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# A STUDY OF ULTRASONIC ABSORPTION OF SOME AMINO ACIDS AND THEIR MIXTURES AT PHYSIOLOGICAL pH

A. Ashraf

*Department of Physics, Ferdowsi University of Mashhad, Mashhad, Islamic Republic of Iran*

**Abstract**

In this paper, the ultrasonic absorption of amino acids was measured using a small cylindrical resonator at physiological pH and 25°C and a concentration of 0.1. M. The absorption of mixtures of some amino acids was also measured. In the case of cysteine-histidine, the absorption of mixtures is much larger (more than twice) than the summation of absorption of the individual amino acid. This may be due to the weak bonds between the side chain of histidine and the SH group of cysteine molecules. As for the absorption of proteins, it could also be due to weak bonds mentioned earlier.

## Introduction

It is well known that the measurement of ultrasonic absorption makes it possible to obtain various types of information on rapid chemical or molecular changes in aqueous solutions of materials. Phenomena such as structural or chemical relaxations in aqueous solutions of amino acids, polypeptides or proteins may easily be studied by such measurements. Various amino acids in aqueous solutions have been studied by several workers [1-6].

Proton transfer at side chain group function has been proposed as a possible molecular mechanism for the absorption of ultrasonics in aqueous proteins and polypeptides [5, 7, 8]. It might be suggested that the absorption of proteins and polypeptides would be predicted by simply summing the absorption of each individual amino acid residue in the chain perhaps with a correction for the contribution due to amino and carboxyl end chain groups which are lost on polymerisation. This turns out not to be the case. For example, a calculation performed for hemoglobin results in a predicted absorption value which is roughly 30 times smaller than that observed experimentally. It seems clear therefore that there is some form of intra-molecular mechanisms contributing to the ultrasonic absorption of these molecules.

The purpose of this study is to provide some further evidence of such mechanisms by studying the concentrating dependence of the absorption of several amino acids in solution and contrasting this with measurements made on a number of simple amino acids mixtures in solution. In the low MHz range, we have found several such mixtures which absorb much more strongly than the sum of their individual components.

**Keywords:** Ultrasonic absorption; Amino acid absorption; Protein absorption; Amino acid mixture absorption

## Theoretical Considerations

The excess sound absorption per wavelength, $\alpha\lambda$, due to perturbation of an equilibrium of a chemical reaction when there is only one relaxation frequency, is given by [9]:

$$(\alpha\lambda)\,\text{excess} = \frac{\pi\rho c^2\Gamma}{RT}\{\beta H'/C_p - V'\}^2 \frac{f/f_r}{1+(f/f_r)^2} \qquad (1)$$

where H` and V` are the changes in enthalpy and standard volume for the reaction; $C_p$ and $\beta$ are the specific heat at constant pressure and the coefficient of thermal expansion; c is the velocity of ultrasound; $\rho$ and R are the density of the solution and the universal gas constant; T is the absolute temperature and $\Gamma$ for a reaction of the form $\Sigma_i \nu_i a_i = 0$, where $\nu_i$ and $a_i$ are the stoichiometric coefficients and the activity of the species i, can be written as [2]:

$$\Gamma = \{\Sigma(\nu_i)^2/C_i\} \quad (2)$$

where $C_i$ are the molar concentrations for species i. The excess absorption per wavelength shows a maximum at frequency $f = f_r$ at a specific pH which may be written as follows:

$$\{(\alpha\lambda)\,\max\}_{f=f_r} = \frac{\pi\rho c^2\Gamma}{2RT}\{\beta H`/C_p - V`\}^2 \quad (3)$$

In aqueous solutions and near room temperature, $\beta$ is relatively small, hence the first term in square brackets, which would alter the result by only a few percent, may be neglected [10]. Then (3) can be written as:

$$\{(\alpha\lambda)_{max}\}_{f=f_r} = \frac{\pi\rho c^2\Gamma}{2RT}(V`)^2 \quad (4)$$

and (1) can be rewritten in the form:

$$\alpha\lambda = 2\,(\alpha\lambda)_{max}\,\frac{f/f_r}{1+(f/f_r)^2} \quad (5)$$

