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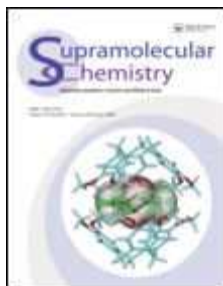
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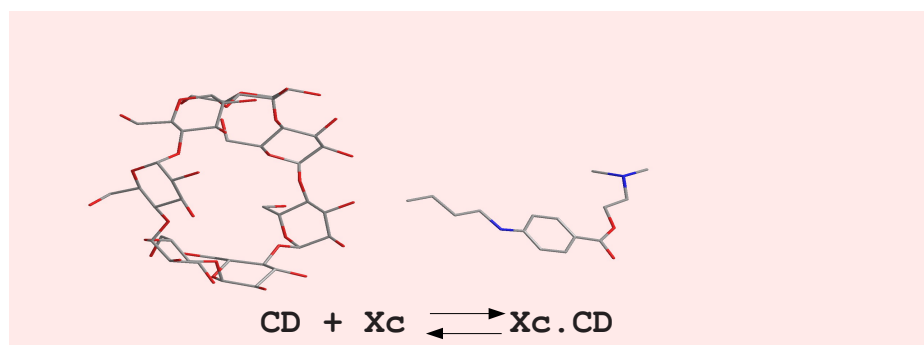
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20 The complexation of several local anesthetics, Xc, with  $\beta$  and  $\gamma$ -cyclodextrins, CD, was studied, tetracaine and  
21 dibucaine complexation constants were determined, while it was found that prilocaine and lidocaine do not  
22 form inclusion complexes.  
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## Potentiometric characterization of cyclodextrin inclusion complexes of local anesthetics

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### Abstracts

The complexation of several local anesthetics by  $\beta$  and  $\gamma$ -cyclodextrins was studied by potentiometry with glass electrode. Tetracaine and dibucaine complexation constants were determined at 25°C in the presence of 0.1 M of NaCl. It was found that prilocaine and lidocaine complexes cannot be detected.

**Keywords:** potentiometric technique; local anesthetics; inclusion complexes; cyclodextrin

### Introduction

Cyclodextrins (CD) form inclusion complexes with a great variety of guests and act as drug-delivery systems. This ability depends on the compatibility of the guest with the size of the conical cylinder cavity of the cyclodextrin and the affinity in terms of polarity with the hydrophobic wall of the cavity (1-3). An interesting group of compounds that form this kind of complexes are local anesthetics that have been studied previously in our laboratory (4-8). Stability constants of procaine were obtained using a wide variety of techniques (fluorescence or UV-vis spectroscopies, electrical conductivity, potentiometry and kinetic experiments) and conditions (4-7); in the same manner, tetracaine was studied by fluorescence spectroscopy (8) and the corresponding stability constants of the complexes were determined.

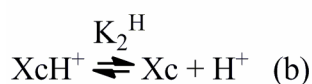
The present paper aims to compare those data with the same constants obtained by potentiometry for the case of tetracaine, with which compound both techniques can be applied, and to determine potentiometrically the stability constants of the anesthetics, dibucaine, lidocaine and prilocaine, whose constants can not be obtained by fluorescence spectroscopy, as it will be discussed below.

**Tetracaine** [TCA, 2-(Dimethylamino)ethyl 4-(butylamino)benzoate] has two amino groups, therefore, depending on the acidity of the medium it may exist as a neutral molecule, a monocation, or a dication. The same is true for dibucaine [DCA, 2-Butoxy-N-[2-(diethylamino)ethyl]-4-quinolinecarboxamide], while in lidocaine [LCA, 2-(Diethylamino)-N-(2,6-dimethylphenyl)acetamide] and prilocaine [PCA, N-(2-Methylphenyl)-2-(propylamino)propanamide] only one amino moiety is present and there is just one acid-base equilibrium between neutral and monoprotonated molecules, see scheme 1. It was that (8) the

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monocation and the neutral molecule of tetracaine are able to enter into the CD cavity with the unprotonated secondary amine group end first. As the aromatic ring of the drug, the hydrophobic part of the molecule, is inside the cavity, the fluorescence emission intensity is enhanced compared to that of the molecule in water, this fact was used to determine the stability constants for the complexes of Tc and HTc<sup>+</sup> with CD. The same approach can not be used for dibucaine, because no appreciable variation in the fluorescence emission was observed in water and in the presence of CD, or with lidocaine and prilocaine, because no fluorescence was observed in the last two cases. Taking this problem into account, a potentiometric method was considered to study the possible complexation of these anesthetics with cyclodextrins. In order to apply this technique, a small amount of sodium chloride (0.1 M) was added to all solutions to keep the ionic strength constant during the titration.

If the acid-base equilibria of an anesthetic, Xc, are represented by,



the complexation equilibrium of anesthetic cation is, then, given by,



and the complexation equilibrium of neutral anesthetic may be represented by,



The constants  $K_2^c$  and  $K_3^c$  are used in accordance with previous notation (4,8) where  $K_1^c$  will represent the stability equilibrium constant of the hypothetical complex between CD and the anesthetic dication. It was also concluded that the fully protonated anesthetic molecule do not form inclusion complexes. These equations are valid for tetracaine and dibucaine. In the cases of lidocaine and prilocaine the first equilibrium,  $K_1^H$ , does not exist because there is only one amine group available to be protonated.

### Experimental

High-purity TCA.HCl, DCA.HCl, LDA.HCl, PDA.HCl and  $\beta$ -cyclodextrin were purchased from Sigma-Aldrich (St Lous, MO, USA), while  $\gamma$ -cyclodextrin were purchased from Cyclolab R & D Laboratory Ltd., Budapest, Hungary.

