Journal of Inclusion Phenomena and Macrocyclic Chemistry **45:** 149–154, 2003.

Application of the MIMS Technique to Study the Stability Constants of Small Organic Guest Molecules into Cyclodextrin Hosts in Aqueous Medium

A.E. BURGOS, R.D. SINISTERRA, RODINEI AUGUSTI and ROCHEL M. LAGO*
Dep. Química, Universidade Federal Minas Gerais, Belo Horizonte, MG, 31270-901, Brazil
rochel@dedalus.lcc.ufmg.br

(Received: 28 March 2002; in final form: 15 August 2002)

Abstract

In this work the potential of MIMS (Membrane Introduction Mass Spectrometry) for studying the inclusion of small organic guest molecules into cyclodextrin hosts in aqueous medium was investigated. MIMS profiles showed that the inclusion of benzene in cyclodextrins is favored in the following order: β-CD $\approx$ HO-propyl-β-CD$>$ α-CD $>$ γ-CD with equilibrium constants of $K_{\beta\text{-CD}} = 404$; $K_{\text{HO-propyl-CD}} = 395$, $K_{\alpha\text{-CD}} = 335$ and $K_{\gamma\text{-CD}} = 210$ M^{-1} at 25 °C. Kinetic experiments suggested that under the conditions employed the inclusion process has a pseudo first-order dependence on the guest benzene concentration with the following order: α-CD $>$ β-CD $\approx$ HO-propyl-β-CD $>$ γ-CD. MIMS inclusion profiles of chlorobenzene and toluene showed that the presence of substituents in benzene makes the inclusion in β-CD more difficult. Experiments with ferrocene-β-CD have also been carried out, showing that the complex rapidly dissociates in water and the resulting free β-CD can complex with benzene present in the solution.

Introduction

Host:guest reactions involving cyclodextrins are highly important for drug delivery systems technology, food and separation industry. These systems are also very relevant as models for understanding general inclusion phenomena, as well as enzyme substrate interactions [1]. Thermodynamic and NMR studies [2–4] suggest that these inclusion compounds are formed by the insertion of the less polar part of the guest into the CD cavity while the more polar portion of the guest molecule is exposed to the solvent. Several techniques have been used to investigate the formation of these inclusion compounds and their stability constants [5], e.g., UV [6], fluorescence lifetime [7], fluorimetry [8], NMR (both in solution and solid state)[1a, 9], powder XRD [1,1a], polarography [1], positron annihilation [10], HPLC [11], gas chromatography [12], mass spectrometry [1], potentiometry [13], partition coefficient [1, 5], conductometric titration [14], calorimetry [5], phosphoresence [15], kinetics (competitive rate constant determination or stopped flow)[16], solubility measurement[17], surface tension measurement[18], vapor pressure measurement [19], and ESR [20]. The use of a cellophane membrane permeation technique to determine the stability constant of the inclusion of phenacetin and several benzoic acids in β-CD [21] has also been reported.

Here, the use of the MIMS technique as a simple and reliable method to study the kinetics and the equilibrium of the inclusion of small organic guest molecules into different cyclodextrins is reported.

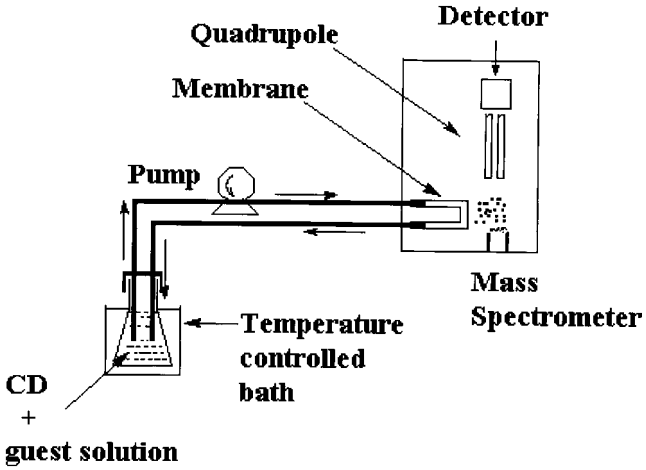

Figure 1. Schematic representation of the MIMS systems to study the inclusion process.

