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The determination of the amphiphilic properties of a prodrug (DDMS) of phenytoin in aqueous media

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Summary

During a previous study it was established that a change in the intrinsic solubility of phenytoin in the presence of its prodrug (DDMS), and a change in the rate of hydrolysis of the prodrug in the presence of higher concentrations thereof, could account for increases in precipitation times of aqueous prodrug solutions. The 50% reduction in the rate of hydrolysis observed at higher prodrug concentrations and the 45-fold increase in the solubility of phenytoin at therapeutic levels of the prodrug were both attributed to the observed increase in precipitation times. In an effort to explain the observed changes in solubility and kinetics a series of experiments were performed to determine the extent of the formation of amphiphilic structures in aqueous media. The presence of associative species in aqueous prodrug solutions was established by tensiometry, conductivity and dynamic light scattering experiments. Electron microscopy showed spherical shapes with sizes which compared favourably with the sizes found with the laser and Kratel particle counter techniques.

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 (Florence, 1968; Felmeister, 1972).

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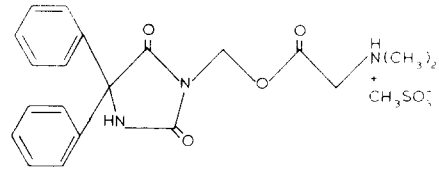
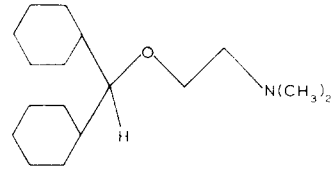
Drugs represent a large variety of amphiphilic structures, ranging at one extreme from the cationic quaternary ammonium germicides, which are easily recognised as typical surfactants, to more complex aromatic or heterocyclic molecules with surfactant characteristics which are not easily recognised. Typical surfactants have hydrocarbon groups which can intertwine during the micellization process to form spheroidal aggregates. Replacement of this flexible hydrophobic moiety with a ridig 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 pirimidine bases of nucleotides which associate by a stacking process. This self-association process is generally continuous, that is, there is no equivalent to a critical micelle concentration (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 a group of drugs which are derivatives of diphenylmethane, such as the prodrug studied (Scheme 1).



N,N-DIMETHYLGLYCINE ESTER OF 3-HYDROXYMETHYL-5,5-DIPHENYLHYDANTOIN MONOMETHANESULFONATE SALT



DIPHENHYDRAMINE

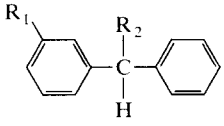
Scheme 1. Chemical structure of the prodrug and diphenhydramine showing the diphenylmethane structure.

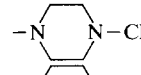
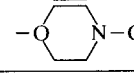
The presence of a CMC may be viewed as a criterion of a micellar type of association. However, it is necessary to examine a system using a variety of techniques to compare the CMC values obtained from inflections of the data. Table 1 (Attwood and Udeala, 1975a–c; Thoma and Siemer, 1976) shows a comparison for several antihistamine drugs. Various techniques were used which resulted in CMC values which differed. The general agreement, however, found between CMC values is evidence that the process of aggregation is indeed micellar. Light-scattering measurements on these compounds (Attwood, 1972) produce plots showing inflections which for conventional surfactants would be identified with the CMC. Aggregation numbers are, however, much lower (usually around 9–12 monomers per micelle in the absence of added electrolyte) than those of flexible chain surfactants.

Changes in the flexible basic diphenylmethane structure have interesting effects on the aggrega-

TABLE 1

Micellar properties of some diphenylmethane antihistamines in water



Drug	R_1	R_2	CMC (mol dm^{-3})	Aggregation number
Diphenydramine 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 techniques including conductivity surface tension and light scattering (Attwood and Udeala, 1975a–c).

tion characteristics. Flexibility of the diphenylmethane group is essential to form micellar aggregates. Replacement of one of the phenyl rings of the diphenylmethane moiety with a cyclohexane ring considerably increases hydrophobicity, as is evident by lower CMC and higher aggregation numbers (Attwood, 1976a,b). When the two phenyl rings of the diphenylmethane moiety are linked as a rigid group, the association no longer shows a monodisperse micellar system (Attwood et al., 1980). The results obtained indicate the influence of the structure of the hydrophobic group on the association pattern. A rigid ring seems to promote non-micellar association.

During a previous study (Müller et al., 1991), it was found that the precipitation time of aqueous solutions of 3-(*N*,*N*-dimethylglycyloxymethyl)-5,5-diphenylhydantoin methanesulfonate (DDMS), a prodrug of phenytoin, was increased due to a decrease in rate of hydrolysis as well as to an increase in the solubilization capacity of phenytoin by the prodrug solutions. In an effort to explain this behavior of aqueous prodrug solutions, a study was undertaken in the attempt to determine the characteristics of the associative species (if any) of the prodrug which may be present in aqueous media.

