hydrochloride was found to be 99.8% ($n = 5$, $CV = \pm 0.7\%$) and that of 4-amino-2-chlorobenzoic acid, 101.0% ($n = 5$, $CV = \pm 0.7\%$). The reproducibility of the method was determined using a typical lot of bulk drug and a 2% formulation by three analysts over a 6-d period. The results and the statistical data are presented in Table I along with the results obtained by the corresponding assays prescribed by USP.

In conclusion, ion-pair reverse-phase chromatography permits the simultaneous analysis of chloroprocaine hydrochloride and its degradation product, 4-amino-2-chlorobenzoic acid, in bulk drug and injection formulations. The method is specific for the analysis of the bulk drug and stability indicating for the drug in injection solutions.

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Adsorption of Sulfonylureas onto Activated Charcoal *In Vitro*

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Abstract □ Adsorption of carbutamide, chlorpropamide, tolazamide, tolbutamide, glibenclamide (glyburide), and glipizide onto activated charcoal was compared *in vitro* using different charcoal-to-drug ratios. Maximal binding capacities of different sulfonylureas were 0.45–0.52 g/g of charcoal at pH 7.5. The affinity of the second generation derivatives, glibenclamide and glipizide, was considerably higher than that of the first generation derivatives. The affinity of sulfonylureas to charcoal was higher at pH 4.9 than at pH 7.5. Poor water solubility of sulfonylureas at pH 1 prevents the adequate testing in these conditions. Contrary to what has appeared previously, activated charcoal effectively adsorbs different sulfonylureas and can be used to possibly prevent their gastrointestinal absorption.

Keyphrases □ Activated charcoal—adsorption, sulfonylureas, carbutamide, chlorpropamide, tolazamide, tolbutamide, glibenclamide, glipizide □ Sulfonylureas—adsorption to activated charcoal □ Adsorption—sulfonylureas, activated charcoal

The capacity of activated charcoal to adsorb chemicals has been recognized for centuries, and in many countries charcoal is generally used as an antidote for intoxication. Maximal amounts of drugs adsorbed by charcoal of good quality are on the order of 100–1000 mg/g of charcoal (1–4). Decker *et al.* (4) have concluded from their *in vitro* studies that compounds insoluble in aqueous acidic solution, such as tolbutamide, are not adsorbed to any measurable extent onto activated charcoal. Others have cited this and extended the conclusions to include other sulfonylureas as well. Recently, claims have been made that activated charcoal is ineffective (5, 6) or even contraindicated (7) as an antidote in poisonings caused by tolbutamide or sulfonylureas in general. This paper reports the adsorption of six commonly used sulfonylureas onto activated charcoal *in vitro*.

EXPERIMENTAL

Material—Activated charcoal[1], carbutamide[2], chlorpropamide[3], tolazamide[4], tolbutamide[5], glibenclamide[2] (glyburide), and glipizide[4] were used as received. Dichlormethane, acetonitrile, isopropyl alcohol, and methanol were HPLC grade. All other reagents were analytical grade quality and were used as received.

Preparation of Drug Solutions—Solutions containing 700 mg/L of various sulfonylureas (or glipizide 360 mg/L, glibenclamide 100 mg/L) were prepared in 50 mM phosphate buffer as follows. The solutes were first dissolved in a small amount of 0.1 M NaOH, then phosphate buffer (pH 6.5) was added and the pH was adjusted to 7.5 with 1 M NaOH. Gilbenclamide, which is very sparingly soluble, was first dissolved in an ethanol–sodium hydroxide solution.

In addition, carbutamide and chlorpropamide were dissolved in two phosphate–acetate buffers, pH 7.5 and 4.9, containing 50 mM phosphate and 40 mM acetate. The sulfonylureas were maintained in the aforementioned solutions for several days at room temperature and at 4°C.

Adsorption Studies—The adsorption studies were carried out at room temperature (20–24°C) either at pH 7.5 or 4.9 (phosphate or phosphate–acetate buffers; see Preparation of Drug Solutions). A solution of 20 mL of sulfonylureas in different concentrations and 20 mg of charcoal were mixed in 30 mL stoppered glass tubes. When the effect of pH was studied, 16 mg of charcoal was added to 25 mL of the drug solution to achieve lower charcoal-to-drug ratios. The charcoal-to-drug ratio varied from 0.91:1 to 27:1. The tubes were shaken for 10 min, after which time the solutions were allowed to stand for an additional 10 min and then centrifuged (1800×*g* for 10 min). The drug concentration in the supernatant was determined.