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2 An aqueous aliquot containing the anesthetic hydrochloride (TCA, DCA) was titrated with a  
3 standard solution of hydrochloric acid (Merck p.a.) to determine  $pK_1^H$ , and with a standard solution  
4 of sodium hydroxide (MERCK p.a.) in order to determine  $pK_2^H$ . When cyclodextrin was added to  
5 the solution of the anesthetic hydrochloride, the titration with HCl yields  $\log K_2^C$ , whereas the  
6 titration with NaOH yields  $\log K_3^C$ . In the case of LDA.HCl and PDA.HCl only titrations with base  
7 (with and without CD) were performed because there is only one acid-base equilibrium. The  
8 anesthetic concentration was made varied from  $10^{-2} M$  to  $10^{-3} M$ ; the concentration was higher in  
9 acid medium than in basic medium, because in the later medium the anesthetic is in the form of a  
10 neutral molecule that easily precipitates. This is especially true for dibucaine, whose concentration  
11 was kept as low as  $4.5 \cdot 10^{-5} M$  in basic media to avoid precipitation. Under these conditions 0.01 M  
12 NaOH was used as titrant and was freshly prepared before use with water bubbled with nitrogen.  
13 On the other hand, the necessary amount of NaCl (Merck, p.a.) to adjust the ionic strength, I, to the  
14 desired value was added to all solutions (I=0.1 M).  
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28 Titrations were carried out in a dual-wall cell, kept at constant temperature (25.0 °C) by a  
29 thermostat-controlled system. Purified nitrogen was bubbled through the solutions in order to ensure  
30 thorough homogenization and CO<sub>2</sub> removal. A Crison microBu 2030 automatic buret furnished with  
31 a 2.5 mL syringe for dispensing the titrant was used. The buret was controlled with a computer that  
32 was used to read the emf values from a Crison micropH 2000 pH meter connected to two  
33 electrodes: a glass electrode (Radiometer pHG211) and a reference electrode (Radiometer REF201).  
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38 Equilibrium constants were calculated from the potentiometric titration data by means of the  
39 Hyperquad program (9). The electrode response at constant ionic strength is given by  $E = E^{0'} + s$   
40  $\log [H^+]$ , where E is the emf, and the electrode parameters, the formal potential,  $E^{0'}$ , and the slope, s,  
41 are required by the program to determine the constants. A separate experiment was carried out to  
42 obtain the electrode parameters: emf was measured in solutions of known proton concentration, at  
43 the desired ionic strength, s and  $E^{0'}$  were obtained from a linear regression; see reference (10)  
44 Measurements in proton concentration,  $[H^+]$ , allows the determination of stoichiometric or  
45 concentration constants. The values of s and  $E^{0'}$  for the corresponding electrode has been used to  
46 plot  $p[H] = -\log[H^+]$ .  
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## 55 Results

### 56 *Tetracaine complexes.*

57 Acid-base constants of TCA were previously obtained by potentiometry, while stability  
58 constants of tetracaine complexes with  $\beta$ -CD were determined by spectrofluorimetry;  $\log K_2^C$  and  
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2  $\log K_3^C$  are listed in Table 1, together with the values found in this work, for purposes of  
3 comparison. When tetracaine hydrochloride was dissolved in water and titrated with strong acid, an  
4 equilibrium is established between dication and cation, Equation (1a). When  $\beta$ -CD is added to this  
5 system, the titration curve is displaced toward more acidic pH values due to the **cation being taken**  
6 by the CD, Equation (2), shifting equilibrium (1a) to the right. This effect is clearly observed in  
7 Figure 1a, where solutions of equal concentration of tetracaine hydrochloride were titrated with and  
8 without CD, with the same electrode, the same day, and with the same titrant solution. The  
9 difference between both curves of the figure **is proof of** the complexation with the CD, which can  
10 be used to determine the complexation constant when  $pK_1^H$  is known. Several titrations of  
11 tetracaine with cyclodextrin in acid media were introduced together in **Hyperquad** to yield the value  
12 listed in Table 1. It may be argued that these data are not of practical interest **because too low a pH**  
13 is needed to found them, but this is not true because TcH.CD is present at higher pH values, too. In  
14 fact, at slightly basic pH, Equation (1b) governs the system, and the equilibrium is established  
15 between the neutral molecule, Tc, and the cation, TcH<sup>+</sup>; when  $\beta$ -CD is added to this system, it will  
16 remove both species partially to form the complexes Tc.CD and TcH.CD<sup>+</sup>, and  $\log K_2^C$  and  
17  $\log K_3^C$  are needed to explain the system behaviour. In Figure 1b, TCA.HCl is titrated with strong  
18 base, with and without  $\beta$ -CD, the difference between the curves is caused by the formation of the  
19 complexes, and this kind of titration can be used to determine their stability constants. At this point  
20 two options are possible, as  $\log K_2^C = 3.00$  is known from acid titrations, this value can be fixed in  
21 the Hyperquad program to obtain the other complexation constant,  $\log K_3^C = 3.51$  (Table 1).  
22 Another choice considers both constants as unknown parameters in Hyperquad and determines both  
23 values **with basic titrations**. Of course, the error was greater when two constants were determined  
24 instead of only one with the same set of titrations, but the results of these two procedures agree  
25 quite well within the experimental error, Table 1. **In other systems** (when some problems difficult  
26 the experimental determination of the constants) it may be necessary to use both kind of titrations,  
27 for example in dibucaine, as it will be discussed later.