In the case of this study, where the measuring frequency is generally not equal to the relaxation frequency, we have to use the equation (1) in the form of:

$$(\alpha\lambda)_{excess} = \frac{\pi\rho c^2\Gamma}{RT}(V`)^2\,\frac{f/f_r}{1+(f/f_r)^2} \quad (6)$$

For a reaction of type:

$$R\text{-}(NH_3)^+ + OH^- \underset{k_b}{\overset{k_f}{\Longleftrightarrow}} R\text{-}NH_2 + H_2O \quad (7)$$

where $k_f$ and $k_b$ are the forward and the backward rate constant, $\Gamma$ may be written as:

$$\Gamma = \{1/C_+ + 1/C_- + 1/C + 1/C_W\}^{-1} \quad (8)$$

where $C_+$, $C$ , $C_-$ and $C_W$ are the concentrations of protonated amino acid, neutral amino acid, hydroxyl group and water, respectively.

For simplicity, if $C^* = C_+ + C$ and the equilibrium constant is defined as:

$$K_{bf} = k_b/k_f = C_+C_-/C \quad (9)$$

then $\Gamma$ in an alternative form may be written as [2]:

$$\Gamma = K_{bf}C^*C_-/\{K_{bf}C^* + (K_{bf}+C_-)^2\} \quad (10)$$

As can be seen, $\Gamma$ is a pH dependent parameter since the concentration of $(R\text{-}NH_3)^+$, $OH^-$ and $R\text{-}NH_2$ would change if the value of pH changes. In alkaline solutions, $\Gamma$ shows a maximum at the following pH;

$$(pH)_{max} = (28 - pK_{bf} + \log(C^* + K_{bf})/2 \quad (11)$$

where $pK_{bf}$ is the negative logarithm of $K_{bf}$.

## Experimental Section

Measurements of absorption were made with a small volume cylindrical resonator which has been described elsewhere [11]. The method consists of measuring the 3 dB band-width frequencies at some resonance of different consecutive orders. The difference between the two frequencies (upper and lower) of 3 dB is related to the absorption by a relation:

$$a\lambda = \pi f^*/\Delta f = \pi(f_u + f_l)/2\Delta f \quad (12)$$

where $f_u$ and $f_l$ are the upper and the lower limit frequencies of 3 dB half power band; a is the attenuation coefficient; and $\lambda$ is the wavelength. The absorption per wavelength can then be calculated by:

$$\alpha\lambda = (a\lambda)_{solution} - (a\lambda)_{solvent} \quad (13)$$

The chemicals were obtained from Sigma Chemical Company Ltd. and used without further purification. For all solutions, pH was adjusted to the desired value using either NaOH or HCl. The measurements are made at 25°C and usually with a concentration of 0.1 M.

## Results

Ultrasonic absorption for those amino acids which are soluble in water at physiological pH were measured at neutral pH. The results are shown in Table 1. Among the amino acids, cysteine has the largest absorption value. Histidine has an absorption smaller than cysteine but greater than the other amino acids. The other amino acids have a very small absorption coefficient (less than $10^{-5}$/0.1 M) which can usually be negligible.

The absorption of mixtures of some amino acids has also been measured. Table 2 shows the results. For a mixture of cysteine and histidine, the absorption is larger than the summation of absorptions of individual amino acids by a factor bigger than two.

The absorption of histidine, cysteine and their mixtures has also been measured at different frequencies. As can be seen from Figure 2, the value of absorption is greater for each at lower frequencies. It means that there must be a relaxation frequency for histidine, cysteine and for the mixtures at frequencies lower than 5.5 MHz.