Determination of Sulfonylurea Concentrations—The UV absorption of sulfonylureas could not be used for direct determination of these drugs because of the unpredictable amount of impurities absorbing at the UV range eluted from charcoal. The drug concentrations were therefore determined by HPLC[6] with some modification of the original procedure (8). The impurities from the charcoal did not interfere with the HPLC assay. Aliquots of the supernatant were diluted to constant volume with buffer and acidified with hydrochloric acid. The drugs were extracted with dichlormethane-containing internal standard (carbutamide or tolbutamide). The organic phase was separated and evaporated in a nitrogen stream. The residue was dissolved in methanol, and samples of this were injected into the chromatograph. The separation was performed with a C-18[7] column which was eluted with 50 mM phosphate buffer–acetonitrile, 55:45, pH 3.9 at a flow rate of 2.5 mL/min. For analysis of glibenclamide 10% isopropyl alcohol was added to the mobile phase to shorten the retention time. The sulfonylureas were monitored with a variable-wavelength UV detector[8] at 230–240 nm, depending on the optimal ratio of absorption of the derivatives and the internal standard.

The coefficient of variation between runs for the whole process comprising incubations with activated charcoal and drug determination was

[1] Carbomix (Norit A), Medica Ltd, Helsinki.
[2] Orion Ltd, Helsinki.
[3] Farmos Group Ltd, Turku.
[4] Medica Ltd, Helsinki.
[5] Hoechst AG, Frankfurt.

[6] HPLC: M6000A Chromatography pump and 6UK injector; Waters Associates, Milford, Mass.
[7] μBondapak C_{18}; Waters Associates, Milford, Mass.
[8] SF 770 Spectroflow; Schoeffel, Westwood, N.J.

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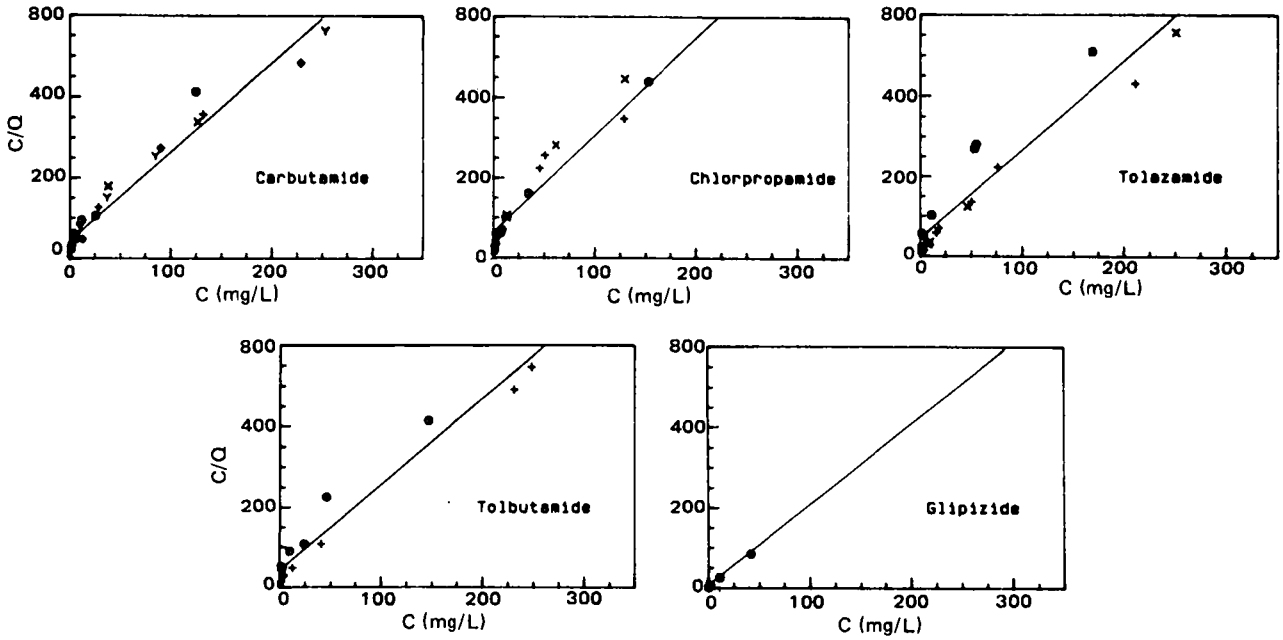

