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# Determination of the position of the ester bond in a micellar prodrug of phenytoin

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## Summary

It was previously determined that the nearly 200-fold increase in the observed precipitation times of aqueous prodrug solutions of 3-(*N,N*-dimethylglycyloxymethyl)-5,5-diphenyl hydantoin methane sulphonate (DDMS) was a result of a decrease in the rate of hydrolysis of the prodrug as well as an increase in the solubility of phenytoin in the presence of DDMS. The formation of a series of associative species by the prodrug are probably responsible for the changes observed in solubility and the rate of hydrolysis. Final proof of the presence of micellar prodrug species was obtained with NMR studies. The major upfield shift shown by the protons of the phenyl groups substantiated the hypothesis that the hydrophobic groups of the prodrug molecules formed the hydrophobic core of the micelles, which enabled the inclusion of the phenytoin, which is hydrophobic, and thereby causes the phenytoin to be solubilized. The NMR results also showed that the labile ester binding of the prodrug was submerged into the micelle which could explain the decrease in the rate of hydrolysis of the prodrug that was previously reported.

## Introduction

Many drugs have been found to exhibit colloidal behavior in aqueous solution in that they accumulate at interfaces, depress surface tension and form aggregates in solution at sufficiently high concentrations (Felmeister, 1972).

Drugs represent a large variety of amphiphilic structures ranging at one extreme from the cationic quaternary ammonium germicides, which are easily recognized as typical surfactants, to more complex aromatic or heterocyclic molecules. Typical surfactants have hydrocarbon groups which can intertwine during the micellization process to form spheroidal aggregates. Replacement of this flexible hydrophobic moiety with a rigid aromatic or heterocyclic ring system can have pronounced effects on the way in which molecules are disposed within the aggregates, to such an extent that the process of association can no longer be regarded as micellization. An example is the association of the cationic dyes and the purine and pyrimidine bases of nucleotides which associate by a stacking process. This self-association process is generally continuous, i.e., there is

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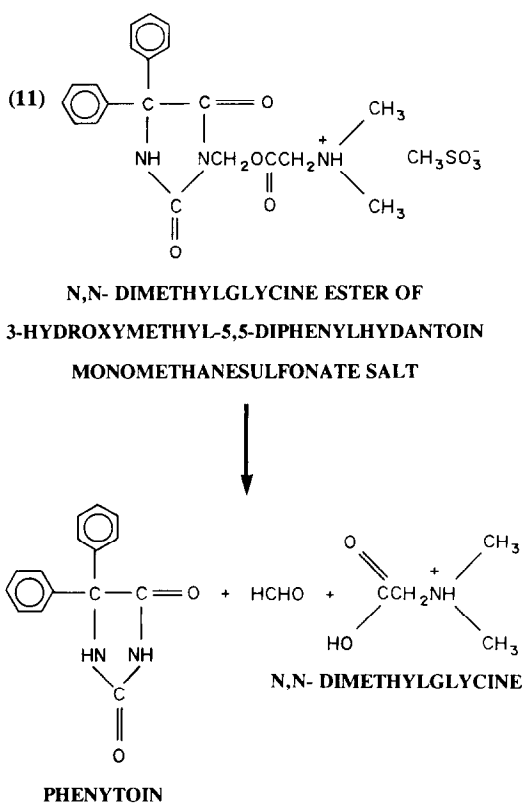
no equivalent to a CMC and there is a wide range of aggregate sizes in solution. The side chains of such molecules are generally small with respect to the hydrophobic ring system and the self-association is controlled by hydrophobic interactions. Although the hydrophobic groups of most drugs are aromatic they may resemble typical surfactants in that these groups have a high degree of flexibility. This is the case for the prodrug DDMS (Scheme 1) and a group of drugs which are derivatives of diphenylmethane (Table 1).