### Calculation of Ultrasonic Parameters $(\alpha\lambda)_{max}$, $f_r$ and $\Delta V$

From (6) it would be possible to calculate the relaxation frequency and the maximum absorption per wavelength, $(\alpha\lambda)_{max}$, if $\alpha\lambda$ were known at two different frequencies. Using the previous data (Tables 1 and 2), the relaxation frequency, $f_r$, and the maximum absorption per wavelength at that frequency may be calculated. The results for cysteine, histidine and mixtures of cysteine and histidine are tabulated in Table 3. The other parameter which may be calculated is the volume change V'. Knowing the parameter $\Gamma$ and the velocity of sound c, the formula (4) allows one to calculate V'. For any specific reaction such as the proton transfer reaction of the SH group of cysteine which may be shown as:

$$R\text{-}S\text{-}H + OH^- \underset{k_b}{\overset{k_f}{\Longleftrightarrow}} R\text{-}S^- + H_2O \qquad (14)$$

equations 10 and 11 may be used to calculate $\Gamma$. As can be seen from Table 4, the value of V' for the cysteine SH group does not seem to be reasonable. The probable reason for this may be the overlapping of the two peaks (amino and thiol groups).

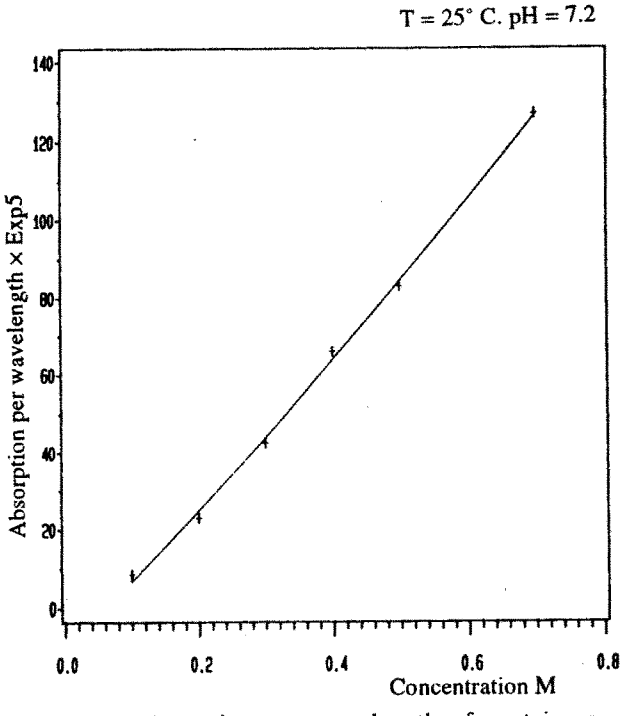


**Figure 1.** Absorption per wavelength of cysteine vs concentration

## Discussion

In general, the absorption of amino acid solutions at physiological pH may be ascribed to classical absorption and perturbation of the equilibrium between bound and free water which is usually called the hydration layer or solute solvent interaction [12].

**Table 1.** Absorption per wavelength for different amino acids at physiological pH, 25°C and for 0.1 M

| Amino acid | pH | Freq. MHz | Absorption per wavelength × $10^5$ |
|---|---|---|---|
| Cysteine | 7.2 | 6.5 | 11.5 |
| Histidine | 7.1 | 6.5 | 2.5 |
| Threonine | 7.1 | 6.5 | .5 |
| Serine | 7.1 | 6.5 | 1 |
| Valine | 7.0 | 6.5 | 0.5 |
| Methionine | 7.1 | 6.5 | 1. |
| Leucine | 7.0 | 6.5 | 1.4 |
| Asparagine | 7.1 | 6.5 | 1.2 |
| Isoleucine | 7.1 | 6.6 | 0.4 |
| Alanine | 7.1 | 6.5 | 0.3 |
| Arginine | 7.1 | 6.4 | 0.5 |
| Glycine | 7.1 | 6.5 | 0.5 |
| Proline | 7.1 | 6.5 | not measurable |
| Lysine | 7.1 | 6.5 | not measurable |
| Cysteine | 7.2 | 8.6 | 8.8 |
| Histidine | 7.1 | 8.5 | 2 |
| Methionine | 7.1 | 8.6 | .75 |
| Threonine | 7.2 | 8.5 | .6 |
| Proline | 7.1 | 8.6 | not measurable |