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Complexation constants of tetracaine with  $\gamma$ -cyclodextrin are also included in Table 1. Titrations in acid and basic media were used to obtain these values. It is interesting to note that when tetracaine was titrated with acid in the presence of  $\gamma$ -CD (four titrations), Hyperquad fitting yields  $\log K_2^C = 2.40$  and  $pK_1^H = 2.26$ , therefore  $pK_1^H$  can be determined simultaneously with  $K_2^C$  in the presence of  $\gamma$ -CD; as it can be seen this result agrees perfectly with  $pK_1^H = 2.24$  on Table 1 obtained without  $\gamma$ -CD. If the complexation equilibrium is omitted from the system, **an incorrect value** of  $pK_1^H = 1.96$  was determined, which clearly shows that the presence of  $\gamma$ -CD affects the

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2 acid-base equilibrium (of course, similar behaviour is observed with  $\beta$ -CD).  
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4       The comparison of the data obtained in this paper with those determined previously from  
5 fluorescence measurements (8), shows that the stability constants with  $\beta$ -CD agree very well, while  
6  $K_3^C$  with  $\gamma$ -CD, obtained in this work, is significantly greater. The explanation may reside in the fact  
7 that concentration of TCA in fluorescence experiments was quite low  $\sim 10 \mu\text{M}$  (it was selected  
8 taking into account that the absorbance should be  $\sim 0.2$ ). But buffer,  $\text{HCO}_3^-/\text{CO}_3^{2-}$ , was added in a  
9 concentration of 0.033 M to adjust the pH, then it was in a much greater quantity than TCA and it  
10 could be able to compete with the anesthetic to form complexes with  $\gamma$ -CD. This result emphasizes  
11 the interest of using different methods to study the complex formation.  
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### 22 *Dibucaine complexes*

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24       It was mentioned in the experimental section that the neutral molecule of dibucaine is less  
25 soluble than the other anesthetics used in this study; solutions as low as  $4.5 \cdot 10^{-5}$  M were used to  
26 avoid precipitation in basic media (when CD was present  $10^{-4}$  M solutions were used). These  
27 concentrations are quite low for the potentiometric technique, (11); for this reason, titrations in acid  
28 media were used to determine  $\log K_2^C$ . With this value and the results of the titrations in basic  
29 media,  $\log K_3^C$  was obtained (Table 1). When only basic titrations are used in Hyperquad to obtain  
30  $\log K_3^C$  and  $\log K_2^C$ , the program is not able to optimize simultaneously the values of both constants;  
31 although it is able to optimize one of them if the other is known. The reason is probably not only the  
32 low concentration of reactants, but the close values of the constants 2.61 and 2.65, that cause no  
33 shift in the titration curves when CD is added to the system, as is explained below. It should be kept  
34 in mind that in basic media there is an equilibrium between neutral molecule, Dc, and cation,  $\text{DcH}^+$ ,  
35 Equation (1b). So, it is possible to calculate the complexation constants of these species, since when  
36 CD is added to the solution, it removes part of these molecules to form the complexes; but if the  
37 constants are quite similar, no shift in the equilibrium is expected: the system *does not detect* the  
38 presence of the CD. To illustrate this point, a simulation has been performed and the result can be  
39 seen in Figure 2. The HySS program (12) was used to reproduce the expected experimental titration  
40 curves for dibucaine in the absence (circles) and in the presence of  $\beta$ -CD (lines from a to g) for  
41 different values of  $\log K_3^C$ . In this simulation process the  $\log K_2^C = 2.60$  was kept constant, while  
42  $\log K_3^C$  was varied with the following values (a)2.60, (b)2.70, .....(g)3.10. The concentrations of  
43 Figure 2a were:  $[\text{DCA}] = 1.5 \cdot 10^{-4}$  M and  $[\beta\text{-CD}] = 1.5 \cdot 10^{-3}$  M titrated with  $[\text{NaOH}] = 0.01$  M. In  
44 Figure 2b these values were increased 10 times to illustrate the influence of concentration, i.e.  
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[DCA]= $1.5 \cdot 10^{-3}$  M and  $[\beta\text{-CD}]=1.5 \cdot 10^{-2}$  M titrated with  $[\text{NaOH}]=0.1$  M. Several remarkable facts can be observed when these graphs are compared. The most evident point is that at high concentrations all features of the curves are more clearly observed: pH variation and separation among curves are bigger (notice that pH-scale is the same in both plots). It is a good way to show the difficulties that appear when low concentrations are used, as it was stated before. Besides, this is the reason why an effect that is almost imperceptible in Figure 2a, becomes clearly observed in Figure 2b i.e., the separation of the curves, at high pH, when  $\beta\text{-CD}$  (curves a to g) is present and when the anesthetic is titrated alone (circles). The explanation is quite simple, the acid-base equilibrium of  $\beta\text{-CD}$  was taken into account in the simulations, and its influence can be noticed at the end of the titrations. It should be noted that  $\beta\text{-CD}$  concentration is higher than that of the anesthetic, not only in the simulation, but in the experimental titrations too, where  $\beta\text{-CD}$  concentration used was equal or several times higher than that of the anesthetic. As this effect was observed in the experimental data, it was considered interesting the determination of the acid-base constants of the cyclodextrins used in this work and it will be discussed later. Although there is a separation of the curves at high pH, because of the reason explained above, at lower pH, when both constants are equal, the plots coincide very well, curves *a* and circles Figure 2a-2b, while the other curves *b-g* increase their separation respect to the circles as long as the difference between  $\log K_3^C$  and  $\log K_2^C$  increases. Then, if both complexation constants are equal, titration curves of the anesthetic remain almost the same in the presence and the absence of CD, unless at high pH, where pK of CD becomes relevant, but this is the same behaviour that would be expected if CD would not form complexes at all with the anesthetic. This is not a problem when one of the constants can be found by another means, in this case the acid titrations yield the complexation constant of the cation and when this value is known, titrations with base yield the complexation constant of the neutral molecule. The values of the constants in this work agree well with others found in the literature (13),  $pK_2^H = 8.73$ , and  $\log K_3^C = 2.8$ , with  $\beta\text{-CD}$ , using drug-sensitive electrodes (14).