Materials and Methods

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 with differential scanning colorimetry (DSCA, Perkin Elmer Corp., Norwalk, CT) and HPLC. The amount of phenytoin contamination present in the prodrug was determined by HPLC (Altex Model 110A pump and a Waters Associates Model 450 variable-wavelength detector).

The water used in the determination of self-association studies was deionized ($< 0.2\ \Omega^{-1}$ conductance) and distilled from an all-glass still. All glassware used in these studies were thoroughly rinsed with deionized water and dried.

Determination of the amphiphilic nature of aqueous prodrug solutions

A variety of methods have been described in the literature on the determination of associative species present in solution of various drugs. Typical colloidal behavior is exhibited by many drugs from several pharmacological groups of compounds including the local anaesthetics, antidepressives, tranquilizers, antibacterials and antibiotics (Florence, 1968; Felmeister, 1972). Self-association of antihistamines has been studied by Farhadieh et al. (1967) and Attwood (1972). The methods used included vapor pressure osmometry, conductivity, tensiometry, light scattering and spectrophotometry (Kan, 1980; Menozzi et al., 1984; Yanuka et al., 1986). Even infrared spectroscopy has been used to determine associative species of hydantoins (Sohar, 1968).

Determination of critical micelle concentrations (CMC)

Experiments were performed to determine whether various aqueous solutions of prodrug showed amphiphilic characteristics.

Conductivity The molar conductivity was determined at 25 ± 0.1°C. The conductivities of prodrug solutions at various concentrations were determined in water. The apparatus (Electro Mark Conductivity analyzer, Markson Science Inc., Demar, CA) was standardized using standard solutions of potassium chloride in water.

Tensiometry The surface tensions as a function of initial prodrug concentration were determined using a Du Nouy tensiometer (Central Scientific Co., Chicago). After calibration of the tensiometer a correction factor of 0.36 was determined for the apparatus.

Determination of the presence of associative species in aqueous prodrug solutions

Determination of the quantity and size of the associative species of prodrug formed in water Several methods exist to measure the distribution of particle sizes in solution, when the particle

diameter is more than 1 μm. These include conventional light microscopy, electron microscopy, resistive-pore (Coulter) counting and diffaction analysis. However, for particle diameters significantly smaller than the wavelength of light (i.e., less than 500 nm), the choices of instrumentation become much more limited.

Recently advances in microelectronics have made possible the design of compact integrated instrument systems which can discriminate between particles sizes easily and accurately. Two of these methods, scattering and light blockade using microelectronics, have been carried out to determine whether some or other form of self-association of the prodrug as a function of prodrug concentration, could be detected. Two methods, namely the Kratel particle counting system and Nicomp laser light scattering, were used in this study.

Kratel particle counting system — Introduction: The particles to be tested pass one at a time through an illuminated optical cell. Each particle passing the cell extinguished partially a continuous collimated light beam, causing a reduction in the amount of light reaching the photodiode placed directly opposite the light source. The resultant voltage pulse is directly proportional to the maximum projected area of each individual particle interrupting the light beam. The pulses are amplified and classified by a Data Evaluation System and used for particle size determination (diameter of a spherical particle which produces the same extinction effect) in the module EDM 32 or the Minicomputer. Measurements are unaffected by variations in color, density and refractive index of particles and the suspending fluid.

The smallest detectable size and the highest possible concentration of particles to be measured with the system depend on the design of the optical cell in the detection system. The cell used was effective over the particle size range of 1–50 μm. The apparatus was calibrated using mono-sized spheres.

Method: The Kratel Partoscope F Model F/SF7, fitted with a sensor, sample feeder, data handler and a printer, was used to determine the amount and size of prodrug particles present in aqueous solutions. Various concentrations of prodrug (1.0, 5.0, 10.0, 20.0, 30.0, 40.0 and 50.0 mg cm^{-3}) were made in double-distilled water which was specially prepared by filtration (Millipore Corp., Bedford, MA) through 0.25 μm filters. Each solution then was again filtered (0.25 μm) three times to remove any possible insoluble material. Three aliquots of each solution were then immediately subjected to analysis. The sample volume for the apparatus was 1.0 cm^3 and a flow rate of 0.8 cm^3 per min was used. The amount of particles detected and the particle size distribution were determined.

Dynamic Light Scattering — Introduction: The technique of dynamic light scattering is available to measure particle size in solutions over a wide range – from a few microns down to a few Ångstroms. Such measurements, requiring only that laser light be passed through the sample solution, can be made automatically and quickly. Unlike classical light scattering, dynamic light scattering is concerned not with the average intensity, but rather with the time behavior of the fluctuations in the scattered intensity. Particles in solution continually undergo random Brownian motion due to solvent collisions. This results in fluctuations in the scattered light intensity at the photomultiplier detector due to changes in the net interference of individual scattered waves originating from each particle. Their phases depend on the precise position of each particle relative to both the incident exciting laser beam and the detector.

