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FG90 chitosan as a new polymer for metronidazole mucoadhesive tablets for vaginal administration

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ABSTRACT

Topical administration of the antibacterial metronidazole (MET) represents the most common therapy in the treatment of bacterial vaginosis (BV). The formulations generally available for BV therapy are creams, gels, vaginal lavages and vaginal suppositories. In this study, a new dosage form, containing MET, was developed with the aim to realize vaginal mucoadhesive tablets by including bioadhesive polymers as chitosan (FG90C), polyvinylpyrrolidone (PVPK90) and polycarbophil (PCPAA1), blended in different ratios. All formulations were characterized by studies of DSC, friability, hardness, hydration, mucoadhesion, in vitro release and antibacterial activity.

All polymer mixtures employed were used to prepare tablets with the compactness and hardness so as allow the application on vaginal mucosa.

FG90C performances improved in particular when mixed to PVPK90 (1:1 ratio). This kind of delivery system is suitable for formulating MET for topical application representing a good alternative to traditional dosage forms for vaginal topical administration.

1. Introduction

Vaginal mucosa is often affected by some infections or inflammations able to change the physiological environment of this area. This region is colonized by several series of microorganisms (*Lactobacillus acidophilus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Candida albicans* and other anaerobic bacteria) which generally maintain the pH value between 4 and 5. Vaginal infections, as bacterial vaginosis (BV), can arise when the normal microbial equilibrium is altered generating an anaerobic bacteria overgrowth; as a consequence *Lactobacillus* bacteria, *Bacteroides*, *Gardnerella vaginalis*, *Peptostreptococci*, *Mobiluncus* and, sometimes, *Mycoplasma hominis* increase (Pescetto et al., 2001).

MET is the drug of choice in BV treatment and it is formulated in many marketed products for local treatment namely creams, gels, vaginal lavages and vaginal suppositories. However, these traditional formulations are not suitable to assure drug permanence on the vaginal mucosa surface for adequate time enough to reduce the possibilities of obtaining the complete bacteria elimination and pathology eradication. The vagina, in fact, thanks to its structural characteristics and easy accessibility, represents a good site for drug administration. However, some factors may interfere with local drug administration (*e.g.* the vaginal fluid wash and the day-time orthostatic posture) and provoke formulation ousting, diminishing the contact time between drug and vaginal mucosa. For this reason and in order to avoid oral therapy, it is useful to design new formulations right to control both drug release and permanence time in the application area. A joint resolution to these problems could be represented by the use of vaginal mucoadhesive formulations as gels, films or tablets. Moreover these particular formulations can offer other numerous advantages as (i) avoiding hepatic first-pass metabolism, (ii) use of small doses, in comparison to oral administration, (iii) side effect minimization, (iv) drug contact increase and daily administration reduction, and (v) easy removal. These goals can be achieved by using mucoadhesive films, tablets and gels (Voorspoels et al., 2002).

The aim of this work was the study of new mucoadhesive vaginal tablets, realized by bioadhesive and swellable polymers, in order to increase MET residence time and to control its topical delivery. Furthermore, the design of this type of swellable drug delivery systems (SDDS) is rather sophisticated because of the necessity to impart two specific properties to the system, *i.e.* immobilization and controlled release characteristics. Among many kinds of immobilized SDDS (Ponchel and Duchene, 1992; Rathbone et al., 1996), monolithic tablets were considered because of the possibility of manufacture using conventional techniques and their ability to hold large amounts of drug.

This goal can also be achieved using mucoadhesive polymers with different physicochemical and mechanical properties. In this

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study a modified chitosan, mixed with a synthetic bioadhesive polymer, such as polivinylpyirrolidone (PVPK90) or polycarbophil (PCPAA1), was employed and investigated.

Chitosan is a hydrolyzed polysaccharide (deacetylated) derivative of chitin, a biopolymer widespread in nature, that is non-toxic, biocompatible and biodegradable (Muzzarelli et al., 1988). It is reported to show antibacterial activity (Ravi Kumar, 2000; Illum, 1998; Kast et al., 2002), and penetration enhancement properties towards pluristratified epithelia by improving the transport of hydrophilic drugs through monostratified mucosa containing tight junctions (Sandri et al., 2005). In addition it has many other properties useful for pharmaceutical applications (Dodane and Vinod, 1998) and, for these reasons, largely employed to prepare buccal and vaginal mucoadhesive dosage forms (Bonferoni et al., 2008; Perioli et al., 2008a,b; Valenta, 2005; Giunchedi et al., 2002; Rossi et al., 2003). The presence of OH and NH_2 groups, together with its cationic character, allows the establishment of hydrogen bonding with mucin chains, resulting in a good mucoadhesive character (Dodane and Vinod, 1998). So, chitosan appeared to be an excellent candidate to prepare formulations applicable on a vaginal surface, rich in numerous folds and microridges.

Chitosan polymers have been sporadically used for tableting (Picker-Freyer and Brink, 2006), however, to date, no detailed compression and compaction characterizations exist and, generally, it necessitate the addition of other ingredients to facilitate compression (Rege et al., 1999).

In this study, the chitosan derivative FG90 was employed. It is a highly deacetylated chitosan (degree of deacetylation 99.97%) with good tableting properties and suitable to obtain highly mechanically stable tablets (Picker-Freyer and Brink, 2006).