### ***Lidocaine and prilocaine complexes***

Lidocaine and prilocaine have only one amino group and only one acid-base equilibria, Equation (1b). Titration with base of the prilocaine hydrochloride alone, with  $\beta\text{-CD}$  and  $\gamma\text{-CD}$ , is presented in Figure 3. The concentration of the anesthetic was the same in the three experiments, and were performed with the same electrode, the same day, and with the same titrant solution. As it can be appreciated all the curves coincide quite well except at high pH, where the influence of the acid-base equilibrium of the CD is appreciable; the same behaviour can be observed in Figure 2b.

At the end of the titration, prilocaine has been converted from the protonated to the neutral form, while CD begins to ionize causing the separation of the curves. Similar behaviour was observed for lidocaine. Several titrations in the presence of CD ( $\beta$ -CD or  $\gamma$ -CD) were fitted with Hyperquad by fixing the pK of CD (Table 2), the pK<sub>H</sub> of the anesthetic was obtained and the results are listed in Table 2, and it agrees with literature reports (13, 15). Titration curves and pK<sub>H</sub> values are equal in the presence and in the absence of CD, therefore it is impossible to determine the stability constant of the inclusion complexes because no separation in the curves appears. There are various possible explanations. One of them is evident and may be attributed to the absence of complex formation; on the other hand, a more subtle explanation is that complexes were formed, but the stability constants are approximately equal and no variation is expected in the curves (see Figure 2) or even the anesthetic does not penetrate very deeply in the cavity of the CD and no disturbance in the acid-base equilibrium is observed. Previously (16), inclusion complexes of the neutral molecules of some anesthetics, including prilocaine and lidocaine, with  $\beta$ -CD were determined from solubility measurements. Quite low values for the stability constants were obtained such as  $K_3^C = 26M^{-1}$  for lidocaine and  $K_3^C = 96M^{-1}$  for prilocain. To illustrate the expected shifts in the titration curves that would be originated by such low complexation constants, a new simulation with the HySS program (12) was performed. The results appear in Figure 3b, when the concentrations used were the same that those appearing in the experimental titrations of Figure 3a for the case of prilocaine, i.e. 40 ml of [PCA]= $1.0 \cdot 10^{-3}$  M and [ $\beta$ -CD]= $4.9 \cdot 10^{-3}$  M titrated with [NaOH]=0.02 M, while the constants used were  $pK^H = 8.01$ ,  $\log K_3^C = \log(96) = 1.98$  was kept constant, and  $\log K_2^C$  was changed with the following values (a)1.98, (b)1.88, .....(g)1.58. The new fact in this plot, compared to the other simulations, is that the curves are very close one to another, showing the difficulties that appear when the constants are too low, which it seems to be the case here, and, of course, this precludes the potentiometric determination of the constants.

#### *Acid-base constants of $\beta$ -cyclodextrin and $\gamma$ -cyclodextrin*

As it has been discussed before, the last points of the titration curves in basic media are influenced by the presence of CD. This fact encouraged us to estimate the pK of  $\beta$ - and  $\gamma$ -cyclodextrins, under the experimental conditions of this work, 25°C and I=0.10 M NaCl. The results are listed in Table 2. Obviously with these pK values, high pH (more than 12) has to be reached in the titrations, which is inconvenient for the potentiometric technique (11). Besides, in this situation the ionization constant of water has to be considered, a value of  $pK_w^C = 13.79$  at 25°C and I=0.10 M NaCl was used (17). If pK<sub>w</sub> is not fixed in the fitting program and is determined together with the

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2 pK of the CD, the obtained results were 13.77 for  $\beta$ -CD and 13.79 for  $\gamma$ -CD, which agree very well  
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4 with the values found using a hydrogen electrode (17). On the other hand, slightly higher values for  
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6 the pK of both cyclodextrins (0.3 units more) can be found in the literature (18). This difference can  
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8 be ascribed to the experimental error at high pH and besides to the calibration procedure that is  
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10 quite different from ours. Previously (18) calibration of the electrode was performed with standard  
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12 buffers of known pH, after that, pH was measured and the proton concentration was obtained, using  
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14 the proton activity coefficient, calculated from Debye-Hückel equation with a certain value of the  
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16 size parameter in this equation. In fact, it is no necessary, to have any supposition about activity or  
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18 activity coefficients of individual ions to perform a calibration in concentration (19). Solutions of  
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20 known concentration can be used to obtain a calibration line without any kind of supposition, that  
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22 could affect the obtained values in an indeterminate way.

## 23 Discussion

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25 Cyclodextrins are truncated cone-shaped molecules with a hollow, tapered cavity of 7.9-Å  
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27 depth. The top and bottom diameters of the cavity are 6.8 and 7.8 Å for  $\beta$ -cyclodextrin, and 8.3 and  
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29 9.5 Å for  $\gamma$ -cyclodextrin, respectively (1).

