



ELSEVIER

Journal of Chromatography A, 770 (1997) 3–11

JOURNAL OF
CHROMATOGRAPHY A

Optimisation of selectivity in the separation of metallo–cyanide complexes by ion-interaction liquid chromatography

Qiong Huang, Brett Paull, Paul R. Haddad*

Department of Chemistry, University of Tasmania, GPO Box 252C, Hobart, Tasmania 7001, Australia

Abstract

A study into the optimisation and selectivity of a reversed-phase ion-interaction liquid chromatographic method for the separation of metallo–cyanide complexes is described. A stable ion-interaction system was developed in which a C_{18} stationary phase was equilibrated with a 60 mM solution of tetrabutylammonium hydroxide ion-interaction reagent in order to saturate the stationary phase and to minimise retention changes caused by adsorption and desorption of this material. The effects on retention of the metallo–cyanide complexes caused by changes in pH and ionic strength were minimised through the addition of a high concentration of a phosphate buffer (150 mM) to the mobile phase. Perchlorate (0.32–5.62 mM) was then added to the mobile phase as a competing anion and its effect upon the capacity factors of each complex determined. A linear relationship between the logarithm of capacity factor and the logarithm of the concentration of perchlorate was observed, although the slopes of these plots were not in accordance with those predicted by a simple ion-exchange model. However, the linearity of the data allowed a simple optimisation procedure to be applied and the concentration of perchlorate could be used to manipulate the separation selectivity of the system. Three differing elution orders of metallo–cyanide complexes were achieved by varying the concentration of perchlorate in the mobile phase within the range 0.94–5.62 mM.

Keywords: Optimization; Selectivity; Metal complexes; Metallo–cyanides

1. Introduction

The use of ion-interaction liquid chromatography for the separation of metallo–cyanide complexes is now a well established technique which generally involves the use of a C_{18} reversed-phase column and a mobile phase containing tetrabutylammonium hydroxide (TBAOH) as the ion-interaction reagent (IIR). In previous works [1–5] we have applied this approach to the analysis of a number of complex sample types derived from the gold mining industry. These include the determination of metallo–cyanides at mg/l levels in gold processing solutions [1], the determination of gold(I) cyanide in mine process

liquors at $\mu\text{g/l}$ levels, using automated on-line preconcentration [2,3], the speciation of metallo–cyanides at $\mu\text{g/l}$ levels in non-saline environmental water samples [4], and the simultaneous determination of both free cyanide and metallo–cyanides in gold cyanidation process liquors [5].

A computer-assisted optimisation procedure has been used to obtain the optimal mobile phase conditions for the separation of metallo–cyanide complexes commonly found in environmental samples [4]. In this optimisation three parameters were considered, namely, the percentage of organic modifier, the concentration of IIR and the mobile phase pH. The conditions for the optimal resolution of six metallo–cyanide complexes were determined and the effects of each of the above parameters upon the

*Corresponding author.

retention times of the complexes illustrated. This study focused upon maximising the chromatographic resolution of the metallo–cyanide complexes and little attention was paid to manipulating the separation selectivity. Indeed, much of the work previously carried out using ion–interaction liquid chromatography for the determination of metallo–cyanide complexes by ourselves and other groups such as Grigorova et al. [6] has resulted in the development of separations in which different selectivity (as reflected by different elution orders) has been exhibited, despite the apparent similarity of the chromatographic systems.

A recent study by Giroux and Barkley [7] investigated the effects of changes in the ionic strength of the mobile phase on the elution order of metallo–cyanide complexes using an ion–interaction system similar to that described above [1–4]. It was found that selectivity was dependent on ionic strength when the pH, concentration of organic modifier and concentration of IIR remained constant. Sodium dihydrogen phosphate was used to adjust the ionic strength of the mobile phase and with ionic strengths less than $6.5 \cdot 10^{-3} M$ the following elution order was achieved, Cu(I), Ag(I), Ni(II), Au(I), Co(III), Fe(III) and Fe(II). Increasing the ionic strength to $14 \cdot 10^{-3} M$, the elution order takes the form Cu(I), Ag(I), Ni(II), Co(III), Fe(II) and Fe(III) (co-eluted) and finally Au(I). However, this study was based on a system in which the effects of changes in ionic strength were not related to the adsorption and desorption of the IIR on the stationary phase.