2. Materials and methods

2.1. Materials

Polycarbophil AA1®NOVEON (PCPAA1) (from Noveon Inc., USA) MW > 10^6; polyvinylpyrrolidone PLASDONE K90 (PVPK90) (from ISP Tecnologies Inc., Wayne, NJ), MW 1.3×10^6 and chitosan (FG90C), a food grade chitosan (manufactured by Primex, Drammen, Norway from shrimp shells and distributed by Faravelli, Milano, Italy) degree of acetylation 0.03%, average MW 100 kDa, viscosity of 1% soln in 1% acetic acid 110 mPa s, ashes 0.3%, were used as mucoadhesive polymers for tablet preparation. Metronidazole (MET) was purchased by Farchemia (Treviglio, Italy). Pig vaginal mucosa, obtained from Large White pigs weighing ~165–175 kg, was obtained from by veterinary service of USL N. 1 (Città di Castello, Perugia, Italy). Before measurements, the mucosa was washed with physiological solution, stored at 4 °C, and used within 12 h from pig euthanasia. Simulated vaginal solution (pH 5.0) was prepared, according to British Pharamacopoeia, with 13.2 g of CH_3COONa, 6 ml of CH_3COOH and ultrafiltered water to 1 l (Milli-Q Plus, Millipore, USA).

Brain Heart Infusion, as dehydrated product (BIOLIFE cod. 401230, Milano, Italy).

2.2. Methods

2.2.1. Manufacturing of tablets

A homogeneous physical blend of polymers (200 mg) and MET (25 mg) was gently prepared by using a pestle and mortar and then compressed, by a 13 mm diameter die on an single-punch, manual hydraulic press (PerkinElmer, England) (Perioli et al., 2008a,b), using a compression force of 1×10^3 kg for a total time of 10 s. Tablet thickness was measured ($n = 5$) by a micrometer (Borletti, Milano, Italy) (Table 1).

Table 1
Tablet composition and characteristics.

Tablets	Polymer composition (mg)			Thickness (mm ± SD) ($n = 5$)	Crushing strength force ((N) mm ± SD) ($n = 10$)	Friability (% ± SD) ($n = 20$)	Ex vivo mucoadhesion time (h ± SD) ($n = 3$)	Ex vivo mucoadhesive force (N ± SD) ($n = 3$)	Inhibition alone (mm ± SD) ($n = 3$)
	PVPK90 ratio (mg)	FG90C ratio (mg)	PCPAA1 ratio (mg)						
1	2 (133.33)	1 (66.66)	–	1.44 ± 0.04	133.50 ± 10.10	0.125 ± 0.067	22 ± 0.15	1.32 ± 0.01	27.33 ± 0.90
2	1 (100)	1 (100)	–	1.41 ± 0.04	91.00 ± 15.50	0.227 ± 0.033	23 ± 0.20	0.92 ± 0.13	40.00 ± 0.35
3	1 (66.66)	2 (133.33)	–	1.34 ± 0.02	87.00 ± 12.23	0.306 ± 0.045	23 ± 0.20	1.43 ± 0.14	27.66 ± 0.61
4	–	1 (66.66)	2 (133.33)	1.33 ± 0.03	138.80 ± 6.54	0.047 ± 0.011	26 ± 0.35	1.47 ± 0.02	–
5	–	1 (100)	1 (100)	1.27 ± 0.03	135.40 ± 9.09	0.054 ± 0.015	>70	1.47 ± 0.16	–
6	–	2 (133.33)	1 (66.66)	1.24 ± 0.03	127.80 ± 11.35	0.039 ± 0.017	21 ± 0.25	1.03 ± 0.07	–

2.2.2. Physical characterization: crushing strength

The crushing strength was analyzed, according to the Farmacopea Ufficiale Italiana (F.U. XII Ed.) using a hardness tester (instrumented uniaxial press ERWEKA TBH 220). Data are reported as an average of 10 measurements and the error expressed as SD (Table 1).

2.2.3. Physical characterization: friability

Friability was determined according to F.U. XII by submitting 20 previously weighed tablets to falling shocks for 4 min in an friabilator (Erweka TA 200), set at 25 rev/min. After 4 min, the tablets were reweighed and the percentage friability was calculated (Table 1).

2.2.4. Physicochemical interaction studies: differential scanning calorimetry (DSC)

DSC thermograms were performed using a thermal analyzer (Mettler Toledo DSC821e). Samples (3–7 mg) were placed into the aluminum pans and the thermal analyses were carried out in the following conditions: two consecutive heating ramps from 15 °C to 220 °C at 10 °C/min scale up rate and cooling in between the two ramps was performed at 5 °C/min (method A); two ramps from 15 °C to 190 °C at 10 °C/min scale up rate with cooling at 5 °C/min (method B); one heating from 15 °C to 150 °C at 10 °C/min (method C) and from 15 °C to 150 °C at 10 °C/min followed by cooling at 5 °C/min and by a second heating until 500 °C at 10 °C/min (method D). Before the analysis all samples were vacuum dried for 24 h.

2.2.5. Swelling studies

Tablet swelling properties (%) and erosion characteristics, matrix erosion or dissolution (DS) were evaluated as previous reported (Perioli et al., 2004). Each tablet was weighed ($W1$) and immersed into a Petri plate (9 cm diameter) containing simulated vaginal fluid at pH 5.0. Plates were thermostated at 37 °C (±0.1) in a ventilate heater (Orbital Incubator, Sanyo Gallenkamp, Japan) for fixed times. These experiments were performed in triplicate and data were calculated using the following Eq. (1) and (2):

$$\%\,\text{of hydration} = \frac{W2 - W1}{W2} \times 100 \quad (1)$$

$$\text{DS} = \frac{W1 - W3}{W1} \times 100 \quad (2)$$

where $W1$ is the dry tablet weight, $W2$ is the weight after immersion in simulate vaginal solution for predetermined time intervals (0.5, 1, 2, 3, 6, 9, and 12 h) and $W3$ is the swollen tablet weighed after drying at 60 °C for 24 h in an oven then 48 h in a desiccator containing $CaCl_2$, times were measured in triplicate.

