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Research Paper

Novel gel formulations with catanionic aggregates enable prolonged drug release and reduced skin permeation

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Abstract

Objectives The aim of this study was to investigate skin permeation rates of a drug substance when applied in novel gel formulations with catanionic aggregates.

Methods Reference gel without catanionic aggregates was compared with formulations with catanionic aggregates composed of tetracaine and either sodium dodecyl sulphate (SDS) or capric acid. Carbomer and SoftCAT were used to compare the effect of different gel types to elucidate if physically cross-linked, 'self-destructing' systems had benefits compared with classical, covalently cross-linked, gels.

Key findings The rheological investigation showed that the interactions between the SoftCAT polymer and tetracaine/SDS aggregates were stronger than when the tetracaine/capric acid aggregates were used. The skin permeation was measured *ex vivo* in horizontal Ussing chambers and the permeation of tetracaine was significantly lower when formulations with tetracaine/SDS aggregates were applied ($P < 0.001$), but not statistically different from the reference when capric acid was used.

Conclusions No morphological differences could be distinguished between the skin samples exposed to the different formulations or the reference. Skin permeation was compared with silicone sheet permeation and the results indicated that silicone sheets could be used as a model of skin when using these formulations.

Keywords catanionic; gel; pig ear skin; silicone membrane; vesicle

Introduction

Gels have a number of pharmaceutical uses, and because of their rheological and bioadhesive properties administration to topical sites are eligible.[1,2] Drug molecules in gels normally diffuse at rates comparable with those in pure water so to benefit from the extended contact time a prolonged drug release is desired. Recently, catanionic aggregates, formed from a drug substance and an oppositely charged surfactant, have been used to prolong the drug release from gels.[3–8] Catanionic aggregates are formed spontaneously upon mixing solutions of a variety of oppositely charged surfactants.[9–11] Polymers and catanionic vesicles may interact in several ways and these interactions may lead to cross-links that result in a gel formation in the system.[12–16] Systems where mixtures of drug containing catanionic vesicles mixed with certain polymers resulted in gel formation have been characterized previously.[7] It was shown that the rheological properties of the systems changed from those typical of an entangled polymer solution to those characteristic of a gel network and the presence of catanionic vesicles was confirmed using cryogenic-temperature transmission electron microscopy (cryo-TEM).

Drug release has been studied *in vitro* from both conventional, preformed, covalently cross-linked gels such as Carbomers (Carbopols) and gels formed through polymer-vesicle interactions, and the apparent diffusion coefficients of drugs can be reduced 10–100 times.[3–8] Gel formulations containing classical liposomes were first explored for cutaneous applications in the early 1980s and they have evolved since then.[17] Vesicular gel formulations have been shown to affect percutaneous penetration for a number of substances, e.g. lipophilic substances, alkaloids and local anaesthetics.[18–20]

The skin is an effective barrier against xenobiotics and the stratum corneum is often described with the brick-and-mortar model.[21] It was suggested that the lipids of traditional vesicles may penetrate the skin's intracellular lipid layer and modify these lamellae.[22] The potential use of vesicles as drug carriers through skin was suggested in the early 1980s by Mezei and Gulasekharamin[23] and since then the research in this area has expanded

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extensively, e.g. see the reviews of Cevc[24] and Maghraby *et al*.[25] It has also been shown that catanionic aggregates affect skin penetration.[26] To study drug penetration through biological or synthetic membranes horizontal Ussing chambers have been used.[27] Pig ear skin is a good model of human skin and silicone membranes have been used successfully as a model of human skin, also when using permeation enhancers.[28–31]

Vlachy *et al*.[32] examined the cytotoxicity of catanionic aggregates as well as the separate surfactant components. The vesicles applied in this study were composed of either the anionic surfactants sodium dodecyl sulphate (SDS) or capric acid and the cationic drug substance tetracaine. SDS is a classical, frequently used synthetic surfactant and known skin irritant; capric acid is a surfactant of natural origin and has been classified as a corrosive on skin, but has also been shown to be less irritating than SDS.[33–36] Both SDS and capric acid have been shown to increase skin penetration of model substances.[37–41] Tetracaine is used in commercially available products for local anaesthesia and was used as a model substance in this study. However, the aim to localize the drug substance to the skin rather than to transfer as much as possible to the systemic circulation was of inferior importance in this study. Numerous studies of tetracaine skin penetration have been carried out both *in vivo* and *in vitro* also using silicone membranes.[29,42–45]

The primary objective of this study was to investigate if the prolonged diffusion rate of drug substance from novel gel formulations, due to formation of catanionic aggregates, would result in a decreased absorption of drug substance through skin. To achieve a more biocompatible formulation, the potential use of natural, possibly less toxic surfactants such as capric acid, has been investigated. In addition, the use of covalently and physically cross-linked gels has been evaluated with respect to skin penetration and morphology. Silicone membranes have been compared with pig skin to investigate if they could be used as a skin model in the Ussing chamber set-up when using these formulations.