Dynamic light scattering possesses several distinct advantages over its classical predecessor. First, it is able to provide information about the particle size distribution, which is lost in a simple measurement of the total scattering intensity. Second, it eliminates the need for precise standard solutions of known concentration and particle size. The calibration for the dynamic measurement is ensured by a crystal-controlled time base, fixed laser wavelength and known solvent parameters. Instrumental drift and warm-up errors are essentially non-existent. Third, reliable particle size measurements can usually be made without regard for particle concentration, over a wide range of concentrations. If there exist significant interparticle interactions (e.g., Coulomb

electrostatic repulsions between charged particles in solutions of low ionic strength), one can measure R_h for a series of sample dilutions and extrapolate the value to zero particle concentration. The larger the particle size, the smaller the concentration needed to produce an acceptable level of scattered intensity. Since the latter is proportional to the product of concentration (w/v) and molecular mass (i.e., R^3), it is easy to arrive at some approximate benchmarks. For globular proteins of molecular mass 50000 Da the typical concentration required is 0.5–1% w/v (i.e., 5–10 mg cm^{-3}). For latex spheres of diameter 0.1 μm, less than 0.002% w/v is required to produce a reliable mean size and width.

Method: Like the methods used with the Kratel Partoscope, various concentrations of prodrug solutions in specially prepared dust-free (through filtration) water were subjected to analysis. Concentrations from 20.0 to 50.0 mg cm^{-3} were analyzed in the hope of finding a drastic change in size of the associative species around the expected CMC. The 32.0 mg cm^{-3} samples were analyzed twice, the second analysis being performed after 24 h to determine whether time has an influence on the size of the associative species formed. The same sample was subjected to extensive (three times) filtration (0.45 μm filters) to establish this influence on the sizes of the associative species. After each solution was made, it was put into special cuvettes into the Nicomp automatic analyzer.

Visualization of the associative species with electron microscopy Because the methods used to determine particle size and distribution showed the presence of unexpected large structures, use was made of electron microscopy to visualize the structures.

Introduction: Transmission electron microscopic methods can be used (Schoefl, 1968; Pamperl and Kleinberger, 1982) to study intralipid liposomes. Although it is not a general method to determine the sizes of drops in emulsions, it can give valuable information since the drops are visualized so effectively. A considerable problem with such a method is to fix the structures in solution without changing the original size and structures of the species involved. In both works

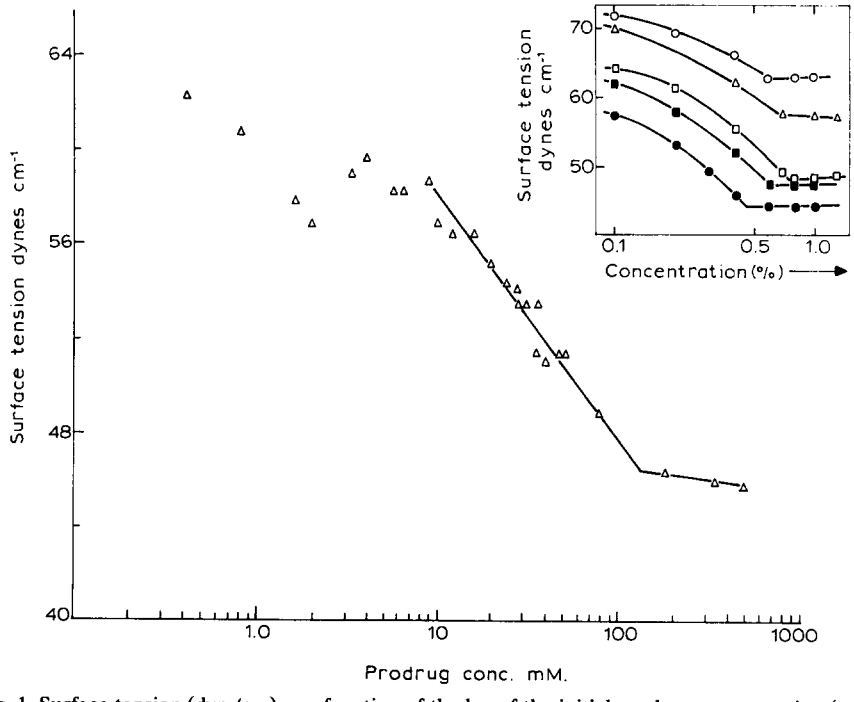


Fig. 1. Surface tension (dyn/cm) as a function of the log of the initial prodrug concentration (mM).

mentioned above, centrifugation was used as well as fixation.

Since centrifugation, being an additional force in the solution, may alter existing structures in the solution, the use thereof was questioned. In a study by Du Plessis (1986) the two methods mentioned, and that of Henstra and Schmidt (1974) were evaluated for their efficacy and reproducibility to determine the size of liposomes.

It was also shown by Du Plessis (1986) that unbuffered 2% OsO_4 was a better alternative as a fixative than those previously mentioned.

