Acoustic nonlinearity parameter B/A of aqueous solutions of some amino acids and proteins

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A precision ultrasonic velocimeter and a new differential method of calculation of specific concentration increments were employed to study the nonlinear acoustic properties of aqueous solutions of ten amino acids and six proteins. The greater precision of the velocity measurements allows much more dilute solutions to be treated, providing for a wider range of amino acids and proteins to be studied. The specific increment of B/A of amino acids was found to exhibit a significant sensitivity to change of position or replacement of a single atomic group of the solute molecule. A strong influence of the character of the solute–solvent interaction on the B/A value is revealed. It is shown that the specific increment of B/A of a solute could be much more sensitive to the molecular structural features than other parameters such as the specific increments of sound velocity and density.

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INTRODUCTION

Data on the acoustic nonlinear parameter B/A of aqueous solutions of organic and inorganic compounds suggest that it is dependent upon the chemical composition of solutions and upon the molecular structure of the solute.[1–10]

Current methods and instruments employed for measuring the nonlinearity parameter B/A contribute relatively large errors and, therefore, high concentrations of solute in solutions are required in order to make reasonable determinations. This difficulty limits the number of solutions of biological compounds that can be studied. In studies of the molecular physics of solutions it is usually not necessary to know the absolute values of corresponding physico-chemical, thermodynamic, and acoustic parameters, but rather the increments of these parameters; that is, the difference between solution and solvent. Thus, a differential method for calculating the concentration increment of the nonlinearity parameter B/A of the solute is developed, which requires only knowledge of concentration slopes of the corresponding solution parameters. The accuracy of the relative measurements of the nonlinearity parameter is better than 0.3%, which makes it possible to carry out systematic investigations to find correlations between the nonlinearity parameter B/A of dilute aqueous solutions of biological substances and molecular structure of the solute.

I. REQUIRED SENSITIVITY FOR INVESTIGATION OF THE NONLINEAR PROPERTIES OF BIOMOLECULAR SOLUTIONS AND THE ERRORS OF THE CONVENTIONAL METHODS

Most biological compounds cannot be obtained in high concentrations in aqueous solution because of low solubility, e.g., nucleic bases, nucleosides, some amino acids, and proteins, or because of aggregation processes, e.g., in the case of many proteins, nucleic acids, lipids, etc. Thus, the typical range of concentrations for biochemical investigations is not greater than approximately 1–30 mg/cm^3. The contribution of such amounts of solute to the nonlinearity parameter of the aqueous solution is very small; of the order of 1%. This estimate is easily obtained from the published nonlinearity data of highly concentrated protein solutions,[8] i.e., 300 mg/cm^3 of BSA in aqueous solution yields only about a 20% increase in the nonlinearity parameter of the solvent. Therefore, methods of measurements that do not provide precision of measurements better than 1% have a limited usefulness for biomolecular studies.

There are two principal methods for determining the nonlinearity parameter B/A: the finite amplitude method and the thermodynamic method. The finite amplitude method is based on the measurement of the amplitude of the second harmonic generated due to distortion of the propagating sinusoidal wave. The literature contains descriptions of various versions of this method.[1,6,8,11–13] Analysis of the finite amplitude method carried out by Law *et al.*[8] has shown the total systematic error to be of the order of $\pm$ 8%.

The thermodynamic method is based on the following expression obtained by Beyer:[14]

$$\left(\frac{B}{A}\right) = 2\rho_0 U_0 \left(\frac{\partial U}{\partial p}\right)_T + \frac{2U_0 T\alpha}{C_p}\left(\frac{\partial U}{\partial T}\right)_p, \tag{1}$$

where ρ_0 is the equilibrium density, U_0 is the infinitesimal amplitude sound wave velocity, T is the absolute temperature, C_p is the specific heat capacity at constant pressure, α is the volume coefficient of thermal expansion, and $(\partial U/\partial p)_T$ and $(\partial U/\partial T)_p$ are, respectively, the change of the sound velocity with pressure at constant temperature and the change of sound velocity with temperature at constant pressure. It has been shown that an accuracy for the determination of the nonlinearity parameter B/A in liquids by means of the conventional thermodynamic method could be about 5%.[8] Such accuracy of both the finite amplitude and the thermodynamic methods limits the usefulness of direct application of these methods to biomolecular investigations.

