Drug Development and Industrial Pharmacy, 2009; 35(7): 769–779

informa
healthcare

RESEARCH ARTICLE

Formulation studies of benzydamine mucoadhesive formulations for vaginal administration

Luana Perioli, Valeria Ambrogi, Lorena Venezia, Stefano Giovagnoli, Cinzia Pagano and Carlo Rossi

Dipartimento di Chimica e Tecnologia del Farmaco, Università degli Studi di Perugia, Perugia, Italy

Abstract

Background: Vaginal cavity represents a good site for drug administration and delivery. *Aim*: The aim of this work was the design of new mucoadhesive semisolid dosage forms for vaginal delivery of benzydamine. *Method*: Simple gels, obtained by using sodium carboxymethylcellulose (NaCMC) and hydroxyethylcellulose (HEC), were employed as water phase of an oil-in-water emulsion (O/W cream) to obtain emulgels, more stable and manageable than gels. Successively, in order to modify the emulgel consistency, the ingredient cetostearylic alcohol was replaced by the same amount of gel or vaseline. All the preparations were submitted to mucoadhesion and rheological, extrusion, and release studies and compared to market vaginal cream Tantum Rosa®. *Results*: HEC formulations showed good drug release profiles and good rheological behavior but low mucoadhesion strength, whereas NaCMC (4% gel) formulations had better drug release and very high mucoadhesive strength. However, the presence of NaCMC 4% conferred too much viscosity to the preparation. Taking into consideration all performances, the most suitable formulations for vaginal applications resulted in those containing NaCMC (3% gel) and with gel replacing cetostearylic alcohol as they showed good ex vivo performances in terms of manageability and high bioadhesion to vaginal mucosa.

Key words: *Benzydamine; emulgels; mucoadhesion; rheology; vaginal inflammation*

Introduction

Normally, most medical doctors and patients refer to the term vaginitis as general vulvovaginal irritations or itching, especially when accompanied by abnormal secretions. Common infectious forms of vaginitis include bacterial vaginosis (BV), vulvovaginal candidiasis (VVC), and trichomoniasis[1]. Vaginitis by *Trichomonas vaginalis* is the most common sexually transmitted infection[2], but vaginitis can be also the result of candidiasis and infection by mixed bacterial flora[3]. Specific diagnostic criteria for BV include a thin homogenous vaginal discharge, vaginal pH higher than 4.5, release of fishy odor from the vaginal discharge, presence of abnormal amines in the vaginal fluid, and vaginal epithelial cells heavily coated with bacilli, also referred to as 'clue cells[3].'

A pharmacological approach is the use of benzydamine that has been successfully employed for a long time in this type of pathology[4]. Currently benzydamine can be useful, particularly in the treatment of the *T. vaginalis* and *Gardnerella vaginalis* infections[5], and it could be reformulated and presented in the form of new preparations. Benzydamine hydrochloride is a nonsteroid anti-inflammatory drug (NSAID), whose application as topical dosage form has been recently successful in gynecology. This drug, besides its efficacy against inflammatory processes of the lower female genital tract, possesses a fairly good antiseptic action, directly proportional to the environmental pH, against different species of germs at 1–1.5 mg/mL concentration in topical applications[4].

The vagina, as a site of drug delivery, offers certain unique features that can be exploited in order to achieve desirable therapeutic effects and constitutes a potentially valuable route for systemic and local drug administration. Traditionally, the vaginal cavity has been used for the delivery of locally active drug such as antibacterial, antifungal, antiprotozoal, antiviral, spermicidal agents, and steroids. Marketed vaginal formulations

Address for correspondence: Dr. Luana Perioli, PhD Researcher, Dipartimento di Chimica e Tecnologia del Farmaco, Università degli Studi di Perugia, Via del Liceo,1 06123 Perugia, Italy. Tel: +39 075 5855133, Fax: +39 075 5855163. E-mail: luanaper@unipg.it

(Received 6 Mar 2008; accepted 30 Oct 2008)

ISSN 0363-9045 print/ISSN 1520-5762 online © Informa UK, Ltd.
DOI: 10.1080/03639040802592435

as tablets, tampons, films, sponges, ointments, pessaries, ovules, douches, creams, foam, and gels suffer from poor retention in the vaginal tract and are easily removed by vaginal self-cleansing mechanisms[6]. Therefore, the treatment requires multiple daily application, thus decreasing patient's compliance. For the above reasons, it is necessary to have at one's disposal a new vaginal delivery system overcoming the actual therapy limitations and suitable to satisfy some women's requirements as no leakage, no irritation, odorless and colorless, no adverse effect on coitus[7]. New formulation designs should take into account that the therapy goals are often linked to many variables such as the particular anatomy, age, cyclic hormonal changes, self-cleaning action of the vaginal tract, and the kind of drug formulation chosen[8]. In this regard, mucoadhesive formulations, able to extend drug residence time may be advantageous indeed by remarkably increasing patient's compliance as well[9,10].

Among the diverse polymers used to formulate mucoadhesive devices, sodium carboxymethylcellulose (NaCMC) and hydroxyethylcellulose (HEC) are being considered very suitable because of their properties such as film-forming ability, swellability, biodegradability, no toxicity, tolerability, and bioadhesivity[9,11–16].

The aim of this study was the development of new mucoadhesive semisolid formulations (emulgels O/W), containing mucoadhesive polymers (HEC and NaCMC) for benzydamine vaginal delivery. In this context four kinds of gels and emulgels were prepared.

Emulsions are extensively used for their therapeutic properties as vehicles to deliver various drugs; they possess a certain degree of elegance and are easily washed off whenever desired. They also have a high ability to enhance drug penetration, a property necessary in the case of irregular surfaces (vaginal mucosa). In addition, it is possible to control better viscosity and to ensure a prolonged contact time with mucosa if compared to the simple gel, thanks for the presence of the internal oil phase. Emulsions efficiency and stability can be increased by replacing the outer water phase with an aqueous-gelled phase (emulgels). Therefore, in this work, simple gels (HEC 5% and NaCMC 3% and 4%) were formulated and then added as outer water phase (Wphase) of an oil-in-water emulsion (O/W cream), named 'cetomacrogol-based cream,' as described in the Farmacopea Ufficiale Italiana XI Ed. (F.U.XI).

Successively, formulation composition was changed by replacing cetostearyl alcohol, constituting the emulsion oil phase together with white vaseline and liquid paraffin, with the same amount of gel or white vaseline. All formulations (gels and emulgels) were loaded with 0.5% (wt/wt) of benzydamine, loaded and characterized in terms of mucoadhesion force, rheological properties (viscometry), and release behavior and compared to the market vaginal cream Tantum Rosa®, containing the same amount of anti-inflammatory drug and HEC as mucoadhesive polymer.

