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Interaction of indomethacin with a new penetration enhancer, dodecyl 2-(*N*,*N*-dimethylamino)propionate (DDAIP): its effect on transdermal delivery

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Abstract

The new biodegradable penetration enhancer DDAIP (dodecyl 2-(*N*,*N*-dimethylamino)propionate) shows a striking enhancement of the acidic drug indomethacin through shed snake skin. This enhancement appears to be due partially to some interaction between the drug and the enhancer. This interaction was examined using UV, IR, ^{1}H- and ^{13}C-NMR spectrometry and differential scanning calorimetry (DSC). The existence of preferential hydrogen bonding between the carboxylic acid group of indomethacin and the tertiary amine group of the enhancer and a dipole-dipole interaction was demonstrated.

Keywords: Indomethacin; Dodecyl 2-(*N*,*N*-dimethylamino)propionate; Azone; Interaction; UV; IR; ^{1}H-NMR; ^{13}C-NMR; Differential scanning calorimetry; Transdermal delivery; Shed snake skin

1. Introduction

The stratum corneum layer of the skin is an excellent barrier to most chemicals. Relatively few drugs can be transported through this layer to deliver an adequate therapeutic dose. Since many therapeutic agents are weak acids or bases, they exist as charged species under physiological conditions. Therefore, their permeation across the lipophilic horny layer by simple diffusion is quite limited. By the use of different reagents it is possible to alter the partition characteristics, e.g. lipophilicity of drugs. Such approaches include ion pair formation (Green et al., 1989) and complexation (Ibuki, 1985; Bhattachar et al., 1992). Another alternative involves the interaction of an enhancer with different domains of the membrane to increase the permeation of drugs (Nishihata et al., 1988a,b; Takahashi et al., 1991; Araki et al., 1992; Sugibayashi et al., 1992).

We reported that dodecyl 2-(*N*,*N*-dimethylamino)propionate (DDAIP) (Büyüktimkin et al., 1993) is a very effective biodegradable penetration enhancer and facilitates the permeation of various drugs through the skin. The transport of indomethacin was found to be 430 times higher than control, whereas it was 28 and 265 times with clonidine and hydrocortisone respectively.

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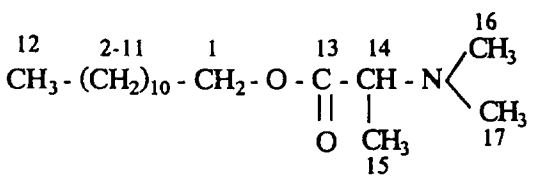
Striking differences between these enhancements suggested that the interaction of DDAIP with indomethacin, an acidic drug, is possible. The interaction of enhancers to facilitate the permeation of indomethacin has already been reported (Ibuki, 1985; Bhattachar et al., 1992).

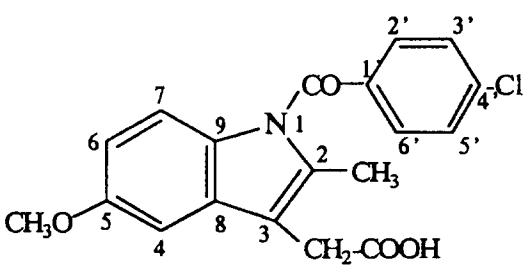
In the present study the possibility of an interaction between DDAIP and indomethacin was examined using UV, IR, ^{1}H- and ^{13}C-NMR spectrometry and differential scanning calorimetry (DSC).

2. Materials and methods

2.1. Materials (Scheme 1)

Indomethacin (Sigma, lot 75F-0557) was supplied by Sigma Chemical Co., St. Louis. Azone was obtained from Nelson Research and Development Co., Irvine. All solvents were purchased from Aldrich and they were analytical grade. Dodecyl 2-(*N*,*N*-dimethylamino)propionate (DDAIP) was prepared as described (Büyüktimkin et al., 1993).





Scheme 1. Structures of DDAIP (top) and indomethacin (bottom). Nomenclature of DDAIP is not systematic by convenience.

2.2. Preparation of the complex

The complex was prepared by mixing equimolecular amounts (1 mmole) of indomethacin and DDAIP in 10 ml ethanol free chloroform at 32°C for 5 min. After the complete evaporation of the solvent under vacuum, the remaining residue was used for NMR, IR and DSC studies.

