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Deoxycholate-hydrogels: novel drug carrier systems for topical use

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Abstract

Na-deoxycholate (Na-DOC) forms a viscous thixotropic gel when in contact with excess buffer systems. The resulting gels have been tested as novel drug carrier systems for topical use. The influence of differing amounts of mannitol, glycerol and xylitol on the viscous modulus (G''/Pa) was evaluated by oscillatory measurements. Na-DOC (0.5%) in phosphate buffered saline (PBS) with 5% mannitol was chosen as an optimised formulation, taking into account viscosity, distribution and appearance. The release rate of the model drug rutin through an artificial membrane was higher than those from hydroxyethylcellulose- (HEC) and sodium polyacrylate (NaC934)-gels; permeation through excised rat skin was also highest for the Na-DOC systems. The results indicate that Na-DOC significantly increases the membrane permeability. The microbial stability was in the same range as HEC- and NaC934-gels, making a preservation necessary. Na-DOC-gels are novel low molecular weight, multifunctional drug carriers, which also act as penetration enhancers. Their thixotropy is an additional advantage for better application to large skin areas, nasal, vaginal and buccal membranes. Therefore, Na-DOC-gels can be considered promising, alternative drug carrier systems for topical pharmaceutical as well as cosmetic use.

Keywords: Sodium-deoxycholate aqueous gels; Rutin; Skin permeation; Excised rat skin; Microbial stability

1. Introduction

Low molecular weight substances have generated considerable interest as alternatives to polymeric matrices for developing drug carrier systems (Chang and Bodmeier, 1997a,b; Geraghty et al., 1996). The major advantages of low molecular weight components, when compared to polymers, include their low melt viscosity, potential biocompatibility and biodegradability and the absence of toxic impurities such as synthesis-residues like organic solvents, catalysts and initiators. Sodium-deoxycholate (Na-DOC), a naturally occurring bile salt is a low molecular weight substance (mol. wt.: 414.5) which is able to form gels. In the late 1950s the formation of the helical complex of macromolecular dimensions from the bile acid steroid Na-DOC was described as a substance

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which is able to form gels in aqueous solution. If they contain glycylglycine there is a large viscosity increase within a few minutes. The resulting clear, mucous-like gel was caused mainly by hydrogen bonding (Rich and Blow, 1958). In addition a detailed pattern of association of the trihydroxy bile salts in aqueous electrolyte solutions was investigated utilising the light scattering technique (Chang and Cardinal, 1978a,b). Comparison of the light scattering data with a monomer micellar model showed that qualitative agreement was obtained. Further examination showed that the results were in good agreement with a model that includes dimers, trimers and a higher aggregate containing approximately eight monomeric units. Early viscosimetry studies on an Ostwald viscometer showed that the complex had thixotropic behaviour. The results suggested the formation of a highly specific complex, since closely related compounds did not show this effect (Blow and Rich, 1960).

To date, however Na-DOC-gels have not been evaluated with regard to their pharmaceutical relevance. Since the importance of Na-DOC to the physicochemical properties of biological membranes is well known, it is worth investigating to see if Na-DOC-gels can be used as drug carrier systems for dermal delivery. This study was set up to evaluate the effects of various excipients on the viscosity of Na-DOC-gel systems and to compare the release and permeation profiles and microbial stability of Na-DOC-gels and established polymeric-based hydrogels.

2. Materials and methods

2.1. Materials

Na-DOC, sorbitol, xylitol, glycerol and rutin were purchased from Sigma (St. Louis, MO), whereas mannitol was from Merck (Darmstadt; Germany). Hydroxyethylcellulose (HEC) corresponded to Natrosol 250 MR (Aqualon, NL). Carbopol 934 (Goodrich; UK) was neutralised with NaOH as previously reported (Valenta et al., 1998a) and designated NaC934.

2.2. Screening of flow properties of Na-DOC preparations based on various compositions

Na-DOC (50 mg) and 200 mg of auxiliary agent, as shown in Table 1, were dissolved in 10 ml of the designated buffer. This mixture (5.0 ml) was transferred into a stoppered, plastic bottle, which was then shaken. After 12 h incubation at room temperature (RT) the consistency of all preparations was tested.

