

# Application of Ultrasonic Attenuation Measurements in the Studies on Macromolecular Conformational Behaviors ——Phase Behavior of the Aqueous Solution of Poly(vinyl methyl ether) Sensitive to Temperature and the Modification of the Behavior by Using Poly(acrylic acid)

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The phase behavior of the aqueous solution of poly(vinyl methyl ether) (PVME) sensitive to temperature and the modification of the behavior by using poly(acrylic acid) (PAA) have been studied by ultrasonic attenuation measurements and fluorescence probe techniques. It has been observed that PVME solution is transparent at room temperature and becomes turbid upon heating. The solution turns clear again as soon as the temperature is decreased to room temperature. The heating and cooling process can be repeated for many times. The phase behavior of the solution sensitive to temperature is attributed to the conformational changes of the polymer. PVME may adopt an open coil conformation at room temperature. With this conformation, the polymer is well miscible with the solvent, water, and thereby the system is a real solution. The polymer may adopt a compact coil conformation when the temperature is higher than a specific value, which is called the LCST (the lower critical solution temperature) of PVME. In this case, the polymer tangles to each other and forms various aggregates, which can scatter incident light and ultrasonic waves greatly, resulting in the phase separation. Introduction of PAA decreases the temperature sensitivity of the phase behavior of the polymer. The nature of the inhibition is attributed to the complexation of PAA with PVME and the strong hydrophilicity of PAA. Results from fluorescence probe studies are in accordance with those from ultrasonic attenuation measurements, indicating again that the ultrasonic attenuation method can be successfully used for the qualitative studies of polymer conformations and complexation between polymers.

**Keywords** ultrasonic attenuation, conformation, poly(vinyl methyl ether), poly(acrylic acid), complexation

## Introduction

Studies on the conformational and aggregation behaviors of macromolecules in aqueous solution and at solid/liquid interfaces have been highlighted in colloid and interface science since the early 1990s.<sup>1-3</sup> It was mentioned in the first part of this series of studies that development of new methods, which are characterized by being in time and non-destructive and can be used for monitoring the conformational and aggregation behavior of macromolecules, is of critical importance for the promotion of this field of research.<sup>4</sup> Ultrasonic attenuation measurement is one of this kind of methods, and it has been used for the measurement of viscosity of gels,<sup>5</sup> monitoring flocculation in slurries,<sup>6</sup> and study of molecular interactions in solutions<sup>7</sup> due to its mature in theory, simple in operation and non-destructive in nature. On the basis of these considerations and the work con-

ducted in our laboratory, ultrasonic attenuation measurement has been employed in the studies of the phase behavior of the aqueous solution of poly(vinyl methyl ether) (PVME) sensitive to temperature and the modification of the behavior by using poly(acrylic acid) (PAA). The results obtained from ultrasonic attenuation measurement have been confirmed by fluorescence probe study, which is a technique established well in chemical and biological research.

## Experimental

### Materials and instruments

PVME (50% in aqueous phase) was purchased from Aldrich and purified by dialysis with a semi-penetrating membrane (entrapping molecule weight: 6000) for 5 d. The purified solution was maintained as a stock solution.

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The concentration of the polymer in the solution was determined by weighing the residual, which was obtained from freeze-drying of a given volume of the purified PVME solution. Pyrene (Py 96%) was a product of Acros and purified before use by extraction with ethanol in a Soxhlet's extractor. The crystals collected from the extraction are pure Py. PAA was synthesized by polymerization of acrylic acid (AA). The monomer, AA was purified by irradiation first and then distilled under reduced pressure. The details of the preparation, purification and characterization were described in a previous paper.<sup>2</sup> The number average molecular weight of the polymer is 164000 as measured by GPC. Azo-bisobutyronitrile (AIBN) was re-crystallized from methanol prior to use. Other chemicals are of analytical grade, and water was doubly distilled after ion exchange before use.

Mode 5052 Ultrasonic Analyzer was used for the ultrasonic attenuation measurement, and fluorescence measurement was conducted on a Perkin-Elmer LS-50B luminescence spectrometer. The sample tube used in the ultrasonic measurement was a lab-made tube of a flat bottom.

