Kinetics of Paclitaxel 2'-N-methylpyridinium Mesylate Decomposition

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ABSTRACT

This study was designed to examine the kinetics of decomposition of paclitaxel 2'-N-methylpyridinium mesylate (PNMM), a derivative of paclitaxel. Further, the potential for PNMM to act as a prodrug of paclitaxel was assessed in vitro. Stability studies of PNMM were conducted over a pH range of 4.0 to 8.0 at 25°C. The critical micelle concentration (CMC) of PNMM was determined by pulsating bubble surfactometry. Studies of the conversion of PNMM to paclitaxel were conducted in vitro in human plasma. Decomposition of PNMM followed apparent zero-order kinetics. The pH-rate profile exhibited no evidence of acid catalysis down to pH 4.0, while the rate was accelerated under base conditions. Surface tension studies suggested that PNMM formed micelles with a CMC of approximately 34 µg/mL. Conversion studies in phosphate buffer showed that no more than 5% of PNMM converted to paclitaxel, while in human plasma the conversion was about 25%. The degradation of PNMM was via apparent zero-order kinetics and was dependent upon pH. The observed apparent zero-order kinetics of decomposition of PNMM was consistent with the formation of micelles in phosphate buffer. In buffered aqueous media alone or in human plasma, PNMM did not convert quantitatively to paclitaxel. Thus, the limiting factor in the application of PNMM as a prodrug would appear to be the poor potential to convert to paclitaxel.

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INTRODUCTION

Paclitaxel is an antineoplastic agent, isolated in the late 1960s from the western Pacific yew tree *Taxus brevifolia*.[1] Paclitaxel has been approved for the treatment of breast and ovarian cancers and is currently under investigation for the treatment of other types of cancer.[2,3] The use of paclitaxel, in spite of its potency, has been hampered by its poor water solubility of about only 12 µg/mL. Currently, paclitaxel is administered in an intravenous formulation that utilizes Cremophore oil and ethanol as cosolvents in a 1:1 ratio prior to dilution with 5% dextrose.[4] Unfortunately, this formulation may trigger side effects such as severe hypersensitivity reaction that could lead to the discontinuation of the medication.[5] Considerable efforts have been expended to improve the water solubility of paclitaxel, including synthesizing salts of ionizable ester prodrugs at the 2' or 7' position[6-10] or polyethylene glycol prodrugs,[11] and employing lipid assemblies.[12] Prodrugs have exhibited varying success in terms of solubility enhancement, release of the parent compound, and cytotoxicity.

Paclitaxel 2'-N-methylpyridinium mesylate (PNMM) is an N-methylpyridinium derivative of paclitaxel recently prepared by our group[13] with the intent of enhancing water solubility. We observed that the water solubility of the N-methylpyridinium mesylate derivative of paclitaxel is more than 1.0 mg/mL (data not shown). Reports in the literature indicate that mesylate salts can exhibit superior aqueous solubilities compared with the other salt forms.[14] These observations, coupled with the success of the prodrug efforts prompted us to explore the possibility that compound PNMM may be a useful water-soluble prodrug of paclitaxel. In the present work we describe results generated in the course of our studies with PNMM concerning its chemical stability and initial efforts in determining the potential for conversion to the mother compound.

MATERIALS AND METHODS

IVAX Corporation (Miami, Florida) kindly provided paclitaxel (purity greater than 98% by high-peformance liquid chromatography (HPLC), nuclear magnetic resonance (NMR), and mass spectrometry). PNMM was prepared by reacting paclitaxel with 2-fluoro, N-methylpyridinium tosylate for 70 minutes in CH_2Cl_2 in the presence of triethylamine, followed by HPLC purification and ion-exchange (acetonitrile, aqueous sodium mesylate as the mobile phase) over a C^{18} μBondapak preparative HPLC column (Waters Corporation, Milford, MA) (purity greater than 97%). Sodium mono- and dibasic phosphate, and sodium mesylate were all purchased from Aldrich (Milwaukee, WI). All solvents used (methanol, acetonitrile, methylene chloride) were HPLC grade and purchased from Fisher Scientific (Pittsburgh, PA). Deionized water was produced by a Nanopure System (Millipore, Inc, Billerica, MA). Critical micelle concentration determinations were carried out using a pulsating bubble surfactometer (Electronetics Corporation, Amherst, NY). An HPLC system from Spectra-Physics (San Jose, CA) consisting of a 400 solvent delivery system, a 783 UV detector, an 878A autosampler, and a 429A integrator was used.

