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Liposomal gels for vaginal drug delivery

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Abstract

The aim of our study was to develop a liposomal drug carrier system, able to provide sustained and controlled release of appropriate drug for local vaginal therapy. To optimise the preparation of liposomes with regards to size and entrapment efficiency, liposomes containing calcein were prepared by five different methods. Two optimal liposomal preparations (proliposomes and polyol dilution liposomes) were tested for their in vitro stability in media that simulate human vaginal conditions (buffer, pH 4.5). To be closer to in vivo application of liposomes and to achieve further improvement of their stability, liposomes were incorporated in vehicles suitable for vaginal self-administration. Gels of polyacrylate were chosen as vehicles for liposomal preparations. Due to their hydrophilic nature and bioadhesive properties, it was possible to achieve an adequate pH value corresponding to physiological conditions as well as desirable viscosity. In vitro release studies of liposomes incorporated in these gels (Carbopol 974P NF or Carbopol 980 NF) confirmed their applicability as a novel drug carrier system in vaginal delivery. Regardless of the gel used, even 24 h after the incubation of liposomal gel in the buffer pH 4.5 more than 80% of the originally entrapped substance was still retained.

Keywords: Liposomes; Polyacrylate gels; Stability; Vaginal application; Viscosity

1. Introduction

The effectiveness of the vaginal cavity as a site of drug administration for systemic and local effects has been well established (Brown et al., 1997; Jain et al., 1997). Pharmaceutical dosage forms destined for vaginal delivery are usually applied in the local treatment of specific gynaecological diseases, such as candidiasis, vaginosis, as well as genital *Herpes*. Despite the variety of formulations for intravaginal therapy (tablets, creams, suppositories, pessaries, foams, solutions, ointments and gels), their efficacy is often limited by a poor retention at the site of action due to the self-cleansing action of the vaginal tract. Furthermore, intravaginal route of drug administration may lead to a systemic rather than a localised effect due to the remarkable absorption of drug

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from the vaginal wall (Robinson and Bologna, 1994). To overcome these limitations, novel delivery systems (microspheres, bioadhesive gels and tablets) have attracted considerable attention for their opportunity to prolong the contact of drug with a mucosal surface, without inducing adverse local effects on the epithelium (Richardson et al., 1996).

As a valuable, biocompatible drug delivery system, liposomes are widely administered for topical treatments of diseases, especially in dermatology (Lasch and Bouwstra, 1995). Due to their ability to provide prolonged release of incorporated material, they also have a potential for vaginal application (Jain et al., 1996; Foldvari and Moreland, 1997; Jain et al., 1997; Pavelić et al., 1999). However, the major disadvantage in using liposomes topically and vaginally is the liquid nature of the preparation. Suitable viscosity and application properties of liposomes can be achieved by their incorporation in an appropriate vehicle. It has been confirmed that liposomes are fairly compatible with viscosity increasing agents such as methylcellulose, as well as polymers derived from acrylic acid (Carbopol resins) (Foldvari, 1996; Škalko et al., 1998). Due to the good bioadhesive properties of some Carbopols and the prolonged retention of the formulation at the site of administration (Knuth et al., 1993), these hydrogels can be good vehicles for incorporation of liposomes destined for vaginal delivery.

The purpose of the present study was to develop a liposomal carrier system, able to provide controlled and sustained release of appropriate drugs for the local treatment of gynaecological diseases. In order to optimise liposomal preparation, liposomes containing calcein as a hydrophilic model substance were prepared by several preparation methods: proliposome (Perrett et al., 1991), polyol dilution (Kikuchi et al., 1994), film, detergent dialysis and high pressure homogenisation (Gregoriadis, 1993). As lipid membrane components egg phosphatidylcholine (EPC) and egg phosphatidylglycerol-sodium (EPG-Na) in the molar ratio 9:1 were used. All liposomal preparations were characterised for particle size, trapping efficiency and the two optimal preparations tested for their in vitro stability in buffer pH 4.5 (corresponding to normal human vaginal pH (Robinson and Bologna, 1994)). As in vivo application in humans would require an appropriate vehicle, liposomes were incorporated in Carbopol gels and examined for in vitro release of entrapped calcein.