Materials and Methods

Materials

Sodium chloride, SDS, tetracaine hydrochloride, capric acid sodium salt, sodium hydroxide, borax, toluidine blue, hydrochloric acid and Trizma buffer were purchased from Sigma Aldrich Chemical Company (St Louis, MO, USA). Bouin's fixative solution was made from saturated picric acid and 37% formaldehyde (Histolab, Göteborg, Sweden) and glacial acetic acid (Merck, Darmstadt, Germany) (in a 15 : 5 : 1 ratio). Acetonitrile for chromatography was purchased from VWR International (West Chester, PA, USA). Ethanol was purchased from Solveco AB (Rosersberg, Sweden). All substances were of analytical grade or ultra quality. The SoftCAT SK-MH polymer was a kind gift of The Dow Chemical Company and Carbomer 940 was a kind gift of Noveon Inc (Breeksville, OH, USA). The polymers were used as received, without further purification. Millipore water (Millipore, France) was used in all experiments. The Technovit 7100 skin embedding system was purchased from Heraeus Kulzer GmbH (Werheim, Germany). Silatos silicone sheeting (0.13 mm thickness) was purchased from Atos Medical AB (Hörby, Sweden).

Sample preparation

Catanionic aggregate preparation

Catanionic aggregate mixtures were prepared by mixing solutions of tetracaine and either SDS or capric acid. Stock solutions of tetracaine and the respective oppositely charged surfactants with the total concentration of 80 mM were prepared in 150 mM sodium chloride solution. Thereafter, different volumes of the stock solutions were used to achieve the different ratios of tetracaine and oppositely charged surfactants desired to achieve vesicle formation. The stock solutions were carefully mixed, and no excessive shaking or vortexing was conducted to induce aggregate formation or manipulation. The catanionic aggregate mixtures were allowed to equilibrate for at least 24 h before further experimental use.

Gel preparation

Gel samples were prepared with either Carbomer or SoftCAT. The Carbomer powder was dispersed in 150 mM sodium chloride solution for at least one hour and upon addition of 1 M sodium hydroxide solution to adjust the pH to 7.4 $\pm$ 0.1 the polymer swelled. To adjust the polymer concentration to 2%, 150 mM sodium chloride solution was added and finally the pH was controlled again and, when needed, adjusted. SoftCAT polymer solution was prepared with 150 mM sodium chloride solution and the polymer concentration was set at 4%. The final polymer concentration was set at 2% when mixed with an equal volume of the vesicles, to obtain formulations with sufficient viscoelasticity. No reference formulation, i.e. with only tetracaine, could be prepared with SoftCAT, as this polymer only forms gels in the presence of vesicles.

Gels containing catanionic aggregates were prepared by mixing equal volumes of catanionic aggregate mixtures with either Carbomer or SoftCAT, thereby rendering desired experimental concentrations. Magnetic stirrers were used to blend the gels and the samples were centrifuged before use to remove trapped air. The formulations studied were: tetracaine/SDS (in a 35 : 65 ratio) at a total concentration of 40 mM, or 0.4 (w/v) % tetracaine, in 150 mM saline solution, and in 1% Carbomer 940, and in 2% SoftCAT; tetracaine/capric acid (in a 2 : 8 ratio) at a total concentration of 40 mM, or 0.4 (w/v) % tetracaine, in 150 mM saline solution, and in 1% Carbomer 940, and in 2% SoftCAT. As reference formulations, 14 mM, or 0.4 (w/v) %, tetracaine solution, and in 1% Carbomer 940 was applied.

Rheology

A rheological investigation was conducted to investigate the possibility of a gel formation in the tetracaine/capric acid vesicles mixed with SoftCAT polymer. The viscoelastic measurements were performed with a Bohlin VOR rheometer (Bohlin Reologi AB, Lund, Sweden), which is a controlled rate rheometer of Couette type. A concentric cylinder (C14) measuring system was used for all samples. Before the experiments the samples were centrifuged until no visible air bubbles were present, and silicone oil was applied to the

surface to prevent evaporation of fluid during the measurements. The temperature was set to 20°C for all measurements. Strain sweeps were used to find the linear viscoelastic region, where the oscillation measurements were carried out. The storage modulus (G′) and the loss modulus (G″) were characterized, and a gel formation is considered to have taken place when G′ > > G″ at all frequencies in the linear viscoelastic region, and G′ is frequency independent.[46,47]