Materials and methods

Materials

HEC NATROSOL 250 HHX® (viscosity in 1% solution 3.500–5.500 centipoises, M.W. 2.7×10^4– 10.5×10^4) was a gift from Aqualon (Hercules Inc., Hopewell, VA, USA). NaCMC sodium salt (viscosity in 1% solution 3.000–6.000 centipoises, M.W. 1×10^6– 4×10^6) was purchased from Aldrich Chemical Company Inc. (Milwaukee, WI, USA). White vaseline was purchased from Farvima Medicinali (Perugia, Italy). Vaseline oil was furnished by Caelo (Hilden, Germany). Cetomacrogol and cetostearyl alcohol (mixture of solid aliphatic alcohols consisting mainly of stearyl, $C_{18}H_{38}O$, and cetyl, $C_{16}H_{34}O$, alcohols) were purchased from Galeno (Milan, Italy). Benzydamine hydrochloride was purchased from Comifar (Perugia, Italy). Tantum Rosa® 0.5% vaginal cream (containing: benzydamine hydrochloride 0.5%, propylene glycol, saturated neutral triglycerides, cetomacrogol 1000, HEC, monohydrate citric acid, bi-hydrate sodium citrate, benzoic acid, and deionized water) was kindly gifted by ACRAF-Angelini (Rome, Italy). All other materials were of reagent grade.

Methods

Preparation of gels and emulgels

Gels and emulgels (O/W emulsions in which the W phase is constituted by gelled water) were carefully prepared according to the F.U.XI instructions 'semisolid preparations for skin applications' and, instead of water, citrate buffer (pH 5.0; F.U.XI) was used in order to have formulation pH values similar to those of vagina (pathological vaginal pH) and ensure stability of the polymers employed[12]. The buffer composition was the following: citric acid 20.1 g, sodium hydroxide 8.0 g, deionized water until 1000 mL; the pH value was adjusted to 5.0.

In order to obtain drug homogeneous distribution a mixer Cito Unguator®[17] was used (800 rpm for 2 minutes at room temperature).

The simple gels **1a**, **2a**, and **3a** (Table 1) had the following composition:

mucoadhesive gelling polymer (3% and 4%)	HEC (5%) or NaCMC
glycerol 85%	10.0 g
citrate buffer	until 100.0 g.

Cetomacrogol-based cream (F.U.XI), chosen as model cream, has the following composition:

oil phase (O)	
white vaseline	15 g

Table 1. Composition of mucoadhesive formulations.

	Formulations			
Polymer	Gels (polymer %)	Emulgels Cetostearyl alcohol presence	Cetostearyl alcohol substituted with gel	Cetostearyl alcohol substituted with white vaseline
HEC	1a (5)	1b	1c	1d
NaCMC	2a (3)	2b	2c	2d
NaCMC	3a (4)	3b	3c	3d

liquid paraffin	6 g
cetostearyl alcohol	7.2 g
water phase (W)	
cetomacrogol 1000	1.8.0 g
deionized water	70.0 g

Emulgels **1b**, **2b**, and **3b** were prepared by employing gels **1a**, **2a**, and **3a**, respectively, instead of deionized water amount as in simple cream. In order to modify the emulgel characteristics in terms of hydrophilicity/lipophilicity and consistency, further modifications were made on the oil phase. Cetostearyl alcohol was replaced by the same amount of gel (**1a**, **2a**, or **3a**) obtaining more hydrophilic emulgels **1c**, **2c**, and **3c**, respectively, or by white vaseline obtaining more lipophilic formulations **1d**, **2d**, and **3d**, respectively, (Table 1). Benzydamine hydrochloride was dissolved in citrate buffer (pH 5.0) in order to obtain a final 0.5% (wt/wt) drug matching the drug loading in the Tantum Rosa® product.

Mucoadhesion studies

Ex vivo adhesion strength was measured as the force needed to pull out formulations from the mucosa. Porcine vaginal mucosa was obtained from Large White pigs provided by veterinary service of USL N 1 Città di Castello (Perugia, Italy). It was washed with saline solution, stored at 4°C, and used within 12 hours from pig euthanasia.

Bioadhesion strength was assessed by a dynamometer[18] (Lehrmittelbau, Bonn, Germany) and cyanoacrylate glue was used to fix the porcine vaginal mucosa to the upper support connected to the dynamometer. The formulation was kept in a vessel placed in a thermostatic bath at 37°C (±0.1). The free surface of the vaginal mucosa (2 × 2 cm) first was wetted with citrate buffer (100 µL) and then was put in contact with the formulation at a constant pressure of 0.22 kPa for 30 seconds. The force required to separate the formulation from the mucosa surface was determined using a dynamometer with a strain rate of 0.2 in seconds. All values are the average of three measurements ($n = 3$) and the error was expressed as SD.

Rheological studies

Viscometry measurements (viscosity 20°C and 37°C, yield stress 25°C) of formulations were performed by a Stresstech HR (Reologica Instruments AB, Milan, Italy) rheometer with plate–plate geometry (diameter of 40 mm). Samples were carefully applied to the lower plate using a spatula to avoid formulation shearing and air bubble formation.

Filling and extrusion studies

All formulations were (handy) inserted in 30 mL aluminum ointment tubes (APONORM®; Karl Höll GmbH & Co. KG, Langenfeld, Deutschland) by an appropriate tube filler (APONORM®); then all tubes were sealed by pincers (APONORM®). Successively the formulations were transferred, by squeezing the tubes, into the vaginal applicator, plunger up provided, a holded tube (high 8.5 cm, internal diameter 1 cm). The necessary force to extrude the formulation (from applicator into the vagina) by pressing the plunger was measured by a dynamometer (Lehrmittelbau, Bonn, Germany) connected to it and under tension.

Release studies

In vitro drug release was assessed by means of a Franz cell diffusion (PermeGear Inc., Bethlehem, PA, USA; diameter 20 mm). It was formed by a water-jacketed receptor chamber (15mL), thermostated at 37°C, and a donor chamber. The receptor solution (citrate buffer) was maintained at 37°C and constantly stirred at 600 rpm. The two chambers were separated by a cellulose membrane (Filter paper Whatman 41, 20–25 µm; Whatman GmbH, Dassel, Germany) and each formulation (200 mg) was spread on a circular portion of the membrane. After the two chambers were clamped, 2 mL of deionized water was added into the upper donor chamber and the donor cell was sealed with parafilm®. At predetermined intervals, 500 µL aliquots were removed and replaced with the same amount of release medium. The collected samples were diluted with citrate buffer, filtered with SWINNEX system (Millipore, Cork, Ireland) using filter paper Whatman 41, porosity 20–25µm (Whatman GmbH) and analyzed. The benzydamine content was determined at 306.0 nm by a spectrophotometer (UV-Visible Agilent model 8453) by using a drug calibration curve in citrate buffer ($r = 0.9995$) and citrate buffer as blank. At this λ value only drug absorbance was present and no interferences of excipients were observed. The drug percentage released at each time point was expressed as a fraction of the total amount of benzydamine in the formulation. The experiments

were carried out in triplicate under sink conditions and the error expressed as SD.

