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Trace enrichment on a metal-loaded thiol stationary phase in liquid chromatography: effect of analyte structure and pH value on the (de)sorption behaviour*

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Abstract: In order to obtain more information on the (de)sorption processes on a metal-loaded thiol phase, various compounds containing heterocyclic nitrogen atoms have been studied. Adenine and adenosine, which contain no carbonyl groups in a position *ortho* to the ring nitrogen, were chosen to study the role of the binding donor atoms and, therefore, of the 'minimum' structure required for efficient pH-dependent (de)sorption. The results indicate that a single heterocyclic nitrogen atom, preferably with a high pK_a value, is sufficient to provide pH-dependent (de)sorption on chelating phases loaded with soft metal ions, such as Ag(I) or Hg(II), which can profitably be used in an on-line combination with column liquid chromatography. Barbiturates were chosen on the basis of their structural similarity with the earlier studied (halogenated) uracil derivatives. The (de)sorption behaviour of the barbiturates was studied as a function of the pH, the metal ion [Cu(II), Ag(I), Hg(II), Pd(II)] bound to the stationary phase and the type of stationary phase. The combined data show that the pK_a value of an analyte and the stability constant of the complex formed with the metal ion sorbed on the stationary phase give a reliable indication of the optimum (de)sorption pH. Application of this principle is demonstrated by the determination of four barbiturates in plasma.

Keywords: *Trace enrichment; metal-loaded phases; pH dependence of (de)sorption; complex formation; reversed-phase LC.*

Introduction

In two recent papers [1, 2] the suitability of silver-loaded stationary phases for the trace enrichment of pyrimidine nucleobases such as uracil, thymine and cytosine, and derivatives such as uridine, 5-fluorouracil, bromacil and azidothymidine (AZT) combined with on-line reversed-phase liquid chromatography (LC) has been demonstrated. All compounds investigated, with the exception of uridine, form sufficiently

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strong complexes with Ag(I), Hg(II) and Pd(II) to be retained on a thiol stationary phase loaded with these metal ions. Both sorption and desorption of the ligands were mainly influenced by the pH value of the sample solution; the optimum sorption pH was about two to three units higher than the pK_a value of the corresponding compound. This confirmed that complex formation requires the deprotonation of at least one of the nitrogen atoms of the pyrimidine ring.

Complete desorption of the uracil derivatives from the Ag(I)-thiol phase was obtained at a pH value of 5–6 which is about three units lower than the pK_a value of these analytes, resulting, however, in a rather bad peak shape; this indicates also that the desorption kinetics are strongly pH dependent. Much narrower peaks were obtained if a small volume of a strongly acidic solution (pH 1.2) was injected onto the Ag(I)-thiol pre-column to achieve desorption. As regards desorption, the metal ions investigated behaved differently: Pd(II) and Hg(II) formed much stronger complexes with the uracil derivatives than Ag(I), which resulted in lower recoveries even if 0.6 M perchloric acid (60 µl) was used for desorption.

From these experiments several questions arose concerning more fundamental aspects of the (de)sorption process such as, for example:

(i) does a 'minimum structure' exist for analytes to make them suitable candidates for efficient (de)sorption?

(ii) is the (de)sorption behaviour of an analyte predictable from its pK_a value and the stability constant of its complex with a suitable metal ion and how can it be related to the metal ion chosen?

(iii) which restrictions are imposed on the system, e.g. during injection of a small volume of a strongly acidic solution, by the available chelating stationary phases?

The compounds investigated in the earlier studies possess a carbonyl group in a position *ortho* to a ring nitrogen; that is, coordination can occur through the nitrogen and oxygen atoms [3]. In order to find out whether or not both atoms are involved in complex formation, the purine derivatives adenine and adenosine (see Fig. 1) were investigated. These compounds contain no carbonyl group in an *ortho* position to a ring nitrogen; therefore, no chelation can take place [4–7].