High-Pressure Liquid Chromatography Assay

In the 2 separate HPLC assays used in this work, C^{18} μBondapak columns (3.9 × 300 mm, Waters Corporation, Milford, MA) were employed. The mobile phase in the first assay, which was used for the aqueous media stability studies of PNMM (assay I), consisted of 6 mM of sodium mesylate ($NaCH_3SO_3$) in a 24% aqueous acetonitrile solution. Detection was at 254 nm. The retention times for paclitaxel and PNMM were 2.8 and 12.2 minutes, respectively. The detection limit (95% confidence) was found to be 0.04 mg/mL. Inter- and intra-day variations (coefficient of variance) were 3.7% and 1.0%, respectively. In the second assay, which was used for plasma studies (assay II), the mobile phase consisted of 6 mM $NaCH_3SO_3$ water, acetonitrile, methanol in the following proportions, 35: 30: 35, respectively. Detection was at 254 nm. The retention times for paclitaxel and PNMM were 10.3 and 25.2 minutes, respectively. Both peaks were well separated from the plasma peak.

Stability of PNMM in Aqueous Buffers

In a 20-mL vial, a weighed amount of PNMM was dissolved in a 1.0-mM phosphate buffer at a known pH value. The solution was filtered through a 0.22-μm filter (Millipore, Inc, Billerica, MA), and the filtrate was subsequently transferred to a 2.0-mL autosampler vial, capped, and placed into the autosampler (at room temperature) to be analyzed by HPLC (assay I) at specified time periods. The disappearance of PNMM from solution was studied over a range of pH values of 4.0 to 8.0 at room temperature. The pH values were adjusted by using sodium mono- and dibasic phosphate stock solutions of the same concentration as the buffer used, and pH measurements were taken by the use of Corning pH/ion meter 135 (Corning Medical and Scientific, Medfield, MA).

To study the effect of ionic strength, the loss of PNMM from solution was studied at pH 8.0 at ionic strength values of 4 and 8 mM at room temperature. NaCl was used to adjust the ionic strength.

