ELSEVIER International Journal of Pharmaceutics 134 (1996) 59–70

international
journal of
pharmaceutics

Characterization of the self-association properties of a leukotriene D_4 receptor antagonist, MK-0476

Roch Thibert[a,*], Henryk Mach[b], Sophie-Dorothée Clas[a], Dale R. Meisner[b], Elizabeth B. Vadas[a]

[a]*Pharmaceutical Research and Development, Merck Frosst Centre for Therapeutic Research, P.O. Box 1005, Pointe Claire-Dorval, Quebec, Canada, H9R 4P8*

[b]*Department of Pharmaceutical Research, Merck Research Laboratories, WP78-302, West Point, PA, USA 19486*

Received 6 June 1995; revised 25 October 1995; accepted 13 December 1995

Abstract

The development of a liquid dosage form requires a comprehensive understanding of physicochemical properties unique to solutions. The objective of this study was to investigate the behaviour of MK-0476, a potent leukotriene D_4 receptor antagonist, using determination of solubility characteristics, surface tension measurements, static and dynamic light scattering, and near-UV spectroscopy. More specifically, the self-association behaviour of aqueous solutions of MK-0476 was investigated as a function of ionic strength, pH and temperature. The observed pH-solubility profile deviated markedly from the expected profile suggesting a self-association behaviour. The ionic strength influenced the solubility and surface activity. The critical micelle concentration (cmc) determined by tensiometry at pH 10.0 decreased from 162 μM to 14 μM with increasing ionic strength, and a linear relationship between logarithm of cmc and the logarithm of the total counterion concentration was obtained. The cmc determined from surface tension measurements was marginally affected by pH in the range of 8.8 to 10.8 in a constant ionic strength ($\mu = 0.05$) glycine-KOH buffer. Results from surface tension measurements showed that temperature had no effect on the self-association phenomenon in the range 25°–37°C. Light scattering data indicated that at low ionic strength and basic pH, micelles of an average hydrodynamic radius of 1.6 nm exist. The addition of 150 mM of NaCl increased micelle size to approximately 40 nm. The radius of gyration of these aggregates measured by the angular dissymmetry method were larger than the hydrodynamic radii calculated from dynamic light scattering measurements, indicating an elongated shape. Understanding the surface active behaviour of MK-0476 and establishing the critical parameters affecting its self association provided valuable insight towards the development of a liquid dosage form.

Keywords: Surface active drug; Critical micelle concentration; pH-solubility; Tensiometry; Light scattering; Near-UV spectroscopy

---

* Corresponding author. Université de Montréal, Faculté de pharmacie, C.P. 6128 - Succ. Centre-ville, Montréal (Québec), Canada H3C 3J7. Tel.: + 514 343 6448; fax: + 514 343 2102; e-mail: thibert@ere.umontreal.ca

PII S0378-5173(96)04435-3

1. Introduction

Drug molecules with both hydrophillic and hydrophobic regions can be classified as amphiphiles and, by nature, are surface active. One of their inherent features is to self associate in solution. Examples of amphiphilic drugs include disodium cromoglycate (Cox et al., 1971), diphenylmethane analgesics (Attwood and Tolley, 1980), tricyclic antidepressants (Attwood and Fletcher, 1987), and phenothiazines (Attwood et al., 1993). The surface activity of drugs in aqueous solutions causes them to accumulate at the air-liquid interface at low concentrations, thus depressing the surface tension of water. At higher concentrations, that is, above the critical micelle concentration (cmc), these molecules tend to aggregate in solution to form micelles. The biological and pharmaceutical implications of surface activity may be important to the pharmacokinetic and pharmacodynamic behaviour, and may influence technological development (Thoma and Albert, 1983). Solubilization of preservatives and other excipients, stability of drugs in surfactant systems, the influence of surfactant on the dissolution rate of solids, and the influence of surface activity on bioavailability and toxicity are just a few examples (Florence, 1981; Attwood and Florence, 1983). More specifically, the self-association properties of some molecules cause important changes in solubilization behaviour. Deviations from pH-solubility profiles have been attributed to micelle formation for prostaglandin F_2 (Roseman and Yalkowsky, 1973), clindamycin (Rowe, 1979), 3-(4-heptylbenzoyl) benzoic acid (Pandit and Strykowski, 1989), and XD405 (Maurin et al., 1993). Molecular aggregation via a stacking self association was the probable cause of the deviation in the case of brequinar sodium (King et al., 1989). Self association between molecules can also profoundly affect their chemical stability as in the case of corticosteroids (Flynn and Lamb, 1970; Anderson et al., 1983), penicillin G (Ong and Kostenbauder, 1974) and flupenthixol (Enever et al., 1979). Some leukotriene inhibitors and antagonists are prone to be surface active as demonstrated independently by Pandit and Strykowski (1989) for a benzoic acid derivative and by Vadas et al. (1991) for a propanoic acid derivative. Even though these molecules differ remarkably, they share common structures in as much that they both have a concentration of polar and nonpolar groups at opposite ends of the molecules.

During the preformulation of MK-0476, it was noted that the compound possessed unusual solution characteristics. Aqueous solutions of the compound have high native pH (> 9.5), precipitate at concentration < 5 mg/mL, and foam readily upon agitation. The latter property is usually an indication of surface activity. The structural formula of MK-0476 (Fig. 1) reveals potential regions of hydrophillic and lipophilic interactions with an aqueous environment. The characterization of the physicochemical properties unique to solutions are important in the formulation of compounds in non-solid dosage forms such as oral solutions and injectables. An understanding of the critical parameters that can affect drug solubility or stability such as ionic strength, pH, and temperature will provide the formulator with valuable insight into defining the strategies for the development of a suitable dosage form.

In this study, aqueous solution properties of MK-0476, a potent selective leukotriene D_4 receptor antagonist, were characterized using the determination of its solubility, surface tension measurements, light scattering, and near-UV spectroscopy in order to understand the solution and self-association behaviour of the compound. More specific objectives of the study were to investigate the surface active properties of MK-0476 as a function of ionic strength, pH, and temperature.