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31 As it was stated before, the ability to form inclusion complexes depends on the compatibility  
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33 of the guest molecule with the size of the cavity and the affinity in terms of polarity with the  
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35 hydrophobic wall of the cavity. To explore the possible inclusion of the anesthetic molecules into  
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37 the CD, the size of these compounds was optimized using PM6 method by means of MOPAC (20),  
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39 (identical results were found with PM3). It is expected that the molecule will enter with its more  
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41 hydrophobic part oriented towards the cavity, that is, the aromatic ring. Some relevant distances are  
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43 depicted in scheme 1. It can be shown that tetracaine has a size perfectly compatible with the cavity  
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45 of CDs and give rise to the highest constants. On the other hand, lidocaine is the molecule with the  
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47 biggest diameter because of the methyl groups in the benzene ring, which fact difficults the  
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49 complexation with  $\beta$ -CD, but not with  $\gamma$ -CD that has the biggest cavity. Therefore, the absence or  
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51 the small amount of complex formation may be ascribed to a lack of affinity with the hydrophobic  
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53 cavity of the CD. The same seems to be true for prilocaine, that has a smaller size than lidocaine.  
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55 Both compounds exhibit a much greater solubility in water than dibucaine or tetracaine, and it  
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57 seems they prefer the polar environment of water instead of the hydrophobic cavity of the CDs. On  
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59 the contrary, dibucaine with a large aromatic part, even relatively big, can form complexes in  
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significant amount. A detailed study of the geometry of these molecules, neutral, protonated and in  
different solvents can be found in ref (21).

## Conclusions

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The use of the potentiometric technique to determine inclusion constants of several local anesthetic with CDs has been discussed and the **complexation** constants determined. It was found that tetracaine, neutral or protonated, forms complexes in significant amount with  $\beta$  and  $\gamma$ -cyclodextrins. This system had been studied previously with similar results, in this laboratory, using fluorescence. Although this is a very sensible technique, there are other anesthetics that can not be studied with this method, i.e. no fluorescence or change of fluorescence is observed in the presence of CD, so potentiometry was employed. This is the case of dibucaine that forms complexes with both CDs; also prilocaine and lidocaine, which do not form complexes or do it in a small amount that can not be detected with the potentiometric technique. On the other hand, although the sizes of the cavities of the CDs are adequate to include the molecules of the anesthetics used in this study (with the exception of lidocaine in  $\beta$ -CD); the absence of complexation can be ascribed to different affinity of guest the molecule and the cavity.

### Acknowledgments

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**Table 1.** Acid-base and complexation constants of tetracaine and dibucaine at 25°C and 0.1 M ionic strength of NaCl

| <b>Tetracaine with <math>\beta</math>-cyclodextrin</b>  |           |              |              |  |
|---|-----------|--------------|--------------|--|
| $pK_1^H$  | $pK_2^H$  | $\log K_2^C$ | $\log K_3^C$ | media                                      |
| 2.24*   | 8.39*     | 3.00*        | 3.53*        |  |
|   |           | 3.00±0.01    |              | Acid titration, Equations (1a), (2)        |
|   |           |              | 3.51±0.01    | Basic titration, using $\log K_2^C = 3.00$ |
|   |           | 2.9±0.1      | 3.48±0.07    | Basic titration, Equations(1b), (2),(3)    |
|   |           | 3.01±0.02    | 3.51±0.02    | acid+basic titration together              |
| <b>Tetracaine with <math>\gamma</math>-cyclodextrin</b> |           |              |              |  |
|   |           | $\log K_2^C$ | $\log K_3^C$ |  |
|   |           |              | 1.85*        |  |
|   |           | 2.41±0.05    | 2.84±0.03    | acid+basic titration together              |
| <b>Dibucaine with <math>\beta</math>-cyclodextrin</b>   |           |              |              |  |
| $pK_1^H$  | $pK_2^H$  | $\log K_2^C$ | $\log K_3^C$ |  |
| 1.95±0.01   | 8.77±0.01 | 2.61±0.02    | 2.65±0.02    | acid+basic titration together              |
| <b>Dibucaine with <math>\gamma</math>-cyclodextrin</b>  |           |              |              |  |
|   |           | $\log K_2^C$ | $\log K_3^C$ |  |
|   |           | 2.28±0.04    | 2.58±0.03    | acid+basic titration together              |

\* ref (8)

**Table 2.** Acid-base constants of lidocaine, prilocaine,  $\beta$ -cyclodextrin and  $\gamma$ -cyclodextrin at 25°C and 0.1 M ionic strength of NaCl

|   | $pK^H$     |
|---|------------|
| <b>Lidocaine</b>  | 7.92±0.01  |
| <b>Lidocaine with <math>\beta</math>-cyclodextrin</b>   | 7.89±0.01  |
| <b>Lidocaine with <math>\gamma</math>-cyclodextrin</b>  | 7.90±0.01  |
| <b>Prilocaine</b>                                       | 8.01±0.01  |
| <b>Prilocaine with <math>\beta</math>-cyclodextrin</b>  | 8.03±0.01  |
| <b>Prilocaine with <math>\gamma</math>-cyclodextrin</b> | 7.99±0.01  |
| <b><math>\beta</math>-cyclodextrin</b>                  | 11.95±0.01 |
| <b><math>\gamma</math>-cyclodextrin</b>                 | 11.88±0.02 |

1  
2 **Scheme 1.** Structures of the anesthetics tetracaine, dibucaine, prilocaine and lidocaine.  
3