Figure 1—*Langmuir adsorption isotherms for sulfonylureas. Different symbols represent individual experiments. The correlation coefficients varied from 0.968 (tolazamide) to 1.00 (glipizide).*

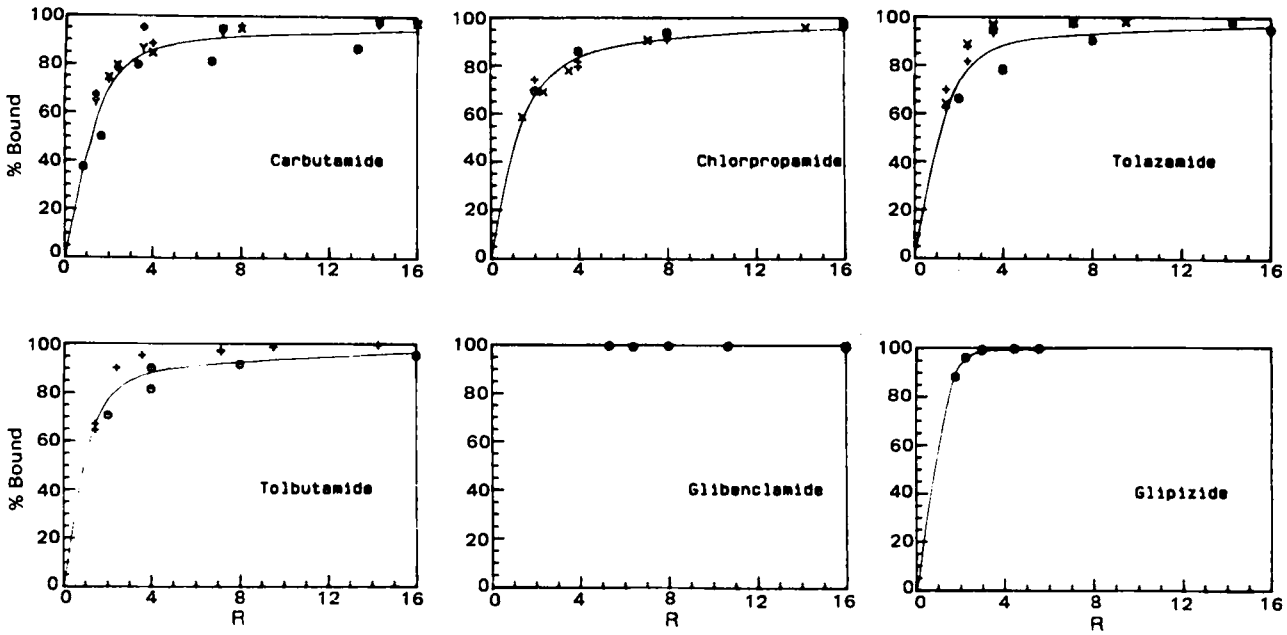

Figure 2—*Correlation of percentage adsorption of sulfonylureas to charcoal–drug ratio (R). Different symbols represent individual experiments.*

9.9% (N = 25). The coefficient of variation within run for drug determination only was 1.9% (N = 8).

Calculations—The Langmuir isotherm was constructed by the following equation (1):

$$\frac{C}{Q} = \frac{1}{KQ_m} + \frac{C}{Q_m}$$

where C is the concentration of free drug in the solution at equilibrium (mg/L), Q is the weight of drug (g) adsorbed per weight unit of charcoal (g), Q_m is the maximal adsorbing capacity, and K is a constant indicating the affinity of the drug to charcoal and physically represents the reciprocal of free drug concentration when half of the maximal binding capacity is used. The plot of C/Q *versus* C yields a straight line, and linear regression analysis was used to calculate the best line through the experimental data and to evaluate the adsorption parameters.

