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Development of a potentiometric kinetic method for drug adsorption studies: the chlorpromazine–charcoal model case

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Abstract

A potentiometric method for the in vitro adsorption kinetic study of an ionic micromolecule to charcoal, based on the continuous direct monitoring of the micromolecule free concentration by means of an ion-selective electrode (ISE), has been developed. A chlorpromazine ISE was constructed and used to study the adsorption kinetics of the drug on pure activated charcoal and two commercial formulations (Ultracarbon tablets and Carbomix powder). The method consists of the rapid addition of a slurry containing the charcoal into the drug solution under stirring at pH 1.2 (to simulate a gastric fluid environment) and continuous recording of the electrode potential until the establishment of equilibrium. The drug free concentration at appropriate time intervals was calculated from the recorded adsorption curve and the apparent adsorption rate constant was estimated assuming first order kinetics. Within run RSD of the estimates ranged from 0.3 to 12% (mainly less than 5%), while between run RSD ($n=3$) ranged from 1 to 19% (mainly less than 10%). A linear relationship was found between the apparent adsorption rate constants and the amount of charcoal used with slopes following the rank order activated charcoal>Ultracarbon tablets>Carbomix powder. These results were explained on the basis of different surface areas of the adsorbents. The work proved the usefulness of ion-selective potentiometry in adsorption studies and can be extended to other ionic drugs for which selective electrodes can be constructed.

Keywords: Charcoal adsorption; Chlorpromazine; Ion-selective potentiometry; Adsorption kinetic study

1. Introduction

It is well known that activated charcoal (AC) is the most valuable agent as an emergency antidote in many cases of intoxications because of its adsorptive properties. An in vitro adsorption technique has been developed and used routinely to assess drug adsorption under conditions that simulate the conditions in vivo (Oderda et al., 1987 Favin et al., 1988; Akintowa and Orisakwe, 1990; Hoffman et al., 1991 Arimori et al., 1993 Makosiej et al., 1993). The technique is based upon an adsorption measurement at equilibrium, whereas the rate of adsorption and desorption are equal. This "single point" measurement is accomplished by separating solid (charcoal) and solution and estimating the loss in concentration of adsorbate from solution. The technique is time consuming since it involves a separation (e.g. filtration) step and the equilibration time needs to be determined. Moreover, it cannot be used to record the adsorption kinetic profile for rapid processes.

It is conceivable that an automated technique, which is not time consuming and can also be (i) adaptable to the experimental system used in the literature; and (ii) capable of providing the entire adsorption kinetic profile, will undoubtedly be of value for the study of adsorption phenomena. Automation has offered great advances in the relevant

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topic of drug–macromolecule interactions by the application of ion-selective electrodes to the binding studies of drugs with cyclodextrins (Valsami et al., 1990 Valsami et al., 1992) and proteins (Valsami et al., 1991 Angelakou et al., 1993 Sideris et al., 1994). Ion-selective electrodes (ISEs), known also as ion chemical sensors, are electrochemical transducers that respond selectively, directly and continuously to the activity of the free ion in solution. The electromotive force of the electrochemical cell (E_{cell}), consisting of an ISE for an ion and a reference electrode, is described by the Nernst equation:

$$E_{cell} = E_{cons} + 2.303RT/zF \times \log \alpha$$
$$= E'_{cons} + S \times \log C \quad (1)$$

where E_{cons} is a constant term (the sum of the constant potentials of the internal and external reference electrodes, and the liquid junction), R the ideal gas constant, T the absolute temperature, F the Faraday constant, z the charge of the ion with its algebraic sign, and α is the activity of the ion. At low concentrations ($C < 10^{-2}$ M) or when the ionic strength of the solution is kept constant, the concentration values (C) can be used instead of the activity in Eq. (1). In the latter case the activity coefficient of the ionic analyte is incorporated in the constant potential term E'_{cons}. The prelogarithmic factor $2.303RT/zF$ is known as the slope (S) of the ISE. In the case of a monovalent cation, such as the chlorpromazine ion, the theoretical value of the ISE slope at 25°C is −59.16 mV/pC (Nernstian response), where $pC = -\log C$. The potential use of ISEs in adsorption studies is due to their ability to directly measure the free adsorbate ion in solution in the presence of the adsorbent and the adsorbate adhering to the adsorbent.