In-vitro drug release from gels

The in-vitro drug release from gels was investigated using a modified USP paddle method. Each formulation was investigated in triplicate. The volume of the gel containers was 6 cm^3 with a surface area of 21 cm^2. The filled containers were covered with a mesh size plastic net and a coarse plastic net. A detailed description of this equipment can be found elsewhere.[6] The filled gel containers were submerged in 500 ml 37 ± 0.5°C 150 mM sodium chloride solution, which was stirred at 20 rev/min using a Pharma Test PTW II USP bath (Pharma Test Apparatebau, Hainburg, Germany). Sink conditions were maintained throughout the experiment. A peristaltic pump (IPC Labinett) and ismaprene tubing (both from Ismatec, SA, Zurich, Switzerland) was used to continuously sample the receiving media, using a UV-vis spectrophotometer (Shimadzu UV-1601, Shimadzu, Kyoto, Japan). The wavelength used was 310 nm and drug release was studied for 13.5 h with more frequent measurements during the first 40 min. These measurements were used to calculate the apparent diffusion coefficient using:

$$Q = 2C_0\left(\frac{Dt}{\pi}\right)^{1/2} \quad (1)$$

where Q is the amount of drug released per unit area, C_0 is the initial concentration of drug substance in the gel formulation, D is the apparent diffusion coefficient in the gel and t is the time since the experiment started. The equation is valid when less than 60% of the drug substance has been released.[48,49] A control for a linear fit, when plotting the released amount vs the square root of the time, was conducted for each experiment. The apparent diffusion coefficient measures the rate at which a substance randomly moves from one region, with high concentration, to one with lower concentration. It is dependant of such things as vesicle deterioration, drug loading within the aggregates and actual diffusivities.

In-vitro, the release from reference Carbomer gel with only tetracaine was compared with Carbomer gels containing catanionic aggregates, either made from tetracaine and SDS or tetracaine and capric acid, and also the tetracaine/SDS–SoftCAT gel.

Skin preparation

Pig ear skin was obtained from pigs used in experiments conducted by another research group, approved by an ethical committee (application number C 257/6). The experiments carried out before the skin collection were not of such a nature that they should affect the skin. The ears were not removed from the pig until these experiments had been completed and the pig had been killed. Immediately after termination of the experiment the ears were removed using a scalpel. Within an hour the skin was separated from the underlying cartilage using a scalpel and the desired thickness was obtained with a Padgett dermatome (Integra, Plainsboro, NJ, USA). The skin was dermatomized to 0.5 mm and a custom made circular punch with the diameter of 15 mm was used to obtain the desired skin measurements. The skin was frozen at −20°C immediately after preparation until the time of experimental use. Before mounting in the horizontal Ussing chambers the skin was thawed in 150 mM sodium chloride solution for 30 min and the thickness was controlled using a digital slide gauge (Schuchart Maskin AB, Huskvarna, Sweden). For each experiment and formulation, grafts from different pigs were used to obtain a randomized selection of skin.

Ex-vivo skin and silicone sheet penetration experiments

A detailed description of the horizontal Ussing chambers that were used can be found elsewhere.[27] The chamber system used in this study comprised six chambers mounted side by side on a water-heated block (Horizontal Diffusion Chamber System, Costar, Cambridge, MA, USA). The 150 mM sodium chloride solution on the receiving side was maintained at 37°C throughout the experiment. By placing the equipment on a circular shaker (Unimax, Wernerglas, Sweden) set at 145 ± 1 rev/min, stirring was achieved. The area of the exposed skin surface was 0.55 cm^2 in this set-up. Before mounting the skin samples, 1.2 ml sodium chloride solution at ambient room temperature was added to the receiving side and after mounting the skin any air bubbles were removed. The Ussing chambers were covered with Parafilm to prevent evaporation of receiving media and placed on the heat block for 30 min to allow the receiving media and skin samples to reach physiological temperatures before the experiment was started. A 200-μl sample of a formulation was applied to each chamber and 100-μl samples were withdrawn from the receiving side at 30, 60, 90 min and 2, 3, 4, 5, 6, and 24 h, and immediately replaced with fresh sodium chloride solution. The withdrawn samples were diluted with HPLC mobile phase before analysis. The apparent penetration was calculated from the amounts of transferred tetracaine during the first five hours.

The conditions and sample withdrawal time points in the silicone sheet experiments were the same as in the skin experiments. The size of the silicone sheeting was obtained using the same punch as for preparing the skin. The sheets were washed in Millipore water before use and mounted in the chambers, and the samples were applied to the donor side 30 min after mounting on the heat block. To evaluate the potential of the silicone sheets, these experiments were limited to the SDS-containing and reference formulations.