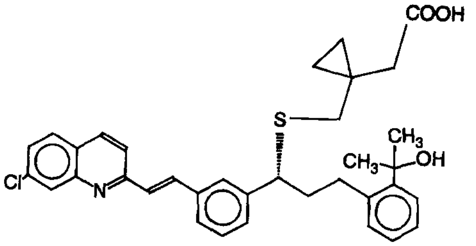

Fig. 1. The structural formula of the free acid form of MK-0476.

2. Materials and methods

2.1. Materials

MK-0476, a crystalline sodium salt (mol. wt. = 608.2), was received from Process Chemistry (Merck Research Laboratories, Rahway) and used without further purification. The purity stated was 99.4% as determined by area percent HPLC. All other chemicals used were reagent grade.

2.2. Solution characteristics

A pH-solubility profile was determined in aqueous buffers from pH 1.4 to 8.4. A series of buffer system (e.g., glycine, acetate, phosphate, TRIS) with constant ionic strength ($\mu = 0.05$) were prepared to cover the desired pH range. Teflon lined screw-cap borosilicate 15 mL vials containing exactly 10 mg of MK-0476 and clean glass beads were filled with 10 mL of the buffer. The glass vials were fitted on a rotating wheel shaker (Glas-Col, model RD4512), covered with an aluminium foil to protect the solutions from light, and mixed at 20 rpm. After 16 h of mixing at room temperature, 3 mL aliquots were centrifuged at 14000 rpm (Brinkman Instruments, model Eppendorf 5415C) for 15 min, and the pH of the supernatant was measured (Fisher Scientific, model Accumet 50). The solubility was determined by a reversed-phase HPLC method which consisted of a HPLC HP1050 (Hewlett-Packard), a MetaChem Inertsil-ODS2 column, and a mobile phase containing acetonitrile, trifluoroacetic acid and water. The data are averages of duplicate or triplicate determinations.

The effect of ionic strength on the physical stability of a 0.1% w/v MK-0476 aqueous solutions was studied using a qualitative evaluation. Solutions containing MK-0476 and sodium bicarbonate, used to adjust ionic strength, were appraised visually for signs of instability (i.e. cloudiness) initially after mixing and after 24 h at room temperature.

2.3. Tensiometry

Surface tension measurements were made with a Cahn Dynamic Contact Analyzer (Cahn Instruments, model DCA-322) based on the Wilhelmy plate method. The inner chamber of the instrument was flushed with nitrogen at 100 mL/min and protected from light. The temperature was controlled with a Haake D-8G circulation bath (Haake GmbH). Unless otherwise noted, all studies were conducted at 25 ± 0.02°C. The investigation of surface activity was made with fresh solutions prepared in degassed distilled water (Milli-Q — reagent grade) adjusted to the desired pH using NaOH or HCl. After dissolution, the pH was verified using an Accumet 50 pH meter (Fisher Scientific). Sodium chloride (reagent grade) was used to adjust the ionic strength. The surface tension data were plotted against the logarithm of the concentration as the abscissa, and the intersection of a descending line and another one close to the horizontal was taken as the cmc (Murkerjee and Mysels, 1970). Each data point represents the average of a duplicate or triplicate experimental run. The effect of pH using a glycine-KOH buffer at a constant ionic strength ($\mu = 0.05$) was studied using the AutoCMC accessory of the Dynamic Contact Analyzer (Cahn Instruments). In this mode, an automatic titrator controls the addition of the surface active molecule to a test solution and records the surface tension. A regression program calculates the cmc as described above.

2.4. Light scattering

Light scattering experiments were performed with a Malvern 4700 Spectrometer (Malvern) equipped with a 5 W argon laser (Spectra Physics) operating at 488 nm and a photomultiplier detector capable of multiangle measurements (i.e. 21 angles from 20 to 120 degrees). The temperature of the sample compartment was maintained at 25 ± 0.02°C. The parameters for most sensitive detection were 250 mW and a detector aperture of 300 μm while a lower aperture was used for comparative measurements of the strongly scattering aggregates. Aqueous solutions of MK-0476 (10 mg/mL) were prepared at different pH values (7.5–12.5) and ionic strengths (0–200 mM NaCl) in degassed distilled water. Solutions containing aggregated material were filtered through 0.1 μm filters. Quartz cuvettes, thoroughly washed in distilled water and dried under vacuum, were used to ensure the absence of interfering dust particles. Square cuvettes (0.5 mL) were used for ionic strength and pH studies, while round cuvettes (5 mL, diameter = 2 cm) were used for the measurement of the dependence of intensity on scattering angle. Polystyrene spheres, used as particle size standards, were purchased from Duke Scientific.

Analysis of the autocorrelation function of the variations of the scattered light arising from Brownian motions of micelles in solution yielded an average diffusion coefficient (Koppel, 1972). The Stokes-Einstein equation was then employed to calculate the z-average hydrodynamic radius, R_h (Candau, 1987). The static light scattering data were analyzed using:

$$Hc/R_0 = 1/M_w (1 + 1/3 * K^2 * R_g)\ Eq.\quad 1$$

where H is a light scattering constant, c is solute concentration, R_0 is the Rayleigh ratio, M_w is the weight-average molecular weight, K is the scattering vector which depends on $\sin^2(\theta/2)$, and θ is the scattering angle, and R_g is the root mean square radius of gyration, corresponding to the average distance from the centre of mass. Since the Rayleigh ratio is proportional to the scattered light intensity, it can be obtained by comparing the intensity from the sample with the intensity of some reference solvent of known Rayleigh ratio such as toluene (Chu, 1991). By extrapolation of the intensity data to zero angle, the term containing K and R_g becomes zero, and the resulting intensity becomes proportional to the molecular weight. The refractive index increment (dn/dc) value for MK-0476 was found to be 0.264 mL/g employing an Optilab 903 interferometric refractometer (Wyatt Technology Corp.) calibrated with a sodium chloride solution (dn/dc = 0.181 mL/g).