Regarding the relationship between the pK_a value of an analyte, the stability constant of the complex formed during reaction and the optimum (de)sorption pH, it is necessary to consider the competition of divalent metal ions for instance and protons for the binding site of a ligand, L^- (formation of a 1:1 complex):

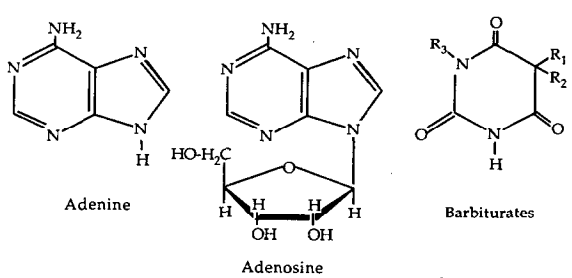

Figure 1
Structure of adenine, adenosine and barbiturates.

$$MeL^+ + H^+ \rightleftarrows Me^{2+} + HL \quad (1)$$

with the corresponding equilibrium constant

$$K = \frac{[Me^{2+}][HL]}{[H^+][MeL^+]}. \quad (2)$$

For the formation of the 1:1 complex according to

$$Me^{2+} + L^- \rightleftarrows MeL^+ \quad (3)$$

it is possible to write the stability constant, K_{MeL}, as:

$$K_{MeL} = \frac{[MeL^+]}{[Me^{2+}][L]}. \quad (4)$$

For the association of the free ligand according to

$$H^+ + L^- \rightleftarrows HL \quad (5)$$

the corresponding expression is:

$$K_{HL} = \frac{[HL]}{[H^+][L]}. \quad (6)$$

The equilibrium constant, K, of reaction (1) is the ratio of the acidity constant of HL (equation 6) and the stability constant of MeL^+ (equation 4):

$$K = \frac{K_{HL}}{K_{MeL}}. \quad (7)$$

Because, by definition, K_a equals $1/K_{HL}$, this equation also can be written as

$$\log K = pK_a - \log K_{MeL}. \quad (8)$$

Under ideal conditions — that is, if steric hindrance and competition with other ligands do not occur and the kinetics are fast — it is possible to predict, that ligands with a high stability constant and a low pK_a value will be efficiently preconcentrated, but are difficult to desorb. On the other hand, ligands with a low stability constant and a high pK_a value should bind less strongly to the metal-loaded phase, but should be more easily desorbed. In order to study the relationship which exists between the pK_a value, the stability constant of a complexing analyte and the optimum (de)sorption pH, 5,5-disubstituted barbiturates (see Fig. 1) were chosen as test compounds. They possess pK_a values in a relatively narrow range (pK_1 = 7.0–8.2 [8]) similar to those of the (halogenated) uracil derivatives. Barbiturates furthermore are easy to separate by reversed-phase LC and a great number of differently substituted compounds is available.

Experimental

Preconcentration/LC system

The analytical set-up is shown in Fig. 2. The LC system consisted of three home-made six-port Valco-type injection valves, a 1.0 ml sample loop, a 60 μl loop to contain the perchloric acid needed for the desorption of analytes, a 10.0 × 4.0 mm home-made stainless-steel pre-column and holder (Chrompack, Middelburg, The Netherlands) and a 200 × 4.6 mm I.D. Hypersil ODS 5 μm (Shandon Southern, Runcorn, UK) analytical column. The carrier solution for preconcentration was delivered by a Gilson (Villiers-le-Bel, France) Model 302 single-head reciprocating pump and the LC eluent by a Waters (Milford, MA, USA) model 510 dual-head reciprocating pump. Pulse dampers are incorporated into the system for each pump. 40–63 μm Spheron (Lachema, Brno,

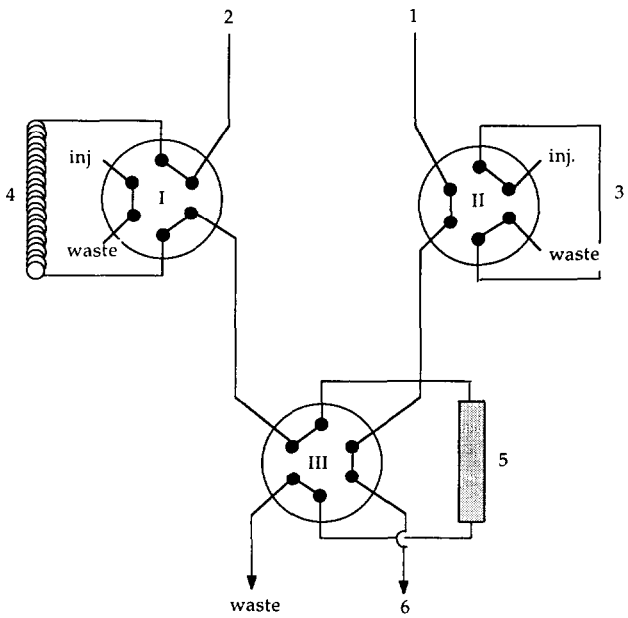

Figure 2
Schematic representation of the analytical system. 1: LC pump; 2: preconcentration pump; 3: injection loop for perchloric acid; 4: sample injection loop (1.0 ml); 5: Ag(I)-thiol pre-column; 6: to analytical column/detector; I, II, III, six-port injection valves.