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II. A RELATIVE THERMODYNAMIC METHOD FOR DETERMINATION OF THE NONLINEAR PROPERTIES OF SOLUTIONS

A significant reduction in the error of evaluation of the relative contribution of a solute to the nonlinear properties of a solution can be obtained by a differential method of calculation of specific increments of the B/A parameter, described below. The most important feature of this method is that only differential values (derivatives) are needed such as the concentration slopes of sound velocity, density, heat capacity, coefficient of thermal expansion, and derivatives of $(dU/dp)_T$ and $(dU/dT)_p$.

The problem of the investigation of the nonlinear properties of solutions of biological substances can be treated by measuring the relative difference between the nonlinearity parameters B/A of the solution and of the solvent. The ratio of that difference to the concentration of the solution could be defined as a specific increment of the B/A of a solute and used to characterize its ultrasonic nonlinearity properties. The relationship for the specific increment of B/A may be found by differentiating Eq. (1),

$$\frac{\Delta(B/A)}{C}\cdot\frac{1}{2\rho_0 U_0} = \frac{1}{C}\Delta\left(\frac{\partial U}{\partial p}\right)_T + ([U]+[\rho])\left(\frac{\partial U}{\partial p}\right)_{T_0} + \frac{\alpha T}{\rho_0 C_p} \times\left[\frac{1}{C}\Delta\left(\frac{\partial U}{\partial T}\right)_{p_0} + ([U]+[\alpha]-[C_p])\left(\frac{\partial U}{\partial T}\right)_{p_0}\right], \tag{2}$$

where C is the concentration of the solution, values related to solvent are denoted by the subscript 0, and Δ means the difference between solution and solvent for the corresponding parameter. Here $[U]$, $[\rho]$, $[\alpha]$, and $[C_p]$ are the relative specific increments of, respectively, sound velocity, density, coefficient of thermal expansion, and specific heat capacity at constant pressure, defined as

$$[U]=\frac{\Delta U}{U_0 C},\quad [\rho]=\frac{\Delta\rho}{\rho_0 C},\quad [\alpha]=\frac{\Delta\alpha}{\alpha_0 C},\quad [C_p]=\frac{\Delta C_p}{C_{p_0} C}.$$

The values of $[\rho]$, $[\alpha]$, and $[C_p]$ for aqueous solutions of some biological compounds can be found in the literature and/or calculated from the literature data on apparent molar volume ϕV; expansibility ϕE, and heat capacity ϕC_p, respectively, that is,

$$[\rho]=M/\rho_0-\phi V, \tag{3}$$

$$[C_p]=\frac{\phi C_p/C_{p_0}-M}{\rho_0[1+C(M/\rho_0-\phi V)]}, \tag{4}$$

$$[\alpha]=\phi E/\alpha_0-\phi V, \tag{5}$$

where

$$\phi C_p=\frac{C_p'-n_1\overline{C}_{p_0}}{n_2},\quad \phi V=\frac{V-n_1\overline{V}_0}{n_2},$$

$$\phi E=\left(\frac{\partial\phi V}{\partial T}\right)_p=\frac{\alpha V-\alpha_0\overline{V}_0 n_1}{n_2}.$$

Here, M is the molecular weight of solute, n_1 is the number of moles of solvent, n_2 is the number of moles of solute, C_p' is the solution heat capacity, $\overline{C}_{p_0}$ is the molar heat capacity of the pure solvent, V the solution volume, and $\overline{V}_0$ the molar volume of pure solvent. Measurements of $[U]$, $\Delta(\partial U/\partial p)_T$, and $\Delta(\partial U/\partial T)_p$ are required for determination of the specific increment of the nonlinearity parameter B/A, provided the corresponding parameters of the solvent, viz., U_0, ρ_0, α_0, C_{p_0} $(\partial U/\partial p)_{T0}$, and $(\partial U/\partial T)_{p_0}$, are known.

Therefore, an ultrasonic velocimeter for precise relative measurements of solutions, in a sufficient range of pressures and temperatures, is required for investigation of the acoustical nonlinearity of solutions of biological substances.

