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Formulation and Evaluation of *In Situ* forming Polymeric Drug Delivery Systems for Mixed Vaginal Infection

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Authors' contributions

This work was carried out in collaboration between both authors. Author HAA designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author RK managed the analyses of the study as well as the literature searches. Both authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: Vaginal preparations are still associated with number of problems including frequent administration and escape from vagina causing discomfort to patient. For efficient vaginal delivery of drugs, the delivery system should reside at the site of infection for a prolonged period of time therefore this work aims to prepare Vaginal Capsules containing sustained release *in situ* forming polymeric particles containing broad spectrum antibiotics to cover all the common pathogen associated with vaginal infections.

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Study Design: Characterization for the developed beads, such as determination od, particle size, drug entrapment yield, and drug release profiles, were characterized prior to determining intracellular uptake profile, *in vitro*, and *in vivo* tissue distribution patterns of the particles.
Place and Duration of Study: Department of pharmaceutical technology in German university in Cairo and Department of Pharmaceutical Technology in National Research Center, Cairo, Egypt between June 2012 and March 2014.
Methodology: Calcium alginate, chitosan and mixed polycarbophil beads containing fluconazole (antifungal) and metronidazole (antiprotozoal) were packed in hard gelatin capsule and evaluated as new vaginal drug delivery forms beads were characterized by size, Scanning Electron Microscopy (SEM), weight uniformity and drug entrapment efficiency as well as *in –vivo* bioadhesion test
Results: Results of the *in vitro* antimicrobial study indicated that the M5 and F4 mixed beads had better antimicrobial action than the commercial intravaginal drug delivery systems and retention was prolonged in an *ex vivo* retention study showed that the bioadhesion of the beads were 68.88 to 84.3%.
Conclusion: Developed beads were found to be more effective in *in vitro* conditions. The average zone of inhibition of the developed M5 the formulae that contain (Metronidazole: Chitosan: Polycarbophil) in (1:1:1 ratio)and F4the formulae that contain (Fluconazole: Chitosan: Polycarbophil) in (1:1:1 ratio) mixed beads *Candida albicans* was 28.3±0.6 mm compared to 24.5±1.7 mm of commercial Amrizol®, indicating significantly higher efficacy of M5 and F4 mixed beads (p>0.001). Average zone of inhibition of the developed M5 and F4 mixed beads against *E. coli* was 31.5±2.2 mm compared to 28.6±1.8 mm of Amrizol®. The developed M5 and F4 mixed beads was more effective than the tested commercial formulations and last for12, 18 and 24 hr.

Keywords: Beads; sustained release; fluconazole; metronidazole.

1. INTRODUCTION

Vaginitis is a common worldwide ambulatory problem in women and it is responsible for more than 10% of visits to providers of women's health care [1]. It generally occurs under predisposing conditions such as such as, diabetes, antibiotic therapy, oral contraceptive and pregnancy (2). Disease is a common disorder among women and nearly 75% of women suffer once in their lifetime of genital candidiasis [2]. In addition, about 5-10% of women suffer from recurrent vaginal candidiasis during their reproductive age [3]. There are mainly three types of infectious of the vagina: *candidiasis, trichomoniasis*, and bacterial Vaginosis [4,5]. Approximately 30% of all cases of vaginitis are caused by simultaneous infections with at least two or more pathogens (e.g. bacterial vaginosis in patients of vulvovaginal *candidiasis*) [6].

The therapy for vaginitis usually consists of Combination therapy which may provide immediate and effective treatment for vaginal infections, irrespective of single or multiple types or even when the diagnosis is not precisely accurate [6] the conventional formulations of vaginal drug delivery systems (VDDS) are associated With poor retention due to the self-cleansing action of vaginal tract, leading to poor Compliance [7] Bioadhesive polymers can hold the drug delivery systems in the vaginal tube by interactingwith vaginal mucosa and thereby increase patient compliance as well as delivery System efficiency [8,9].

Bioadhesive polymers like sodium alginate showed good stability in the wide range of pH 3–10 and are hence good candidates for vaginal drug delivery systems at pH 3.8–4.5, moreover chitosan and polycarbophil demonstrated potential candidate for controlled release of drugs in buccal, vaginal and rectal pH with optimum swelling approaching zero order release [10]. The drugs fluconazole and metronidazole were selected because they are broad-spectrum antimicrobial agents [11,12]. Calcium alginate gel beads have been developed in recent years as a unique vehicle for oral drug delivery due to their excellent biocompatibility, biodegradability, simple method of preparation, abundant sources, low cost and minimal processing requirements [13]. Moreover though these selected polymer achieve prolong contact time and have some ability to modulate drug release from gels, water-soluble drugs particular release from such system quickly [13].