In order to achieve controlled manipulation of selectivity it is necessary to operate with a chromatographic system in which changes in a minimum number of parameters produce predictable effects on selectivity. This paper details a study into selectivity effects of metallo–cyanide complexes using a stabilised ion–interaction mechanism in which changes in resolution and selectivity were achieved solely through control of the competing-ion (perchlorate) concentration in the mobile phase. The inclusion of a high concentration of a phosphate buffer into the mobile phase ensured that retention effects due to changes in ionic strength and pH were minimised. Further, the use of a high concentration of the IIR in the mobile phase was used in order to saturate the surface of the stationary phase, so that small changes

in the composition of the mobile phase would have little or no effect upon retention resulting from adsorption and desorption of the IIR. A simple computer-assisted optimisation of the mobile phase composition was carried out and used to predict the optimal mobile phase conditions for the separation of selected groups of metallo–cyanide complexes.

2. Experimental

2.1. Instrumentation

All instrumentation used in this study was supplied by Waters (Milford, MA, USA) and comprised a conventional HPLC system consisting of a Model 510 pump, connected to a Model U6K injector and a Model 481 UV–visible absorbance detector operated at 215 nm. The analytical column was a Nova-Pak C₁₈ reversed-phase column (150×3.9 mm I.D.), used with a C₁₈ Guard-Pak insert (5.0×3.9 mm I.D.), housed in a Guard-Pak pre-column module. A Maxima 820 data station was used to record the resulting chromatograms.

2.2. Reagents

All reagents used were of analytical-reagent grade unless stated otherwise. De-ionised water was obtained from a Millipore (Bedford, MA, USA.) Milli-Q water-treatment system. TBAOH was supplied by Aldrich (Milwaukee, WI, USA). Potassium salts of the cyanide complexes of Ni(II), Cr(III), Cu(I), Co(III), Mo(IV) and Pd(II) were synthesised according to published methods [8–10]. The following complexes were obtained commercially, KAu(CN)₂ (Johnson and Matthey Chemicals, London, UK), K₄Fe(CN)₆ (By-Products and Chemicals, Sydney, Australia), K₃Fe(CN)₆ (Hopkin and Williams, London, UK) and K₂Pt(CN)₄·3H₂O (Pfaltz and Bauer, Waterbury, USA). The purities of all the metallo–cyanide complexes used were determined experimentally using elemental analysis. Purity was found to be greater than 94% of the expected value for all of the complexes used in this study. Complexes were stored in a vacuum desiccator in the absence of light. Standard solutions (500 mg/l) were prepared by dissolving the appropriate amount of each complex

in alkaline potassium cyanide solution (10 mM NaOH and 10 mM KCN) and were further diluted to 20 mg/l each day prior to injection. Chromatographic grade acetonitrile was obtained from Waters Corporation. The mobile phase used in the final system contained 150 mM phosphoric acid, 60 mM TBAOH, 25% (v/v) acetonitrile and sodium perchlorate ranging from 0.32 to 5.62 mM. The pH of the mobile phase was 7.64 ± 0.02 . The mobile phase was filtered through a 0.45 μm membrane filter and degassed using an ultrasonic bath prior to use. The flow-rate used was 1 ml/min in all cases.

3. Results and discussion

3.1. Development of a stable ion-interaction system

The retention behaviour of metallo–cyanide complexes when using ion-interaction chromatography can be influenced by a number of parameters, including the nature of the stationary phase, the lipophilicity of the IIR (which affects the amount of IIR adsorbed onto the stationary phase), the concentration of organic modifier in the mobile phase, the concentration of IIR in the mobile phase, the ionic strength of the mobile phase, the nature and concentration of the competing-ion and finally, the mobile phase pH. In this study a C_{18} reversed-phase stationary phase was used with TBAOH as the IIR and the concentration of acetonitrile added to the eluent was maintained at 25% (v/v). The pH of the system was stabilised by using a high concentration (150 mM) of phosphate buffer in the mobile phase which maintained the system at pH 7.6 and also provided a high background ionic strength.