2.3. Preparations for rheological experiments

Na-DOC (25 mg) was dissolved in 5.0 ml phosphate buffered saline (PBS). PBS consisted of 0.1 M phosphate buffer (pH 7.2) and 0.9% NaCl. Various amounts of mannitol, xylitol and glycerol were added in final concentrations of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10% (w/w). All preparations were stored at RT.

2.4. Rheological experiments

Studies of rheological properties of these thixotropic preparations, were carried out using a Haake rheometer Rotovisco RT20 (Haake, Karlsruhe, Germany, thermocontroller Haake F6/8). The rheometer was based on a thermostatically-controlled cone/plate with 60 mm diameter and 1° angle. Throughout the experimental period, the cone/plate temperature was maintained at 4°C. Twelve hours after application, the rheological measurements were performed on the samples at 4 ± 1.5°C. The sample volume was approximately 1.5 ml. In oscillation experiments the sinusoidally-varying strain is monitored as a function of time. Shear strain, the stress and the phase angle were determined: the parameters obtained are the complex modulus G^*, and the phase angle δ. The elastic modulus G', the viscous modulus G'' and the dynamic viscosity η' are calculated by:

$$G' = G^* \cos(\delta)$$

$$G'' = G^* \sin(\delta)$$

$$\eta' = G''/\omega$$

where ω is the angular frequency, which was varied from 10 to 1 Hz and the share rate was 10 s^{-1} (± 0.2500) in all experiments.

Table 1
Screening of buffer systems, electrolytes, sugar alcohols and carbamide related to optical and flow properties of Na-DOC-gels[a]

Buffer systems, containing 0.5% (w/v) Na-DOC						
Auxiliary agent 2% (w/v)	0.5 M phosphate (pH 7.2)	0.1 M phosphate (pH 7.2)	0.5 M citrate–phosphate (pH 6.8)	0.5 M Tris–HCl (pH 6.8)	0.1 M Tris–HCl (pH 6.8)	0.5 M NH_3–NH_4Cl (pH 9.0)
NaCl	++	++	+	P	+	+
KCl	++	++	+	P	P	−
$(NH_4)_2SO_4$	++	++	+	+	P	+
Carbamide	++	−	+	−	−	−
Sorbitol	++	+	+	+	P	−
Mannitol	++	+	+	P	P	−
Glycerol	+	+	−	−	P	−
Xylitol	+	+	+	+	P	−

[a] −, Unaffected; ++, high thickness; +, medium thickness; P, precipitation.

2.5. Preparations for diffusion studies

First, 25 mg of Na-DOC and 250 mg of mannitol were dissolved in 5.0 ml 0.1 M PBS. HEC-hydrogels (3% (w/v)) and 1.0% (w/v) NaC934-gels were prepared using demineralised water. In each hydrogel 0.2% (w/v) rutin was incorporated as a model drug.

2.6. Diffusion studies

The diffusion of the model drug rutin across artificial membranes and excised rat skin were investigated.

Dialysis tubing (Sigma, St. Louis, MO), which was heated for 30 min at 100°C before fixing it onto the cell, was used as an artificial membrane. The diffusion cell was made of glass with donor and receptor compartments. The effective area available for release was 5.72 cm^2. The receptor compartment was filled with 15.0 ml demineralised water which was stirred by a magnetic bar at RT. Each formulation (5.0 g) was loaded onto the membrane. Aliqot volumes of 200 μl were withdrawn every 15 min from the receptor compartment and replaced with fresh medium. Prior to spectrophotometric (Perkin-Elmer, Lamda 16) analysis at 405 nm, 50 μl 5 N NaOH were added to each sample, which was then analysed for rutin release. NaOH was necessary in order to intensify the colour caused by a bathocromic shift. Analysis was based on a calibration curve with known amounts of rutin. Linear regression analysis gave a correlation of 0.9999. All experiments were done at least three times.