Membrane Introduction Mass Spectrometry (MIMS) is one of the most efficient, simple and sensitive techniques for the analysis of volatile organic compounds in matrices such as water, soil and air. MIMS is based on the selective permeation of an organic compound through a membrane, which acts as the interface between a mass spectrometer and the sample solution (Figure 1).

The most common membrane used is silicone rubber (PDMS-polydimethylsiloxane) which due to its hydrophobic character shows a much higher permeability to organic compounds compared to water [22].

The high sensitivity of MIMS allows detection limits in the order of low ppb's [23] with no need for preconcentration steps. The relatively short response time and

* Author for correspondence.

the possibility of simultaneous analysis of various components and continuous monitoring allow on-line, *in-situ* [24], *in-vivo* [25] analysis and kinetic experiments [26–31].

In this work the potential of the MIMS (Membrane Introduction Mass Spectrometry) technique for studying inclusion processes is demonstrated. From the MIMS experiments not only equilibrium thermodynamic information can be obtained, but also a detailed kinetic description of the inclusion phenomenon by continuous monitoring of the concentration of the free guest in solution. The experiment is based on the fact that only small organic molecules free in aqueous solution can be detected by MIMS. On the other hand, MIMS is not sensitive to cyclodextrins nor to their inclusion compounds due to their large volume and polarity which make the permeation through the silicone membrane very difficult (Figure 2). Therefore, as the inclusion compound is formed the concentration of free organic molecules in solution decreases, which can be continuously monitored by MIMS.

Experimental

Experimental section

The chemicals were obtained from Aldrich and Merck and were used without further purification. The aqueous solution of the organic compounds prepared with Millipore MilliQ water were sonicated for 1 h and stirred overnight prior to use.

The experiments were carried out on a HP 5989A II mass spectrometer equipped with a membrane probe that has been described in detail elsewhere [2]. Mass spectra were obtained by electron ionization at 70 eV. The most intense fragment of the mass spectrum was used for monitoring each compound [benzene (m/z 78), chlorobenzene (m/z 112), toluene (m/z 91), cyclohexane (m/z 84), and ferrocene (m/z 186)]

In a typical run, the membrane probe was connected to the mass spectrometer as illustrated in Figure 1 and 0.088 mmol (or 0.176 or 0.352 mmol) of the cyclodextrin were added to 100 mL of a benzene solution (17 mg.L^{-1} , 0.022 mmol.L^{-1}) and the m/z 78 signal continuously monitored. The solution was vigorously stirred and kept at 25 $\pm$ 1 °C with a temperature-controlled recirculating bath in a well-closed vessel to avoid evaporation of the organic molecule from the aqueous solution.

The ferrocene-CD complex was prepared [32] from finely ground crystals of ferrocene (0.372 g, 2 mmol) added to an aqueous solution of β-CD (0.657 g, 0.5 mmol) at 60 °C with stirring. The product was extensively washed with THF to remove the uncomplexed ferrocene and extensively washed with water to remove any free β-CD.

Results and discussion

MIMS response to organic molecules in aqueous medium

The linearity and response time of the MIMS signal was investigated for each organic compound using aqueous solution at different concentrations. A typical calibration curve is shown in Figure 3 where the ion of m/z 78 was monitored for aqueous solutions of benzene. Excellent linearity and reproducibility were observed over the concentration range studied.

For the kinetic measurements another important effect studied was the MIMS response time which depends on the diffusion and permeation of the organics from solution through the membrane. Experiments with solutions of benzene and chlorobenzene showed a response time (fall time) of ca. 30 s for the signal intensities to decrease 60%. This response time can be considered negligible compared with the inclusion experiments that are much slower taking typically 40 min for the signal intensities to decrease 15–20%.

Benzene inclusion into different cyclodextrins

The inclusion profiles of benzene in α-, β-hydroxypropyl- and γ-cyclodextrins in aqueous medium are shown in Figure 4.