Under these conditions the concentration of the IIR in the mobile phase was varied with a view to determining a region wherein retention reached a maximum. Fig. 1 shows the effect upon the capacity factor (k') of each metallo–cyanide complex caused by changes in the concentration of the IIR, ranging from 5.0 to 90.0 mM. The results for most of the complexes illustrate typical ion-interaction chromatographic behaviour. As the concentration of the IIR was increased from 5.0 to 30 mM there was a considerable increase in capacity factor due to

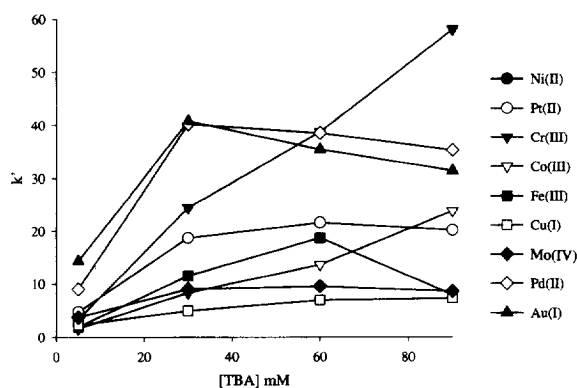


Fig. 1. Capacity factors of metallo–cyanide complexes as a function of IIR concentration within the mobile phase. Other conditions: 150 mM H_3PO_4 , 25% CH_3CN and pH 7.64.

increased adsorption of the IIR onto the stationary phase, thereby increasing the effective ion-exchange capacity of the stationary phase. However, as the concentration of IIR was increased further the stationary phase became saturated with IIR, causing the capacity factor to level off and then decrease progressively due to displacement of the adsorbed solutes by the counter-anion of the IIR (in this case, hydroxide ion). For the majority of the metallo–cyanide complexes shown in Fig. 1, this maximum adsorption occurs between IIR concentrations of 30 and 60 mM. Anomalous retention behaviour was observed for Co(III) and Cr(III), which showed no maximum over the range of conditions used in Fig. 1. The reason for this is unclear, although the rather broad peak shape obtained for the Cr(III) complex indicated that additional interactions with the stationary phase may have occurred. Nevertheless, an IIR concentration of 60 mM was selected since this gave maximum retention for the majority of the complexes and could therefore be assumed to provide approximate saturation of the stationary phase with IIR. The effect of this on the stability of the system for Cr(III) and Co(III), in terms of retention time reproducibility and predictability, will be discussed later.

3.2. Retention model

Many authors have proposed retention models for ion-interaction chromatography [11,12]. However, if

one makes the assumption that the surface of the stationary phase is at a point close to saturation with IIR, it can be expected that the main mechanism of retention would be simple ion-exchange on the surface of the dynamically coated substrate. In this case an ion-exchange retention model [13] could be used to enable prediction of capacity factors. A straightforward model of this type is given in eqn. (1) for a solute A^{x-} being eluted from an anion-exchanger by an eluent containing the competing-ion E^{y-} . Eqn. (1) predicts that a linear relationship exists between the logarithm of k' and the logarithm of the eluent concentration $[E^{y-}]$, having a slope equal to the negative ratio of the analyte charge to eluent charge. Here C_1 must be determined experimentally.

$$\log k'_A = C_1 - \frac{x}{y} \log [E^{y-}] \quad (1)$$

If all other mobile phase and stationary phase conditions remained constant and ion-exchange was the only retention mechanism in operation, the above retention model should allow calculation of the slope for a plot of $\log k'$ versus $\log [E^{y-}]$. This would mean the effect of $[E^{y-}]$ on k' could be predicted from a single chromatogram. To determine if eqn. (1) was applicable to the system under study, perchlorate was added as a competing anion and the effect of changing the perchlorate concentration on k' plotted. The slopes of these plots should approximate x/y in the above model, particularly for singly and doubly charged ions. For ions of greater charge the slope can deviate from the expected value due to activity effects.

Fig. 2 shows the plots obtained for $\log k'$ versus $\log [ClO_4^-]$ for nine metallo–cyanide complexes and the slopes obtained for each complex over the range of 0.32–5.62 mM ClO_4^- are shown in Table 1. It can be seen that for the majority of metallo–cyanide complexes, with the exception of Cr(III) and Cu(I), the slope of $\log k'$ versus $\log [ClO_4^-]$ increases with the charge of the complex. The smallest slope is obtained with the Au(I) complex, for which the capacity factor remains relatively unchanged over the perchlorate concentrations investigated. The complexes of Pt(II), Pd(II) and Ni(II), each being doubly charged, all exhibit similar slopes and this is also the case for the triply charged complexes of Fe(III) and Co(III). The second largest slope was shown by

