Water–Oil Partition Profiling of Ionized Drug Molecules Using Cyclic Voltammetry and a 96-Well Microfilter Plate System

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Purpose. A new experimental set-up for studying partitioning of ionizable drugs at the interface between two immiscible electrolyte solutions (ITIES) by amperometry is presented. The method is quite general, as it can be applied to any charged drug molecule.

Methods. The procedure is based on 96-well microfilter plates with microporous filters to support 96 organic liquid membranes. The new methodology is first validated using a series of tetra-alkylammonium ions and subsequently used to construct the ion partition diagrams of 3,5-*N*,*N*-tetramethylaniline and 2,4-dinitrophenol. The lipophilicity of these drugs was examined by potentiometry and cyclic voltammetry in the NPOE/water system.

Results. Cyclic voltammetry resulted in potential-pH profiles of the studied drugs. When the aqueous phase pK_a is already known, the logP_{NPOE} of lipophilic drugs could be determined using a very little amount of solvents and drugs. The values of the partition coefficients for the neutral forms agree well with those obtained by potentiometry.

Conclusions. The procedure based on commercially available 96-well microfilter plates is shown to be useful for determining log*P* of ionized drugs in a rapid and efficient way.

KEY WORDS: lipophilicity; liquid membranes; cyclic voltammetry.

INTRODUCTION

The partition of neutral and ionic species between two immiscible liquid phases is an important parameter for the understanding of the physicochemical mechanisms of drugs *in vivo* (1–4). For neutral species, the partition depends only on the molecular structure of the solute and on the nature of the two solvents (5,6). For ionic species, an additional condition is imposed by the electroneutrality condition of the two phases (1).

Amperometry is a well-established technique that enables the determination of the Gibbs free energy of transfer of ions between immiscible electrolyte solutions by measurement of formal transfer potentials (7). In particular, cyclic voltammetry can be used to determine the standard partition coefficients of ionic forms of drug molecules (8–10), which is an important parameter in drug partition diagrams. The ionic partition diagram of a drug is a representation of the conditions corresponding to a predominance of a specific form of the solute (e.g., basic, neutral or acidic) as a function of the Galvani potential difference and the pH of the aqueous phase (1,11).

The partition coefficients of drugs vary according to the properties of the solvent used. Many studies have been carried out using water–octanol, water–alkane, and water–1,2-dichloroethane (DCE), respectively (12–15). However, biologic interpretations based on cyclic voltammetric data have only been achieved for the water–DCE system (1,16). Therefore, it would be profitable to expand this methodology to other solvent systems, such as nitrophenyloctyl ether (NPOE), which can be incorporated in a polyvinylidene difluoride membrane (17). We have to say that DCE as a solvent is attractive as suggested by previous solvatochromic analysis (18,19). It has been shown that for a series of small ions (17,20) that the Gibbs energy of ion transfer from water to DCE directly correlate with the Gibbs energy of ion transfer from water to NPOE. Therefore, NPOE can encode the solvation properties correctly. Furthermore, the physical characteristics of NPOE (viscosity, vapor pressure, and hydrophobicity) make it suitable in the present setup. Other solvent systems (octanol, DCE, etc.) are problematic to use in the current set-up, mainly due to fast solvent evaporation.

Recently, Faller *et al.* (15) reported a high-throughput method to measure the ability of drug molecules to diffuse from a donor to an acceptor compartment separated by a hexadecane liquid layer. From the intrinsic membrane permeabilities it is possible to determine alkane–water partition coefficients.

In this article, we describe a new electrochemical method based on a liquid layer immobilized between two aqueous compartments. By using 96-well microfilter plates, the optimization of the method for high-throughput applications is described. The methodology is validated using tetra-alkylammonium ions, fully investigated at the water–NPOE interfaces by many authors (17,20) and can therefore be used to calibrate the new methodology.

It is shown in two examples (i.e., using the ionizable drugs 3,5-*N*,*N*-tetramethylaniline [TMAN] and 2,4-dinitrophenol [DNPh]) that this method can be used to produce a large amount of reliable experimental data on potential pH profiles and water–NPOE partition coefficients in a short time.