Binding study

A binding study was conducted to investigate whether tetracaine would bind to the surface of the Ussing chambers. The binding to the receiving chamber was of most interest as the surface area was quite large and the concentration of tetracaine in the receiving chamber was quite low. Silicone

sheeting was mounted in the chambers and two solutions with different concentrations of tetracaine were added to both the receiving and donor sides. Samples (100 μl) were extracted at 0, 30, 60 and 90 min from the receiving side, and at 0 and 30 min from the donor side, and immediately replaced with fresh tetracaine solution. The samples were diluted with mobile phase and analysed with HPLC.

HPLC sample analysis

The quantification of tetracaine in the samples taken from the receiving side of the Ussing chambers was performed using a HPLC system consisting of a Shimadzu pump LC-10AD, a Waters 717 Plus Autosampler and a Spectra 100 UV detector (Thermo Separation Products). All samples were analysed on a C-18 Hypersil Gold column, 250 mm × 46 mm (5 μm) (Thermo Scientific, UK), with a Universal Uniguard Holder for 5.0/4.6 mm (Thermo Scientific, UK) and Hypersil Gold 5 μm 10 × 4 mm drop-in guards inset (Thermo Scientific, UK). The detector was set at 288 nm and verapamil was used as an internal standard. Spiked samples of known concentrations were used for calibration and validation of the method and three quality control concentrations were used ($n = 3$). A calibration curve was established (2–230 μM) using linear regression of the chromatographic peak areas and ratio of the peaks (tetracaine/verapamil) as a function of tetracaine concentration.

Skin morphology

Skin samples from experiments, exposed to each formulation, as well as reference skin samples, exposed to saline solution or air only while placed in Ussing chambers for 24 h, were used. Each skin sample was dehydrated in ethanol infiltration solution (Historesin, Leica Microsystems, Germany) according to a previously described method.[27] The dehydrated skin samples were embedded in the infiltration solution using hardeners, as instructed by the manufacturer. A motorized microtome (Leica RM 2156, Leica Microsystems, Germany) was used to prepare 2-μm thick slices of each skin sample, which were stained and examined microscopically. Further details regarding this method can be found in Östh *et al*.[27] Slices from various parts of the skin samples were examined at several degrees of magnification and any morphological deviations were noted.

Statistical analysis

The diffusion coefficients from the in-vitro release study as well as the apparent penetration from the ex-vivo skin penetration study were analysed statistically using analysis of variance and a post hoc test Bonferroni's multiple comparisons, with a significance level of $P < 0.05$ considered as statistically significant. The tetracaine contents of the samples withdrawn from the binding study were also compared statistically. The software used was Prism for Windows 4, GraphPad Software Inc. (San Diego, CA, USA).

Results and Discussion

Rheology

A previous study showed gel formation in mixtures of catanionic drug containing vesicles and the SoftCAT polymer.[7] When tetracaine/SDS vesicles were added to the SoftCAT solution, the rheological behaviour changed considerably, as displayed in Figure 1a. The SoftCAT solution had the typical rheological profile of an entangled polymer solution and when tetracaine/SDS vesicles were added the properties became more gel-like, $G' >> G''$ and the G' was frequency independent.[46,47] When tetracaine/capric acid catanionic aggregates were added to the SoftCAT solution, the rheological properties were only slightly changed, see Figure 1b. The crossing over point of the elasticity and viscosity modules occurred at a lower frequency than for the pure polymer solution, which implied that the structure of the material had increased, and that there were more long-lasting interactions, but the interactions were not as strong as with the tetracaine/SDS vesicles.

Many factors may have affected the rheological properties of these mixtures. To gain a deeper understanding of the gel formation process, a study regarding vesicle properties, such