Critical Micelle Concentration Determination

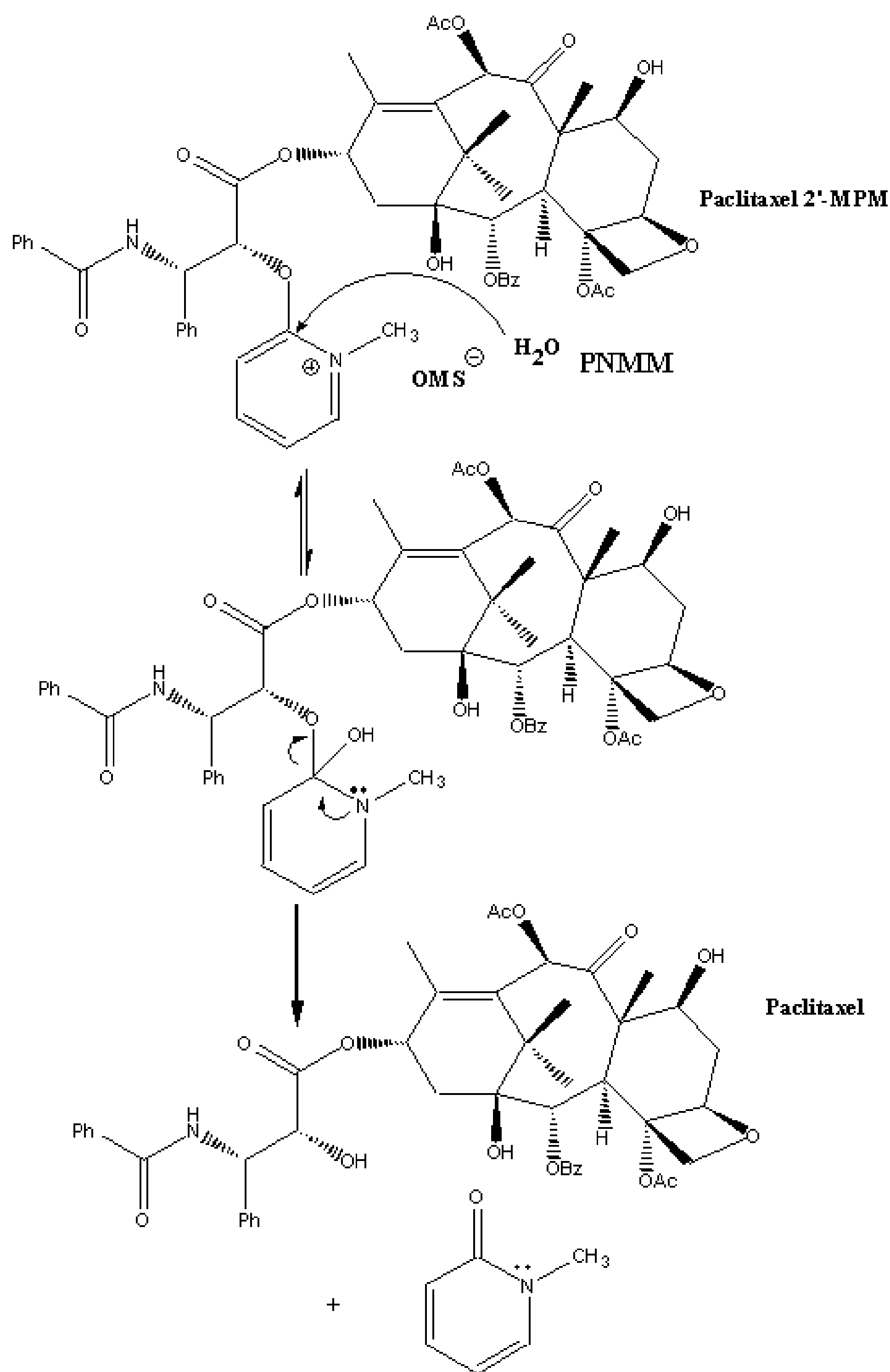
Serial dilutions were made from a stock solution of PNMM (1.0 mg/mL) to obtain concentrations ranging from 1.0 to 0.001 mg/mL. Surface tension measurements were obtained for all concentrations, as well as for deionized water as a control. Because of the high cost of paclitaxel and, therefore, limited amount of PNMM, a pulsating bubble surfactometer (50 μL sample size) was used for the measurements. The measurements were taken at 25 ± 0.5°C.

Plasma Conversion Studies with PNMM

A 1.0-mL aliquot from a 0.4-mg/mL stock solution of PNMM was added to 1.0 mL human plasma and incubated in a reciprocating water bath at 37°C. Samples (100 μL) were taken at different time periods and added to 400 μL of acetonitrile. The mixture was centrifuged at 1×10^4 rpm for 5 minutes. The supernatants were immediately frozen using dry ice. Subsequently, the samples were thawed, filtered through 0.22-μm Millipore filters, and 100-μL aliquots were analyzed by HPLC using assay II. Percentage recovery was approximately 87%.

RESULTS AND DISCUSSION

The structures of paclitaxel and PNMM are shown in **Scheme 1**, along with a possible reaction pathway. The loss of PNMM from phosphate buffer solution (in the range of pH values from 4.0 to 8.0) was followed as a function of time. **Figure 1** shows the typical results for the loss of PNMM in phosphate buffer solution at pH = 8.0. Surprisingly, a zero-order rate of disappearance of



Scheme 1. Structures of paclitaxel and PNMM.

PNMM was observed for all aqueous solutions. In all cases, the correlation coefficient (r^2) of fitted lines was equal to or greater than 0.99. The observed apparent zero-order rate constants and corresponding reaction half-lives are listed in **Table 1**. The values in **Table 1** indicate that the half-life of the disappearance of PNMM in phosphate buffer is strongly dependent upon the pH of the solution in the region studied. **Figure 2**