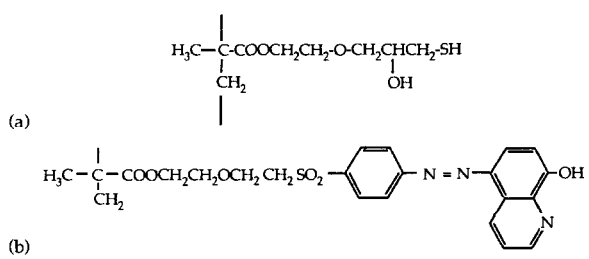

Figure 3
Structure of (a) the Spheron thiol and (b) the Spheron oxine (8-hydroxyquinoline) phase.

Czechoslovakia) thiol (Fig. 3a) and Spheron oxine (Fig. 3b) were used as chelating stationary phases.

The carrier and sample solutions were identical regarding their pH value, type and buffer concentration (10 mM phosphate for pH 5–8, 10 mM borate for pH 8–11, KOH for a pH higher than 11) and methanol percentage (30%, v/v). The preconcentration carrier flow-rate was 0.6 ml min^{-1}. The LC mobile phase (flow-rate, 1.2 ml min^{-1}) for the separation of pheno-, amo-, hexo- and butobarbital was acetate buffer 20 mM (pH 5.0) methanol (42:58, v/v). The UV detection wavelength was 220 nm. Adenine and adenosine were chromatographed using acetate buffer 10 mM (pH 6.0)–acetonitrile (5:95, v/v) and (8:92, v/v), respectively, as mobile phase with UV detection at 269 nm.

Chemicals

Barbituric acid, adenine and adenosine were supplied by Merck (Darmstadt, FRG). All the 5,5-disubstituted barbiturates were supplied by the Academic Hospital of the Free University. All other chemicals were of analytical grade purity.

Procedure for trace enrichment

The chelating stationary phase for trace enrichment was slurry packed via a 5 ml syringe into the stainless-steel pre-column, loaded off-line with 5 ml of a 10 mM solution of the respective metal nitrate and flushed with 5 ml deionized, distilled water and 5 ml HPLC-grade methanol. A 1.0 ml sample plug was flushed through the pre-column at a carrier flow-rate of 0.6 ml min^{-1}. Plasma samples were deproteinized according to the procedure described previously [2]. On-line desorption to the analytical column was carried out by injecting a plug of perchloric acid into the LC eluent stream and, after an appropriate time delay (7 s, the time needed to displace the LC mobile phase from the capillary connecting valve II and III with perchloric acid), switching to the pre-column to desorb the preconcentrated analytes and transfer them to the analytical column.

Results and Discussion

All recovery experiments described in this section were performed in the following way. First, breakthrough curves were recorded as a function of the sorption (sample) pH in order to evaluate whether the analyte is sorbed on the metal-loaded phase. Next, the dependence of the recovery on the sorption (sample) pH was investigated by preconcentration of 1 ml samples of various pH values, with subsequent on-line desorption to the analytical column at the optimum desorption pH. This optimum pH had been determined by preconcentration of 1 ml samples at the optimum sorption pH found in the breakthrough experiments, and subsequent desorption at various pH values.

'Minimum' structure required for effective (de)sorption

Sorption of adenine and adenosine on Ag(I)-thiol. Breakthrough experiments showed that adenine and adenosine are readily sorbed on Ag(I)-thiol over a broad pH range. The sorption pH providing the highest breakthrough volumes (V_B) was 9 (V_B = 47 ml) and 12 (V_B = 3 ml), respectively. Furthermore, it was found that sorption of adenine on Ag(I)-thiol starts to decrease rapidly at a pH of 10 (V_B = 12 ml). The difference in the sorption behaviour of adenine and adenosine may be explained by the fact that N(9) — the most basic site of adenine — is not available for coordination in adenosine, where it is

blocked due to the glycosyl binding. Binding of adenosine has to occur via the less basic nitrogen atoms in the 1 and 7 positions, which probably results in a lower complex stability. This is confirmed by stability data for homogeneous systems where Cu(II), for example, forms considerably stronger complexes with adenine (log K_1 = 7.4) than adenosine (log K_1 = 0.9). The nitrogen of the amino group in the 6 position is not expected to be involved in complex formation [7]. The decrease of V_B for adenine at pH values higher than 9 may be explained by competition with hydroxide ions or slower reaction kinetics.