The prodrug of phenytoin, 3-(*N,N*-dimethylglycyloxymethyl)-5,5-diphenyl hydantoin methane sulphonate (DDMS) (**II**), can be used to increase the aqueous solubility of the poorly water soluble drug phenytoin (**I**). During a previous study (Muller et al., 1991) it was found that the solubility of phenytoin was increased and the rate of hydrolysis of the prodrug (DDMS) was decreased in the presence of the prodrug. The presence of




Scheme 1.

associative species formed by DDMS in aqueous media could account for the observed phenomena. In an effort to explain the unexpected solubility and kinetic behaviour of aqueous DDMS solutions, experiments were performed to determine the position of the ester bonds in the micellar prodrug species. Information of this nature could cast more light on the structure of the micellar species formed, which could explain the unexpected solubility and kinetic behaviour previously observed.

## Experimental

*Materials*

All chemicals, unless otherwise stated, were at least A.C.S. reagent grade and used without further purification.

The prodrug was synthesized according to the method reported by Varia et al. (1984) while the purity was determined by differential scanning calorimetry (DSC4, Perkin Elmer Corp., Norwalk, CT) and HPLC. The amount of phenytoin contamination present in the prodrug was found to be 0.13% as determined by HPLC (Altex Model 110A pump and a Waters Associates Model 450 Variable Wavelength Detector).

The water used was deionized ($< 0.2$ mho conductance) and distilled from an all-glass still. All glassware used in these studies were thoroughly rinsed with deionized water and dried.

*Differential scanning calorimetry (DSC)*

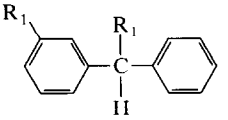
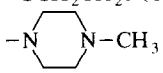
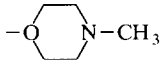
DSC is a known method to predict drug-excipient or drug-drug compatibility (Botha et al., 1986). The use of DSC to predict drug-excipient compatibility was discussed by Hardy (1982) and Smith (1982).

Phenytoin, its prodrug and a combination of the two compounds were subjected to DSC. The instrumentation consisted of a Du Pont 910 DSC system equipped with a Du Pont Series 99 Thermal Analyzer programmer. A Hewlitt-Packard X-Y recorder was used.

Thermograms were obtained by heating at a constant rate of 5°C per min and recorded at a constant chart speed of 5 cm per min. Samples

TABLE 1

*The micellar properties of some diphenylmethane antihistamines in water*

| Drug |  | | CMC (mol $dm^{-3}$) | Aggregation number |
|---|---|---|---|---|
| | $R_1$ | $R_2$ | | |
| Diphenhydramine HCl | $-OCH_2CH_2N(CH_3)_2$ | H | 0.140 [a], 0.127 [b] | 3 |
| Bromodiphenhydramine HCl | $-OCH_2CH_2N(CH_3)_2$ | *p*-Br | 0.042 [a], 0.048 [b] | 11 |
| Chlorcyclizine HCl |  | *p*-Cl | 0.040 [b] | 9 |
| Diphenylpynaline HCl |  | H | 0.090 [b] | 9 |

[a] Mean value at 20°C from several techniques including cryoscopy, spectrophotometry, surface tension, potentiometry and conductivity (Thoma and Siemer, 1976).

[b] Mean value at 30°C from several techiques including conductivity, surface tension and light scattering (Attwood and Udeala, 1975a–c).

were weighed into aluminum pans and hermetically sealed. The reference was a sealed empty aluminum pan. The individual substances and 1:1 physical mixtures of the drugs, prepared with mortar and pestle, were heated over a concentration range of 50–320°C. At least two replicas were made for each thermogram.

*X-ray crystallography*

In an effort to gain more information on the steric configuration of the prodrug molecule in solution, the prodrug was recrystalized from ethanol/acetone and subjected to X-ray crystallography.

*$^1$H-NMR spectroscopy*

NMR has previously been used to obtain evidence of self-association of molecules (Thakkar et al., 1970; Kan, 1980; Yanuka, 1986). NMR spectroscopy has provided useful information about the mode of association, which has led to the proposition of several models to account for the results observed (Yanuka, 1986).

