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Inclusion Complexes of Atrazine with α-, β- and γ-Cyclodextrins. Evidence by Polarographic Kinetic Currents

L. POSPÍŠIL* and R. TRSKOVÁ
J. Heyrovský Institute of Physical Chemistry, Academy of Sciences of the Czech Republic, Dolejškova 3, 18223 Prague, Czech Republic

M.P. COLOMBINI and R. FUOCO
Dipartimento di Chimica e Chimica Industriale, Università degli Studi, via Risorgimento 35, 56126 Pisa, Italy

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Abstract. The reduction of atrazine in acidic aqueous media on mercury electrodes proceeds only after the protonation reaction. In fact, the efficiency of the reduction process is very low at pHs greater than 4. However, the addition of cyclodextrins (CDs) to neutral aqueous solutions of atrazine yields a kinetically controlled polarographic reduction wave, whose limiting current depends on the size of the CD cavity, and increases with the concentration of the CD itself. In particular, the size of the increase follows the order β-CD $<$ γ-CD $<$ α-CD for the same CD concentration. The half-wave potential shifts toward more negative values when the concentration of CDs increases. These findings lead to the conclusion that atrazine and CDs form an inclusion complex, whose stability constant we have estimated, and also that atrazine undergoes protonation facilitated by complexation with CDs. The stability constants of 1:1 complexes evaluated from polarographic data in 0.1M-KCl and at neutral pH for α-CD, β-CD and γ-CD are 4900, 1970 and 19000, respectively. The formation of the inclusion complex was indirectly confirmed by UV-Vis measurements in the presence of methylred and phenolphthalein, which both compete with atrazine in the formation of the corresponding inclusion compounds with CDs.

Key words: cyclodextrins, atrazine, inclusion complex, electrochemistry, preceding reaction, stability constants, kinetic currents.

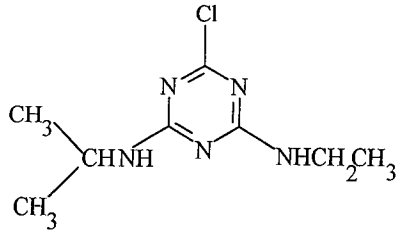
1. Introduction

The complexation of pesticides with polysaccharide-like molecules may be one of many possible ways of their harmful accumulation *in biota*. Our previous study on difenzoquat, a cationic fungicide, [1,2] utilized polarography, conductimetry and fluorescence techniques, and demonstrated its weak binding to cyclodextrins (CDs). CDs are macrocyclic glucose oligomers, which are able to bind a variety of guest molecules inside their torus-shaped cavity. The cyclodextrin molecule contains six, seven or eight glucose units linked by α-(1,4)bonds, and the respective notation is α-CD, β-CD and γ-CD. The rims of the CD skeleton contain the primary

* Author for correspondence.

hydroxy groups, O(6)H, on one side, and the secondary hydroxy groups, O(2)H and O(3)H, on the opposite side of the torus [3]. The inner cavity has a hydrophobic character. The different number of OH groups results in a dipole moment for the CD molecule. The nature of the driving force for the inclusion of a guest molecule from its aqueous solution in the inner cavity has not yet been fully resolved. The complexation is generally explained in terms of the predominant hydrophobic host-guest interactions driven by entropy effects in the displacement of the guest inner water molecules by the host compound. However, the proven inclusion of a series of substituted aromatic molecules indicates that dipolar forces may be predominant and hence the complexation could also be enthalpy driven [4,5].

The present study reports the interaction of atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine)



with α-CD, β-CD and γ-CD in neutral solutions. Atrazine has a hydrophobic character, and is only slightly soluble in water. It is also readily adsorbed at interfaces, and undergoes redox and photo-degradation processes, which yield mainly the corresponding dechloro- and desethyl-atrazines.* The essential role of the protonation reaction of atrazine in reduction pathways was previously described [6,7], and unprotonated atrazine was proved to be redox inactive. The present paper highlights the fact that atrazine becomes electroactive in the presence of cyclodextrins which provide the necessary protonization environment at neutral pH by the formation of an inclusion complex. Redox activity induced by the complexation reaction is used here for the determination of stability constants and kinetic parameters. Electrochemical methods were used to determine stability constants, K_S, of inclusion complexes [8] by measuring the change of diffusion coefficients when increasing CD concentration in solution. The present study deals with kinetically controlled polarographic currents and the evaluation of K_S is based on the shift of the reduction potentials.