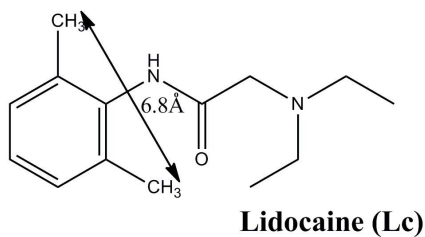
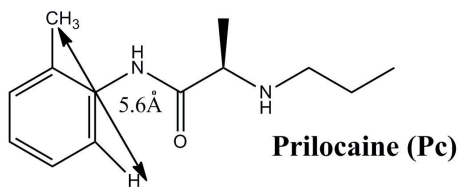
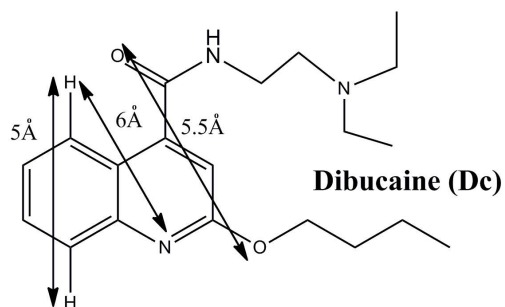
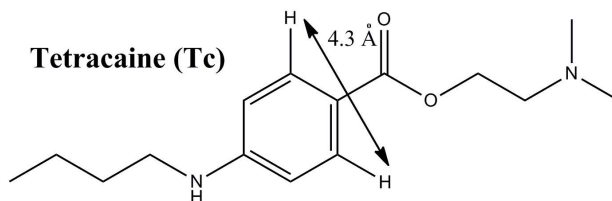
4 **Figure 1.** Tetracaine hydrochloride titration without (circles) and with  $\beta$ -CD (squares) a) in acid  
5 media, [TCA]=3 mM, [ $\beta$ -CD]=3 mM, [HCl]=0.1,  $v_0$ =40 mL b) in basic media, [TCA]=1 mM, [ $\beta$ -  
6 CD]=1 mM, [NaOH]=0.010 M,  $v_0$ =40 mL ( $v_0$  is the initial volume of titrand)  
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9 **Figure 2a.** Simulation of the expected titration curves for dibucaine in the absence (circles) and in  
10 the presence of  $\beta$ -CD (lines) with constants:  $pK_1^H = 1.95$ ,  $pK_2^H = 8.77$ ,  $pK_{\beta-CD}^H = 11.95$ ,  
11  $\log K_2^C = 2.60$ , and  $\log K_3^C$  changing with the following values 2.60, 2.70, 2.80, 2.90, 3.10 and  
12 3.10 (lines a-g); concentrations are [DCA]= $1.5 \cdot 10^{-4}$  M and [ $\beta$ -CD]= $1.5 \cdot 10^{-3}$  M titrated with  
13 [NaOH]=0.01 M,  $v_0$ = 100 mL  
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17 **Figure 2b.** Simulation of the expected titration curves for dibucaine under the same conditions of  
18 figure 2a, except that concentrations were increased 10 times: [DCA]= $1.5 \cdot 10^{-3}$  M and [ $\beta$ -CD]= $1.5$   
19  $\cdot 10^{-2}$  M titrated with [NaOH]=0.1 M  
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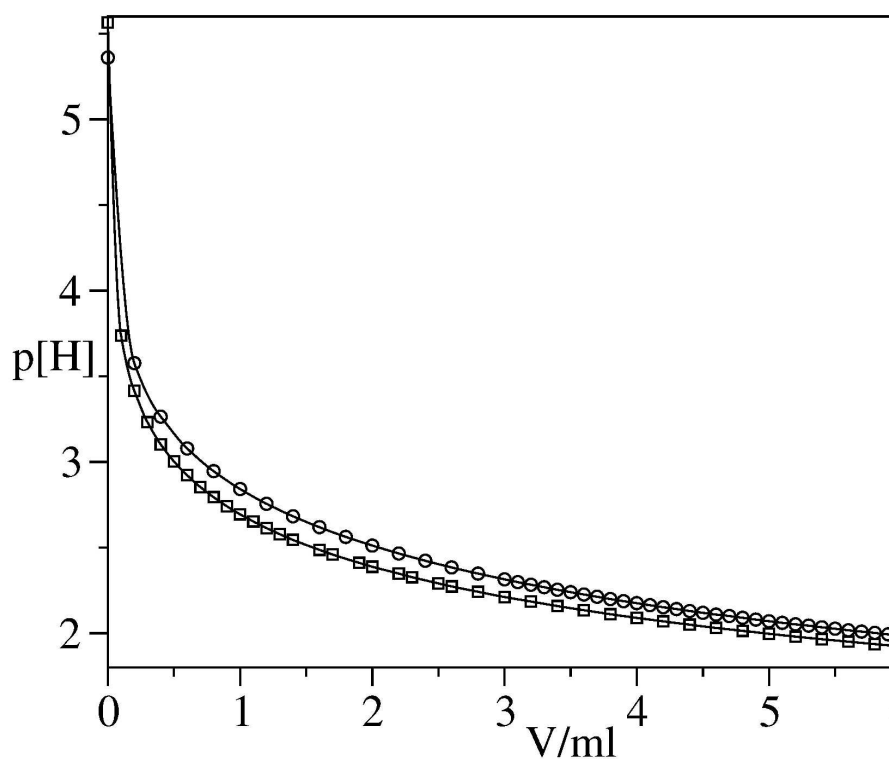
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23 **Figure 3a.** Prilocaine hydrochloride titration alone (line), with  $\beta$ -CD (+) and with  $\gamma$ -CD (x),  
24 [PCA]=1 mM, [ $\beta$ -CD]=5 mM, [ $\gamma$ -CD]=6 mM, [NaOH]=0.02 M,  $v_0$ = 40 mL  
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27 **Figure 3b.** Simulation of the expected titration curves of 40 ml of prilocaine with concentration 1  
28 mM and [ $\beta$ -CD]=4.9 mM titrated with [NaOH]=0.02 M, the constants are  $pK^H = 8.01$ ,  
29  $\log K_3^C = 1.98$ , and  $\log K_2^C$  changing with the following values 1.98, 1.88, 1.78, 1.68 and 1.58  
30 (lines a-g), circles correspond to the titration in absence of CD.  
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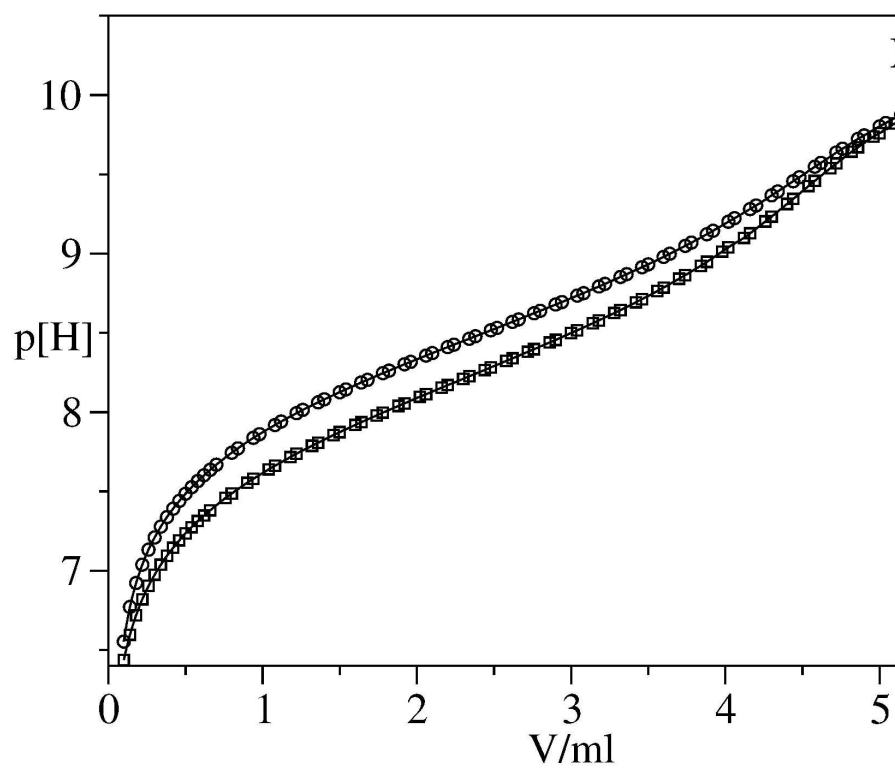