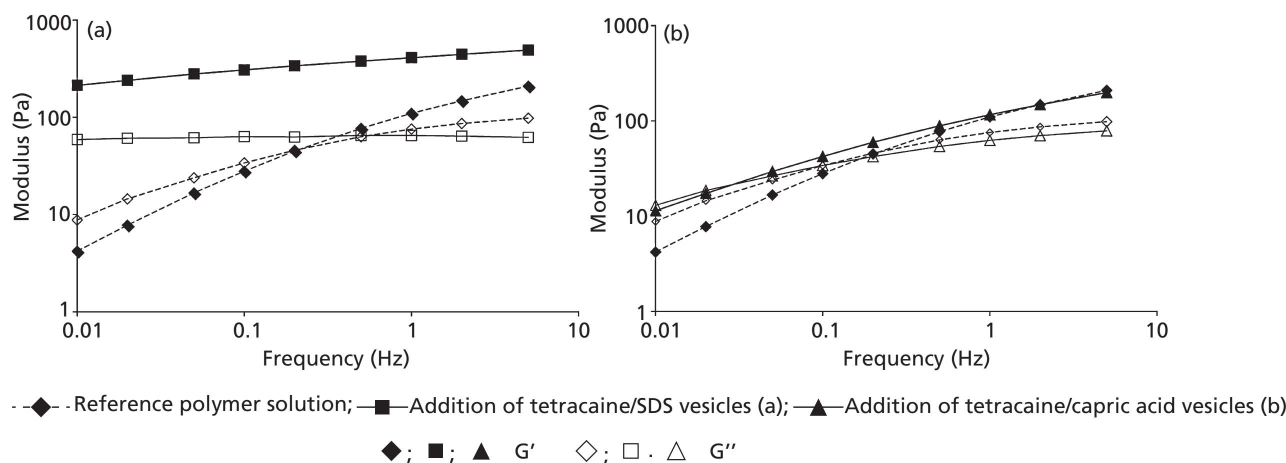

Figure 1 Rheological characterizations of SoftCAT SK-MH (2%) formulations. Tetracaine/sodium dodecyl sulphate (SDS) vesicles, at a 35 : 65 ratio, at a total concentration of 40 mM in (a). Tetracaine/capric acid vesicles, at a 2 : 8 ratio, at a total concentration of 40 mM in (b). G', the storage modulus; G'', the loss modulus.

as charge density of the bilayer, vesicle size and polydispersity, could be of use. There are structural differences between capric acid and SDS, which may have produced differences in the aggregates formed, as could the molar ratios of the components in the respective catanionic mixtures. The amount of vesicles most likely also affected the rheological properties and might have differed in the respective formulations.

Penetration through skin

The drug penetration rate through skin was significantly lowered from gel formulations with catanionic vesicles composed of tetracaine and SDS, compared with the reference gel formulation ($P < 0.001$). The penetration rate of tetracaine from the formulations with capric acid was not significantly lowered compared with the reference gel formulation. A summary of the penetration experiments is presented in Table 1. The in-vitro drug release from gels and ex-vivo skin penetration of tetracaine is displayed in Figure 2, for relevant formulations. The Ussing chamber set-up used in this study proved to be of use when studying the formulations containing polymers. However, the set-up was less suitable for the solutions. The results observed were most likely not caused by effects derived from the properties of the catanionic aggregates.

The drug diffusion from the formulations and skin penetration chain of events are complex, and there are many factors that could have affected the amount of drug substance that reached the acceptor compartment of the Ussing chambers. Such things as vesicle disintegration, drug substance loading within the aggregates, aggregate–monomer equilibration, vesicle and tetracaine interaction with the gel polymer and the diffusivity of the substance in the formulation affect the release rate of tetracaine from the gel formulations. As tetracaine reaches the skin surface the amount that penetrates into the membrane depends on how much the formulation affects the lipid structure of the skin. The stability of the catanionic aggregates and the monomer concentration in the formulation will determine how much of the surfactants that are available to interact with the membrane. There needs to be a sufficient amount of unaggregated drug substance in the donor compartment to drive the diffusion to the acceptor side. The amounts of charged substance was most likely differing in the different formulations, as pH was not adjusted in the SoftCAT formulations. The pH values in the Carbomer gels were adjusted to 7.4 ± 0.1 and measured to 6.8–7.0 in tetracaine/SDS–SoftCAT gels and to 7.9–8.0 in tetracaine/capric acid–SoftCAT formulations. The lack of strong interactions in the capric acid SoftCAT formulation may be explained by a low amount of catanionic vesicles due to a low amount of charged drug substance. A charged substance is necessary for catanionic aggregate formation, but only the fraction of uncharged drug substance present at the interface is available to penetrate the skin surface. The possibility that smaller aggregates or ion pairs may diffuse through the membrane, as uncharged units, is not unreasonable, but only a very small amount of substance is likely to penetrate the skin in this way.[50,51]

When catanionic vesicles composed of tetracaine and SDS were applied in both Carbomer and SoftCAT gels, the skin apparent permeability values were significantly lower than when the reference gel formulation was used, as shown in Table 1 and the penetration profiles are shown in Figure 2a. Similarly, the in-vitro apparent drug diffusion coefficient was reduced when using Carbomer as well as SoftCAT, as displayed in Figure 2c. When SDS was exchanged with capric acid the apparent permeability coefficients were not statistically different from the reference, and the in-vitro apparent drug diffusion coefficient from the tetracaine/capric acid–Carbomer 940 formulation was not as lowered as when SDS was used, which is displayed in Figure 2d. The apparent diffusion coefficients of the formulations with SDS were significantly lower than the one with capric acid ($P < 0.001$ for both polymers) as displayed in Table 2. These results suggested that when the gel formulations containing catanionic vesicles made with SDS were used, the skin penetration rate was controlled by the diffusion rate of tetracaine from the gel.