The benzene concentration in the aqueous phase in the beginning of the experiment is constant, producing a stable m/z 78 signal. As the CD is added to the reaction vessel the m/z 78 signal immediately decreases showing that the concentration of the free benzene molecule in solution decreased due to the inclusion process. Typically after 80 min the MIMS signal stabilizes showing that the system reached equilibrium. From the MIMS signal at the equilibrium it is possible to calculate the amount of the benzene included into the cyclodextrin and therefore the equilibrium constant for the inclusion process.

These inclusion experiments have been carried out at low concentrations of both benzene and CD in order to guarantee the complete solubilization, avoiding solid compounds formation and favoring the 1:1 complex.

The equilibrium constant for 1:1 inclusion is defined by Equation 1.

$$\begin{aligned} K &= \mathbf{a}_{R-CD} \times [\text{G-CD}]/\{\mathbf{a}_G \times [\text{G}] \times \mathbf{a}_{CD} \times [\text{CD}]\} \\ &\approx [\text{G-CD}]/[\text{G}] \times [\text{CD}], \end{aligned} \quad (1)$$

where **a** is the activity coefficient of each specie, G = guest, G-CD = inclusion compound. Since the CD and the organic molecules studied in this work can be considered non-electrolytes, the activity coefficient is close to unity at low concentration. Therefore, the equilibrium constant is calculated by the concentration of the different species in the equilibrium, which can be directly determined by the MIMS signal intensity. The obtained equilibrium constant for the complexation of benzene with the different CDs is shown in Table 1.

From Table 1 the following stability order for the benzene-cyclodextrin complexes can be observed: β-CD $\approx$

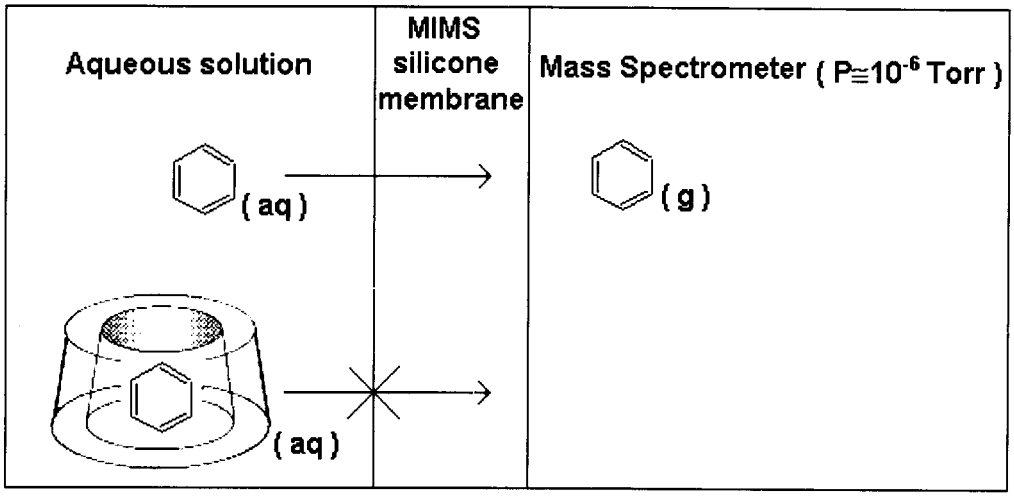

Figure 2. Schematic representation of the permeation processes of free benzene and its cyclodextrin complex in a MIMS system.

