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In vitro evaluation of a series of sodium carboxylates as dermal penetration enhancers

K.I. Cumming *, A.J. Winfield

Department of Pharmacy, The Robert Gordon University, Schoolhill, Aberdeen AB1 9FR, UK

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Abstract

The influence of a series of sodium carboxylates on the percutaneous penetration across neonatal rat stratum corneum (SC) has been investigated. SC was pretreated with surfactant solutions for 24 h at the critical micelle concentration and also at 1 mM concentrations. Permeability studies were carried out on pretreated SC using flow through dissolution cells and [^{14}C]propan-2-ol as the penetrant. Enhancement ratios were calculated from the permeability coefficient (K_p) of the treated samples compared to untreated controls. Differential scanning calorimetry (DSC) has been used to monitor changes in the lipids within the SC following pretreatment with the surfactants. All the surfactant treated samples showed an increase in K_p with a maximal effect being seen with the C_{12} member of the series. In addition, there is a good correlation between the enhancement ratio and the decrease in lipid peak temperatures as measured by DSC. The data suggest that the surfactants increase permeability by modifying the lipid structure of the SC and that DSC may prove useful as a predictor of the effects of such compounds.

Key words: Transdermal delivery; Surfactant; Neonatal rat skin; DSC; Critical micelle concentration

1. Introduction

The principal barrier to drug permeation through the skin is the stratum corneum (SC). In recent years there have been many attempts to increase the flux of drugs through this layer using compounds known as penetration enhancers (Barry, 1987; Ruland and Kreuter, 1992; Michniak et al., 1993). Examples of these include a wide range of compounds such as alkanols (Hori et al., 1992), azone (*N*-dodecylazacycloheptan-2-one) (Stoughton and McClure, 1983; Beastall et al., 1988), azone analogs (Okamoto, et al., 1988; Bouwstra et al., 1992) alkyl *N,N*-dialkyl-substituted aminoacetates (Wong et al., 1989), and terpenes (Williams and Barry, 1991; Cornwell and Barry 1993). In addition to these there are numerous reports of surfactants affecting the permeability of drugs across the stratum corneum (Ashton et al., 1986; Walters, 1990; Kushla and Zatz, 1991; Ashton et al., 1992; Tan et al., 1993). Several attempts have been made to relate structure to activity of dermal enhancers (Okamoto et al., 1988; Tayar et al., 1991) as this would allow the selection of chemicals to be screened as potential enhancers. It has been noted by Hadgraft

* Corresponding author.

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et al. (1992) that the structural features required to produce disorder in the skin lipids and thus provide increased flux, are an alkyl chain length of around 12 carbon atoms and a polar head group. These features are common to many of the chemicals which are known as surfactants and would help to explain why they act as penetration enhancers.

In the present study, a series of sodium carboxylates which are anionic surfactants and commonly known as 'soaps' have been used to pretreat samples of SC to ascertain if they have a direct effect on skin lipids and can produce an increase in skin permeability. Permeability studies have been carried out using flow-through dissolution cells with [^{14}C]propan-2-ol as the penetrant. Differential scanning calorimetry (DSC) has been used to monitor changes in the lipids of the SC after exposure to the surfactants.

2. Materials and methods

2.1. Materials

All the sodium carboxylates with a declared purity of at least 99% were purchased from Sigma Chemical Co. The [^{14}C]propan-2-ol (specific activity 1.0 mCi/mmol) which was used as the model penetrant was purchased from Amersham International. All other chemicals used were of analytical grade.

2.2. Methods

2.2.1. Preparation of stratum corneum

Neonatal rat SC was used in the study because the pilosebaceous units are undeveloped at this age, thus providing an intact barrier. The method of SC preparation was based on one developed by Kligman and Christophers (1963). Following decapitation, the skin was removed from neonatal Sprague Dawley rats which were less than 24 h old. The epidermis was removed after soaking the whole skin in distilled water at 60°C for 45 s. The viable epidermis was then removed by digestion, using phosphate-buffered saline at pH 7.4 containing 0.1% w/v trypsin, then soaking for 45 min at 37°C. Any digested tissue was then removed by washing in distilled water five to six times followed by soaking in distilled water for 1 h. The sheets of SC were then dried on a fine wire mesh, in a dessicator over silica gel at room temperature, for a period of 2 weeks.