Ion selective electrodes have been successfully used for reaction kinetic studies and kinetic methods of analysis by continuous monitoring of the rate of production or consumption of an ion (Efstathiou et al., 1985). In such applications the response time of the ISE used is a critical characteristic.

In this paper, we describe the development of a potentiometric kinetic method for the study of adsorption phenomena based on the continuous potentiometric monitoring of the nonadsorbed (free) ion in the reaction mixture of adsorbate–adsorbent. Chlorpromazine (CHP) was used as a model drug because intoxications by accidental overdoses have been reported (Baldesarini, 1992 Dreisback, 1980 Rumack and Peterson, 1980). The proposed method was applied to the adsorption studies of CHP to three different types of charcoal, namely, activated charcoal, and two commercially available formulations, Ultracarbon tablets and Carbomix powder. The adsorption process was continuously monitored potentiometrically by means of a chlorpromazine ion-selective electrode (CHP-ISE) of the polyvinyl chloride (PVC) type constructed in our laboratory for the purposes of this study, following a modified procedure of a previously published work (Mitsana-Papazoglou et al., 1985).

2. Materials and methods

2.1. Reagents

Chlorpromazine hydrochloride was obtained from the Rhone Poulenc local representative and was used without any further purification.

Activated charcoal: Three different types were used: (a) Active charcoal (AC) (Merck, GR-Nr 2186) pure powder, produced for general laboratory purposes, dried in 10-g portions at 140°C for 1 h. (b) Carbomix®, produced by Norit (Netherlands, Nr 1057), containing 81.3% (w/w) of activated charcoal, 2.4% (w/w) citric acid monohydrate, 8.1% (w/w) acacia (gum arabic) and 8.1% (w/w) glycerol. (c) Ultracarbon® 400-mg tablets, produced by Merck, containing 250 mg of activated charcoal, 119 mg of bentonite and 31 mg of starch maze per tablet.

2-nitrophenyloctylether (2-NPOE) was obtained from Fluka, sodium tetraphenylborate (TPB) from Ferak Berlin, PVC of very high molecular weight from Janssen Chimica (Beerse, Belgium), Na_2SO_4 and concentrated hydrochloric acid from Merck, and tetrahydrofuran (THF) from Labscan. The chemical reagents used were of analytical grade.

All solutions were prepared in aqueous acidic medium (buffer) pH 1.2, simulating a gastric fluid environment. This buffer solution was prepared by adding 12 M hydrochloric acid to deionized water under magnetic stirring and monitoring the pH with a pH-meter. Chlorpromazine 0.100 M stock solution and more dilute working solutions were prepared in the acidic medium pH 1.2 and stored in amber glass bottles.

2.2. Electrode construction

The CHP-ISE was of the PVC membrane type and constructed by attaching the electroactive PVC membrane (diameter of 6 mm) to a used sensing module of the commercially available nitrate electrode (Orion model 93-07). The sensing module was prepared by careful removing of its old membrane, emptying the internal reference solution, washing the internal compartment and drying with air.

The internal reference solution for the CHP-ISE was 0.010 M with respect to CHP in 0.10 M NaCl saturated with AgCl. A 250-μl portion of this solution was used for filling the internal compartment of the ISE.

The electroactive PVC membrane was constructed by entrapping the CHP liquid ion exchanger in a PVC matrix according to the method of Craggs et al. (1975). The liquid ion exchanger for the CHP-ISE was the ion pair of CHP cation with tetraphenylborate (TPB) anion in 2-NPOE at a concentration of about 0.05 M and was prepared as follows: 2 ml of 0.05 M TPB and 1.5 ml of 0.10 M CHP aqueous solutions were mixed and the precipitate was extracted with 3 ml of 2-NPOE. The organic layer was washed three times with water and dried with 0.5 g of anhydrous sodium sulfate to remove any water traces. For the construction of the electroactive membrane the PVC powder (0.085 g) was gradually dissolved in 3 ml of THF and 0.5 ml of the above-described CHP liquid ion exchanger was added to the solution under stirring. The solution obtained was left for 48 h at room temperature for THF evaporation. The resulting membrane was cut and attached to the end of the electrode body using cyclohexanone as adhesive. After construction, the ISE was conditioned for 24 h before use in a 0.10 M CHP solution in water. The same solution was used for the storage of the ISE when not in use.

