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An in vitro investigation for vaginal bioadhesive formulations: bioadhesive properties and swelling states of polymer mixtures

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Abstract

Bioadhesive tablet formulations have been developed for mucosal application. Sixteen different bioadhesive tablet formulations were prepared and evaluated. Their bioadhesion to vaginal mucosa were studied by tensile testing method. The swelling behaviour of the tablets in three different solutions was also investigated. In addition, the effect of the formulations on pH of the medium was followed. The most favorable formulation resulted a mixture of Carbopol 934 and Pectin (2:1). The highest bioadhesive strength, the highest swelling volume and the lowest pH reduction were obtained with this formulation.

Keywords: Bioadhesion; Swelling volume; Vaginal administration; Carbopol 934; Pectin

1. Introduction

In recent years, there has been a growing interest in the use of various absorptive mucosa administrations for systemic delivery of therapeutic agents [1–4]. Transmucosal routes of drug delivery (i.e. the mucosal linings of the nasal, rectal, vaginal, ocular or oral cavity) offer distinct advantages over per oral administration for systemic drug delivery [5]. The bioadhesion mechanism is also used to solve bioavailability problems resulting from a too short stay of pharmaceutical form at the absorption site [6]. Bioadhesion is described as the adhesion of artificial substances to biological substrates, such as adhesion of polymers to skin or other tissues [7]. Adhesion to tissue and swelling state of polymer contributes to its bioadhesive behaviour and various polymers have extensively been employed in bioadhesive formulations [8–10]. In the literature, there are various studies on the bioadhesive formulations prepared with only polymer mixtures to investigate of the polymer behaviours [11,12].

In this study, the bioadhesive strength and swelling state of tablets prepared from the mixtures of polymers such as Carbopol 934, pectin, polyvinylpyrolidon, guar gum, ethylene maleic anhydride resins were studied. There are several experimental techniques for the determination of the adhesive bond strength and degree of swelling of bioadhesive formulations [12–14]. Bioadhesive strength was carried out using a tensile method and the swelling properties of the same tablets were examined in distilled water, lactate (pH 5) and phosphate (pH 7) buffer solutions, respectively. In addition, the effects of the formulations on the pH of the medium were followed during the swelling studies.

2. Experimental

2.1. Materials

Carbopol 934(Cp)(Merck, Dormstadt, Germany), polyvinylpyrolydone (PVP) (BDH Chemicals Ltd, Poole, Dorset), pectin (Pc) (BDH Chemicals Ltd,), GG (Droguan Alfred L. Wolf GmbH/HAMBURG), ethylene maleic anhydride resins (EMA31 (linear), EMA81 (cross-linked) (Cilag and Monsanto, respectively) were

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used as received. All other chemicals were an analytical grade.

2.2. Procedure

2.2.1. Preparation of bioadhesive tablet formulations

Tablets were prepared using pure polymer or polymer mixtures (Table 1) by direct compression using a single punch tablet machine (Carver Laboratory Press) fitted with flat-faced punches. Test substances were compressed by applying 4-tons force for 10 s.

2.2.2. In vitro bioadhesive studies

2.2.2.1. Selection of model mucosa. Several types of mucosa have been used as model biological tissues for the evaluation of bioadhesion, which included rat intestine, pig oral, bovine sublingual, cow vaginal mucosa [14,15]. In our study, cow vaginal mucosa was preferred. Vaginal mucosa removed from newly sacrificed cows was used as a biological matrix. It was stored at $-30\,^{\circ}C$ until bioadhesion studies. For bioadhesive studies, the samples were thawed and cut to a suitable size. Investigation of the bioadhesive strength of tablets was done with a tensile-tester apparatus (ZWICK D-7900). For adaptation of the apparatus to the bioadhesive tablet test, two metallic supports were constructed: the lower one supports the tablet and the upper one for the vaginal mucosa. The lower one was mobile while the upper one was stationary. The tablet and mucosa were attached to the metallic clamps with a cyanoacrylate type glue. A sample of 10 μl of distilled water was placed on the tablet surface using a Hamilton syringe and two surfaces were brought into contact for 10 min to maintain a stronger contact between the tablet and the mucosa. The descending speed of the lower support was set to 20 mm min^{-1} and the detachment test was carried out. All tests were done at room temperature.