Statistics

A paired *t*-test was applied on the mucoadhesion force data in order to highlight differences between the batches produced and the marketed formulation Tantum Rosa®.

Results and discussion

Some bioadhesive polymers were evaluated in order to design and prepare vaginal semisolid formulations able to (i) increase drug residence time improving the efficacy of local therapy, (ii) respect vaginal environment, and (iii) guarantee patient's tolerability. Gels are the easiest formulations to make, therefore in the first step of this study the most suitable polymers, and their respective amounts to obtain consistent gels, were chosen on the basis of the following characteristics:

- water solubility: to produce hydrogels;
- gelling capacity not dependent on alkaline pH value: to respect the vaginal acidity level;
- stability at pH 5.0 (vaginal pH in pathological conditions).

On the basis of a preliminary screening the best polymers were HEC and NaCMC, and by using them, gels **1a**, **2a**, and **3a** and emulgels **1b**, **1c**, **1d**, **2b**, **2c**, **2d**, **3b**, **3c**, and **3d** (Table 1) were prepared as described in the method section. In this way, it is possible to obtain a 'special formulation' stabilized by the formation of a polymer network able to slow down coalescence process. Moreover, plasticity and consistency of the system are mainly related to the gelled water phase rather than to the oil phase ingredients.

All formulations were 0.5% (wt/wt) benzydamine loaded, in analogy to Tantum Rosa®. Gels and emulgels showed good mucoadhesive forces between 0.33 and 0.57 N (Table 2). Gels **1a** (0.37 N) and **2a** (0.37 N) and emulgel **1d** (0.33 N) showed the lowest mucosa adhesion forces. Gels **1a** and **2a** together with **2c** were not significantly different (α = 0.05%) from the marketed formulation (0.38 N). In the case of formulation **1a**, the interaction between HEC and mucin chains is rather weak because of (i) nonionic character of the polymers, able to establish only hydrogen bonds and (ii) the molecular weight that is lower than the minimum value required for bioadhesion[9]. Unexpectedly, gel **2a** presented low bioadhesion power through the NaCMC affinity for mucin. Probably this behavior is likely to be related to small polymer amount[19]. When NaCMC percentage increases from 3% to 4% (gel **3a**) the mucoadhesive force (0.49 N) was also significantly increased (α = 0.05%).

Table 2. Ex vivo mucoadhesion force.

Groups	Formulations	Force (N)±SD (n = 3)
1	1a	0.37 ± 0.010
	1b	0.47 ± 0.010*
	1c	0.38 ± 0.020
	1d	0.33 ± 0.020*
2	2a	0.37 ± 0.010
	2b	0.57 ± 0.020*
	2c	0.41 ± 0.020
	2d	0.413 ± 0.006*
3	3a	0.49 ± 0.020*
	3b	0.53 ± 0.030*
	3c	0.54 ± 0.020*
	3d	0.54 ± 0.020*
Control	Tantum Rosa®	0.383 ± 0.006

*Significantly different from Tantum Rosa (paired *t*-test; α = 0.05).

The physico-chemical characteristics of HEC combined to the increase of emulgel lipophilicity, due to introduction of white vaseline in place of cetostearyl alcohol, were responsible for the low bioadhesion of emulgel **1d** which is significantly lower than that of the market formulation (α = 0.05%). All remaining formulations showed significantly higher bioadhesion than Tantum Rosa® (α = 0.05%).

The presence of NaCMC improved bioadhesion performances that increased proportionally to its percentage (4% > 3%). NaCMC is a negatively charged polymer and this property guarantees good bioadhesion because of the large hydrogen bonding sites and also for the open expanded conformation that the carboxylic ionized groups provide[20]; so it adheres strongly in comparison to nonionic polymers, such as HEC, as observed by other authors[21]. In all cases, the emulgels exhibited higher mucoadhesivity, if compared to the correspondent gel, and the cetostearyl alcohol substitution (with both gel and white vaseline) caused the decrease of the mucoadhesive force with the exception of **3c** and **3d** (4% NaCMC) that showed similar values. The higher emulgel mucoadhesivity can be related to the presence of an internal oil phase responsible for decreasing the number of intramolecular bonds occurring among polymer chains (very numerous in the case of simple gel), thereby more groups are available to interact with mucin. Emulgel **2b** showed the highest adhesion force (0.57 N). In this case the presence of an oil phase among the chains of the polymer network allows a more stretched and open structure able to offer many groups to link the mucosal surface. At the same time cetostearyl alcohol plays an important role for emulsion stability because of its emulsifying properties.

Formulation mucoadhesive properties can be affected by polymer concentration[19]. In fact a high number of chains create a tight network capable of increasing formulation viscosity. In these conditions, only few groups are available to interact with mucin chains. This is the possible explanation of gel **3a** and emulgel **3b** behaviors, containing 4% NaCMC.

When cetostearyl alcohol was replaced by vaseline (more hydrophobic emulgel) or other gel (more hydrophilic emulgel) the obtained formulations formed a stable emulsion, they became thicker and more viscous maintaining, however, their adhesiveness to the mucosa.

Viscometry studies were carried out in order to check formulation flow properties and qualitative/quantitative polymer influences. The measurements (shear stress versus shear rate) were performed at 20°C and 37°C to simulate storage and application conditions, respectively (Figures 1–3). However, since formulation behaviors were detectable in different range values, it was necessary to use different scale for graphic representations.

From rheograms at 20°C (Figure 1A–D) it is possible to note that some formulations exhibited pseudoplastic flow. They were the marketed formulation Tantum Rosa®, formulation **2a**, NaCMC (3%) gel, and formulation **1b**, emulgels containing cetostearyl alcohol. Preparations **2c** and **2d** had similar behaviors but they showed a higher viscosity.

Generally, when cetostearyl alcohol was not present in the preparation, the emulgels showed a decreased viscosity. This effect was observed when cetostearyl alcohol was substituted both with gel (e.g., in the case of **1c**) and with white vaseline (e.g., in the case of **1d**).