Table I—Parameters for Langmuir Adsorption Isotherm of Various Sulfonylureas, Phosphate Buffer pH 7.5

Parameter	Carbutamide	Chlorpropamide	Tolazamide	Tolbutamide	Glipizide
Q_m (g/g)	0.46	0.42	0.45	0.47	0.50
K (L/mg)	0.045	0.036	0.062	0.052	1.204

RESULTS

The Langmuir adsorption isotherms at pH 7.5 for carbutamide, chlorpropamide, tolazamide, tolbutamide, and glipizide are given in Fig. 1 and the calculated parameters in Table I. Due to the poor water solubility of glibenclamide the charcoal–drug ratio in the conditions used remained too high to allow the determination of its adsorption parameters.

Figure 2 shows the percentage adsorption of six sulfonylureas as a function of the charcoal–drug ratio. The adsorption of glibenclamide was

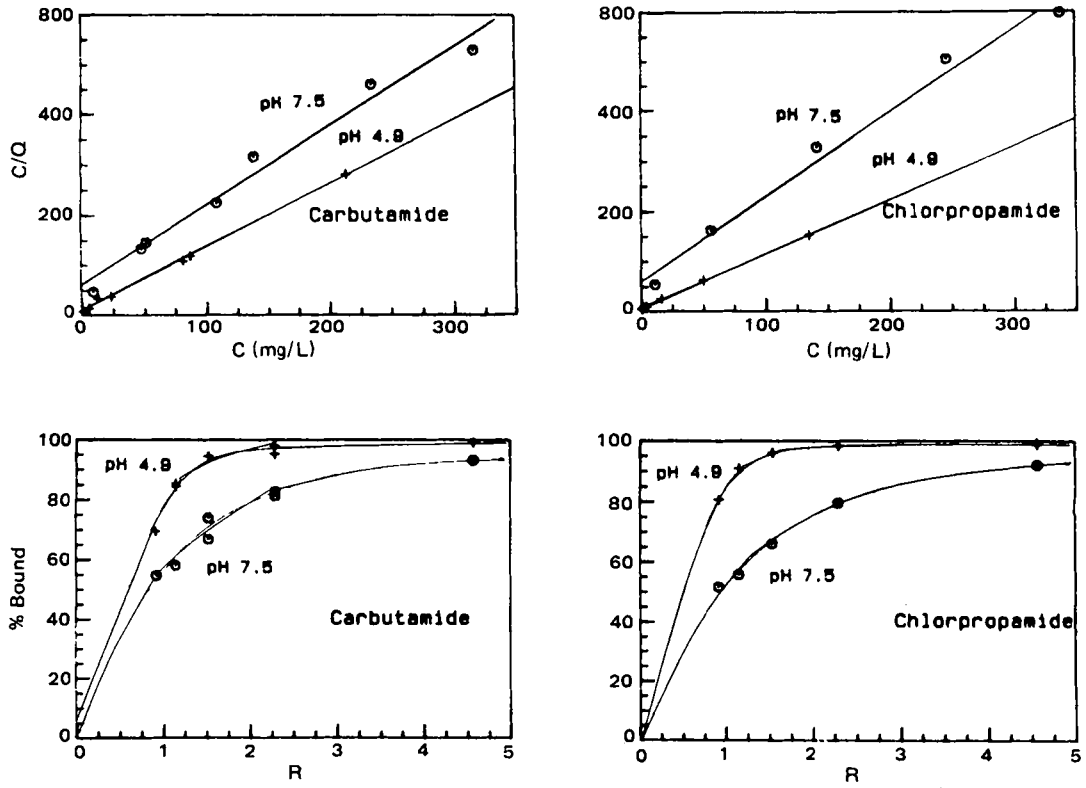

Figure 3—*Langmuir adsorption isotherms (top) and percentage adsorption (bottom) of carbutamide and chlorpropamide at pH 7.5 and 4.9.*

more than 99% at all charcoal–drug ratios tested (5.3:1–16:1). For other sulfonylureas the adsorption of 90% was attained at charcoal–drug ratios from ~2 (glipizide) to 6 (chlorpropamide).