2.2.3. Swelling studies

Since the pH of cow vagina was found to be between 4.5 and 5.0, tablet-swelling tests were performed in lactate buffer at pH 5. In addition, swelling states of the tablets were also evaluated in distilled water and phosphate buffer ($pH = 7$). Tablet formulations were placed in 25 ml of the buffer solution and allowed to swell at 37 °C for at least 24 h. The tablets were removed at selected times and their volume changes were measured before and during swelling. The diameter and thickness of the tablets were measured using a micrometer to calculate the ratio of the volume in the swollen state to the dry state.

3. Results and discussion

The diameter and thickness of tablets as well as their weight variation were measured. All physical properties of tablets met the USP XX criteria.

Table 1
Composition of formulations and detachment force between tablet and cow vaginal mucosa (mean ± S.D., $n = 5$)

Codes of formulations	Polymers	Weight fraction	Average detachment force (N)	Force/Area $\times 10^{-3}$ (Nm^{-2})
F1	Cp	1:0	0.50 ± 0.05	3.71 ± 0.37
F1*	Pc	1:0	0.20 ± 0.05	1.48 ± 0.37
F1**	PVP	1:0	0.20 ± 0.07	1.48 ± 0.58
F1***	EMA31	1:0	0.40 ± 0.08	2.96 ± 0.58
F1****	EMA81	1:0	0.40 ± 0.05	2.96 ± 0.37
F1*****	GG [a]	1:0		
F2	Cp:PVP	2:1	0.10 ± 0.05	0.74 ± 0.37
F3	Cp:PVP	1:2	0.10 ± 0.04	0.74 ± 0.26
F4	PVP:GG	2:1	0.13 ± 0.03	0.96 ± 0.20
F5	PVP:GG	1:2	0.11 ± 0.04	0.81 ± 0.31
F6	PVP:Pc	2:1	0.11 ± 0.04	0.81 ± 0.31
F7	PVP:Pc	1:2	0.12 ± 0.04	0.89 ± 0.33
F8	Pc:GG	2:1	0.20 ± 0.06	1.48 ± 0.45
F9	Pc:GG	1:2	0.05 ± 0.04	0.37 ± 0.26
F10	Cp:Pc	2:1	0.57 ± 0.06	4.23 ± 0.42
F11	Cp:Pc	1:2	0.16 ± 0.07	1.18 ± 0.55
F12	Cp:GG	2:1	0.23 ± 0.12	1.70 ± 0.85
F13	Cp:GG	1:2	0.17 ± 0.04	1.26 ± 0.33
F14	Cp:E31	2:1	0.20 ± 0.11	1.48 ± 0.83
F15	Cp:E31	1:2	0.50 ± 0.15	3.71 ± 1.08
F16	Cp:E81	2:1	0.20 ± 0.09	1.48 ± 0.69
F17	Cp:E81	1:2	0.45 ± 0.08	3.34 ± 0.58

[a] To press pure GG as a tablet form was impossible.

3.1. In vitro bioadhesive studies

To investigate the development of the bioadhesive bonding between the formulations and the cow vaginal mucosa we conducted a series of experiments using a tensile-tester.

Table 1 shows composition of bioadhesive tablet formulations prepared from polymer mixtures and their detachment force values.