2.2.6. Ex vivo mucoadhesion time and behaviour

Ex vivo mucoadhesion times were detected (triplicate) after application of tablets on fresh cut porcine vaginal mucosa. The porcine vaginal tissues, used within 12 h from pig euthanasia, were fixed in the internal side of a beaker with cyanoacrylate glue. Each tablet side was wetted with 50 μl of simulated vaginal fluid and subsequently put in contact with vaginal mucosa surface by applying a finger tip force for 20 s. The beaker was filled (800 ml) by using simulated vaginal fluid and kept at 37 °C (±1) (Han et al., 1999). Tablet behaviour and mucoadhesive times were monitored until complete detachment or dissolution occurred (Table 1).

2.2.7. Ex vivo mucoadhesion force

Ex vivo adhesion strength (expressed as force required to remove a tablet from pig vaginal mucosa) was measured by using a dynamometer (Lehrmittelbau, Bonn, Germany) (Perioli et al., 2004). Measurements started after 2 min from tablet application; the maximum adhesive forces are the expression of the average of three measurements ($n = 3$) and the confidence interval was determined at 0.05 significance level (Table 1).

2.2.8. In vitro drug release studies

Tablet drug release was evaluated using a modified standard basket apparatus (F.U. XII) (Bernkop-Schnürch and Hornof, 2003). A tablet side was wetted with 50 μl of simulated vaginal fluid and fixed to the bottom flat end of the stirring rod instead of the basket fixture. After 2 min, the vessel was filled with simulated vaginal fluid at 37 °C and stirred at 100 rpm speed. Samples (4 ml) were collected at predetermined time intervals and replaced with an equal volume of simulated vaginal fluid. MET concentration in each sample was determined by UV at $\lambda_{max} = 320.0$ nm with a spectrophotometer (Agilent model 8453) after having performed a calibration curve ($r = 0.9991$) and by using simulated vaginal fluid as blank. The % released at each time point was expressed as a fraction of the total drug amount in the tablet. MET concentration was reported as an average of three determinations and the error expressed as SD.

2.2.9. In vitro bacterial inhibition studies

These studies were performed through the tablet diffusion method in agar (Hoel and Casals, 1993). The method was conveniently modified in order to evaluate antimicrobial activity and consequently to confirm drug release from tablets. In this case *Bacteroides fragilis* was chosen as the bacterium strain sensitive to MET.

B. fragilis ATCC 25285, from collection of Istituto Zooprofilattico Sperimentale (IZS) of Umbria and Marche (Perugia, Italy), was employed. The lyophilized strain was suspended in a sterile physiological solution (1 ml), harvested in Brain Hearth Infusion (BHI) broth and placed in an incubator (model MIR 553, Sanyo Europe Ltd., Hertfordshire, UK) at 37 °C (±0.1) for 24 h in anaerobic conditions (AnaeroGen™, Oxoid S.p.A., Milano, Italy). One batch of this culture was harvested on nutrient agar slant and it was incubated at 37 °C (±1) for 48 h in anaerobic conditions (AnaeroGen™, Oxoid S.p.A., Milano, Italy). Three different culture broths (A, B, and C) with the following compositions were used:

Broth A (culture broth): deionized water (1000 ml), agar noble (20 g), meat extract (3 g), glucose (4 g), meat peptone (5 g), potassium phosphate dibasic (1 g) and sodium chloride (10 g) at pH 7.2 ± 0.2 at 25 °C.
Broth B (nutritive broth): deionized water (1000 ml), agar (15 g), soy peptone (5 g) and sodium chloride (5 g) at pH 7.3 ± 0.2 at 25 °C.
Broth C (BHI): deionized water (1000 ml), Brain Hearth Infusion (37 g), pH 7.4 ± 0.2 at 25 °C.

The broth, after testing the pH, was distributed in a series of bottles (broth A) or tubes (broths B and C) that, after filling were sterilized by autoclave (Mod. V D/G, De Lama S.p.A., Pavia, Italy) for 15 min at 121 °C.

Before the use, all broths were submitted to sterility and fertility checks according to ISO 11133-2:2003 "Microbiology of food and animal feeding stuffs – Guidelines on preparation and production of culture media – Part 2: Practical guidelines on performance testing of culture media".

Agar plates containing the chosen strain were prepared as follows: the bottle containing broth A (100 ml) was inoculated with *B. fragilis* suspension (2 ml) as to obtain 3.5×10^7 UFC/ml final concentration. This suspension was accurately mixed and poured (25 ml) into Petri plates (90 mm diameter) and left to cool and solidify by placing the Petri dishes on a cool horizontal surface. After agar cooling and consolidation, a 13 mm diameter well was holed on the centre of each agar plate by using a sterilized hollow cylinder as template. A tablet was placed into the well and then wetted with physiological solution (100 μl) to permit the swelling. All plates

were incubated at 37 °C (±1) for 48 (±2) h in anaerobic conditions (AnaeroGen™, Oxoid S.p.A., Milano, Italy); the test was performed in triplicate. For all incubation time (48 h), the tablet behaviour was observed and the diameter of the inhibition zone was measured with a gauge and expressed in mm (±SD).

3. Results and discussion

The aim of this study was the design of mucoadhesive vaginal tablets by using, as a matrix, a mixture of deacetylated chitosan and synthetic polymers, having improved performances if compared to traditional formulations employed in BV therapy.

3.1. Manufacturing of tablets and technological assays

Six kinds of tablets were prepared by using different mucoadhesive polymer percentages as shown in Table 1. Each tablet contains FG90C mixed, in different ratios, to PVPK90 (tablets **1**–**3**) or to PCPAA1 (tablets **4**–**6**). Observing tablet thickness values (Table 1), it is possible to note that their width is mainly affected by the amounts of the synthetic polymer. In fact, it increased as the synthetic polymer content rises (PVPK90 > PCPAA1), while it was inversely proportional to chitosan amount.