### Measurement principle and method

In liquid, both absorption by visco-elastic medium and scattering caused by micro-particles in the medium are main factors leading to attenuation of acoustic waves.<sup>8</sup> For a polymer solution, the attenuation efficiency of the solution to ultrasonic waves depends upon the frequency and amplitude of the segmental motions of the polymer, and even the average diameters of the polymer coils in aqueous phase, which are well related with the conformation of the polymer chain. The decay of a flat acoustic wave propagating through a homogeneous medium obeys an index law, as shown in Eq. (1).

$$P_x = P_0 e^{-\alpha x} \quad (1)$$

where  $P_0$  stands for the acoustic pressure at the starting point, and  $P_x$  is the pressure at a propagation distance  $x$ .  $\alpha$  is a parameter describing ultrasonic attenuation efficiency. Actually,  $\alpha$  is often expressed as:

$$\alpha = \frac{20}{x} \lg \frac{P_0}{P_x} \quad (2)$$

where the unit of  $\alpha$  is dB. It is well known that in an acoustic field, the acoustic pressure  $P$  can be expressed as:

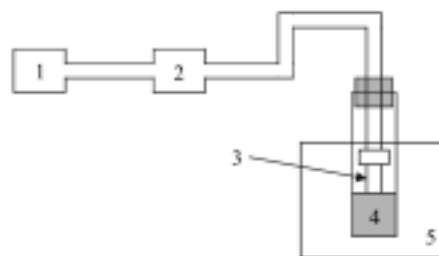
$$P = 2\pi\rho C f A \quad (3)$$

where  $\rho$  is the density of the medium,  $C$  the propagation velocity of the wave in the medium,  $f$  the acoustic frequency, and  $A$  is the amplitude of the wave. Clearly, for a given system, the acoustic pressure is directly proportional to the amplitude of its wave, and thereby ultrasonic attenuation can be expressed as:

$$\alpha = \frac{20}{x} \lg \frac{A_0}{A_x} \quad (4)$$

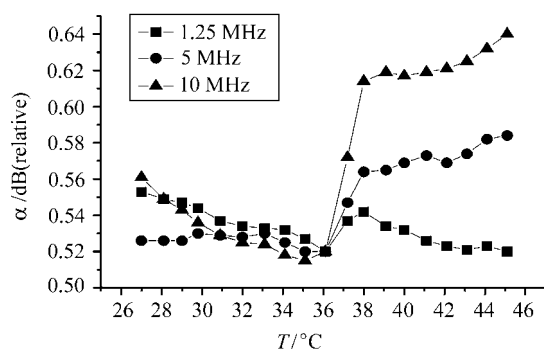
where  $x$  is the thickness of the sample,  $A_0$  and  $A_x$  are the amplitudes of the input wave signal and the reflected wave signal, respectively.

The experimental setup was shown in Figure 1. Ultrasonic signal emitted by transducer (3) propagates through a sample solution (4) to the bottom of the tube and is reflected by glass, it comes back into the solution and arrives the ultrasonic analyzer (2). On the screen of the oscillograph (1), appear two obvious waveforms, corresponding to the emitting signal and the reflecting signal, respectively.  $A_1$  and  $A_2$  stand for the amplitudes of the two signals, respectively. With the distance between the surface of the bottom of the sample tube and the surface of the transducer, and the two  $A$  values, the ultrasonic attenuation caused by the polymer solution can be calculated by using Eq. (4). The ultrasonic wave frequencies produced by the transducers are 10, 5 and 1.25 MHz, respectively. During the measurement, the transducer was fixed properly to ensure that there is no air between the sample and the transducer. The reflectance of the glass to the ultrasonic wave is very effective because its density is much greater than that of the solution, and thereby, the attenuation is mainly caused by the solution. On the basis of this consideration, the calculations may be carried out.



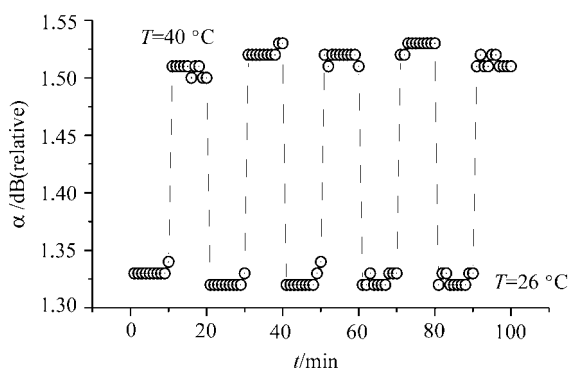
**Figure 1** Schematic diagram of the apparatus for ultrasonic attenuation measurements. 1, oscillograph; 2, ultrasonic analyzer; 3, ultrasonic transducer; 4, sample; 5, thermostat.