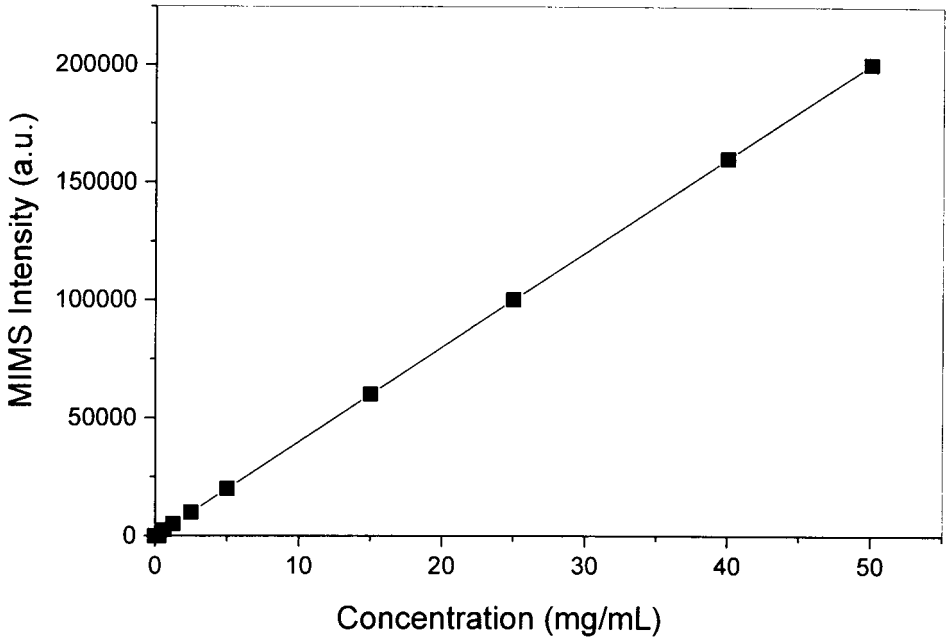

Figure 3. Benzene MIMS calibration curve.

Table 1. Calculated equilibrium constant for the benzene-CD complexes formation

Cyclodextrin	K (M^{-1})
β-CD	404 ± 20
β-hydroxypropyl-CD	395
α-CD	335
γ- CD	210

β-hydroxypropyl-CD > α-CD > γ-CD, with log K values of 2.68, 2.60, 2.52, 2.32. The same stability order was obtained by vapor pressure studies [19], showing that intermediate cavity size, i.e., β-CD, produces more stable inclusion compounds. Similar K values were obtained for the β-CD and β-HO-propyl-CD complexes suggesting that the inclusion of benzene depends mainly on the cavity and it is not significantly affected by the presence of the HO-propyl group. The obtained equilibrium constant, log K_β = 2.68 and log $K_{\beta-HO-propyl}$ = 2.60, are similar to those reported in the literature (log K_β = 2.28) [19]. For the other cyclodextrins lower log K values, i.e., 2.52 and 2.32, for α- and γ- CD, respectively, were observed for the 1:1 benzene-CD complex formation [3].

Kinetic analysis of the benzene inclusion

For the kinetic treatment a pseudo-first order process was considered with relation to the benzene concentration as the inclusion experiments were carried out with different CD:benzene ratios (4:1; 8:1 and 16:1):

$$\text{CD} + \text{benzene} \rightarrow [\text{CD-benzene}]$$
$$\text{inclusion rate} = \boldsymbol{k}.[\text{benzene}] \quad [1]$$

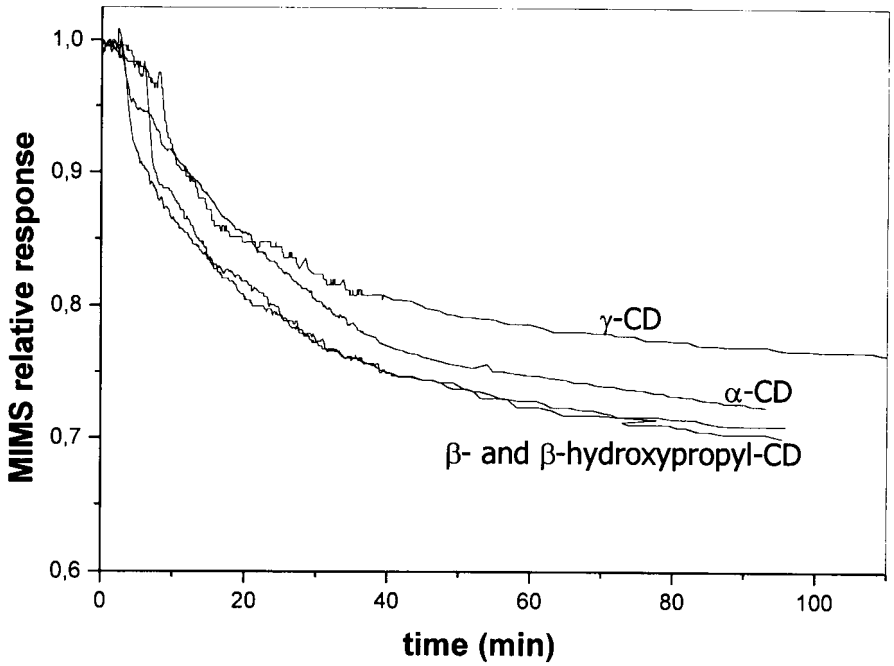

Figure 4. Inclusion MIMS profiles of benzene in α-, β-, β-hydroxypropyl- and γ-cyclodextrins in aqueous medium.