All NMR experiments were performed using a Bruker NMR spectrometer operated at 500 MHz. All experiments were conducted at a constant temperature of 30°C. Prodrug concentrations of 1.0, 5.0, 10.0, 20.0, 30.0, 40.0 and 50.0 mg $cm^{-3}$ (sodium phenytoin equivalents) were made in $D_2O$ and analyzed. The use of 2,2,3,3-tetradeutero-3-(trimethylsilyl)propionic acid, sodium salt (Merck 90424) as an internal standard was found to be ineffective due to undergoing chemical shifts itself. With the use of an external standard as well as chloroform, it was established that the peaks in the spectra designated to $D_2O$ were fit to be used as the reference peaks.

## Results and Discussion

*DSC*

If the thermogram (Fig. 1) is considered, definite shifts in the peaks are found which indicate some or other interaction taking place between phenytoin and its prodrug. The thermogram of phenytoin shows a sharp peak at 295°C, its melting point. The thermogram of the prodrug (iii) shows a sharp peak at 175°C, its melting point, as well as a fairly broad flat peak from 240 to 300°C. This undefined peak is probably due to the fact that the prodrug degrades during the heating process with the formation of phenytoin and other degradation products. Melting of phenytoin in the presence of the other degradation products probably resulted in the broad peak obtained.

The thermogram of the combination of the two compounds (ii) resulted in a small peak cor-

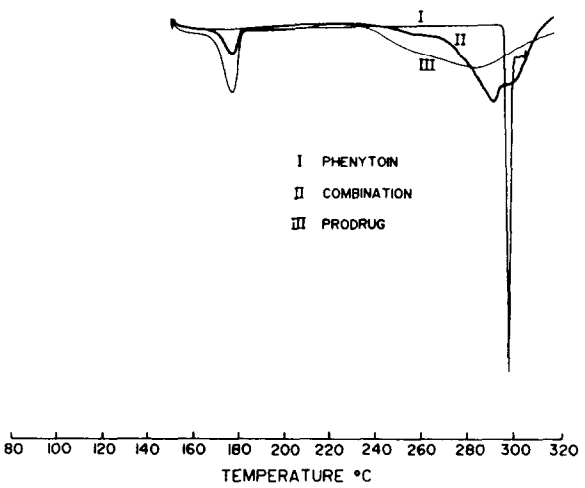



Fig. 1. A thermogram showing the melting points of phenytoin, its prodrug and a combination of the two.

responding with the prodrug, while a shift in the second peak to lower temperatures than those of phenytoin indicates that some or other interaction occurred between the two compounds. This method can only indicate an interaction between two or more compounds. No information on the type of the interaction, however, can be obtained.

The only conclusion to be made from these results is that the characteristics of both phenytoin and its prodrug are influenced when both of them are present in a mixture. The forces between the two types of molecules have a decrease in the melting point as a result. Although this method does not add to our knowledge of the location of phenytoin in the presence of the colloidal prodrug, it at least shows that both molecules have an influence on each other.

*X-ray crystallography*

Fig. 2 shows the relative positions and angles of the atoms of the prodrug molecule as determined with X-ray crystallography.

*$^1$H-NMR spectroscopy*

Fig. 3 shows a typical spectrum obtained from the prodrug in $D_2O$. This particular spectrum was obtained from a 50.0 mg cm$^{-3}$ sample, showing only one peak at about 7.1 ppm representing the protons of the two phenyl rings. Fig. 3 also shows the other signals representing the other proton-containing groups in the prodrug molecules as well as the chemical shifts for the protons in each group.

Fig. 4 shows a similar spectrum of a 1.0 mg cm$^{-3}$ prodrug solution. Compared to the spectrum at the higher concentration, the most outstanding difference is the two signals of the two phenyl groups found between 7.2 and 7.4 ppm. At 50.0 mg cm$^{-3}$ these two signals have shifted upfield merging into one at 7.1 ppm.