A carrier medium consisting of nucleopore filters (Nucleopore Corp., Pleasanton, CA; 13 mm in diameter) with pore sizes of 0.4 μm was used to fix the aqueous prodrug solutions. These filters were found to be adequate for use in electron microscopy because of their uniform pore size while the structure of the filters was found to show good contrast with the species observed in solution.

Method: A prodrug solution (50.0 mg cm^{-3}) in double distilled water was filered (as before) three times before another filter was dipped into the solution. The prodrug solution which adsorbed onto the filter was then fixed in unbuffered 2.0% osmium tetroxide (Taab Laboratories Equipment Ltd, Reading, U.K.) solution for 10 min.

After this the fixated product was removed and left in water until photographed.

Results

In an effort to determine whether associative species exist in solution, and their numbers and sizes, several experiments were performed to determine the colloidal properties of various aqueous prodrug solutions.

Determination of critical micelle concentrations

Tensiometry

The results of this experiment are presented in Fig. 1. The surface tension (dyn cm^{-3}) was plotted as a function of the log of the prodrug concentration. The curve shows a general decline in surface tension with increase in prodrug concentration, with two breaks: one at 1.5–3 mol dm^{-3} and a breakpoint at around 0.11 mol dm^{-3} (30.0

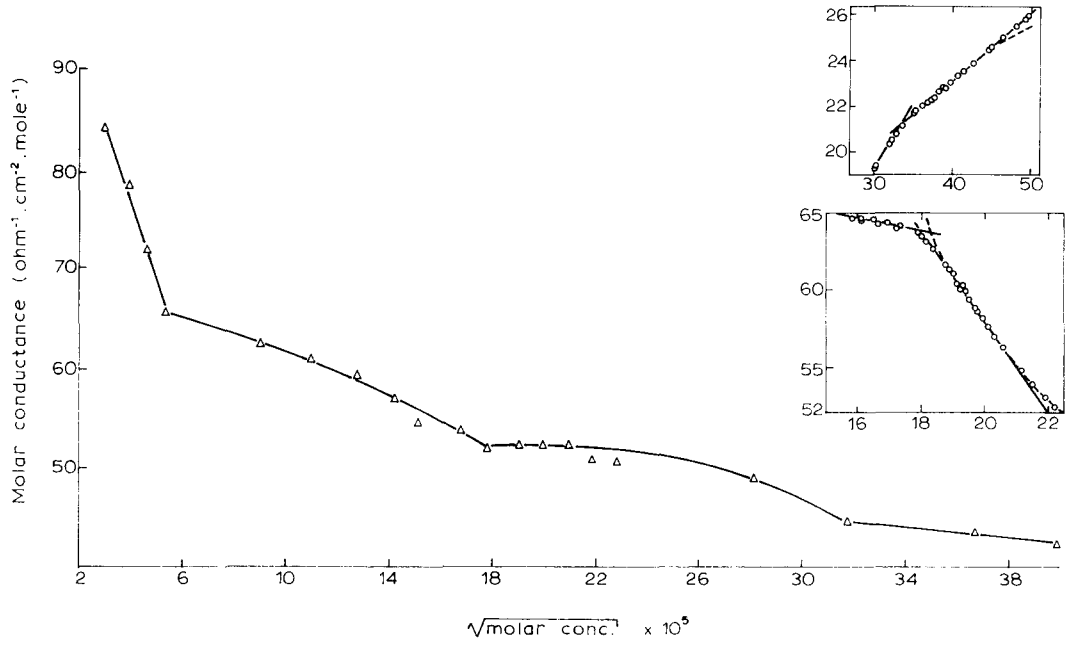


Fig. 2. Conductivity measured as a function of the molar concentration of the prodrug.

mg cm^{-3}) concentration. The break in the curve is an indication of a CMC or phase transition for the prodrug at 0.11 mol dm^{-3}. This value corresponds excellently with that of 0.14 mol dm^{-3} found for diphenhydramine HCl (Thoma and Siemer, 1976) which has a similar diphenylmethane structure (Scheme 1) and a branch consisting of four groups compared to the five groups of the prodrug.

The insert in Fig. 1 shows similar curves of the surface tension found for various antihistamines in 0.9 sodium chloride solutions (Thoma and Siemer, 1976). The curve in Fig. 1 obtained for the prodrug shows similar characteristics, with a curved decline in surface tension with increase in the prodrug concentration.

Conductivity

Changes in the conductivity of a solution as a function of concentration, like surface tension, can be used to measure a potential CMC for a certain compound in a particular solvent.

Conductivity experiments were performed and the results were plotted (Fig. 2) as a function of the square root of the molar concentration. The insets in Fig. 2 demonstrate the use of molar conductance vs the square of the concentration to show a possible CMC.