The recovery as a function of the sorption pH for adenosine (preconcentration of 1 ml samples; desorption, 180 µl of 1 M perchloric acid; cf. below) is shown in Fig. 4. Such data cannot be presented for adenine due to the incomplete desorption of this compound from the Ag(I)-thiol phase under all conditions studied (see below).

On-line desorption of adenine and adenosine. The desorption behaviour of adenine and adenosine also reflects the difference in stability of their Ag(I) complexes. The recovery for adenine (preconcentration of 1 ml; sorption pH, 9) after injection of 180 µl of 1 M perchloric acid was only 70–75%. A second injection of 180 µl fo 1 M perchloric acid on the same Ag(I)-thiol pre-column provided an additional recovery of 20–25%; this means that adenine is strongly retained on Ag(I)-thiol and will not be desorbed in significant amounts by the LC mobile phase (pH 6) which is flushed through the pre-column between the first and second perchloric acid injection.

Adenosine, on the other hand, is completely desorbed after one injection of 180 µl of 1 M perchloric acid. Desorption of adenosine also can be performed using solutions of a higher pH (see Fig. 5). However, as shown in the same figure, this resulted in increased peak broadening which indicates that the desorption kinetics are strongly pH dependent.

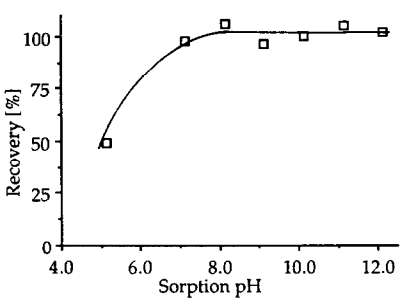

Figure 4
Dependence of the recovery of adenosine, retained on Ag(I)-thiol, on the sorption (sample) pH (recoveries based on peak area measurements were calculated by comparison with direct loop injections of 50 µl). LC conditions: preconcentration of 1 ml; sample pH, as indicated on the *X*-axis; desorption, 180 µl of 0.5 M perchloric acid; other conditions, see Experimental.

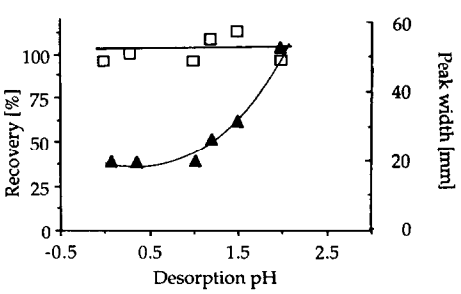

Figure 5
Desorption of adenosine from Ag(I)-thiol as a function of the pH of the desorption solution (▲, peak width ot 10% peak height [mm]; □, recovery [%]). LC conditions: preconcentration of 1 ml; sorption pH, 12; desorption, 180 µl of perchloric acid (pH as indicated on the *X*-axis); other conditions, see Experimental.

Influence of the pH value on the (de)sorption behaviour of barbiturates

Sorption of barbiturates on Ag(I)-thiol. Breakthrough experiments with allo-, apro-, pheno-, buto- and hexobarbital showed that complete retention (V_B > 10 ml) of all analytes occurred already at pH 7.5, that is, at a pH which is equal or lower than the pK_a value of the corresponding compound. The recoveries of the four barbiturates as a function of the sorption pH obtained by preconcentration experiments (sample volume, 1 ml; desorption solution, 180 µl of 1 M perchloric acid) are shown in Fig. 6. Recoveries of over 85% were obtained already at sorption pH of 6.0, a pH value which is about two units lower than the pK_a value of the various compounds. An optimum sorption pH of 7–7.5 was obtained for all barbiturates investigated, a value which was used in all further experiments. As with adenine, recoveries decreased for pH values higher than 8 and were only about 40% for pH 11.5, i.e. the optimum pH for uracil compounds. Barbituric acid which has a pK_a value of 4.4, also is readily retained by Ag(I)-thiol but could not be desorbed with 180 µl of 1 M perchloric acid. This indicates that the pK_a value of barbituric acid is too low and/or the Ag(I)-barbituric acid complex is too stable to allow the desorption from Ag(I)-thiol by means of protonation.