2. Experimental

The electrochemical measurements were made using a laboratory built electrochemical system consisting of a fast rise-time potentiostat interfaced to a personal computer via the IEEE-interface card PcLab model 748. A three-electrode electro-

* This atypical nomenclature, used in environmental literature, comes from the parent pesticides and indicates a substituent that has been cleaved and replaced by the hydrogen atom.

chemical cell was used with a Ag/AgCl in 1M-LiCl reference electrode separated from the test solution by a salt bridge. The working electrode was either a valve operated static mercury electrode SMDE2 (Laboratorní Přístroje, Prague) or a dropping mercury electrode (DME) with a mechanical drop-time regulator. The auxiliary electrode was a platinum net. Oxygen was removed from the solution by flushing with a stream of argon. Atrazine with a certificate of purity was purchased in a crystalline form from Ehrenstorfer, Augsburg (Germany). The other reagents used as supporting electrolytes were of reagent grade. Water was distilled twice from an all quartz still or was de-ionized by means of an Elgastat UHQ purifier. Due to the low solubility of triazines in water, 9.4×10^{-5} M aqueous stock solutions of atrazine were prepared by sonification of the corresponding amount in 500 mL of water. Samples were prepared by dissolving a suitable quantity of CD and/or KCl in 10 mL of the atrazine stock solution. KCl was used as a supporting electrolyte for polarography because it complexes negligibly with CDs. Buffers were not used in order to avoid competitive complexation with CD. Spectral measurements were made using a UV-Vis spectrometer, Cary 4 (Varian, USA). The competitive inclusion of a dye (methyl red or phenolphthalein) and atrazine [9] was investigated by performing the titration of 3 mL solutions in 1 cm wide quartz cuvettes. In order to avoid the contamination of dye solutions with atmospheric carbon dioxide, samples were prepared from boiled water in an inert atmosphere of argon using the Schlenck technique.

3. Results and Discussion

Atrazine in acidic aqueous media at the mercury electrode undergoes electrochemical reduction [6,7] and shows an irreversible reduction wave at about -1 V for concentrations above 0.1 mM and pH lower than 2. The reduction process involves the uptake of two electrons and two protons. The limiting current i_{lim} and the half-wave potential $\mathrm{E}_{1/2}$ of the polarographic reduction wave, strongly depends on the pH of the solution. In particular, $\mathrm{E}_{1/2}$ shifts towards more negative values at increasing pH. The limiting current is diffusion controlled only at pHs lower than 2, while it diminishes at decreasing acidity due to the kinetic control of the preceding protonation reaction [6]. The irreversible character of the reduction process of atrazine is caused by follow-up chemical reactions leading to final reduction products. These reactions involve predominantly the cleavage of the C-Cl bond and to a much lesser extent the cleavage of the ethyl group. At 1×10^{-5} M concentration level of atrazine the separation of a single two-electron reduction wave in two strongly overlapped waves can be observed in the acidic media. Different protonation sites of the atrazine molecule (three nitrogens of the triazine ring and two amino groups) are responsible for this separation [7]. The calculation of electron densities [10] suggests equivalent proton affinity of nitrogen atoms of the triazine ring, while a 37% lower electron density on -NH- substituents indicates an increased proton affinity.

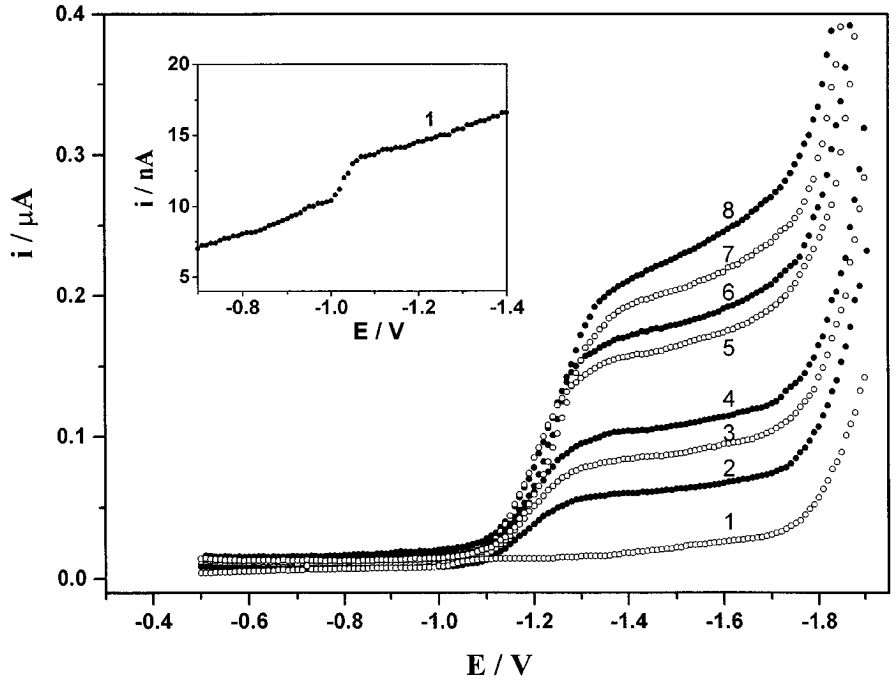


Figure 1. Polarographic kinetic currents of 92.7 mM atrazine in neutral aqueous 0.1 M-KCl at various concentrations of α-CD: 1. 0; 2. 0.8 mM; 3. 1.63 mM; 4. 1.93 mM; 5. 2.33 mM; 6. 3.2 mM; 7. 3.6 mM and 8. 6.2 mM. The inset is an expansion of curve 1.