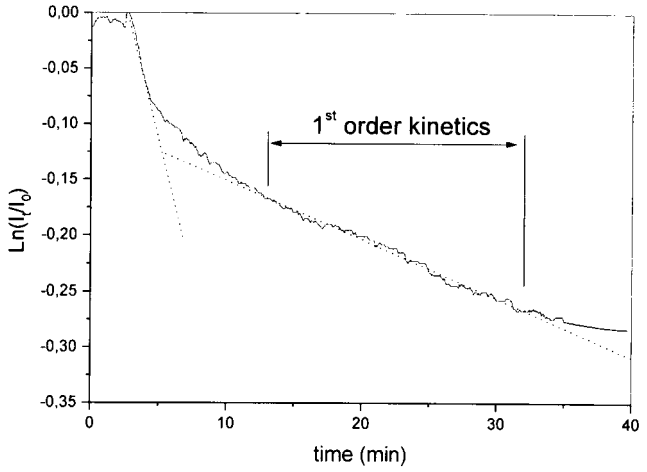

Figure 5. First order kinetic plot for the inclusion of benzene into β-CD.

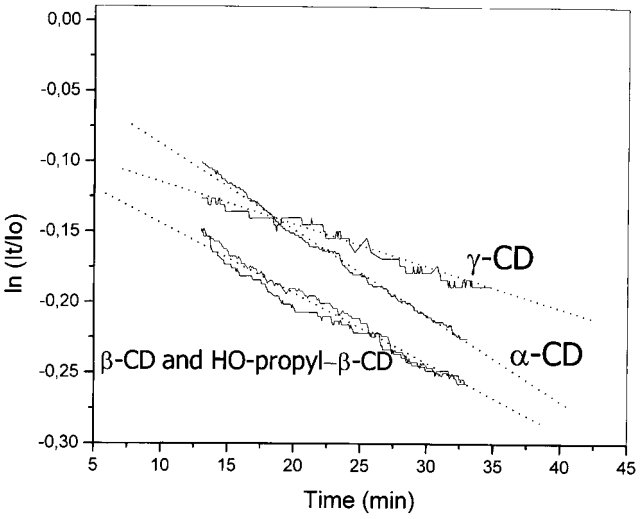

Figure 6. First order ln (I_t/I_0) *versus* time kinetic plots.

From the MIMS profile a first-order kinetic plot ln (I_t/I_0) *versus* time can be obtained, where I_t and I_0 are the MIMS intensities, which are directly related to the benzene concentration, at the time t and $t = 0$. A typical plot is displayed in Figure 5.

It can be observed from Figure 4 that upon the addition of CD the benzene concentration strongly decreases in the first 3 min. This is probably related to the high concentration of CD produced momentarily by the solid CD added to the reaction flask, which favors the benzene inclusion. After the CD concentration homogenizes throughout the system, the process will apparently enter in a first-order kinetic regime, producing a linear ln (I_t/I_0) *versus* time plot from ca. 13 to 33 min (Figure 5). No significant difference on the kinetics was observed for the different CD concentrations suggesting an excess and therefore a zeroth order dependence on the cyclodextrin concentration. After 33 min a deviation was observed, likely related to the significant decrease in the CD concentration up to that point. Therefore, for the kinetic analysis of the inclusion process the data obtained between 13 and 33 min was used (Figure 6)

From Figure 6 the following rate constants were obtained: $\boldsymbol{k}_{\beta\text{-CD}}$ = 0,0050; $\boldsymbol{k}_{\beta\text{-HO-propyl-CD}}$ = 0.0050, $\boldsymbol{k}_{\alpha\text{-CD}}$ = 0.0053 and $\boldsymbol{k}_{\gamma\text{-CD}}$ = 0.0030 min^{-1}. The same $\boldsymbol{k}$ values for benzene inclusion in β-CD and β-HO-propyl-CD were obtained, suggesting that the HO-propyl group does not affect the inclusion rate. On the other hand, the rates observed for α-CD and γ-CD were significantly different with the following order: α-CD > β-CD $\approx$ β-HO-propyl-CD $\gg$ γ-CD. The benzene inclusion rate in γ-CD is much smaller compared to the inclusion into other CDs whereas the α-CD shows the highest rate.

