

Ultrasonic Relaxation Associated with Inclusion Complex of Drugs and β-Cyclodextrin

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The dynamic interactions between β-cyclodextrin (β-CD) (host) and salicylic acid (guest) at pH ≈6 and ≈3 and between β-CD and benzoic acid (guest) at pH ≈6 were investigated in aqueous solutions in terms of ultrasonic absorption in the frequency range from 0.8 to 95 MHz. A single relaxational absorption was found when the host and the guest were coexisting in water. Ultrasonic relaxation parameters were determined as a function of the guest concentration. From the concentration dependence of the parameters, the cause of the relaxation was attributed to a perturbation of an equilibrium associated with an inclusion complex formed by the host and the guest. The forward and backward rate constants, the equilibrium constants and the standard volume changes for the host–guest complexation reaction were determined from the acid concentration dependences of the relaxation frequency and the maximum absorption per wavelength. The results were compared with those in solution containing aspirin and β-CD. It was found that the substituent effect on the rate and thermodynamic parameters was not remarkable in the dissociated forms of the ortho-substituted benzoic acid. However, the charge effect on a carboxylic group on the parameters was determined to contribute significantly to stability of the complexes formed by the acids and β-CD and to the rate constants for the departure of the acids from β-CD cavity.

Cyclodextrins (CDs) with specific cavities are target compounds which can be used for drug-delivery systems. There are many publications associated with the complexation of drugs with CDs.[1–3] A lot of reports have also been published on the applications of CDs in foods, cosmetics, etc.[4–6] Junquera et al.[7] have reported that undissociated form (carboxylic form) of carboxylic acids binds CDs with higher affinities than the dissociated partners (carboxylate form) from the stability constants obtained by pH potentiometric measurements. Liu and Guo[8] have shown correlations between the experimental stability constant and the calculated one for the inclusion complexes formed by CDs and benzene derivatives. However, most of these studies are associated with the static properties of the interaction between CDs and organic or inorganic molecules. Murphy and Bohne[9] have stressed the importance of the dynamics of entry and exist of guest molecules into or out of CD cavity for the applications of CDs to drug-delivery systems.

In our dynamic studies on the inclusion complexes by CDs (host) and several organic molecules (guest) in terms of an ultrasonic relaxation, we have extended the examinations to the complexation reaction between host and drug (guest). That is, we have reported in a previous paper[10] the results of the ultrasonic relaxation in aqueous system containing aspirin and β-cyclodextrin (β-CD). It has been found that benzene moiety is included in the cavity of β-CD and the effect of charge on a carboxylic group is important for the release rate of the guest molecule into a bulk phase. In order to obtain a more precise understanding of the complexation reaction between β-CD and related drugs with a benzene ring, we have chosen two guest compounds, i.e., salicylic acid and benzoic acid, in this study. By comparing the kinetic and thermodynamic results obtained with those for solution with both β-CD and aspirin, it is desired to clarify the dynamics of how the complex is stabilized and how the rate constants of the formation and disruption of the complex are affected when the structures of the guest molecules are different. This type information is also desirable for understanding more complex biological reactions and the interaction reactions between host and guest, because the inclusion complex reactions are models for enzyme substrate binding.

Experimental

Chemicals. β-CD was purchased from Wako Pure Chemical Co., Ltd. and recrystallized once from distilled water. Salicylic acid (2-hydroxybenzoic acid) and benzoic acid were also obtained from the same company as their purest reagent grades, and they were used without further purification. Water distilled and filtered by using a Milli-Q SP-TOC filter system from Japan Millipore Ltd. was used as solvent, and it was degassed under a reduced pressure. Aqueous solutions of salicylic acid and benzoic acid as the ionized form (pH ≈6) were obtained by adding a concentrated aqueous solution of sodium hydroxide. The nonionized form of salicylic acid and benzoic acid in aqueous solution was prepared by an addition of concentrated hydrochloric acid. All sample solutions were freshly prepared by weighing just before the experimental measurements.