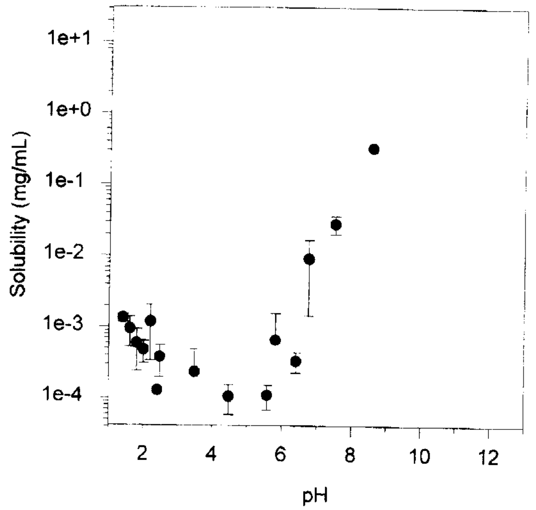

Fig. 2. The pH-solubility profile of MK-0476 at room temperature.

2.5. Near-UV spectroscopy

Near-ultraviolet absorption measurements were performed using a diode-array double beam Hewlett-Packard 8450A spectrophotometer (Hewlett-Packard). Quartz cuvettes of 1, 2 and 10 mm pathlengths were used depending on the concentration. The instrument's software was used for derivative calculation and smoothing. Spectral band positions were defined as the X-intercept of the second derivative plots with wavelength axis near 342 nm. The intecepts were calculated by linear interpolation between discrete, 1-nm spaced data points. The data are averages of at least triplicate determinations.

3. Results and discussion

3.1. Solution characteristics

The solubility of MK-0476 as a function of pH is presented in Fig. 2. The shape of the pH-solubility profile is U-shaped indicative of a zwitterion. It is possible to determine the apparent pKas and intrinsic solubility of the compound using a curve fitting procedure. The apparent pKas found were 2.8 and 5.7, and the intrinsic solubility of the free acid form of MK-0476 is equal to 0.2 μg/mL. The intrinsic solubility of the free acid was determined independently and found to be equal to 0.5 μg/mL which is in close agreement with the value extrapolated from the pH-solubility profiles. The apparent pKas are different from the expected values for a nitrogen in a quinoline ring which is usually closer to 3.9 and for the carboxyl group which is usually closer to 5. This observation has been made for a precursor of MK-0476 (Vadas et al., 1991), and it was hypothesized that the orientation of the molecule and local effects at the molecular levels were probably responsible for the observed differences.

The effect of ionic strength adjusted with sodium bicarbonate on the physical stability of MK-0476 solutions is presented in Table 1. The pH of these solutions varied between 8.5 and 8.6. The maximum ionic strength for physical stability appears to be $0.07 < \mu < 0.08$. At $\mu > 0.08$ aqueous solutions of MK-0476 were physically unstable.

3.2. Tensiometry

The measurement of surface tension is one of the preferred methods to investigate surface activity of solutes. As shown in Fig. 3, there is a sharp break in the surface tension versus concentration curves for the aqueous solutions of MK-0476 indicative of micelle formation.

The effect of increasing the ionic strength by adding NaCl (up to 200 mM) leads to a decrease in cmc to 14 μM (Table 2). The cmc is known to be affected by the concentration of counterions present in aqueous solutions, therefore the decrease in cmc was anticipated (Attwood and Florence, 1983). The relationship derived from the mass-action theory of micelle formation can be expressed in the following form:

$$\log cmc = -(1 - \alpha)\log X^- + \Delta G^\circ_m/2.303RT + 1/N \log F(M_p^+) \quad Eq.\ 2$$

where α is the degree of ionization of the micelle, X^- is the counterion concentration, ΔG°_m is the standard free energy of micellization (per mole of monomeric drug ion), R is the gas constant, T

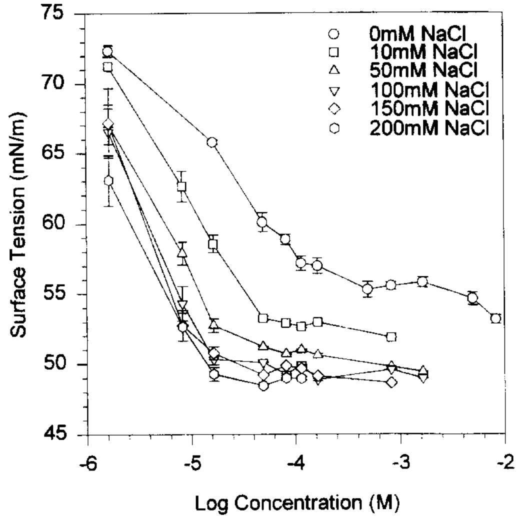

Fig. 3. The effect of ionic strength adjusted with NaCl on the surface activity of MK-0476 in aqueous solutions at pH 10.0 and 25°C.

Table 1
The effect of ionic strength on the physical stability of a 0.1% w/v solution of MK-0476

Ionic Strength (mM $NaHCO_3$)	Appearance of Solution	
	Initial	24 h
30	Clear	Clear
60	Clear	Clear
70	Slightly cloudy	Clear
80	Cloudy	Cloudy
90	Cloudy	Cloudy
120	Cloudy	Cloudy

Table 2
The influence of ionic strength on the cmc determined by tensiometry of MK-0476 at 25°C and pH 10.0

Ionic Strength (mM NaCl)	cmc (μM)
0	161.6
10	54.4
50	19.8
100	15.6
150	13.8
200	14.1

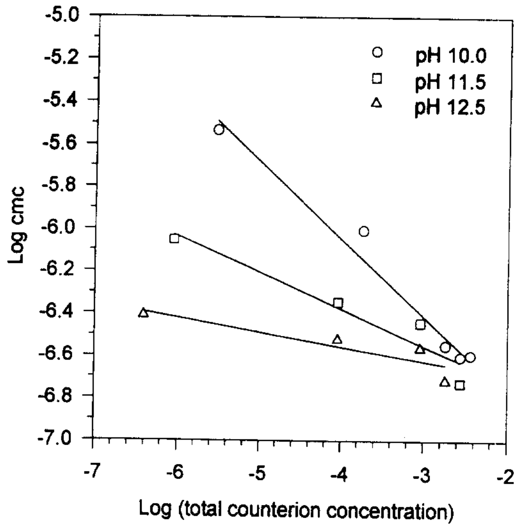

Fig. 4. The relationship between the logarithm of the cmc and the logarithm of total counterion concentration for aqueous solutions of MK-0476 at 25°C.