2.3. Apparatuses

The system used for the potentiometric measurements consists of an electrometer (Orion Ionanalyzer, Model 801 pH/mV meter) with a readability of ± 0.1 mV, connected to a Radiometer Chart Recorder Model 61. The emf values were measured against a Ag/AgCl reference electrode (Orion, single junction, Model 90-01) filled with the reference electrode filling solution (saturated KCl solution, Orion 90-00-01). All measurements were carried out in a 100-ml double-walled glass cell, thermostated at a temperature of 37 ± 0.5°C with an Edmund Buhler 7400 Tubingen type UKT30 water bath, with constant magnetic stirring of solutions.

2.4. Procedures

2.4.1. Construction of the calibration curve of the CHP-ISE

The pair of electrodes was immersed in the measurement cell which contained 25 ml of the aqueous acidic medium pH 1.2 and after the potential was stabilized (± 0.1 mV), a series of successive aliquots of the 0.10 M CHP stock solution (5 μl–1.67 ml) were added to cover the concentration range 2.0×10^{-5}–6.26×10^{-3} M. The emf values (potential $-E$ in mV) were recorded and measured after stabilization (± 0.1 mV) following each addition. The potential values E_i were plotted against the negative logarithm of the total molar CHP concentration in solution for each addition, according to the Nernst equation, Eq. (1).

The equation of the calibration curve (or response curve) of the CHP ISE was obtained by linear regression of the E_i, pC_i data using a least-squares fitting program. Corrections for the changes in volume after each addition were performed automatically by this program.

2.4.2. Kinetic experiments

The pair of electrodes was immersed in the measurement cell, which contained 25 ml of a 4×10^{-3} M CHP solution under continuous stirring (maximum stirring rate without causing turbulent flow) and the potential was measured and recorded after stabilization. A 4.00-ml slurry containing in each case the appropriate amount (0.125, 0.250, 0.375, and 0.500 g) of activated charcoal in an aqueous solution of pH 1.2 was then injected in the measurement cell using a glass syringe under continuous monitoring of the potential. Potential recordings were obtained up to equilibrium (stabilisation of the potential).

The same type of adsorption experiments were performed using a 4.00-ml slurry containing various amounts of Carbomix powder (equivalent to 0.200, 0.300, 0.400, and 0.500 g of charcoal) dispersed in all cases in an aqueous solution of pH 1.2. Ultracarbon

tablets were mechanically disintegrated prior to the experiment; a weighted amount of the resulting powder equivalent to 0.250, 0.300, 0.375, and 0.500 g of charcoal was then used to prepare a 4.00-ml slurry in an aqueous solution of pH 1.2. Experiments were run in triplicate in all cases.

The potential difference ΔE_i between the readings at the commencement of the experiment and at any time t_i, respectively, can be used to calculate the decrease in the free concentration relying on Eq. (1). Consequently, the CHP concentration adsorbed to charcoal expressed in molar units, $C_{ad,i}$, at each time point t_i, can be obtained from Eq. (2):

$$C_{ad,i} = C_T(1 - 10^{-\Delta Ei/S}) \quad (2)$$

where C_T is the total CHP concentration in the solution, 3.45×10^{-3} M, and S the slope of the calibration curve. The values of ΔE_i were estimated graphically from the adsorption curve and the known calibration factor in mV/mm of chart recorder. In all calculations, corrections were made to account for the changes in the potential readings due to the initial dilution of the CHP 4×10^{-3} M solution to 3.45×10^{-3} M after the addition of the slurry.

Estimates for the adsorption rate constants were calculated from the adsorption data ($C_{ad,i}$,t_i) assuming first-order adsorption kinetics using the relevant equation (Eq. (3)):

$$C_{ad,i} = C_{ad,eq}(1 - \exp(-k_{app}t_i)) \quad (3)$$

where $C_{ad,eq}$ is the adsorbed CHP concentration at equilibrium, and k_{app} is the apparent adsorption first-order rate constant. The estimates for k_{app} were derived from the linearized form of Eq. (3):

$$\ln(1 - (C_{ad,i}/C_{ad,eq})) = -k_{app}t_i \quad (4)$$

applying linear regression analysis. Although this transformation changes the distribution and variances of the errors, and the optimized solution (minimum) may be shifted, the linear and nonlinear solutions for k_{app} were found to be almost identical.