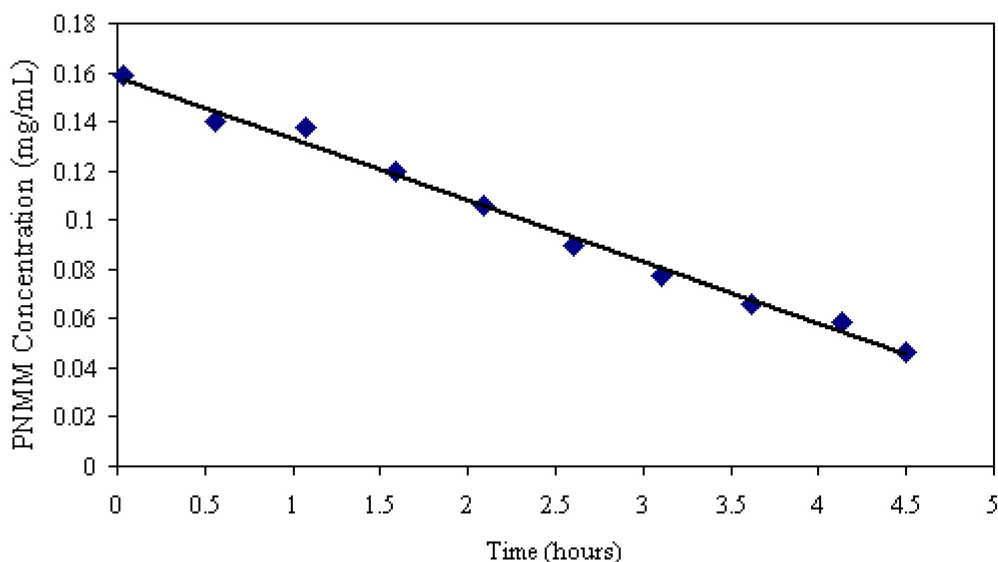


Figure 1. Typical plot of concentration of PNMM in 1.0-mM phosphate buffer (pH = 8.0) as a function of time.

Table 1. K_{obs} and $t_{1/2}$ Values for PNMM Decomposition in 1.0-mM Phosphate Buffer at pH Values of 4.0 to 8.0*

Medium	pH 4.0	pH 5.0	pH 6.0	pH 7.0	pH 8.0
$k_{obs\ zero}$	8.39×10^{-6}	8.67×10^{-6}	1.06×10^{-5}	2.77×10^{-5}	1.89×10^{-4}
Mh^{-1} (± SD)	(2.31×10^{-6})	(2.64×10^{-6})	(3.71×10^{-6})	(3.07×10^{-6})	(2.08×10^{-5})
$t_{1/2}$ (h)	11.5	11.1	8.0	3.5	0.5

*k_{obs} indicates the observed rate constant; PNMM, paclitaxel 2'-N-methylpyridinium mesylate; and $k_{obs\ zero}$, the observed zero-order rate of loss of PNMM from solution.

shows the pH-rate profile for the disappearance of PNMM from phosphate-buffered solutions. The results presented indicate that the degradation of PNMM appears to show some evidence of base catalysis (either hydroxide ion, phosphate ion, or both), while showing no acid catalysis down to a pH of 4.0. Obviously, acid catalysis at pH values below 4.0 cannot be ruled out. Ionic strength had no effect on the rate of degradation (data not shown).

A reasonable starting point for the study of the rate of a (putative) hydrolysis reaction is to assume pseudo first-order kinetics. The general equation for the rate of disappearance (dC/dt) of PNMM from solution can thus be written as follows:

$$-dC/dt = k_{obs} \times C \qquad (1)$$

Where k_{obs} is the observed rate constant, and C is the concentration of PNMM. Several potential chemical reactions can contribute to the observed rate constant, including water-catalyzed hydrolysis, hydrogen ion–catalyzed hydrolysis, hydroxyl ion–catalyzed hydrolysis, and buffer-catalyzed hydrolysis. Assuming that, under buffer conditions, the concentrations of hydrogen ion, hydroxide ion, and buffer components remain constant, the contributions of these various chemical reactions are also constant and combined together give k_{obs}.

If the reaction is indeed pseudo zero order as shown in **Figure 1**, C is constant (which would occur, for example, if the drug is present at or above the critical micelle concentration (CMC) and only drug in the monomer form degraded) and Equation 1 could be rewritten as

$$(-dC/dt) = k_{obs\ zero} \qquad (2)$$

Where $k_{obs\ zero}$ is the observed zero-order rate of loss of PNMM from solution.