is the absolute temperature, N is the aggregation number of the micelle, M_p^+ is the mole fraction of micelle, and F is a term involving the activity coefficient of all species present in solution (Attwood and Florence, 1983). In the strictest sense, the micellization constant should be expressed in terms of activity instead of concentration of the chemical species when the mass-action model of micelle formation is observed. However, because of electroneutrality constraints which can only be expressed in terms of concentration, the term F includes the activity coefficients of the species involved (Attwood et al., 1993; Moroi, 1988). As seen in Fig. 4, plots of the logarithm of cmc (mole fraction) as a function of the logarithm of the total counterion concentration (mole fraction) are linear for the aqueous solutions in the range of pH 10.0 to 12.5. The degree of ionization, α, calculated from the slopes of these linear plots yielded values of 0.64, 0.83 and 0.93 for pH 10.0, 11.5 and 12.5, respectively. The degree of ionization is high which corresponds to the rich ionic environment of the basic solutions. The intercept obtained on extrapolation to log $X^- = 0$ may be equated with $\Delta G_m^\circ/2.303RT$ thus enabling ΔG_m° to be estimated (Eq. 2). The free energy of micelle formation calculated from the regression analysis converge to a value of -17.7 ± 0.9 kJ/mol.

The effect of pH on the self association of the compound was initially studied by adjusting the pH of aqueous solutions with NaOH to the desired values. Using this approach, the surface tension profiles of aqueous solutions of MK-0476 indicate that the surface activity was influenced by the pH (Fig. 5). The cmc changes from 162 μM at pH 10.0 to 21 μM at pH 12.5. However, in these experiments the ionic strength was not uniform and undoubtedly contributed to changes in the cmc values. This was confirmed in subsequent experiments in which the cmc was determined at a constant ionic strength (μ = 0.05) with a glycine-KOH buffer in the pH range 8.8 to 10.8. Under these conditions, the cmc remains relatively stable at 11.8 ± 1.9 μM (Table 3).

The effect of temperature on the surface activity of MK-0476 was also investigated. At pH 10.0 and 150 mM NaCl, the cmc at 25°C and 37°C are essentially identical, 13.7 μM and 12.9 μM, respectively. A slight decrease in surface tension from *ca.* 50 mN/m at 25°C to *ca.* 48 mN/m at 37°C was observed yielding a dγ/dT = 0.167. However, this may be caused by thermal effects on the water and is not considered to be signifi-

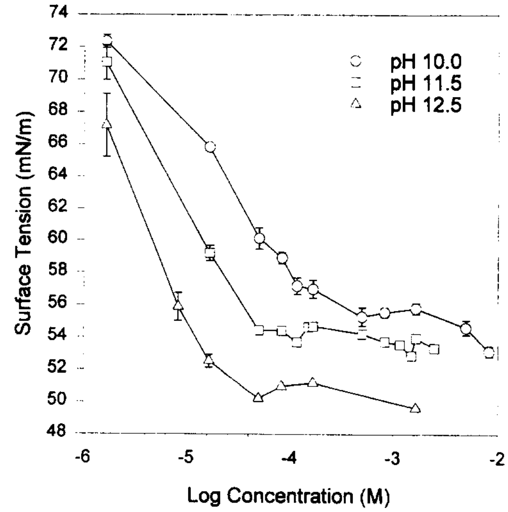

Fig. 5. The effect of pH on the surface activity of aqueous solutions of MK-0476 where the pH is adjusted with NaOH at 25°C.

Table 3
The effect of pH on the cmc (± standard deviation, $n = 2$ or 3) of aqueous solutions of MK-0476 in a constant ionic strength ($\mu = 0.05$) glycine-KOH buffer at 25°C

pH	cmc (μM)
8.8	8.8 ± 0.7
9.4	10.0 ± 0.9
9.8	11.2 ± 0.2
10.0	13.9 ± 3.2
10.2	11.3 ± 2.7
10.4	11.9 ± 2.3
10.6	13.6 ± 3.2
10.8	13.8 ± 0.7

cant since the literature value $d\gamma/dT$ for pure water at 25°C and 40°C is equal to 0.161 (Weast, 1984).

3.3. Light scattering

The results of light scattering studies on the influence of ionic strength and pH at 25°C on the micellar size of MK-0476 are shown in Fig. 6. The formation of insoluble material at pH < 8.5 is marked in the plot by the appearance of aggregates with $R_h > 100$ nm. Nuclear magnetic resonance (NMR) measurements carried out on the precipitate showed that it was the free acid form of MK-0476 (L. Trimble, personal communication). It can be seen that at low ionic strength ($\leq$ 50 mM NaCl) and pH 10, the compound exists in solution as micelles with a $R_h = 1.6$ nm. The micellar size appears to be in the range of approximately 2 nm in moderate amounts of sodium chloride (50–100 mM) at pH 9.5–10.5. A sodium chloride concentration of 150 mM or more induced the formation of aggregates with a R_h of approximately 40 nm. The same effect is observed in 100 mM NaCl at pH values above 11, where the ionic strength from the added NaOH exceeds 50 mM. This ionic strength effect may be attributed potentially to the electrostatic shielding of repulsive forces between negatively charged micelles allowing short-range forces to take effect (i.e. hydrophobic, van der Waals or hydrogen bond interactions). The structure of the compound (Fig. 1) shows several areas which lend themselves to these types of interactions.