2.3. UV studies

For the UV studies, 0.05 mmoles DDAIP, accurately weighed, was suspended in 5 ml of a saturated indomethacin solution in pH 7.0 phosphate buffer and stirred for 24 h at 32°C. The solubility of indomethacin (916 μg/ml) was obtained from Fleeker et al., 1989. The mixture was centrifuged and a 1 ml aliquot of the aqueous phase was diluted to 100 ml with the same buffer and the amount of indomethacin was assayed by UV using a standard calibration curve. A Perkin Elmer Lambda 5 spectrometer was used.

2.4. Infrared spectrometry

The infrared spectra of the drug, enhancer and drug/enhancer complex was measured by dissolving the compounds in spectroscopic grade $CHCl_3$ using a Perkin Elmer 1420 Ratio Recording IR spectrometer (sample concentration: 10 mg in 1 ml $CHCl_3$).

2.5. 1H- and ^{13}C-NMR spectrometry

The NMR spectra of the samples were recorded on a General Electric QE 300 instrument using $CDCl_3$ as solvent and TMS as the internal standard. For ^{1}H-NMR spectral measurements the concentration of the enhancer and indomethacin were ~ 0.1 mmole in 0.6 ml, whereas for ^{13}C-NMR ~ 0.2 mmoles in 0.6 ml solutions of the samples were used. For chemical shift assignments stoichiometric amounts of indomethacin were successively added to the enhancer samples.

2.6. Differential scanning calorimetry

Thermal analyses of the enhancer and the complex were performed using a Perkin Elmer 4 differential scanning calorimeter. Aluminum pans and lids were used for all samples. Temperature calibration was made using indium as standard. All samples were run at a scanning rate of 10°C/min using nitrogen as the effluent gas.

3. Results

3.1. UV absorption

The spectra of indomethacin before and after mixing with enhancer are shown in Fig. 1. Although no shifts of the λ max were observed, a hypsochromic effect indicated an interaction of the drug with DDAIP. The diminution of the absorption intensity may be due to the reduced solubility of the resulting complex in aqueous solution.

3.2. IR spectrometry

IR spectra of indomethacin, DDAIP and the complex are depicted in Fig. 2. Infrared spectroscopy showed that they were several distinct differences in the absorption characteristics of the drug/DDAIP complex, drug and enhancer. Marked differences are visible in 1400–1500, 1000–1100 and 900–1000 cm^{-1} regions.

3.3. 1H-NMR spectrometry

^{1}H-NMR spectra of indomethacin, DDAIP and the resulting complex and the shifts of the major signals in the complex as a function of concentration modification are depicted in Fig. 3. As expected the methyl group at the 15 position of DDAIP was shifted significantly by ~ 0.1 ppm. The singlet due to the dimethylamino group showed a deshielding of 0.232 ppm. The proton signal at the 14 position was also considerably shifted upfield by 0.55 ppm. The high shifts exhibited by protons at the 14 and 15 positions suggest that these groups were particularly influenced by