2. Materials and methods

2.1. Materials

Phospholipids (EPC and EPG-Na), as well as Carbopol 974P NF and Carbopol 980 NF were generous gifts from Lipoid GmbH (Ludwigshafen, Germany) and BFGoodrich (Brussels, Belgium), respectively. Calcein was purchased from Sigma Chemicals (Deisenhofen, Germany).

Phosphate buffer, pH 7.4 was composed of 8 g/l (137 mM) NaCl, 0.19 g/l (1.4 mM) KH_2PO_4 and 2.38 g/l (16.7 mM) Na_2HPO_4. Phosphate buffer, pH 4.5 was made of 13.61 g/l (100 mM) KH_2PO_4 in demineralised water.

All chemicals used in experiments were of analytical grade.

2.2. Preparation of liposomes

To find optimal conditions with regards to size and trapping efficiency of liposomes, different preparation methods were applied, whereas total lipid amount (12.8 mM) and calcein concentration (200 μM) were kept constant in all preparations. pH value of calcein in phosphate buffer solution was adjusted to 7.4.

The first applied method was the proliposome method of Perrett et al. (1991). Lipid components, EPC (88 mg) and EPG-Na (12 mg), were dissolved in warm ethanol (80 mg) and 200 μM calcein solution (200 mg) was added to yield an initial proliposome mixture. This mixture was stirred for a few minutes at 60°C, cooled to room temperature and then converted to a liposomal suspension by drop-wise addition of 10 ml of phosphate buffer pH 7.4. During the last stage of this procedure the suspension was stirred continuously (600 rpm) for 1 h.

The modified method of Kikuchi et al. (1994) was used as the second preparation method. Briefly, EPC and EPG-Na (as shown above) were dissolved in propylene glycol (500 mg) at 60°C and 500 mg of 200 μM calcein solution (previously warmed to the same temperature) poured to the lipid–polyol solution. The suspension was diluted by addition of buffer, pH 7.4 (10 ml), magnetically stirred (600 rpm) for 45 min at 60°C and then cooled to room temperature.

Liposomes containing calcein were also prepared by the conventional film method. First, phospholipids (100 mg) were dissolved in concentrated ethanol (3 ml) in a round bottomed flask. The solvent was then completely removed on a rotary vacuum evaporator and the lipid film deposited on the flask wall was hydrated by the addition of 10 ml of 200 μM calcein solution. Dispersion was hand-shaken for 20 min and left overnight prior to the characterisation.

Controlled detergent removal according to Milsmann et al. (1978) was applied as the fourth preparation method. Lipid components and detergent (Na-cholate; in 1:1.8 lipid to detergent molar ratio) were dispersed in a buffer solution of calcein. The solution of such preformed mixed micelles containing calcein and phospholipids was then dialysed extensively (24 h) against freshly prepared 200 μM calcein solution (400 ml), using a commercially available dialysis apparatus (Mini-Lipoprep, Dianorm, Munich, Germany) and a highly permeable dialysis membrane with a cutoff of 10 kDa (Diachema, Langnau, Switzerland). Dialysis resulted in spontaneous formation of homogeneously sized and unilamellar vesicles, essentially free of detergent.

The fifth procedure used for preparation of liposomes was high pressure homogenisation. In brief; dry powdered lipids were mixed with calcein solution and allowed to swell for 1 h under magnetic stirring. To form liposomes, the raw dispersion was fed into a APV Micron Lab 40 high-pressure homogeniser (Gaulin Homogeniser, Lübeck, Germany) and homogenised five times at pressures of 70 MPa. For details see Brandl et al. (1997) and Brandl et al. (1998).

Finally, on all pre-formed liposomes prepared by described methods a freeze-thawing procedure was applied. Preparations were rapidly frozen in liquid nitrogen then brought to room temperature to thaw completely (approximately 15 min). The procedure was performed five times (New, 1990).

Prior to their characterisation, all liposomal preparations (before and after freeze-thawing) were extruded once through 0.4 μm polycarbonate membrane filters (LiposoFast, Avestin, Ottawa, Canada).

Mean diameter (z-average) and size distribution (polydispersity) of liposomes were determined by photon correlation spectroscopy (Zetamaster, Malvern Instruments, Malvern, UK) 24 h after their preparation.