The present investigation deals with development and evaluation of Vaginal Capsules containing sustained release *in situ* forming polymeric particles containing broad spectrum antibiotics to cover all the common pathogen associated with vaginal infections bioadheve polymers for the formulation of vaginal beads of fluconazole and metronidazole. Present dosage form includes thermosensitive polymers and pH activated polymers for *in situ* gel formulation. The prepared dosage regimens provided ease in administration along with good bioadhesion and retention properties.

2. MATERIALS AND METHODS

2. 1 Material

2.1.1 Materials

Chitosan, highly viscous % deacetylation>75 and viscosity 800 – 2000(cps) and sodium alginate from brown algae were purchased from Fluka, BioChemika, Japan. Polycarbophil was a gift from (POLY, Noveon AA-A, Goodrich Chemicals, England). Metronidazole (M) and Fluconazole (F) where kindly supplied by(EPICO Company Egypt) Sabouraud Dextrose Agar was purchased from Oxoid, England; its typical formula (g/L) mycological peptone 10.0; glucose 40.0; Agar *15.0,* pH 5.6±02 Lot/CH, -B: 340 *53683.* Sabouraud liquid medium was purchased from Oxoid, England *Candida albicans* was isolated from vaginal swab of female patient with vaginal candidiasis who had received no antifungal or antimicrobial therapy for three weeks .The available commercial product Amrizol® suppository Amrya Pharm, Each suppository contains 500 mg Metronidazole.

Potassium dihydrogen phosphate and disodium monohydrogen phosphate, Sigma-Aldrich (USA).

2.1.2 Equipment

PH meter, (Schott-Geräte, GmbH, Germany), Centrifuge, Remi laboratory centrifuge R32 A, (Remi Equipment, Bombay, India), Vortex mixer (Paramix II, Julabo, Germany), Autoclave (systec 5075ELV, Germany) Incubator Eocell Medcenter (Einrichtungen GmbH MMM Med Center D-82166 Grafelfing, Germany). Scanning electron microscopy (Model Quanta 250 FEG Field emission gun, FE I company, Netherlands).

2.1.3 Tested animals

Eight adult New Zealand female albino rabbits weighing *2.5±0.25* Kg were used.

2.2 Preparation of the Beads

The beads where prepared by the previously discussed ionotropic gelation and extrusion method [14] which involves an “all-aqueous” system and avoid the presence of residual solvents. Briefly, the beads were produced by dissolving the polymer (s) in an aqueous solution, suspending the active ingredient in the mixture under heating and extruding through a precision device (18 G needle syringe) at constant rate (1mL/min) ,producing microdroplets which fall into a slowly stirred hardening bath containing cross-linking agent, the homogeneous solution of alginate and chitosan was dripped into CaCl2 solution (2%), the resultant calcium cross linked beads were dipped in glutaraldehyde (2%) solution sequentially to prepare dual cross linked beads (s), spherical beads were formed and allowed to harden for before washing with distilled water and then drying under vacuum at room temperature until attaining constant weight. In case of the mixed polymeric preparations, the mixture was subjected to high speed stirring then homogenization at 20.000 rpm for 5 min before extrusion; the composition of the different beads is shown in Table 1.

Table 1a. Composition of both metronidazole and fluconazole loaded beads

Formula	Composition	Ratio
M1	Drug: Alginate	1:1
M2	Drug: Chitosan	1:1
M3	Drug: Alginate: Chitosan	1:1:1
M4	Drug: Alginate: Polycarbophil	1:1:1
M5	Drug: Chitosan: Polycarbophil	1:1:1
F1	Drug: Alginate	1:1
F2	Drug: Chitosan	1:1
F3	Drug: Alginate: Chitosan	1:1:1
F4	Drug: Alginate: Polycarbophil	1:1:1
F5	Drug: Chitosan: Polycarbophil	1:1:1

2.3 Morphology and Particle Size Analysis

The shape and surface morphology as well as Particle size of beads prepared, were characterized by scanning electron microscopy (Model are mentioned in the Material and methods part).

2.4 Determination of Drug Encapsulation Efficiency

To determine the total drug content of prepared beads a known amount of beads were ground to fine powder, 500 mg of beads were soaked in 50 mL of distilled water and sonicated for 2 h, then solution was to remove the insoluble parts of the polymers, then was washed twice with fresh solvent (water) to extract any adhered drug finally clear solution was filtrated filter then analyzed UV spectrophotometer.