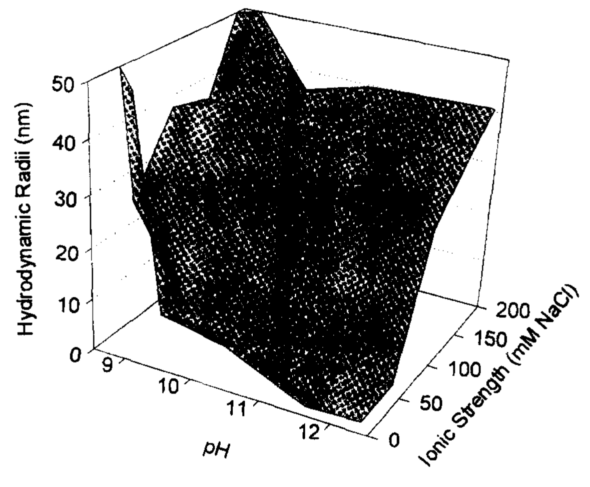

Fig. 6. Influence of ionic strength and pH on the hydrodynamic radii of MK-0476 micelles in aqueous solutions at 25°C.

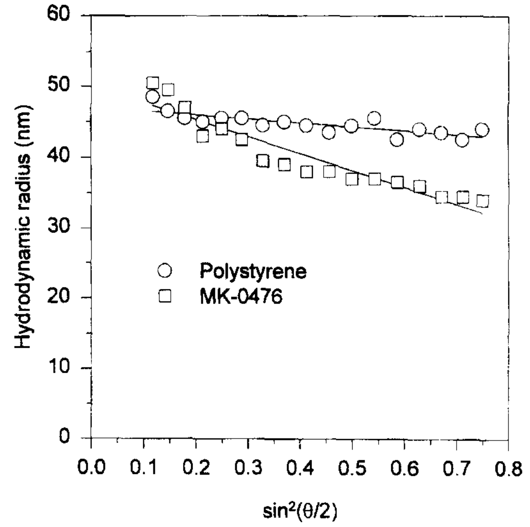

Fig. 7. Angular dependence of hydrodynamic radius of polystyrene size standard particles and MK-0476 (straight lines represent least-squares fits).

In order to assess the size of the aggregates observed at high ionic strength, R_h and the scattering intensity at a range of angles were measured at the following conditions: 150 mM NaCl, pH 11.5, and MK-0476 = 0.14 mg/mL (Figs. 7 and 8A, respectively). As standards, monodisperse polystyrene spheres of comparable dimensions were also analyzed to provide reference data. As expected, the hydrodynamic size of the polystyrene standard spheres varied little when observed at various scattering angles indicating a homogenous population (Fig. 7). However, the more pronounced angular dependence of the R_h of MK-0476 suggests some size variability. Since calculations of the average diffusion coefficient from the autocorrelation function yields a z-average value which is heavily weighted towards the aggregates, the increase of R_h at small angles may potentially arise from a relatively small fraction of aggregates (i.e. 40–50 nm). For aggregates in this size range, the intensity of scattered light rapidly decreases with increasing angles (Fig. 8A). Thus, the high scattering intensities at small angles may be partially due to a subpopulation of aggregates.

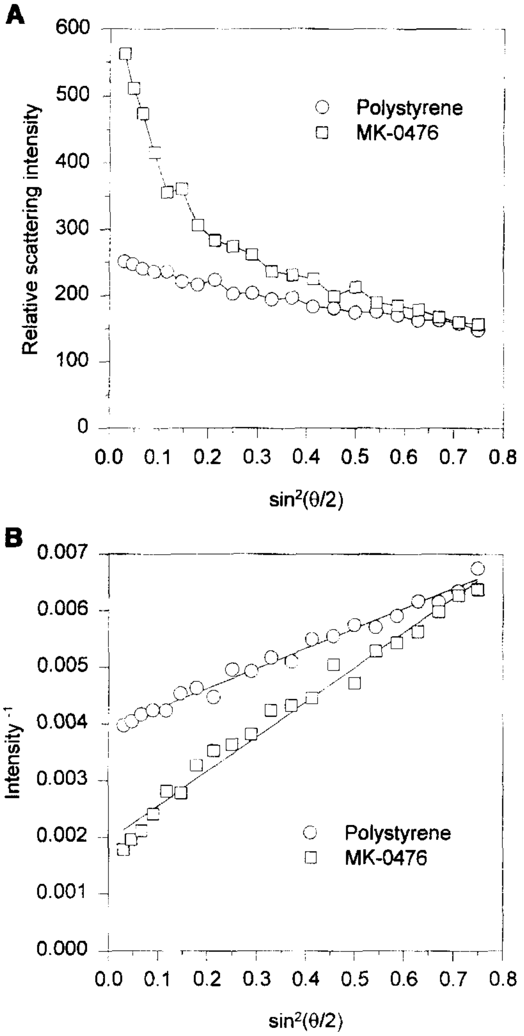

Fig. 8. Static light scattering of MK-0476. (A) Angular dependence of the relative intensity of scattered light for polystyrene size standard particles and MK-0476. (B) Data plotted as reciprocal and fitted to Eqn. 3.

Using theoretical equations predicting particle scattering factors viz. their angular intensity dependence (van Holde, 1985), it can be shown that for particles with R_h in the 10–50 nm range, spherical shape particles produce roughly linear plots of intensity as a function of $\sin^2(\theta/2)$, whereas rod-like particles yield well defined curvatures at low angles and their reciprocal plots tend to be linear (H. Mach, unpublished results). Thus, the lack of similar intensity variations at low angles for the polystyrene standard may be explained not only by size homogeneity but also by its spherical shape. Angular dissymmetry was used to determine R_g of the micelles and, by comparison to the R_h, the shape of the micelle can be inferred (van Holde, 1985). In order to assess R_g, the reciprocal of the intensity was plotted against $\sin^2(\theta/2)$, and R_g values were calculated from the expression (Slayter et al., 1985):