Fig. 5 shows the results obtained (Table 2) when the chemical shifts (Hz) of the proton-containing groups of the prodrug were measured as a function of the prodrug concentration. As mentioned before the $D_2O$ peak was identified as reliable enough to be used as a reference peak.

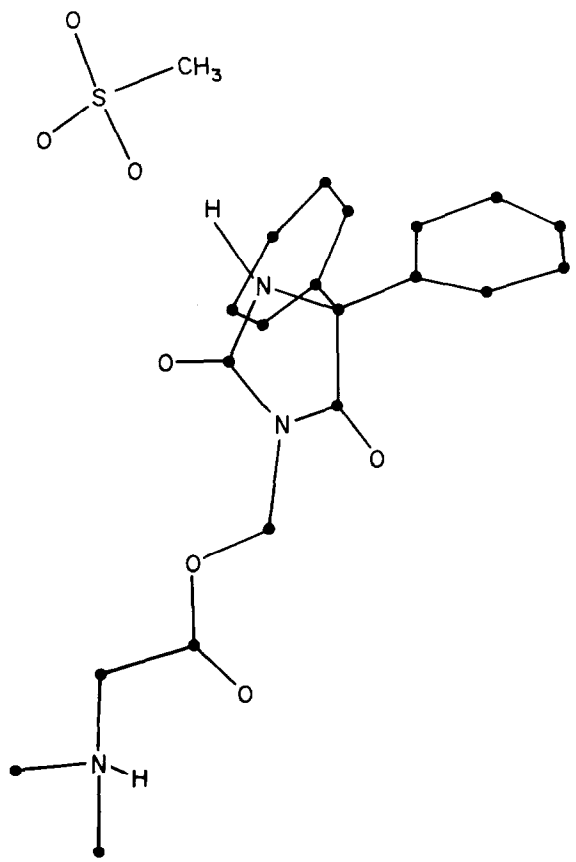



Fig. 2. The relative positions and angles of the atoms of the prodrug molecule as determined with X-ray crystallography.

Using the $D_2O$ signal as a reference the chemical shifts of the other signals were determined.

The change in chemical shifts ($\Delta$Hz) for the protons of each group was calculated as the differences found at high and at low prodrug concentrations. Since the chemical shifts can be taken as indications of the environment in which each species of proton finds itself, a change in that environment will be reflected in the chemical shift. The greater the change in the environment the larger the change in the chemical shift of the particular proton species.

The chemical shift for the protons of one of the phenyl groups was found to be the largest (111 Hz), followed by the protons of the -N-$CH_2$-O- group (85.6 Hz). As is shown by Fig. 2, one of the phenyl groups is situated sterically closer to the -N-H- group in the five-membered ring. It is possibly the protons of this phenyl group that are influenced the most by an increase in prodrug concentration and which showed the greatest increase in chemical shift. It is interesting to note that at the 50.0 mg $cm^{-3}$ concentration both phenyl groups showed the same chemical shift, which is an indication that both found themselves in a fairly similar environment.

Other valuable information to be obtained from Fig. 2 is the fact that the protons of the -N-$CH_2$-O- group show such a large chemical shift (85.6 Hz), even more than the protons of the second phenyl group.

Both these observations confirm the formation of micelles of prodrug molecules, probably due to hydrophobic forces in the aqueous environment. The magnitude of the chemical shift is an indication of the amount of inclusion into the micelle of each particular group.