3. Results and discussion

3.1. Electrode characteristics

The CHP-ISE showed a slightly sub-Nernstian response in the linear concentration range (1.4×10^{-4}–6.3×10^{-3} M) with a slope at 37°C varied from −54 to −56 mV/pC ($r>0.9998$). The detection limit [concentration, which is estimated from the calibration curve twice as large as its real value, due to the background signal, i.e. the concentration for which the measured potential deviates 18 mV from the expanded calibration curve (at 25°C, $59.16/z\times\log2=18$) (Cammann, 1979)] was found to be 2×10^{-5} M. The electrode membrane was found to have an operative life of about one month, after which a new one was attached to the electrode body. The slope of the electrode remained relatively constant and the potential for the same aqueous solution varied ±0.5 mV during its operative life.

The response time was studied by recording the potential changes of stepwise increases in CHP concentration. The potential was restabilized, in the whole concentration range, in less than 1 s after each addition. Therefore, the kinetics of the electrode response has no practical effect on the adsorption kinetic profile with a duration of at least 50 s (Fig. 3).

The electrode was free from serious drift and therefore adequate for reliable measurements even in prolonged adsorption experiments.

Fig. 1 shows a calibration curve of CHP-ISE (E versus the negative logarithm of total concentration, pC_t) in the acidic medium. In the field of ISEs, both the detection and least linear concentration limits (2×10^{-5} and 1.4×10^{-4} M, respectively, for the CHP-ISE) are subjected to limitations imposed by the solubility of the electroactive ion exchanger from the

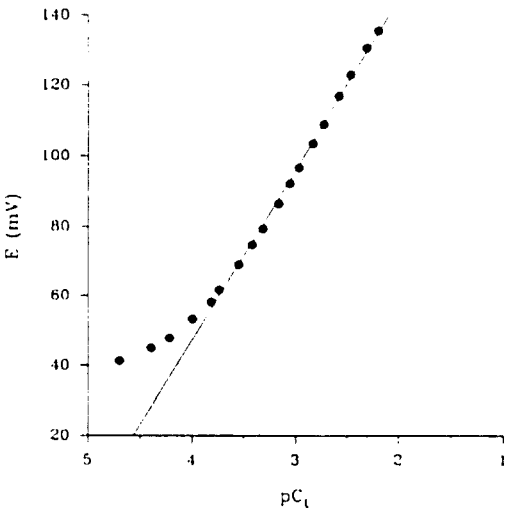

Fig. 1. Calibration curve of CHP-ISE in the aqueous solution pH 1.2.

PVC membrane in the sample solution. However, in the presence of a complexing agent the free ion concentration is decreased considerably, down to $pC = pK_d + 1$ where K_d is the dissociation constant of the complex formed. Thus, an expansion of the least linear limit of the calibration curve has been achieved for various ISEs by the use of complexing agents, known as ion buffers. For example, the copper ISE shows Nernstian response down to $pC = 19$ in the presence of ethylenediamine (Avdeef et al., 1983), and calcium ISE down to $pC = 8$ in the presence of various ligands (Otto et al., 1985). The same behaviour has also been observed in protein binding studies performed with direct potentiometry; again, the expansion is associated with the buffering effect of the macromolecule on the micromolecule concentration (Angelakou et al., 1993). This phenomenon increases the usefulness of the ISE potentiometric technique in binding and adsorption studies since it permits the measurement of low concentrations of the micromolecule even less than the least linear concentration limit of the ISE. In this study, all adsorbents used caused an expansion of the least linear concentration limit of CHP-ISE down to 3×10^{-5} M. The expansion was verified with parallel experiments (not included in this work) carried out to study the effect of polyethylene glycol to the adsorption of CHP to the adsorbents. The free CHP concentration calculated from the determined adsorption affinity constants of CHP to the adsorbents and the total CHP concentration utilized resulted in potential readings lying exactly on the expansion of the calibration curve. The good fitting of all the data from the complete reaction curve to Eq. (4) is an additional indirect proof that free CHP concentrations can be measured validly in the expanded linear range (3×10^{-5}–1.4×10^{-4} M).