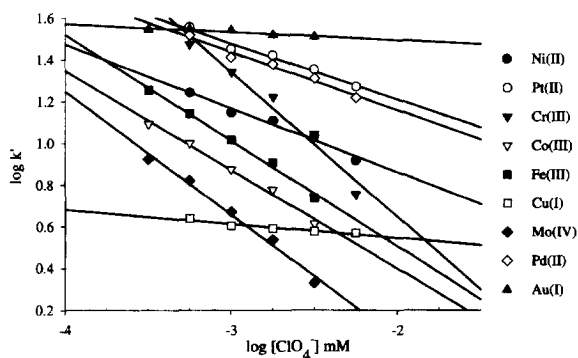


Fig. 2. Dependence of $\log k'$ on the concentration of perchlorate added to the mobile phase. Other conditions: 150 mM H_3PO_4 , 60 mM TBAOH, 25% CH_3CN and pH 7.64.

Mo(IV) which also has the largest charge of the complexes investigated, -4 . The reasons for the deviation from this pattern shown by the complexes of Cu(I) and Cr(III) are uncertain, although it is noteworthy that both complexes still exhibited a linear relationship.

Fig. 2 shows that the linearity of the data was good, but from Table 1 it is evident that the experimental slopes were very much smaller than those predicted using the ion-exchange model. These outcomes suggested that whilst ion-exchange certainly makes a significant contribution to retention, a second retention mechanism must also be in operation. A possible mechanism may be a “salting out” effect on the IIR caused by the increase in the ionic strength of the system as the perchlorate concentration was increased, leading to increased adsorption

Table 1
Theoretical and experimental slopes of retention plots achieved for individual metallo–cyanide complexes

| Complex | Theoretical slope | Experimental slope |
|-----------------|-------------------|--------------------|
| $Au(CN)_2^-$ | -1 | -0.038 |
| $Pt(CN)_4^{2-}$ | -2 | -0.26 |
| $Pd(CN)_4^{2-}$ | -2 | -0.28 |
| $Ni(CN)_4^{2-}$ | -2 | -0.31 |
| $Cu(CN)_3^-$ | -3 | -0.07 |
| $Fe(CN)_6^{3-}$ | -3 | -0.47 |
| $Co(CN)_6^{3-}$ | -3 | -0.51 |
| $Cr(CN)_6^{3-}$ | -3 | -0.69 |
| $Mo(CN)_8^{4-}$ | -4 | -0.59 |

of the IIR on the surface of the stationary phase and hence increased retention of the metallo–cyanide complexes. This would to a certain degree counteract the reduction in retention caused by the increase in the perchlorate concentration, producing lower than expected slopes being exhibited by the complexes. Such a “salting out” effect has been shown in reversed-phase HPLC by Horváth and Melander [14]. In offering this explanation, it should be remembered that the conditions had been chosen so as to ensure as complete a coverage of the stationary phase by the IIR as possible, so that further adsorption of the IIR must occur either on relatively inaccessible sites on the stationary phase or by formation of a multi-layer of adsorbed IIR. This behaviour is partly supported by the results obtained for Cr(III) and Co(III) in Fig. 1.

Despite the above deviation from the expected ion-exchange model, it was most significant that a linear relationship between $\log k'$ and $\log [\text{ClO}_4^-]$ was observed for each of the complexes investigated. This behaviour makes it possible to use addition of perchlorate to the mobile phase as a method to both optimise the separation of metallo–cyanide complexes and to predict and manipulate selectivity.

3.3. Optimisation of mobile phase composition

An iterative optimisation procedure was used to determine the mobile phase conditions providing a uniform spread of peaks in the separation of metallo–cyanide complexes. For each metallo–cyanide complex, retention times were determined experimentally at perchlorate concentrations of 0.32 mM and 5.62 mM. The retention behaviour intermediate between these two points was shown above to be linear if the data are plotted as $\log k'$ versus $\log [\text{ClO}_4^-]$. An entropy-based measure (see below) was calculated from the normalised distance between consecutively eluting solutes for all perchlorate concentrations intermediate between the two end points. The concentration of perchlorate resulting in the maximum value of the calculated criterion represented the optimal value in terms of obtaining maximum uniformity of peak spread. The entropy measure H is calculated from the following expression [15]:

$$H = \omega[\max(y_i) - \min(y_i)] - (1 - \omega) \sum_{i=1}^{n-1} P_i \log P_i \quad (2)$$

where, $y_i = a_i + b_i x$ is the retention equation for solute i ; $i = 1, 2, \dots, n$; y_i is the expected $\log k'$ for given $\log [\text{ClO}_4^-]$; $y_1 \leq y_2 \leq \dots \leq y_n$, for each value of x ; a_i and b_i are estimates of the intercept and slope of the retention plot for solute i ; and:

$$P_i = \frac{(y_{i+1} - y_i)}{[\max(y_i) - \min(y_i)]} \quad (3)$$

ω is a weighting factor which governs whether the optimisation criterion is maximised for the maximum absolute spread of peaks (here $\omega = 1$) or for the uniform spreading of peaks (here $\omega = 0$). In this work ω was taken as zero.