The phenyl groups probably form the hy-

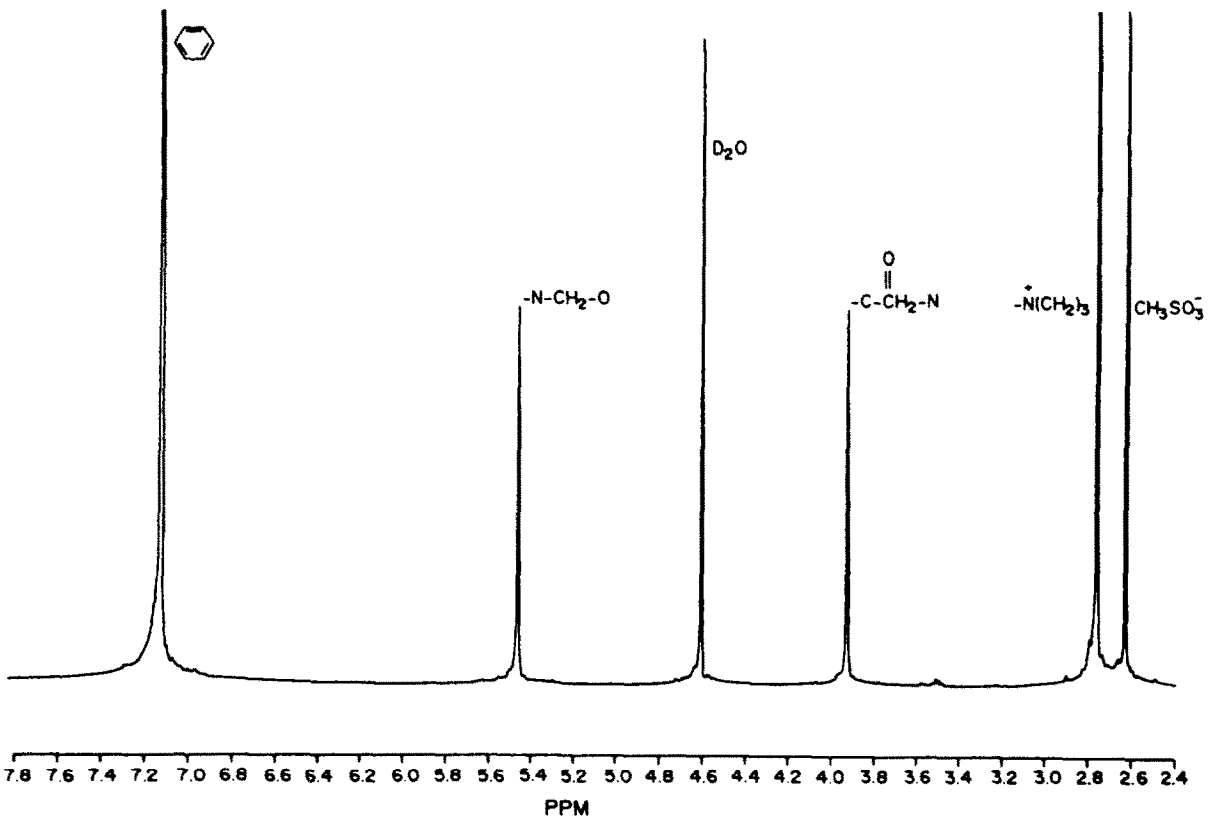



Fig. 3. NMR spectrum of a prodrug sample (50 mg $cm^{-3}$).

drophobic core of the micelles. The protons of the -N-$CH_2$-O- group, showing such an extensive chemical shift, are probably also included into the micellar interior although more to the surface of the micelle. The rest of the molecule probably forms the Stern layer while the quaternary ammonium groups are probably responsible for the formation of the cation exterior of the micelle. The fact that a trend can be recognized in the chemical shifts, from the highest found for the phenyl groups declining with each group situated further from the hydrophobic phenyl groups, most probably substantiates the theory that micelles are formed.

If the shape of the curves in Fig. 5 is compared with the curves obtained when the same method was applied for aqueous solutions of caffeine (Thakker et al., 1970), another difference is observed. In the case of caffeine, molecular stacking probably brings about the formation of the associated species. The curves for this type of self-association show large initial drops in the chemical shifts at low caffeine concentrations with a lesser decline at higher drug concentrations.

In the case of the prodrug the initial change in chemical shift is small, followed by an increased decline in chemical shift at higher prodrug concentrations. The difference can probably be attributed to differences in the type of associated species formed. Large chemical shifts for micellar type molecules obviously will only be possible at concentrations above the CMC.