When considering the solution state, the most frequently encountered mechanism resulting in apparent

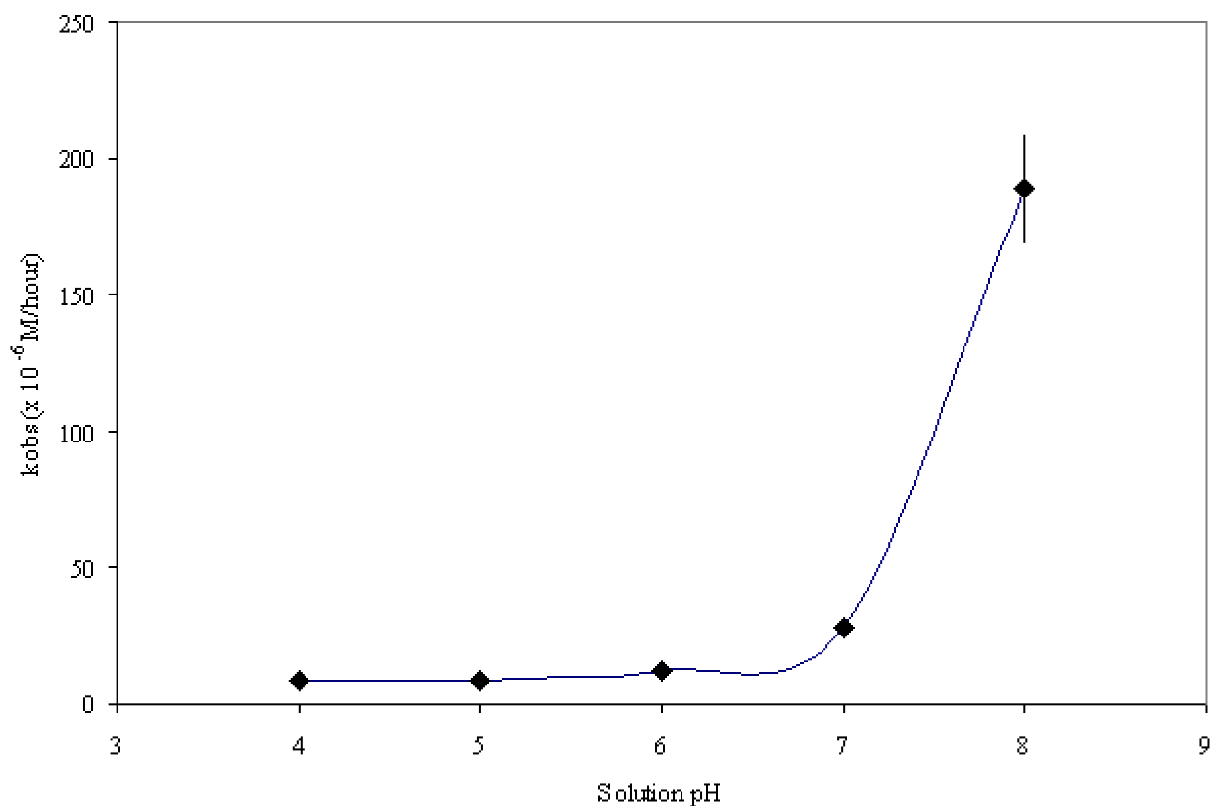


Figure 2. pH-rate profile for PNMM decomposition in 1.0-mM phosphate buffer. Error bars indicate standard deviation.