In neutral aqueous solutions of potassium chloride a very small faradaic current (typically 5–10 nA) can be detected (see the inset in Figure 1), though it is generally unnoticed in a routine experiment. This current substantially increases as the solution increases in acidity (Figure 2). A similar effect, i.e. the increase in the reduction current, is observed when one of the cyclodextrins is present in the neutral solution, and the efficiency follows the order α-CD $>$ γ-CD $>$ β-CD. In particular, a well defined reduction wave is obtained at CD concentration above 2 mM, whose limiting current is smaller than the one corresponding to a diffusion controlled heterogeneous reduction. Figure 1 shows a few dc polarograms obtained at various concentrations of α-CD, which gives the most pronounced effect. The addition of γ-CD enhances less efficiently the wave of atrazine, whereas the effect of β-CD is by far the lowest. The kinetic control of limiting currents was confirmed by their dependence on the time constant of the electrochemical experiment [11]. The comparison of limiting kinetic currents for all three CDs at a constant concentration of atrazine is given in Figure 3.

All these polarographic findings lead to the conclusion that the interaction between atrazine and CD changes the redox inactive neutral molecule of atrazine to a redox active form, that is the protonated form. Thus, either the presence of free protons or the presence of CDs is required in order to observe the reduction wave of

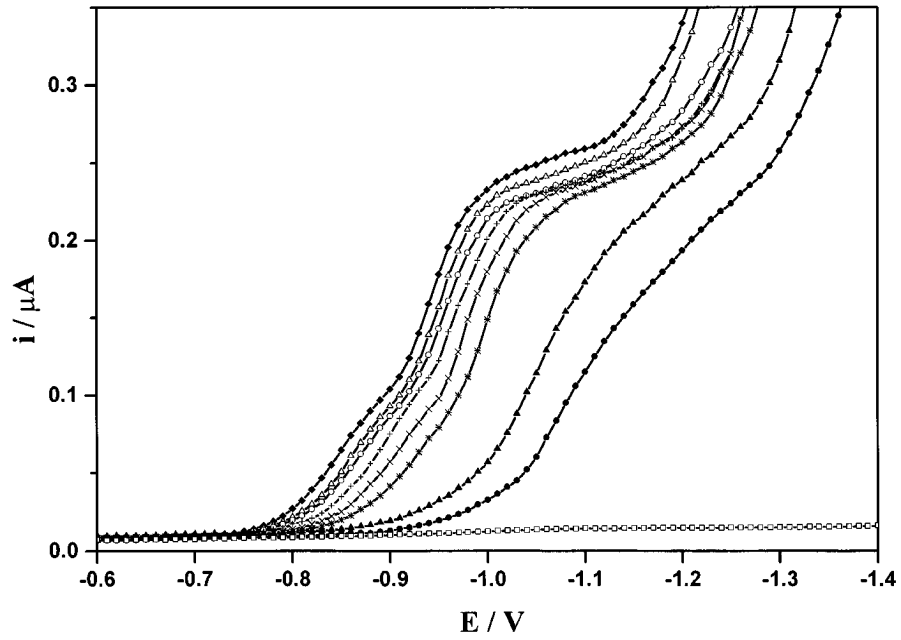


Figure 2. Polarographic kinetic currents of 92.7 mM atrazine in aqueous 0.1 M-KCl at various concentrations of HCl: 0 (□), 0.2 mM (●), 0.4 mM (▲), 2.4 mM (∗), 4.4 mM (×), 6.4 mM (+), 10.4 mM (○), 24 mM (△) and 36 mM (⧫).

atrazine. We assume that an atrazine-cyclodextrin inclusion complex is formed and that the hydrogen bond interaction provides the protonation of the guest molecule. The kinetic character of limiting polarographic currents and their increase with an increasing concentration of CD indicates that the overall process is controlled by the complex formation.

The $E_{1/2}$ of the reduction wave relating to the inclusion complex [atrazine.γ-CD] is almost 200 mV more negative than that of the small reduction wave of atrazine obtained in a neutral solution of KCl. The shift of $E_{1/2}$ with the 'ligand' concentration is another proof that the formation of an inclusion complex is involved (Figure 4). In fact, the half-wave potential of atrazine shifts toward less negative values at lower pH, i.e. in the opposite direction (Figure 2). The determination of stability constants from the shift of the electrochemical half-wave potentials is a standard procedure in the case of the reversible redox reduction of free species in equilibrium with a ligand and the relevant complex [12,13].