Scheme 1  
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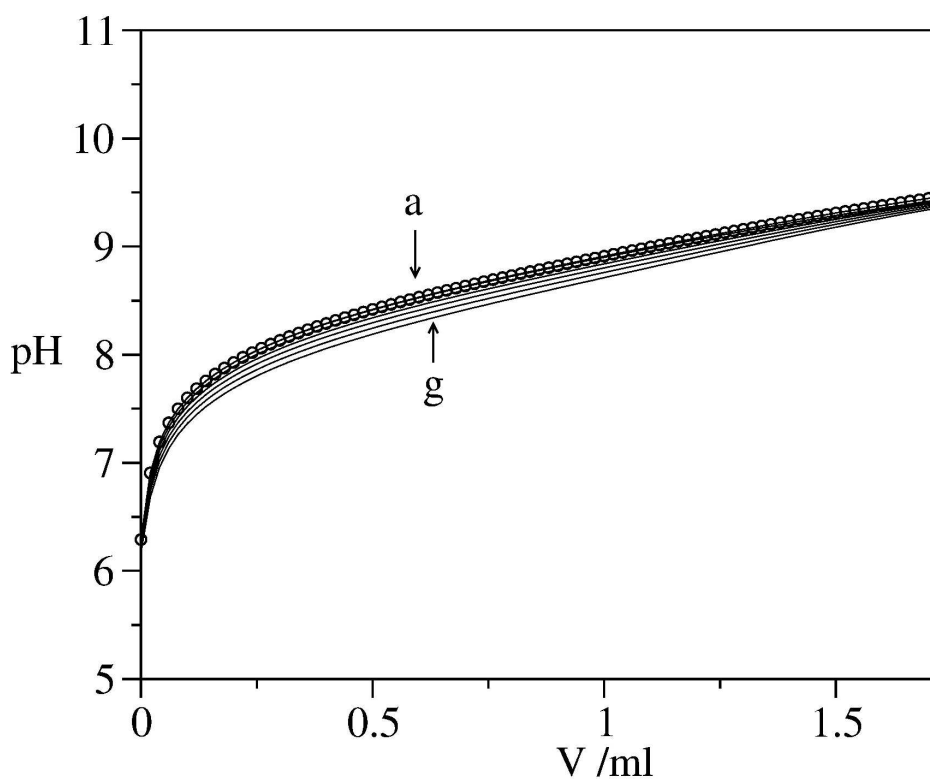


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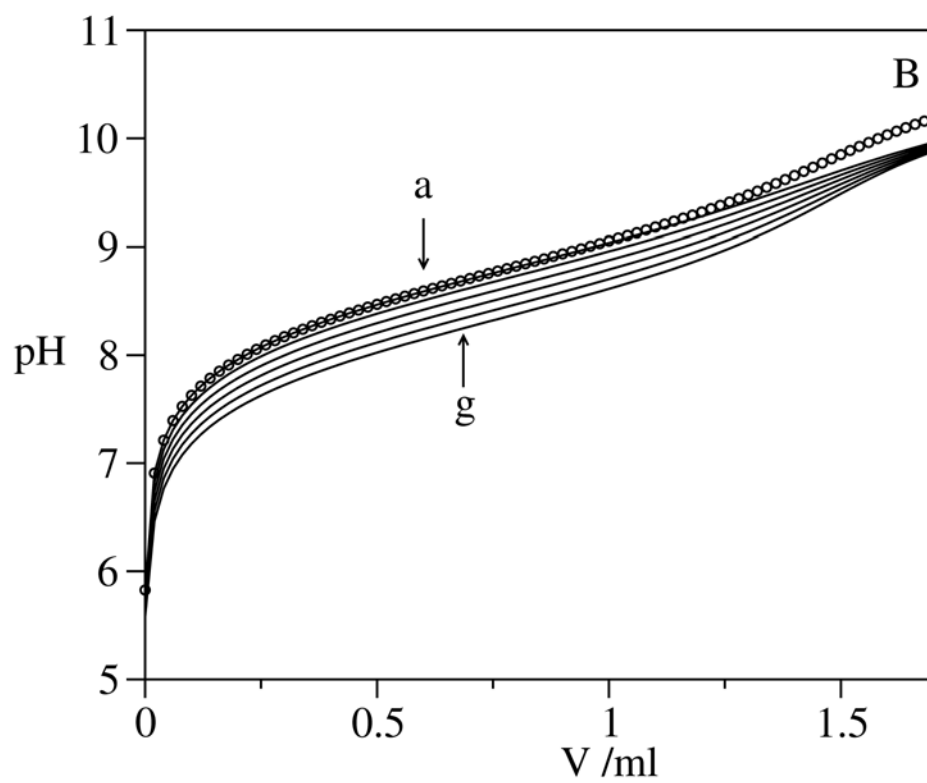