The testing process: a given volume of the PVME solution (0.2 wt% in water) was put into the sample tube, and an ultrasonic transducer was inserted slowly into the tube. The position of the transducer was adjusted and fixed to let the surface of it be just in contact of the solution. Then, the distance between the surface of the transducer and the bottom of the tube ( $x/2$ ) was measured accurately. The ultrasonic attenuation efficiency was measured at each temperature from 22 to 44 °C with a gap of 1 °C. The amplitudes of the input and the reflected wave signals were recorded for each measurement, and used for calculating ultrasonic attenuation. Figure 2 shows the relative ultrasonic attenuation of the PVME solution as a function of temperature at frequencies of 1.25, 5 and 10 MHz, respectively.



**Figure 2** Temperature dependence of the relative ultrasonic attenuation of the PVME aqueous solution measured with different ultrasonic transducers.

A similar method was employed for monitoring the reversible phase behavior of the polymer solution. The solution was thermo-stated at 26 °C for 30 min, and then the ultrasonic attenuation efficiency was measured. The measurement was repeated for 10 times. After the measurements, the temperature of the solution was raised to 40 °C and thermo-stated for another 30 min. Again, the ultrasonic attenuation efficiency was measured for another 10 times. The whole process was repeated for a number of times, and the result was shown in Figure 3.



**Figure 3** Time dependence of the relative ultrasonic attenuation of the PVME solution at two different temperatures (40 and 26 °C).

For complexation measurements, all sample solutions with various ratios (0.03 : 1; 0.15 : 1; and 0.45 : 1) of PAA to PVME, of which PVME concentration was fixed to 0.2 wt%, were prepared in a similar manner. The method may be described by taking a solution of PAA and PVME (the ratio of the two polymers is 0.03 : 1 in monomer units) as an example. To prepare the solution, 1.6 mL of PAA stock solution (0.05 wt%) and 4.4 mL of PVME solution (0.45 wt%) were added, sequentially, to a 10 mL volumetric flask under shaking. The mixture was diluted to 10 mL with water and equilibrated at room temperature for 20 min. After the equilibration reached, the ultrasonic attenuation of the mixture was measured as a function of temperature. The mixtures of other ratios (0.15 : 1 and 0.45 : 1) were prepared and studied in a similar manner.

The polymer solutions used for Py probe studies were prepared in the following way. A stock solution ( $10^{-3}$  mol/L) of Py in ethyl ether was prepared, and then diluted to  $10^{-5}$  mol/L with ethyl ether before using. 100  $\mu$ L of the Py solution ( $10^{-5}$  mol/L) was injected into a 10 mL volumetric flask. The solvent, ethyl ether was evaporated at room temperature overnight. Then, a required volume of the solution of PAA and PVME mixture was added to the flask, and diluted with water to 10 mL. To ensure solubilization and equilibration, the polymer/probe solution was sonicated for 20 min, and left at room temperature for more than 12 h. Each fluorescence measurement was repeated for more than 10 times, and the results shown in the figures are average values. The ratio of  $I_3/I_1$  is an indication of the micro-polarity of the medium of the probe, the larger the ratio, the lower the polarity of the medium.  $I_3$  and  $I_1$  are the intensities of the emission at 400 and 380 nm, respectively, with 340 nm as an excitation wavelength.<sup>9,10</sup>

## Results and discussion

### Thermo-sensitivity of PVME solution

Similar to the behavior of poly(*N*-isopropylacrylamide) (PNIPAM),<sup>11</sup> the aqueous solution of PVME is transparent at temperatures below a specific value, but turbid at temperatures above that.<sup>12,13</sup> The phase behavior of the solution is reversible, and the specific temperature is called the lower critical solution temperature (LCST) of the polymer. The phase behavior of the PVME solution has been attributed to the conformational changes of the polymer.<sup>13</sup> It is believed that the polymer adopts an open coil conformation at temperatures below its LCST, but a compact coil structure at temperatures above that. Phase separation occurs due to aggregation of polymer chains.