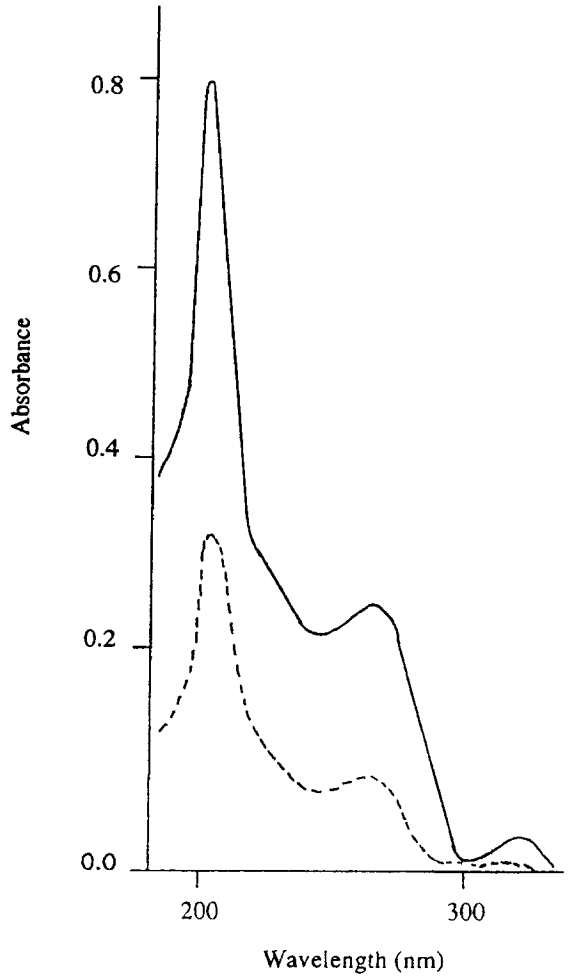


Fig. 1. UV spectra of indomethacin before treatment with DDAIP (solid line) and after treatment with DDAIP (dotted line).

bonding between DDAIP and indomethacin. The singlets due to the CH_3 and OCH_3 groups of indomethacin were also shifted by ~ 0.03 ppm. The spectrum of the interaction product also exhibited a broad peak exchangeable with D_2O which may be attributed to the formation of N.....H bonding with the carboxylic acid group of indomethacin and the tertiary functionality of DDAIP. This proton showed an extensive upfield shift of 3.38 ppm by the increased addition of indomethacin. Similar bonding involving carboxylic acids and amine groups has been reported by Hosono et al., 1979, Sanders and Hunter, 1989 and Beten et al., 1992.