The wave shape of atrazine has a reversible character at low concentrations in neutral solutions (the log-plot slope is 30 mV). However, the log-plot analysis of polarographic waves obtained in the presence of CD, gives a typical value of the slope of about 97 mV. This shows quite a high degree of irreversibility of the reduction process. In addition, the limiting kinetic currents are controlled by a preceding complexation reaction. This entails evaluating the stability constants of

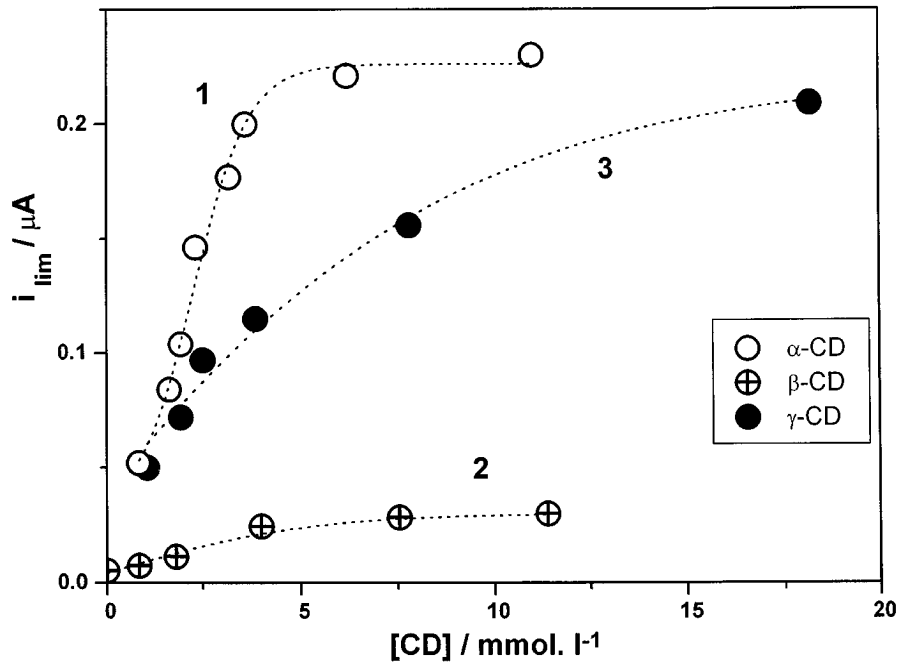


Figure 3. The dependence of polarographic kinetic limiting currents on the concentration of α-CD, β-CD and γ-CD in 92.7 mM atrazine in neutral aqueous 0.1 M-KCl.

a complex according to a more general theoretical treatment given by Nashihara and Matsuda [14]. They solved the dependence of $E_{1/2}$ on homogeneous and heterogeneous parameters for the reaction scheme 1

$$\mathrm{X} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{Ox} \underset{\mathrm{ne}}{\overset{k^0}{\rightleftharpoons}} \mathrm{Red} \tag{1}$$

X, Ox and Red, refer to the inactive, oxidized and reduced form, respectively. The Nakashira-Matsuda theory takes into account the rate of the preceding chemical reaction and also the finite rate of the electron transfer step. The rate constant of a monomolecular preceding reaction is k_1 and the equilibrium constant is K' = [X]/[Ox]. The finite charge transfer of n electrons is characterized by the transfer coefficient α, and the standard heterogeneous rate constant k^0. Denoting free atrazine as A, one can consider the following reaction sequence:

$$\mathrm{A} + \mathrm{CD} \underset{k}{\rightleftarrows} \begin{matrix} \mathrm{HO} \\ \vdots \quad \backslash \\ [\mathrm{A.\ CD}] \end{matrix} \xrightarrow[k^0]{+2\mathrm{e}} \mathrm{Products} \tag{2}$$

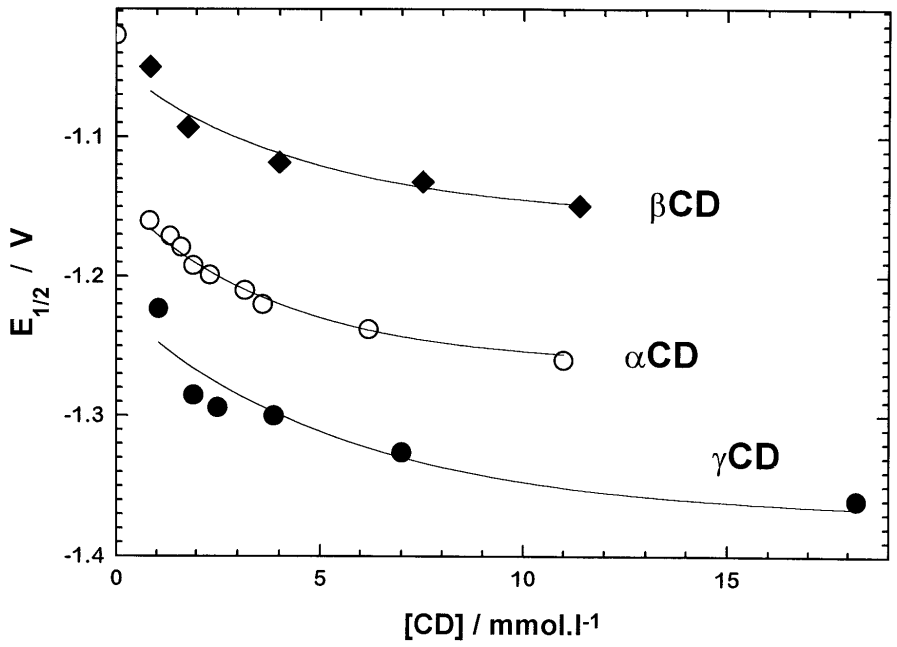


Figure 4. The dependence of the half-wave potential of polarographic waves of 92.7 mM atrazine on the concentration of cyclodextrins. Full curves indicate the best fit according to Equation (3) for parameters given in Table 1.