**Table 2.** Absorption per wavelength for mixtures of some amino acids at 25°C and physiological pH

| Name | Concentration M | Freq. MHz | pH | Absorption per wavelength $\times 10^5$ |
|---|---|---|---|---|
| Cysteine | 0.2 | 8.6 | 7.2 | 17.5 |
| Cysteine | 0.2 | 6.6 | 7.2 | 23 |
| Arginine | 0.1 | 6.5 | 7.1 | 0.25 |
| Histidine | 0.1 | 8.6 | 7.1 | 2. |
| Methionine | 0.2 | 8.6 | 7.1 | 1.5 |
| Proline | 0.2 | 8.6 | 7.1 | not measurable |
| Threonine | 0.2 | 6.6 | 7.1 | 0.9 |
| Cys-His | 0.2-0.1 | 8.6 | 7.15 | 46 |
| Cys-Arg | 0.2-0.1 | 6.6 | 7.15 | 37 |
| Cys-Thr | 0.2-0.2 | 6.6 | 7.15 | 30 |
| Cys-Met | 0.2-0.2 | 6.6 | 7.15 | 26 |
| Cys-Pro | 0.2-0.3 | 8.6 | 7.15 | 21.6 |
| Met-His | 0.2-0.1 | 8.5 | 7.25 | 4 |

At neutral pH, the number of $OH^-$ and $(OH_3)^+$ ions is so low that one cannot take them into account as a proton transfer reaction. That is, it cannot be supposed that there exists an equilibrium between R-$(NH_3)^+$ or R`-$COO^-$ ions with R-$NH_2$ or R'-COOH, respectively. Hence the proton transfer cannot be, in general, an adequate mechanism which is responsible for the absorption of amino acids at neutral pH.

However, the absorption per wavelength of cysteine is the only one which is considerable at physiological pH. The only difference between cysteine and other amino acids is the existence of an SH group in cysteine which has a pK of 8.33 [13]. Therefore, at physiological pH the absorption of cysteine may partially be ascribed to the perturbation of proton transfer between the SH group of cysteine and water. Histidine also has a pK of between 6 and 7, but it seems that the proton transfer does not make a significant contribution to histidine absorption at neutral pH.

Table 2 shows the effect of mixing some amino acids. For some of them, e. g. methionine-histidine mixture, there does not seem to be any difference between the absorption of the mixture and the summation of the absorptions of the components. But for some of them, the absorption of the mixture is larger than the sum of individual ones. This effect is very strong in the case of cysteine-histidine mixtures.

The side chain of histidine at neutral pH is partially positive. Some of the molecules have a positive side chain and some are neutral [14]. On the other hand, cysteine at neutral pH is a very weak acid (SH group), and it may be guessed that a weak bond between the side chain of each histidine molecule (those which are neutral) and the SH group of a cysteine molecule may

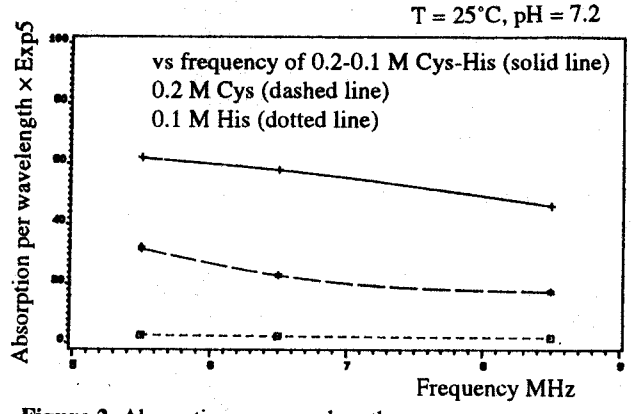


**Figure 2.** Absorption per wavelength

**Table 3.** Calculated relaxation frequency and maximum absorption per wavelength for some amino acids

T = 25°C; pH = 7.1-7.2

| Name | Concen. (M) | Freq. (MHz) | $(\alpha\lambda)_{max} \times 10^5$ |
|---|---|---|---|
| Cys (SH) | 0.1 | 1.12 | 34 |
| His | 0.1 | 2.47 | 4 |
| Cys-His | 0.2-0.1 | 1.99 | 103 |