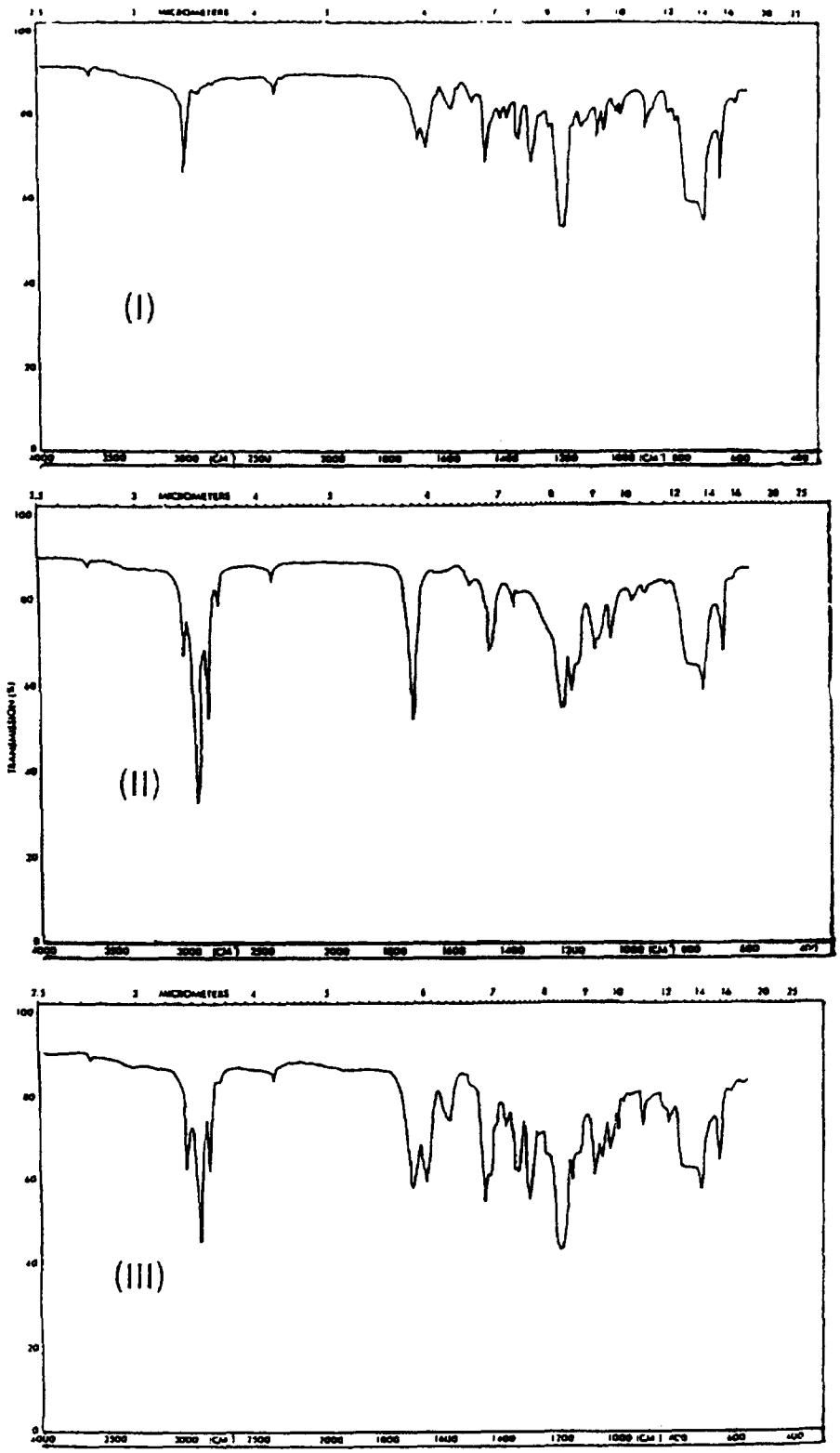


Fig. 2. IR spectra of indomethacin (I), DDAIP (II), and DDAIP/indomethacin complex (III).

and Beten et al., 1992.

3.4. 13C-NMR spectrometry

The assignments of chemical shift signals of indomethacin were based on those reported in the literature (O'Brien et al., 1984). Chemical shifts of DDAIP and the complex were also evaluated using multiple pulse sequence and 2-D spectra techniques such as APT and HETCOR. An edited DEPT technique was also employed to obtain separated spectra for CH_3, CH_2 and CH carbons. Fig. 4 shows the ^{13}C NMR spectra of indomethacin, DDAIP and their interaction product. Chemical shifts of the representative carbons in the complex are listed in the Table 1. In the indomethacin moiety, as was observed by proton NMR spectrometry, the carbon atoms most strongly affected by complexation are CH_3, $-OCH_3$, $\underline{C}H_2-CO$, C=O, and N–CO–. In the DDAIP moiety those are at the 1,12,14,15 and 16-17 positions. Strong shielding of the C=O group in indomethacin confirms that this carbon, together with those of the 16 and 17 positions in DDAIP were involved in the interaction.

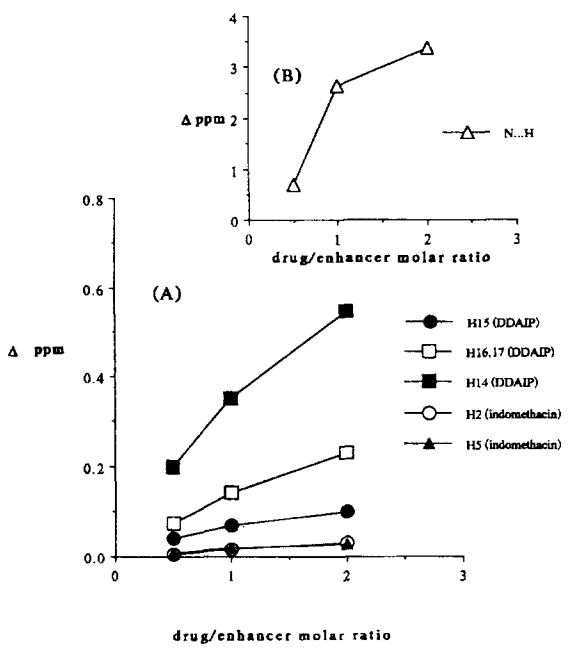


Fig. 3. Chemical shifts in ^1H-NMR spectra of DMP/indomethacin complex (A), NH proton (B) of the same complex as a function of the molar ratios of DDAIP to indomethacin in $CDCl_3$ at room temperature.

3.5. Differential scanning calorimetry

The sample scans obtained by DSC are shown in Fig. 5. It is observed that the melting point of the complex is substantially lower than that of indomethacin. The endotherms were quite sharp indicating a distinct complex rather than a broadening due to an intimate mixture. The melting point of the complex was 1.45°C. The enhancer alone showed a melting point −9.49°C and indomethacin melted at 163°C.