When NaCMC was used, the gel behaviour was closely dependent on the mucoadhesive polymer

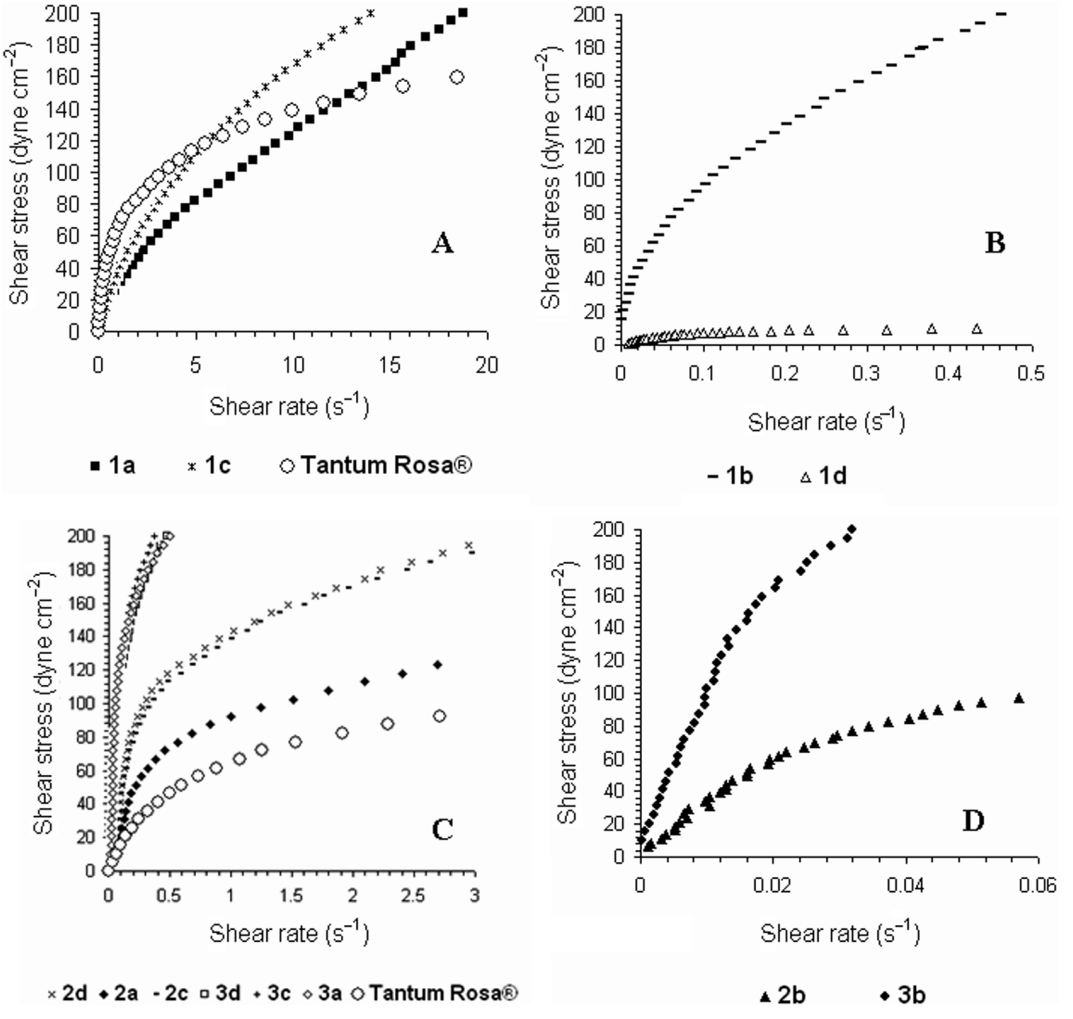

Figure 1. Viscosity measurements at 20°C.

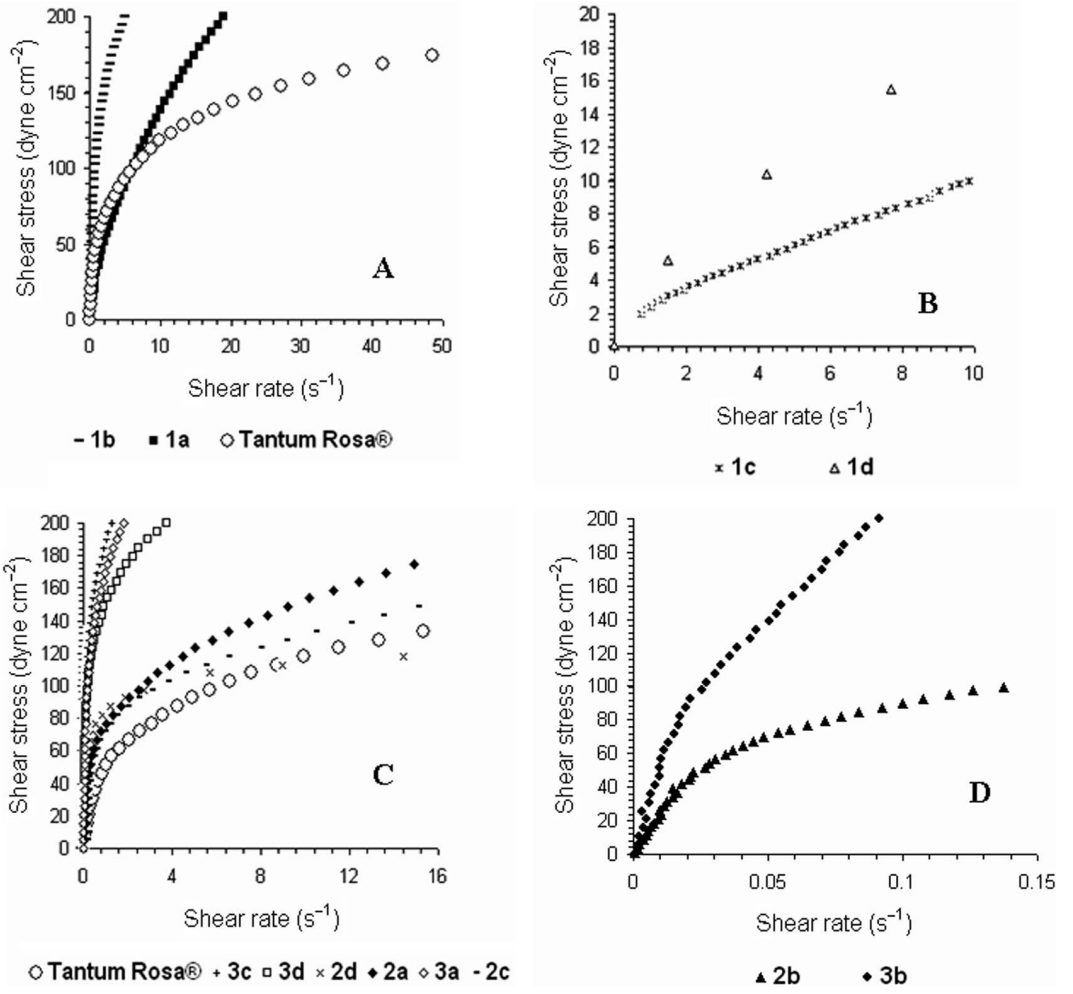

Figure 2. Viscosity measurements at 37°C.

amount. In fact 3% NaCMC gel (**2a**) and 4% NaCMC gel (**3a**) showed a higher viscosity than the marketed cream. All emulgels containing NaCMC, with or without cetostearyl alcohol, showed pseudoplastic flow and the viscosity was significantly increased raising the polymer amount to 4% (**3a**, **3c**, and **3d**).