Apparatus. Ultrasonic absorption coefficients, α, were measured by using a resonance method in the frequency range from about 0.8 to 7.5 MHz using x-cut crystals with 3 and 5 MHz x-cut fundamental frequencies. The temperature for the resonator

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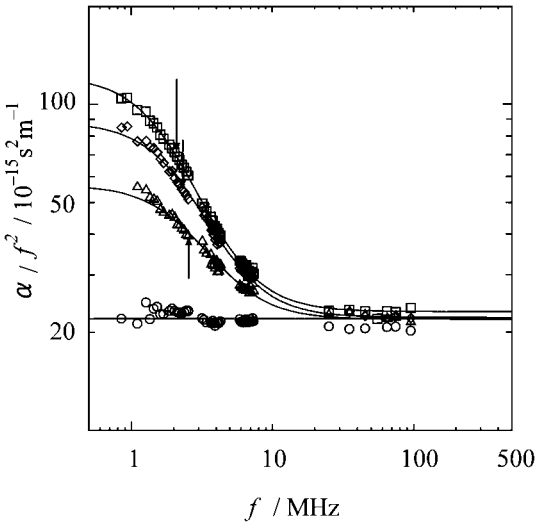

Fig. 1. Ultrasonic absorption spectra in aqueous solution of salicylic acid in the presence and absence of β-cyclodextrin at 25.0 °C and at pH ≈6. (○): 0.012 mol dm^{-3} salicylic acid only, (△): 0.0030 mol dm^{-3} salicylic acid + 0.0087 mol dm^{-3} β-CD, (◇): 0.0070 mol dm^{-3} salicylic acid + 0.0087 mol dm^{-3} β-CD, (□): 0.010 mol dm^{-3} salicylic acid + 0.0087 mol dm^{-3} β-CD. The arrows are the positions of the relaxation frequency.

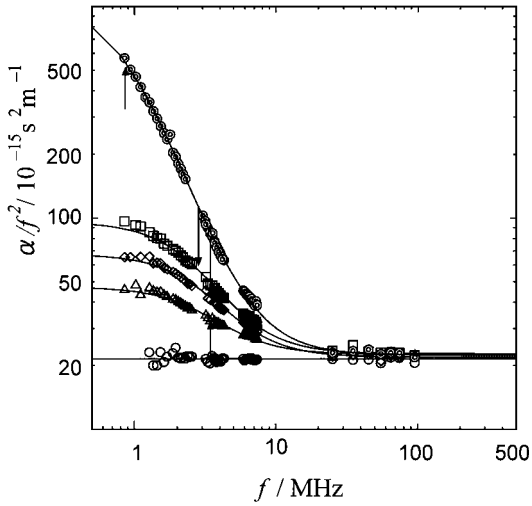

Fig. 2. Ultrasonic absorption spectra in aqueous solution of benzoic acid in the presence and absence of β-cyclodextrin at 25.0 °C. (○): 0.010 mol dm^{-3} benzoic acid only, (△): 0.0020 mol dm^{-3} benzoic acid + 0.0087 mol dm^{-3} β-CD at pH 6.01, (◇): 0.0040 mol dm^{-3} benzoic acid + 0.0087 mol dm^{-3} β-CD at pH 6.04, (□): 0.0060 mol dm^{-3} benzoic acid + 0.0087 mol dm^{-3} β-CD at pH 6.07 and (◎): 0.010 mol dm^{-3} benzoic acid + 0.0087 mol dm^{-3} β-CD at pH 1.9.

cells was precisely maintained within ±0.01 °C (Lauda RM20). A pulse method equipped with 5 MHz fundamental x-cut crystals was applied at the odd overtone harmonic frequencies in the range from 25 to 95 MHz, and the temperature for the pulse cell was maintained within ±0.1 °C (EYEYA UNI ACE BATH NCB-2200). More details for the absorption apparatus have been described elsewhere.[11,12] Sound velocity values were obtained by the resonance method at around 3 MHz using the same absorption apparatus with 5 MHz crystals. From the two different resonance frequencies, the sound velocity was calculated, the details of which have been reported elsewhere.[13] Solution densities were measured on a vibrating density meter (Anton Paar NMA 60/602). Solution pHs were received through a glass electrode (HM-60S Toa Denpa pH meter) in the same thermostatic bath for the pulse method. All experiments were carried out at 25.0 °C.