The chemical shift for the prodrug was found to be more pronounced (0.222 ppm) over a com-

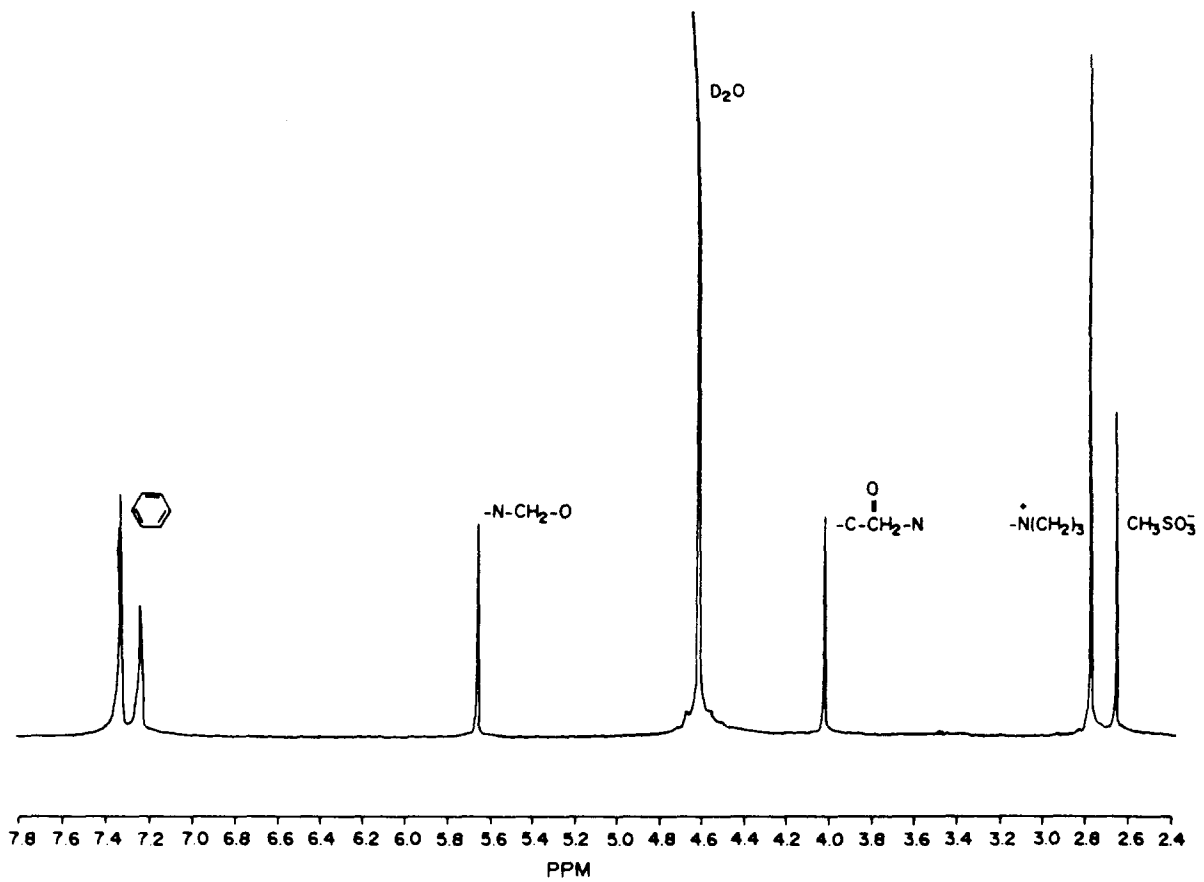



Fig. 4. NMR spectrum of a prodrug sample (1.0 mg $cm^{-3}$).