Penetration through silicone membranes

To evaluate the silicon membranes the tetracaine/SDS formulations were chosen, as these displayed lower drug permeability through skin than the reference formulation. The silicone membrane permeability rate of tetracaine from the

Table 1 Summary of penetration experiments

Formulation	Pig skin		Silicone	
	Amount transferred after 5 h (%)	Apparent permeability (μmol/cm²/h)	Amount transferred after 5 h (%)	Apparent permeability (μmol/cm²/h)
Tetracaine C940 reference gel	12.69	0.150	8.14	0.107
Tetracaine/SDS/C940	2.64	0.053***	2.52	0.026**
Tetracaine/SDS/SK-MH	2.33	0.016***	2.14	0.022**
Tetracaine/capric acid/C940	12.11	0.088[n.s.]	–	–
Tetracaine/capric acid/SK-MH	8.40	0.060[n.s.]	–	–
Tetracaine reference solution	3.63	0.036	2.22	0.018
Tetracaine/SDS solution	1.16[v]	0.010[v]	24.30	0.364
Tetracaine/capric acid solution	16.32	0.103	–	–

$n = 6$. Asterisks indicate statistical difference from the tetracaine Carbomer 940 (C940) reference gel sample in relevant formulations: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. [n.s]Statistically not significantly different. [v]$n = 5$ for this measurement. SDS, sodium dodecyl sulphate; SK-MH, SoftCAT polymer.