The hardness test (Table 1) showed that tablets had a crushing strength range between 87.00 and 138.80 N. In particular, tablets **4**–**6** (PCPAA1/FG90C) presented similar behaviour and higher strength than tablets **1**–**3** (PVPK90/FG90C). For all these, hardness increases as chitosan content decreases. These data, in conjunction with the noted effect on tablet thickness, confirm that FG90C is a very pressure sensitive material and that it facilitates soft tableting (Picker-Freyer and Brink, 2006).

When new formulations are studied and new polymer mixture are proposed, it is very important to know tablet friability in order to evaluate tablet compaction and resistance. Friability tests were performed according to the specific monograph of the F.U. XII and 20 tablets were tested by using a friabilometer and were observed at predetermined time: if none of these is cracked or broken, all tablets, after re-weighting, must show a mass loss less than 1%. From friability data (Table 1), it is apparent that all values were under this limit and particularly: (i) tablets **4**–**6** were less friable than **1**–**3**, probably because the PCPAA1 presence; (ii) tablets **1**–**3**, showed a friability proportional to chitosan amount confirming that FG90C facilitates soft tableting.

Friability and hardness data demonstrated that the polymers employed are able to confer compactness to the formulations producing resistant and stable tablets suitable for manufacturing.

3.2. Physicochemical interaction studies: differential scanning calorimetry (DSC)

Glass transition (Tg) of amorphous polymers is the passage from glassy state to rubbery state, depending mainly on chain stretching and temperature, and can change physical and mechanical properties of compounds. For this reason, it is very interesting to know if the presence of MET (crystalline compound) and the compression force influence the Tg of FG90C, PVPK90, PCPAA1 and their blends. DSC thermograms of each polymer were carried out (data not reported) and two heating scans were performed: FG90C using method A, PVPK90 and PCPAA1 using method B to underline polymer Tg. In all cases, during the first heating scan, an endothermic peak between 90 °C and 100 °C, due to water elimination, was observed. This peak was partially superimposed to polymer Tg. During the second heating, it is possible to note the following Tg: FG90C around 100 °C (Zerrouk et al., 2004), PVPK90 around 180 °C (Albertini et al., 2003; Khoo et al., 2003) and PCPAA1 over 140 °C (Gòmez-Carracedo et al., 2004), as described by other authors.

PVPK90/FG90C blends (ratios 2:1, 1:1, 1:2) and PCPAA1/FG90C blend (2:1) DSC thermograms (Fig. 1A and B) showed no variations of Tg temperatures. While PCPAA1/FG90C blends (1:1 and 1:2) (Fig. 1B) showed a light Tg decrease (~130 °C). FG90C Tg, independently from weight ratios, was in all cases around 100 °C.

Possible interactions between polymers and MET were investigated too. MET DSC thermogram was performed using method C (data not reported) and an endothermic peak at 161.20 °C ($\Delta H = -106.17$ J/g) (DeSouza et al., 2003), due to drug fusion, was observed (data not reported).

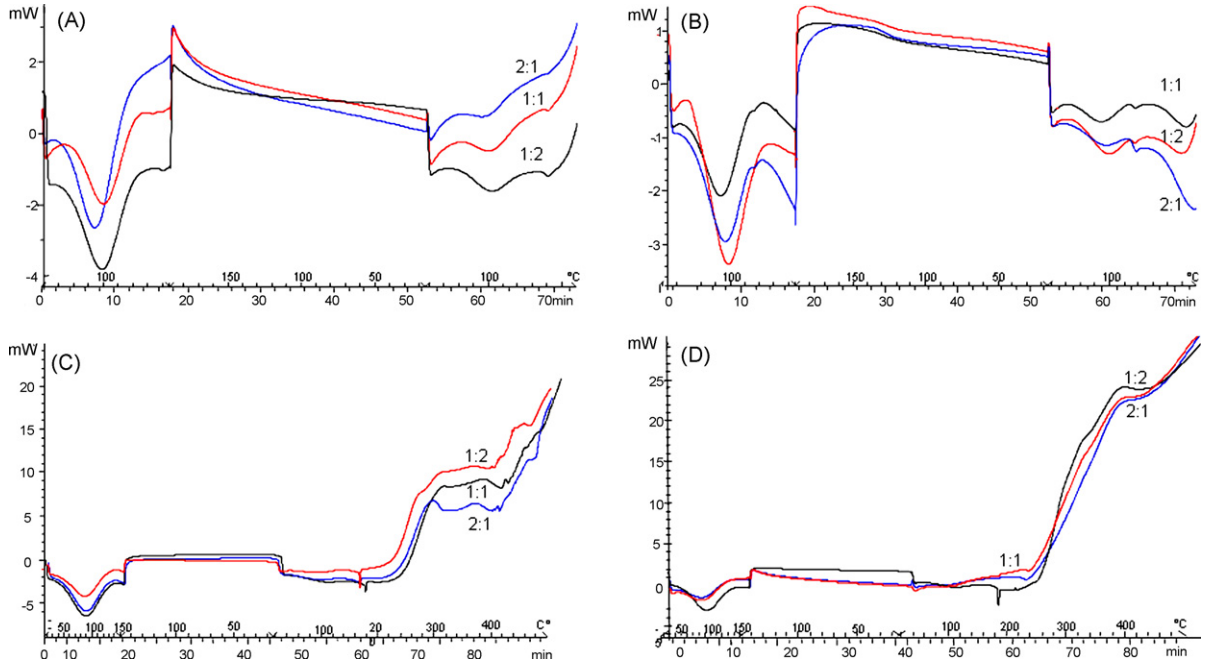

Fig. 1. DSC analysis of (A) PVPK90/FG90C mixtures (1:2; 1:1; 2:1), (B) PCPAA1/FG90C mixtures (1:2; 1:1; 2:1), (C) PVPK90/FG90C mixtures (1:2; 1:1; 2:1) in the presence of MET, and (D) PCPAA1/FG90C mixtures (1:2; 1:1; 2:1) in the presence of MET.