Results and Discussion

Ultrasonic absorption measurements were carried out in aqueous solution of salicylic acid at 0.012 mol dm^{-3} and of benzoic acid at 0.010 mol dm^{-3}. The absorption coefficients divided by the square of the measurement frequency, α/f^2, were independent of the frequency as can be see in Figs. 1 and 2. These results indicated that no relaxation occurred in these acid solutions. Ultrasonic relaxation in the MHz frequency range did not exist in aqueous solution of β-CD, when the concentration was less than 0.013 mol dm^{-3}.[14,15] In order to make the experimental condition simple, the concentration of β-CD was fixed at 0.0087 mol dm^{-3} in the present study, where any relaxation was not observed in solution of β-CD in our frequency range. When β-CD (host) was added to aqueous solutions of salicylic acid (at pH ≈6 and pH ≈3) or to solution of benzoic acid (at pH ≈6), the ultrasonic relaxation was clearly observed, and the representative results are shown in Figs. 1 and 2. A Debye-type single relaxational equation, $\alpha/f^2 = A/\{1 + (f/f_r)^2\} + B$, was applied to analyze the frequency dependence of the absorption coefficients, where f_r is the relaxation frequency, A and B are the constants. The ultrasonic relaxation parameters, f_r, A, and B, were determined by using a nonlinear least-mean square computer program so as to receive the best fit of the experimental data to the above equation. The parameters thus determined are listed in Table 1 along with the experimental values of the sound velocity (v), the density (ρ), and the pH. The solid curves in Figs. 1 and 2 are the calculated values by using the determined parameters. The experimental data could be fitted to the calculated lines, the results of which support the observation of the single relaxational absorption. The ultrasonic absorption measurements were also carried out in solution with both β-CD and benzoic acid at pH ≈1.9, and the results are shown in Fig. 2 and Table 1. The position of the relaxation frequency shifted to a considerably lower frequency. At this condition, the most of the acid molecules exist as the nonionized forms.

The fact that the relaxation was only found when the host and the guest were both in solution suggested that the observed relaxation is related to an interaction between the host and the guest. Thus, the perturbation of the following equilibrium by the applied sound wave is thought to be a cause of the observed relaxation.

$$\mathrm{CD} + \mathrm{GT} \underset{k_b}{\overset{k_f}{\rightleftharpoons}} \mathrm{CDGT}, \quad (1)$$

where CD is the host molecule, GT is the guest one and CDGT is the inclusion complex formed by the host and guest molecules. The relationship between the relaxation frequency and the concentrations is as

Table 1. Ultrasonic and Thermodynamic Parameters for Aqueous Solutions of Salicylic Acid and Benzoic Acid with 0.0087 mol dm^{-3} β-CD at 25.0 °C

C_{GT} /mol dm^{-3}	f_r /MHz	A /10^{-15} s^2 m^{-1}	B /10^{-15} s^2 m^{-1}	v[a] /m s^{-1}	ρ[b] /kg dm^{-3}	pH
		Salicylic acid				
0.0007	1.91 ± 0.13	60.4 ± 5.2	22.1 ± 0.1	1505	1000.91	3.74
0.0010	1.84 ± 0.10	87.8 ± 6.1	22.7 ± 0.1	1505	1000.94	3.67
0.0025	1.89 ± 0.06	168 ± 7	21.9 ± 0.1	1505	1001.01	3.38
0.0050	1.49 ± 0.06	353 ± 18	23.0 ± 0.1	1506	1001.04	3.18
0.0075	1.31 ± 0.05	568 ± 31	22.4 ± 0.2	1506	1001.08	3.02
0.0100	1.25 ± 0.05	626 ± 32	23.6 ± 0.2	1506	1001.23	2.86
0.0120	1.30 ± 0.04	601 ± 25	23.0 ± 0.1	1505	1001.24	2.90
0.0010	2.41 ± 0.11	22.1 ± 1.3	22.1 ± 1.3	1505	1001.13	6.33
0.0030	2.72 ± 0.15	34.7 ± 2.4	21.9 ± 0.1	1505	1001.45	6.47
0.0050	2.43 ± 0.11	53.4 ± 3.1	21.9 ± 0.1	1506	1001.49	6.13
0.0070	2.40 ± 0.05	66.1 ± 1.7	22.2 ± 0.1	1506	1001.78	6.26
0.0080	2.19 ± 0.06	76.4 ± 2.7	21.9 ± 0.1	1505	1001.49	6.40
0.010	1.95 ± 0.08	99.0 ± 5.3	23.1 ± 0.1	1505	1001.62	6.87
		Benzoic acid				
0.0015	3.51 ± 0.31	19.6 ± 1.9	22.0 ± 0.1	1494	1001.04	6.01
0.0020	3.12 ± 0.17	24.5 ± 1.5	22.6 ± 0.1	1493	1001.05	6.06
0.0030	3.15 ± 0.20	34.8 ± 2.5	22.5 ± 0.0	1493	1001.16	6.06
0.0040	3.05 ± 0.12	45.5 ± 2.0	21.9 ± 0.4	1495	1001.20	6.04
0.0050	2.74 ± 0.11	62.7 ± 3.1	22.1 ± 0.1	1494	1001.20	6.02
0.0060	2.62 ± 0.09	72.9 ± 3.0	22.8 ± 0.1	1494	1001.23	6.07
0.0070	2.93 ± 0.15	72.4 ± 4.3	22.2 ± 0.1	1495	1001.35	6.02
0.0080	2.99 ± 0.07	77.1 ± 2.1	22.7 ± 0.0	1494	1001.46	6.08
0.0090	3.39 ± 0.14	78.2 ± 3.4	21.2 ± 0.9	1494	1001.53	5.96
0.0100	3.35 ± 0.10	87.8 ± 2.1	21.5 ± 0.1	1494	1001.56	6.10
0.0100	0.87 ± 0.02	1030 ± 30	22.5 ± 0.1	—	—	1.90