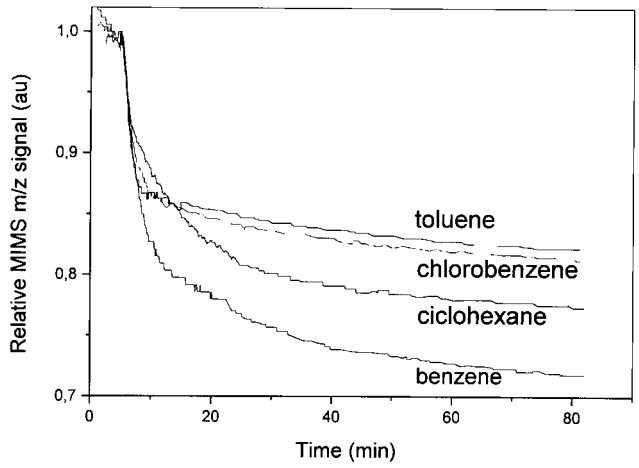

Figure 7. MIMS inclusion profile of benzene, cyclohexane, chlorobenzene and toluene in β-CD.

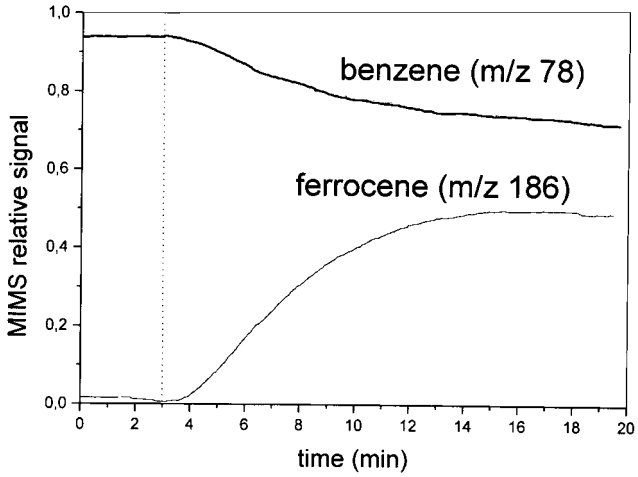

Figure 8. MIMS m/z 78 and 186 profiles when ferrocene-β-CD complex was added to an aqueous benzene solution.

The inclusion-rate-determining step depends mainly on two factors: (i) the diffusion of the guest and CD molecules in water and (ii) the collapse of the hydrate shell surrounding both molecules, CD and guest, prior to the inclusion step [1, 2, 33].

Diffusion coefficients in aqueous solutions at 40 °C reported for α-, β- and γ-CD are 3.443, 3.224, 3.000 $\times$ 10^5 D/cm^2 s^{-1}, respectively [1], which can explain, at least partially, the higher inclusion rate of benzene in α-CD. The small cavity diameters of α-CD of 4.7–5.3 Å, compared to β-CD (6.0-6.5 Å) and γ-CD (7.5–8.3 Å) [1]. should also be considered. The small cavity of α-CD can accommodate a small number of water molecules compared to β-CD and especially to γ-CD. This probably facilitates the process of the water molecules exit from the CD cavity allowing the entrance of the benzene guest molecule.

Inclusion of other organic volatile molecules in β-CD

The inclusion of other organic volatile molecules such as toluene, chlorobenzene and cyclohexane was also studied. The MIMS inclusion profiles are shown in Figure 7.

From the profiles in Figure 7 the following inclusion order can be observed: benzene > cyclohexane > toluene $\approx$ chlorobenzene. The inclusion profiles of toluene and chlorobenzene are similar, suggesting that the nature of the substituent in this case, –CH_3 and –Cl, does not significantly affect the complex formation. These results also show that the inclusion of benzene in solution is more favorable compared to chlorobenzene and toluene, suggesting that when benzene is substituted its inclusion becomes more difficult. Cyclohexane also showed a less stable inclusion complex compared to benzene.