EXPERIMENTAL

Chemicals

Hydrochloride acid, lithium chloride, tetrapropylammonium chloride, tetraethylammonium chloride, and tetramethylammonium chloride were purchased from Fluka (CH). The organic salts used were tetrabutylammonium tetrakis (4-chlorophenyl) borate (TBA^+TPBCl^-) and bis (triphenylphosphoranylidene) ammonium tetrakis (4-chlorophenyl) borate (BTPPA$^+$TPBCl–). These salts were obtained by metathesis of potassium tetrakis (4-chlorophenyl) borate (Lancaster) with tetrabutylammonium chloride (Fluka) and bis (triphenylphosphoranylidene) ammonium chloride (Fluka), respectively. The water was obtained from a Millipore Milli-Q 185 (CH) system and NPOE was purchased from Fluka (CH).

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The drugs, 3,5-N,N-tetramethylaniline and 2,4 - dinitrophenol, were from Fluka.

Cyclic voltammograms were recorded using a classic four-electrode potentiostat with feedback *IR* compensation. The cell diagrams of Cell I and Cell II are shown below:

Ag | AgCl | 5 mM TBACl | 5 mM TBATPBCl in NPOE

Reference plate PVDF membrane

10 mM LiCl
0.4 mM XCl or
0.8 mM TMAN | AgCl | Ag Cell I

Microwell

Ag | AgCl | 5 mM BTPPACl 10 mM LiCl | 5 mM BTPPATPBCl 1 mM DNPh in NPOE

Reference plate PVDF membrane

10 mM LiCl
0.8 mM TMACl | AgCl | Ag Cell II

Microwell

Methods

Ion transfer experiments were carried out in 96-well microfilter plates obtained from Millipore (CH). The polyvinylidene difluoride membrane has the following specifications: 0.45-μm pore size, 135-μm thickness, and diameter 0.5 cm. Each well of the filter plate was impregnated with ca. 5 μL of *o*-NPOE that contained a supporting electrolyte and left at least 10 min to allow a complete absorption of the solvent into the pores of the membrane. Subsequently, the wells were filled with 250 μL of 10 mM lithium chloride of various pH. In this way one data point was produce for each well. The pH of the wells was controlled by adding either Hydrochloride acid or LiOH in the aqueous phase. The reference plate contained an aqueous solution with supporting electrolyte (see cell I and II). The resulting sandwich structure (Fig. 1) was used in the electrochemical study of the drug transfer. The potential difference was applied between the well and the reference compartment, following the procedure described in the literature (21). To optimize the system a Luggin capillary filled with electrolyte solution was used for the aqueous electrodes to facilitate the movement of the electrodes between the wells. Further optimization could be done with an automated electrode positioning system. This could for instance be achieved using a laboratory robot system.

Electrode Fabrication

Two Ag/AgCl and two Pt electrodes were used. One of the Ag/AgCl electrodes was a leaf, which covers the entire surface of the acceptor plate. The Ag/AgCl electrode leaf has been prepared by a screen-printing technique. The surface of the substrate (PET) was covered by an ink layer of the Ag/AgCl paste (ERCON). The impressed tape was dried in the oven for 2 h at 80°C.

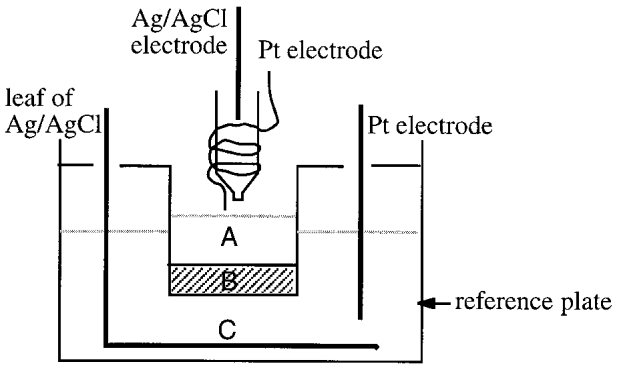

Fig. 1. Schematic representation of the principle of the 96-well microfilter plates system. A, Aqueous phase 250 μL at fixed pH containing the drug; B, polyvinylidene difluoride membrane with nitrophenyloctyl ether solvent; C, Aqueous reference phase

The partition coefficients of the neutral species were also determined by potentiometry, using the GLpK_a apparatus of Sirius Analytical Instruments (Forrest Row, East Sussex, U.K.). The detailed experimental procedures can be found in the literature (22).