The percent drug loading was calculated according to the following Equation:

$$\text{Percent LE} = (\text{drug load- drug loss})/\text{drug load} \times 100$$

The percent entrapment efficiency (%EE) was calculated according to the following Equation:

EE (%) = (actual drug content/ theoretical drug content) ×100

2.5 Measurement of *In vitro* Bioadhesion

In- vitro bioadhesion (in triplicate) was determined by following a previously reported method. [15,16]. The vagina of the overnight fasted rabbits were removed and cut into pieces 2 cm long and 1 cm wide and were rinsed using 2 ml of physiological saline and 50 mg of beads were placed the vaginal mucosa. Both were placed at 80% R.H, and temperature of 25±0.5ºC to keep it with sufficient hydration for 25 min. The mucosal lumen was rinsed with simulated vaginal fluid angle of 45º [15,16], the washings were dried at 60℃ in a hot air oven, and then ratios of adhered and applied beads were calculated as percent bioadhesion.

2.*6 In-vitro* Release of Metronidazole and Fluconazole from Different Vaginal Beads

In- Vitro Release of Metronidazole (MTZ) and Fluconazole (FLZ) from different beads was carried out by using glass cylindrical tubes opened from both ends and having a diameter of 2.5 cm and was tightly covered with a semipermeable membrane (Albet® Cellulose nitrate membrane filter, 0.45 urn pore size) *a certain amount of beads equivalent to either to 500 mg* MTZ or 150 mg FLZ was placed in the cylindrical tube covered with the semipermeable membrane. The tube was suspended so that the membrane was just below the surface of 50 ml phosphate buffer pH 4.8 and magnetically stirred at approximately 150 rpm in water bath maintained at 37± 0.5ºC. Samples, each of three ml were withdrawn from the beaker at 0.5, 1, 2, 3, 4, 6, 8, 12 and 24 hours time intervals and replaced by equal volumes of fresh buffer, then concentration of Metronidazole in the samples was measured spectrophotometrically at λ_{max}316 nm and λ_{max} 260 nm for Metronidazole (MTZ) and Fluconazole (FLZ) respectively.

2.7 *In-vitro* Disintegration Capsules

Simulated vaginal fluid [17] was used as test medium to evaluate the disintegration properties hard gelatin capsule using the watch glass method to simulate vaginal disintegration [18,19]. One capsule was placed in the centre of a watch glass (diameter: 11 cm), which floated on a water bath at 37ºC. Simulated vaginal fluid of 4 ml (37ºC) was poured on the capsule and. The disintegration time was defined as the time point at which the beads were released from the capsules.

2.8 Kinetic Modeling of Drug Release

Curve fitting was performed using Microsoft Excel 2007 version. The dissolution data were fitted to the following equation. Release exponent 'n' was calculated [20].

$$Mt / M\infty = Ktn$$

Where, Mt / M∞ is the fraction of the drug released at time t, k is the kinetic constant of the system, and n is the exponent characteristic of the mode transport. The release exponent takes various values depending upon different geometries, for the drug release from a cylindrical or a flat swellable polymer, if n approaches to 0.89, the release mechanism could be Case-II transport and if n is close to 0.45, the release mechanism can be Fickian. On the other hand if 0.45 <n <0.89, non-Fickian transport could be obtained [20].

2.9 *In- vitro* Antifungal Study

In-vitro antifungal study was performed against *Candida albicans and Escherichia coli* microorganisms by the cup plate method [21].

Sterilized Sabouraud's agar/nutrient agar medium (25 mL) was poured into sterilized Petri plates (diameter 15 cm) under laminar air flow and allowed to solidify, the 0.4mL aqueous suspension of *Candida albicans/Escherichia coli* was spread uniformly on solidified Sabouraud's agar/nutrient agar medium. The cups were cut and formulations were filled into different cups using sterilized syringes, under laminar air flow the cups cut in the inoculated solidified media were filled with different formulations using sterilized syringes. The marketed suppository Amrizol® formulation was crushed and dissolved in 2 mL of sterilized water was applied using sterilized syringe. The developed M5 and F4 mixed beads were swelled in 2 mL of sterile water applied into the cups.

The comparative antimicrobial efficacy of the antimicrobials was studied on the basis of the zone of inhibition. The study was continued for 24 h for the both so as to check the sustained antimicrobial activity of the mixed beads which last for12, 18 and 24 h.