$$R_g = (2550 * \text{slope/intercept})^{1/2} \quad \textit{Eq. } 3$$

The results obtained were $R_g = 47.9$ nm for the polystyrene spheres and $R_g = 80.2$ nm for MK-0476 (Fig. 8B). The relation R_h to R_g is dependent on the overall size of the micelle. However, equations have been derived for the theoretical prediction of values for both R_h and R_g in the case of spheres, ellipsoids, random coils and thin rods (van Holde, 1985). Although only two shape-describing parameters are being used for the non-spherical particles, there is a trend in the predicted R_h/R_g ratios ranging from 1.29 for spheres to approximately 0.6 for thin rods (Young et al., 1978). In this experiment, the values of R_h extrapolated to zero angle (Fig. 7) are 47 nm for polystyrene, and 50 nm for MK-0476 yielding R_h/R_g ratios of 1.0 and 0.6, respectively. These results indicate notable elongation of the MK-0476 aggregates. These data are to be treated with caution, however, since several approximations are made. The solvent non-interacting spherical shape is assumed when calculating hydrodynamic radii based on the diffusion coefficient. In addition, the radius of gyration is estimated from the intensity reciprocal plot. Furthermore, when the intensity data are analyzed by an equation which includes more than one term from a power series, thus avoiding several approximations, and a heterogenous population is assumed, the R_h/R_g values are comparable to those obtained for spheres (not illustrated). Despite the many uncertainties regarding the validity of the shape estimation from light scattering, the method has been utilized in studies of many surfactant and macromolecules (Young et al., 1978; Slayter et al., 1985; van Holde, 1985; Shorgren et al., 1986; Murphy et al., 1990; Schurtenberger and Augusteyn, 1991). Similar results to those seen here for MK-0476 were obtained for sodium dodecyl sulfate at high ionic strength, where micelles with R_h of about 1nm formed aggregates having R_h as large as 20 nm (Young et al., 1978). The data were found to be in agreement with predictions based on a rod-like shape. Whether or not the aggregates are composed of defined micelles is under investigation.

By measuring molecular weights of micelles, the aggregation number (n) can also be determined from light scattering data. Eq. 1 was used to obtain estimates of average aggregation numbers for micelles (R_h = 1.6 nm), and aggregates (R_h ≈ 40 nm). There is no angular dependence of the scattered light for the initial micelles, thus Eq. 1 can be rearranged to the following expression to calculate the molecular weight (M_w) for incident light wavelength at 488 nm using toluene as a standard solvent:

$$M_w = 1240 * I/(I_t * c * (dn/dc)^2) \quad Eq. 4$$

where I is the scattered light intensity of the solute, I_t is the scattering light intensity of the reference solvent, c is concentration (mg/mL), and dn/dc is refractive index increment (mL/g). A molecular weight of 11 000 was obtained, equivalent to an aggregation number of 18. In order to estimate the aggregation number of the 40 nm aggregates, the value of the scattering intensity at zero angle was found by extrapolation of the reciprocal intensity plot (Fig. 8B). Eq. 4 was then used, assuming that the amount of primary micelles at high ionic strength is negligible. An aggregation number of 16300 was obtained, equivalent to an assembly of approximately 900 primary micelles.

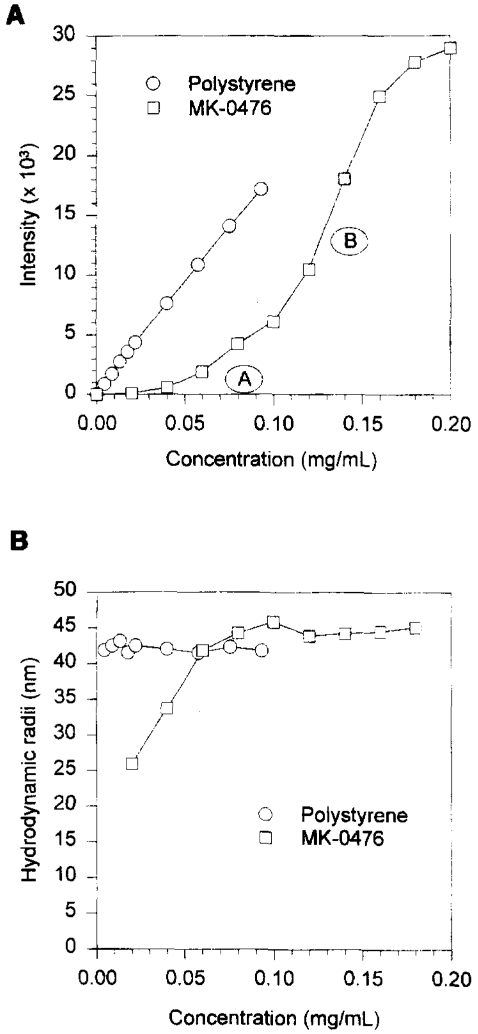

Fig. 9. (A) Static light scattering studies showing the intensity of scattered light as a function of concentration for a polystyrene size standard and MK-0476 in aqueous solutions at 200 mM NaCl, pH 10.5 and 25°C. (B) Dynamic light scattering studies showing the z-average hydrodynamic radii as a function of concentration for a polystyrene size standard and MK-0476 in aqueous solutions at 200 mM NaCl, pH 10.5 and 25°C.

The dependence of micellar size on the concentration at pH 10.5 and 25°C of MK-0476 in aqueous solutions containing 200 mM NaCl can be seen in Fig. 9A. In the case of polystyrene spheres, a linear response of scattered light intensity as a function of concentration is obtained with the monodisperse non-interacting particles. On the other hand, MK-0476 manifests a strong concentration dependence on scattered light intensity. Extrapolation of the linear portion of the plot (region B — Fig. 9A) leads to a cmc of 164 μM for the 40 nm species. Fig. 9A and B illustrate the qualitative differences between static and dynamic light scattering and help to explain the previous observations. The static intensity measurements yield a weight-averaged molecular weight which strictly reflects the aggregation phenomenon occurring in solution. The dynamic R_h determinations yield a z-average where a few large species skew the results. This discrepancy can be seen by observing the z-average results in the 0.02–0.06 mg/mL range where R_h reaches an asymptomatic 40–45 nm value while the intensity results indicate that primary micelles are still present.