After these considerations it is clear that **2c** and **2d** had the best behavior because:

- **3a**, **3c**, and **3d** had too high viscosities;
- also **2b** and **3b**, containing cetostearyl alcohol, were highly consistent and waxy so as that it was very difficult to measure their rheological properties (plate slipping).

The rheological studies at 37°C (Figure 2A–D) demonstrated that Tantum Rosa® and gel **2a** maintained similar flow to that observed at 20°C, whereas in the case of **2c** and **2d** a higher flow threshold was measured at 37°C. **3a**, **3c**, and **3d** conserved the same flow with a higher viscosity at 37°C as well. Conversely, **1c** viscosity was significantly lower at 37°C than at 20°C; in the case of **2b** and **3b** no considerable variations were observed with temperature and their measurements were rather difficult owing to plate slipping.

Viscometry measurements in pharmaceutical field can offer important information about formulation extrudability from packaging, squeezing, and spreading properties. Extrudability information is very important in the case of semisolid vaginal formulations because of their packaging (tube) and administration modality (vaginal applicator). In this regard viscosity versus shear stress studies at 25°C were performed (Figure 3A–D).

The comparison of the yield stress values, point of rheogram corresponding to the maximum force (Pa/sec) necessary to obtain formulation flow, allows classification of all preparations according to the easiness or toughness of extrusion. In the case of formulations containing HEC, extrudability was better than market cream with the exception of **1b**. It is clear that HEC can

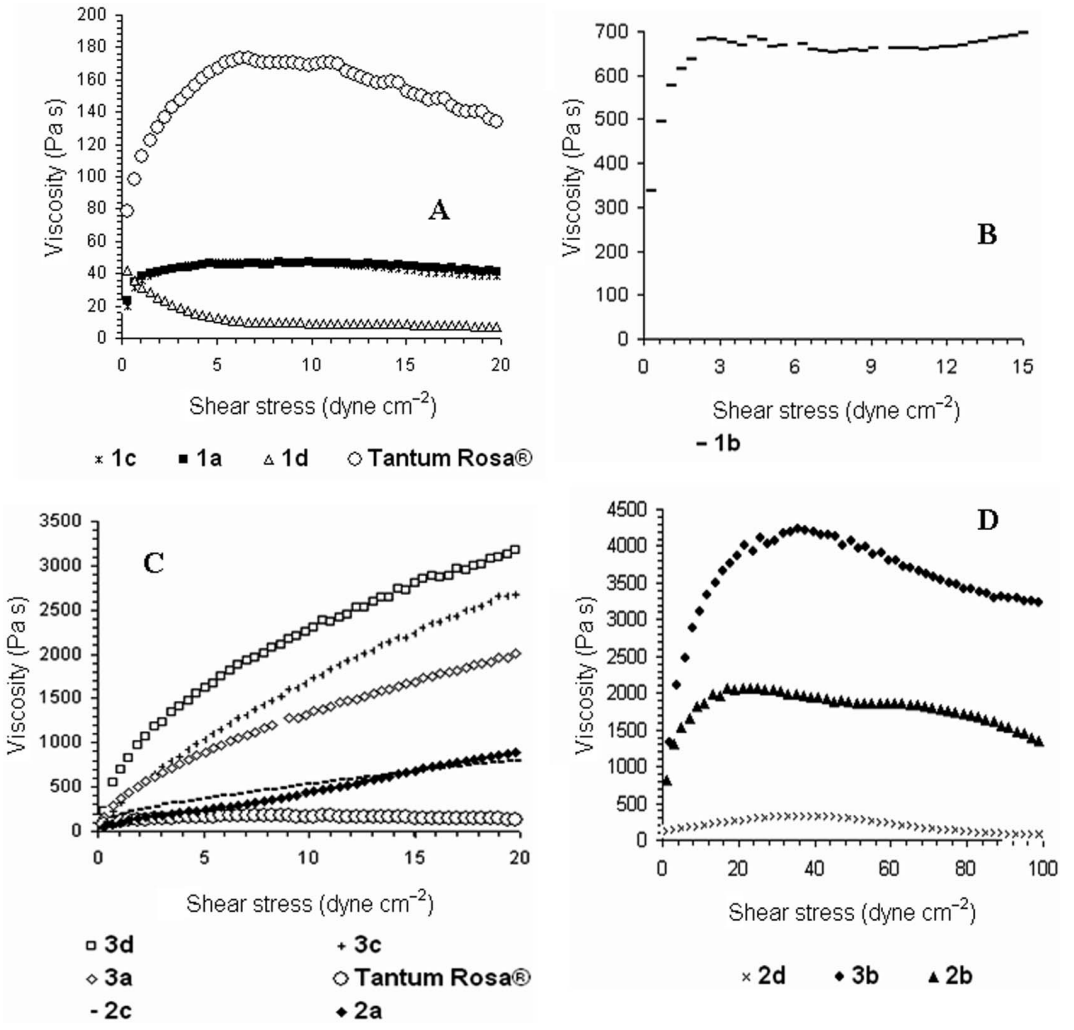

Figure 3. Extrudibility measurements.

permit a better flow rate from tube to applicator and an easier vaginal administration. Only in the case of simultaneous presence of HEC and cetostearyl alcohol viscosity increased, thus affecting extrudability (**1b**). When cetostearyl alcohol was substituted by white vaseline, the viscosity decreased (viscosity is very low either at 20°C or at 37°C) and this is evident from the very high extrudability of **1d** (Figure 3A). On the other hand NaCMC made the preparations less flowing (Figure 3), all formulations had higher extrudability than Tantum Rosa® (**3b**>**2b**>**2d**>**3d**>**3c**>**3a**>**2c**>**2a**> Tantum Rosa®). Formulations **3b** and **2b**, containing cetostearyl alcohol, showed big extrusion problems.

Filling and extrusion studies were further performed in order to understand the composition influence on drug administration easiness and to compare these formulation features to their rheological characteristics (extrudability). Aluminum ointment tubes were filled manually as reported previously and, successively, a sample (5 mL) was transferred into an apposite vaginal applicator with the attached plunger up. Therefore, the cap was removed from the tube, the threaded end of the applicator was attached by screwing onto the tube, that was squeezed from the bottom until the formulation filled the applicator. After removal of the applicator from the tube in order to simulate the administration into the vagina, the internal plunger was connected to a dynamometer and the force and time necessary to eject the gel/emulgel were determined (Table 3). Only **1a** showed the same extreme filling easiness as Tantum Rosa®, whereas it was rather difficult for **1b**, **2d**, **3a**, and **3b**. In the case of industrial manufacturing such a feature does not present a problem; so the most important steps in order to administer drugs are the tube-applicator filling and the administration into the vagina. The applicator filling was easy for all gels/emulgels with the

Table 3. Filling and extrusion force measurements.