The precision measuring technique, together with the differential method for calculating $\Delta(B/A)$, provides an approach to understanding the physical nature of the dependence of B/A on biomolecular structural features.

III. PRECISION ULTRASONIC MEASURING INSTRUMENT

An instrument for high-precision ultrasonic measurement in biological liquids, in a wide range of temperatures and pressures, has been developed and will be described in greater detail elsewhere. Briefly, the velocimeter is comprised of a four-channel resonator cell in a high-pressure vessel, an electronic unit, a high-pressure control system, and a thermostated water bath. The device provides simultaneous measurements of sound velocity in the liquids filling the four parallel measuring chambers. Each of the four measuring chambers has a volume of 0.2 cc.

Measurement of the sound velocity is made by the so-called resonator method.[15,16] The resonator cell is made of the inert metals titanium or stainless steel. The piezoelectric transducers are made of lithium niobate and backed by benzene, which transmits the hydrostatic pressure from the high-pressure control system. The resonance frequency of the transducers is about 10 MHz. All measurements reported herein were carried out in the 7.5 to 8.0-MHz frequency range.

The high-pressure vessel, containing the measuring cell, is placed in the thermostated water bath, where the temperature is maintained with an accuracy $\pm$ 0.05 °C. In the entire range of temperature and pressure changes, respectively, 0 to 100 °C and 1 to 2500 bar, the accuracy of the relative sound velocity measurements is better than 10^{-3}%. Analysis of possible errors in the method of relative measurements of the nonlinearity parameter B/A shows that an accuracy better than $\pm$ 0.3% is achievable for measurements of small changes of the nonlinearity parameter.

IV. MATERIALS AND METHODS

All the amino acids and proteins studied herein were obtained from Sigma Chemicals and were used without further purification, except collagen, which was isolated from porcine skin.

Solutions were prepared using distilled, degassed water. Concentrations of the compounds were determined by weighing 20–30 mg of the solute material, with precision of $\pm$ 0.05 mg, and the necessary amount of water. Concentration of the collagen in solution was determinated from the dry weight, i.e., by weighing some amount of solution, drying it at the temperature of 110 °C, and weighing the dehydrated residue.

TABLE I. Specific increments of the nonlinearity parameter B/A of amino acids in aqueous solution at 25 °C.

Amino acids	$\Delta(B/A)/C$ (cm³/g)	Specific concentration (mg/cm³)
Glycine	6.4 ± 0.3	10–50
Glutamine	5.0 ± 0.4	25
Histidine	4.6 ± 0.4	10–25
Alanine	3.0 ± 0.3	50
Phenylalanine	2.4 ± 0.6	10–15
Valine	2.0 ± 0.2	50
Proline	1.6 ± 0.2	50
Norvaline	1.5 ± 0.2	50
Isoleucine	1.4 ± 0.3	15–35
Leucine	0.4 ± 0.2	20

The relative specific increment of sound velocity was calculated from the equation:

$$[U] = (U - U_0)/U_0C = (f - f_0)/f_0C,$$

where f and f_0 are the resonance frequencies for the cell filled with solution and solvent, respectively.

The values of $\Delta(\partial U/\partial p)_T$ in Eq. (2) were determined by measuring $[U]$ at 1, 50, 100, 150, and 200 bar. The measurements of sound velocity of the solutions at the five pressures allow determination of the dependence of the sound velocity on pressure with high accuracy and calculation of the initial slope $\Delta(\partial U/\partial p)_T$.

V. RESULTS AND DISCUSSION

Tables I and II show the experimental data for specific increments of the nonlinearity parameter B/A at 25 °C for some amino acids and proteins, respectively.

The data on the apparent molar volumes and heat capacities of amino acids published by Jolicoeur *et al.*[17] have been used for determination of the specific increments of the nonlinearity parameter B/A of amino acids by means of Eq. (2). The values of temperature slopes of apparent molar volumes of amino acids equal to their apparent molar thermal expansibility at 25 °C were obtained from the data of Kharakoz.[18] The apparent specific heat capacities do not differ much at 25 °C for most of the globular proteins and, according to Privalov and Khechinashvili,[19] are equal to 0.31 J K^{-1} g^{-1}.