Pectin is employed in mucoadhesive formulations and delivery systems [5] and Carbopol 934, a polymer of acrylic acid, is reported to have good mucosa-adhesive properties [16]. When they were used together in F10 formulation, the bioadhesive strength of the tablet prepared with the mixtures of Cp:Pc (2:1) reached the highest adhesion forces to cow vaginal mucosa. Presence of Carbopol 934 in the formulations provided higher bioadhesive strength than other polymers. Although PVP and Cp showed strong interpolymer complex formation [8], formulations including PVP provided the lower detachment values than other formulations. The force of detachment from the cow vaginal membrane for tablets including higher amount of GG was significantly reduced. In addition, polymers ethylene maleic anhydride resins had nearly strong bioadhesive effect as Carbopol 934. The bioadhesive measurements

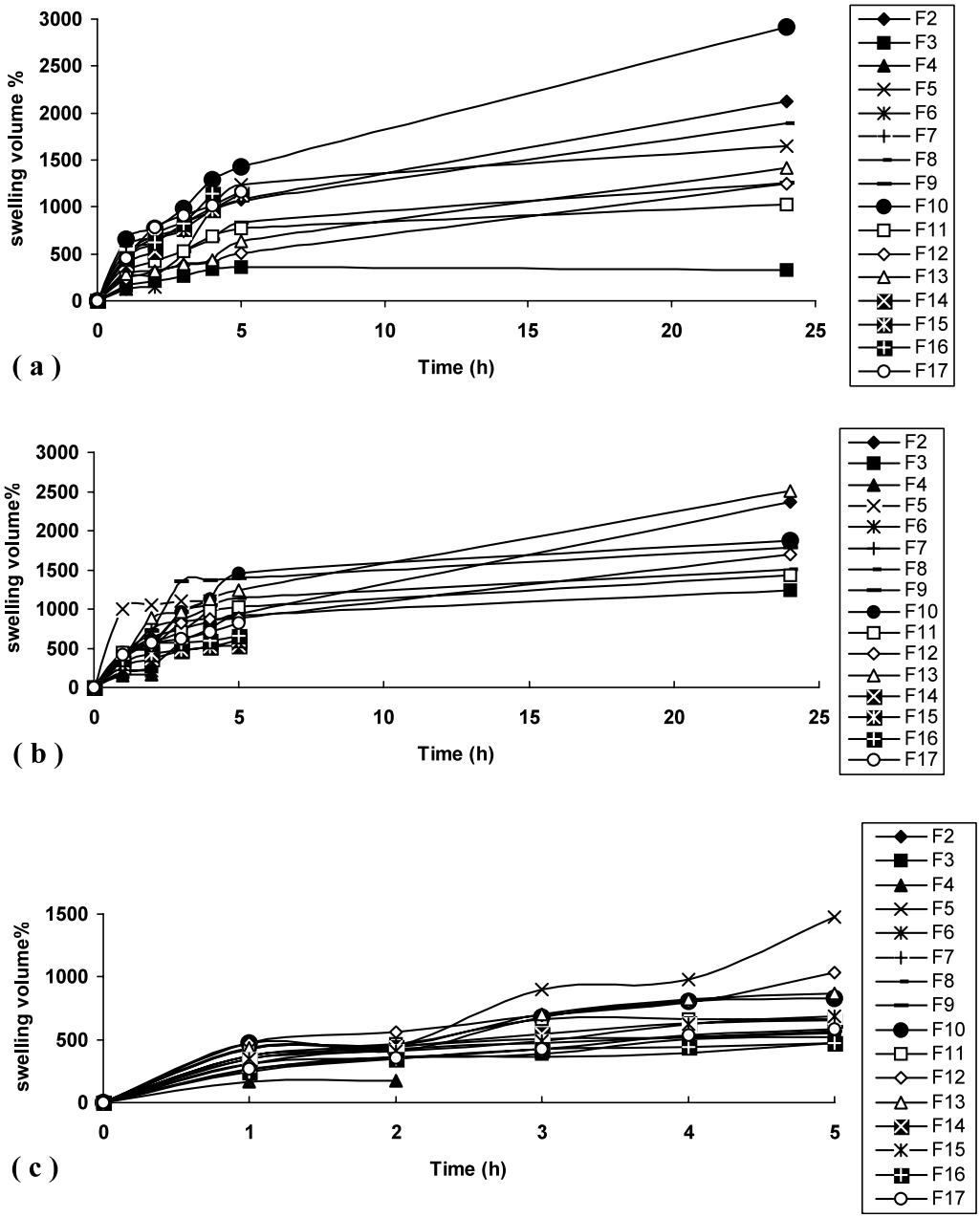

Fig. 1. Variation of swelling volume of the blend tablets against time in lactate buffer (a), distilled water (b) and phosphate buffer (c), respectively.

of our formulations prepared with polymer mixtures mentioned above showed that the ratio and the polymer types influenced the detachment forces.