2.2.2. Pretreatment with surfactants

SC sheets were soaked in the appropriate concentration of the test surfactant at 20°C for a period of 24 h. Excess surfactant was then removed by washing the treated sheets in distilled water, followed by soaking in distilled water for 1 h, before drying over silica gel as above.

2.2.3. Permeability experiments

The permeability was determined using flow-through diffusion cells of a design similar to that of Akhter et al. (1984). The cells were custom made from aluminium with the area of exposed SC being 0.2 cm^2. The cells held 80 μl of receptor and up to 6 ml of donor solution. Freshly distilled water was used as the receptor solution and was pumped through the cells at a rate of approx. 6 ml h^{-1}, using a peristaltic pump with a multiple pump head. This flow rate changed the solution in the receptor chamber more than 70 times per h, thus giving sink conditions (Barry, 1983), but did not produce excessive quantities of fluid for analysis.

For each experiment 1 ml of 1.5% v/v aqueous [^{14}C]propan-2-ol was added to each cell and the chamber was covered with a glass slide to prevent evaporation. The receptor solution was collected in plastic scintillation vials at 30 min intervals over a period of 8 h. Following the addition of 10 ml of 'Instagel', a liquid scintillation cocktail, the activity of the samples was measured using a Packard Tri-Carb 4430 liquid scintillation counter.

Using a Lotus 1-2-3 spreadsheet package the steady-state flux was calculated from a plot of the cumulative count versus time using regression analysis. From this value the permeability coefficient (K_p) was then calculated. The enhancement

ratio (ER) as defined below was then calculated for the treated samples:

$$ER = (K_p \text{ of pretreated sample}) / (K_p \text{ of control sample})$$

2.2.4. Determination of critical micelle concentration (CMC)

The surface tension of aqueous solutions of the surfactants was measured using a torsion balance (White Electrical Instrument Co. Ltd) and the CMC was then determined from a plot of surface tension vs log concentration.

2.2.5. DSC analysis

DSC was performed on a Perkin Elmer DSC7 instrument coupled to an IBM compatible PC. Pretreated samples from the same sheets as those used in the permeability studies were used to allow for a direct comparison. The SC samples (2–5 mg) were folded and placed in aluminium pans and sealed with a second pan to provide a good thermal contact. The samples were heated from 25 to 140°C at 10°C/min followed by rapid cooling back to 25°C before a second heating run was carried out.

3. Results and discussion

3.1. Determination of CMC

The results of the CMC determinations from the surface tension measurements are shown in Fig. 1. It is clear that the concentration drops sharply as the chain length of the carboxylate increases. The relationship between CMC and chain length is approximately logarithmic, as is common for surfactants and has been previously reported by Shinoda (1963).

3.2. Permeability studies

The results of the permeability studies for the samples pretreated with the carboxylates at the CMC are presented in Table 1. All the pretreated samples showed an increase in permeability and

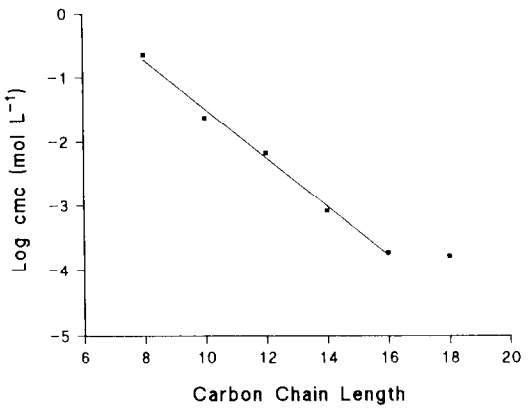

Fig. 1. A plot of log CMC against carbon chain length for the sodium carboxylate surfactants.

were highly significantly different ($p < 0.001$) from the control samples. The C_{12} member of the series showed the maximum increase in permeability.

