CONCENTRATION DEPENDENCE OF ULTRASONIC ABSORPTION IN AQUEOUS SOLUTIONS OF BOVINE SERUM ALBUMIN

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ABSTRACT. Previous investigations have considered the ultrasonic absorption in macromolecular solutions to be a linear function of concentration, at least for concentrations less than about 10% by weight. In the present study, absorption measurements were made at six frequencies, from 3.4 to 15 MHz, at a temperature of 20°C, in aqueous solutions of bovine serum albumin (M_w66,000) in the concentration range to about 40%. These measurements reveal a distinctly nonlinear dependence of the absorption on concentration and provide an assessment of the degree to which linearity, in the low concentration range, can be considered. Intermolecular interactions are believed to be the source of the nonlinear concentration effects.

Introduction

The mechanism(s) by which acoustic energy is absorbed in biological materials is usually attributed to processes occurring at the macromolecular level.[1,2] Since tissues and organs are overly complex media in which to study such absorption processes, useful measurements can be made in aqueous solutions of pertinent and available biomacromolecules. Concentrations of less than about 10% by weight provide simple macromolecular systems for investigation, as it is generally assumed that intermolecular events do not occur. It is uncertain, however, whether the absorption mechanism(s) responsible in the biopolymer solution case are the same processes involved in the absorption of sound in tissue, since tissue is a highly structured medium for which typically about 15% of the wet weight is attributed to proteins, 75% to water, and the remaining 10% to other constituents. In addition, the ultrasonic absorption exhibited by tissue is three to five times greater than that observed in, for example, a 10% protein solution.

More recent studies have indicated that absorption values typical of soft tissues may be obtained by increasing the biomacromolecular concentration of specimen solutions to about 45%.[3] The concentration of biopolymer under investigation is seen then to have a substantial effect on the observed ultrasonic absorption, and possibly on the mechanism responsible for that absorption. Previous study concerning the concentration dependence of ultrasonic absorption in biopolymer solutions have suggested a linear dependence for dilute solutions (less than about 15%), and a deviation from linearity in concentrated solutions (greater than 15%).[4,5,6] In the case of dilute solutions, absorption is attributed to processes involving solvent-solute interaction, whereas for more concentrated solutions, absorption seems to arise from intermolecular interaction processes. It is the purpose of this study to examine the concentration dependence on the ultrasonic absorption coefficient as a function of frequency and concentration. Such a relation should aid in understanding, and realistically viewing, the dependence of ultrasonic absorption as a linear function of concentration for dilute biopolymer solutions, and in defining more precisely the nature of the nonlinearity observed at the higher concentration.

Experimental Techniques

The globular protein bovine serum albumin (BSA) used in this study was obtained from Calbiochem Corporation, San Diego, California, in crystalline form (Fraction V, B Grade, 98% pure by electrophoresis). Since ultrasonic measurements were to be made in both forward and back concentration titrations, solutions were prepared by adding the appropriate amount of either BSA or singly deionized and distilled water to each test solution, and the combination mixed with a magnetic stirrer while being refrigerated at 7°C until complete solution was achieved. The solutions of this study were not filtered because the high concentrations, above about 15%, made this process very difficult. It has been found, however, that impurities which would be filtered with a 5.0μ filter do not contribute significantly to the observed ultrasonic velocity or absorption.[7]

Upon completion of the mixing process the solution was placed in the ultrasonic measuring tank (minimum volume about 500ml), allowed to reach thermal equilibrium, and the ultrasonic measurements performed. The concentration of BSA by weight of the specimen solution was determined after each ultrasonic measurement by evaporation over air until dry and storing in a vacuum dessicator for 24 hours before weighing. It has been found that concentrations measured in this way have an uncertainty of ± 0.5%.

Ultrasonic measurements were made using an automated version of the Pellam and Galt method,[8,9] consisting of a transmitting and receiving transducer, and employing standard pulse techniques. System details have been described elsewhere.[7,10] The technique assumes that the absorption of the fluid under investigation behaves exponentially as,

$$P(x) = P(0)^{-\alpha x} \qquad (1)$$

where P(0) is the sound pressure amplitude at x=0, x is the acoustic path length traversed by the pulse, and α is the amplitude absorption coefficient per unit path length. Speed of sound measurements were made by comparing the phase of a received pulse with coherent reference signal, as the transducers are moved relative to each other at a constant known velocity. This comparison results in a periodic interference signal used to evaluate the phase velocity of the sound wave.