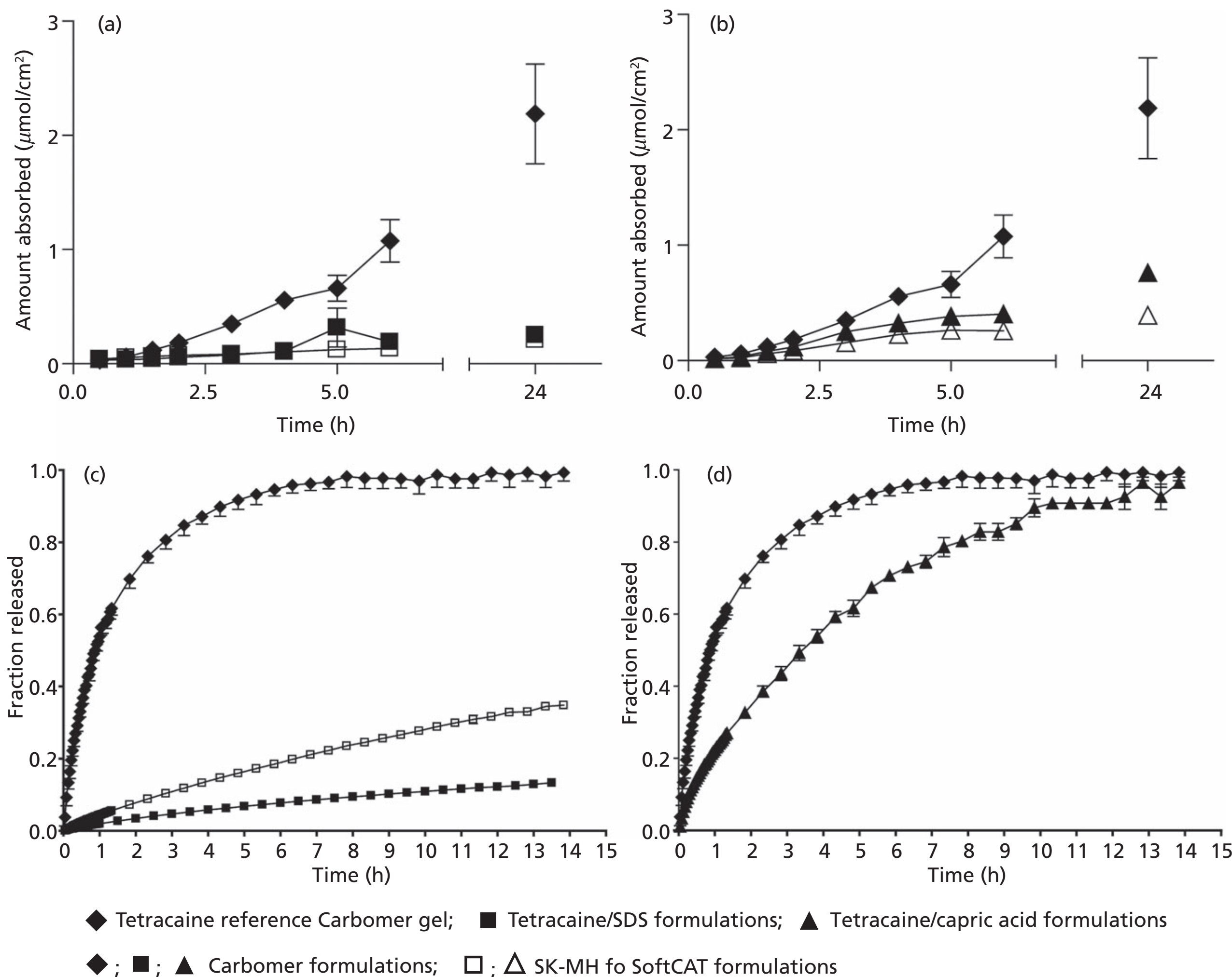

Figure 2 Skin permeation of drugs (a and b) compared with drug release from gels (c and d). The data in (c) have previously been published in Dew *et al*.[7]

Table 2 Apparent diffusion coefficients of drug substances from Carbomer 940 and catanionic vesicle–SoftCAT SK-MH gels

Formulation	D (cm^2/s)	CI
Tetracaine C940 reference gel	5.78×10^{-6}	3.03×10^{-7}
Tetracaine/SDS/C940[a]	2.78×10^{-8}***	5.49×10^{-8}
Tetracaine/SDS/SK-MH[a]	$4{,}33 \times 10^{-8}$***	7.05×10^{-9}
Tetracaine/capric acid/C940	1.03×10^{-7}***	3.11×10^{-8}

$n = 3$. CI, confidence interval; D, apparent diffusion coefficient; SDS, sodium dodecyl sulphate; SK-MH, SoftCAT polymer. Asterisks indicate statistical difference from the tetracaine Carbomer 940 (C940) reference gel sample in relevant formulations: *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$. [a]Data was previously published in Dew *et al*.[7]

formulations with catanionic vesicles were slower than from the reference formulation ($P < 0.01$), see Table 1. No statistically significant differences were noted between the vesicle formulations with Carbomer and SoftCAT.

The silicone membranes and the skin samples rendered similar apparent penetration, unlike previous studies where a 10-fold difference was displayed using similar substances.[30] However, the percentages of applied drug substance found in the acceptor chambers at the end of the experiment were not in the same range (data not shown). The main differences between the skin samples and the silicone sheets were that the skin structure deteriorated during the experiment, while the silicone sheet properties were not affected as much. The lipid structure of the skin may have been altered by capric acid and SDS, and the properties of silicon membranes might have been affected by skin penetration enhancers.[31] In this study, no penetration enhancing effect was seen by the surfactants on the skin, but could account for some of the differences observed, especially at later times.

The silicone and skin membranes gave rise to similar amounts of permeated drug substance after five hours, and similar apparent penetration; the differences were not statistically significant. The use of silicone sheets as a skin model

can therefore be advocated when studying these gel formulations with drug containing catanionic aggregates.

Binding study and skin morphology

The binding study revealed that a nonsignificant amount of tetracaine was bound to the Ussing chambers, so no alterations to the measured amounts in the penetration study were made.

After evaluation of images of the skin samples, reference skin and skin exposed to all formulations, no morphological differences between them could be distinguished. The formulations did not visibly affect the skin during the 24 h of experiments. A selection of representative images of skin samples exposed to relevant formulations is displayed in Figure 3. In studies with similar drug substances SDS increased the systemic absorption when the skin was pretreated with SDS, while SDS was found to leave upper layers of the stratum corneum unaffected in another study.[34,52] Capric acid has been shown also to increase the skin permeability of drug substances, but no correlation was seen between irritation and flux enhancement when using fatty acids similar to capric acid.[38,39,41] When catanionic mixtures of classical surfactants, one being SDS, have been investigated, the mixture has less skin irritation potential than the separate surfactants respectively similar to what has been shown considering cytotoxicity.[32,34] The skin is a tissue much more resilient to degenerative substances and processes than mucosa, and formulations similar to the ones used in this study have been shown to be harmful to porcine nasal mucosa.[27] It is likely that use of a more sensitive tissue or model would have resulted in distinguishable morphological changes, which were not visible in this study.