RESULTS AND DISCUSSION

Experimental Validation Based on Simple Ion Transfer Reactions

To ensure that the present electrochemical microfilter plates system is reliable, the transfer of tetra-alkylammonium ions from water to NPOE has been studied. A potential is applied between water and NPOE using a waveform generator and a four-electrode potentiostat resulting in a Galvani potential difference, $\Delta_o^w\phi$, being established between the two phases. If it is assumed that the ion transfer of the ion, *i*, across the interface is controlled by linear diffusion, the corresponding Nernst equation at the half-wave potential is given by (23):

$$\Delta_o^w\phi_{1/2} = \Delta_o^w\phi_i^{o'} - \frac{RT}{z_iF}\ln\xi_i \quad (1)$$

where

$$\xi_i = \sqrt{\frac{D_i^o}{D_i^w}} \quad (2)$$

The formal transfer potential, $\Delta_o^w\phi_i^{o'}$, is related to the standard transfer potential, $\Delta_o^w\phi_i^{o'}$, through

$$\Delta_o^w\phi_i^{o'} = \Delta_o^w\phi_i^o + \frac{RT}{z_iF}\ln\left(\frac{\gamma_i^o}{\gamma_i^w}\right) \quad (3)$$

where γ_i^o and γ_i^w are the activity coefficients of *i* in oil and water respectively. The standard transfer potential is related to the standard Gibbs energy of transfer:

$$\Delta_o^w\phi_i^o = \frac{\Delta G_{tr,i}^{o,w\to o}}{z_iF} \quad (4)$$

Figure 2 shows the cyclic voltammograms obtained with Cell I in presence of $TPrA^+$, TEA^+, and TMA+ in the aqueous wells. Using a value of $\Delta G_{tr,i}^{o,w\rightarrow o} = 10.7$ kJ mol^{-1} (17) for the transfer of TMA^+ from water to NPOE, the values of the standard transfer potentials for $TPrA^+$ and TEA^+ were evaluated as follows: $\Delta_o^w\phi^o_{TPrA^+} = -100$ mV, $\Delta_o^w\phi^o_{TEA^+} = 20$ mV using a value of 0.06 for ξ (Waldens rule). These values correlate rather well with previous measurements: $\Delta_o^w\phi^o_{TPrA^+} = -90$ mV and $\Delta_o^w\phi^o_{TEA^+} = 26$ mV (17), indicating that diffusion in the membrane is similar to the diffusion in free-standing NPOE. It can therefore be concluded that the microfilter plate system is adequate for the measurement of formal transfer potentials by voltammetric methods.

Ion Partition Diagrams

Let us first consider the ion-partition diagram of the monobasic compound, BH^+. The corresponding dissociation equilibrium is:

$$BH^+ \rightleftarrows B + H^+$$

The corresponding acid dissociation constant in water (w) is given by:

$$K_a^w = \frac{a_B^w a_{H^+}^w}{a_{BH^+}^w} \tag{5}$$

A similar constant can be defined for the organic phase (o). At low pH, the only species present is BH^+. Consequently, the response in cyclic voltammetry is simply that of the simple ion transfer of BH^+. The experimentally measurable parameter is the half-wave potential, which is given by equation (1).

As the pH is increased the situation becomes more complex as the concentration of BH^+ starts to be dependent on the pH of the aqueous phase (24,25). The exact transition between the two situations is dependent on the acid dissociation constants and the standard partition coefficient of the neutral form, B. The latter parameter is simply defined as follows:

$$P_B = \frac{a_B^o}{a_B^w} \tag{6}$$

The half-wave potential for $pH > pK_a^w - \log(1 + P\xi_B)$ is given by the following (26):

$$\Delta_o^w\phi_{1/2} = \Delta_o^w\phi^{o'}_{BH^+} - \frac{RT\ln 10}{F}\log\xi_{BH^+} + \frac{RT\ln 10}{F}(\log(1 + \xi_B P_B) - pK_a^w + pH) \tag{7}$$

Because the diffusion coefficients of B and BH^+ can be assumed to be similar, we get $\xi_{BH^+} = \xi_B = \xi$. Furthermore, for $\xi P_B >> 1$, (i.e. lipophilic B and / or large ξ) Eq. (7) reduces to the following:

$$\Delta_o^w\phi_{1/2} = \Delta_o^w\phi^{o'}_{BH^+} + \frac{RT\ln 10}{F}(\log P_B - pK_a^w + pH) \tag{8}$$

Obviously, at very positive potentials the proton will transfer to the oil phase, but this reaction cannot be observed under the current conditions because of the interference of the ion transfer reaction of the supporting electrolyte. The half-wave potential and the pH defines a boundary line between a predominance of BH^+ in oil and B in oil, respectively.