TABLE II. Specific increments of the nonlinearity parameter B/A of proteins in aqueous solution at 25 °C.

Proteins	$\Delta(B/A)/C$ (cm³/g)	Specific concentration (mg/cm³)	Molecular weight (kD)
Collagen	4.3 ± 1.0	4	130
Rabbit serum Albumin	4.1 ± 0.3	25–70	64 (human)
Ribonuclease	3.7 ± 0.3	30	14
Hemoglobin	3.6 ± 0.3	25–50	64 (16/subunit)
Lysozyme	3.3 ± 0.3	35	14
Myoglobin	2.8 ± 0.3	35	17

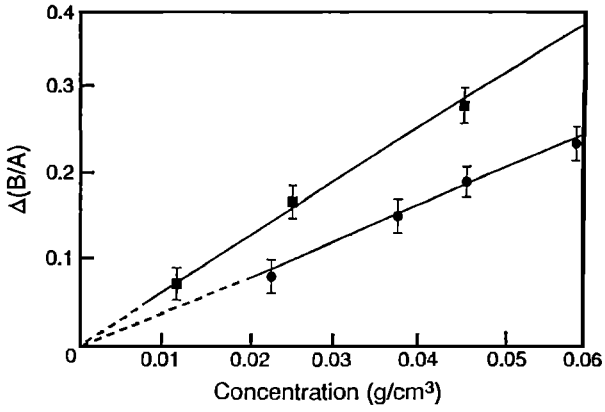


FIG. 1. Concentration dependence of the nonlinearity parameter B/A increment of glycine and rabbit serum albumin in aqueous solution at 25 °C. ■—glycine, ●—RSA.

The concentration dependences of the increment of the nonlinearity parameter B/A for all the investigated samples of amino acids and proteins are linear. Figure 1 shows an example of this linearity, viz., the concentration dependencies of the increment of B/A for one amino acid (glycine) and one protein (rabbit serum albumin). The linearity of these dependences indicates that the parameter B/A of the solution is not sensitive to solute–solute interactions, and that only solute–solvent interactions contribute to the B/A increment. This assumption is supported by comparison of these data with the data on the nonlinearity parameter B/A of bovine serum albumin (BSA) solutions at the much greater concentrations up to 0.4 g cm^{-3} by Law *et al.*[8] (see Fig. 2). The agreement between these two independently obtained sets of data is striking considering the many differences in experimental conditions and in the precision of the methods employed. Figure 2 also illustrates the advantages of the methods of measurement and calculation used in the present study, compared with that of the earlier work. The considerably smaller error of evaluation, as well as the much

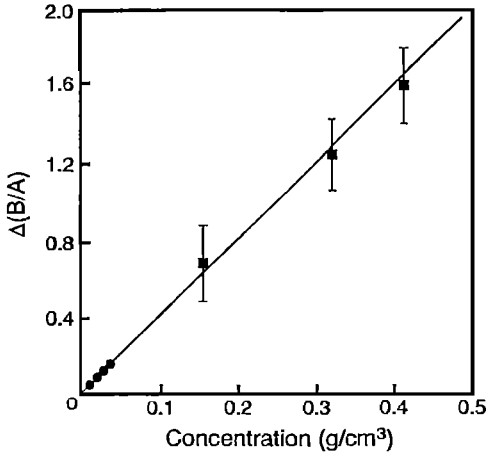


FIG. 2. Concentration dependence of the nonlinearity parameter increment of rabbit serum albumin and bovine serum albumin at 25 °C. ●—RSA, ■—BSA (8).

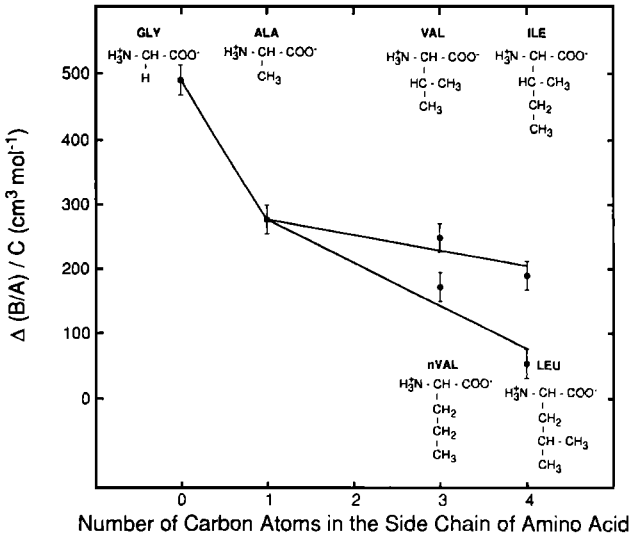


FIG. 3. Molar specific increments of the nonlinearity parameter B/A of aliphatic amino acid dependencies on the number of carbon atoms in the side chain, at 25 °C.