a) Error of the sound velocity is ±1 m s^{-1}. b) Error of the density is ±0.01 kg dm^{-3}.

$$2\pi f_r = k_f\{[CD] + [GT]\} + k_b \quad (2)$$

$$= k_b\{(KC_{CD} + KC_{GT} + 1)^2 - 4K^2C_{CD}C_{GT}\}^{1/2}, \quad (2')$$

where k_f and k_b are the forward and backward rate constants, C_{CD} and C_{GT} are the analytical concentration of the host and that of the guest, respectively, and K is defined as $K = k_f/k_b$. As can be seen in Table 1 and in Fig. 3 (the lines are explained later), the change in the relaxation frequency with the guest concentration was very small for both systems with salicylic acid and benzoic acid. In the case of the system for aspirin at pH ≈1.7, Eq. 2′ was directly applied to obtain the most probable values of K and k_b along with the most probable errors for them. However, it was crucial at this stage to use Eq. 2′ in order to determine the rate and equilibrium constants. In this case, another analytical procedure should be applied to receive the constants, K and k_b. The maximum absorption per wavelength (μ_{max}), is defined as $\mu_{max} = 0.5Af_rv$. This quantity is related to the equilibrium concentrations of the reactants as $\mu_{max} = \pi\rho v^2\Gamma^{-1}(\Delta V)^2/2RT$, where $\Gamma = 1/[CD] + 1/[GST] + 1/[CDGST]$, ΔV is the standard volume change of reaction, R is the gas constant, and T is the absolute temperature.[16] If the equilibrium constant has been determined, the equilibrium reactant concentrations in Γ term can be calculated. Assuming various values of K, a trial and error procedure was used iteratively to acquire the best straight line that goes through a zero intercept in the plots of $2RT\mu_{max}/\pi\rho v^2$ vs Γ^{-1}. When the best linear fit was obtained for the plots, a linear least-mean square method was applied to obtain the standard volume change of the reaction. The above plots are shown in Fig. 4, and the best adjusted K values are listed in Table 2. The value of k_b was calculated by rearranging Eq. 2 as $k_b = 2\pi f_r/[K\{[CD] + [GST]\} + 1]$. The mean values of k_bs are listed in Table 2 along with the error which is defined as $0.674\{\Sigma(k_{b\,exp} - k_b)^2/(n-1)\}^{1/2}$, where n is the number of experimental data. Then, the value of k_f was obtained from the definition of the equilibrium constant, and the error was also calculated in a similar procedure as that for k_b. They are tabulated in Table 2. As the errors for k_f and k_b were estimated, the region of the probable relaxation frequency could be calculated by Eq. 2′, and they are indicated by the lines in Fig. 3. The experimental relaxation frequencies were located in the calculated regions for the three systems. It is seen that the relaxation frequency increases with the analytical concentration of the acid or goes through a minimum with a decrease in the concentration, depending on the values of the equilibrium constant.