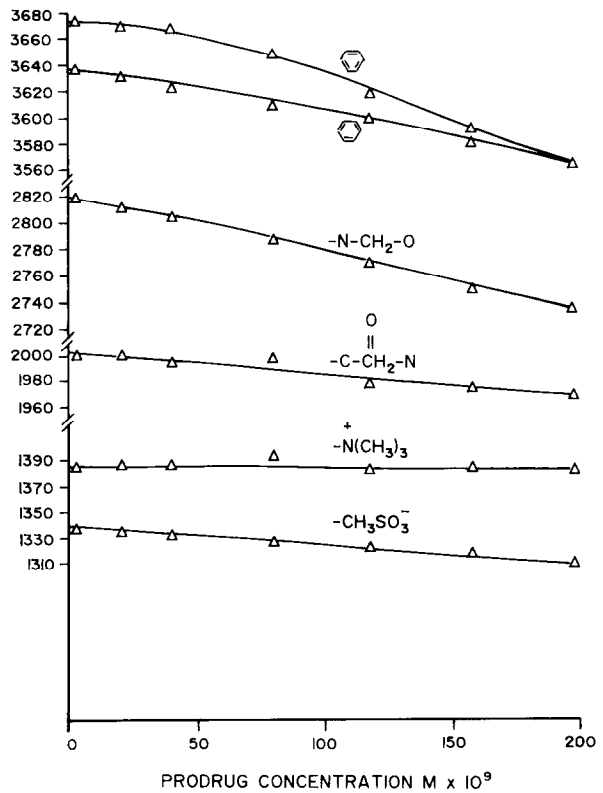



Fig. 5. Chemical shifts (Hz) of functional groups in the prodrug as a function of prodrug concentration.

parable concentration range than for aqueous caffeine solutions (Thakker et al., 1970).

## Conclusion

These results present ample proof that the prodrug of phenytoin, DDMS, probably forms a series of higher associative species at increasing concentrations. The major upfield chemical shift shown by the protons of the phenyl groups of the prodrug molecules demonstrates that the hydrophobic groups of the prodrug probably form the hydrophobic core of the micelles or aggregates, which enables the micelles or aggregates to include phenytoin, which in itself is fairly hydrophobic, into the species and thereby solubilize it.

The NMR results also showed that the labile ester binding of the prodrug was also submerged into the micelle which could have an effect on the hydrolysis rate. As was shown, the nearly 50% reduction in the rate of hydrolysis of the prodrug at higher prodrug concentrations can be attributed to the inclusion of the ester bond into the micelle.

TABLE 2

*Chemical shifts (Hz) of various protons in the prodrug molecule as a function of the initial prodrug concentration (M)*

| Concentration | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| mg/cm$^3$ | M | $-CH_3SO_3$ | $-N(CH_3)_3$ | $-\overset{O}{\overset{\|}{C}}-CH_2-N$ | $D_2O$ | $-N-CH_2-O$ | Phenyl | Phenyl |
| 0.5 | 0.0009 | 1337.6 | 1384.7 | 2000.3 | 2300.73 | 2818.5 | 3637.3 | 3674.3 |
| 1.0 | 0.0018 | 1336.4 | 1388.3 | 2004.3 | 2300.7 | 2817.4 | 3636.1 | 3672.9 |
| 2.0 | 0.0036 | 1336.3 | 1393.3 | 2009.8 | 2300.8 | 2817.3 | 3654.4 | 3672.4 |
| 3.0 | 0.0055 | 1334.9 | 1392.7 | 2008.8 | 2300.8 | 2815.2 | 3633.3 | 3672.4 |
| 4.0 | 0.0073 | 1334.8 | 1393.9 | 2009.6 | 2300.7 | 2814.1 | 3632.5 | 3671.6 |
| 5.0 | 0.0092 | 1334.4 | 1385.0 | 1997.9 | 2300.8 | 2811.1 | 3630.8 | 3670.4 |
| 10.0 | 0.0184 | 1332.3 | 1384.1 | 1994.2 | 2300.8 | 2804.4 | 3624.8 | 3667.6 |
| 15.0 | 0.027 | 1331.5 | 1394.6 | 2003.1 | 2300.7 | 2799.7 | 3620.2 | 3661.8 |
| 20.0 | 0.036 | 1327.8 | 1391.7 | 1996.2 | 2300.0 | 2788.6 | 3608.2 | 3647.5 |
| 30.0 | 0.055 | 1322.3 | 1381.0 | 1977.0 | 2300.0 | 2767.4 | 3590.0 | 3618.0 |
| 40.0 | 0.073 | 1318.6 | 1382.8 | 1974.1 | 2300.7 | 2751.7 | 3580.0 | 3591.3 |
| 50.0 | 0.091 | 1309.7 | 1381.6 | 1968.1 | 2299.1 | 2734.9 | 3565.4 | 3565.4 |
| $\Delta$Hz | | $\pm$27.9 | 7.0 | 38.3 | – | 85.6 | 74 | 111 |
| $\Delta\delta$ (ppm) | | +0.056 | +0.014 | +0.076 | | +0.171 | +0.148 | +0.222 |