2.3. Determination of trapping efficiency

The trapping efficiencies were measured in order to compare different liposome preparation methods. To separate unentrapped calcein from liposomes containing calcein, liposomal suspensions were gel chromatographed on a Sepharose CL-4B (Pharmacia, Uppsala, Sweden) column and eluted with buffer, pH 7.4. The concentration of the model substance, both in liposomes and free, was determined in all collected fractions by measuring fluorescence intensity on Perkin-Elmer luminescence spectrometer (excitation wavelength 496 nm, emission wavelength 524 nm). Prior to the measurements, liposomal fractions were treated with detergent (10% Triton X-100 solution) to dissolve phospholipids and were then diluted with buffer pH 7.4 to achieve a final concentration of the detergent of 1%. All measurements were performed at pH 7.4 to avoid differences in the intensity of calcein due to different pH.

The influence of Triton X-100 on fluorescence intensity of calcein was avoided by performing blank tests (1% Triton X-100 buffer solution, pH 7.4).

Recovery of calcein for all preparations was between 92–97% of the amount taken into preparation.

2.4. Preparation of carbopol gels

As a vehicle for the incorporation of liposomes destined for vaginal application, two types of Carbopol resins were used, i.e. Carbopol 974P NF with a more pronounced adhesive property and Carbopol 980 NF. The gels (1% w/w) were prepared by the following procedure (Škalko et al., 1998): Carbopol resin (1 g) was dispersed in distilled water (88 g) in which glycerol (10 g) was previously added. The mixture was stirred until thickening occurred and then neutralised by dropwise addition of 50% (w/w) triethanolamine, until a transparent gel appeared. Quantity of triethanolamine was adjusted to achieve gel pH 5.5.

2.5. Incorporation of liposomes into 1% carbopol gels

Liposomes were incorporated into Carbopol gels following the procedure described by Škalko et al. (1998). Briefly, liposomes (free from unencapsulated calcein) were mixed into vehicles by an electrical mixer (25 rpm, 5 min), with the concentration of liposomes in the gel being 10% (w/w, liposomal suspension/total). Prior to in vitro studies, samples were examined microscopically by image analysis to check the morphology of incorporated vesicles (Škalko et al., 1996, 1998).

Control gels (Carbopol gels mixed with 200 μM calcein solution in buffer instead of liposomes) were prepared under the same conditions. Concentration of calcein solution in the gel was 10% (w/w).

2.6. In vitro stability studies

Two optimal liposomal preparations were examined for their in vitro stability in the pH 4.5 phosphate buffer (Pavelić et al., 1999). Liposomes (2 ml), separated from unentrapped calcein, were dispersed in pH 4.5 phosphate buffer (10 ml) and incubated at 37°C (water bath). Samples were taken at certain time intervals (1, 2, 4, 6 and 24 h), separated from released calcein as described earlier, and concentration of calcein was determined fluorimetrically.

To determine stability of liposomes incorporated in Carbopol gels under in vitro conditions (pH 4.5) the method by Peschka et al. (1998) was used. Samples of gels containing liposomes were put in glass vials and were separated from receptor solution (phosphate buffer, pH 4.5) by a second layer of 2% agarose gel. The vials were incubated at 37°C and receptor solution was completely replaced at certain time intervals (1, 2, 4, 6 and 24 h). The amount of released calcein was determined fluorimetrically, as described earlier. Control tests (Carbopol gels containing free calcein) were performed simultaneously and under exactly the same conditions.

2.7. Rheological evaluation of gels containing liposomes

Flow properties of Carbopol gels with incorporated liposomes were determined on a CS-rheometer (RheoStress RS 100 1 Ncm, Peltier TC81, Haake, Germany). Measurements were performed at 20°C by using the cone/plate C 35/1° (0.05 mm) measuring system.

Under the same conditions the flow properties of liposome-free control gels (i.e. a gel prepared in water and the other one mixed with buffer), were examined.

3. Results and discussion

3.1. Characterisation of liposomes

To deliver a sufficient amount of drug providing therapeutic effect, a high trapping efficiency of drug in liposomes is required. One of the limits connected with almost all preparation methods is the poor entrapment of water-soluble compounds into liposomes (Brandl et al., 1997). Usually, only a minor proportion of hydrophilic substances ends up inside the liposomes. On the contrary, trapping is more easily achieved with lipophilic and amphiphilic molecules, due to their tendency to be accommodated in the liposomal membrane (Gregoriadis, 1993).