3. RESULTS AND DISCUSSION

3.1 Morphological Properties and Size of Metronidazole and Fluconazole Beads

SEM showed that prepared beads were as a uniform units and spherical in shape with a rough outer surface because of the accompanied drug (Fig. 1b). The surface associated drug adsorbed on the surface of the beads might give an immediate release this is coincide with Paruvathanahalli et al. [22] and help enhance the MTZ as well as FLZ concentration for the effective *candida albicans* clearance shortly after vaginal administration.

Incorporating a combination of polycarbophil improved the undesired irregular shape of beads caused by incorporation of the drug [23] the scanning electron micrograph (SEM) of the beads obtained is shown in Fig. 1a.

It was observed that the addition of chitosan to the coagulation solution produced beads with a smoother surface than that of alginate alone, this is coincide with Bazigha et al. [24]. The yield of prepared beads was almost 80 to 90% for all the formulations.

It was noticed that the bead yield was slightly lower (70–75%) in case of mixed polymer formulations. Sizes of the prepared beads of different formulations are shown in Table 1. The mean diameter of the microspheres was found to be in the range of 631.4 um to 935.6 um.

3.2 Incorporation Efficiency of Beads

The incorporation efficiency of the prepared beads is shown in Table 1. The incorporation efficiency of the prepared beads varied from 59.2% for F5 to 78.2% for M5 The incorporation efficiency increased with formulae containing both Alginate and chitosan and was higher inalginate containing formulae than other formulae this is coincide with Paruvathanahalli et al. [22].