As mentioned earlier, the propagation of ultrasonic wave in solution could be attenuated by scattering and absorption of the medium. The attenuation efficiency of a polymer solution to a given ultrasonic wave with specific frequency depends upon the size and size distribution of the aggregates. It is not difficult to understand that the size and size distribution of the aggregates in a polymer solution are dependent upon the conformation of the polymer. Therefore, the polymer conformation could be studied qualitatively, at least, by ultrasonic attenuation measurement. On the basis of this consideration, we successfully studied the conformational behavior of PNIPAM doped in chitosan hydrogels as a function of temperature, and that of poly(methacrylic acid) (PMAA) in aqueous phase as a function of pH.<sup>4</sup>

Figure 2 shows the plots of the ultrasonic attenuation efficiencies of the PVME solution measured with different transducers against temperature. Interrogation of the figure reveals that (1) for all plots, there is a transition around 37 °C, which corresponds to the LCST of the polymer; (2) the difference in the attenuation efficiency before and after the sharp change depends on the

frequency of the transducer employed, and the larger the frequency, the more sensitive the technique to the change in phase behavior. The first observation may be rationalized by considering the conformational change of the PVME chain below and above its LCST, that is, the polymer adopts compact coil conformation above its LCST and loose coil conformation below the temperature. The second finding is the same as that observed in the studies of the phase behavior of PNIPAM.<sup>4</sup> Considering that the transducer with larger frequency is more sensitive to the change in the phase behavior of polymer solution, the ultrasonic transducer of 10 MHz was employed for the following studies.

Further interrogation of Figure 2 reveals that the change of the attenuation efficiency around 37 °C is not a real "sharp" process. It lasts for a few degrees. This observation is in coincidence with the result reported by Karayanni and co-workers,<sup>13</sup> by using an UV absorption technique. The phenomenon is not difficult to understand because the phase separation occurs only when the polymer coil is compact enough that the density of the aggregates is greater than that of the medium. Clearly, the compactness of the polymer coil is a function of temperature, and it is a result of comprehensive interactions, including intra-polymer interactions, inter-polymer interactions, and the interactions between the polymer and the solvent. Therefore, the phase separation occurs gradually. The finding that change in polymer conformation is a progressive process was also reported by Thomson and his co-workers,<sup>14</sup> who studied the conformational changes of PMAA in aqueous phase as a function of pH by combining fluorescence quenching and stop flow techniques.

### Reversibility of the phase behavior of the PVME solution

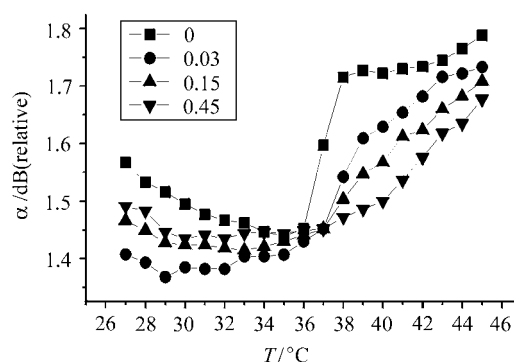
Figure 3 depicts the result from ultrasonic attenuation studies of the phase behavior of the PVME solution by alternating the temperature of the solution between 26 and 40 °C. As that expected, the value of the parameter  $\alpha$  is about 1.33 dB at 26 °C, and increases to 1.53 dB when the solution is heated to 40 °C. The value recovers to about 1.33 dB again when the temperature of the system is decreased to 26 °C. As that indicated in the former section, the lower  $\alpha$  value corresponds to clear solution, and larger one corresponds to turbid solution. Alternation in temperatures between 26 and 40 °C causes alternation in the values of  $\alpha$  indicates that the phase behavior of the polymer solution is reversible, and the phase separation occurring in the system is a pure physical process. The change may be employed to design new smart materials like "smart gels" and "smart films".<sup>15,16</sup> Related studies are under progress.

### Inhibition effect of PAA to the phase behavior of PVME solution

It is expected that the phase behavior of PVME may be altered by the presence of other polymer, especially when specific interactions exist between the two poly-

mers. As that reported by Karayanni and Staikos,<sup>13</sup> the complexation of PVME with PAA makes the phase behavior of PVME change a lot when PAA was introduced into the system. A similar finding was observed in the present study. Figure 4 shows the phase behavior of the PVME solution as a function of temperature in the presence of various concentrations of PAA. Clearly, with increasing PAA concentration (or the ratio of PAA to PVME), the transition in the attenuation efficiency around 37 °C is more diffused, demonstrating that introduction of PAA inhibits the phase separation of PVME. This observation may be understood by considering the interaction between PVME and PAA, and the dependence of the conformation of PAA on temperature. Karayanni<sup>13</sup> and Cowie<sup>17</sup> discovered the complexation of PAA with PVME within a wide pH range. Furthermore, it was reported also that PAA adopts an open coil conformation within a wide pH and temperature range. Thus, the complexation of PVME with PAA makes the chain of PVME less flexible, and thereby difficult to change from a loose coil conformation at lower temperatures to a compact coil conformation at higher temperatures. A direct result of this effect is the alteration of the phase behavior of the PVME solution.