Rat skin permeation profiles were determined using Franz-type (LG -1083-PC; ERWEKA) diffusion cells. The effective area available for permeation was 0.785 cm^2. The membranes were prepared from abdominal rat skin using a previously reported method (Valenta and Wedenig, 1997). The excised skin was set in place with the stratum corneum uppermost, and the dermal side facing the receptor compartment which contained demineralised water. The Na-DOC formulations (1 g) were placed on the skin surface in the donor compartment which was occluded with plastic film (parafilm). The geometry of the cell required the application of a greater amount of formulation than usually seen in vivo. Each experiment was carried out for 6 h and repeated twice.

Analysis of rutin was done by HPLC. The analytical system consisted of a pump (series 10 LC, Perkin-Elmer) a UV-detector (LC 235 C diode array, Perkin-Elmer) set at 350 nm and an autosampler (ISS-100; Perkin-Elmer). The column (250 × 4 mm. i.d.) packed with Nucleosil 100-5C-18 (5 μm particle size) was eluted at a temperature of 40°C. Gradient elution was performed as follows: flow rate 1.0 ml min^{-1}; a 0–10.0 min linear gradient from 82% A/18% B to 55% A/45% B (eluent A: 0.1% trifluoroacetic acid in water; eluent B: acetonitrile). Rutin concentration was established by comparing the peak area of the unknown with a standard calibration curve. Standard solutions contained between 1.5 and 154 μg ml^{-1} rutin. Linear regression analysis of the peak areas gave a correlation coefficient of 0.9999. Rutin concentration was calculated automatically using Turbochrom 4.0 software (Perkin-Elmer) via an interface (ISS 900) connected to a workstation. The samples (20 μl) withdrawn from the receptor chamber were directly injected by the autosampler.

2.7. Microbial stability

The inoculum contained the complete microbial spectrum occurring in demineralised water, which was determined in advance with 1.5×10^4 colony forming units (cfu) per ml according the method of Porter and Feig (1980). For Na-DOC formulations 0.1 M PBS (pH 7.2) was prepared with this demineralised water. Na-DOC hydrogels (0.5% (w/v)) containing 5% mannitol in 0.1 M PBS, 0.5% (w/v) HEC-hydrogels and 0.17% (w/v) NaC934-hydrogels prepared with demineralised water were stored at RT in glass cups during the test period of 28 days.

The cfu count of each sample was obtained immediately and 1, 7, 14, 21 and 28 days after preparation. Each gel (1 g) was mixed vigorously with 9.0 ml sterile NaCl–peptone buffer (see the Pharmacopoeia Europaea, 1991). The resulting solution (1 ml) was plated onto a petri dish containing bacterial medium B and the cfus were

counted after incubation at 37°C. Where necessary, the samples were diluted until a quantifiable number of cfus were obtained. Five independent replicates were counted out from each sample.

2.8. Statistical data analysis

The results are expressed as the means of at least three experiments ± S.D. Statistical data analysis was performed using the *t*-test with $P < 0.1$ as a minimal level of significance.

3. Results

3.1. Physical stability

The properties investigated were the compatibility with different buffer systems of various pH and ionic strength. Visual observation and rheological testing were also performed. The influence on the flow properties were characterised by indications of no effect (−), medium thickness (+), high thickness (++) and precipitation (P), as listed in Table 1. Whether or not a thick gel can be formed, depends on the type of buffer in which the Na-DOC is dissolved and the type of auxiliary agent (Table 1). All tested auxiliary agents caused a viscosity increase in 0.5 M phosphate buffer whereas in citrate–phosphate buffer the gels formed were less viscous. The Tris–HCl buffer was not suitable because it caused precipitation. Addition of sodium chloride, potassium chloride, ammonium sulfate and carbamide to the Na-DOC systems containing 0.5% Na-DOC led to suitable hydrogels. Addition of either mannitol, xylitol, sorbitol and glycerol also had the same effect to the viscosity and the final products were also clear aggregates. These preliminary studies identified the best formulation with a transparent appearance and good viscosity as 0.5% Na-DOC in 0.1 M PBS pH 7.2. Sorbitol, mannitol, glycerol and xylitol were the most interesting compounds with regard to possible topical use. In order to determine their influence on the viscosity of Na-DOC-gels, precise viscosity measurements were performed. No viscosity differences could be detected between Na-DOC–sorbitol and Na-DOC–mannitol mixtures, which may be due to the structural similarity of mannitol and sorbitol. Therefore in further investigations only mannitol, xylitol and glycerol were used.