4. Discussion

Recently we have reported that the new biodegradable enhancer DDAIP significantly increased the penetration of indomethacin, clonidine and hydrocortisone through shed snake skin (Büyüktimkin et al., 1993). The enhancement of indomethacin was 430 times higher than that of control, whereas the enhancements of clonidine and hydrocortisone were 28 and 265 times respectively. When compared to Azone, a nearly 5 fold enhancement of indomethacin was obtained by DDAIP. It has already been reported that the penetration of some drugs is considerably increased in the presence of enhancers which may interact with them. Green et al., 1989 have reported that the lipophilicity of cationic drugs can be increased by forming an ion-pair with the carboxylate anion of fatty acids. It has also been suggested that enhanced flux could be accounted for by an increase in lipophilicity through ion-pairing (Falk, 1991). Lee et al., 1987 showed that ionic drugs form electrically neutral ion-pairs in non aqueous media. These ion-pairs can permeate through hydrophobic membranes, such as stratum corneum, due to their lipophilicity. The transport of an ionized drug in water such as indomethacin, with a pKa of 4.12 (ionized over 99%) (Hayashi et al., 1992) is strongly reduced. Ionic drugs can only penetrate through hydrophobic pores after wetting. The impermeability of a hydrophobic mem-

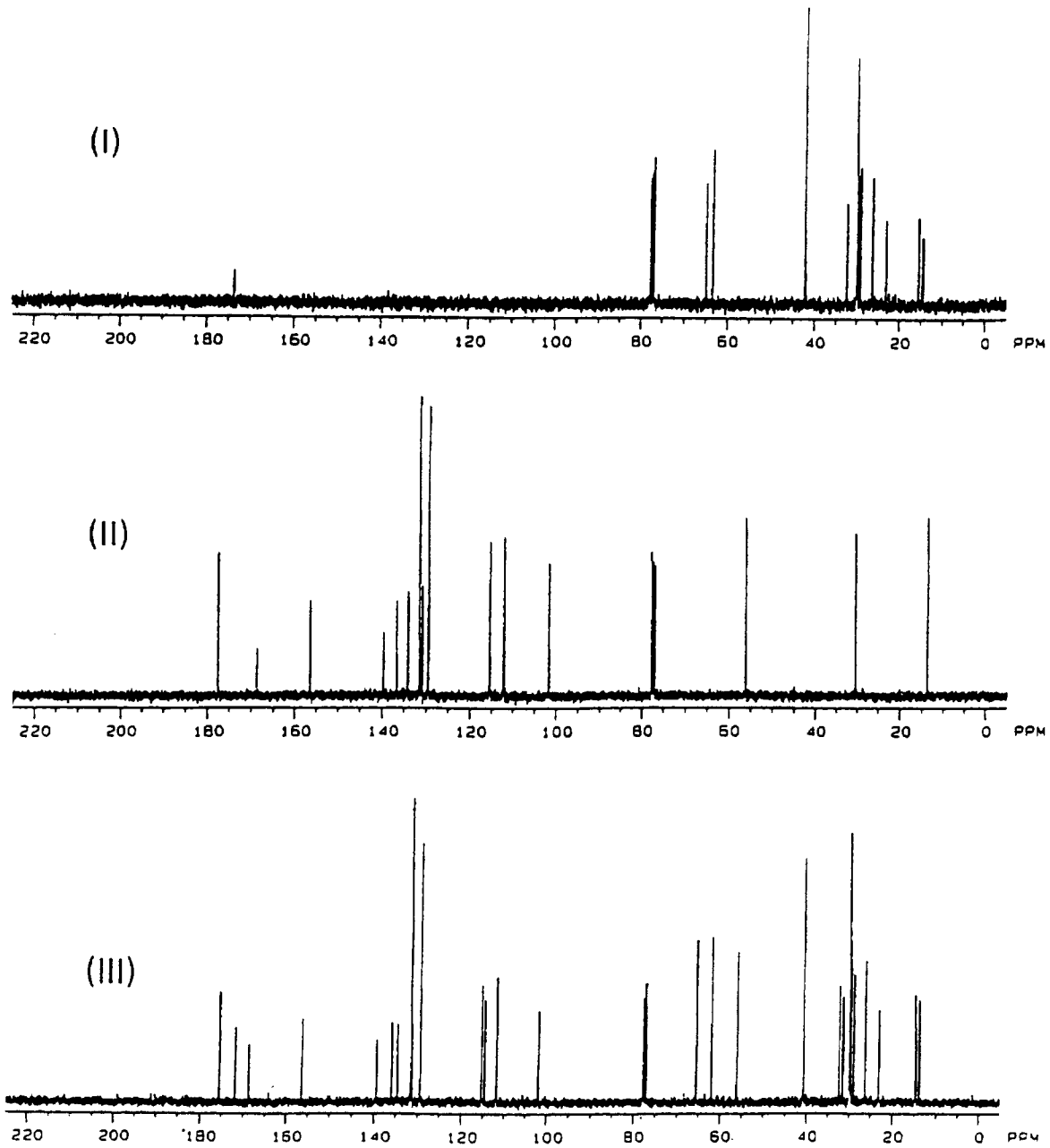


Fig. 4. ^{13}C-NMR spectra of DDAIP (I), indomethacin (II), DDAIP/indomethacin complex (III).

brane such as stratum corneum to the ionized form of the drug may be explained by the Born energy of charging (Parsegian, 1969). According to this theory a considerable energy difference exists between an ion in an aqueous system and an ion in an apolar medium. Thus, the activation energy for neutral ion-pairs to partition into a hydrophobic membrane is lower than for the ions. Therefore, the permeation of ion-pairs through a membrane is greater than the permeation of the drug itself.