Absorption coefficients and sound velocities in distilled water at 20°C obtained by this system averaged over four trials are within ± 3%[11] and ± 0.05% of their respective accepted values.[12] The system is capable of maintaining test liquid temperatures to ± 0.05°C.

Results

The ultrasonic absorption and velocity in aqueous solutions of bovine serum albumin (BSA) was measured as a function of concentration at a temperature of 20°C. Velocity measurements were carried out at a frequency of 8.02 MHz, while absorption measurements were made at six frequencies, in the range 3.41 to 14.93 MHz. The BSA concentration was varied in both forward and back concentration titrations to assure that the process of preparing the more highly concentrated solutions did not alter irreversibly the acoustic properties of the solution.

The absorption data is presented in terms of the excess frequency-free absorption coefficient, A, defined as,

$$A = \frac{\text{solution absorption - solvent absorption}}{(\text{frequency})^2} \quad (2)$$

$$= \frac{\Delta\alpha}{f^2}$$

The solution absorption has been corrected for diffraction effects using the technique given by Del Grosso.[13] The solvent is water and at 20°C has a value for A of 25.3×10^{-17} sec^2 cm^{-1}.[11]

The ultrasonic absorption (in terms of A) of aqueous solutions of BSA is shown as a function of BSA concentration in Figure 1, for the frequency 3.41 MHz, with data points plotted for forward and back concentration titration. Figure 2 is a composite (with data points omitted) of data taken at the six frequencies of measurement. Two observations can be readily made from these plots. First, the absorption data obtained as the BSA concentration was gradually increased to about 38% (forward concentration titration), within experimental error, was the same as that obtained as the BSA solution was diluted (back concentration titration). This implies that the solution preparation procedure very likely did not influence irreversibly the acoustic properties of the solution. While data of this type is only shown for one frequency, this result was typical of all frequencies measured. Second, the relation between the absorption and BSA concentration is nonlinear throughout the frequency range of observation.

Figure 3 is a log-log plot of the 3.41 and 14.93 MHz data, from which the slope 1.2 is determined. The ordinate intercepts are frequency dependent. From such curves the empirical relation between ultrasonic absorption and BSA concentration may be written as,

$$A = K\,C^{1.2} \quad (3)$$

where C is the BSA concentration and K is a function of frequency.

Table 1 shows values of K for each frequency investigated, for the concentration in units of gm/100cc and A in units of 10^{17} sec^2 cm^{-1}. Plotting the values for K as a function of frequency on a log-log plot, as shown in Figure 4, yields a straight line of slope -0.55 and intercept of 53.21, for frequency, f_m, in units of MHz. This provides the empirical equation

$$K = 53.21\, f_m^{-0.55} \quad (4)$$

Combining (3) and (4), the empirical equation for the excess frequency-free absorption as a function of BSA concentration is

$$A = 53.21\, f_m^{-0.55} C^{1.2} \quad (5)$$

Comparison of measured absorption data with that predicted by equation (5) is shown in Figure 5, for the frequencies 3.41, 8.02, and 14.93 MHz. Acceptable agreement is obtained. To test the empirical relation over a more extended range of these parameters, absorption data of a number of investigators, for 10% BSA solutions, at temperatures near the 20°C measurement temperature of this study, are shown in Figure 6 as a function of frequency, over the frequency range 1 to 165 MHz. While some deviation of the measured absorption values from that of the empirical equation occur at the higher frequencies (greater than about 100 MHz), good agreement (to within 10%) is obtained between from 1 to 50 MHz.

While the absorption exhibits a very nonlinear dependence upon concentration, it is uncertain whether such behavior is exhibited by the sound velocity. As shown in Figure 7, it appears that a linear dependence on concentration prevails, although some deviation from linearity (about 1%) seems to be evident at concentrations greater than about 30%.

Discussion

The ultrasonic absorption of aqueous solutions of bovine serum albumin has been found to increase in a nonlinear manner with increasing solution concentration. This alters the previous belief that the concentration dependence of absorption could be described as linear for concentrations less than about 15%. It thus appears that intermolecular interaction contributes appreciably to measured absorption throughout the entire concentration region of measurement. However, nonlinearity is, not exceptionally pronounced at the low solute concentrations and this may justify the assumption of linearity by earlier workers.

The empirically obtained frequency dependance, viz., $f^{-0.55}$, is in agreement with that obtained for dextran,[17] ($f^{-0.54}$) and is similar to that obtained for hemoglobin[4] ($f^{-0.7}$).