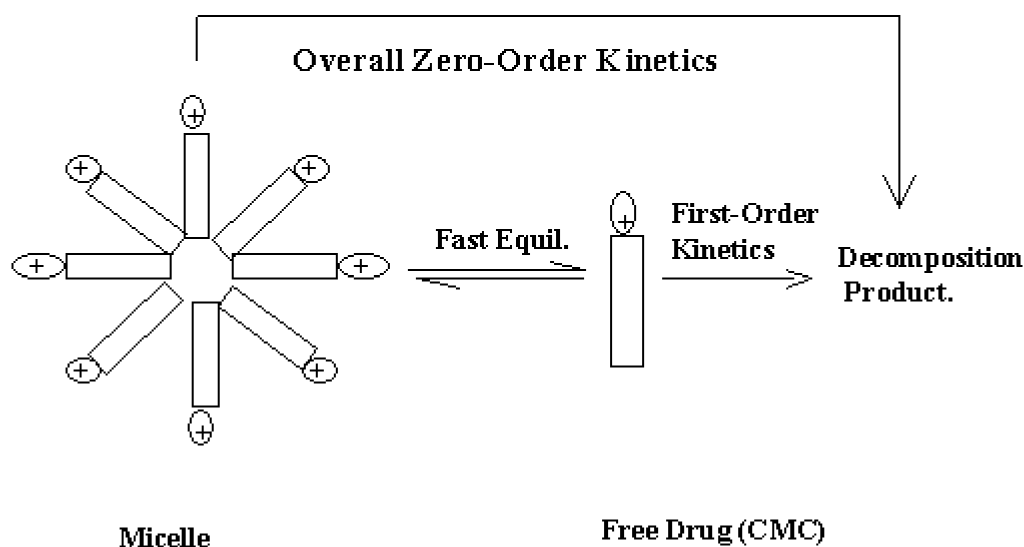


Figure 3. Schematic presentation of PNMM decomposition in aqueous buffers above the CMC.

zero-order kinetics involves self-association of the drug molecules. **Figure 3** shows a possible scheme by which this effect may be understood. The mechanism assumes that the drug is stable when it exists in the aggregate form and degrades only when the drug exists in the monomer form. Drug monomer typically exhibits a