Samples were also pretreated with 1 mM concentrations of the surfactants to allow for a more direct comparison of the effect of chain length, since there was such a variation in the CMC values for the series, the results for these pretreated samples are presented in Table 2. Again all the pretreated samples showed a highly significant increase in the permeability with the C_{12} member having the maximum effect. It is apparent that there is an optimum effect for the 12-membered carbon chain and that longer and shorter chain lengths have a decreased effect.

These findings are similar to those of Kushla and Zatz (1991) who showed that for a series of

Table 1
Effect of surfactants on permeability; pretreatment 24 h at CMC

Sample	K_p ($\times 10^5$) (cm min^{-1})	ER	SE	n	p value
Control	1.10	1.00	0.05	27	–
C_8	3.74	3.40	0.14	9	$p < 0.001$
C_{10}	3.20	2.91	0.11	9	$p < 0.001$
C_{12}	5.23	4.75	0.54	6	$p < 0.001$
C_{14}	2.56	2.33	0.10	9	$p < 0.001$
C_{16}	1.90	1.73	0.16	9	$p < 0.001$

p values are for treated sample compared to the control using Student's t-test.

Table 2
Effect of surfactants on permeability; pretreatment 24 h at 1 mM

Sample	K_p ($\times 10^5$) (cm min^{-1})	ER	SE	n	p value
Control	1.10	1.00	0.05	27	
C_6	1.53	1.39	0.08	8	$p < 0.001$
C_8	1.82	1.65	0.04	5	$p < 0.001$
C_{10}	2.30	2.09	0.06	5	$p < 0.001$
C_{12}	3.45	3.14	0.11	9	$p < 0.001$
C_{14}	2.78	2.53	0.12	6	$p < 0.001$
C_{16}	2.97	2.70	0.07	8	$p < 0.001$
C_{18}	1.90	1.73	0.08	7	$p < 0.001$

p values are for treated sample compared to the control using Student's t-test.

alkyl trimethylammonium halide cationic surfactants the optimum enhancement was seen for chain lengths of 12 or 14 carbons. They have suggested that due to the conformational structure of the alkyl chains when the chain length is 12 carbons, an optimum interaction with water leads to an enhanced migration into the skin. It is widely accepted that molecules which affect the lipid order within the SC can act as penetration enhancers (Goodman and Barry, 1986; Bouwstra et al., 1989; Schuckler and Lee, 1992). The structural features required to impart this disorder are an alkyl chain length of around 12 carbon atoms and a polar head group (Hadgraft et al., 1992). Results from the DSC analysis presented below show that the surfactant pretreatment has affected the lipid order within the SC and it is likely that this has thus led to the increase in the permeability of these samples.

3.3. DSC analysis

The DSC profile of control SC for both the first and second heating runs is shown in Fig. 2.