To draw conclusions about toxicity or skin lipid modification would be premature from the results presented here, but the penetration data could be interpreted as that the formulations with SDS affected the skin to a lesser extent than capric acid, due to the higher amounts of tetracaine transferred from the capric acid formulations. The effects seen were rather a result of a low SDS monomer concentration due to durable catanionic aggregates. Combined with a lower amount of vesicles in the capric acid formulations this could have been a reason for the high amounts of transferred tetracaine from the capric acid formulations. The rheological study and the in-vitro drug release data also suggested a higher vesicle content or more stable aggregates formed in the SDS formulations than in the capric acid formulations. It was probable that the amount of monomers in the SDS formulations was lower, and less surfactant may have affected the membrane quality in these cases. An interesting future study with measurements of monomeric SDS and capric acid could increase the understanding of the drug release and membrane permeation. A study of the deposition of tetracaine in the different layers of the skin might be required to elucidate how the formulations and the catanionic aggregates affect the tissue.

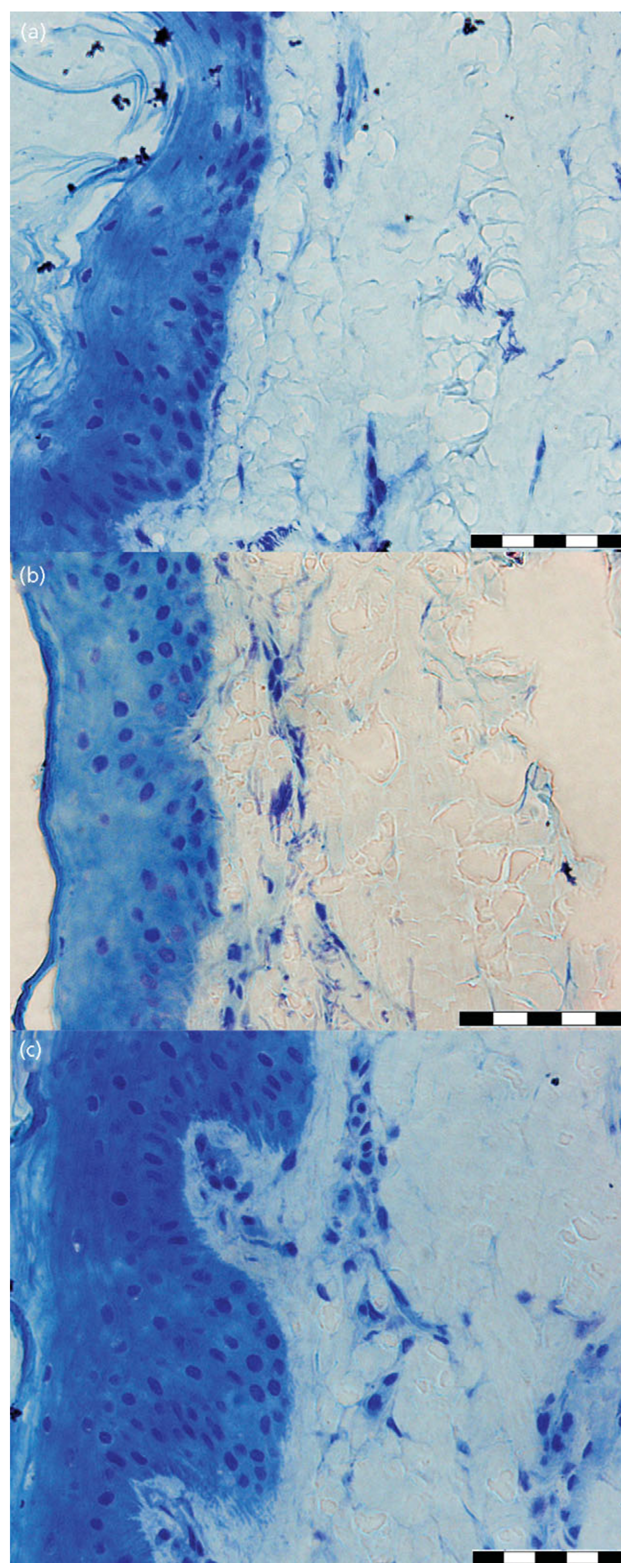

Figure 3 Images of skin samples after their exposure to various formulations. (a) Saline; (b) tetracaine/sodium dodecyl sulphate in SoftCAT SK-MH; (c) tetracaine/capric acid in SoftCAT SK-MH. Size bars indicate 50 μm.

Conclusions

This study has shown that the skin penetration rate could be controlled by the diffusion rate from the gel formulations containing catanionic vesicles composed of tetracaine and

SDS. Catanionic aggregates composed with SDS rendered a more prolonged in-vitro release and ex-vivo skin permeation than aggregates made with capric acid. Silicone membranes could be used as a model of skin when studying skin penetration rates of drug substances and when using these novel gel formulations with drug containing catanionic aggregates.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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