Fluorescence probe experiment was also conducted in order to verify the reliability of the result from ultrasonic attenuation studies.

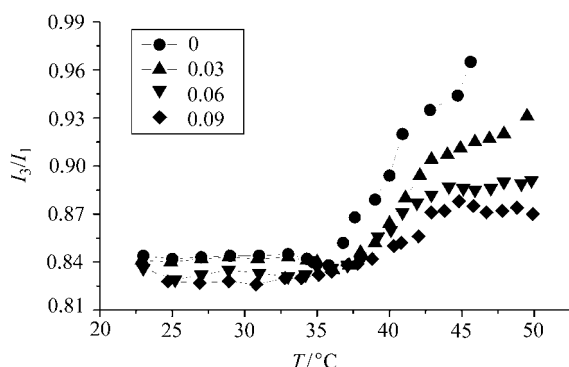


**Figure 4** Dependence of the relative ultrasonic attenuation of the PVME solution on temperature under different concentrations of PAA (the values inserted in the figure are the ratios of PAA : PVME in monomer units).

### Fluorescence probe studies

It is well known that only a limited number of water-soluble polymers show LCST property. This is because both hydrophobic structures and hydrophilic structures exist within the chain of this kind of polymers. As that of surfactant in aqueous phase, the hydrophobic part of the polymers tends to avoid the solvent—water, but the hydrophilic part tends to dissolve in it. Therefore, the conformation adopted by a polymer having LCST property in aqueous phase is a balance of hydrophobic interaction and hydrophilic interaction. For PVME, ether-oxygen is the hydrophilic structure, and the carbon chain and the side methyl group are the hydrophobic structures. As the solvent is water, the hydrophilic part

of the polymer will expose itself to the medium if it takes a compact coil conformation, and the inside of the coil should be relatively hydrophobic. This is to say that the polymer solution is of micro-multiphase property. Introduction of a polarity sensitive fluorescence probe like Py into the system will result in partition of the probe between the two phases. Clearly, more Py will stay inside of the coil due to its hydrophobic nature. A direct result of this partition is that the fine structure of the emission spectrum of the probe is characterized by a larger  $I_3/I_1$  values. This is because the monomer emission of Py is composed of five sharp peaks. The one around 384 nm is assigned as peak III, and the one at 373 nm is peak I. It is well documented that the ratio of  $I_3/I_1$  is closely related to the polarity of its microenvironment, and  $I_3/I_1$  is called Pyrene scale.<sup>9,18</sup> It has been found that the greater the ratio is, the less polar the micro-environment of the probe. Based upon this principle, Py has been used widely to measure the multi-phase property of polymer solutions, and from the result, the conformation adopted by the polymer may be deduced.



**Figure 5** Temperature dependence of  $I_3/I_1$  of pyrene dispersed in the PVME aqueous solution in the presence of different concentrations of PAA (the values inserted in the figure are the ratios of PAA : PVME in monomer units).

Figure 5 shows the plots of  $I_3/I_1$  against temperature at various ratios of PAA/PVME. Comparing the characters of Figure 5 and those of Figure 4, it may be found that both parameters vary with temperature in a similar way. The larger values of  $I_3/I_1$  at higher temperature indicate that more probe partitioned in the inner part of the polymer coil at these temperatures, suggesting that the polymer adopts a compact coil conformation. Further examination of the figure reveals that at a given temperature above 37 °C, the ratio decreases along with increasing PAA concentration, indicating that the polarity of the polymer coil increases. This finding may be also rationalized by assuming the complexation of PVME and PAA, and supported the results obtained by ultrasonic attenuation studies.

## Conclusion

Ultrasonic attenuation measurements and fluorescence probe studies demonstrate that the phase behavior

of the aqueous solution of PVME sensitive to temperature is caused by the conformational changes of the polymer, which takes an open coil conformation at temperatures below its LCST and a compact coil conformation at temperatures above its LCST. In addition, the phase behavior of the polymer sensitive to temperature is reversible, and may be inhibited by introduction of PAA. The nature of the inhibition has been attributed to the complexation of PVME with PAA. The results from ultrasonic attenuation measurement are in accordance with those from fluorescence probe studies, indicating again that the ultrasonic attenuation method is an in time and non-destructive technique for monitoring phase behavior of polymers and complexation between polymers in solution. Compared with that of fluorescence techniques, the sensitivity of ultrasonic attenuation method may be low, but it may be used for the studies of some special systems of which fluorescence techniques can not be used.

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