lesser concentrations of the investigated samples, are seen clearly in Fig. 2.

Tables I and II relate the sensitivity of B/A to molecular structure of the solutes and to differences in their hydration. For example, an increase in the number of hydrophobic methyl groups in the side chain of the aliphatic amino acids causes a decrease in the value of the specific increment of the nonlinearity parameter B/A. This phenomenon becomes more evident if the specific values of the B/A increment are recalculated in terms of the molar values. The use of molar values of the B/A increment is necessary for the quantitative estimations of contributions of different atomic groups, in the discussion below. Figure 3 shows the correlation between the nonlinearity parameter molar increment $\Delta(B/A)/C$ of aliphatic amino acids and the number of methyl groups in the side chain. The correlation exhibits discontinuity at the point corresponding to alanine. Beyond alanine the curve bifurcates passing (1) through the points corresponding to valine and isoleucine, molecules that contain a methyl group at the carbon atom in the β position, and (2) through the points corresponding to norvaline and leucine, molecules that exhibit differences only at the carbon atom in the γ position.

The values of the B/A molar increments of amino acids are positive, i.e., B/A of aqueous solutions of amino acids is greater than that of free water. The data in Fig. 3 show significant differences in the structure of the hydration shell of charged $-NH_3^+$ and $-COO^-$ groups and methyl—CH_2— groups. (The nature of these differences is discussed below in the analysis of the data of Tables III and IV.) As seen in Fig. 3, the increase of the number of aliphatic —CH_2— groups in the side chain of amino acids causes a decrease in the B/A molar increment. The contribution of a single methyl group to the B/A molar increment of aliphatic amino acids is negative and approximately -30 to -70 cm^3/mol. The contribution to the nonlinearity parameter B/A by the hydration shell of charged groups being positive is greater than that of bulk water, while the contribution of the hydration shell of —CH_2— groups is less. This means that an increase of the amount of charged groups of the solute molecules increases the nonlinearity parameter B/A of the solution, while the increase of the amount of —CH_2— groups accessible to the solvent decreases it.

Tables III and IV lend support to this view. In the case of glycine (see Table III) the hydration shell is formed by electrostatic solute–solvent interactions. Dipoles of water molecules are attracted and oriented in the electric field of $-NH_3^+$ and $-COO^-$ atomic groups. Glycine differs from all the amino acids by the absence of a side chain and, therefore, by the greater accessibility of the charged $-NH_3^+$ and $-COO^-$ group to water molecules. In the case of alanine, the hydrophobic $-CH_3$ group changes the structure of the hydration shell and limits accessibility of the charged groups of solute to water molecules. It can be seen in Table III that

TABLE III. Sensitivity of the concentration increment of the nonlinearity parameter B/A to changes in the character of solute-solvent interactions and to the structure of the hydration shell.

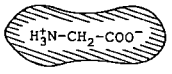
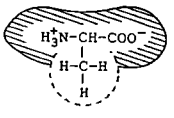
AMINO ACID	GLY	ALA
Diagram of the hydration shell		
Physical nature of the solute–solvent interactions	Hydration shell is formed by electrostatic solute–solvent interactions. Dipoles of water molecules are attracted and oriented in the field of $-NH_3^+$ and COO^- atomic groups.	Hydrophobic $-CH_3$ group changes the structure of the hydration shell and limits accessibility of water molecules to the charged groups of the solute.
$\Delta(B/A)/C$ (cm^3/mol)	480 ± 20	270 ± 20

TABLE IV. Sensivity of the nonlinearity parameter to the replacement of a single atomic group within a molecule. (Replaced group is shown by a dashed-circle.)