The DSC thermograms of MET mixed to PVPK90 and FG90C (ratios 2:1, 1:1, 1:2) (D method) (Fig. 1C) showed that MET fusion peak decreased with the increase of PVPK90. Probably interactions occurring between MET and PVPK90 (between PVP carbonylic group and alcoholic function or nitrogen of MET) are responsible for this behaviour. Thermal profiles of MET, mixed to PCPAA1 and FG90C, have been registered as well (Fig. 1D) and revealed the presence of the endothermic peak only in the blend PCPAA1/FG90C 1:2 allowing to suppose that the PCPAA1 content increase could cause MET crystallinity decrease. The interactions probably occurring between MET and acrylic polymer (between PVP carbonylic group and MET alcoholic function or nitrogen) could explain these results.

Successively, the same drug–polymer mixtures, after compression (tablets **1**–**3** and **4**–**6**), were investigated (using D method) with the aim to evaluate if compression force influenced their thermal behaviour (data not reported). Thermal profiles of tablets **1**–**3** resulted the same of those of drug–polymer mixtures before compression. However, in the case of tablets **4**–**6**, it was possible to underline that, after compression, MET crystallinity increased as shown by the endothermic peak at ~160 °C (fusion).

3.3. Swelling studies

All tablets have been submitted to hydration studies as this phenomenon is directly connected to bioadhesion capability of swellable formulations. In fact, bioadhesive polymers, when in contact with water, can hydrate, produce gel layer. If the hydration level is excessive, the bioadhesive property is reduced because water molecules bind the polymer groups necessary to link mucin chains.

The hydration ability of formulation is important because it influences (i) tablet size, (ii) drug retention time, and (iii) drug release kinetics. Tablet behaviours, represented as hydration percentage *vs.* time, have been reported (Fig. 2). Error bars have not been indicated for graph clarity as these may be confusing since many profiles overlap.

All tablets swelled quickly with high hydration percentage (54.54–73.09%) after only 30 min, reaching values between 70 and 90% after 2 h. It was clearly evident that tablets **4**–**6** (PCPAA1/FG90C) hydrated more and faster than tablets **1**–**3** attaining the maximum of hydration in 2 h, while the latter (PVPK90/FG90C) hydrated very slowly showing hydration <60% after 30 min.

As swelling and gel formation can contribute to tablet erosion and their wholeness lost, the evaluation of DS (or matrix erosion) is essential. The polymer tendency to erosion/solubilization, in fact, is responsible for tablet integrity. Fig. 3 that reports tablets **1**–**3** had bigger DS values. In particular, tablet **1** registered 40% weight loss after 8 h because of its high PVP content (PVPK90/FG90C 2:1).

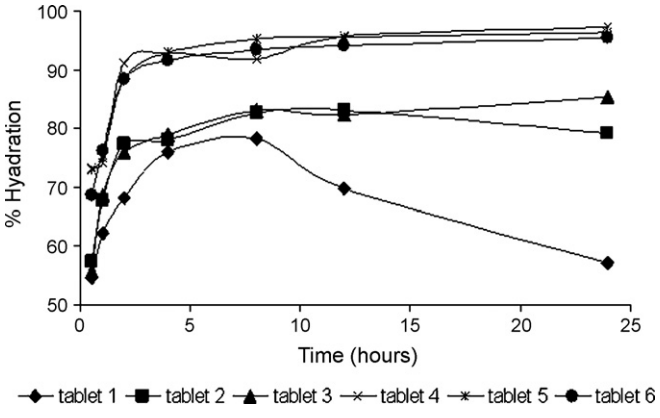

Fig. 2. Hydration percentage (%) *vs.* time of each tablet at pH 5.0 and at 37 °C (±0.1).

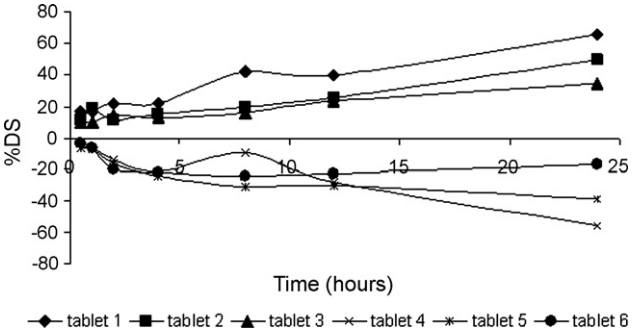

Fig. 3. Matrix erosion or dissolution (DS) of tablets ($n = 3$; $\alpha = 0.05$).

On the contrary, tablets **4**–**6** were more compact and intact and, tablet **4** (PCPAA1/FG90C 2:1), showed the highest hydration level. The difference in behaviour between the two groups is related to the type and quantity of the synthetic polymer present in the formulation.

PVPK90 is an hydrophilic synthetic homopolymer, constituted by N-vinylpyrrolidone, soluble in water. So, tablets **1**–**3** can absorb water, swell easily and slowly; the formed gel can dissolve and diffuse in the medium decreasing tablet weigh.

PCPAA1, instead, is a polyacrylic derivative water insoluble, for the presence of divinylglicole cross-linked chains, whose shaped network can swell in aqueous environment up to thousand folds its original volume (Noveon Bulletin, 2002). This swelling takes place at those pH values (4.0–6.0) in which their polymeric carboxylic groups are ionized. For this reason, tablets **4**–**6** showed higher hydration and more integrity (low DS values) at the same time.

Although these data do not permit a complete evaluation of tablet properties, however are very useful to select the most suitable formulation for vaginal MET mucoadhesive administration.