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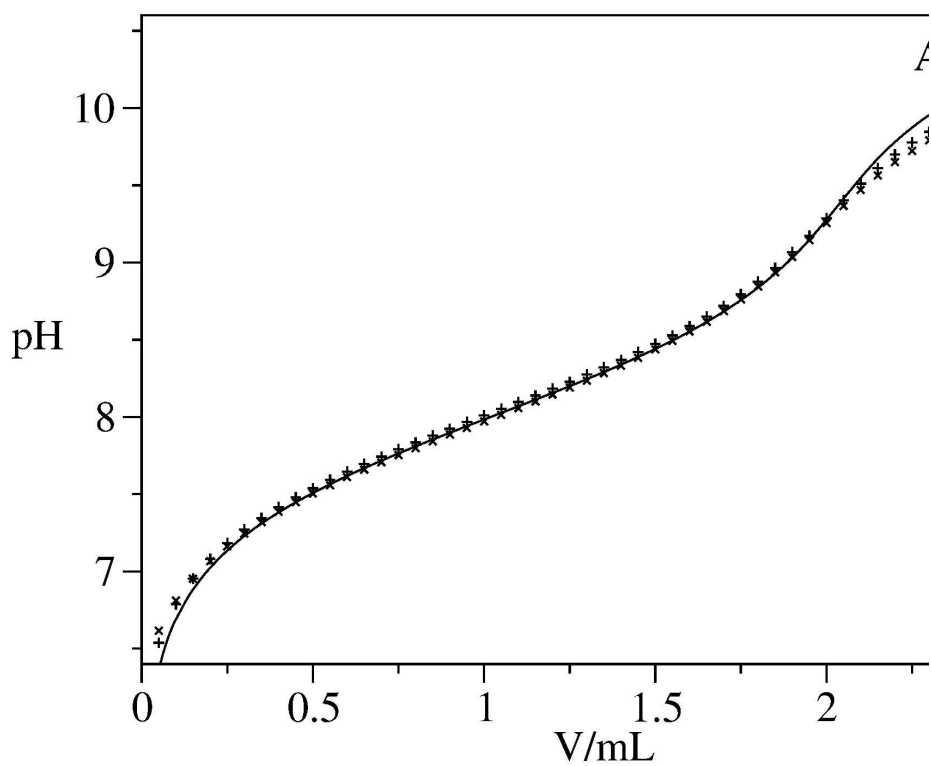
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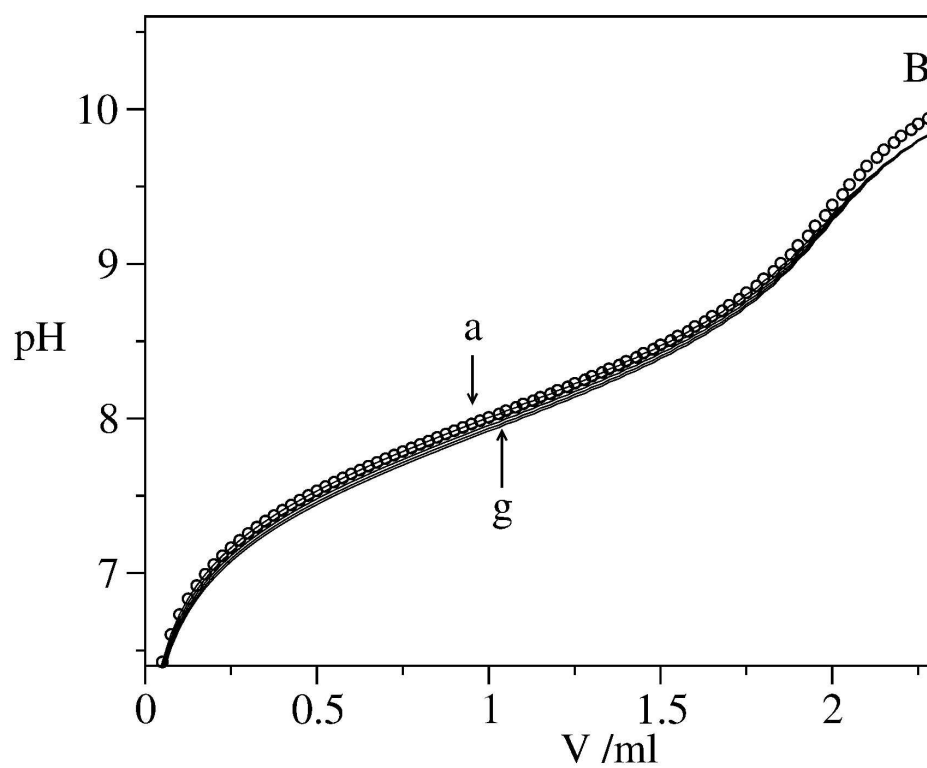
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