where the OH group symbolizes the hydroxy groups of CDs participating in H-bonds with the guest molecule. The expression for the shift of $E_{1/2}$ according to [14] is the following:

$$\Delta E_{1/2} = \frac{RT}{\alpha n F} \log\left[\sqrt{\frac{t}{D}}\left(\frac{k^0}{1+(K[CD])}\right)\right] + \frac{RT}{\alpha n F} \log\left[\frac{K[CD]\tanh(1.5k[CD](1+k[CD]t^{1/2}))}{\sqrt{k[CD](1+K[CD]t)}} + C_{irr}\right] \quad (3)$$

$$C_{irr} = 1.33 - \left[0.8\frac{\sqrt{k[CD](1+K[CD])t}}{k[CD]} + 2.0\right]^{-1} \quad (4)$$

where D is the diffusion coefficient, t is the time at which the current is measured, k is the bimolecular rate constant of the complex formation and K = [A][CD]/[A.CD] is the dissociation constant of the inclusion complex. Assuming that the first reaction in (2), which refers to the complex formation, is sufficiently mobile [13] under the conditions of the electron transfer control we fitted the shift of $E_{1/2}$ as a function of [CD] according to Equation (3). The relationship for $\Delta E_{1/2}$ is not sensitive for large values of k and hence these constants were more accurately estimated

Table I. Kinetic and equilibrium constants of inclusion complexes of atrazine and cyclodextrins estimated from polarographic data.

Cyclodextrin	k^0/cm.s^{-1*}	α^*	K_S/M^{-1*}	k/s$^{-1\dagger}$
α-CD	6.8×10^{-4}	0.32	4900	2.9×10^{8}
β-CD	6.3×10^{-3}	0.33	1970	5.9×10^{5}
γ-CD	4.0×10^{-4}	0.29	19000	2.1×10^{8}

* Calculated according to Nashihara-Matsuda Equation (3).
† Calculated according to Koutecký Equation (5).

from the limiting kinetic currents. Table I shows the values of these parameters for each CD considered, while full curves in Figure 4 refer to the goodness of fit indicated. The application of Equation (3) is justified by the negative shift of $E_{1/2}$ with increasing concentrations of all three CDs [13]. The evaluated stability constants ($K_S = 1/K$) increase in the following order β-CD $<$ α-CD $\ll$ γ-CD. The interpretation of the observed polarographic behavior has to resolve a controversy on the influence of CD concentration. The increasing concentration of CD has the following effects:

- shift of the complexation reaction in favor of $[A.CD]$, which causes a higher kinetic current;
- suppression of dissociation of the protonized form AH^+, that is the only redox active form, from the cavity of CD.

The dual effect of CD concentration could be better understood from examination of both the K_S value and the rate of the preceding chemical reaction, k, discussed below.

The rate of the preceding reaction was evaluated from polarographic limiting kinetic currents (Figure 3) using the Koutecký equation [15]. The ratio of the limiting kinetic current i_{lim} to limiting diffusion current i_{dif} yields the Koutecký function $F(\chi) = i_{lim}/i_{dif}$ for each CD concentration. For the reaction scheme (2) the variable χ is related to the rate and equilibrium constants by the following relationship:

$$\chi = \sqrt{\frac{12}{7}\frac{k[\mathrm{CD}]^2 t}{K}}. \tag{5}$$

The resulting values of $\chi^2 \sim k[\mathrm{CD}]^2$ are shown in Figure 5. The observed quadratic dependence on [CD] confirms the applicability of the suggested reaction mechanism given by Equation (2). An alternative mechanism based on a rate-determining dissociation of the redox-active form of atrazine should yield a linear dependence $\chi^2 \sim k$[CD], that was not experimentally found. The rate constants k calculated from the rate parameter χ are listed in Table I. The rates follow the order α-CD $>$ γ-CD $>$ β-CD, which does not match neither the change of the cavity size nor