			Extrusion of applicator	
Formulations	Tube filling	Tube-applicator filling	Force (N)±conf. ($n = 3$; $\alpha = 0.05$)	Time (s) (±1s) ($n = 3$)
1a	+	+	0.173 ± 0.02	28
1b	++++	+	0.453 ± 0.01	36
1c	++	++	0.250 ± 0.03	28
1d	+++	+	0.267 ± 0.03	34
2a	+++	+	0.257 ± 0.02	32
2b	++	++	0.700 ± 0.08	36
2c	+++	++	0.390 ± 0.04	34
2d	++++	+	0.467 ± 0.03	38
3a	++++	++	0.683 ± 0.03	28
3b	++++	+++	1.300 ± 0.34	42
3c	++	++	0.540 ± 0.06	25
3d	++	++	0.557 ± 0.02	27
Tantum Rosa®	+	+	0.153 ± 0.03	35

+, very easy; ++, easy; +++, difficult; ++++, very difficult.

only exception of **3b**. All formulations, only **3b** required a longer time, were completely squeezed from the tube in about 28–36 seconds (Table 3).

According to extrudability studies of formulations containing HEC, **1b** needed the highest extrusion force while **1a**, **1c**, and **1d** were more easily extrudible. In the case of preparations containing NaCMC, the highest forces were required for the emulgels **2b** and **3b** while they decreased for the formulation not containing cetostearyl alcohol. According to our studies **2a**, **2c**, and **2d** were the most extrudible.

All formulations were then submitted to in vitro drug release experiments and compared to the marketed formulation. The drug release was monitored for 24 hours. Since from the 10th to the 24th hour no significant variations were observed, the release profiles were recorded between the 1st and the 10th hour. It is evident (Figure 4A–C) that all formulations showed a release profile similar to Tantum Rosa®, with the only exception of **1a**.

Taking into account the formulation compositions some conclusions can be drawn:

- Formulations **1a**–**1d** containing HEC as gelling agent (Figure 4A) did not show the same release profiles. The simple gel (**1a**) released more benzydamine and more quickly, showing a behavior very similar to Tantum Rosa® and different from the emulgels **1b**–**1d**.
- When NaCMC (3% and 4%) was used (Figure 4B and 4C) no significant differences were observed between gels and emulgels and this was probably due to the high polymer concentration.

Notwithstanding emulgel release profiles showed very little differences. It was possible to note that the presence of cetostearyl alcohol enhanced drug release and its substitution by gel or vaseline reduced it. The decrease was highest in the presence of vaseline that, because of its lipophilicity, did not promote drug diffusion in the acceptor fluid.

The preparations (gels and emulgels) under study are hydrophilic formulations containing polymeric chains able to build a network where both water and drug molecules can be entrapped influencing gel structure, viscosity, and drug release mechanism. In vitro drug release data have been employed after that to investigate what kind of mechanism was involved; the Ritger and Peppas's kinetic mathematical model[22] and a first-order kinetic model were used to fit experimental release profiles (linear regression) (Table 4). From the calculated r values (correlation coefficient) it is possible to assert that in no case the release fitted the first-order and zero-order ($n = 1$, case II transport) kinetics, meaning that the drug transport mechanism is not dependent on drug concentration and that it is not a linear function of time. For all formulations (Tantum Rosa® included), the r value increased as the release exponent n decreased from 1 to 0.5 and, with the only exception of **2c** and **2d**, when the highest values were achieved for $n = 0.5$. This means that the employed polymers did not play an important role on drug release and that displacement of benzydamine from the polymeric network was mainly due to a Fickian diffusion (following Higuchi kinetics). On the contrary, an anomalous transport (not Fickian) was observed in the case of **2c** and **2d**, probably because:

- NaCMC is able to control drug release more than HEC. It is particularly highlighted when NaCMC gel/cetostearyl