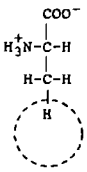
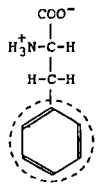
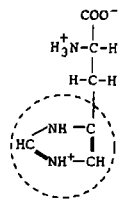
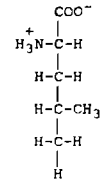
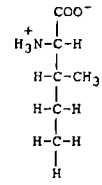
AMINO ACID	ALA	PHE	HIS
Chemical structure			
$\Delta(B/A)/C$ (cm^3/mol)	270 ± 20	400 ± 100	710 ± 70

TABLE VI. An example of striking sensitivity of $\Delta(B/A)/C$ to a change of the position of one atomic group within the same molecule.

AMINO ACID	LEU	ILE
Chemical structure		
$\Delta(B/A)/C$ (cm^3/mol)	50 ± 20	180 ± 20

such changes in the hydration shell of solute cause a decrease of the molar increment of the nonlinearity parameter B/A by about 40%, which can be explained by the greater value of B/A of the hydration shell of charged groups, rather than that of bulk water.

Table IV shows the sensitivity of the nonlinearity parameter B/A to the replacement of a single atomic group within a molecule. It is seen that histidine, at neutral pH having a side chain with charged and polar atomic groups, is characterized by the greatest value of the B/A molar increment. The presence of additional hydrogen bonds and electrostatic solute-solvent interactions in the hydration shell of histidine considerably increases the B/A molar increment. In contrast to the methyl group, the water-insoluble aromatic ring increases the B/A molar increment by 130 cm^3/mol, as is seen from comparison of B/A increments of alanine and phenylalanine, presented in Table IV.

The argument regarding the increase of the nonlinearity parameter B/A in the hydration shell of the polar groups, where strong solute-solvent interactions by hydrogen bonding are observed, is supported by the data of Table V, where amino acids having the same amount of carbon atoms in the side chain are compared. Here, the only amino acid having a polar side chain is glutamine, which is characterized by a maximum value of the molar increment of the nonlinearity parameter B/A. Other amino acids, presented in Table V, with three carbon atoms in the side chain, such as glutamine, but without polar or charged atomic groups, have nearly the same values of the B/A molar increment. Thus, it is shown that the polar groups, as well as the charged groups, increase the acoustic nonlinearity of the solution.

The concentration increment of the nonlinearity parameter B/A could be much more sensitive to the molecular structural features than other acoustic parameters used in biomolecular studies, such as relative concentration increments of density or sound velocity. An example of striking sensitivity of $\Delta(B/A)/C$ to a change of the position of one atomic group within the same amino acid is shown in Table VI, wherein replacement of a single $-CH_3$ group from the γ position (in leucine) to the β position (in isoleucine) increases the $\Delta(B/A)/C$ from 50 to 180 cm^3/mol. A similar phenomenon is observed for valine and norvaline, viz., the presence of a $-CH_3$ group in the β position in valine leads to an increase of $\Delta(B/A)/C$ compared with norvaline with the nonbranched side chain.

It is interesting to observe that, in contrast to the $\Delta(B/A)/C$, relative concentration increments of speed of sound and of density are not sensitive to structural differences exhibited between leucine and isoleucine and valine and norvaline, as shown in Table VII.

TABLE V. Sensivity of $\Delta(B/A)/C$ of amino acids to rearrangements in the side chains having the same amount of carbon atoms.

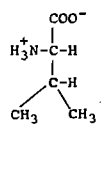
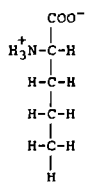
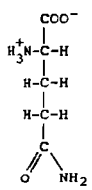
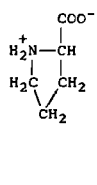
AMINO ACID	VAL	nVAL	GLN	PRO
Chemical structure				
$\Delta(B/A)/C$ (cm^3/mol)	230 ± 20	170 ± 20	730 ± 40	190 ± 20

TABLE VII. Comparison of relative sensitivities of different acoustic parameters to the change of the relative position of atomic groups in solute molecules of the same atomic composition.