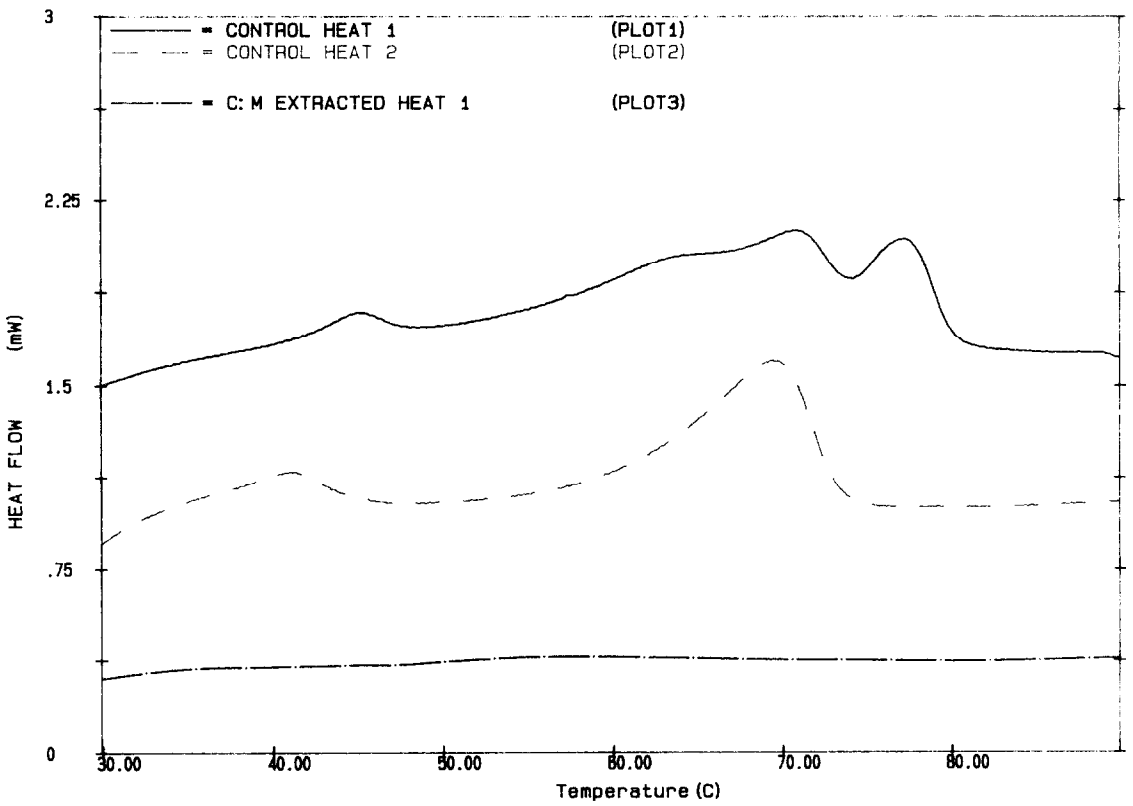

Fig. 2. DSC profiles for control SC for first and second heating runs and for C:M lipid extracted SC.

Three peaks were recorded on the first heating run, a small peak at 45°C, followed by two major transitions at 70 and 77°C. During the second heating the small peak was measured at a lower temperature of 41°C and the two major transitions had merged to produce a single peak at 68°C. Further heating runs always produced a profile identical to that for the second heating with no further changes in the transitions. Also shown in Fig. 2 is the thermal profile for a SC sample which had the lipids extracted using a chloroform : methanol (2 : 1 v/v) solvent system (C : M), the extracted SC showed no peaks on the first or subsequent heating runs. A profile of the extracted lipids (not shown) showed two peaks, a small transition at 42°C and a much larger transition at 68°C. All these findings strongly suggest that the three transitions seen in the control SC sample are lipid related, as previously reported by other researchers.

It has been suggested by Al-Saidan (1985), following thermal analysis on SC and extracted lipids, that the initial transition at 42°C is due to the melting of lipids within the SC. Further studies using data from small-angle X-ray scattering together with thermal analysis allowed Bouwstra et al. (1992) to propose that this first transition is due to a change from crystalline state bilayers to gel state bilayers for the lipids.

The two major transitions which occur in the region 65–80°C have also been reported by many researchers including Golden et al. (1986) and the two previously cited authors, all of whom agree that the first of these transitions is lipid only in nature as it remains in reheating profiles of SC (although present at a slightly lower tem-