Once the value for the optimal concentration of perchlorate had been established, a mobile phase at this composition was applied experimentally and the observed retention times were compared with those calculated for this particular perchlorate concentration. Calculated retention times could be obtained by applying the following expression:

$$t_R = (10^b [\text{ClO}_4^-]^a + 1) t_0 \quad (4)$$

Where a and b are the intercept and slope obtained from the retention equation for each complex, t_R is the retention time and t_0 is the elution time for an unretained solute. If the observed and calculated retention times differ by more than a predetermined percentage (e.g., 5%), the measured retention data are added to the data file used for the original calculation of the optimum and the process is repeated. As this iterative procedure progresses, a closer approximation to the true retention behaviour will be obtained, leading to closer agreement between calculated and experimentally observed retention times. In the first iteration (i.e., when just two data points at extreme concentrations of perchlorate were used to model retention), the variation between the two sets of data was relatively large (5–38%). This error was reduced to less than 3% for all complexes except Cr(III) after six iterations of the procedure and the final variation between the ex-

Table 2

Calculated and experimentally observed retention times for metallo–cyanide complexes obtained using a perchlorate concentration of 2.34 mM

| Complex | t_R | | Error (%) |
|-------------------------------|------------|----------|-----------|
| | Calculated | Observed | |
| $\text{Au}(\text{CN})_2^-$ | 30.20 | 30.09 | 0.34 |
| $\text{Pt}(\text{CN})_4^{2-}$ | 24.98 | 24.89 | 0.34 |
| $\text{Pd}(\text{CN})_4^{2-}$ | 22.80 | 22.63 | 0.73 |
| $\text{Ni}(\text{CN})_4^{2-}$ | 12.56 | 12.53 | 0.24 |
| $\text{Cu}(\text{CN})_4^{3-}$ | 4.69 | 4.63 | 1.28 |
| $\text{Fe}(\text{CN})_6^{3-}$ | 7.87 | 7.63 | 3.00 |
| $\text{Co}(\text{CN})_6^{3-}$ | 6.21 | 6.05 | 2.46 |
| $\text{Cr}(\text{CN})_6^{3-}$ | 15.89 | 14.91 | 6.21 |
| $\text{Mo}(\text{CN})_8^{4-}$ | 3.82 | 3.79 | 0.77 |

perimentally determined and calculated retention times is given in Table 2.

It is interesting to note that the three complexes which showed the largest deviation from the predicted retention times were those of Cr(III), Co(III) and Fe(III). These were the three complexes which

deviated from the pattern shown by the remaining complexes when k' was plotted against the concentration of IIR (Fig. 1). It is likely that the relatively large error shown in Table 2 could be due to slight changes in the amount of IIR adsorbed onto the stationary phase, which from Fig. 1 would cause appreciable changes in the retention times of these particular complexes.

The optimisation procedure was applied to two groups of metallo–cyanide complexes. The first group included all of the nine complexes discussed previously and the optimised chromatogram obtained for this mixture is shown in Fig. 3. A perchlorate concentration of 2.34 mM was added to the mobile phase to obtain the separation. The second group of complexes included those metals likely to be present in environmental samples, namely Fe(II), Cu(I), Co(III), Fe(III), Ni(II) and Cr(III). The optimised chromatogram obtained for this mixture is shown in Fig. 4, for which a perchlorate concentration of 0.48 mM was used. Under these conditions Ni(II) showed some evidence of peak splitting. The reason for this