Acoustic parameter	$\Delta(B/A)/C$	$\Delta\rho/C$[a]	$\Delta U/C$[a]
A ratio of values for valine and norvaline	1.3 ± 0.1	1.000 ± 0.005	0.980 ± 0.005
A ratio of values for leucine and isoleucine	3.6 ± 0.1	1.000 ± 0.005	0.990 ± 0.005

[a]Calculated from the data of Kharakoz.[18]

It is possible to estimate the polar group contribution to the B/A molar increment of a solute from the entries of Table V. It is seen that glutamine differs from norvaline by the polar atomic =O and $-NH_2$ groups at the carbon atom in the δ position. The contribution of a polar group to the B/A molar increment is estimated then from comparison of the B/A molar increments of glutamine and norvaline from

$$\left(\frac{\Delta(B/A)}{C}\right)_{\text{polar}} = 0.5\left[\left(\frac{\Delta(B/A)}{C}\right)_{\text{GLN}} - \left(\frac{\Delta(B/A)}{C}\right)_{\text{nVAL}} + \left(\frac{\Delta(B/A)}{C}\right)_{\text{CH}_3}\right],$$

where $(\Delta(B/A)/C)_{\text{CH}_3}$ is the contribution of the $-CH_3$ group in the nonbranched side chain of aliphatic amino acids. It is considered that contributions of polar groups in glutamine are equal. The methyl $-CH_3$ group in the nonbranched side chain of aliphatic amino acids contributes to the B/A molar increment approximately $-50\ \text{cm}^3/\text{mol}$, as seen from comparison of alanine and norvaline. Thus, contribution of one polar group is equal to $0.5(730 - 170 - 50) = 255\ \text{cm}^3/\text{mol}$.

Contribution of one charged group to the B/A molar increment can be assumed approximately equal to half of the glycine B/A molar increment, viz., $240\ \text{cm}^3/\text{mol}$.

Taking into account errors of estimation, contributions of both polar and charged groups are approximately equal to $200–300\ \text{cm}^3/\text{mol}$.

The molecular interpretation of the B/A specific increments of proteins is a more complex problem and more experimental data are necessary to analyze it. However, one can see from Table II that different proteins are characterized by significantly different values of B/A specific increments. This suggests that the sensitivity of the nonlinearity parameter B/A depends upon the type of biopolymer and its conformation, structure, hydration, and size. For example, Table II shows the correlation between the molecular weight of a protein and the specific increment of B/A. In contrast to amino acids, proteins of increasing molecular weight exhibit increasing values of $\Delta(B/A)/C$, except hemoglobin for which $\Delta(B/A)$ seems to be related to the major subunits, rather than to the molecule as a whole. The largest value of $\Delta(B/A)/C$ in Table II is that of collagen, the only fibrillar protein in the tabulation. It should be noted that the concentration increment of the ultrasound velocity for fibrillar proteins is considerably greater than that of globular proteins.[18,20]

VI. SUMMARY

Experimental data on the specific increments of the nonlinearity parameter B/A of ten amino acids and six proteins are presented. These data were obtained using high-precision velocimetry for measurements at high pressures and a new differential method of calculation of the B/A specific increments that provide an accuracy of relative measurements of the nonlinearity parameter B/A better than $\pm 0.3\%$. Such high precision of the measurements makes it possible to carry out molecular interpretation of the data obtained on the acoustical nonlinearity of investigated substances. In comparison with the literature data, measurements were made in much more dilute solutions and, therefore, the results are more artifact-free.

A number of dependences of B/A upon biomolecular structural features are observed. It is shown that strong intermolecular solute–solvent interactions, such as electrostriction in the hydration shell of charged atomic groups or hydrogen bonding in the hydration shell of polar groups, lead to an increase of the nonlinearity parameter B/A of solution. Each charged or polar group contributes to the B/A molar increment of the solute about $200–300\ \text{cm}^3/\text{mol}$. In contrast, contribution of a hydrophobic methyl group is negative and equal to -30 to $-70\ \text{cm}^3/\text{mol}$.

Replacement of a single atomic group within a molecule may cause large changes in the B/A concentration increment, while other acoustic parameters of solutions may not be sensitive to the replacement. Therefore, measurements of the nonlinearity parameter B/A may provide additional information about structure and hydration of biomolecules in solutions.

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