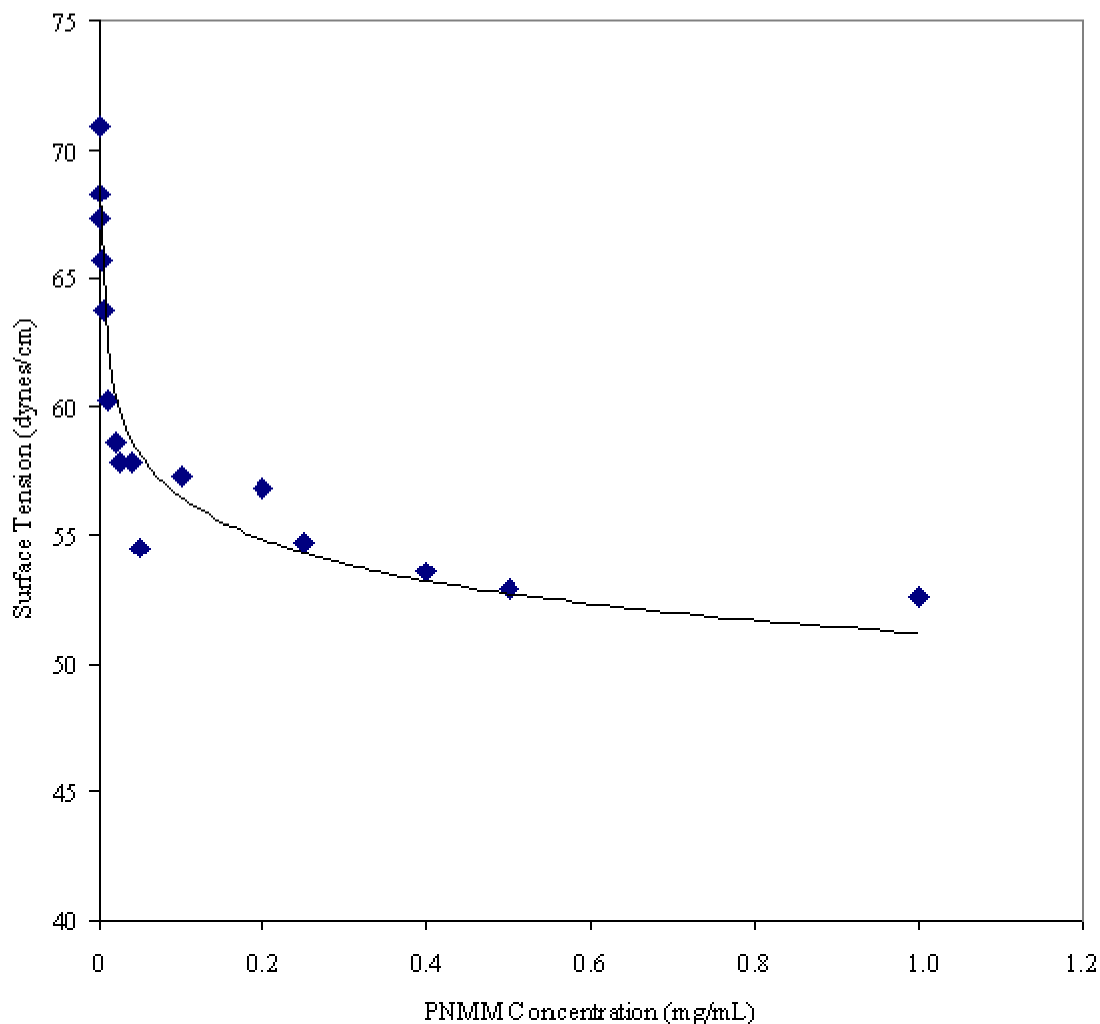


Figure 4. Surface tension as a function of PNMM of concentration in deionized water using a pulsating bubble surfactometer.

fast equilibrium with the aggregate, such as a micelle. If the rate of decomposition of PNMM in solution is slow compared with the dissociation of PNMM from the aggregate, the monomer concentration of the drug in solution will remain constant as long as the aggregate exists. Even if the degradation reaction is first order with respect to monomer, the rapid replenishment of the monomer in solution will result in the observation of an overall zero-order reaction.[15]

Given the putative mechanism outlined in **Figure 3**, and the observation of pseudo zero-order kinetics in **Figure 1**, studies were carried out to determine if PNMM was capable of self-associating in solution. Critical behavior in surface activity is a common means of observing self-association. Pulsating bubble surfactometry was employed to measure the dynamic surface tension of solutions of PNMM as a function of concentration. **Figure 4** shows that the surface tension values initially drop sharply with increasing concentration of PNMM and rapidly approach a plateau value of about 55 dynes/cm. This behavior is consistent with the self-association of PNMM into micelles.[16] The intersection point of the extrapolations of the 2 linear regions observed in **Figure 4** results in a CMC of approximately 34 µg/mL, indicating that any solution concentration higher than this value will contain drug that has self-associated into aggregates such as micelles. More importantly, these results suggest that any solution of PNMM with a concentration greater than 34 µg/mL may exhibit zero-order loss of this compound. Unfortunately, because of sensitivity limitations of the HPLC assay, it was not possible to verify whether or not solutions with concentrations of PNMM less than 34 µg/mL exhibit first-order degradation kinetics.