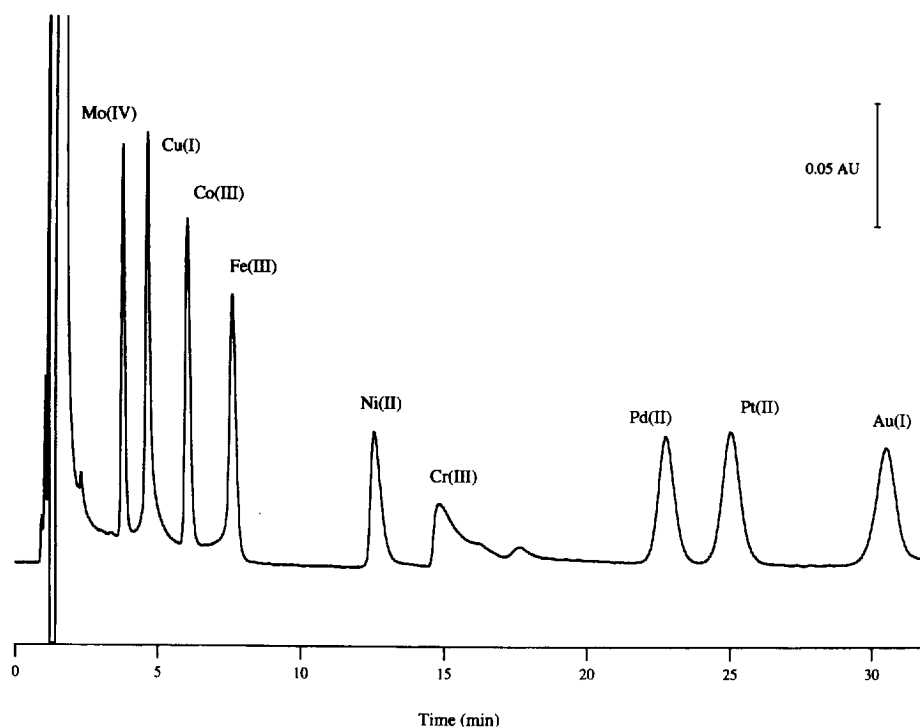


Fig. 3. Chromatogram showing the optimised separation of nine metallo–cyanide complexes using a mobile phase containing 150 mM H_3PO_4 , 60 mM TBAOH, 25% CH_3CN and 2.34 mM NaClO_4 .

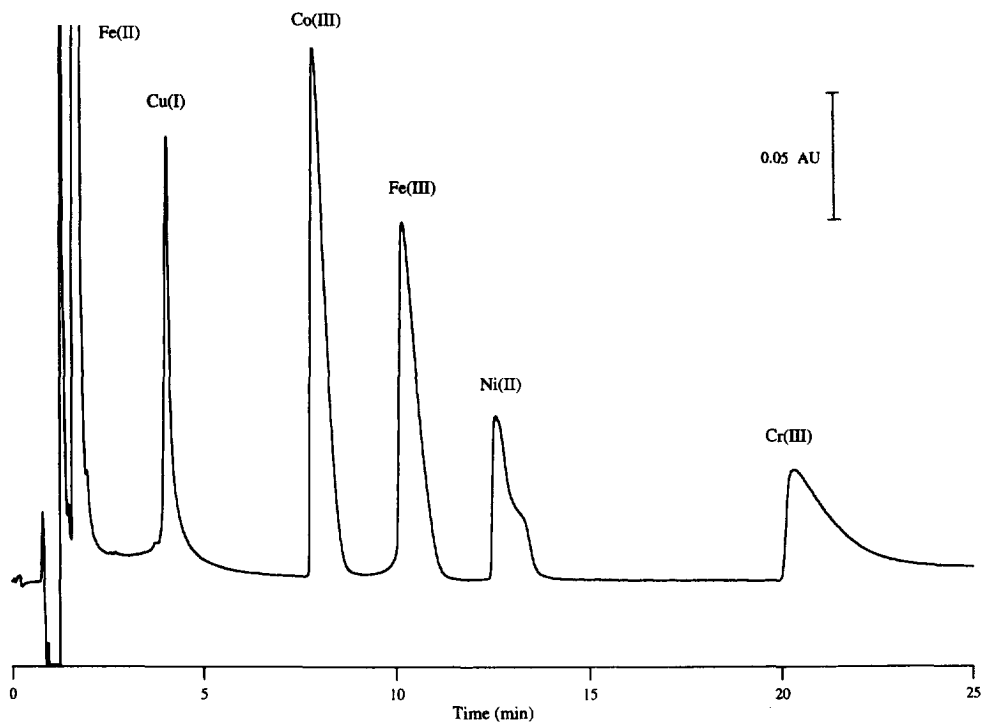


Fig. 4. Chromatogram showing the optimised separation of six environmentally significant metallo-cyanide complexes using a mobile phase containing 150 mM H_3PO_4 , 60 mM TBAOH, 25% CH_3CN and 0.48 mM NaClO_4 .

is again unclear although the effect was found to be reproducible at this concentration of perchlorate.