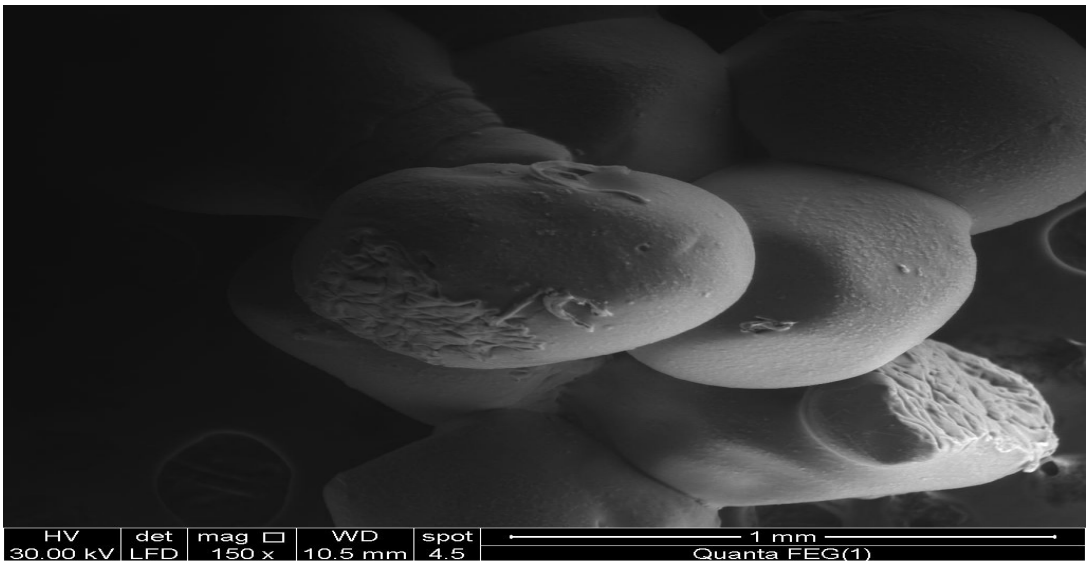

Fig. 1a. SEM Photo graph of prepared bioadhesive beads

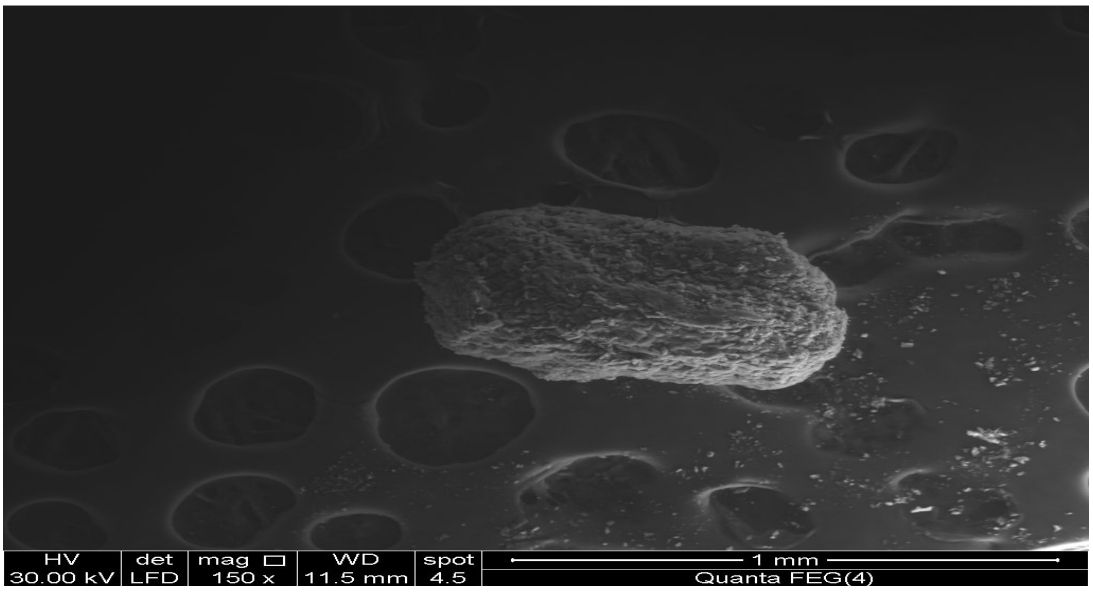

Fig. 1b. SEM Photo graph of prepared bioadhesive beads morphological surface

3.2 Bioadhesivity

The study of *in vitro* bioadhesion revealed that all the batches of prepared beads had good bioadhesive property ranging from 68.88 to 84.3%.

The formulation F1 showed the highest bioadhesive property (84.3) These studies suggest that the spherical matrix of microspheres can interact with muco substrate on the surface of the vagina, and adhere to mucosa more strongly and could stay in vagina for prolong period

for more effective *against vaginal* clearance characterized by an initial phase of high release (burst effect) followed by a second phase of moderate release. Coincide with Paruvathanahalli et al. [22]. Also the beads with a coat consisting of sodium alginate and mucoadhesive polymer exhibited good mucoadhesive property in the ex vivo wash off test [25] values are listed in Table 1.

3.3 *In –vitro* Drug Release

The dissolution profiles of the different MTZ and FLZ batches of the beads are depicted in Figs. 2 and 3, where T50% is time required for 50% drug release, T75% is time required for 75% drug release and T90% is time required for 90% drug release also the dissolution parameter of the different formulations is indicated in Tables 2 and 3 based on the T50% the release of MTZ from the different formulation can be arranged as M5> M1> M4> M2> M3 and for FLZ as F5>F4>F2>F1>F3. Similarly, based on the T75% (h) values (time taken for 75% of drug to release) the drug release from different batches can be arranged as M5>M1=M2>M4>M3 for MTZ containing beads and arranged as F4>F5>F2>F3>F1 for FLZ containing beads, after 24(h) of dissolution study the formulation M2,M5and F4 achieved 90% of drug release in less than 24 hours, all other formulation did not attain T90% (h) values time taken for 90% of drug release even after 24.hs of dissolution study.

The differences in drug release characteristics of various spheres are due to differences in the porosity of the coat formed and its solubility in the dissolution fluid [26,27]. Among all batches, the M5 and F4 Batches are considered to be optimized formulation (T50%2.5, T90%20 h for M5 and T50%4.5 and T90% 22 h for F4) because among all the batches it shows better extent of drug release ,good entrapment efficiency 78.2% for M5 and 76.6% for F4 and the in vitro wash off test showed good bioadhesion (71.3% for M5 and 72.6% for F4) MTZ and FLZ release from the optimized formulae M5 and F4 was slow and extended over a period of (24)h and these drug loaded spheres were found suitable for vaginal controlled release. The initial release of MTZ and FLZ (drug) appears to depend on the concentration of alginates smaller concentration of alginate in spheres may have produced more porous spheres which release drug (MTZ or FLZ) more quickly [28]. Moreover, spheres containing smaller alginate concentration may have produced a relatively weaker network which broke down faster than the relatively weaker network which broke down faster than the relatively stronger network formed spheres containing a larger concentration of alginate [29] In most release studies dealing with muliparticulate systems, an initials burst effect is reported due to migration of drug to the surface of the particles. In this investigation, a burst effect was exhibited by spheres containing low concentration of the polymer. The initial slow release was followed by a linear rate of release until almost90% of drug release [30] as the degree of cross linking increases, the porosity decreases and the reduced porosity will further retard the release of drug from alginate spheres [31].