3.4. Near-UV spectroscopy

In principle, near-UV spectroscopy can be utilized to detect changes of the microenvironment of aromatic residues of MK-0476 which result from incorporation of parts of the molecules into the hydrophobic interior of the micelles. As mentioned previously, analysis of the scattering intensity versus concentration plot at pH 10.5 (Fig. 9A) indicates that self association starts at a concentration of MK-0476 of approximately 50 μM (extrapolation of region A to the X-intercept). When a 200 mM NaCl solution was employed, the aggregates were detectable at 15 μM of MK-0476 (not shown), implying involvement of electrostatic shielding in the formation of these aggregates. In order to monitor the changes of the microenvironment of aromatic rings of MK-0476 upon incorporation into micelles, near-ultraviolet absorption band positions of the drug were measured at different conditions (Fig. 10A). A spectral shift is observed upon micelle formation, which can be seen in a plot of the second derivative of the absorbance plot at the wavelength axis near 342 nm (Fig. 10B). A decrease of ionic strength dramatically increases the cmc from 10.2 μM at $\mu = 0.150$, to 41 μM at $\mu = 0.010$, and 164 μM at $\mu = 0$ (Fig. 11). The spectral bands of MK-0476 are shifted approximately 3.5 nm to higher wavelengths upon the change of the microenvironment from the aqueous solution to the interior of the micelle. At MK-0476 concentration of 50–500 μM, where the aggregates are formed, further shifts are small, reflecting less burial of hydrophobic surfaces. Whether this behaviour is

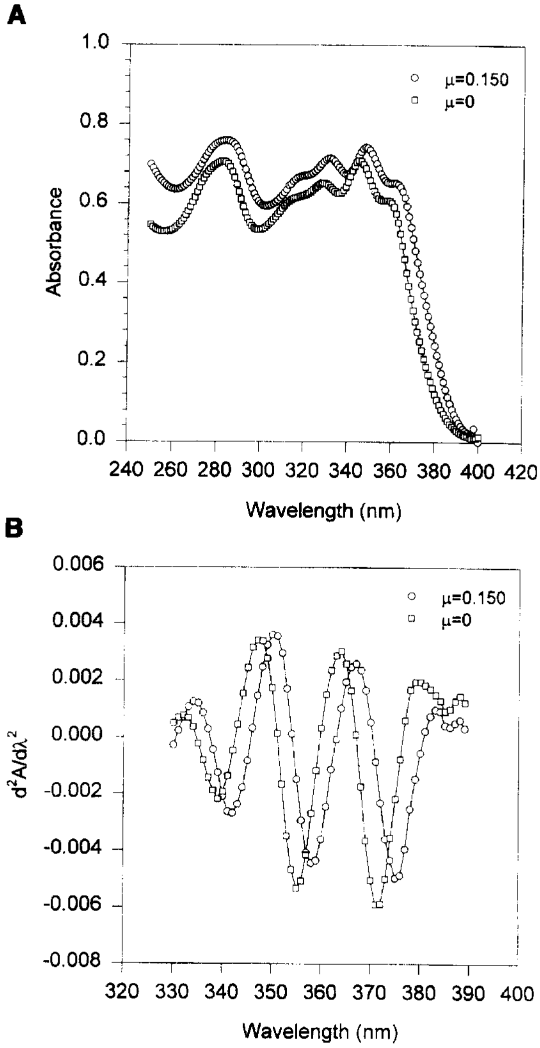

Fig. 10. (A) Ultraviolet spectra of MK-0476 at different ionic strength in aqueous solution. (B) Second derivative spectra of 320–380 nm region based on data shown in (A).

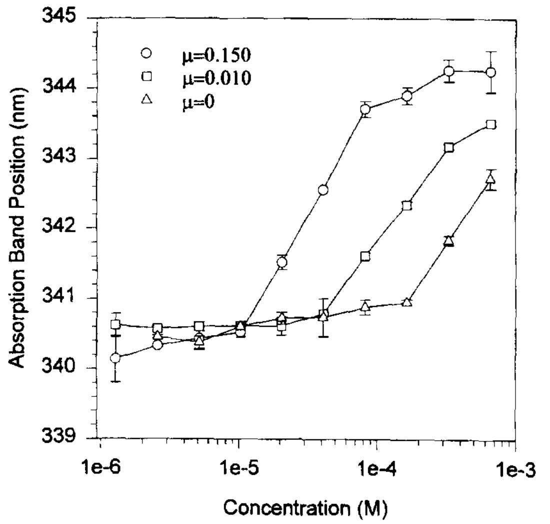

Fig. 11. MK-0476 spectral band positions as a function of concentration at various ionic strength.

due to the free energy decrease per residue upon aggregation or a larger involvement of polar interactions remains to be determined.

4. Conclusions

It was demonstrated that MK-0476 showed significant surface activity in aqueous solution. Tensiometry showed that the cmc of MK-0476 was 162 μM at pH 10.0 in aqueous solution at 25°C. Ionic strength strongly influenced self association. The self-association properties of MK-0476 appeared to be only marginally affected by pH (range 8.8–10.8) and temperature (range 25°–37°C). Increases in ionic strength lowered the cmc of micelles (R_h = 1.6 nm) and promoted the aggregation to high molecular weight complexes (R_h ≈ 40 nm). The aggregation numbers were found to be approximately 18 for micelles, and 16 300 for the aggregates. These experiments have helped to characterize the critical parameters affecting the behaviour of MK-0476 in solution and have provided useful insight into the development of non-solid dosage forms. The understanding of the critical role of ionic strength and pH effects have made it possible to formulate stable solutions where the ionic strength and pH have been optimized to provide maximum stability and solubility.

Acknowledgements

We would like to thank Dr. E. Kwong and Ms. S. Spagnoli (Pharmaceutical R and D, Montreal) for their help with HPLC, Dr. L. Trimble (Medicinal Chemistry, Montreal) for the NMR analysis, and Dr. E. Vargha-Butler (Dalhousie University, Halifax, Nova Scotia) for her valuable comments. We would also like to thank Drs C.R. Gardner, C.R. Middaugh, and C.J. Burke for their interest and support of this work.