3.4. Manipulation of separation selectivity

The retention data obtained and shown in Fig. 2 could also be used to manipulate separation selectivity since the concentration of perchlorate in the mobile phase could be easily varied and its effect upon selectivity of the ion-interaction system could be predicted accurately. The elution order obtained in the optimised separation given in Fig. 3, (at 2.34 mM perchlorate) was as follows: Mo(IV), Cu(I), Co(III), Fe(III), Ni(II), Cr(III), Pd(II), Pt(II) and Au(I). This corresponds to that observed in our previous studies [1–5] and also by Grigorova et al. [6]. However, with a reduction in the perchlorate concentration from 2.34 to 0.94 mM, the elution order can be changed to the following, Cu(I), Mo(IV), Co(III), Fe(III), Ni(II), Cr(III), Pd(II), Pt(II) and Au(I), as shown in Fig. 5. It is interesting

to note the substantial increase in retention of the Cr(III) complex compared to the other complexes investigated, which corresponds to the retention data in Fig. 2 where Cr(III) exhibits a larger slope than the other complexes of similar charge. The resolution of the Pd(II), Pt(II) and Au(I) was decreased with a reduction in perchlorate concentration, although the retention time of Au(I) remained almost unaffected throughout the concentration range investigated.

With an increase in perchlorate concentration to 5.62 mM, a third retention order can be achieved. Fig. 6 shows the chromatogram obtained when using the above eluent concentration and exhibits the following elution order: Mo(IV), Co(III), Fe(III) and Cu(I) (co-eluted), Cr(III), Ni(II), Pd(II), Pt(II) and Au(I). From Fig. 6 it can be seen that the retention time of the Cr(III) complex is greatly reduced so that it was eluted prior to Ni(II) and also gave improved peak symmetry.

In addition to the above selectivities, extrapolation of the retention slopes shown in Fig. 2 indicates that

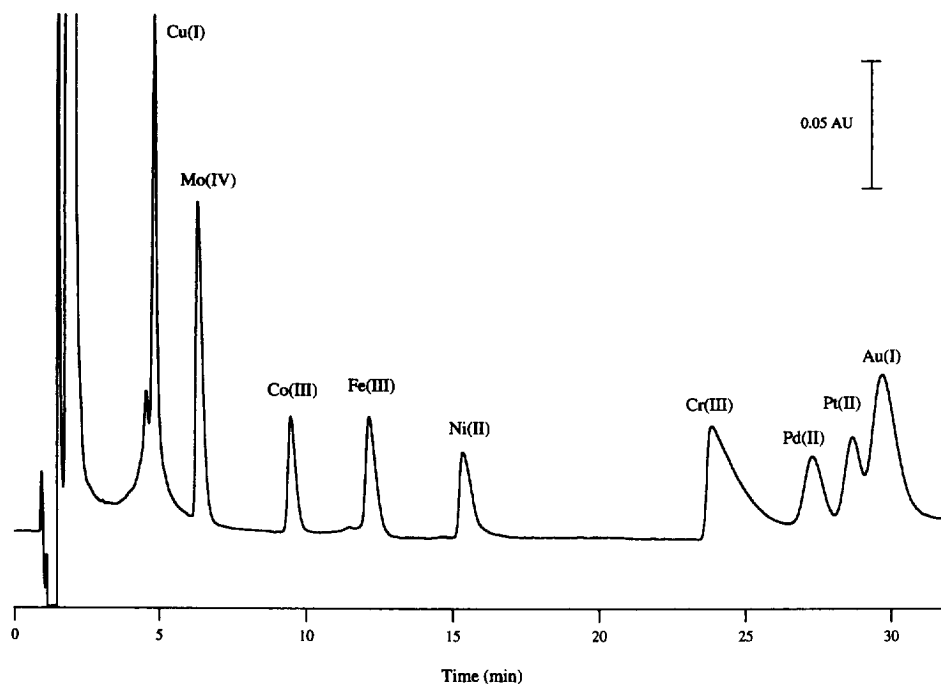


Fig. 5. Chromatogram showing the selectivity of metallo-cyanide complexes using a mobile phase containing 0.94 mM NaClO₄. Other conditions as in Fig. 4.

further changes in selectivity should be achievable if the perchlorate concentration were increased still further. In particular, a perchlorate concentration between in the range 15–25 mM should result in Cu(I) being eluted after Co(III), Fe(III) and Cr(III).