3.2. Kinetic experiments

Fig. 2 shows typical kinetic adsorption profiles (potential versus time) of CHP to 0.5 g of activated charcoal and appropriate amounts of Carbomix powder and Ultracarbon tablets equivalent to 0.5 g of charcoal. The plots of Fig. 2 demonstrate that the rate of CHP adsorption to the adsorbents studied follows the rank order, activated charcoal > Ultracarbon tablets > Carbomix powder. This is in accord with the higher surface area of AC and justifies that "a real time" adsorption kinetic profile can be monitored using ISEs. However, the approach to the steady state is very rapid for all three adsorbents examined under the experimental conditions used. The experimentally determined $C_{ad,eq}$ value for all three cases examined in Fig. 2 was found to be identical (under the experimental conditions used, i.e. large excess of adsorbents) and equal to 3.42×10^{-3} M, which corresponds to 99.1% of the total CHP concentration used. This means that CHP is adsorbed almost completely to the adsorbents.

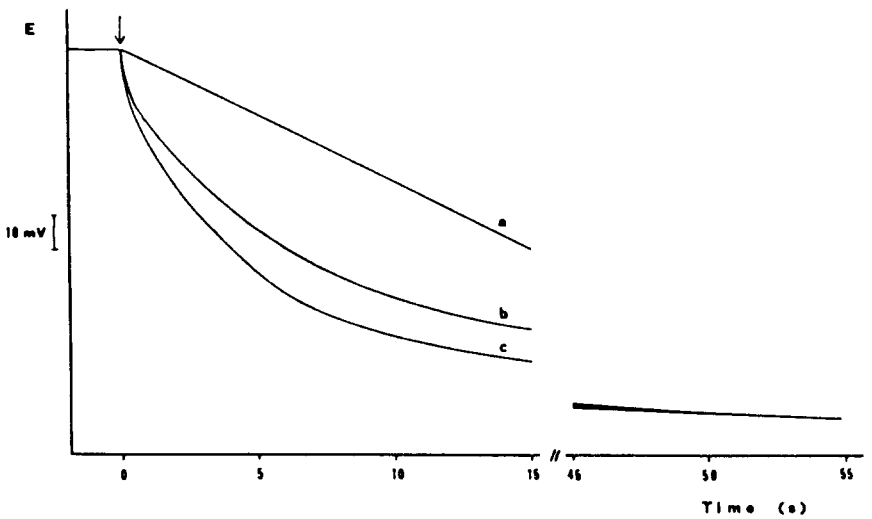

Fig. 2. Plot of potential versus time for the adsorption of CHP to (a) an amount of Carbomix powder equivalent to 0.5 g of charcoal (b) an amount of Ultracarbon tablets equivalent to 0.5 g of charcoal and (c) 0.5 g of activated charcoal. The arrow indicates the time of the addition of the slurry.

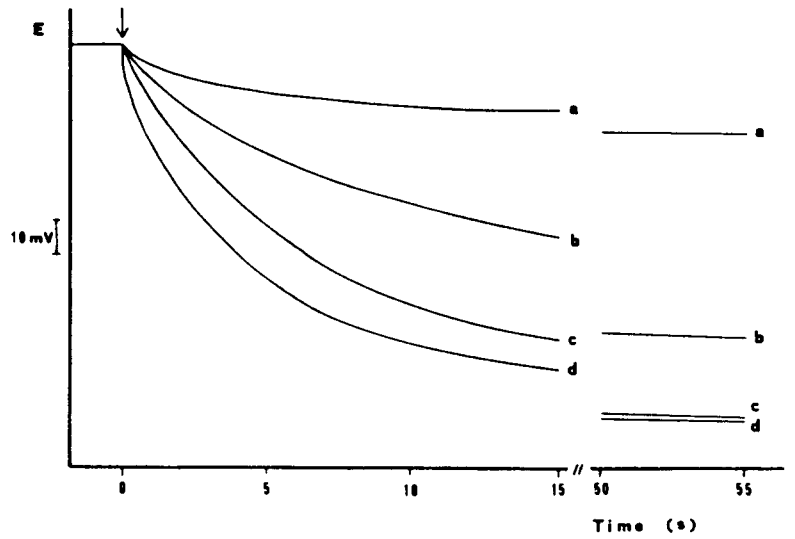

Fig. 3. Plot of potential versus time for the adsorption of CHP to various amounts of activated charcoal. Key: (a) 0.125 g (b) 0.250 g (c) 0.375 g and (d) 0.500 g. The arrow indicates the time of the addition of the slurry. The free percent CHP concentration at equilibrium was found: (a) 38.3, (b) 3.8, (c) 1.4, and (d) 0.9%.

