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Effect of Human Serum on the pH-Induced Rheological Properties of Polymethacrylic Acid

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ABSTRACT

The use of polyelectrolytes have been proposed for the production of pH sensitive microcapsules and liposomes. Polymethacrylic acid (PMA) has been synthesized for this purpose and has been shown to be extremely effective in increasing the release from these delivery systems a pH's below 6.0 in distilled water. The current studies show that serum and ionic strength conditions dramatically effect the rate and extent of any PMA conformational changes. The rate of conformational change was found to be proportional to time in contrast to the cube root of time reported within molecular probe investigations. Conformational changes in this study have been monitored using a cone/plate rotational viscometer. The results of this study suggest that under biologically relevant conditions, PMA (Mv 400,000) may not be suitable for use in pH sensitive delivery systems but may have other important uses.

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INTRODUCTION

Macromolecular polyelectrolytes have been proposed as away of achieving pH-controlled drug delivery or targeting[1,2,3]. The ability to achieve a pH-sensitive delivery system is considered to be advantageous for the delivery of medicinals to tissues or interstitial spaces having an elevated or depressed pH compared to physiological pH, i.e. infection sites[4], tumors[5] and some endosomes[6,7]. There are two basic designs which have been proposed: (i)a polyelectrolyte bound to a physically stable capsule wall, e.g. nylon[8] or (ii) a polyelectrolyte is bound to a fluid membrane wall, e.g. liposome[9]. Both systems are dependent on a pH-induced conformational transition[10] of macromolecular polyelectrolytes, i.e. linear to coiled or coiled to linear. Polymers such as poly (2-ethylacrylic acid) (PEAA)[11], polymethacrylic acid (PMA)[12,13], polyacrylic acid (PAA)[14] and poly-L-glutamic acid[14] undergo such a transition. The characteristics of these two basic systems differ only slightly in that the physically stable nylon capsule systems are reversible, while those systems using liposomes are irreversible. The release characteristics for these systems are determined by measuring the flux or release of material from the capsule or liposome as a function of pH using techniques such as florescence[15], conductance[16] and calorimetry[17]. Although the methods of detecting the release of marker compounds differ substantially, the release of a marker compound into a buffer solution (routinely at ionic strengths below that of physiological

fluids) is a commonly accepted and approved experimental design[1,2,3]. While this basic experimental design does provide justification for evaluating these systems, if such systems are to be used in vivo, the release properties should be tested under the appropriate bio-conditions

The present work was initiated in order to characterize the transitional properties of PMA based systems under biologically relevant conditions. In order to keep the results of this study generalized, the release of a marker compound from liposomes or another encapsulated system will not be used. Instead the conformational change of PMA will be determined by characterizing the rheological properties of this molecule.

MATERIALS AND METHODS

Materials. Methacrylic acid (Kodak, USA) was distilled in order to remove p-methoxyphenol, a polymerization inhibitor added by the manufacturer, and partially polymerized methacrylic acid. Anhydrous ethyl ether (Mallinckrodt, Analytical Reagent Grade, USA), methanol (J.T. Baker, HPLC Grade, USA), hydrogen peroxide (Sigma, USA, 30% solution), sodium phosphate monobasic and sodium chloride (Sigma, Molecular Biology Reagent, USA) were used without further purification. Lyophilized human serum (Sigma, USA) was reconstituted with sterile water for injection as required. In all other cases, water was double distilled in a borosilicate glass apparatus.

Synthesis and Characterization of Polymethacrylic Acid. Polymethacrylic acid (PMA) was synthesized and purified

according to the method of Leyte and Mandel[18]. Basically, the process involves heating a 20% by weight solution of methacrylic acid containing 0.5% hydrogen peroxide under nitrogen for two hours at 87°C. The resulting mass was dissolved in methanol and precipitated in multiple steps with ethyl ether. The viscosity molecular weight (Mv) was determined[19] to be 510,000 grams per mole.

Determination of Viscosity. A Brookfield Model LVTDV-IICP cone and plate viscometer was used throughout this study. A CP-42 cone was used in all cases. Since the theta condition of PMA in water is reported to be 26°C[20], the sample cup was thermostated to 26 ± 0.2°C. The sample (1 ml) was introduced through a luer lock sample port and allowed to equilibrate to the experimental temperature. PMA concentrations of 0.5% and 0.8% have been used throughout this study and represent the lowest and highest concentrations, respectively, which would enable the viscosity to be determined throughout the range of shear rates (greater than 10% but not 100% of the instrument spring torque).

RESULTS

An aqueous solutions of 0.8% PMA was prepared and the pH recorded as a function of the volume of titrant (1N NaOH) in order to determine the pK_a for PMA. The titration results are shown in Fig. 1. Analysis of Fig. 1 suggests that the pK_a for our material was approximately 6. Analogous results for PMA have been reported by Leyte and Mandel[18]. A pK_a of 6

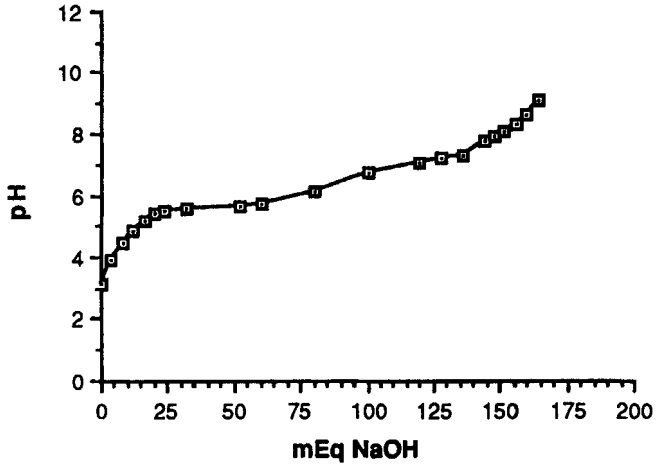

FIGURE 1

pH of polymethacrylic acid in solution (0.8%) at 26°C as a function of mEq sodium hydroxide.

suggests that the coil to chain transition for this sample should occur around pH 6.