The significant difference in the sorption behaviour of barbiturates and uracil derivatives is demonstrated in Fig. 7. However, both groups of compounds behave

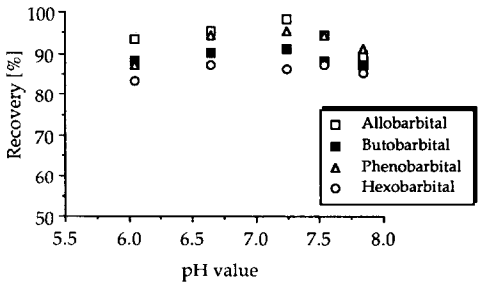

Figure 6
Dependence of the recovery of allo-, buto-, pheno-, and hexobarbital on the sorption pH. LC conditions: preconcentration of 1 ml; sorption pH, as indicated on the *X*-axis; desorption, 180 µl of 1 M perchloric acid; other conditions: see Experimental.

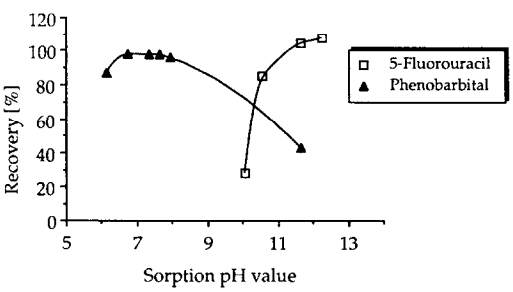

Figure 7
Comparison of the sorption behaviour of phenobarbital and 5-fluorouracil. LC conditions: for phenobarbital, see Fig. 9 (i.e. total recovery of two desorption steps); for 5-fluorouracil, cf. ref. [1].

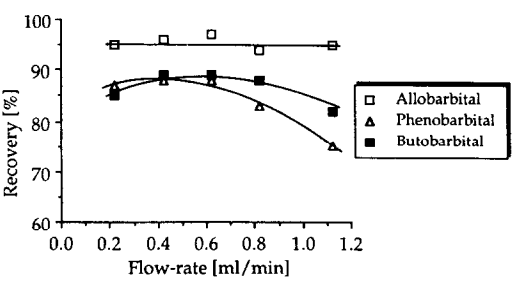

Figure 8
Percentage recovery of allobarbital, phenobarbital and butobarbital (sorption pH, 7.2) as a function of the carrier flow-rate. LC conditions: preconcentration of 1 ml samples; sorption pH, 7.2; desorption, 180 µl of 1 M perchloric acid; other conditions, see Experimental.

almost identically regarding the flow dependence of the sorption process (see Fig. 8; for uracil derivatives, see Fig. 4 in ref. [1]).

The results suggest that barbiturates form stronger complexes with Ag(I) than uracil derivatives: Ag(I) can compete more effectively with H^+ for the pyrimidine nitrogen in barbiturates than in uracils at a pH equal to the pK_a value. In other words, the pK_a value can give only a rough indication of the optimum sorption pH. As was the case with bromacil [1], alkyl substituents on one pyrimidine nitrogen, as in, e.g. hexobarbital, have no significant negative effect on the recovery.

On-line desorption of barbiturates. The dependence of the recovery (black boxes) on the desorption pH (sorption pH, 7.2; desorption, 180 µl of perchloric acid) for buto- and phenobarbital are shown in Fig. 9(a and b). Obviously, the desorption behaviour of the barbiturates and uracil derivatives differs strongly. At the pH of the LC mobile phase (pH 5) no barbiturates are desorbed at all, while the uracil compounds are completely desorbed under these conditions (although a bad peak shape was obtained at this pH; cf. ref. [1]). At a desorption pH of 1.2, the recoveries for the barbiturates still were only about 70%, and as low as 40% for phenobarbital. Best results were obtained when desorbing with 0.8–1 M perchloric acid (pH 0–0.1).

Phenobarbital is more strongly retained on Ag(I)-thiol than the other four barbiturates studies, exemplified by butobarbital in Fig. 9. It is only 75% desorbed with 0.8 M perchloric acid compared to 98% for butobarbital, the 20–25% difference remaining the same for all desorption pHs investigated. In all experiments, the fraction of the barbiturates still bound to Ag(I)-thiol after the first desorption could be recovered almost quantitatively with a (second) injection of 180 µl of 1 M perchloric acid on the same Ag(I)-thio, pre-column (see Figs 9a and b, white boxes). The total recovery for all barbiturates was higher than 90%.