The effect of the amount of the adsorbent on the rate of CHP adsorption is shown in Fig. 3 using the activated charcoal as an example. As it was expected, the rate (as well as the extent) of CHP adsorption increases with the amount of activated charcoal used. The kinetic analysis of data was based on Eq. (4). A typical example based on the linearized Eq. (4) is shown in Fig. 4 for the adsorption data of CHP to an amount of Carbomix powder equivalent to 0.2 g of charcoal. The estimates of apparent adsorption rate constants obtained from the slope of Eq. (4) are given in Table 1. For all cases examined, the values of the correlation coefficients were found to be higher than 0.99. The validity of the estimates for the apparent rate constant is further substantiated from the low within and between run SD values (Table 1). It

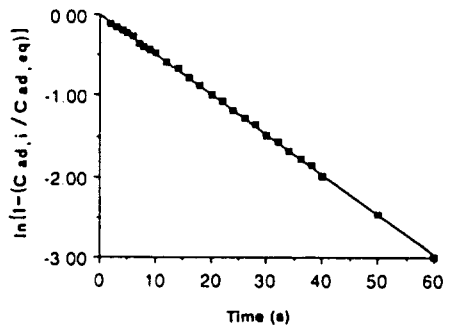

Fig. 4. Typical regression line for the estimation of the apparent adsorption rate constant of CHP to an amount of Carbomix powder equivalent to 0.200 g of charcoal.

should be noted that the estimates for the adsorption rate constant listed in Table 1 are apparent and not intrinsic, k_{int}. This means that the surface area, A, of the adsorbents has been incorporated into k_{app} when the latter was defined in the context of the fundamental equation (Eq. (3)), i.e. $k_{app} = A \times k_{int}$. Therefore, the values reported in Table 1 reflect the kinetic sensitivity of the CHP towards the adsorbents as well as the effect of the surface area on the kinetics of adsorption. Obviously, the smaller mean particle size of AC (larger surface area) is a determining factor for the results quoted in Table 1. Stirring effects, which are also very important in kinetic studies, should not be taken into consideration here since all studies were carried out with identical stirring conditions. However, if one wishes to extrapolate these results to the in vivo conditions, the much lower agitation prevailing in the GI tract should be seriously taken into account. Unfortunately, the need for homogeneous solutions, in order to carry out the in vitro measurements, is in contrast with the heterogeneity resulting from the limited mixing of GI fluids.

A linear relationship was found when the estimates for the apparent adsorption rate constants were plotted against the amounts of adsorbents used, Fig. 5. The linearity observed reveals that an increase of amount is associated with a proportional increase in the apparent adsorption rate constant. The slopes of the regression lines for the activated charcoal, Ul-

Table 1
Values of apparent adsorption rate constants of chlorpromazine to the various types of charcoal for three experiments[a]

Amount (g)	K_{app} s^{-1}	K_{app} s^{-1}	K_{app} s^{-1}	Mean value, s^{-1}
Activated charcoal				
0.125	0.173(0.009)	0.167(0.02)	0.154(0.015)	0.165(0.010)
0.250	0.219(0.010)	0.236(0.013)	0.240(0.016)	0.232(0.011)
0.375	0.395(0.016)	0.353(0.006)	0.447(0.025)	0.398(0.027)
0.500	0.582(0.024)	0.560(0.039)	0.558(0.033)	0.567(0.013)
Carbomix[b]				
0.200	0.050(0.0002)	0.061(0.0002)	0.050(0.0002)	0.054(0.006)
0.300	0.099(0.0004)	0.105(0.0007)	0.103(0.0005)	0.102(0.003)
0.400	0.134(0.0006)	0.126(0.0007)	0.148(0.0008)	0.136(0.011)
0.500	0.184(0.0007)	0.189(0.0009)	0.185(0.0008)	0.186(0.003)
Ultracarbon[b]				
0.250	0.125(0.002)	0.124(0.003)	0.119(0.001)	0.123(0.003)
0.300	0.198(0.004)	0.191(0.003)	0.174(0.003)	0.188(0.012)
0.375	0.271(0.007)	0.276(0.006)	0.300(0.018)	0.273(0.003)
0.500	0.380(0.006)	0.271(0.014)	0.290(0.011)	0.314(0.060)