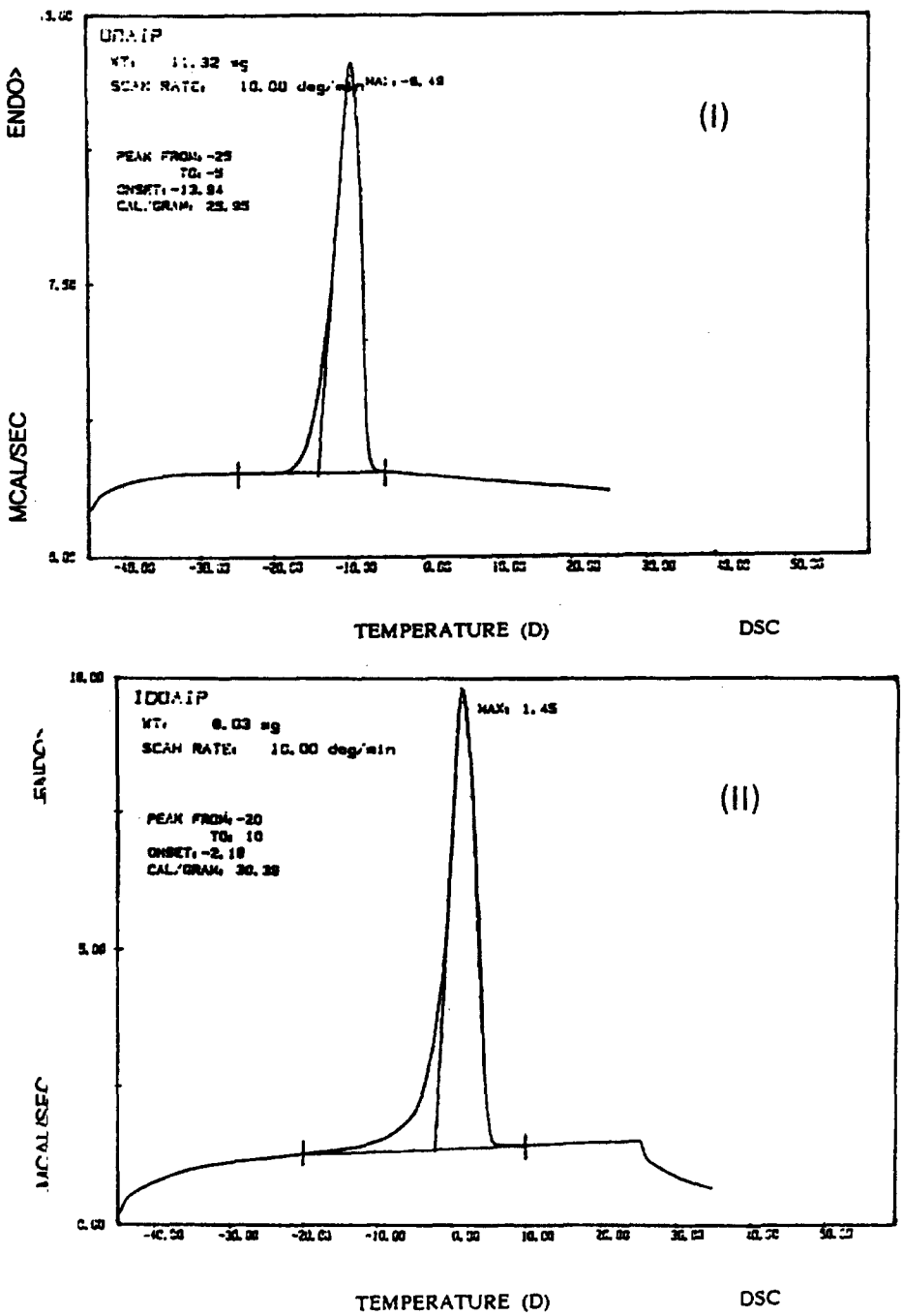


Fig. 5. DSC thermograms of DDAIP (I), DDAIP/indomethacin complex (II).

Table 1
Chemical shift (ppm) assignments in ^{13}C-NMR spectra of DDAIP, indomethacin and the complex (1:1) in $CDCl_3$

Carbon	Chemical shift (ppm)			
	DDAIP (δ)	Indomethacin (δ)	Complex (δ')	δ-δ'
2-CH_3		13.74	13.85	+0.11
5-CH_3O–		56.14	56.05	−0.09
3-$\underline{C}H_2CO$		30.43	31.41	+0.98
–N-CO-		168.66	166.72	−2.46
3-$CH_2\underline{C}O$		175.0	177.61	+2.61
15-CH_3	15.53		14.83	−0.7
16,17-CH_3	42.18		40.73	-1.45
14-CH–	63.4		61.96	−0.6
1-CH_2-O	64.91		65.51	+0.6

Furthermore Kato and Iwata, 1988 also showed that the corneal transport of bunazosin was considerably increased by its interaction with caprylic and capric acids. Ibuki, 1985 reported that the penetration enhancement of indomethacin in the presence of cyclic urea type enhancers may also be due to such interaction. Bhattachar et al., 1992 have also shown that the complexation of indomethacin with hydrogenated phospholipids considerably increased the penetration across shed snake skin.

Our present investigations suggest that an interaction mechanism may also be effective on the transport of indomethacin in the presence of DDAIP. The interaction seems to occur by bonding between the tertiary amine group of DDAIP and the carboxylic acid group of indomethacin. Such complexation is suggested especially by ^{1}H- and ^{13}C-NMR spectrometric studies. However, the extensive upfield shift of the N–CO– group of indomethacin in the ^{13}C-NMR spectrum may not be explained only by hydrogen bonding, because there is no activated hydrogen on this position. It might be suggested that