As seen in Fig. 3 and Table II, the decrease of the incubation medium pH from 7.5 to 4.9 increased the affinity of carbutamide and chlorpropamide to charcoal. The differences of K were significant ($p < 0.001$), whereas the maximal binding capacities were only insignificantly increased ($p < 0.10$). The differences were tested by comparing the regression lines (9).

DISCUSSION

The adsorption of different drugs onto activated charcoal *in vitro* has been studied by several authors (1–4), but there have been no systematic studies on the adsorption of sulfonylureas. However, the statement, "The adsorptive capacity of activated charcoal for ferrous sulphate, sulfonylureas and organo-phosphorous insecticides is too low to be useful," has appeared (5), and activated charcoal has even been claimed to be contraindicated in poisonings caused by tolbutamide (7). To our knowledge, these conclusions are based on very limited *in vitro* studies in acidic conditions, in which the water solubility of tolbutamide was rather limited, so that its adsorption onto charcoal could not be measured (4).

Quite recently, the effect of activated charcoal on the absorption of tolbutamide and chlorpropamide has been studied in humans (10, 11). Activated charcoal (50 g), ingested 5 min after these drugs, reduced their absorption by ~90%. The charcoal–drug ratio was rather high in these two studies. However, *in vivo* the absolute amount of charcoal is probably more important than the charcoal–drug ratio, as has been demonstrated earlier (12, 13).

The present results demonstrate that sulfonylureas are adsorbed onto charcoal at pH 7.5 *in vitro*. In an acidic medium they seem to be even more effectively adsorbed, provided that they are soluble at that pH. The affinities of carbutamide and chlorpropamide were considerably higher at pH 4.9 than at pH 7.5, which was predictable because of the shift in equilibrium toward nondissociable, nonpolar species of these weak acids. Similar behavior of other acidic substances has been reported (6, 14).

Table II—Parameters for Langmuir Adsorption Isotherm of Carbutamide and Chlorpropamide, Phosphate–Acetate Buffer, pH 7.5 and 4.9

Parameter	Carbutamide		Chlorpropamide	
	pH 7.5	pH 4.9	pH 7.5	pH 4.9
Q_m (g/g)	0.62	0.78[a]	0.58	0.91[a]
K (L/mg)	0.028	0.160[b]	0.026	0.189[b]

[a] The differences from the respective value at pH 7.5, $p < 0.10$, [b] $p < 0.001$.

In the present study the adsorption isotherms in phosphate–acetate buffer, pH 7.5 differed from those in phosphate buffer, pH 7.5 (Tables I and II). This might be due to the buffers or to the fact that a different charcoal concentration (16 mg/25 mL instead of 20 mg/20 mL) was used in the experiments with phosphate–acetate buffer.

The affinity of the second generation sulfonylureas, glibenclamide and glipizide, to charcoal is considerably higher than that of the older derivatives (Table I, Fig. 2). Clinically used doses of glipizide and glibenclamide are only about $^1/_{100}$ of those of the first generation derivatives. Thus, most probably, the generally recommended doses of activated charcoal (50 g), given soon enough after the ingestion of glipizide and glibenclamide, would be effective in preventing their absorption. In massive intoxication caused by the older sulfonylureas, the adsorption capacity of charcoal may be saturated and the dose of charcoal should, therefore, be as high as possible.

Although poisoning due to oral hypoglycemic agents is relatively uncommon, several deaths and cerebral damage have been reported. Of 16 patients who had deliberately ingested toxic doses of a sulfonylurea, 6 died (15). The gastrointestinal absorption of many sulfonylureas is slow, at least partially due to their poor solubility at the gastric pH. Therefore, their absorption in acute intoxication may be delayed for several hours so that they may be adsorbed onto charcoal given even several hours later.

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Equilibrium Dialysis of Barbituric Acids up to High Concentrations of Aqueous Sodium Alkylsulfonate Solutions

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Abstract □ The partition coefficient K of four barbituric acids has been determined by the equilibrium dialysis method in aqueous solutions of sodium alkylsulfonate at 25°C. The results obtained from dilute solutions to 0.3 M surfactant concentration are compared with solubility data for the same systems. The K values as deduced from Langmuir or Freundlich isotherms decrease with increasing surfactant concentration. The detergent used was an impure commercial product of known composition, which could be considered as a mixed surfactant. The change of partition coefficient with surfactant concentration as obtained from equilibrium dialysis experiments has been interpreted by assuming a continuous change of micelle composition.