[a]Standard deviations in parentheses.
[b]The amount stated is equivalent to pure activated charcoal.

tracarbon tablets, and Carbomix powder were 1.10 (±0.14), 0.75 (±0.17) and 0.43 (±0.02) $s^{-1} \cdot g^{-1}$, respectively, while the correlation coefficients were found to be in the range 0.95–0.99. Again, the ranking of slopes in Fig. 5 follows the pattern observed in Fig. 2 and it is associated with the larger surface area of AC.

In conclusion, the study of the adsorption of CHP to three types of charcoal was accomplished with the ISE technique. The continuous monitoring of the CHP in the slurry without need for separation of the adsorbed CHP species enabled us to observe the entire kinetic adsorption profile. The proposed application of ISEs to the adsorption studies furnishes new features in this field of research which in summary can be delineated: (1) unattended operation—once an adsorption experiment is initiated, the potential is continuously recorded; (2) automated data analysis—the complete kinetic adsorption profile is available at the end of the experiment while the completion of the adsorption process (i.e. when equilibrium has been achieved) is easily and visually identified (stabilisation of the potential); and (3) versatility in methodology—the technique can be easily modified to obtain the conventional adsorption profile (adsorption isotherm) by adding the drug gradually into the adsorbent solution in a manner similar to this described in the studies of drug–macromolecule interactions (Angelakou et al., 1993 Sideris et al., 1994 Valsami et al., 1990 Valsami et al., 1991 Valsami et al., 1992). Apart from all above, the kinetic method developed offers a new approach to study adsorption phenomena from solutions in relation to morphology irregularities. Up to now, this kind of study has been mainly focused on reaction of adsorbents with molecules like N_2 in the vapour state (Avnir, 1987). Thus, properly designed adsorption experiments in solutions can be carried out using the method developed in order to establish log–log relationships between the rate of adsorption and the size of the radius of the adsorbent's particles e.g. silica, carbon etc. (Farin and Avnir, 1992). This kind of analysis can provide the fractal reaction dimension of adsorption on the solid surface which takes place in solution. Similar studies in the field of drug dissolution have been started recently (Farin and Avnir, 1992 Valsami and Macheras, 1995).

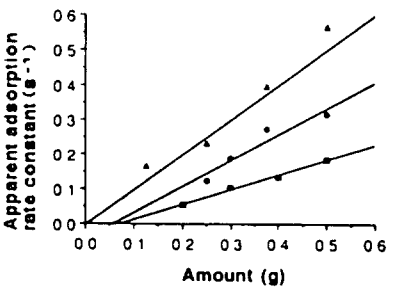

Fig. 5. Plots of the apparent adsorption rate constant of CHP versus the amount of (▲) activated charcoal, and the equivalent amount of charcoal of (●) Ultracarbon tablets and (■) Carbomix powder. Each data point corresponds to the mean of three experiments.

References

Akintowa, A. and Orisakwe, O.E. (1990) The adsorption of quinine and quinidine to activated charcoal with and without magnesium sulfate. Vet. Hum. Toxicol. 32(6), 567–568.

Angelakou, A., Valsami, G., Koupparis, M. and Macheras, P. (1993) Use of 1-anilino-8-naphthalenesulphonate as an ion probe for the potentiometric study of the binding of sulphonamides to bovine serum albumin and plasma. J. Pharm. Pharmacol. 45, 434–438.

Arimori, K., Deshimaru, M., Furukawa, E. and Nakano, M. (1993) Adsorption of mexiletine onto activated charcoal in macrogol-electrolyte solution. Chem. Pharm. Bull. 41(4), 766–768.

Avdeef, A., Zabronsky, J. and Stuting, H. (1983) Calibration of copper ion selective electrode response to pCu 19. Anal. Chem. 55, 298–304.