3.2. Swelling studies

The swelling time is also important for assessment of adhesion. Shortly after the beginning of swelling, adhesion does occur, but the bond formed is not very strong. To develop maximum adhesion strength, an optimum water concentration was needed for polymer particles [7]. When the tablets were examined for their swelling behaviour, F2, F5, F8, F10, F15, F17 formulations swelled with a 1000% increase in the volume within 5 h and F10 provides the highest swelling volume with a 1427 and 2917% in lactate buffer at the end of the 5th

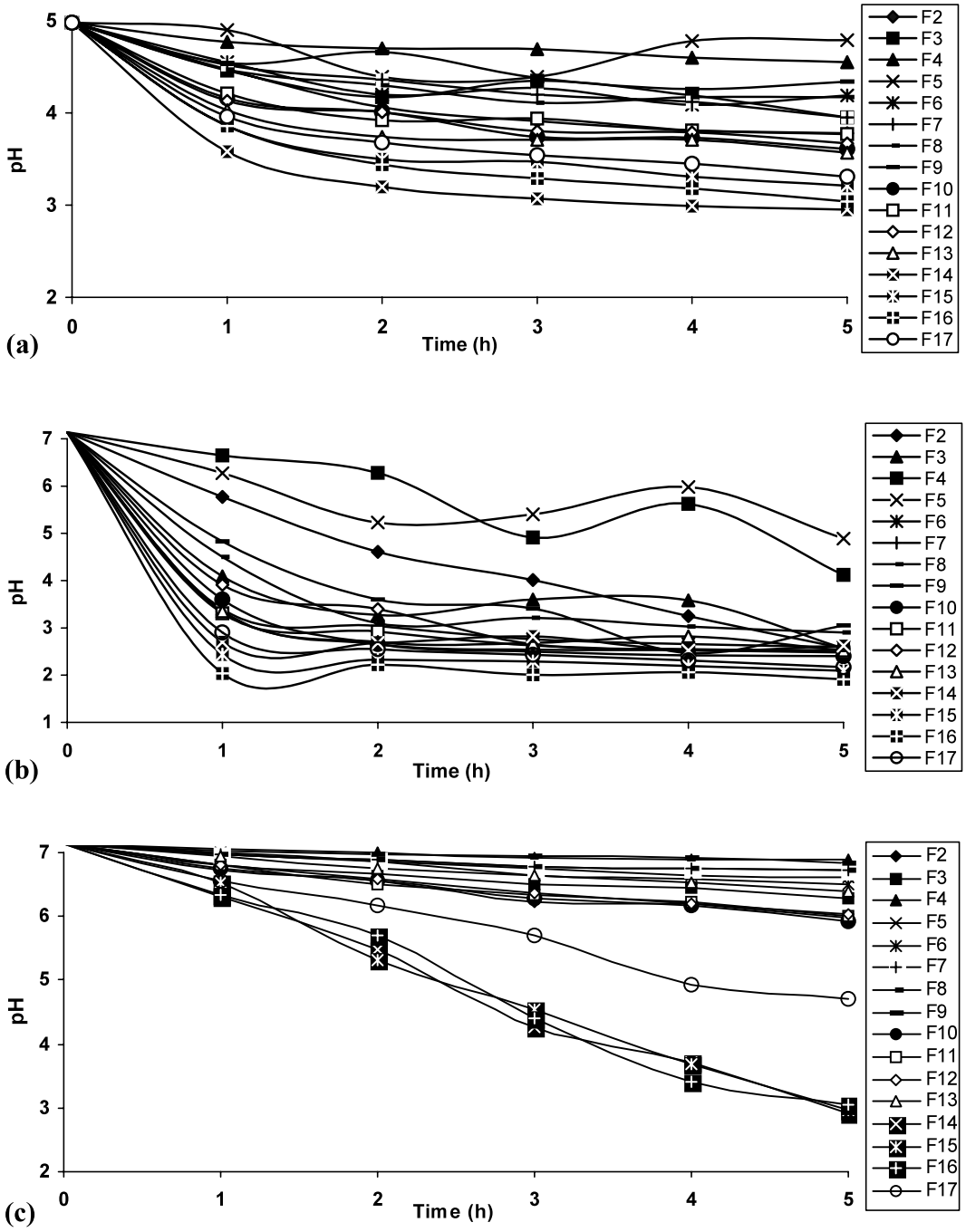

Fig. 2. pH versus time graph in lactate buffer (a), distilled water (b), phosphate buffer (c), respectively.

and 24th h, respectively. The swellings of 2:1 mixtures of Cp usually showed higher swelling volume than 1:2 mixtures. On the contrary, only F12 had slightly lower swelling capacity than F13. It may be related with higher amount of GG, which can be hydrated easily [17]. F14-17 formulations swelled in a short time due to high hydrophilic capacity of EMA but they disintegrated easily.

The swelling state of polymer contributes to its bioadhesive behaviour [18]. However, the general idea that increased swelling contributes to stronger bioadhesive bonds is not quite correct. Although the wet adhesive strength (measured as stress at break) which developed as the polymer absorbed water, increased with increasing degree of swelling, excessive water content led to an abrupt drop in the adhesive strength [19,20]. So excessive swelling of the bioadhesive polymer is undesirable. In addition, this can cause cracking of the outer cap and unwanted drug loss into the tissue [21]. However, F10 remained intact during the test despite it's the highest swelling capacity. It has also high detachment force. Consequently, it is preferable polymer mixture for bioadhesive tablet preparations.

In distilled water, all formulations showed higher swelling volume than 1000% except F4, F6 and F14-F17. F4, F6 and F14-F17 formulations began to disintegrate after the 3rd and the 5th h, respectively. F10 provides the highest swelling also in this medium at the end of the 5th h.