As an indicator of conformational changes which can occur, the viscosity of PMA was determined as a function of pH in phosphate buffer (0.01M) in order to confirm that our sample of PMA does exhibit a characteristic transition similar to that observed via release properties of entrapped substances by other investigators[3,8]. The results of these studies are shown in Fig. 2.

Ionic Strength Properties. The rheological properties of 0.5% and 0.8% PMA in various buffer strengths (pH 6) and serum were determined. The results are shown in Fig. 3 as a function of ionic strength.

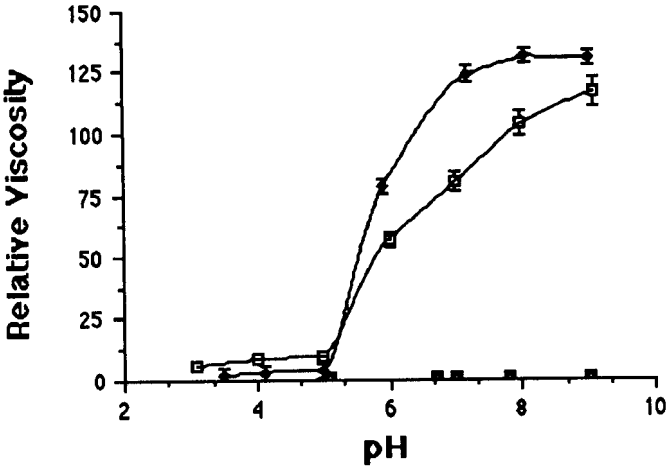

FIGURE 2

Relative viscosity of polymethacrylic acid in water and serum as function of pH at 3 rpm and 26°C (N=3). Legend:◆0.8% PMA ;▫0.5% PMA;▪0.5% and 0.8% PMA in serum.

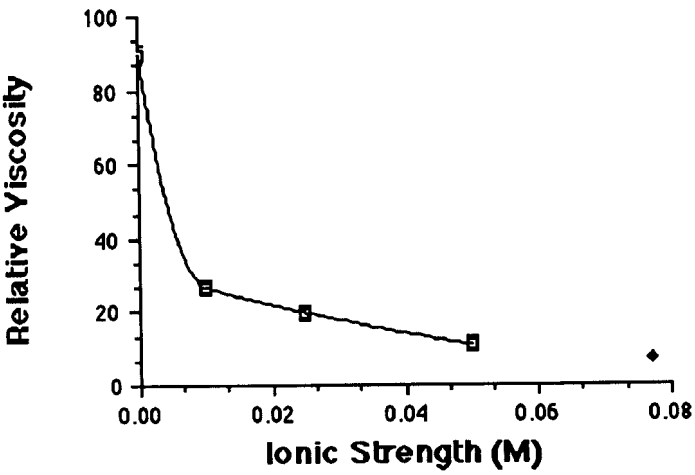

FIGURE 3

Relative viscosity of PMA in buffer and serum as a function of ionic strength. Each sample contained 0.5% PMA at 26°C, pH 6.0. The viscosity was determined at 3 rpm (N=2). Legend: ▫PMA in buffer;◆PMA in serum.

DISCUSSION

Analysis of Fig. 2 indicates that the viscosity of PMA undergoes a transition between pH 5 and pH 7. In general, the comparison of the results shown in Fig. 2 to previous marker release studies[3,8] is very similar. Analysis of Fig. 2 also indicates that the viscosity of PMA (0.5% and 0.8%) in serum above pH 6.0 was essentially no different from the control serum at this and higher pH's. A critical evaluation of the PMA in serum data actually indicates a small increase in the relative viscosity at increasing pH, just the opposite of the expected result. Due to serum denaturation at pH's below pH 5.8 as evidenced by precipitation, the rheological properties of such solutions were not determined. The results suggest that serum may interact with this polyelectrolyte to inhibit the pH induced transition. Due to the size of PMA in this study, there is a potential for multiple sites of interaction between serum and the polymer (M_V = 550,000) which may result in a lack of any notable pH-induced rheological or conformational alterations. Future studies are planned using PMA of lower M_V to delineate the mechanism..

Analysis of Fig. 3 indicates a dependency between viscosity and buffer strength for PMA solutions. With serum present, such is not observed. Analysis of Fig. 3 indicates that as the ionic strength was increased, the relative viscosity rapidly decreased and approached that of the PMA/serum systems. Since normal saline has an ionic strength of 0.077 M NaCl, Fig. 3 suggests that minimal rheological and

conformational changes should be expected in biological fluids, even in the absence of serum components.

In summary, the results suggest that serum can influence the conformational changes of PMA. If polyelectrolytes are to be utilized for the development of a parenteral pH-sensitive delivery system, the effect of the biologic mileu should be determined.

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