The curve obtained shows four distinct linear or semi-linear regions with three possible breakpoints which can be interpreted as possible CMCs. It is interesting to note that the breakpoint at higher prodrug concentrations correspondence to a CMC of 0.102 mol dm^{-3} which is very similar to that determined by surface tension measurements. The other two breakpoints observed are probably caused by the formation of associates of a lower order due to self-association of the prodrug. Those associations formed are probably non-micellar associations.

Determination of the quantities and sizes of species in aqueous prodrug solutions

Two methods were used to measured the particle sizes and the distribution of prodrug molecules in water. Each aqueous prodrug solution was subjected to extensive filtration before analysis. The rationale for this approach was that the filters used (0.25 μm) allowed possibly only monomeric or smaller prodrug molecules to pass through the pores. A structure or 'particle' observed after completion of the filtration process must have been due to the reformation of self-associative species.

TABLE 2

Particle size distribution and the amount of particles detected as a function of the initial prodrug concentration

Prodrug concentration (mg/cm^3)	Amount of particles	Particle size (μm)		
		Min	Mean	Maximum
1.0	–	–	–	–
5.0 a	364	1.08	1.89	3.5
b	109	1.02	1.25	5.4
c	210	1.07	1.37	4.6
10.0 a	382	1.11	3.32	23.3
b	352	1.07	2.41	12.3
c	345	1.02	2.65	13.2
20.0 a	85	1.10	2.06	16.7
b	40	1.50	5.50	19.1
c	76	1.11	3.64	18.5
30.0 a	887	1.14	2.53	13.2
b	624	1.09	3.44	19.2
c	756	1.06	3.21	17.3
50.0 a	1797	1.15	1.82	9.5
b	2419	1.07	5.08	22.5
c	2205	1.09	4.64	19.5

Kratel particle counting system

The particle size distribution as well as the amounts detected with this method are listed in Table 2. The first important observation to be made is the fact that particles are found in solution, even after extensive filtration. The size of the particles is unexpectedly large when the pore size (0.25 μm) of the filters used is taken into consideration. This observation is proof that the prodrug molecules self-associate to form self-associative micellar or non-micellar species in aqueous solutions.

Another important observation to be made is that no associative species was found at prodrug concentrations lower than around 5.0 mg cm^{-3}. From this finding it must be concluded that ei-

ther the particles present are so small that observation by the apparatus is impossible, or that formation of associative species at prodrug concentrations lower than 5.0 mg cm^{-3} does not occur. The minimum particle size detectable by this counting system is 1 μm with a maximum of 50 μm.

The particle counting system which is controlled by a computer program is assisted by a statistical program. Apart from registering the amount of particles, it is also capable of determining the average particle size present, as well as minimum and maximum particle sizes registered. The mean sizes recorded seem not to be prodrug concentration dependent, since all the species ranged from 1.25 to 5 μm in size. It is only at prodrug concentrations of 5.0 mg cm^{-3} where sizes in the range of 1 μm were recorded, compared to sizes of 5.50 and 5.08 μm registered at 20.0 and 50.0 mg cm^{-3} prodrug concentration, respectively. It thus can be concluded that the standard sizes recorded for the associative species ranged from around 1.0 to 5.0 μm and that no clear relationship exists between the sizes of associations and prodrug concentration. The sizes of associations recorded are unexpectedly high as sizes of 1–5 μm for the associative species are extraordinary and may be an indication of phase transition and higher aggregates. One should expect a gradual increase in species size with increase in prodrug concentration.

If the number of particles observed are considered, some trend of the amount of particles as a function of prodrug concentration can be found. At 5.0 mg cm^{-3} the average amount of particles recorded per sample aliquot (0.8 cm^3) is 227. A higher average amount (350) of particles is found at 10.0 mg cm^{-3} prodrug concentration. However, at 20 mg cm^{-3} concentration an unexpected drop in the amount of particles was recorded. At 30.0 mg cm^{-3} the amount increases again as expected with an average of 2140 particles registered at the 50.0 mg cm^{-3} prodrug concentration. The relatively small number of particles observed at the 20.0 mg cm^{-3} concentation is probably because less but larger species are formed at this level. Although the average particle size does not indicate an increase in species sizes, the maximum species sizes recorded seem to be higher than at 10.0 and even at 30.0 mg cm^{-3}.

TABLE 3

Particle size distribution as a function of the initial prodrug concentration as determined with the Nicomp Laser Computing Autocorrelator

Prodrug concentration (mg/cm^3)	Particle size distribution		
20.0	639 nm	2.0 μm	2.6 μm
30.0	700 nm	1.0 μm	1.4 μm
32.0	800 nm	1.4 μm	2.0 μm
32.0 (24 h)	–	1.4 μm	2.0 μm
34.0	480 nm	575 nm	800 nm
36.0	900 nm	1.0 μm	2.8 μm
40.0	982 nm	1.4 μm	2.02 μm
45.0	846 nm	1.2 μm	–
50.0	621 nm	–	4.0 μm

From these results it is evident that at all prodrug concentrations, except at levels lower than 5.0 mg cm^{-3}, associative species were formed in solution. Except for prodrug concentrations around 20.0 mg cm^{-3}, a gradual increase in the amount of associative species with increase in prodrug concentration was registered. The average associative species sizes were found to vary between 1.0 and 5.0 μm.