Na-DOC-gels display thixotropic behaviour. Common rheological studies assess viscosity at a defined shear rate, which destroys the structure of such gels. Oscillatory measurements with a low oscillating angle can slow down immediate gel destruction. The disadvantage of using this low phase angle is the large variation seen in G'' (Calfors et al., 1998). After a storage time of 12 h at 4°C, the gel structure has built up again. To enable a comparison of gel-viscosities using different concentrations of mannitol, xylitol or glycerol the applied gels had to be mounted on the measuring plate of the viscometer at 4°C 12 h prior to measurement.

In Fig. 1 the dependence of the viscous modulus (G''/Pa) on the concentration of mannitol, xylitol and glycerol is compared at 9 Hz oscillation frequency. The biggest difference of G'' among the additives is in the concentration range between 4 and 5% (w/v), whereas glycerol and xylitol have similar high values. The G'' of gels with 5% mannitol is 1.6–2.1-fold higher when compared to that of glycerol and xylitol. Na-DOC

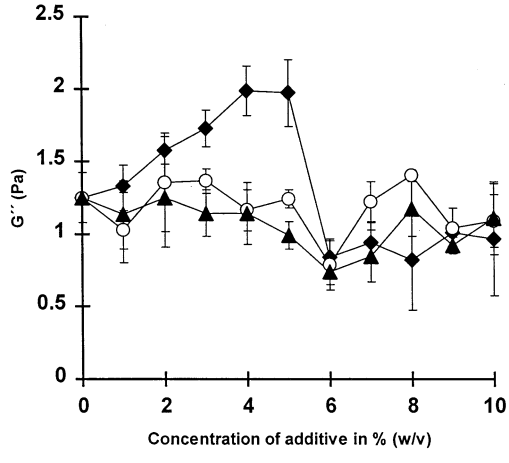

Fig. 1. Comparison of the viscous modulus G''/Pa of Na-DOC-gels containing different concentrations (w/v) of: ◆, mannitol; ○, xylitol; and ▲, glycerol. The viscous modulus is plotted vs. concentration of additive at the oscillating frequency of 9 Hz (means ± S.D.; $n = 3$).

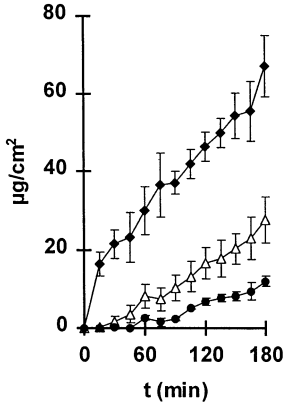

Fig. 2. Comparison of the release profiles of rutin from: ◆, Na-DOC-gels; △, HEC-gels; and ●, NaC934-gels (means ± S.D.; $n = 3$).

(0.5%) in 0.1 M PBS (pH 7.2) with 5% mannitol displayed the highest consistency and was therefore used for all further investigations.

3.2. Diffusion studies

Diffusion experiments using an artificial membrane were run for 3 h. In Fig. 2 rutin-release from Na-DOC-, HEC- and NaC934-gels is compared. The cumulative amount of drug released through the artificial membrane, Q/t, at any time, was determined from the following formula: $Q = (C \times V)/A$ where C is the rutin concentration in the receiver compartment in µg ml^{-1} for the corresponding sample time t. V is the volume of fluid in the receptor phase and A is the diffusional area of the cell. The slope of the best fit line gives the steady state flux J (µg cm^{-2} h^{-1}). The highest flux of rutin within 3 h was measured from the Na-DOC preparation (0.381 µg cm^{-2} h^{-1}). It was 7.1 times higher than from NaC934-gels (0.053 µg cm^{-2} h^{-1}) and 2.8 times higher than from HEC-gels (0.134 µg cm^{-2} h^{-1}).