Avnir, D. (1987) The geometry factor in photoprocesses on irregular (fractal) surfaces. 1. Static considerations. J. Amer. Chem. Soc. 109, 2931–2938.

Baldesarini, R. (1992) Drugs and the treatment of psychiatric disorders. In: Gilman, A.G., Rall, T.W., Nies, A.S. and Taylor, P. (Eds.), The Pharmacologic Basis of Therapeutics, 8th edn. Mc Graw Hill International, New York, pp. 383–435.

Cammann, K. (1979) Working With Ion-Selective Electrodes, Springer-Verlag, Berlin, p. 122.

Craggs, A., Moody, G.J. and Thomas, J.D. (1975) PVC matrix membrane ion-selective electrodes. J. Chem. Educ. 51, 541–544.

Dreisback, R.H. (1980) Depressants. In: Handbook of poisoning, 10th edn. Lange Medical Publications, Los Altos, CA.

Efstathiou, C.E., Koupparis, M.A. and Hadjiioannou, T.P. (1985) Applications of ion-selective electrodes in reaction kinetics and kinetic analysis. Ion-Sel. Rev. 7(2), 203–259.

Farin, D. and Avnir, D. (1992) Use of fractal geometry to determine effects of surface morphology on drug dissolution. J. Pharm. Sci. 81, 54–57.

Favin, F., Klein-Schwartz, W., Oderda, G. and Rose, R. (1988) In vitro study of lithium carbonate adsorption by activated charcoal. J. Toxicol. Clin. Toxicol. 26(7), 443–450.

Hoffman, R., Chiang, W., Howland, M., Weisman, R. and Goldfrank, L. (1991) Theophylline desorption from activated charcoal caused by whole bowel irrigation solution. J. Toxicol. Clin. Toxicol. 29, 191–202.

Makosiej, F., Hoffman, R., Howland, M. and Goldfrank, L. (1993) An in vitro evaluation of cocaine hydrochloride adsorption by activated charcoal and desorption uppon addition of polyethylene glycol electrolyte lavage solution. J. Toxicol. Clin. Toxicol. 31(3), 381–395.

Mitsana-Papazoglou, A., Christopoulos, T., Diamandis, E. and Hadjioannou, T. (1985) Construction of ion-selective electrodes for chlorpromazine, amitriptyline, propantheline and meperidine: Analytical study and application to pharmaceutical analysis. Analyst 110, 1091–1094.

Oderda, G., Klein-Schwartz, W. and Insley, B. (1987) In vitro study of boric acid and activated charcoal. J. Toxicol. Clin. Toxicol. 25(1 and 2), 13–19.

Otto, M., May, P., Murray, K. and Thomas, J. (1985) Calibration of ionized calcium and magnesium with ligand mixtures for intracellular ion-selective electrode measurements. Anal. Chem. 57, 1511–1517.

Rumack, B. and Peterson, R. (1980) Clinical toxicology. In: Doull, J., Klaassen, C.D. and Amdur, M.O. (Eds.), Toxicology: The Basic Science of Poisons, 2nd edn.,, Macmillan, New York, Toronto, London, pp. 677–698.

Sideris, E., Koupparis, M., and Macheras, P. (1994) Effect of cyclodextrins on protein binding of drugs: The diflunisal/hydroxylpropyl-β-cyclodextrin model case. Pharm. Res. 11(1), 90–95.

Valsami, G., Macheras, P. and Koupparis, M. (1990) Binding studies of ions with cyclodextrins using ion selective electrodes. J. Pharm. Sci. 79(12), 1087–1094.

Valsami, G., Macheras, P. and Koupparis, M. (1991) Binding study of the fluorescence probe 1-anilino-8-naphthalene-sulfonate to human plasma and human and bovine serum albumin using potentiometric titration. Pharm. Res. 8(7), 888–892.

Valsami, G., Koupparis, M. and Macheras, P. (1992) Complexation studies of cyclodextrins with tricyclic antidepressants using ion-selective electrodes. Pharm. Res. 9(1), 94–100.

Valsami, G. and Macheras, P. (1995) Determination of fractal reaction dimension in dissolution studies. Eur. J. Pharm. Sci., 3, 163–169.