The intercept between the horizontal line and the oblique line can be calculated from Eqs. (1) and (8). It corresponds to a pH of:

$$pH = pK_a^w - \log P_B - \log\xi \tag{9}$$

The parameter ξ can be estimated from the ratio of the viscosities of water and oil (Walden rule) (17) as validated above.

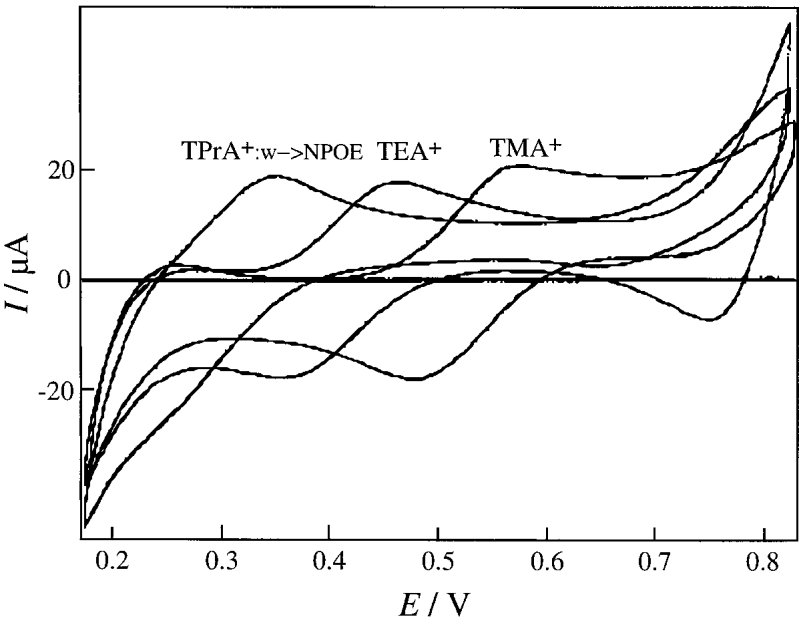

Fig. 2. Cyclic voltammograms obtained for $TPrA^+$, TEA^+, and TMA^+ (0.4 mM of each one) ion transfer across the water–nitrophenyloctyl ether interface using cell I. Scan rate was 80 mV s^{-1}.

The ion partition diagram of a monoacid, AH, can be constructed by following a similar procedure. At high pH the Nernst equation at the half-wave potential is given by equation (1) for A^-. As the pH is lowered the presence of AH is more and more pronounced and the half-wave potential ultimately becomes dependent on the pH. For $pH < pK_a^w + \log(1 + \xi_{AH}P_{AH})$, the half-wave potential is given by the following (25):

$$\Delta_o^w\phi_{1/2} = \Delta_o^w\phi_{A^-}^{o'} - \frac{RT\ln 10}{F}\log\xi_{A^-} + \frac{RT\ln 10}{F}\log\left(\frac{a_{H^+}^w}{K_a^w(AH)}(1 + \xi_{AH}P_{AH})\right) \quad (10)$$

which for $\xi_{AH} = \xi_{A^-} = \xi$ and $\xi P_{AH} >> 1$ (i.e., lipophilic AH and/or large ξ) reduces to the following:

$$\Delta_o^w\phi_{1/2} = \Delta_o^w\phi_{A^-}^{o'} + \frac{RT\ln 10}{F}(pK_a^w(AH) - pH + \log P_{AH}) \quad (11)$$