Also, drug release from a hydrophilic matrix is controlled by the formation of a hydrated viscous layer around the matrix which act as a barrier to drug release by opposing penetration of water in to the matrix and also movement of dissoluted solutes out of the matrix [32]. The release of drug was considered to occur mostly by diffusion but could be accelerated by the weight loss of the mucoadhesive polymer. The alginate mucoadhesive polymeric gel might have acted as a barrier to the penetration of the dissolution medium, there by suppressing the diffusion of the drug from the swollen alginate-mucoadhesive polymeric matrix [33]. The release of the drug was modulated by diffusion of the drug through the swollen polymeric matrix [34]. The release of Metronidazole from Formulae M1and M4 mainly batches were Characterized by an initial phase of high release (burst effect) followed

by a second phase of moderate release. This bi-phasic pattern of release is a characteristic feature of matrix diffusion kinetics [35,36]. But in case of Fluconazole the burst effect were from formulae F4 and F5 (polycrpophil) containing formulae the slow release of fluconazole from chitosan beads may be due to the higher swelling profile and slower erosion rate of chitosan based beads [37,38].

Increasing the polymer concentration cause reduction in the initial burst effect although that increasing the polymer concentration resulted in better incorporation efficiency could be the reason for the observed decrease in burst effect since the amount of surface associated drug decreases with an increase in incorporation efficiency [22].

These polymers showed high swelling resulting in an increase in diffusional pathlength of drug and then cause reduction of drug release [39]. Moreover, the thick gel layer formed on the swollen film surface will prevent matrix disintegration and controlling additional water penetration [40].

Table 1b. Characterization of beads metronidazole and fluconazole vaginal beads

Formula	Mean Particle size in UM±S.D	Incorporation efficiency (%) Mean ± S.D	Bioadhesion (%) Mean ±S.E.M
M1	678.32±2.11	71.2±2.1	82.5±1.62
M2	744.1±1.6	73.7±1.03	78.66±2.13
M3	755.7±0.34	66.3±1.63	79.12 ±1.23
M4	935.6±2.2	76.5±0.89	74.32±1.78
M5	825.7±1.3	78.2±1.8	71.3±1.4
F1	759.6±0.78	72.1±1.24	84.3±1.32
F2	703.3±0.67	66.2±2.3	79.2±1.88
F3	641.1±0.35	63.5±2.26	77.3±1.9
F4	631.4±1.04	76.2±2.05	72.6±2.1
F5	712.1±2.35	59.2±1.36	68.88±1.73

Table 1b Characterization of beads 1

3.4 Release Kinetics

The obtained release data were kinetically evaluated by either zero order, first order or Higuchi model. [41] the release data was ideally characterized by Higuchi model According to the determination coefficients (*r*2) suggesting a similarity to release from a matrix [Higuchi] which suggesting that the diffusion and erosion are playing an essential role in extending the drug release [42]. The linear regression analysis is summarized in Table 4. The examination of coefficient of determination (*r*2) values indicated that drug release followed the diffusion control mechanism from the microspheres. Further, to understand the drug release mechanism, the data were fitted to Peppas exponential model [34].

$$Mt/M\infty\infty = K^{n}_{t} \text{ where}$$

Mt/*M*∞ is fraction of drug released after time 't' and '*K*' is kinetic Constant and '*n*' is release exponent which '*n*' is release exponent which characterizes the drug transport mechanism. Values for release exponent '*n*' are listed in Table 4. The values of '*n*' were in the range of 0.143 to 0.234, which was further indicative of the drug release following Fickian diffusion (n<0.45).