3.4. Ex vivo mucoadhesion time and behaviour

Ex vivo mucoadhesive tests were performed in order to evaluate tablet residence time on the application site, to observe tablet behaviour when in contact to mucosal surface and vaginal fluid, and to identify a formulation able to avoid numerous daily administrations.

All kinds of tablets adhered immediately to mucosal surface and showed high adhesion times (21–70 h), but tablets **4**–**6** had more prolonged times than **1**–**3**. From mucoadhesion time data (Table 1), it is possible to assess that the presence of PCPAA1 offered a prolonged mucosa-tablet contact, particularly when mixed to an equal amount of FG90C. When the latter polymer predominated, mucoadhesion time decreased. Gel formation on tablet surface was very rapid, faster in the case of tablets **4**–**6**, according to hydration times. In tablets **1**–**3** gelled layer was very evident and compact and it was aimed to disperse in (simulated) vaginal fluid causing the loss of tablet edges and their diameter reduction. No fragment loss was observed. These data are in agreement with DS data.

From observation of swelled tablets, it was possible to note the external gelled layer and a clear boundary between the area containing polymers in glassy state (not hydrated part) and the area containing polymers just hydrated (rubbery phase). In the case of tablets **4**–**6**, the external layer swelled very fast and the formed gel differed from classic gel. In fact, it was spongy and not homogeneous, did not solubilize in the vaginal fluid and did not release big and semisolid fragments. Tablets **1**–**3** hydrated and swelled very slowly and it was possible to observe water penetrating into the tablet. All tablets (**1**–**3**) showed the presence of a not hydrated inner

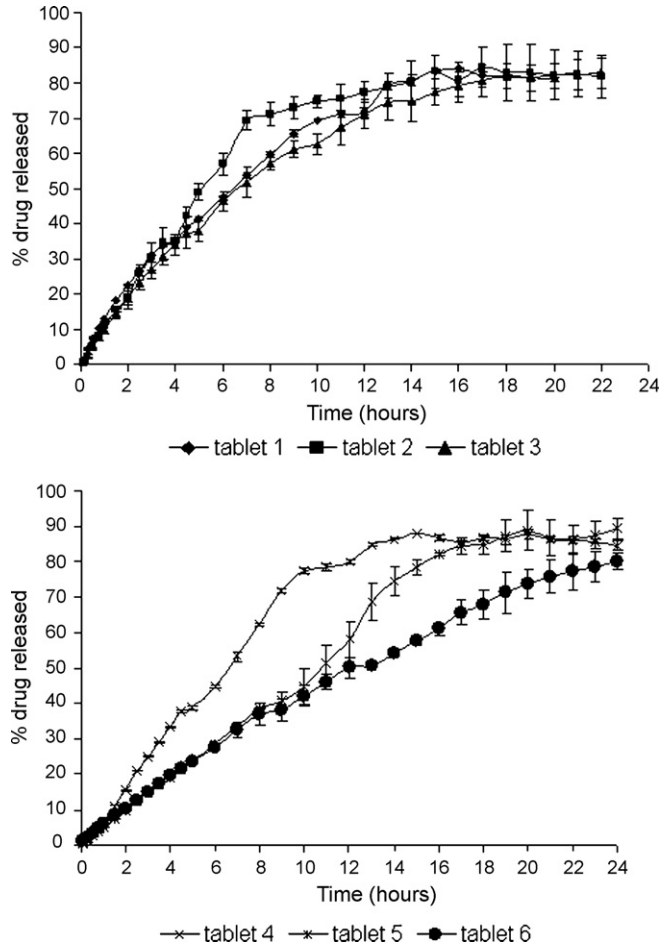

Fig. 4. MET release profiles (% released at each time) in simulated vaginal fluid at 37 °C for 24 h ($n = 3$; $\alpha = 0.05$).

core that gradually disappeared (after 3.5 h). As seen in previous tests, tablets **4**–**6** hydrated very quickly and the inner core was not present.

This different behaviour is attributable to the polymer characteristics; PVPK90 and FG90C are water-soluble and this is the reason because tablets **1**–**3** hydrated quickly and produced water-soluble gel. On the contrary, when a swellable polymer, PCPAA1, was present, the entrapped water produced tablet swelling, but the formed gel did not dissolve in the medium. When the cross-linked network did not further retain the entrapped water, the gel broke and released numerous fragments. In fact, tablet **4**, having the highest PCPAA1 amount (PCPAA1/FG90C 2:1), presented very high swelling level, and its edges and margins became filamentous and released fragments.

3.5. Ex vivo mucoadhesion force

All tablets showed good mucoadhesive forces with values ranging between 0.92 and 1.47 N (Table 1).

In this case, no exact relationships between bioadhesion force and polymer blends could be drawn. Tablets **3**–**5** resulted to have the strongest adhesivity.

As regards to PVPK90/FG90C blend (tablets **1**–**3**), the highest force was observed (tablet **3**) in presence of low amounts of the hydrophilic polymer PVPK90. In fact, the bioadhesion capacity of these tablets seemed to be dependent mainly on chitosan presence. It is interesting to note that tablet **2**, PVPK90/FG90C (ratio 1:1), showed the lowest force and this was probably due to the inter-

Table 2
Ritger and Peppas's kinetic mathematical model and first order kinetics model fitting.