The intercept can then be calculated as:

$$pH = \log P_{AH} + \log\xi + pK_a^w(AH) \quad (12)$$

From Eq. (9) and (12), it follows immediately that for a lipophilic neutral form (i.e., B or AH) electrochemistry at ITIES can provide information on $\Delta_o^w\phi_i^{o'}$, $\Delta G_{tr,i}^{o,wo\rightarrow o}$ and log*P*, provided that the aqueous phase pK_a and ξ are known. In practice, a series of experiments are carried out in which the half-wave potential is measured as a function of pH. The experimental points are then used to construct ion partition diagrams as defined by Eqs. (1) and (9) for BH^+ or Eqs. (1) and (12) for A^-. Usually, such an investigation is carried out using a simple electrochemical cell where only a few points can be determined at a time, resulting in a long analysis time and a large solvent and drug consumption. With the microfilter plate up to 96 points in a single drug partition diagram can be determined using just one set-up and minute amounts of drugs and solvents. In practice, 96 wells are sufficient for studying seven to eight drugs as each well is used for one data point. In practice, the accessible dynamic range of log*P* for the neutral form and pK_a values depends on the specific drugs. As long as a signal is observed in cyclic voltammetry, the compound can be studied using this method. This condition is fulfilled when one of the protonated forms is charged and less hydrophobic than the supporting electrolyte in the aqueous phase and less hydrophilic than the supporting electrolyte in the oil phase.

In the case of a charged drug molecule, the accessible dynamic range of log *P* values depends only on the potential window. In practice, the accessible Galvani potential differences are from –400 mV to 400 mV resulting in dynamic range from approximately –4 to 4 in log P_I.

To illustrate the methodology with a practical example, the transfer of a monobasic compound, TMAN, was studied experimentally at various pH values. The analyte is added to the donor compartments, already filled with aqueous electrolyte solutions of different pH. To obtain an absolute value, the ion transfer half-wave potentials are corrected using the tetrapropylammonium ($TPrA^+$) ion as a reference.

The resulting ionic partition diagram is shown in Fig. 3. The aqueous pK_a value of $TMAN^+$ is known to be 5.5 from literature (9) and the intercept corresponding to Eq. (9) can be determined from Fig. 3 to be 2.5. The logarithm of the partition coefficient of the neutral form can be calculated to be 3.6. It is assumed that ξ can be obtained from the viscosities of water and NPOE (Walden rule) as validated above.

The lipophilic monoacidic compound, 2,4-dinitrophenol (2,4-DNPh) was also studied. The half-wave potential for the transfer of the DNPh ion measured by cyclic voltammetry shifted with increasing pH. In this case the absolute values of the ion transfer half-wave potentials were calculated by using the half-wave potential of the tetramethylammonium (TMA^+) ion as a reference. From the aqueous dissociation constant ($pK_a = 4.1$) and an intercept of 5.5, the above theory predicts a value of the partition coefficient of 2.0 (using Eq. 12). The complete ionic partition diagram is shown in Fig. 4. The amount of TMAN and 2,4-DNPh used for each well were 0.037 mg and 0.0092 mg, respectively.

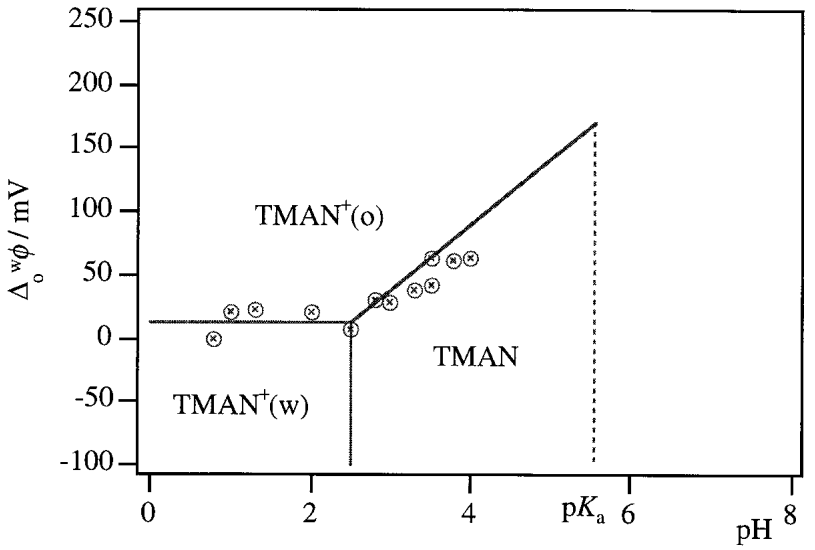

Fig. 3. Ionic partition diagram of tetramethylaniline (BH^+) at the water–nitrophenyloctyl ether interface obtained with the microfilter plate system. The dotted line at the right side shows the aqueous pK_a value.