Permeation studies with excised rat skin were performed to investigate whether these results can be confirmed using a biological membrane. The cumulative amounts diffused across rat skin for each formulation are shown in Fig. 3. As with the dialysis membrane, there was an increase in the total amount of drug diffused from Na-DOC-gels.

Using a non-linear curve fitting method as described by Pellett et al. (1997) an estimate of the flux values can be determined by application of the following equation:

$$u(t) = KhC_v\left[D\frac{t}{h^2} - \frac{1}{6} - \frac{2}{\pi}\sum_{n=1}^{\infty}\frac{(-1)^n}{n^2}\exp\left(\frac{-Dn^2\pi^2t}{h^2}\right)\right] \quad (1)$$

Where $u(t)$ is the amount permeated over time (t), D is the diffusion coefficient, K is the partition coefficient, h is the diffusional pathlength and C_v is the donor phase concentration.

As K, D, and h are all unknown, the products Kh and D/h^2 were replaced in Eq. (1) by Q and R to give Eq. (2):

$$u(t) = QC_v\left[Rt - \frac{1}{6} - \frac{2}{\pi}\sum_{n=1}^{\infty}\frac{(-1)^n}{n^2}\exp(-Rn^2\pi^2t)\right] \quad (2)$$

Using a computer fitting program (EasyPlot 4.01, Cherwell Scientific Software) for Eq. (2), which is derived according to Fick's second law of diffusion, values for Q and R were estimated. QR is equal to the permeability coefficient and was used to estimate steady state fluxes (Table 2).

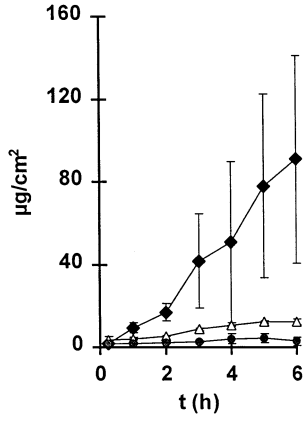

Fig. 3. Permeation of rutin from: ◆, Na-DOC-gels; △, HEC-gels; and ●, NaC934-gels, through excised rat skin (means ± S.D.; $n = 3$).

Table 2
Calculated fluxes (J) for each of the preparations of rutin[a]

System	J (μg cm^{-2} h^{-1}) (mean ± S.D.)
Na-DOC (0.5%)	17.8 ± 9.8
HEC (3%)	2.37 ± 0.1
NaC934 (1%)	1.17 ± 0.23
Control (demineralised water)	8.66 ± 0.9

[a] Concentration of rutin in each preparation: 0.2% (w/v).

The flux of rutin from HEC-gels and from NaC934-gels, respectively, was about 7.5 and 15.2 times lower than that from Na-DOC-gels. The lowest flux was measured from NaC934-gels. Because of the different viscosity values between the compared gel-systems, an aqueous rutin solution was used as reference. Although the viscosity of Na-DOC-gels was comparatively higher than that of the aqueous solution, the cumulative amount of rutin was still two times higher than from the aqueous reference solution. As shown in Fig. 2 and Table 2, the S.D. of Na-DOC are the highest compared to the other systems. The results indicate that Na-DOC significantly increases membrane permeability.

3.3. Microbial stability

The growth kinetics of the complete bacteria spectrum occurring in demineralised water were compared between Na-DOC-, HEC- and NaC934-gels. Immediately after preparation the cfu g^{-1} were counted and indicated as the starting level. The S.D.s were less than 10% in each group. The bacterial counts were in the same range, for all three preparations during the 28 days test period (Fig. 4) and remained constantly high. Because of the high bacterial growth additional preservation is required.