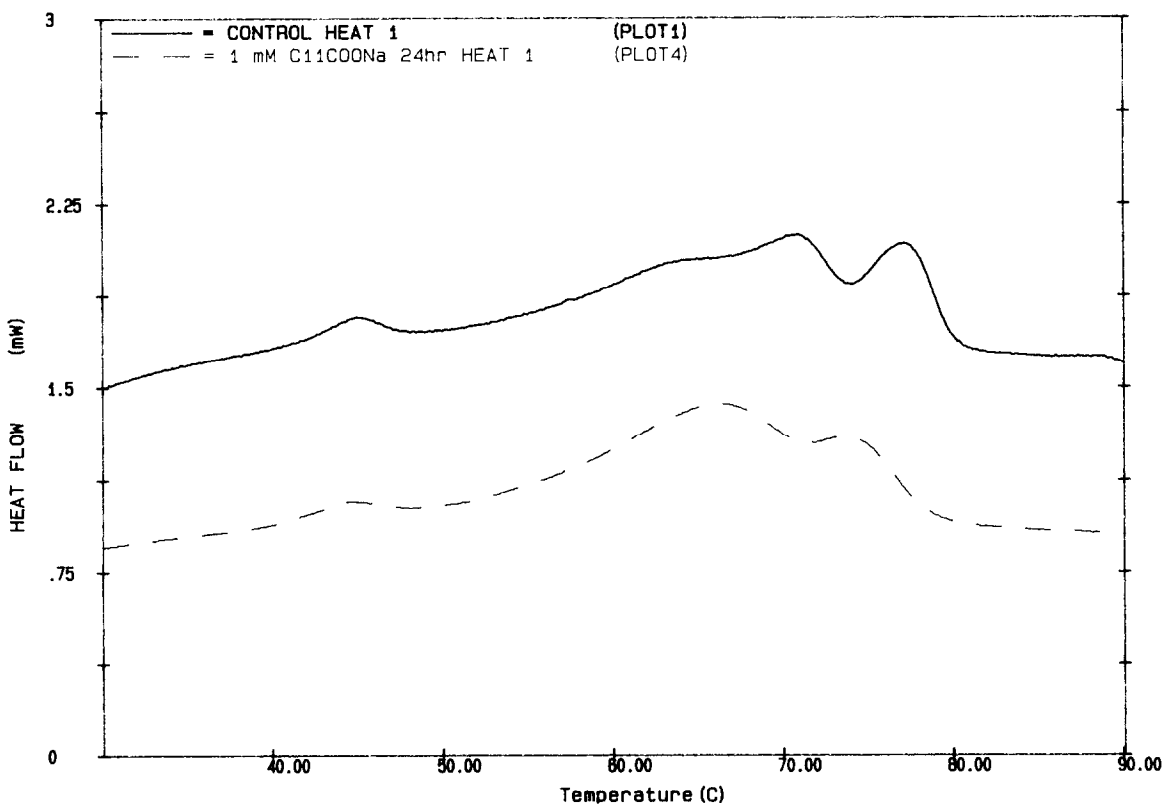

Fig. 3. DSC profiles for the first heating runs of a control SC sample and a sample pretreated with 1 mM sodium dodecanoate for 24 h.

perature) and also there is a peak at this temperature for lipids which have been extracted from SC. The second of these main transitions does not appear on second and subsequent heating runs and is not present for lipids extracted from the SC and it has been proposed that this transition is due to a lipid-protein association in the SC. It has further been suggested by Potts and Francoeur (1992) that the irreversibility of this second major transition is a consequence of a heat-labile association of certain SC lipids and a protein constituent, possibly an element from the corneocyte envelope.

The profile of a surfactant treated sample compared with a control is shown in Fig. 3 and shows that the two main transitions move to a lower temperature following pretreatment. Four samples were analysed from each pretreatment to allow for statistical analysis of the results. The peak temperatures and the total temperature depression for both peaks are presented in Tables 3 and 4. For all samples pretreated with the surfactant at the CMC there was a highly significant temperature depression of these lipid transitions and for the majority of the samples pretreated at 1 mM concentration there was a significant depression. It is again clear that an optimum effect is seen for a carbon chain length of 12 and that longer and shorter chains have a decreased effect.

A similar reduction in peak temperatures has been reported for azone analogs (Bouwstra et al., 1989), for azone and dodecyl-L-pyroglutamate (Schuckler and Lee, 1992) and for oleic acid (Francoeur et al., 1990). Reduction in these transition temperatures has been assigned to increasing disruption of the lamellar bilayer structure of the lipids in the SC (Bouwstra et al., 1989). It is our understanding that these modifications to the lipid structure of the SC which are detected by DSC are responsible for the increase in permeability which has been detected following pretreatment with the surfactants. From TLC studies (results not reported) there was no evidence of any lipid extraction following pretreatment and this is supported by the findings of Froebe et al. (1990) who showed that extraction only occurred at concentrations above the CMC. It is therefore proposed that at the concentrations studied the surfactants have a direct effect on the structure of the skin lipids and their effect is not due to lipid extraction.