We previously reported the preparation of liposomes containing more or less lipophilic antimi-

crobial drugs for the treatment of bacterial vaginosis by the polyol dilution and proliposome methods. Clotrimazole and chloramphenicol were easily incorporated in sufficient amount in liposomes prepared by both methods (Pavelić et al., 1999).

Our concept at the beginning of this project was fairly straight-forward: to find an optimal preparation method for liposomes containing hydrophilic drugs. As a majority of classical drugs are of low molecular weight, calcein was chosen as a model compound.

The different liposome preparation methods proliposome, polyol dilution, film, detergent dialysis and high-pressure homogenisation, were used to entrap calcein and the freeze-thawing procedure was applied on all pre-formed liposomes. As shown in Table 1, liposomes prepared by proliposome, polyol dilution and the conventional film method were of similar mean diameter (between 280 and 300 nm). They were bigger than those prepared by detergent removal (70 nm) and high-pressure homogenisation (155 nm). This was due to the presence of different types of liposomes; multilamellar (first three preparation methods) (Perrett et al., 1991; Gregoriadis, 1993; Kikuchi et al., 1994) and predominantly unilamellar (detergent removal and homogenisation) (Schubert et al., 1991; Gregoriadis, 1993).

Despite the evidence (New, 1990; Gregoriadis, 1993) that larger multilamellar vesicles (MLVs) often entrap a lower amount of hydrophilic substance than smaller large unilamellar vesicles (LUVs), the highest trapping efficiency (Fig. 1) was obtained for liposomes prepared by polyol dilution method (63%). Those prepared by the proliposome method could entrap up to 31.4% of the starting amount of calcein taken into preparation. High trapping efficiency for liposomes prepared by polyol dilution and proliposome method is a result of using a highly concentrated calcein solution in the first steps of the preparation procedure (for details see Section 2). As a consequence of size, trapping efficiency was quite low for liposomes prepared by detergent dialysis and homogenisation method. One of the generally applicable approaches to improve trapping efficiency is to prepare fewer but bigger liposomes instead of many small ones. This can be achieved by using dehydration-rehydration (Kirby and Gregoriadis, 1984) or freeze-thawing techniques (New, 1990; Gregoriadis, 1993). Here we applied freezing and thawing (5 cycles) on pre-formed liposomes prepared by different methods (Table 1, Fig. 1) and confirmed significantly better encapsulation of calcein in liposomes prepared by the detergent (from 1.5 to 6.4%) and homogenisation method (from 0.5 to up to 7.7%, respectively) (Fig. 1). Similar results were obtained for liposomes prepared by the film method, whereas the opposite effect was observed for liposomes prepared by the proliposome and polyol dilution methods. After freeze-thawing of the pre-formed proliposomes and polyol dilution liposomes the amount of entrapped calcein decreased. This could be expected due to the effect of the freeze-thawing procedure. Liposomal membranes were disrupted during rapid freezing in liquid nitrogen

Table 1
Mean diameters (nm) and polydispersity of liposomes prepared by the different methods

Preparation method	Before freeze-thawing		After freeze-thawing	
	Mean diameter (nm)	Polydispersity	Mean diameter (nm)	Polydispersity
Proliposome	282.8 ± 4.4	0.401 ± 0.017	215.1 ± 1.3	0.223 ± 0.030
Polyol dilution	280.6 ± 4.8	0.288 ± 0.075	232.1 ± 0.8	0.215 ± 0.031
Film	303.9 ± 8.0	0.322 ± 0.018	215.1 ± 3.6	0.140 ± 0.043
Detergent removal	70.9 ± 0.5	0.003 ± 0.002	202.5 ± 2.9	0.240 ± 0.060
High pressure homogenisation	155.0 ± 2.4	0.034 ± 0.033	220.6 ± 7.3	0.134 ± 0.062

Liposomes containing calcein were prepared by the different methods and the freeze-thawing procedure (five cycles) was applied on all pre-formed liposomes. Mean diameters and polydispersity were determined by photon correlation spectroscopy ($n = 3$).