While serum albumin and hemoglobin, both globular proteins, may be expected to exhibit similar frequency dependencies, the nearly identical frequency dependence of dextran, a material which differs so markedly in molecular weight, chemical composition, molecular size, and structure indicates that a common intermolecular interaction mechanism may be responsible for the observed absorption in these solutions.

Acknowledgement

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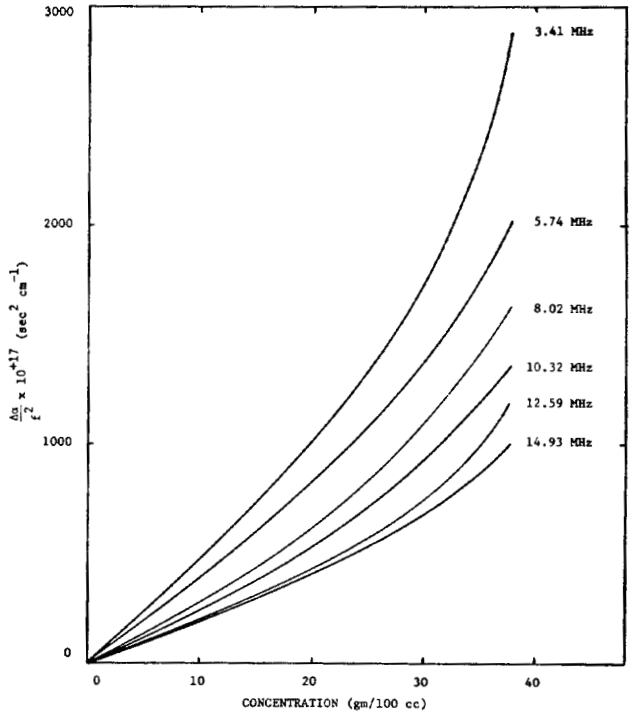

Figure 2 Excess frequency-free absorption as a function of BSA concentration at the six frequencies of measurement, 20°C.

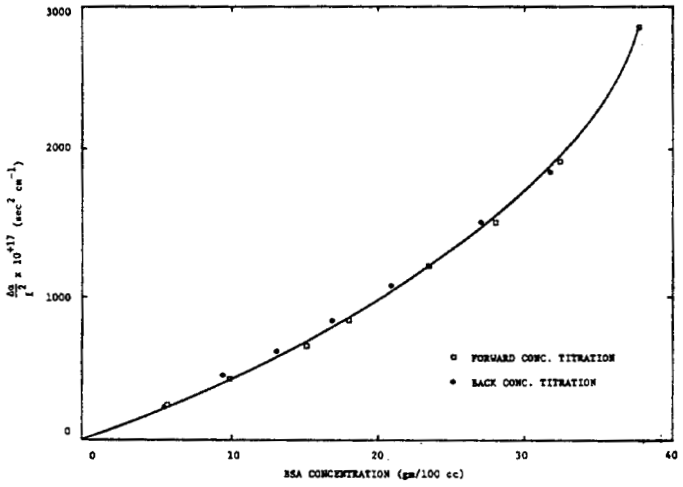

Figure 1 Excess frequency-free absorption versus BSA concentration, 3.41 MHz, 20°C.

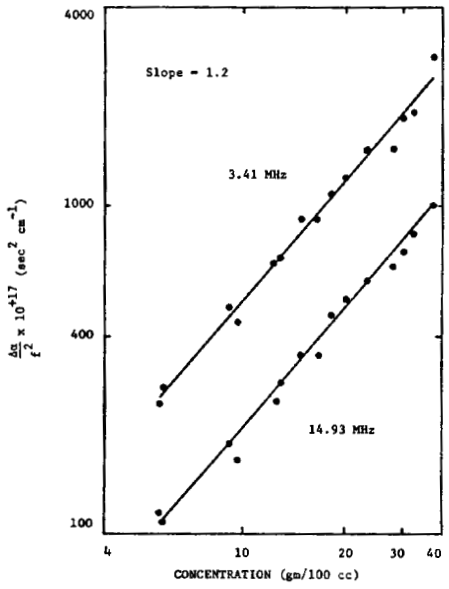

Figure 3 Excess frequency-free absorption versus BSA concentration, 20°C.

f_m (MHz)	K (10^{17} sec^2 cm^{-1})
3.41	27.5
5.74	22.0
8.02	17.9
10.32	14.7
12.59	13.5
14.93	12.3

Table 1 Values of the intercept K at the six measurement frequencies.