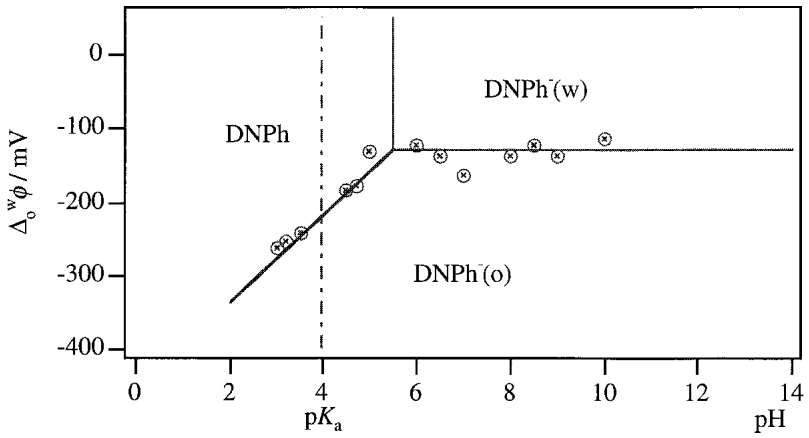

Fig. 4. Ionic partition diagram of 2,4-dinitrophenol at the water–nitrophenyloctyl ether interface obtained with the microfilter plate system. The dotted line at the left side shows the aqueous pK_a value.

The logP_{NPOE} values obtained for the neutral and ionized forms of the studied compounds are given in Table I. The corresponding literature values for the octanol–water and DCE–water systems have been added. The values of the partition coefficients of the neutral forms for the NPOE–water system obtained by cyclic voltammetry compares well with those measured by potentiometry. The comparison between log$P$ for the three different solvent systems shows that the results obtained in the present work using NPOE are between the values obtained with octanol and DCE as the oil phase. This observation correlate rather well with previous investigations in which an empirical relation between logP_{NPOE} and logP_{OCT} was put forward (27). A more exact theoretical analysis is complicated due to the complexity involved in a thorough and detailed description of solvation phenomena.

For both a lipophilic base (B) and acid (AH), the main difference in the ionic partition diagram when ξ is significantly less than unity, is an enlargement of the region where the neutral species predominates. As illustrated by the examples, the method is quite flexible in terms of solvation requirements as the drug can be introduced in the water phase or directly in the membrane. It should also be mentioned that the set-up can be used for multiprotic molecules, although this would require a larger number of data points.

Table I. Physicochemical Parameters Determining the Transfer Behavior of the Monobase, Tetramethylaniline and the Monoacid, Dinitrophenol

	3,5-*N,N*-Tetramethylaniline	2,4-Dinitrophenol
logP_{NPOE}	3.6 ± 0.2	2.0 ± 0.2
logP_{NPOE}	3.83[a]	1.94[a]
logP_{OCT}	3.2[b]	1.55[b]
logP_{DCE}	4.01[c]	2.46[d]
pK_a	5.5 ± 0.2[c]	4.1 ± 0.2[d]
$\Delta_o^w\phi_I^{o'}$/mV	14 ± 10	−130 ± 10
log$P_I^{o'}$ (NPOE)	−0.24	−2.23
log$P_I^{o'}$ (DCE)	−2.8[c]	−1.7[d]
Intercept	2.5	5.5

[a] Measured by potentiometry.
[b] log$P$ values from the SciFinder Scholar data base.
[c] log$P$ value from the Reference 9.
[d] Values from the Reference 19.

CONCLUSIONS

The electrochemical methodology presented in this paper is shown to be useful for determining the log$P$ of drug molecules in a rapid and efficient way. The procedure is based on commercially available 96-well microfilter plates that enables studies on pH lipophilicity profiles of ionizable compounds and establishment of their ionic partition diagrams. If the aqueous phase pK_a is already known, the measurements of logP_{NPOE} of lipophilic drugs using a very little amount of solvents and drugs can be achieved. Inversely, the aqueous phase pK_a can be determined if log$P$ is known or if the neutral form of the drug is hydrophilic. A complete automation may even be envisaged provided that the method is further optimized, and automated using a laboratory robot system.

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