4. Conclusions

A stable ion-interaction chromatographic method has been developed in which the separation selectivity for metallo-cyanide complexes can be manipulated by control of the perchlorate concentration within the mobile phase. A linear relationship existed between the logarithm of the capacity factor of each of the metallo-cyanide complexes studied and the logarithm of the concentration of perchlorate. However, the retention data obtained indicated that ion exchange was not the sole retention mechanism in operation and that the surface of the stationary phase was not fully saturated with the ion-interaction reagent. Despite this, the linear nature of the data allowed a full optimisation procedure to be applied

and the slopes of the retention plots could be used to both predict changes in selectivity and to provide the perchlorate concentrations required to achieve these differing selectivities.

References

- [1] D.F. Hilton and P.R. Haddad, *J. Chromatogr.*, 361 (1986) 141.
- [2] P.R. Haddad and N.E. Rochester, *J. Chromatogr.*, 439 (1988) 23.
- [3] P.R. Haddad and N.E. Rochester, *Anal. Chem.*, 60 (1988) 536.
- [4] P.R. Haddad and C. Kalambaheti, *Anal. Chim. Acta*, 250, (1991) 21.
- [5] P.A. Fagan and P.R. Haddad, *J. Chromatogr.*, 550 (1991) 559.
- [6] B. Grigorova, S.A. Wright and M. Josephson, *J. Chromatogr.*, 410 (1987) 419.
- [7] L. Giroux and D.J. Barkley, *Can. J. Chem.*, 72 (1994) 269.
- [8] G. Brauer (Editor), *Handbook of Inorganic Chemistry*, Academic Press, New York, 2nd ed., 1965.
- [9] J.C. Bailar (Editor), *Comprehensive Inorganic Chemistry*, Pergamon Press, Oxford, 1973.

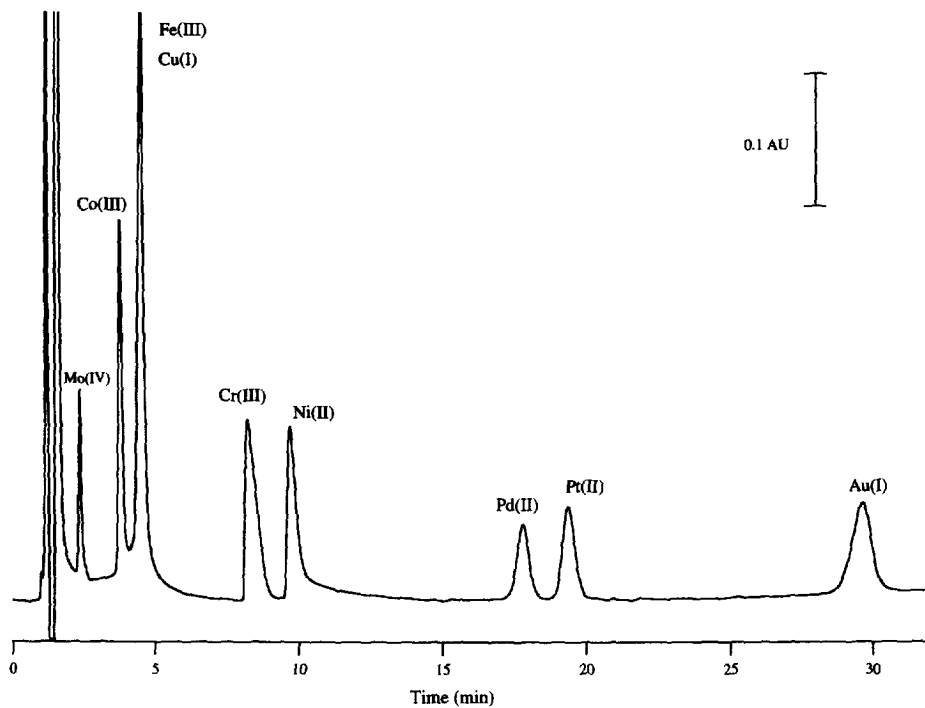


Fig. 6. Chromatogram showing the selectivity of metallo-cyanide complexes using a mobile phase containing 5.62 mM NaClO₄. Other conditions as in Fig. 4.