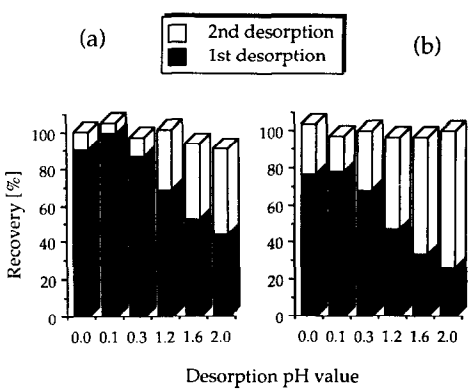

Figure 9
Percentage recovery of (a) butobarbital and (b) phenobarbital as a function of the desorption pH value. LC conditions: preconcentration of 1 ml; sorption pH, 7.2; 1st desorption, 180 µl of perchloric acid (pH, see *X*-axis of the figure); 2nd desorption, 180 µl of 1 M perchloric acid; other conditions, see Experimental.

Influence of the metal ion on the (de)sorption behaviour of barbiturates

Next to Ag(I), Cu(II), Hg(II) or Pd(II) were also tested for their suitability in the described (de)sorption system. Results obtained with uracil derivatives [1] indicate that the order of complex stability is Pd(II) > Hg(II) > Ag(I) > Cu(II). Because barbiturates form stronger Ag(I) complexes than uracil derivatives, it was interesting to know, (i) whether Cu(II)-barbiturate complexes are sufficiently stable to obtain complete retention of barbiturates on Cu(II)-thiol, and (ii) which desorption recoveries will be obtained for Pd(II)- and Hg(II)-thiol.

Breakthrough experiments using Cu(II)-, Ag(I)-, Hg(II)- and Pd(II)-thiol as stationary phase showed that, at a sorption pH of 7.2, all barbiturates investigated had V_B values higher than 10 ml. Recovery data obtained for these metal-loaded phases are presented in Fig. 10. On-line desorption of the barbiturates from Cu(II)-thiol could be performed by the injection of 50 µl of 0.8 M perchloric acid. The desorption efficiency was comparable to that of the Ag(I)-thiol system. If Hg(II)-thiol or Pd(II)-thiol were used, the recoveries were about 10 and 1–2%, respectively (desorption solution, 50 µl of 0.8 M perchloric acid). Because all barbiturates have high V_B values for these phases, the low recoveries must be attributed to inefficient desorption by the protonation mechanism.

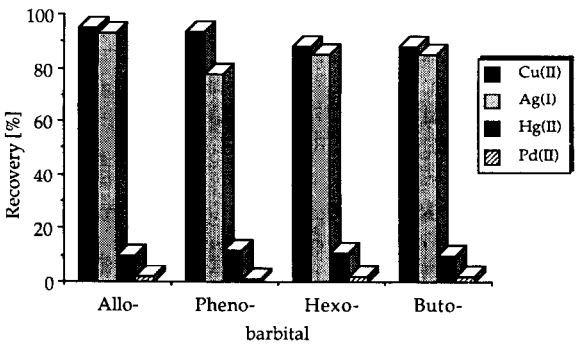

Figure 10
Recoveries for the desorption of allo-, pheno-, hexo- and butobarbital on Cu(II)-, Ag(I)-, Hg(II)- and Pd(II)-thiol. LC conditions: preconcentration of 1 ml; sorption pH, 7.2; desorption, 50 µl of 0.8 M perchloric acid; other conditions, see Experimental.

Effect of the chelating stationary phase on the (de)sorption behaviour of barbiturates

Two stationary phases were tested, namely, Spheron thiol and Spheron oxine. Unlike thiol, the oxine group, which has a pK_a of 11.4, has metal bonding characteristics which are strongly pH dependent. Maximum metal loading is reached at a high pH. In acidic solutions metal ions are rapidly released. Therefore this material is often used for the trace enrichment of metal ions before their determination by AAS or ICP-AES.