The results of the dynamic interaction between the acids and β-CD at the condition of pH ≈6 are considered firstly. The acids in these solutions with salicylic acid, with benzoic acid

or with aspirin exist as the dissociated forms (carboxylate) which was estimated from their acid dissociation constants. For salicylic acid, there are only a few reported values of the equilibrium constant of complexation, and one[17] of which is $90 \pm 20\,\mathrm{mol^{-1}\,dm^3}$. The value obtained in this study is close to it. For benzoic acid, the equilibrium constant ranges from 10 to $36\,\mathrm{mol^{-1}\,dm^3}$,[5,18] and our result in this system was similar. It is seen from Table 2 that the values of k_f and k_b are similar to one another for each guest molecule. These results are interpreted as follows. (1) The forward process for the formation of the inclusion complex is diffusion controlled based on Smoluchowski's equation.[19] Thus, the values of k_f are similar, which have also been obtained in solutions of alcohols,[20] amines,[21] amino acids,[22] and carboxylic acids[23] ($3–8 \times 10^8$ $\mathrm{mol^{-1}\,dm^3\,s^{-1}}$). This is because the diffusion coefficients for these guest molecules are not so different. (2) The values of k_b are, on the other hand, strongly dependent on hydrophobicity of the guest molecules. With an increase in the hydrophobicity, the constant decreases steeply. The guest compounds in the present study are considered to have a similar hydrophobicity due to the benzene ring. As a result, similar values of k_b were obtained from the experiments. Since K is the ratio of the forward rate constant to the backward rate constant, the resultant equilibrium constants are almost the same values. This explanation supports that the benzene ring of the guest molecule was captured.[24] In addition, K and rate constants are scarcely dependent on the ortho-substituents in the dissociated forms of benzoic acid and its related compounds.

The experimental values of the standard volume changes of the reaction are listed in Table 2, and they are almost same for each guest compound. These results also indicate that the same group of the guest molecules is included into the β-CD cavity. The smaller volume changes, which are more than the molar volume of benzene, occurred to compensate for the water molecules expelled from the cavity of β-CD upon inclusion of the guest molecules. The sign of the volume change is thought to be positive on the basis of a precise experimental study for the partial molar volume.[25] Thus, the volume change can be expressed simply as $\Delta V = mV_{H2O} - V_{incl}$ where m is the number of ejected water molecules from the cavity, V_{H2O} is the molar volume of water and V_{incl} is a part of the guest volume inserted into the cavity. If V_{incl} is the molar volume of the benzene ($83 \times 10^{-6}\,\mathrm{m^3\,mol^{-1}}$),[26] the number of water molecules expelled from the cavity is about 5.1, which is close to the number of water molecules that are originally in the cavity when no guest is incorporated.[25,27]

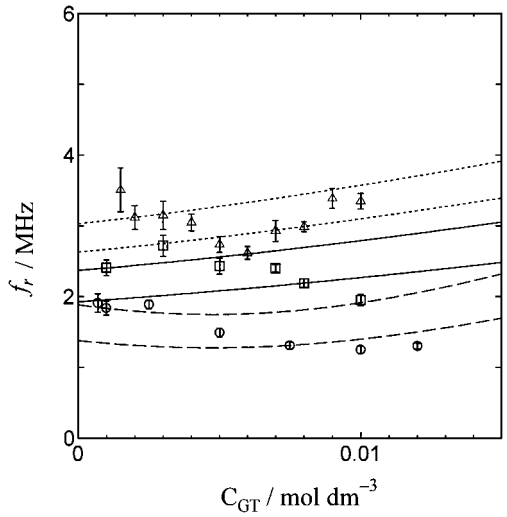

Fig. 3. The guest-concentration dependences of the relaxation frequency. (△): benzoic acid solution with 0.0087 mol dm^{-3} β-CD at pH ≈6, (□): salicylic acid solution with 0.0087 mol dm^{-3} β-CD at pH ≈6, (○): salicylic acid solution with 0.0087 mol dm^{-3} β-CD at pH ≈3. The region indicated by dotted lines (···) is the relaxation frequency range calculated by Eq. 2 for the benzoic acid solution, the region by solid lines (—) is the range for salicylic acid solution at pH ≈6 and the region by dashed lines (– – –) is that for salicylic acid solution at pH ≈3.