$M_t/M_\infty = Kt^n$	Tablets					
	1	**2**	**3**	**4**	**5**	**6**
$n = 1$	$y = 5.244x + 11.055$, $r = 0.9795$	$y = 5.2062x + 12.689$, $r = 0.9418$	$y = 3.8809x + 14.364$, $r = 0.9521$	$y = 3.893x - 14.519$, $r = 0.9768$	$y = 4.2248x + 3.3466$, $r = 0.9818$	$y = 3.4218x + 4.9467$, $r = 0.9942$
$n = 0.9$	$y = 7.0186x + 8.4957$, $r = 0.9862$	$y = 7.0437x + 9.8176$, $r = 0.9528$	$y = 5.3818x + 11.641$, $r = 0.9626$	$y = 3.893x - 14.519$, $r = 0.9768$	$y = 5.8652x + 0.4487$, $r = 0.9852$	$y = 4.748x + 2.6177$, $r = 0.9972$
$n = 0.8$	$y = 9.432x + 5.4012$, $r = 0.9918$	$y = 9.5689x + 6.3489$, $r = 0.9629$	$y = 7.4949x + 8.3518$, $r = 0.9722$	$y = 9.2565x + 3.5814$, $r = 0.9683$	$y = 8.1724x - 3.0352$, $r = 0.9871$	$y = 6.6138x - 0.191$, $r = 0.9988$
$n = 0.7$	$y = 12.751x + 1.5574$, $r = 0.9958$	$y = 13.078x + 2.0472$, $r = 0.9716$	$y = 10.502x + 4.2683$, $r = 0.9806$	$y = 12.833x - 1.0419$, $r = 0.9754$	$y = 11.45x - 7.3422$, $r = 0.9870$	$y = 9.2658x - 3.6735$, $r = 0.9986$
$n = 0.6$	$y = 1.397x - 0.3911$, $r = .9978$	$y = 18.555x - 4.4361$, $r = 0.9801$	$y = 14.855x - 0.9839$, $r = 0.9871$	$y = 3.893x - 14.519$, $r = 0.9768$	$y = 16.182x - 12.867$, $r = 0.9843$	$y = 13.097x - 8.1529$, $r = 0.9961$
$n = 0.55$	$y = 20.423x - 6.4618$, $r = 0.9978$	$y = 21.289x - 6.9019$, $r = 0.9807$	$y = 17.756x - 4.2399$, $r = 0.9896$	$y = 21.339x - 10.649$, $r = 0.9818$	$y = 19.328x - 16.29$, $r = 0.9819$	$y = 15.647x - 10.932$, $r = 0.9938$
$n = 0.5$	$y = 24.088x - 10.086$, $r = 0.9971$	$y = 25.244x - 10.936$, $r = 0.9823$	$y = 21.322x - 8.0795$, $r = 0.9913$	$y = 25.48x - 14.976$, $r = 0.9825$	$y = 23.19x - 20.325$, $r = 0.9785$	$y = 18.778x - 14.212$, $r = 0.9907$
$n = 0.5$, Higuchi (release 0–60%)	$y = 24.088x - 10.086$, $r = 0.9954$	$y = 25.85x - 12.961$, $r = 0.9802$	$y = 22.807x - 10.913$, $r = 0.9915$	$y = 23.205x - 13.388$, $r = 0.9816$	$y = 17.652x - 12.188$, $r = 0.9731$	$y = 16.619x - 10.609$, $r = 0.9876$

actions between polymers, involving hydroxylic or aminic group of chitosan and carboxylic groups of PVPK90, as noted by other authors (Khoo et al., 2003). In these conditions, the possibility to link mucin chains is diminished.

The second tablet group (**4**–**6**), showed the highest mucoadhesion force when the amount of acrylic polymer, PCPAA1 (water insoluble), was twice as much or the same as FG90C. In this case, the capacity to link mucin did not depend strictly on chitosan amount, but it was related to PCPAA1 carboxylic groups responsible for the adhesive force.

It was not possible to evaluate the bioadhesion force of not blended FG90C because tablets prepared with chitosan alone were very friable and mechanically not resistant.

3.6. In vitro drug release studies

Tablets **1**–**3** released only up to 80% of MET in 12 h (Fig. 4). Release profiles were very similar and practically overlapped for the first 4 h. After this period, it was possible to note a small difference: tablet **2** (PVPK90/FG90C ratio 1:1) drug release slightly increased (6–8 h).

At the end of the experiment, tablets were completely dissolved, as observed also in ex vivo mucoadhesion time assays. This could be explained considering that the gelled layer was able to dissolve itself on the aqueous medium.

Tablets **4**–**6** behaviours were less uniform than **1**–**3**. The percentage of drug released was proportional to PCPAA1 content in the polymer blend. MET release from tablet **4** (PCPAA1:FG90C 2:1), in fact, was most rapid, reaching 62.4% after 8 h, 80.0% after 11 h and the maximum of 88.0% after 15 h.

Release profiles of tablets **5** and **6** were overlapped for the first 10 h. After this time, tablet **6** (PCPAA1:FG90C 1:2) showed the lowest profile, while tablet **5** (PCPAA1/FG90C 1:1) had an intermediate behaviour between **6** and **4**.

Finally, tablets presenting highest drug release were those based on PCPAA1/FG90C blend, confirming mucoadhesion time and behaviour tests, because of high swelling due to PCPPA1 presence.

3.7. In vitro release mathematical model

In vitro release data were submitted to statistical investigation. All tablet release profiles were fitted to the Ritger and Peppas's kinetics mathematical model $M_t/M_\infty = Kt^n$ (Ritger and Peppas, 1987), applied to swellable matrices, in order to investigate what mechanism causing for MET release from its diffusional exponent evaluation. This release mechanism can be controlled by water penetration rate, responsible for hydration and drug diffusion, and polymeric chain relaxation time. Diffusional exponent value of one (=1) means that drug release occurs as an apparent zero-order mechanism when is time dependent while value = 0.5 means that release is controlled by a pure Fickian diffusion mechanism. A value between 0.5 and 1 indicates an anomalous mechanism (not Fickian) meaning that both liquid penetration rate and polymeric chain relaxation rate control the release.