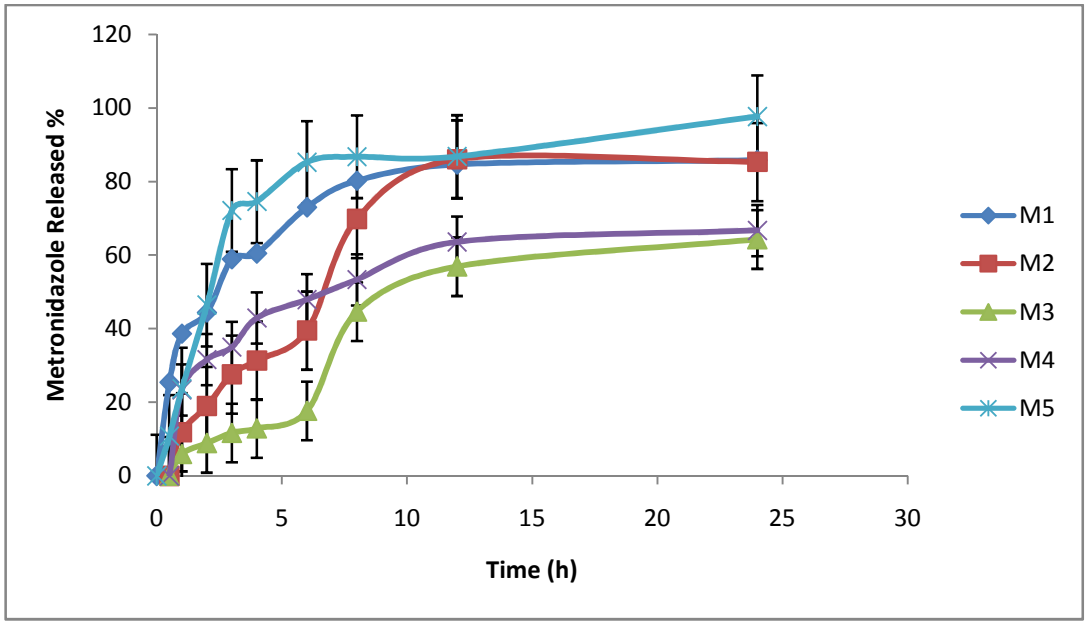

Fig. 2. Release profile of metronidazole from different beads

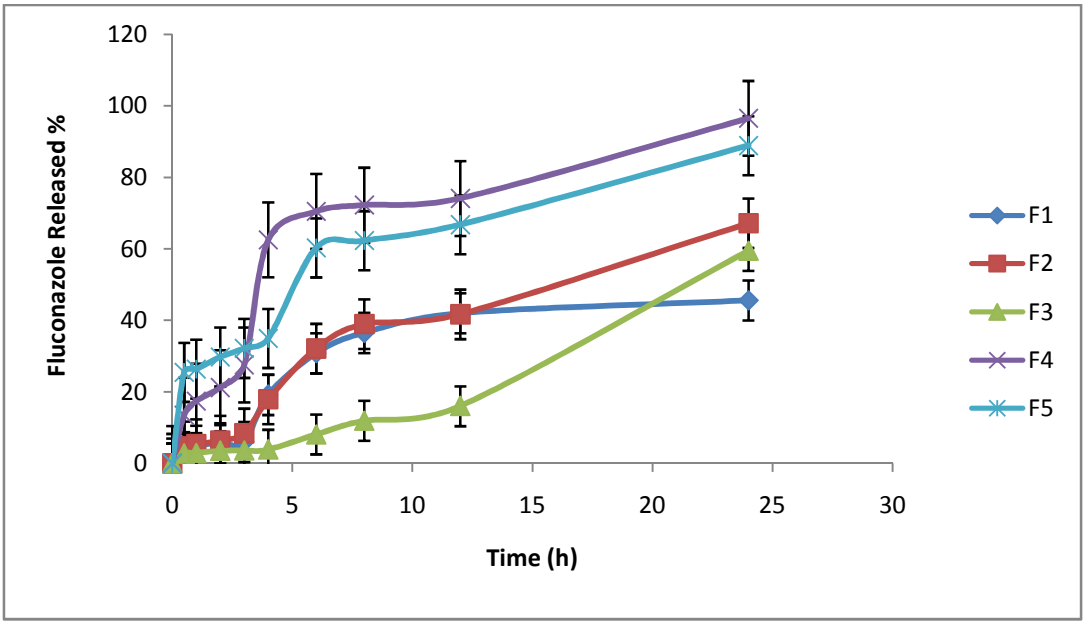

Fig. 3. Release profile of fluconazole from different beads

Table 2. Release profile of metronidazole from different beads

Time (hour)	M1 %	M2%	M3%	M4%	M5%
0	0±SD	0±SD	0±SD	0±SD	0±SD
0.5	25.38±1.2	11.87±1.81	5.93±2.01	23.39±1.32	10.80 ±1.54
1	38.68±1.1	19.02±1.32	8.88±1.9	31.64±1.8	23.67±1.1 2
2	44.40±2.3	27.59±1.76	11.69±1.7	34.98±1.87	46.43±1 .65
3	58.88±1.6	31.36±1.09	12.90±0.98	42.95±2.34	72.16± 1.9
4	60.44±.15	39.53±1.9	17.70±1.4	47.92±1.36	74.55±0. 87
6	72.96±1.3	69.83±1.23	44.65±1.1	53.33±1.3	85.24±1. 5
8	80.21±2.1	86.04±1.52	56.88±2.01	63.57±1.2	86.75±1 .123
12	84.62±2.3	85.33±1.3	64.28±1.72	66.73±1.17	86.81± 1.43
24	85.76±1.6	93.15±1.65	81.35±1.36	77.77±1.87	97.66 ±1.33
T50%	2.5h	5h	7.5h	5h	2.5h
T75%	7h	7h	22h	14h	5h
T90%	>24h	23h	>24h	>24h	20h