Dynamic light scattering

The Nicomp laser computing autocorrelator used was capable of measuring particle sizes much smaller (25 nm–5 μm) than those determined with the Kratel particle counting system mentioned above. Unfortunately, data (Table 3) of sizes of associative species lower than 20.0 mg cm^{-3} of prodrug are not available. The apparatus used was only able to determine the particle size distribution and not the amount of particles present per unit volume. Like the Kratel experiment no general trend is found in particle sizes with respect to prodrug concentration. Species sizes ranging from 500 nm to 4.0 μm were recorded which corresponds excellently with the sizes obtained with the Kratel experiment.

Another interesting observation was that prodrug concentrations (32.0 mg cm^{-3}) left for 24 h after analysis showed the same species size distri-

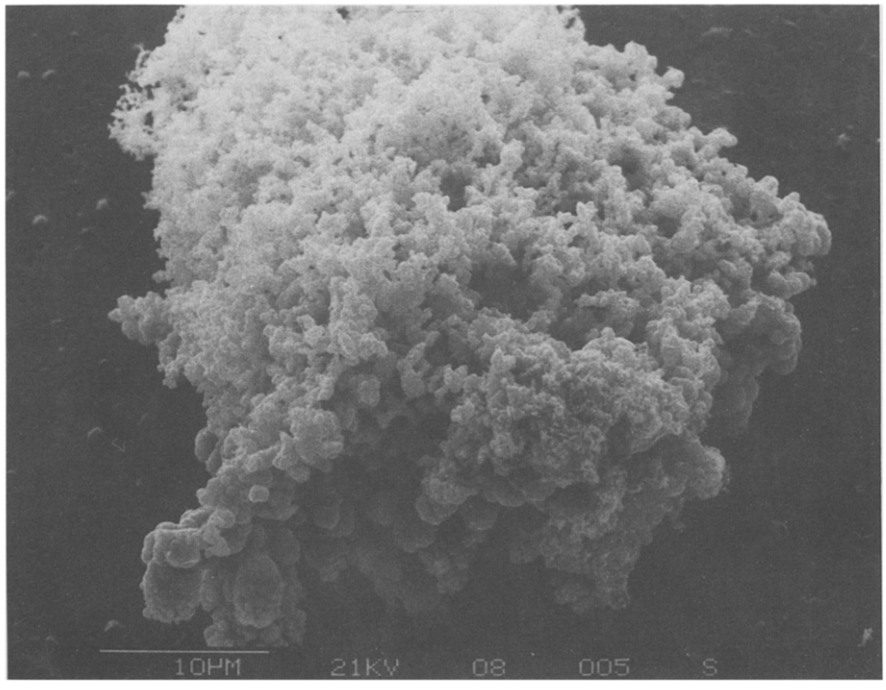


Fig. 3. Electron microscopic photo of the associative species found in aqueous media at $\times 2000$ enlargement.

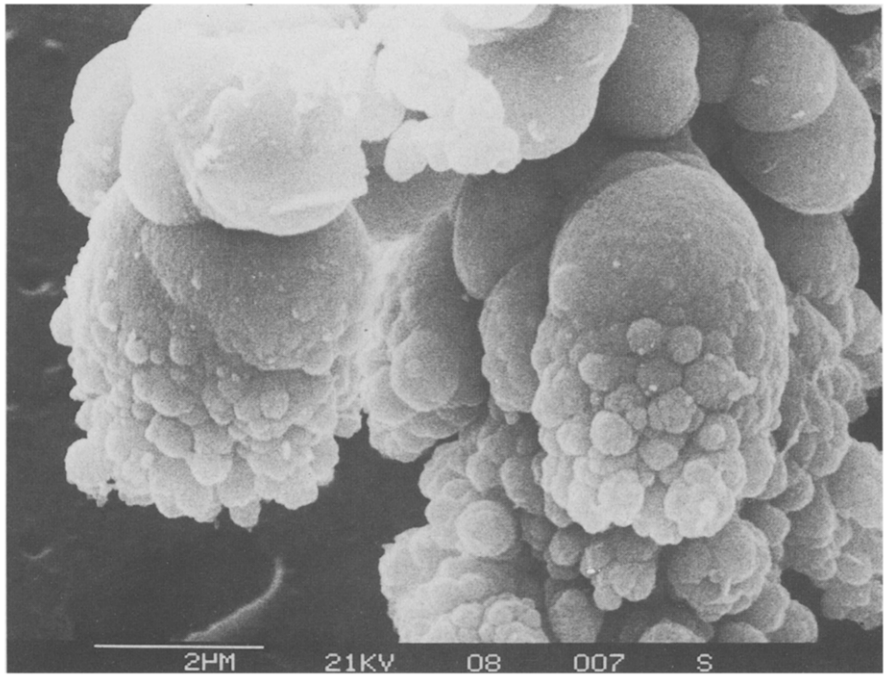


Fig. 4. Electron microscopic photo of the associative species found in aqueous media at $\times 11\,000$ enlargement.