It thus can be concluded that, if a prodrug of a poorly water soluble drug shows micellar characteristics, the possibility will exist that the precipiation times of such formulations will be increased which in turn will increase the shelf lives of such formulations.

## References


Attwood, D. and Udeala, O.K., Aggregation of antihistamines in aqueous solution. The effect of electrolyte on the micellar properties of some diphenylmethane derivatives. *J. Pharm. Pharmacol.*, 27 (1975a) 395–399.

Attwood, D. and Udeala, O.K., The surface activity of some antihistamines at the air-solution interface. *J. Pharm. Pharmacol.*, 27 (1975b) 754–758.

Attwood, D. and Udeala, O.K., Aggregation of antihistamines in aqueous solution showing self-association of some pyridine derivatives. *J. Phys. Chem.*, 79 (1975c) 889–892.

Botha, S.A., Du Preez, J.L. and Lotter, A.P., DSC screening for drug-drug and drug-excipient interactions in polypharmaceuticals intended for the alleviation of the symptoms of colds and flu: I. *Drug Dev. Ind. Pharm.*, 12 (1986) 811–827.

Felmeister, A., Relationships between surface activity and biological activity of drugs. *J. Pharm. Sci.*, 61 (1972) 151–164.

Hardy, M.J., Drug-excipient compatibility prediction by DSC. *Anal. Proc.*, 19 (1982) 556–557.

Kan, L.S., Borer, P.N., Cheng, D.M. and Ts'O, P.O.P., Proton and carbon-13 NMR studies on caffeine and its interaction with nucleic acids. *Biopolymers*, 19 (1980) 1641–1654.

Muller, D.G., Stella, V.S. and Lotter, A.P., Factors influencing the precipitation time of phenytoin in the presence of DDMS, one of its prodrugs. *Int. J. Pharm.*, 75 (1991) 201–209.

Smith, A., Use of thermal analysis in predicting drug-excipient interactions. *Anal. Proc.*, 19 (1982) 559–561.

Thakkar, A.L., Tensmeyer, L.G., Hermann. R.B. and Wilham, W.L., Selfassociation of caffeine in aqueous solution: Nuclear magnetic resonance study. *J. Chem. Soc. D*, 9 (1970) 524–525.

Thoma, K. and Siemer, E., Kolloidassociation von Antihistaminica. 1. Mitterlung beziehungen zwischen chemischer struktur und kritischer mizell bildungs konzentration. *Pharm. Acta Helv.*, 51 (1976) 50–58.

Varia, S.A., Schuller, S., Sloan, K.B. and Stella, V.J., Phenytoin prodrugs: III. Water soluble prodrugs for oral and for parenteral use. *J. Pharm. Sci.*, 73 (1984) 1068–1073.

Yanuka, Y., Zahalka, J. and Dondrow, M., A symmetrical model for the self-association of xanthines in aqueous solution. *J. Chem. Soc. Perkin Trans.*, II (1986) 911–915.