Ferrocene exchange by benzene in a β-CD complex in aqueous medium

In this experiment a ferrocene-β-CD complex was added to an aqueous solution of benzene and the signals m/z 186 for ferrocene and m/z 78 for benzene monitored (Figure 8).

Experiments with the ferrocene-β-CD complex added to pure water showed identical profiles as displayed in Figure 8 (m/z 186), suggesting that the complex dissociates in solution to produce free ferrocene, which can be detected by MIMS. If the experiments are carried out in an aqueous benzene solution, it can be observed that as the ferrocene-β-CD complex is added the signal m/z 186 increases and simultaneously the signal m/z 78 decreases, suggesting the exchange ferrocene-benzene in the β-CD complex.

Kinetic measurements showed a rate constant for the [ferrocene-β-CD] complex dissociation of $\boldsymbol{k}$ = 1.60 min^{-1}, whereas for benzene inclusion (Equation 2) a much lower value of k = 0.03 min^{-1} was obtained. These results indicate that the presence of benzene has no effect on the ferrocene dissociation and that the process occurs by the dissociation (Equation 2) followed by the inclusion of benzene (Equation 3)

$$[\text{ferrocene-}\beta\text{-CD}] \leftrightarrow \text{ferrocene} + \beta\text{-CD} \qquad (2)$$

$$\beta\text{-CD} + \text{benzene} \leftrightarrow [\text{benzene-}\beta\text{-CD}] \qquad (3)$$

Conclusions

The MIMS technique has proved to have a great potential for studying inclusion processes in aqueous medium, showing several advantages over other methods. MIMS experiments allow a continuous monitoring, affording information on the equilibrium and especially on the kinetics of the inclusion process, which cannot be easily obtained by other techniques. MIMS is very sensitive, allowing measurements at very low concentrations, typically in sub-ppm or ppb range. MIMS can also be used to study the dissociation of CD complexes and the competitive complex formation by different guest molecules. MIMS is suitable for studying the inclusion of small apolar organic molecules. On the other hand, the technique might show some limitation for polar, especially high molecular weight molecules.

Acknowledgements

The authors are grateful for the support from FAPEMIG, CNPq and CAPES.