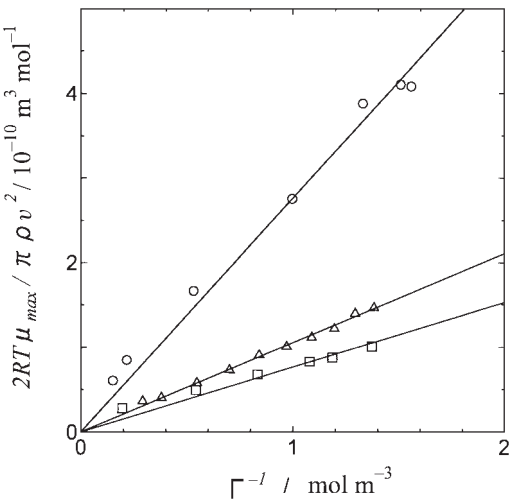

Fig. 4. Plots of $2RT\mu_{max}/\pi\rho v^2$ vs Γ^{-1} (○): the results for solution of salicylic acid at pH ≈3, (△): those for solution of benzoic acid at pH ≈6, (□): those for salicylic acid solution at pH ≈6.

Table 2. The Rate and Thermodynamic Parameters Associated with Dynamic Interaction between β-CD and the Acids at 25.0 °C

Guest	pH	k_f /10^8 mol^{-1} dm^3 s^{-1}	k_b /10^6 s^{-1}	K /mol^{-1} dm^3	ΔV /10^{-6} m^3 mol^{-1}	
Salicylic acid	≈6	4.4 ± 0.5	9.7 ± 1.0	45	9.4 ± 0.9	This work
Benzoic acid	≈6	5.8 ± 0.4	12.7 ± 0.9	46	8.9 ± 2.6	This work
Aspirin	≈6	7.8 ± 0.5	15 ± 1	51	8.5 ± 1.1	Ref. 10
Aspirin	≈1.7	7.21 ± 0.05	1.31 ± 0.03	549 ± 2	15.5 ± 0.1	Ref. 10
Salicylic acid	≈3	8.0 ± 1.1	3.2 ± 0.5	253	17.7 ± 1.1	This work

Next, we consider solutions pH <4 containing benzoic acid or salicylic acid. Most of the acid molecules exist in their the undissociated forms in these solutions. In the salicylic acid solution, the relaxation frequency was lower at pH <4 than that at neutral pH as can be seen in Table 1. This trend was also reflected in the value of k_b which was smaller than that in the neutral solutions Similar results were also obtained for solutions containing aspirin as the guest. This is because the inclusion process is associated with the diffusion controlled reaction. Therefore, the forward rate constants were similar as can be seen in Table 2. The benzene derivatives with carboxylate group appear to exit more quickly from the cavity than those with carboxylic group, which is a clear charge effect on the stability of the inclusion complex between host and guest. The slightly greater value of the standard volume change of the reaction than that in the neutral condition suggests that the number of the expelled water molecules was slightly higher at lower pH. If the same group or the same part of the hydrophobic group of salicylic group at the condition of pH $\approx$6 is included in the cavity, then the number of the expelled water molecules from the β-CD cavity should be $m \approx 5.5$.

In solution of benzoic acid at pH 1.9 and 0.01 mol dm^{-3}, the ultrasonic parameters were obtained experimentally. The ultrasonic spectrum is shown in Fig. 2, and the results are tabulated in Table 1. It should be noticed that the relaxation frequency was 0.87 MHz. If the value k_f is the same as that at pH $\approx$6, because the process is diffusion controlled reaction and the diffusion coefficient of the carboxylic form is not so different from that of carboxylate form, $k_b = 0.96 \times 10^6\,s^{-1}$ for $K = 604$ mol^{-1} dm^3,[28] $k_b = 1.6 \times 10^6\,s^{-1}$ for $K = 358$ mol^{-1} dm^3,[8] and $k_b = 4.5 \times 10^6\,s^{-1}$ for $K = 127$ mol^{-1} dm^3.[4] These values indicate that the relaxation frequency is in the rage from 0.75 to 1.7 MHz, which is the same range as the experimental value observed as can be seen in Table 1. In other words, the observed relaxation is associated with the dynamics of the interaction between the host and the guest.

In conclusion, the ultrasonic relaxation process could only be observed in aqueous solution when β-CD and drug molecule were both present. The cause of the relaxation is due to the complexation reaction between the host and the guest. The ortho-substitution effect on the rate and thermodynamic constants was not remarkable, although the charge effect was important for the departure of the drug from the CDs cavity. The included portion of benzoic acid and the related drugs appeared to be the benzene ring.

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