The results obtained in phosphate buffer solution indicated that the percentage increases in the swelling volume for F5 and F12 ($\sim$1500 and 1000% by 5 h, respectively). Other swelling volume values were below 1000% in the same buffer and all of the formulations disintegrated in 24 h. The swelling results mentioned above were shown graphically in Fig. 1.

During the swelling studies, pH change of the medium was followed. It is pointed out that, generally normal vaginal pH of healthy women of reproductive age remains 3.8–4.4 and during vaginal infection vaginal pH is found between 4.5 and 6.5 [22]. The pH changes with ages, stages of menstrual cycle and infections, and affect the vaginal absorption of drugs [23]. Our study showed that all formulations had an effect on the pH value of the medium. In lactate buffer, F4 and F5 coded tablets slightly reduced the pH values of the buffer solution. F5 reduced the pH to 4.79 from 5. F14 caused the highest reduction of pH with 3.04 in the same solution.

The lowest and the highest pH were observed with F16 and F5 in distilled water at the end of the 5th h, respectively.

There were no significant changes in phosphate buffer with F2-F13 formulations. On the contrary, F14-F17 formulations caused the lowest pH values. All the tablets including Carbopol 934 caused lowering of pH values greater than the other formulations in three different solutions at the end of the 5th h. However, these pH values were in biological vaginal pH limitations in lactate buffer. Fig. 2 showed the pH-time graphs of the formulations in each solution.

A bioadhesive formulation might not necessarily contain a therapeutic agent and can be used as a moisturizer for the treatment of dry vagina or the therapeutic agent might be added to the formulation for therapeutic purpose [24]. However, it contains polymers both synthetic and from natural sources to provide bioadhesive behaviour. Their swelling state, bioadhesive properties and the effect on the medium pH has significant importance for bioadhesive vaginal formulations. In our study, we prepared sixteen formulations with using polymer mixtures and examined their properties. Among the formulations, F10 can be suggested as a bioadhesive preparation due to its high adhesion force to cow vaginal mucosa, high swelling capacity and reasonable pH value.

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