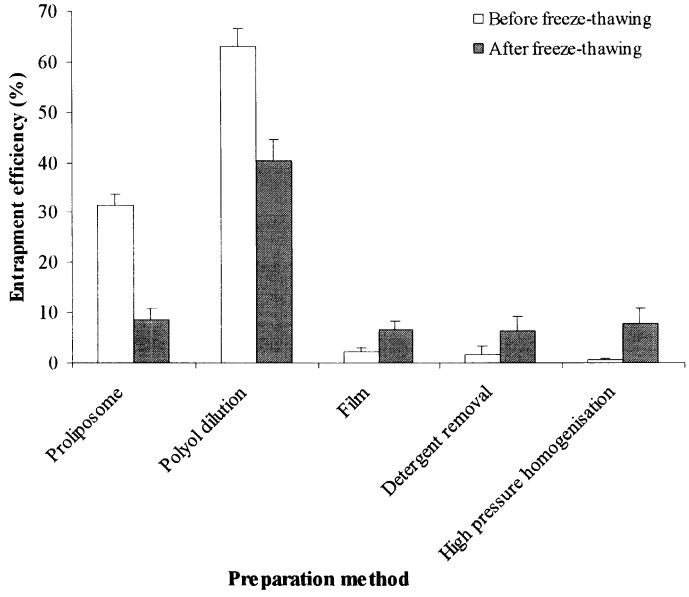

Fig. 1. Trapping efficiency of liposomes. Liposomes containing calcein were prepared by the different methods and freeze-thawing (five cycles) was applied on all pre-formed liposomes. The trapping efficiency of calcein was determined fluorimetrically after separating free and entrapped calcein by column chromatography ($n = 3$).

and originally entrapped calcein has been lost from liposomal aqueous spaces. Upon reforming (re-fusing) of liposomes during the thawing phase, in which the solute has the tendency to equilibrate between the inside and the outside (New, 1990), part of the originally encapsulated calcein was released. Therefore, the proliposome and the polyol dilution methods (without the freeze-thawing step) were used for the preparation of liposomes in further experiments.

3.2. In vitro stability studies

To develop a liposomal drug carrier system able to provide sustained and controlled vaginal delivery, we have tested the stability of liposomes in conditions that mimic the vaginal environment. In comparison with other mucousal tissues, healthy human vaginal mucous is characterised by low pH value; usually ranging between pH 4.0–5.0 (Caillouette et al., 1997). Therefore, in vitro testing was performed in the pH 4.5 phosphate buffer (Jain et al., 1997; Pavelić et al., 1998, 1999). As can be seen in Fig. 2, liposomes containing the hydrophilic model substance and prepared by polyol dilution and proliposome methods were stable in the media chosen to simulate normal vaginal pH. After 24 h of incubation at 37°C (an extreme condition considering the application in humans) more than 60% of the originally entrapped calcein was still retained in liposomes, regardless of the

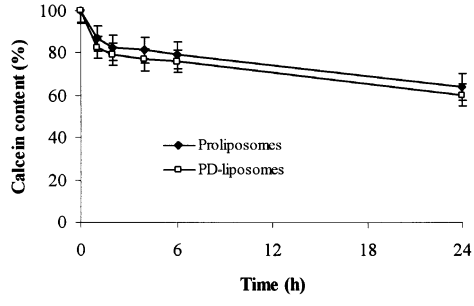

Fig. 2. In vitro stability of liposomes. Proliposomes and polyol dilution (PD) liposomes (prepared as described earlier), free from unentrapped substance, were incubated in the phosphate buffer, pH 4.5 at 37°C. Samples were taken at certain time intervals and concentration of calcein still present in liposomes was determined fluorimetrically. The values denote the average of three preparations ± S.D.

liposome preparation method. The calculated half-life values ($t_{1/2}$) for calcein release from liposomes were 121.6 h (polyol dilution) and 115.5 h (proliposome method).

In our previous experiments (Pavelić et al., 1998, 1999) we examined the stability of liposomes composed of lecithin and containing lipophilic drugs, prepared by the same methods. We observed that, regardless of the preparation procedure, liposomes containing clotrimazole and metronidazole were similarly stable. The only exception were liposomes containing chloramphenicol; those prepared by the polyol dilution method retained less of the originally incorporated drug than proliposomes (30 and 46%, respectively). Comparison of those findings with results in the present study demonstrates that liposomes containing hydrophilic drugs are more stable in conditions simulating the vaginal environment than liposomes containing lipophilic substances. Better stability could be a consequence of the hydrophilic nature of calcein at neutral pH in liposomes; therefore, there is no membrane penetration and liposomes could retain a higher amount of originally entrapped calcein.