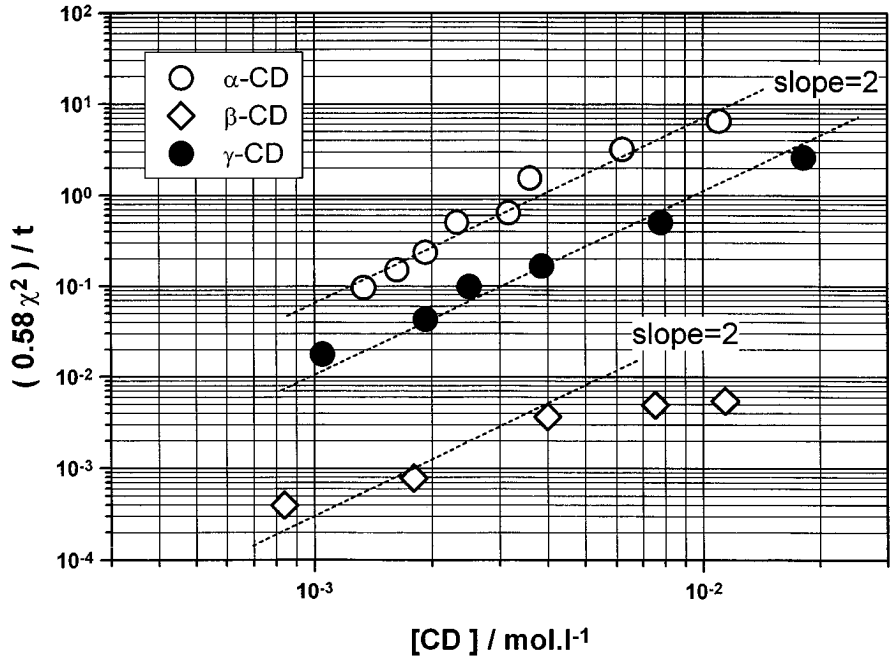


Figure 5. The rate of chemical reaction preceding the reduction of atrazine in the presence of cyclodextrins.

the observed order of stability constants. The explanation of trends found for K_S and k were sought in molecular models of three inclusion complexes. First, the geometry optimization [10] of the atrazine molecule was performed. The lowest energy isomer is shown in Figure 6 together with its molecular dimensions. The size of atrazine suggests that this guest molecule can be completely included in β-CD in a long-wise orientation, e.g. with the C(2) and C(5) atoms of the triazine ring parallel to the cavity axis. The molecular fitting procedure [10] was applied for finding the best configuration of atrazine inside the cavities of the two other CDs. A smaller α-CD will accommodate atrazine with the N-alkyl substituents inside and the Cl atom outside the cavity (Figure 7). The estimated interatomic distances between secondary OH oxygens and —NH— nitrogens of such a complex are favorable for the formation of hydrogen bonds. Since a part of the atrazine molecule is still outside the cavity it is likely that the guest is accessible for the electron transfer reaction at the electrode without dissociating from the cavity. This is in agreement with previous conclusions that the dissociation rate of the redox-active form is negligible. A similar molecular fitting procedure applied to the [atrazine.γ-CD] complex is not unambiguous. The guest can be located in the cavity of γ-CD in several orientations all of them featuring the triazine ring more or less perpendicular to the cavity axis. Figure 8 shows two placements, symmetric and asymmetric, of atrazine in γ-CD. Only the asymmetric configuration favors