Keyphrases □ Barbituric acids—equilibrium dialysis up to high concentrations of aqueous sodium alkylsulfonate solutions, solubility □ Sodium alkylsulfonate—aqueous solutions, equilibrium dialysis of barbituric acids up to high concentrations, solubility □ Equilibrium dialysis—barbituric acids up to high concentrations of aqueous sodium alkylsulfonate solutions, solubility □ Solubility—equilibrium dialysis of barbituric acids up to high concentrations of aqueous sodium alkylsulfonate solutions

The solubility of 13 barbituric acids as a function of sodium alkylsulfonate concentration between 25°C and 55°C shows a rather sharp change in slope at low surfactant concentrations (~0.05 mol/L); the solubility increases less rapidly above this concentration than below it (1, 2). The ionic surfactant used was not pure but was a typical commercial product mixture consisting of 91% monosulfonated and 9% disulfonated ions. A model was suggested (1) to explain the solubility profile, which assumed two types of mixed micelles: predominantly monosulfonated mixed micelles at low surfactant concentration and predominantly disulfonated micelles at high concentrations. To further investigate this micellar system, we decided to study the solubilization behavior at 25°C of four typical barbituric acids (amobarbital, phenobarbital, secobarbital, and allobarbital) with the same surface-active agent using equilibrium dialysis instead of the solubility method.

Table I—Characteristics of Barbituric Acids

Barbituric Acid	Solubility, mol/L[a]	Melting Point, °C
Amobarbital	0.0022	157
Phenobarbital	0.0052	174
Allobarbital	0.0087	172
Secobarbital	0.0044	98

[a] Solubility in water at 25°C.

EXPERIMENTAL

Materials—The barbituric acids[1] were used without further purification. The melting points and the solubility in water at 25°C are presented in Table I. The sodium alkylsulfonate was a commercial product[2] containing monosulfonated ion–disulfonated ion–polysulfonated ion (90.7:8.8:0.5, v/v/v). The monosulfonated product was a mixture of $C_{14}H_{29}SO_3Na$ and $C_{15}H_{31}SO_3Na$, whose average molecular weight was 321.

Methods—The equilibrium dialysis experiments were performed using dilute to saturated solutions of barbituric acids at 25°C thermostatically controlled to within ±0.1°C with polytef cells and a cellulose acetate membrane. The equilibrium was attained in ~4 h. Barbituric acid concentrations were analyzed using a UV spectrophotometer. Each experiment was repeated five times, and at least six barbiturate concentrations were studied for each surfactant concentration.

The solubility of the barbituric acid is dependent on the pH of the medium. The pH of the solutions was measured at each concentration, and a correction applied to the concentration of barbituric acid on both sides of the dialysis using:

$$S_T = S_0(1 + 10^{pH-pK}) \qquad \text{(Eq. 1)}$$

where S_T and S_0 are the total concentration and the concentration of undissolved barbituric acid of a given pK, respectively.

RESULTS

Sodium alkylsulfonate showed a very high degree of adsorption onto the cellulose acetate membrane (Fig. 1). This adsorption was reproducible, and a systematic correction was applied. The barbituric acids were also partially adsorbed onto the membrane. A correction was also applied which amounted at most to 5% of the total concentration of the drug dissolved in the solution.

Two types of isotherms were used to obtain the partition constants. The Freundlich isotherm may be written as:

$$\log r = \log K + \frac{1}{n_f}\log B_{aq} \qquad \text{(Eq. 2)}$$

[1] Amobarbital and secobarbital obtained from: Expandia, 13 Avenue de l'Opéra, 75001 Paris, France. Allobarbital obtained from: Soprotec, 144 Avenue de Malakoff, 75116 Paris, France. Phenobarbital obtained from: Coopération Pharmaceutique Française, 66 Rue du Chemin Vert, 75001 Paris, France.

[2] Société des Produits Chimiques de la Montagne Noire, 81100 Castres, France.

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