Table 3
Effect of surfactants on lipid thermal transitions; pretreatment 24 h at CMC

Sample	Temperature peak A + peak B	Temperature depression	SE	n	p value
Control	147.35	–	0.13	7	–
C_8	143.20	4.15	0.09	4	$p < 0.001$
C_{10}	142.66	4.69	0.27	4	$p < 0.001$
C_{12}	135.79	11.56	0.76	4	$p < 0.001$
C_{14}	143.26	4.09	0.48	4	$p < 0.001$
C_{16}	145.17	2.18	0.55	4	$p < 0.001$

p values are for treated sample compared to the control using Student's t-test.

Table 4
Effect of surfactants on lipid thermal transitions; pretreatment 24 h at 1 mM

Sample	Temperature peak A + peak B	Temperature depression	SE	n	p value
Control	147.35	–	0.13	7	–
C_6	146.36	0.99	0.57	4	$p < 0.1$
C_8	147.64	0.29	0.16	4	n.s.
C_{10}	146.07	1.28	0.12	4	$p < 0.001$
C_{12}	140.26	7.09	0.33	4	$p < 0.001$
C_{14}	144.45	2.90	0.28	4	$p < 0.001$
C_{16}	142.61	4.74	0.75	4	$p < 0.001$
C_{18}	146.29	1.06	0.40	4	$p < 0.02$

p values are for treated sample compared to the control using Student's t-test.

3.4. Correlation of enhancement ratio (ER) with temperature depression

From both the permeability studies and the DSC analysis there was an optimum effect observed with the C_{12} member of the sodium carboxylate series. In addition, an increased permeability appeared to be reflected by a greater temperature depression seen with the DSC analysis. Fig. 4 shows a plot of temperature depression against enhancement ratio for the samples pre-

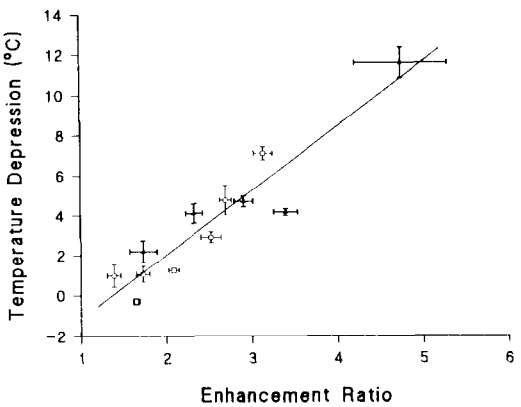

Fig. 4. Correlation of enhancement ratio with temperature depression. (▲) Samples pretreated at the CMC; (□) samples pretreated at 1 mM concentration. Bars indicate SE.

treated at the CMC and also at 1 mM concentration; the regression equation is as follows:

$$ER = 3.20 \times \text{temperature depression} - 4.39$$

with $r^2 = 0.873$. This shows that there is a good correlation between ER and temperature depression from this series of surfactants. However, it is possible that at very low enhancement ratios the DSC analysis is not sensitive enough to detect any changes in the lipid transitions. It has previously been suggested by Winfield and Taylor (1990) that thermal analysis may prove useful as a screen test for possible penetration enhancers. The improved sensitivity and reproducibility obtained from this DSC system would lead us to suggest that DSC can indeed prove useful as a screen test for some penetration enhancers.

4. Conclusion

From the data it can be concluded that the sodium carboxylates investigated in this study were all effective as penetration enhancers and that they had a direct effect on the structure of the SC. The DSC data lead us to conclude that the surfactants have a direct effect on the lipids of the SC and cause a disruption of the lamellar bilayer structure which causes this increase in permeability. Furthermore, from this investigation there was a good correlation between enhancement ratio and temperature depression of the lipid transitions in the DSC analysis which would allow the DSC to be used as a screen test for this type of penetration enhancers. Further work is being carried out to investigate the effects of other surfactants and to ascertain if the DSC analysis will continue to prove useful as a screen test.

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