4. Discussion

The formation of a helical complex of macromolecular dimensions from the bile acid steroid Na-DOC has been described earlier (Rich and Blow, 1958). It has been shown that the complex can be formed simply by adding acid to an aqueous solution of Na-DOC. X-ray diffraction studies of fibres showed that the molecules had assumed an elongated helical configuration of 36 Å in diameter. It has been suggested, that the formation of the complex is highly specific since closely related compounds do not exhibit the same effect. It has also been observed that the viscosity increased when the pH was below 6.8 (Blow and Rich, 1960). Our experiments could not confirm an increased viscosity below pH 6.8. Screening tests identified phosphate and citrate buffer systems with an ionic strength between 0.1 and 0.5 M and a pH between 6.8 and 7.2 as most suitable for forming gels (Table 1). Tris–HCl buffer, although with the same ionic strength and pH, caused precipitation. The pH, temperature and ionic strength play a major role in both forming the gels and in their degree of thixotropic behaviour. When in the form of the viscous complex, Na-DOC behaves like a polymer of high molecular weight. This suggests that the presence of special additives through hydrogen bond formation affects the complexity of these structures, which will grow in all directions forming a matted matrix of high viscosity. The influence of different additives on the gel structure have been tested before. Three factors were found to influence the gel-stability: The type of additive, hydration and

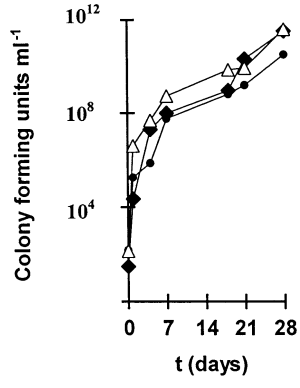

Fig. 4. Viable bacterial counts of the complete spectrum occurring in demineralised water in: ◆, Na-DOC-gels; △, HEC-gels; and ●, NaC934-gels. (means ± S.D.; $n = 5$)

hydrogen bonding (Botré et al., 1967). In agreement with this insight we tested the influence of mannitol, glycerol and xylitol on the gel properties.

Addition of 5% mannitol to an Na-DOC-buffer gel increases its viscous modulus more than glycerol and xylitol. A reason for this observation could be seen in the special formation of the Na-DOC molecules which might be stabilised by a certain number of OH-groups building up intermolecular hydrogen bonds. Mannitol and sorbitol have been used in dermal and cosmetic formulations as conventional humectants (Middleton, 1974). Therefore, preparations with mannitol will have a positive effect on dry skin. The thixotropy of the transparent gels could be an advantage for topical application. The preparations are easy to apply to the skin, eye, nose and vagina. As a surfactant Na-DOC is able to solubilise several drugs by forming mixed micelles (Dangi et al., 1998). Na-DOC enhances the penetration of insulin and acyclovir in nasal preparations (Shao and Mitra, 1992; Wolniak et al., 1993; Mayor and Illum, 1997). It has been reported that the vaginal absorption of calcitonin in rats was affected by Na-DOC (Nakada et al., 1993) as well as the transbuccal permeation of morphine (Senel et al., 1998) and the absorption of progesterone and prednisolone through hairless mouse skin (Carelli et al., 1993). In the last case it was observed that Na-DOC produced structural changes in the stratum corneum of the mouse skin resulting in increased permeability of both steroids, which can be seen as the reason for the high rutin permeation. The release rates of rutin were highest from Na-DOC-gels on artificial membranes as on rat skin. The latter indicates an interaction with parts of the skin.

To investigate the growth of the complete bacterial spectrum occurring in demineralised water in the different systems the number of cfu g^{-1} were counted. The microbial stability of the Na-DOC-gels is the same as that of HEC- and NaC934-gels. The growth kinetics are in the same range, which confirms previous studies (Valenta et al., 1998a). However, additional preservation is required. The problem of preservation of polymer systems is the possible adsorption of preservatives onto the polymers (Valenta et al., 1998b) which might be excluded for low molecular weight compounds such as Na-DOC.

In conclusion, Na-DOC-gels are novel low molecular weight drug carriers, multifunctional systems, in which the drug carrier acts as a penetration enhancer as well. Therefore, high permeation rates for incorporated drugs can be predicted. Their thixotropy and no detectable residue on the application area are additional advantages for better application on large skin areas, the nasal membrane, and the vaginal and buccal membrane. Therefore Na-DOC-gels might be promising alternative drug carrier systems for topical pharmaceutical as well as cosmetic preparations.

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