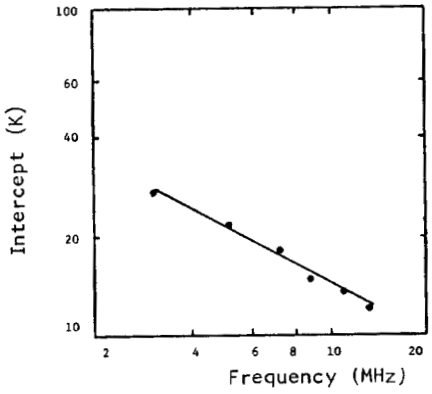

Figure 4 Intercept (K) versus frequency.

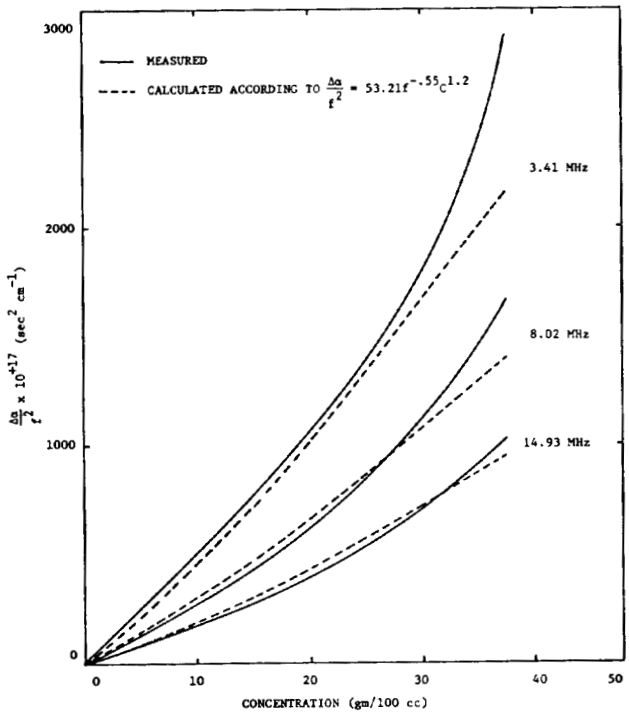

Figure 5 Comparison of calculated and measured concentration dependence on excess frequency-free absorption.

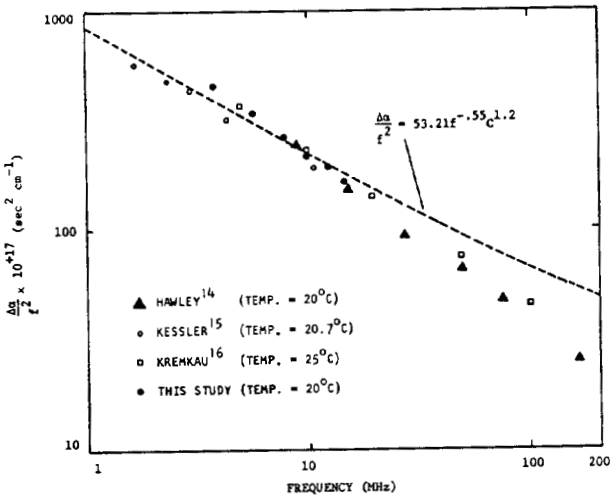

Figure 6 Excess frequency-free absorption versus frequency, 10 gm/100cc concentration.

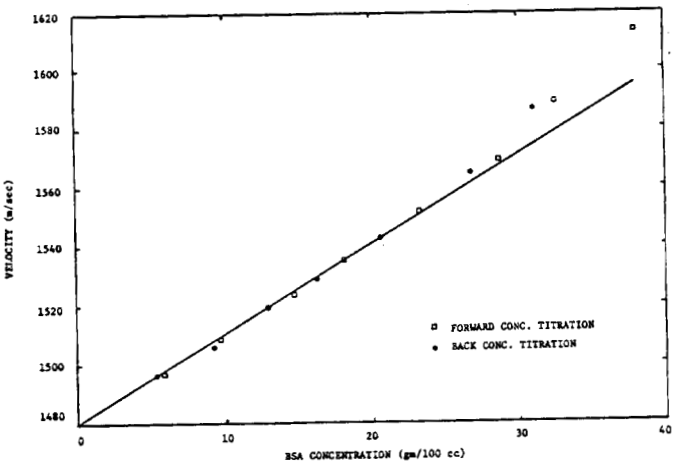

Figure 7 Velocity versus BSA concentration, 8.02 MHz, 20°C.