References

1. J. Szejtli: *Cyclodextrin Technology*, Kluwer Academic Publishers, Dordrecht (1988). 1a. R.D. Sinisterra, O.L. Alves, R. Najjar, P.S. Santos, A.L.C Silva, and C.A.A. Carvalho: *J. Incl. Phenom. Mol. Recogn. Chem.* **22**, 91 (1995).
2. R.J. Bergeron: *Inclusion Compounds*. In J.L. Atwood, J.E.D. Davies and D.D. MacNicol (eds.), Academic Press, London (1984), Vol 3, pp. 231.
3. MV. Rekharsky and Y. Inoue: *Chem. Rev.* **98**, 1875 (1998).
4. K.A. Connors: *Chem. Rev.* **97**, 1325 (1997).
5. M.V. Rekharsky and Y. Inoue: *Chem. Rev.* **98**, 1875 (1998).
6. R.I. Gelb, L.M. Schwartz, and D.A. Laufer: *J. Chem. Soc., Perkin Trans.* **2**, 15 (1984).
7. G. Nelson, G. Patonay, and I.M. Warner: *J. Inclusion Phenom.* **6**, 277 (1988).
8. H. Mwakibete, D.M. Bloor, and E. Wyn Jones: *J. Inclusion Phenom.* **10**, 497 (1991).
9. K. Kano, Y. Kato, M. Kodera: *J. Chem. Soc., Perkin Trans.* **2**, 1211 (1996).
10. L.D. Hall and T.K. Lim: *J. Am. Chem. Soc.* **106**, 1858 (1984).
11. L.A. Blyshak, I.M. Warner, and G. Patonay: *Anal. Chim. Acta* **232**, 239 (1990).
12. K. Ito, K. Kikuchi, N. Okazaki, and S. Kobayashi: *Argric. Biol. Chem.* **52**, 2763 (1988).
13. R.I. Gelb, L.M. Schawartz, R.F. Johnson, and D.A. Laufer: *J. Am. Chem. Soc.* **101**, 1869 (1979).
14. K. Harata, K. Tsuda, K. Uekama, M. Otagiri, and F. Hirayama: *J. Incl. Phenom.* **6**, 135 (1988).
15. N.J. Turro, T. Okubo, and C. Chung: *J. Am. Chem. Soc.* **104**, 1789 (1982).
16. T.S. Straub and M.L. Blender: *J. Am. Chem. Soc.* **94**, 8881 (1972).
17. F.A. Menard, M.G. Dedhyia, and C.T. Rhodes: *Pharm. Acta Helv.* **63**, 303 (1988).
18. H. Jin and L.F. Zhang: *Acta Chim. Sin.* **45**, 159 (1987).
19. E.E. Tucker and S.D. Christian: *J. Am. Chem. Soc.* **106**, 106 (1984).
20. Y. Kotake and E.G. Janzen: *J. Am. Chem. Soc.* **111**, 7323 (1989).
21. N. Ono, F. Hirayama, H. Arima, and K. Uekama: *Eur. J. Pharm. Sci.* **8**, 133 (1999).
22. T. Kotiaho, F. R. Lauritsen, T. K. Choudhury, and R. G. Cooks: *Anal. Chem.* **63**, 875A (1991).
23. (a) S. Bauer and D. Solyom: *Anal. Chem.* **66**, 4422 (1994). (b) M. Leth and F.R. Lauritsen: *Rapid Commun. Mass Spectrom.* **9**, 591 (1995). (c) M.H. Soni, S. Bauer, J.W. Amy, P. Wong, and R.G. Cooks: *Anal. Chem.* **67**, 1409 (1995). (d) M.H. Soni and R.G. Cooks: *Anal. Chem.* **66**, 2488 (1994). (e) A.A. Rivlin: *Rapid Commun. Mass Spectrom.* **9**, 397 (1995). (f) J.A. Shoemaker, T.A. Bellar, J.W. Eichelberger, and W.L. Budde: *J. Chromatogr. Sci.* **31**, 279 (1993). (g) M.A. Mendes, R.S. Pimpim, T. Kotiaho, and M.N. Eberlin: *Anal. Chem.* **68**, 3502 (1996).
24. C. Xu, J.S. Patrick, and R.G. Cooks: *Anal. Chem.* **67**, 724 (1995).
25. J.S. Brodbelt, R.G. Cooks, J.C. Tou, J. Kallos, and M.D. Drysga: *Anal. Chem.* **57**, 724 (1987).
26. M.J. Hayward, T. Kotiaho, A.K. Lister, R.G. Cooks, G.D. Austin, and R. Narayan: *Anal. Chem.* **62**, 1796 (1990).
27. F.R. Lauritsen and S. Gylling: *Anal. Chem.* **67**, 1418 (1995).
28. (a) R.A. Ketola, V.T. Virkki, M. Ojala, V. Komppa, and T. Kotiaho: *Talanta* **44**(3), 373 (1997). (b) M. Ojala, R.A. Ketola, T. Mansikka, T. Kotiaho, and R. Kostiainen: *J. High Resolut. Chromatogr.* **20**(3), 165 (1997).
29. R.V.R.A. Rios, L.L. Rocha, T. Giuliano, R. Augusti, and R. M. Lago: *J. Mass Spec.* **35**, 618–624 (2000).
30. R. Augusti, A.O. Dias, L.L. Rocha, and R.M. Lago: *J. Phys. Chem. A* **102**, 10723 (1998).
31. Ana C.B. Silva, Ilza Dalmázio, R. Augusti, and R.M. Lago: *J. Chem. Soc., Faraday Trans. (PCCP)* **10**, 2501–2504 (1999).
32. A. Harada, Y. Hu, Shyoko Yamamoto, and S. Takahashi: *J. Chem. Soc. Dalton Trans.* **729**, 23 (1988).
33. F. Cramer, W. Saenger, and H.C. Spatz: *J. Am. Chem. Soc.* **14**, 89 (1967).