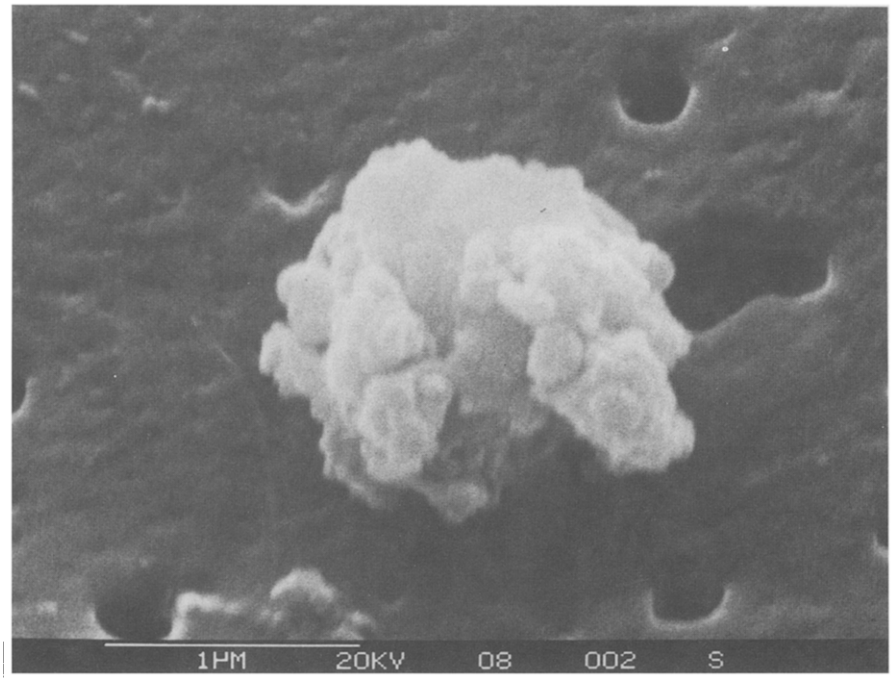


Fig. 5. Sandwich-type stacking of the associative species found in aqueous media at $\times 30\,000$ enlargement.

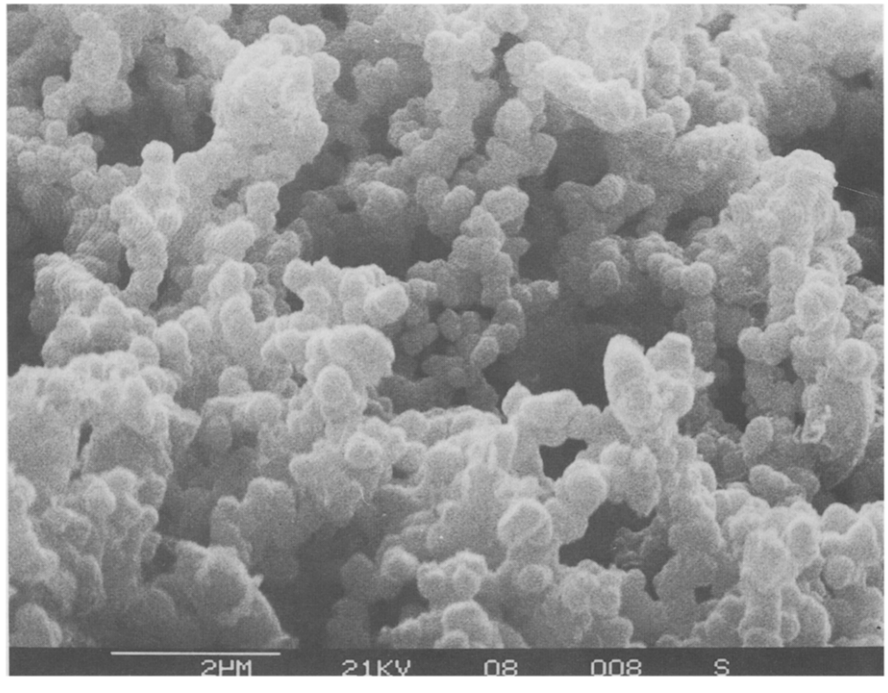


Fig. 6. Chain-like type of associations of DDMS in aqueous media at $\times 21\,000$ enlargement.

bution. It was also found that repeated filtration of the same solution had no effect on the particle size distribution. Formation of the associative species thus was not time dependent and occurred instantaneously.

No significant differences in species sizes was seen around the apparent CMC or phase transition. From these results it can be deduced that the existence of the CMC or phase transition is probably more dependent on the amount of species present and not the sizes thereof.