The barbiturates were sorbed quantitatively ($V_B > 10$ ml) by Cu(II)-thiol as well as Cu(II)-oxine. Recoveries of 80–90% were obtained for both materials after desorption with 50 µl of 0.8 M perchloric acid. With Cu(II)-thiol the recoveries were constant for at least 10 experiments (preconcentration of 1 ml; desorption, 50 µl of 0.8 M perchloric acid); however, for Cu(II)-oxine recoveries decreased below 2% after one injection of 50 µl of 0.8 M perchloric acid. This indicates that injection of strongly acidic solutions on Cu(II)-oxine results in a complete release of Cu(II), in spite of the rather high stability of the Cu(II)/8-hydroxyquinoline complex ($\log K_1 = 18.8$). The same behaviour can be expected for Ag(I)-oxine, because the stability of Ag(I)/8-hydroxyquinoline is considerably lower ($\log K_1 = 6.6$) than that of the Cu(II) complex. Even though the oxine phase probably can be re-used after re-loading with the selected metal ion, the thiol phase should be preferred, since no metal ions are released during desorption. This also prevents interferences during the separation and detection of the analytes.

Applications

In two previous papers [1, 2] the suitability of a silver-loaded sorbent for the trace enrichment of 5-fluorouracil and azidothymidine from plasma samples has been demonstrated. In both cases, the removal of proteins before the preconcentration step was essential, since they interfered with the preconcentration, separation and detection of the analytes. Two different methods have been tested, namely precipitation with perchloric acid or methanol and a dual pre-column system and both techniques have been found to provide recoveries higher than 90% and a satisfactory relative standard deviation (4–6%).

The dual pre-column technique involves the preconcentration of the analytes on a styrene–divinylbenzene copolymer (PRP_1; Hamilton, Reno, NA, USA) pre-column, removal of water-soluble proteins by washing with acetate buffer followed by the transfer of the analytes to the Ag(I)-thiol pre-column by flushing with methanol–KOH (pH 11.5) (20:80, v/v). Apart from the addition of the acetate buffer, no off-line sample pretreatment is required. The method can be used for non-polar or medium polar analytes as was demonstrated by Nielen *et al.* [9] for the determination of barbiturates in urine. In some cases, the PRP_1 pre-column could only be used once; that is, a cartridge exchanger [10] is needed to allow automation of the method.

The precipitation of proteins using perchloric or trichloroacetic acid, or organic solvents such as methanol and acetonitrile has the advantage that it is generally applicable regardless of the polarity of the analytes. It requires, however, several off-line steps such as addition of acid or organic solvent, centrifugation, neutralization and pH adjustment. A chromatogram of the determination of four barbiturates in plasma after deproteination with perchloric acid is shown in Fig. 11. The 1-ml sample was diluted 1:1 due to neutralization and pH adjustment. However, because of the relatively high breakthrough volumes of barbiturates on Ag(I)-thiol ($V_B > 10$ ml), the whole sample volume (2 ml) could be preconcentrated; dilution of the sample therefore does not lead to a loss of sensitivity of the analytical method. If the analytes are of medium polarity, the dual pre-column system also can be applied after protein precipitation in order to retain the strongly apolar compounds present in the sample, which results in a drastic decrease of the analysis time.

Conclusions

Barbiturates as well as purine derivatives such as adenine and adenosine can be enriched quantitatively on a Ag(I)-thiol stationary phase. The sorption behaviour of adenine and adenosine shows that a single heterocyclic nitrogen atom, probably N(9) in adenine and N(1) or N(7) in adenosine, is sufficient to retain these compounds on a Ag(I)-loaded phase to such an extent that an on-line pre-column/column LC system can profitably be set up. Obviously binding on Ag(I)-thiol does not necessarily require a carbonyl group in a position *ortho* to a ring nitrogen. As was the case with bromacil and cytosine [1], the presence of substituents on the most basic nitrogen atom does not prevent sorption on Ag(I)-thiol, if one unsubstituted heterocyclic nitrogen is still available for complex formation. Further experiments with, e.g. substituted purines, will be necessary to evaluate whether compounds which contain only one heterocyclic nitrogen atom, are generally suitable for the (de)sorption system described and how the basicity of this nitrogen atom and steric effects influence the sorption on metal-loaded phases.