To achieve proper viscosity of liposomal preparations for human administration and to improve their stability as well, liposomes were incorporated in a suitable vehicle. Commercially available conventional dosage forms for vaginal delivery, such as creams, suppositories and vaginal tablets have some disadvantages which restrict their effectiveness. One of the major restrictions is the short retention time of drug at the site of application. While a prolonged residence is often required for activity, recent research efforts have been made on using hydrophilic bioadhesive polymers (polyethylene oxide, polyacrylic acid) to improve drug delivery via vaginal route (Brannon-Peppas, 1993). Furthermore, it has been reported that hydrogels prepared from such polymers can alter the hydration level of the vaginal tissue and be very effective in the treatment of vaginal dryness (Robinson and Bologna, 1994). In addition, the compatibility of liposomes with acrylic acid polymers (Carbopol) has been proven (Foldvari, 1996). Therefore, it was worthy to use these gels as vehicles for incorporation of liposomes.

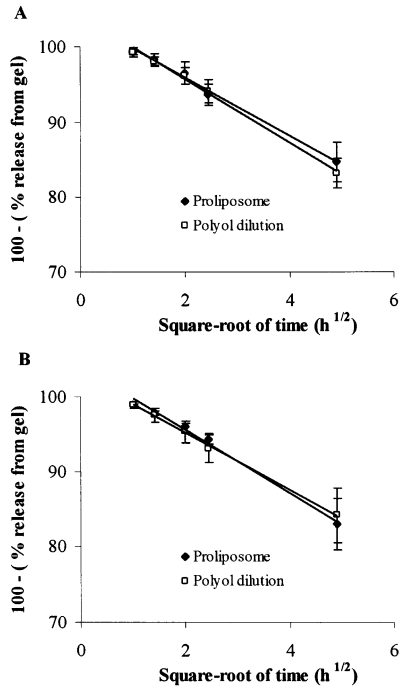

Fig. 3. Calcein release from gels fitted against square-root of time. Liposomes containing calcein and incorporated in Carbopol 974P NF (A) and Carbopol 980 NF (B) gels were tested for in vitro release (pH 4.5). The amounts of released calcein were detected in receptor media before addition of detergent. The values denote the average of four preparations ± S.D.

In the present study, we mixed liposomes into 1% Carbopol hydrogels (namely Carbopol 974P NF and Carbopol 980 NF) under conditions which preserve their original structure. In vitro release of encapsulated hydrophilic marker from liposomes incorporated in gels was tested and compared with liposomes dispersed in buffer.

An elegant and simple method for studying in vitro release kinetics of liposome encapsulated material was developed by Peschka et al. (1998). We reasoned that a similar system could be applied to follow the release of calcein from liposomes incorporated in 1% Carbopol gels. The porosity of the matrix permits intact liposomes and free (released) calcein to diffuse through the matrix in receptor solution. The amount of released encapsulated substance or intact liposomes was determined fluorimetrically before and after the addition of Triton X-100. As shown in Table 2 and Fig. 3 a

Table 2
In vitro release of calcein entrapped in liposomes incorporated in Carbopol gels