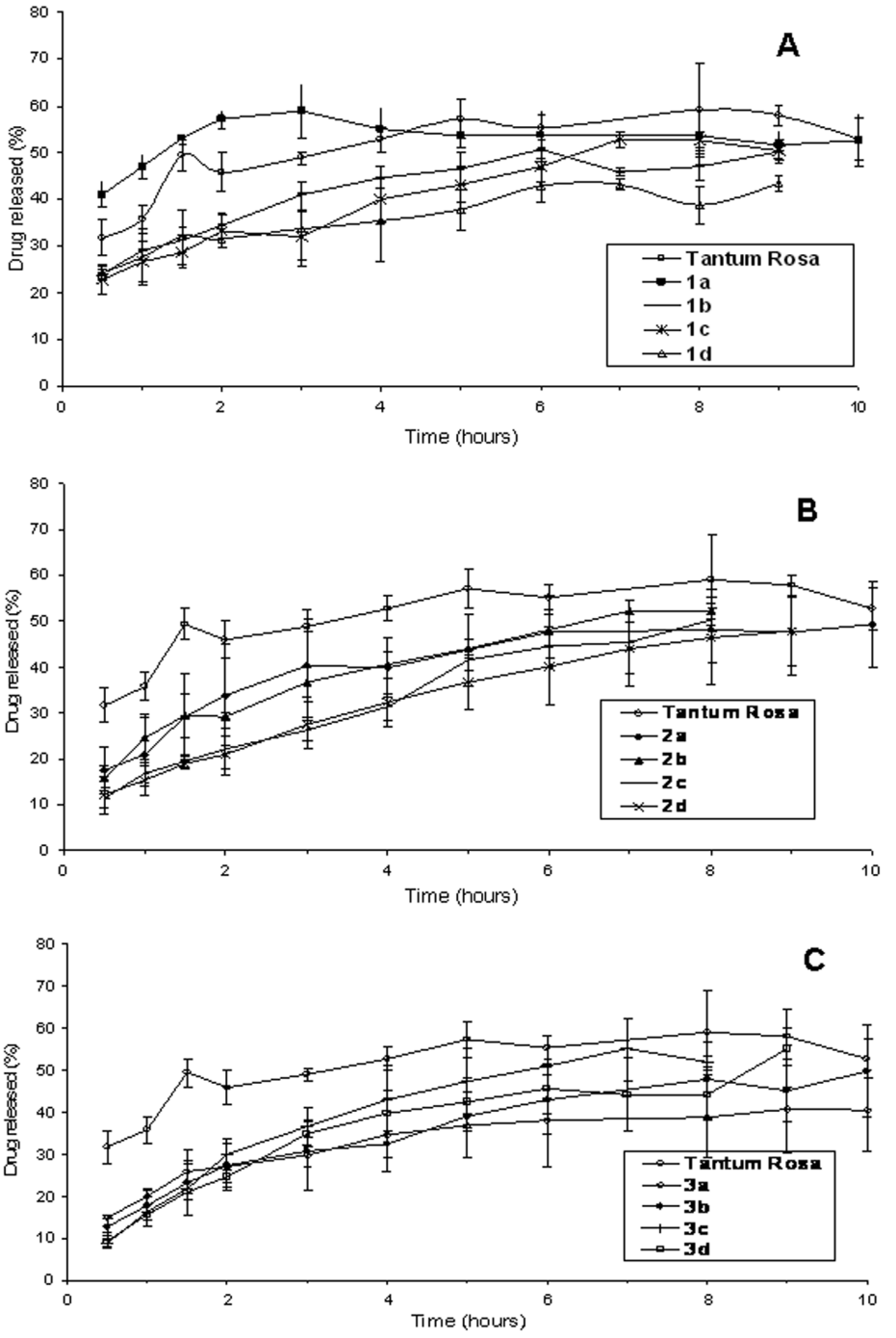

Figure 4. In vitro drug release from formulations, average ± SD ($n = 3$).

alcohol substitution occurred (**2c**) and mainly when its concentration was higher.

- The addition of lipophilic ingredient (vaseline) in the formulation decreases the polymeric chain influence on drug release and increases the prevalence of diffusive mechanism. These effects were more evident for **2d** where cetostearyl alcohol was replaced by vaseline.

Conclusions

Taking into account only the bioadhesive performances, formulations **3a**, **3b**, **3c**, and **3d** containing NaCMC showed the best performances (NaCMC 4% > 3%). Since a good vaginal formulation must also be easy to administer and must possess proper drug release and high storage stability, the polymer choice

Table 4. Ritger and Peppas's kinetic mathematical model and first-order kinetics model fitting.

	$M_t/M_\infty = k_t{}^n$						
Formulations	$n = 1$ (zero-order)	$n = 0.9$	$n = 0.8$	$n = 0.7$	$n = 0.6$	$n = 0.5$ Higuchi (0–60% release)	$M_t/M_\infty = 1 - e^{-kt}$ (first-order)
1a	$y = 0.397x + 50.633$	$y = 0.5518x + 50.325$	$y = 0.7727x + 49.938$	$y = 1.0927x + 49.44$	$y = 1.5673x + 48.776$	$y = 2.2956x + 47.851$	$y = -0.003x - 2.3108$
	$r = 0.2798$	$r = 0.3023$	$r = 0.3262$	$r = 0.3517$	$r = 0.3787$	$r = 0.4071$	$r = 0.2447$
1b	$y = 2.8226x + 28.366$	$y = 3.6452x + 27.248$	$y = 4.7428x + 25.863$	$y = 6.2351x + 24.1$	$y = 8.3187x + 21.769$	$y = 11.343x + 18.526$	$y = -0.0202x - 2.1432$
	$r = 0.9001$	$r = 0.9116$	$r = 0.9227$	$r = 0.9331$	$r = 0.9427$	$r = 0.9514$	$r = 0.9081$
1c	$y = 3.5844x + 23.702$	$y = 4.5913x + 22.417$	$y = 5.9236x + 20.828$	$y = 7.7203x + 18.802$	$y = 10.209x + 16.118$	$y = 13.796x + 12.38$	$y = -0.0259x - 2.1106$
	$r = 0.9673$	$r = 0.9716$	$r = 0.9751$	$r = 0.9776$	$r = 0.9790$	$r = 0.9792$	$r = 0.9698$
1d	$y = 2.0158x + 26.864$	$y = 2.5919x + 26.106$	$y = 3.3577x + 25.166$	$y = 4.3952x + 23.968$	$y = 5.839x + 22.38$	$y = 7.929x + 20.168$	$y = -0.0135x - 2.1344$
	$r = 0.9218$	$r = 0.9295$	$r = 0.9366$	$r = 0.9431$	$r = 0.9488$	$r = 0.9536$	$r = 0.9248$
2a	$y = 3.1177x + 24.237$	$y = 4.0666x + 22.877$	$y = 5.344x + 21.195$	$y = 7.0955x + 19.052$	$y = 9.5604x + 16.219$	$y = 13.164x + 12.279$	$y = -0.0216x - 2.1187$
	$r = 0.9012$	$r = 0.9133$	$r = 0.9251$	$r = 0.9364$	$r = 0.9471$	$r = 0.9571$	$r = 0.9240$
2b	$y = 4.5557x + 19.954$	$y = 5.786x + 18.435$	$y = 7.4052x + 16.549$	$y = 9.5789x + 14.136$	$y = 12.579x + 10.932$	$y = 16.89x + 6.4615$	$y = -0.0319x - 2.0887$
	$r = 0.9690$	$r = 0.9750$	$r = 0.9805$	$r = 0.9853$	$r = 0.9892$	$r = 0.9923$	$r = 0.9840$
2c	$y = 5.1591x + 11.327$	$y = 6.5277x + 9.6867$	$y = 8.321x + 7.6519$	$y = 10.718x + 5.0516$	$y = 14.011x + 1.6006$	$y = 18.722x - 3.2147$	$y = -0.0334x - 2.0416$
	$r = 0.9877$	$r = 0.9901$	$r = 0.9917$	$r = 0.9923$	$r = 0.9918$	$r = 0.99005$	$r = 0.9912$
2d	$y = 4.3465x + 12.544$	$y = 5.5658x + 10.993$	$y = 7.1789x + 9.072$	$y = 9.3541x + 6.6224$	$y = 12.367x + 3.377$	$y = 16.709x - 1.145$	$y = -0.0279x - 2.0499$
	$r = 0.9868$	$r = 0.9909$	$r = 0.9942$	$r = 0.9965$	$r = 0.9977$	$r = 0.9975$	$r = 0.9946$
3a	$y = 2.3821x + 20.829$	$y = 3.1075x + 19.789$	$y = 4.0838x + 18.502$	$y = 5.4222x + 16.865$	$y = 7.3053x + 14.701$	$y = 10.058x + 11.692$	$y = -0.0149x - 2.1005$
	$r = 0.9036$	$r = 0.9159$	$r = 0.9278$	$r = 0.9391$	$r = 0.9499$	$r = 0.9597$	$r = 0.9176$
3b	$y = 3.9337x + 16.479$	$y = 5.0917x + 14.919$	$y = 6.6385x + 12.988$	$y = 8.7434x + 10.526$	$y = 11.684x + 7.2654$	$y = 15.955x + 2.7226$	$y = -0.0267x - 2.0697$
	$r = 0.9725$	$r = 0.9780$	$r = 0.9829$	$r = 0.9869$	$r = 0.9901$	$r = 0.9921$	$r = 0.9841$
3c	$y = 5.8583x + 13.876$	$y = 7.4635x + 11.847$	$y = 9.5814x + 9.3322$	$y = 12.431x + 6.121$	$y = 16.371x + 1.8649$	$y = 22.044x - 4.0652$	$y = -0.0399x - 2.0554$
	$r = 0.9459$	$r = 0.9548$	$r = 0.9631$	$r = 0.9707$	$r = 0.9774$	$r = 0.9832$	$r = 0.9647$
3d	$y = 4.614x + 14.546$	$y = 5.9364x + 12.798$	$y = 7.6955x + 10.631$	$y = 10.08x + 7.8656$	$y = 13.402x + 4.2017$	$y = 18.212x - 0.9019$	$y = -0.0306x - 2.0609$
	$r = 0.9379$	$r = 0.9464$	$r = 0.9543$	$r = 0.9616$	$r = 0.9681$	$r = 0.9736$	$r = 0.9520$
Tantum Rosa®	$y = 2.2136x + 40.264$	$y = 2.9083x + 39.215$	$y = 3.8495x + 37.92$	$y = 5.1478x + 36.273$	$y = 6.9857x + 34.099$	$y = 10.024x + 30.36$	$y = -0.0187x - 2.2256$
	$r = 0.7779$	$r = 0.7940$	$r = 0.8101$	$r = 0.8259$	$r = 0.8414$	$r = 0.8701$	$r = 0.7843$