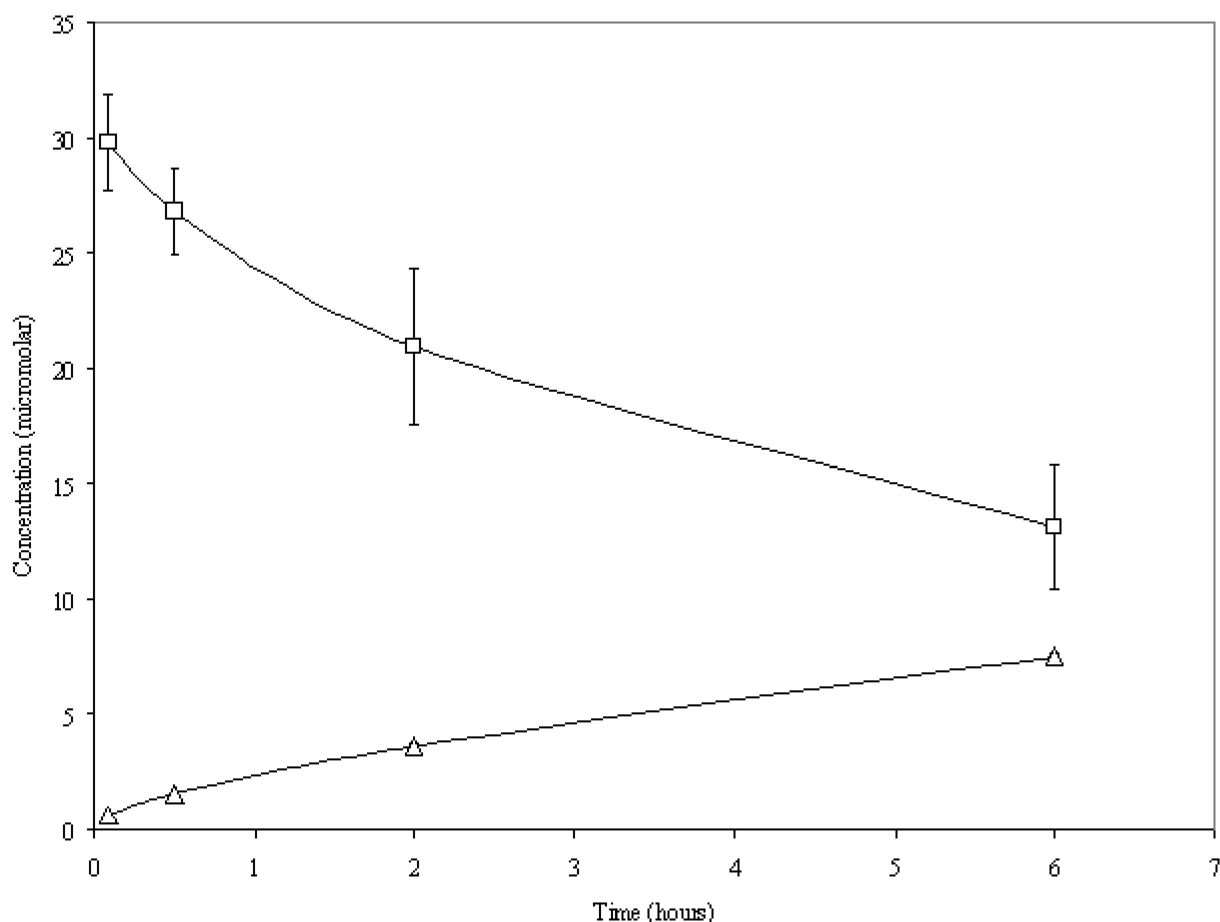


Figure 5. PNMM (□) and paclitaxel (△) concentration, as a function of time, in the conversion study in human plasma, 3 replicates per point. Error bars represent standard deviation.

The nature of the putative PNMM aggregate (such as morphology or aggregation number) is unknown, although it is likely to be driven by hydrophobic interactions. Additional studies would be necessary to clarify why self-aggregation might prevent (or at least slow) the rate of degradation. It is interesting to note that Nicolaou et al also observed micelle-like behavior in a related compound, paclitaxel N-methylpyridinium acetate.[6]

PNMM was synthesized with the intent to create a prodrug of paclitaxel with an enhanced water solubility, and in order for PNMM to be clinically useful, it should convert back to paclitaxel. In studying the stability of PNMM in phosphate-buffered solutions, the main decomposition product was not paclitaxel. In fact, under these conditions, paclitaxel was found to be only a minor product (<5%). This result clearly indicates that PNMM does not behave as a prodrug of paclitaxel in phosphate buffer. Upon limited investigation by mass spectroscopy, the decomposition product was found to possess the same molecular weight as PNMM, suggesting that the degradation reaction is one of rearrangement. One possible rearragement would have the decomposition product resulting from an intramolecular attack from the hydroxyl group in the 1 or 7 position of PNMM on its N-methylpyridinium group.

Plasma conversion studies showed that PNMM did convert to paclitaxel to a greater extent than in phosphate buffer but still only to an extent of 25% over a period of 6 hours (**Figure 5**), again suggesting that PNMM is not suitable as a prodrug of paclitaxel. Admittedly, the initial concentration of PNMM in the plasma conversion study was much higher than that anticipated in clinical application.[3] This high concentration was necessary to remain above the detection limit of the assay throughout the duration of the study. Whether this high concentration had an effect on the pathway of conversion of PNMM to paclitaxel or not remains unknown. If the conversion to paclitaxel was enzyme mediated, too high a concentration of PNMM could saturate the en-

zyme, perhaps permitting more of the putative prodrug to degrade by other mechanisms. Considerable work remains in the study of the mechanism of the bioconversion of PNMM to paclitaxel.

CONCLUSION

Stability studies in phosphate buffer showed that PNMM decomposes by an apparent zero-order process that can be explained by micelle formation. In the pH range studied (from 4.0 to 8.0), the pH-rate profile of the decomposition exhibited base catalysis only. Phosphate buffer was found to influence the decomposition rate of PNMM, while a change in ionic strength did not appear to have a noticeable effect. Less than 5% of PNMM converted to paclitaxel in aqueous buffers, while about 25% converted in vitro in human plasma. Thus, although PNMM is of higher water solubility than the parent compound, it cannot be considered a prodrug of paclitaxel.

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