7	0.170 ± 0.020	63.815 ± 1.14	0.676	0.658	0.871	0.818	0.444
F8	0.140 ± 0.009	48.869 ± 1.05	0.760	0.649	0.892	0.791	1.376
ANOVA							
F	78.023						
df	39						
p	6.12						

n- Diffusion exponent related to mechanism of drug release, according to Korsmeyer and Peppas equation, $\mathbf{m_t / m_\alpha = kt^n}$. Each value represents as mean ± standard deviation, n=3.Standard error mean < 0.756.

Vaginal bioadhesion measurements

Figure 5 and column 2 of Table. 7, indicates the vaginal bioadhesive properties of the prepared gels (F1-F8) in goat vagina and the result showed that all vaginal bioadhesive strengths were found in the following order F5>F6>F7>F8>F4>F2>F3>F1. It was concluded that carbopol 974 (F5) showed the highest bioadhesive property.

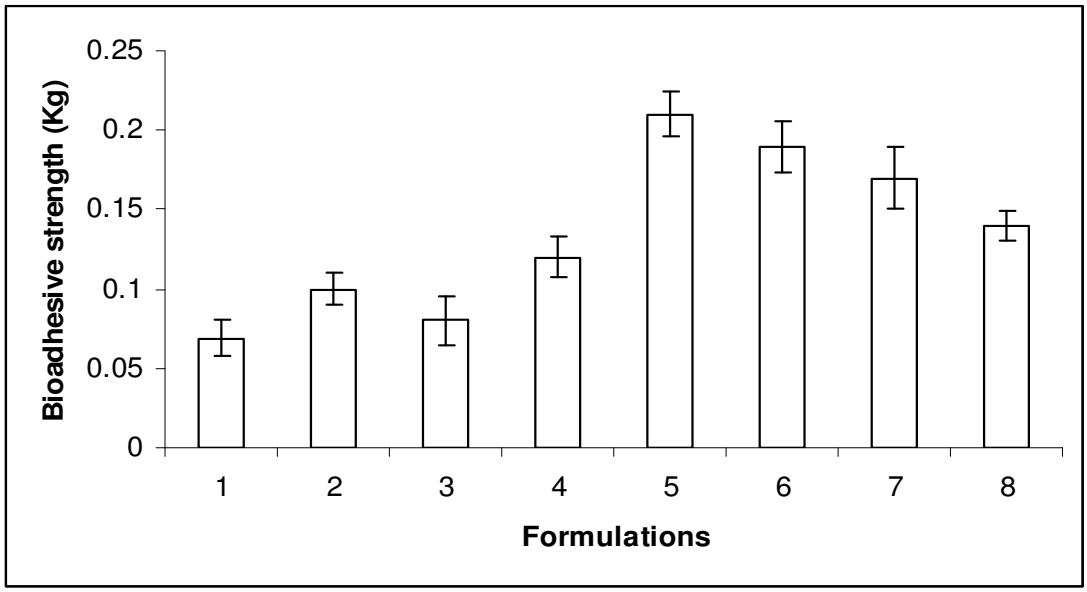

Fig. 5 : It shows vaginal bioadhesion measurement of various microencapsulated bioadhesive vaginal gels. Each point represents as mean ± S.D., (n=3).

Accelerated stability studies of microencapsulated gel

The accelerated stability studies were performed according to ICH guidelines for 12 weeks and the results were found to be stable in varying temperature as shown in Table. 8, which further verified with one way ANOVA method, found to be significant for F (3.395) at 5 % level of significance ($p < 0.05$).

Table 8 : Accelerated stability study of selected microencapsulated bioadhesive vaginal gels

Storage Temp. (°C)	Potency of formulation (%) Period of studies in week						
	1st day	2nd	4th	6th	8th	10th	12th
37 ± 2	99.56	99.31	99.12	99.05	98.87	98.51	98.39
45 ± 2	99.56	99.17	98.94	98.76	98.61	98.29	98.15
60 ± 2	99.56	99.08	98.84	98.54	98.33	98.13	97.98
pH	6.8	6.9	6.7	6.6	6.7	6.8	6.9

CONCLUSION

In conclusion, MC_3 containing drug: polymer ratio 1:4 was found to be the best microcapsule formulation, regarding all the properties evaluated in order to achieve one objective of this study. Formulation MC_3 was selected on basis of its slower release rate, higher entrapment efficiency, excellent flow property and higher wall thickness for its use in next objective.

Another objective was to further incorporation of selected microcapsules in gel by using different carbopol polymers for prolonging the bioadhesion and release of representative drug. The evaluation reports of microencapsulated gel explained F5 gel (containing 1 % w/w of drug loaded microcapsules and 0.6 % w/w of carbopol 974) was found to be the best, releasing about 100 % of metronidazole over a period of 36 hours in SVF successfully. The novel formulation design facilitated the optimization and successful development of MBVG formulations for enhanced vaginal drug delivery by optimum vaginal bioadhesion and longer retention. Our data concluded that MBVG protocol may be an effective strategy for the development of easy, reproducible and cost effective method to prove its potential for safe and effective vaginal delivery therapy. This technique can be further tested for the development of different vaginal carrier therapeutics.

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