influences remarkably all the final product characteristics. Hence it is possible to conclude that:

1. all the formulations containing HEC showed good drug release profiles and good rheological characteristics but low mucoadhesion;
2. formulations prepared by using NaCMC (4%) presented very good mucoadhesive performances and good drug release, but their high viscosity did not make them handy;
3. the most suitable emulgels for vaginal application were those containing NaCMC (3%).

The best formulation was the emulgel in which cetostearyl alcohol was replaced by NaCMC gel. In fact, this preparation showed the best in vitro and ex vivo performances furnishing a product that could be suitable to improve vaginal tolerability and patient's *compliance*. Moreover, a higher vaginal mucosa bioadhesion, which increases drug residence time in comparison to Tantum Rosa®, could improve benzydamine efficacy.

Acknowledgments

Authors are very much grateful to Dr. Giampiero Guerri from USL N 1 Città di Castello (Perugia, Italy) for providing porcine mucosa to perform ex vivo experiments and Mr. Marco Marani from Dipartimento di Chimica e Tecnologia del Farmaco (Perugia, Italy) for the precious collaboration and technical assistance.

Declaration of interest: The authors report no conflicts of interest.

References

1. Sobel JD. (1997). Vaginitis. N Engl J Med, 337:1896–903.
2. Johnston VJ, Mabey DC. (2008). Global epidemiology and control of *Trichomonas vaginalis*. Curr Opin Infect Dis, 21(1):56–64.
3. Kukner S, Ergin T, Cicek N, Ugur M, Yesilyurt H, Gokmen O. (1996). Treatment of vaginitis. Int J Gynaecol Obstet, 52:43–7.
4. Magliano EM, Conti M, Clerici P, Laurita L. (1987). The role of benzydamine in the topical treatment of the so-called non-specific vaginitis. Int J Tissue React, 9(2):151–6.
5. Silvestrini B. (1986). Patent use of benzydamine in the treatment of the *Trichomonas vaginalis* and *Gardnerella vaginalis* infections, EP0195749.
6. Ceschel GC, Maffei P, Borgia SL, Ronchi C, Rossi S. (2001). Development of a mucoadhesive dosage form for vaginal administration. Drug Dev Ind Pharm, 27:541–7.
7. Vermani K, Garg S. (2000). The scope and potential of vaginal drug delivery. Pharm Sci Technol Today, 3:359–64.
8. Knut K, Amiji M, Robinson JR. (1993). Hydrogel delivery systems for vaginal and oral applications — Formulation and biological considerations. Adv Drug Deliv Rev, 11:137–67.
9. Lee JW, Park JH, Robinson JR. (2000). Bioadhesive-based dosage forms: The next generation. J Pharm Sci, 89:850–66.
10. Bonferoni MC, Giunchedi P, Scalia S, Rossi S, Sandri G, Caramella C. (2006). Chitosan gels for the vaginal delivery of lactic acid: Relevance of formulation parameters to mucoadhesion and release mechanisms. AAAPSPharmSciTech, article 104. http://www.aapspharmscitech.org
11. Jones DS, Woolfson AD, Brown AF. (1997). Textural, viscoelastic and mucoadhesive properties of pharmaceutical gels composed of cellulose polymers. Int J Pharm, 151:223–33.
12. Parson D, Harwood RJ. (2000). Carboxymethylcellulose sodium and hydroxyethylcellulose. In: Kibbe H, ed. Handbook of pharmaceutical excipients, 3rd ed. Washington, D.C.: American Pharmaceutical Association, 87–90 and 240–3.
13. Perioli L, Ambrogi V, Angelici F, Ricci M, Giovagnoli S, Capuccella M, et al. (2004). Development of mucoadhesive patches for buccal administration of ibuprofen. J Control Release, 99:73–82.
14. Das Neves J, Bahia MF. (2006). Gels as vaginal drug delivery systems. Int J Pĥarm, 318:1–14.
15. Pavelic Ž, Škalko-Basnet N, Jalšenjak I. (2005). Characterisation and in vitro evaluation of bioadhesive liposome gels for local therapy of vaginitis. Int J Pharm, 301:140–8.
16. Perioli L, Ambrogi V, Giovagnoli S, Ricci M, Blasi P, Rossi C. (2007). Mucoadhesive bilayered tablets for buccal sustained release of flurbiprofen. AAAPSPharmSciTech, article 54. http://www.aapspharmscitech.org
17. Refai H, Müller-Goymann CC. (2002). The influence of dilution of topical semisolid preparations on hydrocortisone permeation through excised human stratum corneum. Eur J Pharm Biopharm, 54:143–50.
18. Prudat-Christiansen C, Arnaud P, Allain P, Chaumeil JC. (1996). Aminophilline bioadhesive tablets attempted by wet granulation. Int J Pharm, 141:109–16.
19. Bertram U, Bodmeier R. (2006). In situ gelling, bioadhesive nasal inserts for extended drug delivery: In vitro characterization of a new nasal dosage form. Eur J Pharm Sci, 27:62–71.
20. Madsen F, Eberth K, Smarth JD. (1998). A rheological examination of the mucoadhesive/mucus interaction: The effect of mucoadhesive type and concentration. J Control Release, 50(1–3):167–78.
21. El-Kamel AH, Sokar MS, Naggar VF, Al Gamal SS. (2002). Bioadhesive controlled release metronidazole vaginal tablets. Acta Pharm, 52:171–9.
22. Ritger P, Peppas NA. (1987). A simple equation for description of solute release. II. Fickian and anomalous release from swellable devices. J Control Release, 5:37–42.