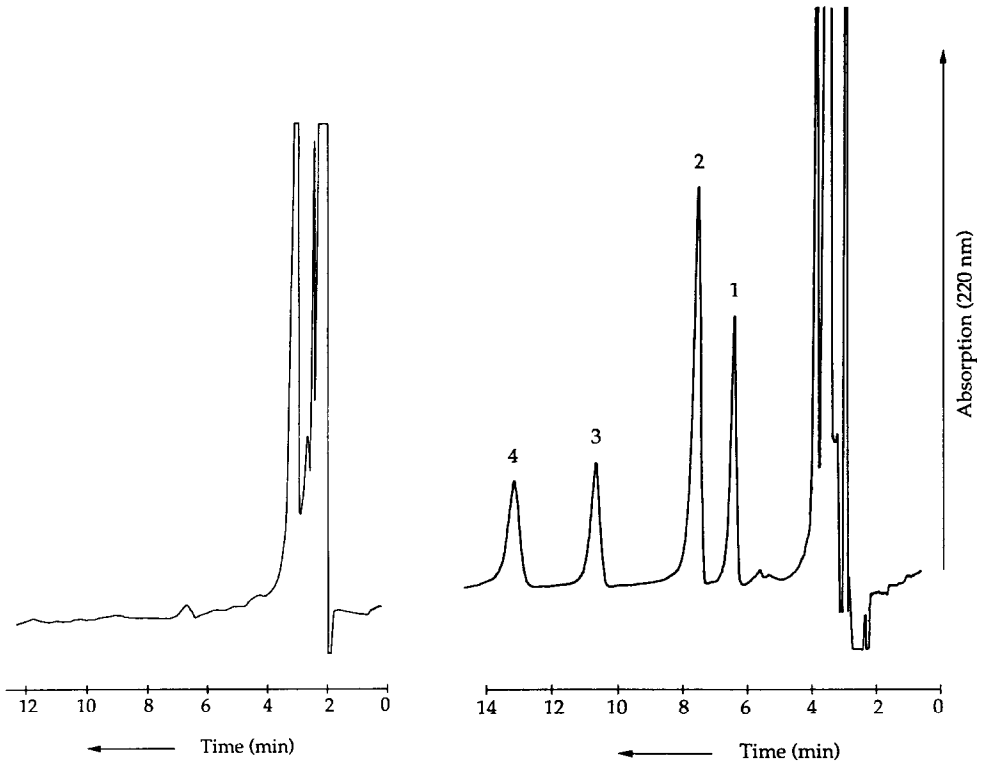

Figure 11
Determination of (1) allo-, (2) buto-, (3) pheno-, and (4) hexobarbital in plasma. (left) blank plasma, (right) plasma spiked with 10^{-6} M of barbiturates. Conditions: deproteination, with 7% perchloric acid as described in ref. [2]; preconcentration volume after neutralization and pH adjustment, 2 ml; other conditions, see Experimental.

The study also reveals that the sorption and desorption behaviour of a compound can, to a first approximation, be estimated from its pK_a value and the stability constant of the sorbed complex. To quote two examples, if the pK_a value of the ligand is distinctly higher than the logarithmic stability constant such as in Cu(II)/uracil (log K_{MeL} = 4.5, pK_2 = 9.7), the pH value has to be at least two units higher than the pK_a in order to retain the analyte quantitatively. If the pK_a value is lower than the logarithmic stability constant such as, e.g. in Cu(II)/phenobarbital (log K_{MeL} = 9.3, pK_a = 7.5), the compound can already be sorbed completely at a pH which is about equal to the pK_a value.

If desorption is considered, it is obviously favourable if the pK_a value of a compound is higher than the logarithmic stability constant, e.g. in Ag(I)/uracil. Desorption becomes more difficult (that is, requires more strongly acidic solutions) if the pK_a is lower than the logarithmic stability constant as, e.g. for Ag(I)/phenobarbital or Pd(II)/uracil. Among complexes with similar stability constants, such as the Ag(I) complexes of pheno-, buto- and hexobarbital, desorption proceeds more efficiently for compounds with higher pK_a values (buto- and hexobarbital). For ligands which form strong complexes with certain metal ions, e.g. Pd(II)/5-fluorouracil (log K_1 = 21.3), or which have a rather low pK_a value, e.g. barbituric acid (pK_a = 4.4), on-line desorption by injection of strongly acidic solutions is impossible.

From the chelating stationary phases tested, the thiol phase should be preferred to the oxine phase since it binds soft metal ions such as Ag(I), Hg(II) or Pd(II) much more strongly at low pH. This prevents interferences of metal ions released during desorption in the LC separation or detection, and makes reloading of the stationary phase after each run superfluous.

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