Table 3. Release profile of fluconazole from different beads

Time	F1 %	F2 %	F3 %	F4%	F5%
0	0±SD	0±SD	0±SD	0±SD	0±SD
0.5	3.15±1.716	4.76±1.543	2.66±88	13.49±1.47	25.46± 1.17
1	4.94±0.895	5.38±1.383	2.910±025	17.42±0.93	26.38± 0.93
2	5.62±0.852	6.36±0.927	3.46±608	21.206±1.1	29.78±1 .605
3	5.99±2.23	8.40±0.98	3.58±648	27.55±1.73	32.19±1.8 3
4	19.15±1.22	17.92±1.353	3.83±785	62.56±1.55	34.97± 1.59
6	30.83±1.38	32.13±1.385	8.099±878	70.53±1.65	60.30 ±1.733
8	36.52±2.05	38.99±1.87	11.93±049	72.29±0.965	62.34 ±1.62
12	42.01±2.28	41.71±0.835	16.00±823	74.150±1.05	66. 79±1.65
24	45.6±1.43	67.227±1.3	59.50±413	96.57±1.76	88.91± 1.33
T50%	>24 h	22h	23h	4.5h	5h
T75%	>24h	>24h	>24h	13h	13h
T90%	>24h	>24h	>24h	22h	>24h

Table 4. *In vitro* drug release kinetic studies of metronidazole and fluconazole beads

Formula code	Zero order		First order		Higuchi model		Korsmeyer-peppas model		
M1	r2	Ko (µg/sec)	r2	K1	r2	KH (µg/√sec)	r2	n	comment
M2	0.76	1.65	0.87	-0.067	0.94	12.87	0.96	0.152	Fickian diffusion
M3	0.66	1.87	0.74	-0.017	0.92	43.86	0.943	0.163	Fickian diffusion
M4	0.53	1.58	0.83	0-.026	0-83	25.78	0.953	0.154	Fickian diffusion
M5	0.508	2.09	0.734	-0.067	0.92	13.7	0.986	0.176	Fickian diffusion
F1	0.834	2.67	0.82	-0.13	0.96	31.8	0.971	0.234	Fickian diffusion
F2	0.86	1.87	0.92	-0.067	0.87	13.6	0.932	0.154	Fickian diffusion
F3	0.56	1.45	0.789	-0.034	0.91	22.7	0.946	0.162	Fickian diffusion
F4	0.557	2.15	0.83	-0.065	0.92	13.7	0.956	0.143	Fickian diffusion
F5	0.46	4.89	0.91	-0.032	0.88	11.87	0.955	0.176	Fickian diffusion

3.5 Antimicrobial Studies

The *in vitro* efficacy of the developed M5 and F4 mixed beads were compared with commercial suppository Amrizol® formulation. Developed beads was found to be more effective in *in vitro* conditions. The average zone of inhibition of the developed M5 and F4 mixed beads *Candida albicans* was 28.3±0.6 mm compared to 24.5±1.7 mm of commercial Amrizol®, indicating significantly higher efficacy of M5 and F4 mixed beads (p>0.001). Average zone of inhibition of the developed M5 and F4 mixed beads against E*. coli* was 31.5±2.2 mm compared to 28.6±1.8 mm of Amrizol®. From the results of *in vitro* antimicrobial activity it is clear that the developed M5 and F4 mixed beads was more effective than the tested commercial formulations and last for12,18 and 24 h [42,43].

4. CONCLUSION

Bioadhesive vaginal beads was developed for the treatment of single as well as mixed vaginal infections. The developed bioadhesive beads were found to have prolonged *ex vivo* retention, *in vitro* results of antimicrobial activity suggest that the bioadhesive beads were more efficacious than the commercial formulations tested. Incorporating a combination of polycarbophil and chitosan improved the undesired irregular shape of beads caused by incorporation of the drug, as in M5 and F4 mixed beads which were compared with commercial suppository Amrizol® formulation and were effective than tested commercial formulations and last for12, 18 and 24 h.

The formulation is, easy to administer along with good bioadhesion and retention property. This formulation has potential for better patient compliance as vaginal formulation. The efficacy of the formulation is recommended to be studied by *In vivo* and clinical experiments.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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