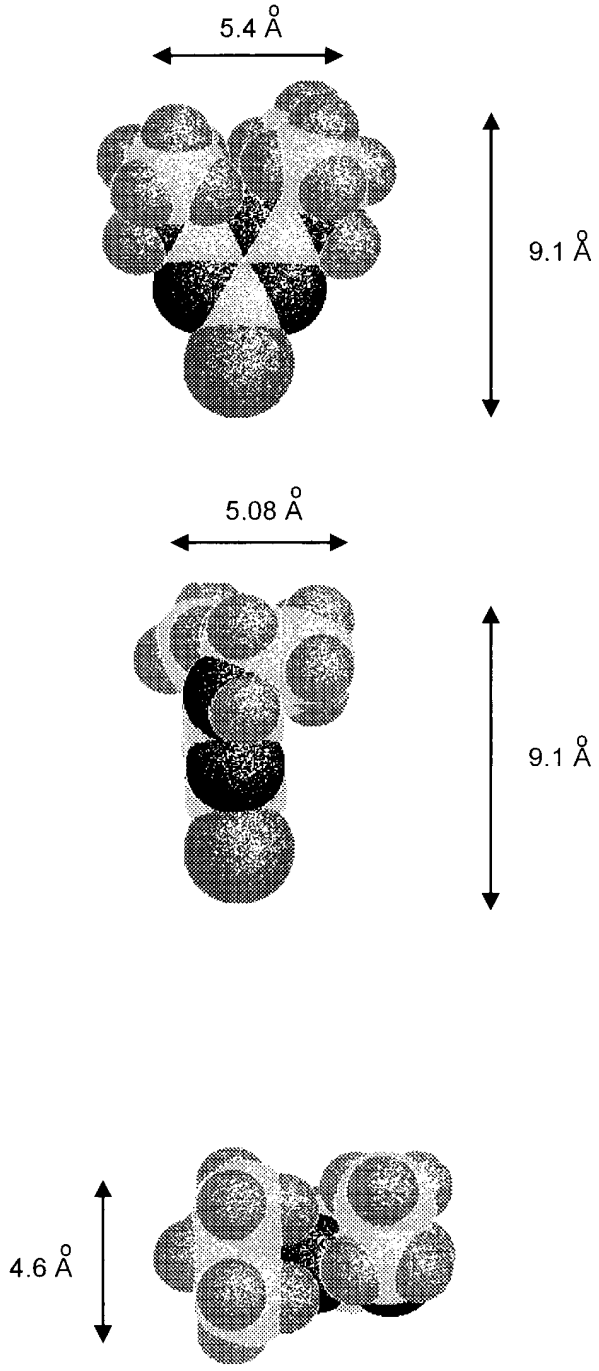


Figure 6. Molecular model of the atrazine molecule and its dimensions: face view (top), side view (middle) and top view (bottom).

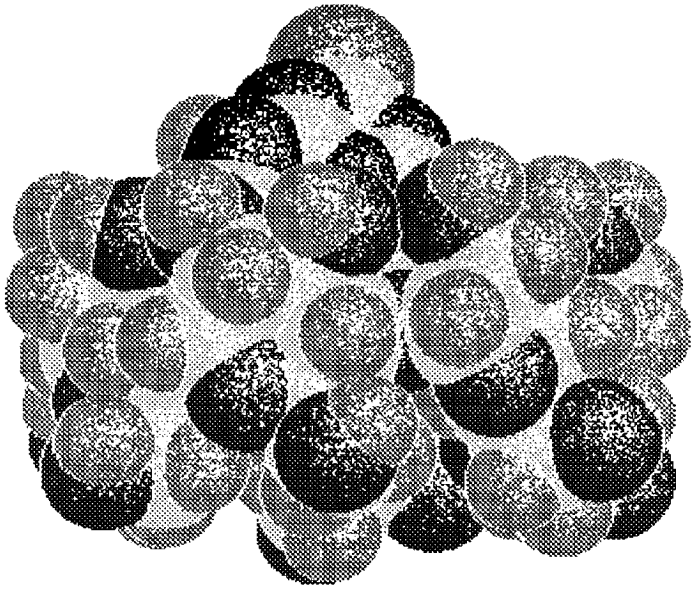


Figure 7. Molecular model of the inclusion complex of atrazine and α-CD.

the formation of hydrogen bonds and at the same time leaves the Cl atom slightly outside the cavity. Molecular modeling suggests that the experimentally observed redox activity requires not only an efficient hydrogen bond formation but also the spatial accessibility of the guest for a contact with the electrode. In view of this, one can explain the low efficiency of β-CD due to a very tight fit with the guest that has to dissociate from the cavity in a protonized form in order to participate in an electron transfer reaction. A higher concentration of β-CD may suppress such dissociation. The rate of the preceding chemical reaction (Figure 5) shows for higher concentrations of β-CD a leveling off, thus confirming the dual effect of this host on the equilibrium involved in the reaction sequence. The lower kinetic currents of γ-CD compared with α-CD very likely reflect the differences in the depth of protrusion of atrazine inside the cavity and the population of hydrogen bonds. Multiple configurations inside γ-CD certainly diminish the protonation rate.

The formation of an inclusion complex in the bulk of the solution is not evidenced by spectrometry: UV-Vis spectra of atrazine solutions in the absence and in the presence of a surplus of CD concentration are identical. This seems to be in contradiction with electrochemical findings. However, a similar striking difference between conductivity and spectral measurements of o-methyl red and α-CD was reported recently [16]. The conductivity clearly indicated the complexation while the spectroscopic data failed to detect the formation of an inclusion complex. In our case the absorption properties may be negligibly influenced by complexation and hence the spectra fail to reflect the complexation itself. The complex formation was at least indirectly confirmed by means of the competition between atrazine and a