Time (h)	Carbopol 974P NF gel				Carbopol 980 NF gel			
	Free calcein (%)	Total calcein (%)	Intact liposomes (%)	Control (%)	Free calcein (%)	Total calcein (%)	Intact liposomes (%)	Control (%)
1	0.78 ± 0.19^{a}	0.80 ± 0.19^{a}	0.02 ± 0.02^{a}	2.73 ± 0.54	1.07 ± 0.18^{a}	1.21 ± 0.18^{a}	0.14 ± 0.07^{a}	2.15 ± 1.06
	0.85 ± 0.16^{b}	0.19 ± 0.15^{b}	0.05 ± 0.07^{b}		1.10 ± 0.38^{b}	1.22 ± 0.42^{b}	0.12 ± 0.08^{b}	
2	1.76 ± 0.38^{a}	1.84 ± 0.36^{a}	0.07 ± 0.02^{a}	6.52 ± 0.92	2.22 ± 0.31^{a}	2.49 ± 0.32^{a}	0.27 ± 0.12^{a}	4.63 ± 1.88
	1.95 ± 0.34^{b}	2.15 ± 0.25^{b}	0.20 ± 0.11^{b}		2.47 ± 0.88^{b}	2.77 ± 0.93^{b}	0.30 ± 0.16^{b}	
4	3.48 ± 0.27^{a}	4.05 ± 0.31^{a}	0.21 ± 0.08^{a}	12.75 ± 1.75	4.04 ± 0.43^{a}	4.48 ± 0.53^{a}	0.44 ± 0.23^{a}	8.48 ± 3.16
	3.84 ± 0.71^{b}	4.14 ± 0.60^{b}	0.30 ± 0.20^{b}		4.68 ± 1.46^{b}	5.13 ± 1.44^{b}	0.45 ± 0.18^{b}	
6	6.31 ± 0.60^{a}	6.64 ± 0.67^{a}	0.33 ± 0.12^{a}	18.32 ± 1.92	5.72 ± 0.51^{a}	6.35 ± 0.58^{a}	0.63 ± 0.24^{a}	13.34 ± 3.17
	5.87 ± 1.16^{b}	6.35 ± 1.08^{b}	0.47 ± 0.31^{b}		6.91 ± 2.08^{b}	7.58 ± 1.95^{b}	0.67 ± 0.24^{b}	
24	15.31 ± 1.03^{a}	16.41 ± 1.26^{a}	1.10 ± 0.34^{a}	41.97 ± 2.60	17.00 ± 3.81^{a}	19.48 ± 3.45^{a}	2.47 ± 0.89^{a}	36.32 ± 5.4
	16.78 ± 2.07^{b}	17.99 ± 2.08^{b}	1.21 ± 0.31^{b}		15.80 ± 4.02^{b}	16.88 ± 3.64^{b}	1.08 ± 0.39^{b}	

Samples of gel containing proliposomes[a] and polyol dilution[b] liposomes were incubated in phosphate buffer pH 4.5 at 37°C (for details see Materials and Methods section). The amount of calcein was determined in release media before and after disruption of liposomes by the addition of detergent. Control experiments (gel containing free calcein, without liposomes) were performed simultaneously. The values denote the average of four preparations ± S.D.

slower release of calcein from liposomes, as seen by their incorporation in gel-vehicles, was confirmed. In vitro release data for liposomes incorporated in Carbopol 974P NF gel show that, regardless of the preparation method used, liposomes retained a similar amount of the originally encapsulated calcein. Even after 24 h of incubation at 37°C we detected only about 16% of released calcein (in the absence of detergent), for both preparation methods (Table 2). Upon the addition of detergent fluorescence intensity increased, thus indicating that intact liposomes are diffusing through the agarose matrix and appearing in the receptor phase (pH 4.5). Similar values were obtained for liposomes incorporated in Carbopol 980 NF gel. Even after 24 h, which would be an extreme condition considering the application in humans, more than 80% of the originally encapsulated calcein was retained in liposomes embedded in gel (both preparation methods), in comparison to the control (60%, respectively). In vitro stability of liposomes dispersed in buffer (Fig. 2), show that about 60% of originally entrapped calcein was retained in liposomes after 24 h incubation. These results prove the protective effect of hydrogel matrix on liposomes. Therefore, with regards to stability and vaginal application of liposomal preparations in humans, Carbopol gels would be chosen as suitable vehicles.

3.3. Characterisation of carbopol gels

A vehicle for incorporation of liposomes should provide adequate pH value, stability and rheological characteristics (Škalko et al., 1998). Due to good physical, chemical and biological properties of hydrophilic polymer gels (Knuth et al., 1993), we have chosen polymers of acrylic acid (Carbopol resins) to prepare hydrogels as an appropriate vehicle for incorporation of liposomes destined for vaginal delivery. Moreover, some Carbopol gels have been shown to be bioadhesive and remain on vaginal tissue for 3-4 days (Robinson and Bologna, 1994). Therefore, we prepared hydrogels from Carbopol 974P NF, a polymer known for its bioadhesivity and Carbopol 980 NF, commonly used in topical preparations. Empty Carbopol hydrogels (blank) and those containing liposomes were tested for basic rheological properties (Fig. 4). Both empty Carbopol gels showed a similar behaviour. The yield point was detected at a shear stress of approximately 40 Pa. Interestingly, up to approximately 130 Pa the 980 NF type showed a constant slope (i.e. constant viscosity) of shear stress vs. shear rate, representing a Bingham-type fluid. The Carbopol 974P NF gel was found to be more of a Casson-type fluid with continuously decreasing viscosity, thus indicating successive loss of polymer entanglement upon increasing shear stress. However, both Carbopol types show a pronounced loss of viscosity for samples with incorporated liposomes and for samples containing buffer instead of liposomes. The changed viscosity is probably due to