Analysis data (Table 2) showed that the 1th group of tablets, had two kinds of kinetics. In the case of tablet **1**, the best fitting ($r = 0.9978$) was found for n value 0.6 and 0.55 indicating an anomalous release mechanism. The polymeric chain relaxation played an important role. In fact, this tablet had low hydration level and slow water sorption. The recorded anomalous non-Fickian transport can be correlated also to tablet erosion, which contributed to MET release from swellable system. Table 1, in fact, proved the highest DS values. When chitosan content increased in the blend, an additional release mechanism was observed. Tablets **2** and **3** showed highest r value for $n = 0.5$, meaning that drug release depended on the square time (Higuchi kinetic for tablet **3**). This demonstrates that the liquid penetration rate constitutes the main process and that drug can easily diffuse among polymeric network of gelled phase.

The 2nd group of tablets, **4**, **5** and **6**, showed different release profiles and mechanisms. Tablet **4**, containing the highest amount of PCPAA1 and presenting a very high hydration level, released the drug mainly through a diffusion mechanism ($r = 0.9825$, $n = 0.5$). When chitosan content increased, polymeric chain relaxation process affected drug diffusion and the n value increased: 0.8 for tablet **5** ($r = 0.9871$) and tablet **6** ($r = 0.9988$). This means that the unfolding and stretching polymer chains of these formulations deeply limited the diffusion of MET among polymeric network suggesting that the release was controlled both by drug diffusion process through the matrix and by polymeric chain relaxation time. A case II transport never occurred meaning that the relaxation process was not slower than diffusion.

3.8. In vitro bacterial inhibition studies

As these formulations need to hydrate to become adhesive and able to release drug, 100 μl of water was added after their introduction into the wells before incubation. During this time (48 h), all plates were observed and finally inhibition zone diameters were measured with a gauge. During the incubation time it was interesting to note that, from 1 to 24 h, all tablets presented very similar behaviours both for swelling level and for alone formation. From 24 to 48 h instead, the behaviour of the first group was very different from the second. Tablets **1**–**3** gradually swelled and the released drug was able to diffuse in agar inhibiting *B. fragilis* growth (Fig. 5). Tablets **4**–**6**, on the contrary, swelled rapidly and heavily and this phenomenon was responsible for fracture and dehydration of surrounding area (Fig. 5). This phenomenon was in agreement with ex vivo mucoadhesion performances that under-

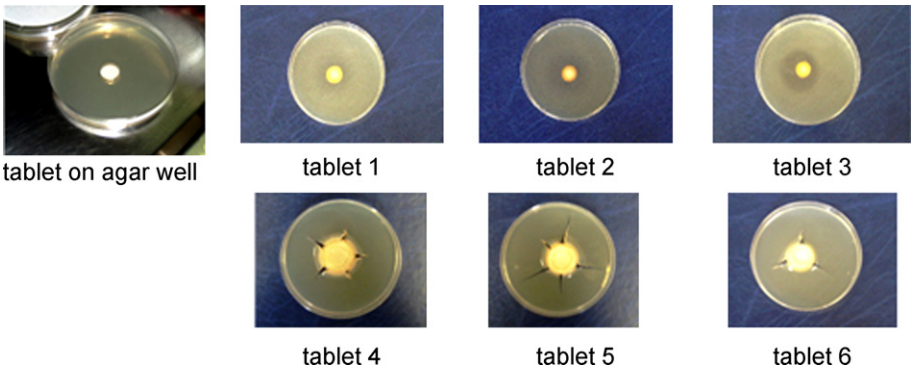

Fig. 5. Effect of tablets on *Bacteroides fragilis* growth. Inhibition zone in agar plates after incubation at 37 °C (±1) for 48 h (±2) in anaerobic conditions ($n = 3$; $\alpha = 0.05$).

lined high swelling levels, due to PCPAA1 presence, and, perhaps, that these tablets are not suitable to be applied in vaginal environment because they could provoke mucosa dryness and undesirable encumbrance.

Inhibition zone diameters of tablets **1**–**3** have been measured (Table 1). Tablet **2** produced the biggest bacterial inhibition. This means that MET was gradually released and it was able to diffuse first among polymeric network of swelled tablet, according to in vitro release mathematical model data, and then through the agar broth demonstrating that PVPK90/FG90C ratio 1:1 was the best blend to control drug release.

4. Conclusion

It is possible to assess that FG90C is a suitable polymer to realize mucoadhesive SDDS. In order to prepare good tablets, chitosan must be blended with other polymers (PVPK90 or PCPAA1) because its direct compression is not achievable. All polymer mixtures employed were useful to prepare tablets; polymer–polymer and drug–polymer negative interactions were not observed. Only in the case of PCPAA1, polymer–drug interactions were noticed, especially after compression. In particular:

1. When FG90C was mixed to PCPAA1, tablets (**4**–**6**) showed high hardness, low friability, high hydration, high mucoadhesion (time and strength) and good drug release. However, the excessive swelling observed, due to PCPAA1 presence, may be a limit and makes them unsuitable for vaginal application because induces vaginal mucosa dehydration, steric obstruction and, reduces patient compliance.
2. When FG90C was mixed to PVPK90, tablets (**1**–**3**) presented more friability, lower mucoadhesion values (time and force) and slow hydration. The last characteristic permitted a controlled drug release and, at the same time, allows the gradual adaptation of tablets on the mucosal surface (without dehydration) by producing a final swelled product with acceptable dimension. In this context and in consideration of high in vitro adhesion time (23 h) and highest inhibition zone diameter presented, tablet **2** (FG90C/PVPK90 1:1) has been considered the best formulation.

On the basis of these results, this SDDS can be employed in the BV treatment directly at the pathological site, by using low MET doses and avoiding fragment release or secretions, with important advantages as:

1. application number reduction in respect to creams, gels and vaginal suppositories;
2. no modification of vaginal environment;
3. good patient compliance because of easy administration.

In conclusion this formulation may offer a new way to perform an efficacious therapy with the possibility to reduce healing times and relapses.

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