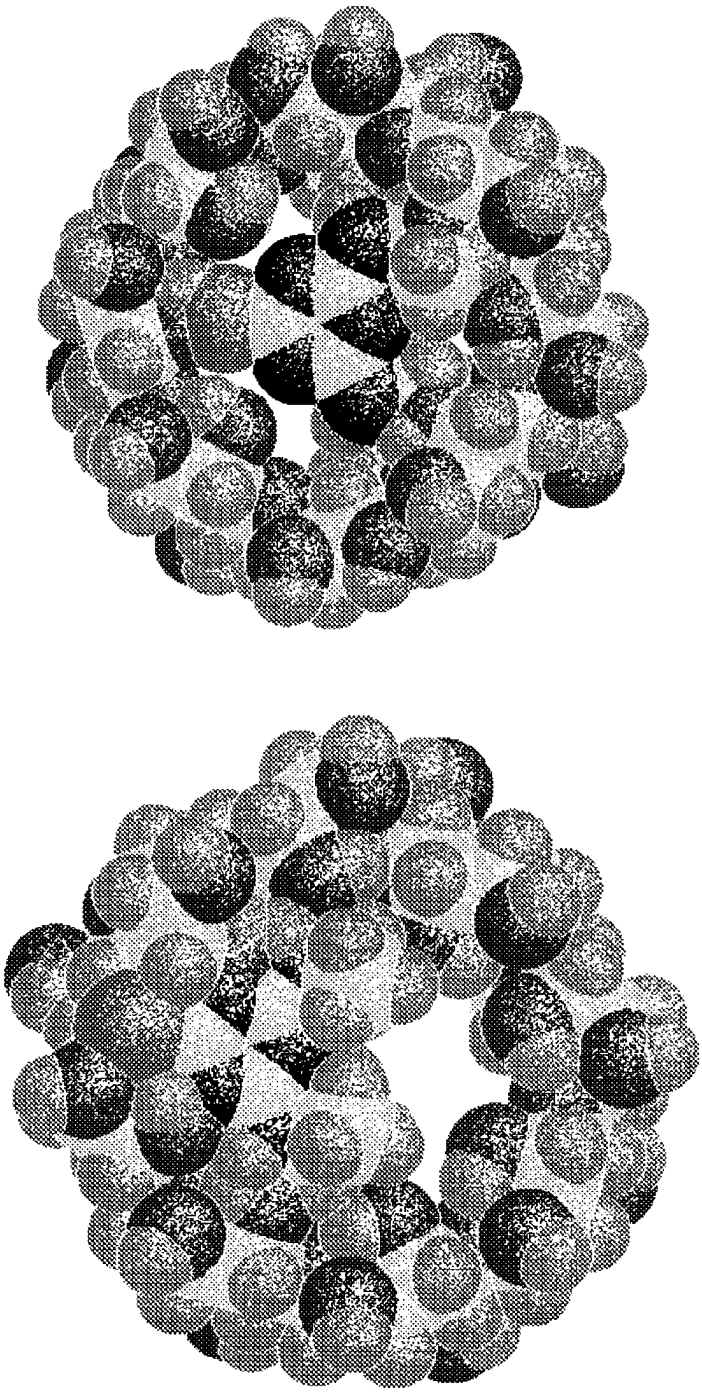


Figure 8. Molecular models of inclusion of atrazine into the cavity of γ-CD: symmetric position in the cavity (top) and asymmetric configuration favoring hydrogen bonding between secondary OH groups of CD and —NH— groups of atrazine (bottom).

dye in the complexation reaction with CD, which yields a change of the absorbance. A series of titrations of either phenolphtaline or methyl red solutions by the addition of γ-CD without and with the addition of a fixed amount of atrazine has been performed. A small difference between two data sets, that can be considered as a qualitative confirmation of complex formation, has been detected.

It seems reasonable to say that the electrode reaction itself drives the sequence of chemical equilibrium from free atrazine to reduced products. The consumption of the protonized form of atrazine at the electrode surface shifts the equilibrium in the reaction layer near the electrode and thus forces the complexation equilibrium to incorporate atrazine in the CD cavity. The surface activity of both cyclodextrins and atrazine can also have a favorable effect, and this is highlighted by the measurements of the double layer capacity [7,17]. Experimental elucidation of the complex structure still remains to be done. Our attempts to prepare the complexes in a solid form for structural analysis and NMR spectroscopy failed, mainly due to the low solubility of atrazine in water. Solvent mixtures result in a higher solubility of atrazine however, they introduce complications due to co-inclusion into the CD cavity.

The interaction of the OH groups of CDs with the guest molecule is quite common, though it has not yet been observed in redox reactions of inclusion complexes [18]. However, there are examples where the OH groups of CDs influence chemical processes. The stereoselectivity of reduction of phenylglyoxalic acid in the presence of CD is very likely to be related to the role of these OH groups [19]. Also, the cleavage of the acyl substituent of phenylacetates [20] proceeds with the participation of the secondary hydroxy groups of CD. The identification of the rate-determining step in the reaction scheme (2) or in any similar reaction pathway which considers both complexation and protonation with the same CD molecule is not trivial. The reaction order with respect to both CD concentration and pH cannot be determined independently because the only variable parameter, the cyclodextrin concentration, influences equally both the complexation and the ‘internal” proton transfer within the complex. Previous applications of electrochemical techniques to host-guest complexation in many cases used the change of mass transport rate towards the electrode that is connected with a much larger size of the resulting complex. The electrochemical approach presented here clearly shows that inclusion phenomena can induce the electrochemical activity of the guest and hence in such cases they can be monitored in a more direct way.

4. Conclusions

1. The herbicide atrazine forms inclusion complexes with CDs in neutral aqueous solutions.
2. The complex formation changes the redox-inactive neutral form of atrazine into a reducible protonated form yielding a well defined polarographic reduction current that is controlled by a preceding homogeneous chemical reaction.

3. The appropriate analysis of electrochemical data allows estimation of both the stability and rate constants of the reaction preceding the heterogeneous electron transfer.

Acknowledgments

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