**Table 4.** Calculated volume change for cysteine and histidine as well as their mixtures

T = 25°C

| Name | $pH_{max}$ | C (M) | r(mole/m³) | V' (ml) |
|---|---|---|---|---|
| His | 11 | 0.1 | 0.5 | 33.9±6 |
| Cys (SH) | 9.2 | 0.1 | 0.017 | 255±57 |
| Cys-His | 10.4 | 0.2-0.1 | 0.24 | 75±19 |

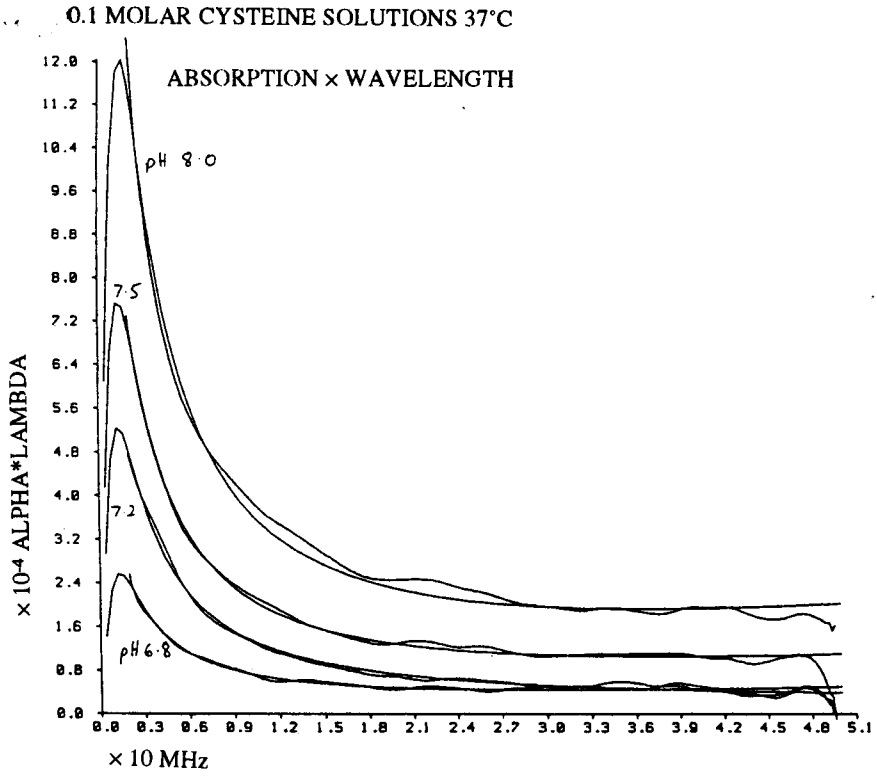


**Figure 3.** Absorption per wavelength

xist. That is:

$$R - N + R` - SH \Longleftrightarrow R - N \ldots H \ldots S - R` \qquad (15)$$

This may be formed at least for a fraction of nolecules and a weak equilibrium may be ascribed to hese bound molecules. Thus, if this explanation is alid, then the excess ultrasonic absorption may be scribed to the perturbation of this equilibrium.

The table also shows that the same effect, but veaker, exists in the case of arginine. From the hemical point of view, it is very hard to say that the ame thing happens for arginine-cysteine mixtures, ince the side chain of arginine has a very high pK alue (greater than 12) which remains protonated nder most conditions [13]. Nevertheless, if one upposes that the same situation exists, albeit weaker n this case, then the same interpretation may be ccepted.

Having accepted the above explanation for nixtures of cysteine and histidine, the absorption of roteins may then be attributed partially to these weak onds. The side chain of amino acids will exist in the ame form in proteins as in the constituent amino cids. Hence, the side chains of amino acids in roteins are free to form the same bonds with each other if the conditions demand. It is interesting to note that the results for the SH group of cysteine are very similar to those which were measured by the Acoustic group of Keele University (Fig. 3) [15].

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