References

Anderson, B.D., Conradi, R.A. and Johnson, K., Influence of premicellar and micellar association on the reactivity of methylprednisolone-21-hemiesters in aqueous solution. *J. Pharm. Sci.*, 72 (1983) 448–454.

Attwood, D. and Fletcher, P., Total intensity and quasielastic light scattering studies on the association of amphiphilic drugs in aqueous electrolyte solutions. *J. Colloid Interface Sci.*, 115 (1987) 104–109.

Attwood, D. and Florence A.T., *Surfactant Systems: Their Chemistry, Pharmacy and Biology*, Chapman and Hall, London, 1983.

Attwood, D. and Tolley, J.A., Self association of analgesics in aqueous solution: micellar properties of dextropropoxyphene hydrochloride and methadone hydrochloride. *J. Pharm. Pharmacol.*, 32 (1980) 533–536.

Attwood, D., Blundell, R. and Mosquera, V., Light scattering studies on the association of phenothiazine drugs in aqueous solutions of low ionic strength. *J. Colloid Interface Sci.*, 157 (1993) 50–54.

Candau, S.J., Light scattering. In: Zana, R, Editor, *Surfactant Solutions: New Methods of Investigation*, Surfactant Science Series Vol. 22, Marcel Dekker, New York, 1987, pp. 147–208.

Chu, B., *Laser Light Scattering*, Academic Press, San Diego, 1991.

Cox, J.S.G., Woodward, G.D. and McCrone, W.C. Solid-state chemistry of cromolyn sodium (disodium cromoglycate). *J. Pharm. Sci.*, 60 (1971) 1458–1465.

Enever, R.P., Li Wan Po, A. and Shotton, E., Flupenthixol dihydrochloride decomposition in aqueous solution. *J. Pharm. Sci.*, 68 (1979) 169–171.

Florence, A.T. Drug solubilization in surfactant systems. In: Yalkowski, S.H. (Editor), *Techniques of Solubilization of Drugs*, Marcel Dekker, New York, 1981, pp. 15–89.

Flynn, G.L. and Lamb, D.J. Factors influencing solvolysis of corticosteroid-21-phosphate esters. *J. Pharm. Sci.*, 59 (1970) 1433–1438.

King, S.Y.P., Basista, A. and Torosian, G., Self association and solubility behaviours of a novel anticancer agent, brequinar sodium. *J. Pharm. Sci.*, 78 (1989) 95–100.

Koppel, D.E., Analysis of macromolecular polydispersity in intensity correlation spectroscopy: the methods of cumulants. *J. Chem. Phys.*, 57 (1972) 4814–4820.

Maurin, M.B., Vickery, R.D., Gerard, C.A. and Hussain, M., Solubility of ionization behaviour of the antifungal α-(2,4-difluorophenyl)-α-[(1-(2-(2-pyridyl)phenylethyl)]-1H-1,2,4-triazole-1-ethanol bismesylate (XD405). *Int. J. Pharm.*, 94 (1993) 11–14.

Moroi, Y. J. Mass action model of micelle formation: its application to sodium dodecyl sulfate solution. *Colloid Interface Sc.*, 122 (1988) 308–314.

Murkerjee, P. and Mysels, K.J., *Critical Micelle Concentration of Aqueous Surfactant Systems*, NSRDS-NBS 36, National Bureau of Standards, Washington, D.C., 1970.

Murphy, R.M., Chemberlin, R.A., Schurtenberger, P., Colton, C.K. and Yarmush, M., Size and structure of antigen-antibody complexes: thermodynamic parameters. *Biochemistry*, 29 (1990) 10 889–10 899.

Ong, J.T.H. and Kostenbauder, H.B., Effect of self association on rate of penicillin G degradation in concentrated aqueous solutions. *J. Pharm. Sci.*, 64 (1974) 1378–1380.

Pandit, N.K. and Strykowski, J.M., Effect of pH and temperature on the solubility of a surface active carboxylic acid. *J. Pharm. Sci.*, 78 (1989) 767–770.

Roseman, T.J. and Yalkowsky, S.H., Physicochemical properties of prostaglandin F_2 (tromethamine salt): solubility behaviour, surface properties, and ionization constants. *J. Pharm. Sci.*, 62 (1973) 1680–1685.

Rowe, E.L., Anomalous solution behaviour of 2-palmitate esters of lincomycin and clindamycin. *J. Pharm. Sci.*, 68 (1979) 1292–1296.

Schurtenberger, P. and Augusteyn, R.C. Structural properties of polydisperse biopolymer solutions: a light scattering study of bovine α-crystalline. *Biopolymers*, 31 (1991) 1229–1240.

Shorgren, R.L., Jamieson, A.M., Blackwell, J. and Jentoft, N., Conformation of mucous glycoproteins in aqueous solvents. *Biopolymers*, 25 (1986) 1505–1517.

Slayter, H., Loscalzo, J., Bockenstedt, P. and Handin, R.I., Native conformation of human von Willebrand protein. *J. Biol. Chem.*, 260 (1985) 8559–8563.

Thoma, K. and Albert, K., Amphiphile Arzneistoffe. Teil 2: Beziehungen zwischen dem kolloidchemischen Eigenschaften und dem pharmazeutisch-technologischen, pharmakokinetischen und pharmakodynamischen Verhalten. *Pharmazie*, 38 (1983) 807–817.

Vadas, E.B., Toma, P. and Zografi, G., Solid-state phase transitions initiated by water vapour sorption of crystalline L-660,711, a leukotriene D4 receptor antagonist. *Pharm. Res.*, 8 (1991) 148–155.

van Holde, K.E., *Physical Biochemistry*, Prentice-Hall Inc., Englewood Cliffs, 1985.

Weast, R.C. (Editor-in-Chief), *Handbook of Chemistry and Physics*, 64th Edition, CRC Press, Boca Raton, 1984.

Young, C.Y., Missel, P.J., Mazer, N.A., Benedek, G.B. and Carey, M.C., Deduction of micellar shape from angular dissymmetry measurements of light scattered from aqueous sodium dodecyl sulfate solutions at high sodium chloride concentrations. *J. Phys. Chem.*, 82 (1978) 1375–1378.