indomethacin becomes polarized in such a way that the carbonyl oxygen is nucleophilic (Higuchi and Connors, 1965). Complexation can occur as a result of dipole-dipole interactions between this group and the electrophilic nitrogen of DDAIP. However, the possibility of salt formation between the drug and the enhancer is precluded. In either case the formed compound may have a better partition coefficient than the drug alone, and the interaction product after facilitating transdermal delivery, will dissociate to the parent compounds. HPLC analyses of flux samples showed no changes in the retention time of indomethacin and no extra peaks appeared on the chromatograms.

To further confirm the interaction, similar studies were also undertaken with Azone as enhancer. No significant interaction was found between indomethacin and Azone using the methods cited above.

We already have demonstrated (Büyüktimkin et al., 1996) that DDAIP similar to ethanol, interacts with stratum corneum proteins by breaking –S-S– bonds of keratin structure. Therefore, some other mechanisms such as an interaction with other components of the stratum corneum (Nishihata et al., 1988a; Nishihata et al., 1988b; Araki et al., 1992; Takahashi et al., 1991) or ion-sequestration (Yata et al., 1983) may also be possible. The extent of these actions on the absorption promotion of indomethacin remains to be further investigated.

Overall the results suggest that the interaction of indomethacin with an enhancer may lead to the formation of new structures which can increase its penetration through the stratum corneum.

Acknowledgements

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