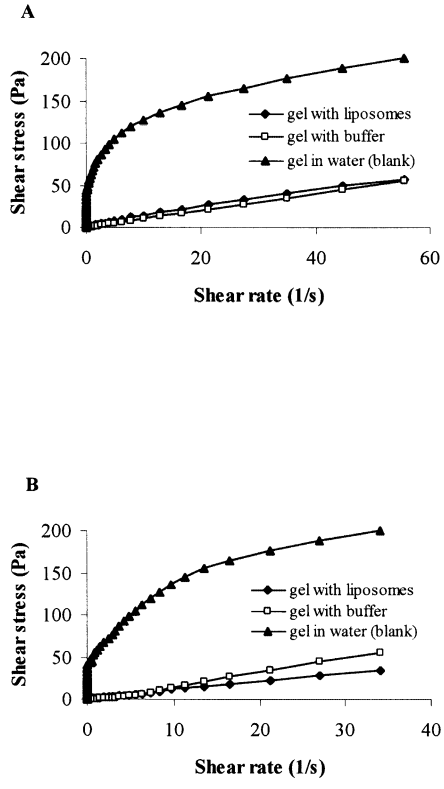

Fig. 4. Flow behaviour of liposomes incorporated in Carbopol gels. Liposomes prepared by the polyol dilution method were incorporated in 1% Carbopol 974P NF gel (A) and 1% Carbopol 980 NF gel (B), as described earlier and tested for flow behaviour on CS-rheometer at 20°C (cone/plate measuring system). Control and blank gels were examined under exactly the same conditions.

the presence of cationic ingredients (sodium ions) in the buffer which are not compatible with the anionic Carbopol resins (Dittgen et al., 1997). Therefore, we decided to prepare 1% gels, instead of the commonly used 0.5% (Knuth et al., 1993; Dittgen et al., 1997), whereupon after addition of 10% liposomes an appropriate viscosity for application was obtained.

The rheological measurements performed in this study were preliminary and for orientation purposes, however, further rheometrical testing would be worthwhile. The knowledge of the rheological properties of such pharmaceutical dosage forms is valuable for their characterisation, especially if one is considering a possible scale-up.

Experiments performed in this study confirm the ability of liposomes as a novel vaginal delivery system for controlled and sustained release of encapsulated drug. In a previously reported study (Pavelić et al., 1999), polyol dilution and proliposome methods have been proven as simple, reproducible and appropriate for incorporation of lipophilic drugs. Continuing those investigations, the same methods were tested for possible encapsulation of hydrophilic drugs and resulted with high trapping efficiency for model substance (calcein). To be closer to in vivo application in humans, we followed stability of liposomes in media chosen to simulate vaginal pH. The retention of calcein in liposomes was further prolonged by their incorporation in hydrogels, which have been suggested to be a suitable vehicle for vaginal delivery. Despite the evidence that both examined gels have yielded similar in vitro stability for incorporated liposomes, Carbopol 974P NF would be taken as the resin of choice for preparing gels for vaginal application because of its good bioadhesive properties. Currently, we are continuing our investigation in the field of vaginal delivery by preparing liposomes containing antiviral drugs for the local treatment of vaginal infections (genital *Herpes*).

4. Conclusion

Proliposome and polyol dilution methods would be the right choice of preparation methods for preparing liposomes due to their high trapping efficiency of model substance. Both methods are simple, reproducible and suitable for mass production of liposomes, stable in conditions chosen to mimic the human vaginal environment (buffer pH 4.5). Incorporation of those liposomes in Carbopol gels further improved their stability and confirmed the applicability of liposome gels as a novel vaginal delivery system, able to provide controlled and sustained release of entrapped drug.

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