As is the case with the Kratel particle counting system, no doubt can exist that aqueous prodrug solutions show colloidal behavior. Aqueous solutions of prodrug probably form small micellar or non-micellar associative species at lower concentrations while at levels above 32 mg cm^{-3} (the CMC for these solutions) higher micellar species or aggregates are probably formed. It can be concluded that the structure of the prodrug is such that it shows both non-micellar as well as micellar characteristics. It is also evident that formation of micellar associations does not depend on a change in the size of the associative species.

Electron microscopy

A variety of excellent transmission electron microscopic pictures were obtained. The electron microscope used was able to show whether or not the structures in the sample contained osmium. This is very helpful to distinguish between organic and inorganic material, because it is only the organic material present which would react with the fixating agent osmium tetroxide.

Figs 3 and 4 show the same associative species at increasing enlargement. The structure in Fig. 3 (×2000 enlargement) shows clusters of spherical structures that are bunched together. Fig. 4 (×11 000 enlargement) clearly shows the spherical nature of the associative species.

Fig. 5 shows at very high enlargement (×30 000) flat, sandwich-type stacking of the structures to form spherical structures. The sizes of the structures found compare favorably with those of the particles measured with the laser and Kratel particle counters.

Fig. 6 taken at 21 000 enlargement shows different chain-like type associations. These associates may be the initial structures formed with the carrier medium in the background. Although much smaller, the associates show spherical units which are grouped together in a chain-like fashion. The units in this slide have an approximate size of 500 nm which is not uncommon for these associates.

Conclusion

Self-association of the prodrug has been demonstrated to occur because only monomers of dimers are allowed through the filters. However, much larger associates, unable to penetrate the pores of the filters, are found in the filtrate. This obviously means that self-association of the prodrug must take place after filtration.

The structure of the associates is spherical in shape, while evidence of flat sanwich-type associates is also present. The sizes of the associates observed with electron microscopy are similar to those determined with the various particle counting techniques.

The formation of large associates seems to occur via the merging of smaller associates into larger associates. In other words, evidence for step-like self-association of the prodrug molecules was found.

Both tensiometry and conductivity experiments confirmed the existence of CMCs that compared favourably with those of similar substances in the literature.

It can thus be concluded with a high degree of certainty that the prodrug (DDMS) of phenytoin forms associative species in aqueous media. These species are probably responsible for the decrease in the rate of hydrolysis of the prodrug as well as for the increase in solubility of phenytoin in the presence of its prodrug, observed in previous experiments.

References

Attwood, D. Micelle formation by some antihistamines in aqueous solution. *J. Pharm. Pharmacol.*, 24 (1972) 751–751.

Attwood, D., Aggregation of antiacetylcholine drugs in aqueous solution showing micellar properties of some diphenylmethane derivatives. *J. Pharm. Pharmacol.*, 28 (1976a) 407–409.

Attwood, D., Micellar and nonmicellar association of antiacetylcholine drugs in aqueous solution. *J. Phys. Chem.*, 80 (1976b) 1984–1987.

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) 395–401.

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.

Attwood, D., Agarwal, S.P. and Waight, R.D, Effect of the nature of the hydrophobic group on the mode of association of amphiphilic molecules in aqueous solution. *J. Chem. Soc. Faraday Trans. I*, 76 (1980) 2187–2192.

Du Plessis, J., Die stabiliteit van emulsies in parenterale voedingsmengsels. Potchefstroom, p. 162, Verhandeling (M.Sc.), PU vir CHO, 1986.

Farhadieh, B., Hall, N.A. and Hammarlund, E.R., Aggregation of certain medicinal amines in aqueous solutions of their salts. *J. Pharm. Soc.*, 56 (1967) 18–23.

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

Florence, A.T., Surface chemical and micellar properties of drugs in solution. *Adv. Colloid Interface Sci.*, 2 (1968) 115–149.

Henstra, S. and Schmidt, D.G., The microcapsule technique, an embedding procedure for the study of suspensions and emulsions. Application note for LKB-produkter AB, Sweden, 1972, pp. 1–4.

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.

Menozzi, M., Valentini, L., Vannini, E. and Acramone, T., Self-association of doxorubicin and related compounds in aqueous solution. *J. Pharm. Sci.*, 73 (1984) 766–770.

Muller, D.G., Stella, V.J. 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.

Pamperl, H. and Kleinberger, G., Morphologic-changes of intralipid 20% liposomes in all-in-one solutions during prolonged storage. *Infusionsther. Klin. Ernabrung*, 9(2) (1982) 86–91.

Schoefl, G.I., The ultra structure of chylomicrons and of the particles in the artificial fat emulsion. *Proc. Roy. Soc. B.*, 169 (1968) 147–152.

Sohar, P., NH stretching vibration bands at wave numbers lower than 3000 cm^{-1}. VI. Cyclic dimeric structures of Hydantoin derivatives. *Acta Chim.*, 57 (1968) 425–444.

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) 911–916.