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Reversed-phase liquid chromatographic separation of platinum(II) complexes of methionine- and histidine-containing peptides using perfluorinated carboxylic acids as ion-pairing reagents

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Abstract

Reversed-phase liquid chromatography with the perfluorinated carboxylic acids trifluoroacetic acid, pentafluoropropanoic acid (PFP) and heptafluorobutyric acid as ion-pairing reagents provides a flexible system for both the analytical and semi-preparative separation of the products of the reaction between $[Pt(en)(H_2O)_2]^{2+}$ and methionine- and histidine-containing peptides. High selectivity can be achieved by variation of the chain length and concentration of the ion-pairing reagent for organic modifiers (methanol, acetonitrile, tetrahydrofuran) at concentrations in the range 0–20% (v/v). In general, employment of PFP (9.5 mmol l^{-1}) and acetonitrile (2–6%, v/v) leads to the best results. The retention behaviour of the peptide complexes is influenced by their charge, hydrophobicity and coordination mode.

Keywords: Ion-pairing reagents; Mobile phase composition; Peptides; Carboxylic acids, perfluorinated; Platinum

1. Introduction

Cisplatin *cis*-$[PtCl_2(NH_3)_2]$ is an extensively employed antitumour agent whose mechanism of action appears to be based on an interaction with guanine bases of DNA [1]. Dosage of the drug is limited by a concentration-dependent nephrotoxicity that has been ascribed to coordination of Pt(II) by sulphur atoms of cysteine or methionine (L-metH) side chains in certain enzymes [2–4]. One of the few characterised metabolites of cisplatin is indeed the methionine complex $[Pt(L\text{-met})_2]$, which has been isolated from urine [5,6]. The intensive investigation of the cisplatin antitumour mechanism in the past 25 years has led to a number of publications dealing with the HPLC separation of products of the reaction of Pt(II) complexes with amino acids and peptides. These may be grouped into studies involving plasma and urine from test animals or cancer patients [7–9] and those concerned with individual model systems for amino acids and small peptides [10–14]. The ionic nature of the products has led to an emphasis on reversed-phase liquid chromatography often with the use of ion-pairing reagents (IPRs) for the aqueous solutions involved. For instance sodium dodecyl sulphate [7,8], various C_6–C_{12} alkyl sulphonates [9] and hexyl sulphate [5] have all been employed for the separation of the products of the cisplatin/methionine reaction system. However, such long chain IPRs, though suitable for analytical purposes, are in general, less useful for preparative separations, as they often interfere with the subsequent IR and

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NMR spectroscopic characterisation of the products. As a result some previous separation studies on Pt(II) complexes of amino acids and peptides have ignored the question of characterisation [7–9]. Other publications have presented either a less satisfactory reversed-phase system for preparative separations [5,13] or a time-consuming work up of the preparative fractions [13].

The goal of our present work has been the development of a simple relatively universal reversed-phase chromatographic system for Pt(II) complexes of peptides. Such a system should be suitable for both analytical and semi-preparative separations and should allow high selectivity for reasonable times of analysis.

Volatile IPRs and buffers such as trifluoroacetic acid (TFA), ammonium bicarbonate, ammonium acetate and pyridinium formate have often been employed in the preparative separation of peptides and proteins [15]. The use of TFA for the analytical separation of $(NH_3)_5Co(III)$ and $(\eta^6\text{-}C_6H_6)Ru(II)$ amino acid and peptide complexes has also been reported [16,17]. Reversed-phase chromatography with TFA or the related derivatives, pentafluoropropanoic acid (PFP) and heptafluorobutyric acid (HFB), as IPRs proved to provide a flexible well-suited system for the separation of the Pt(II) complexes of peptides considered in this work. On-column coordination by the hard O donor atoms of these IPRs in competition to the softer peptide N and S atoms is not to be expected. NMR and mass spectrometry (MS) data [18] provide unequivocal evidence that the IPRs are only present as counterions in the separated complexes. Further support for this finding is provided by the fact that the number and size of the chromatographic peaks are in all cases independent of IPR concentration and chain length. Characteristics of this system are presented for complexes of $[Pt(en)(H_2O)_2]^{2+}$ with methionine-containing peptides of the types met*x*H (metglyH, metglyglyH, methisH; glyH=glycine, hisH=L-histidine) and *x*metH (glymetH, glyglymetH, glymetglyH) at various molar ratios and pH values. Fig. 1 shows the structures of these complexes. The cyclopeptides cyclo-alahis (alaH=L-alanine) and cyclo-glyhis were also included in the investigations. Details of the spectroscopic characterisation [fast atom bombardment (FAB)-MS, ^1H, ^{13}C and ^{195}Pt NMR] of the complexes are presented in a second publication [18].

2. Experimental

2.1. Reagents

$[PtCl_2(en)]$ and [PtI(dien)]I were prepared according to literature procedures [19–21]. Peptides were purchased from Bachem and used as received. Methanol, acetonitrile and tetrahydrofuran (THF) of liquid chromatography (LC) grade were obtained from Baker and Riedel de Haën. The IPRs TFA, PFP and HFB exhibited minimum purity grades of respectively 99, 97 and 99%. All other reagents were of analytical-reagent quality. LC water was obtained by double distillation.

2.2. Procedures

Stock solutions of $[Pt(en)(H_2O)_2](NO_3)_2$ and $[Pt(dien)(H_2O)](NO_3)_2$ were prepared at respective concentrations of 4 mmol l^{-1} and 15–40 mmol l^{-1} for analytical and semi-preparative separations by addition of the required equivalent quantities of $AgNO_3$ (1.95:1) to aqueous suspensions of $[PtCl_2(en)]$ or [PtI(dien)]I. The resulting precipitates of AgCl or AgI were removed by centrifugation after stirring the suspensions for 15 h in the dark. Peptide solutions were prepared in LC water at the required molar concentrations. Analytical reaction systems (50 ml, 0.8–1.2 mmol l^{-1}) at various stoichiometries [Pt(II):peptide=1:0, 2:1, 1:1, 1:2 and, 1:3 and 0:2] were prepared from these stock solutions with adjustment of the final pH value being achieved by addition of 0.1 *M* NaOH or 0.1 *M* HNO_3. The reaction mixtures were incubated at 37°C in the dark for a period of 3–7 days until the species distribution and the pH value of the solution remained constant. Such equilibrium systems may then be stored at 4°C in the dark for a period of months without further change. Reaction systems (50 ml) for semi-preparative separations were prepared at concentrations in the range 5–10 mmol l^{-1} under analogous conditions. Immediately before use, such solutions were reduced in volume, centrifuged and filled up to 7 ml with LC water. Portions of 0.3–1.0 ml were then

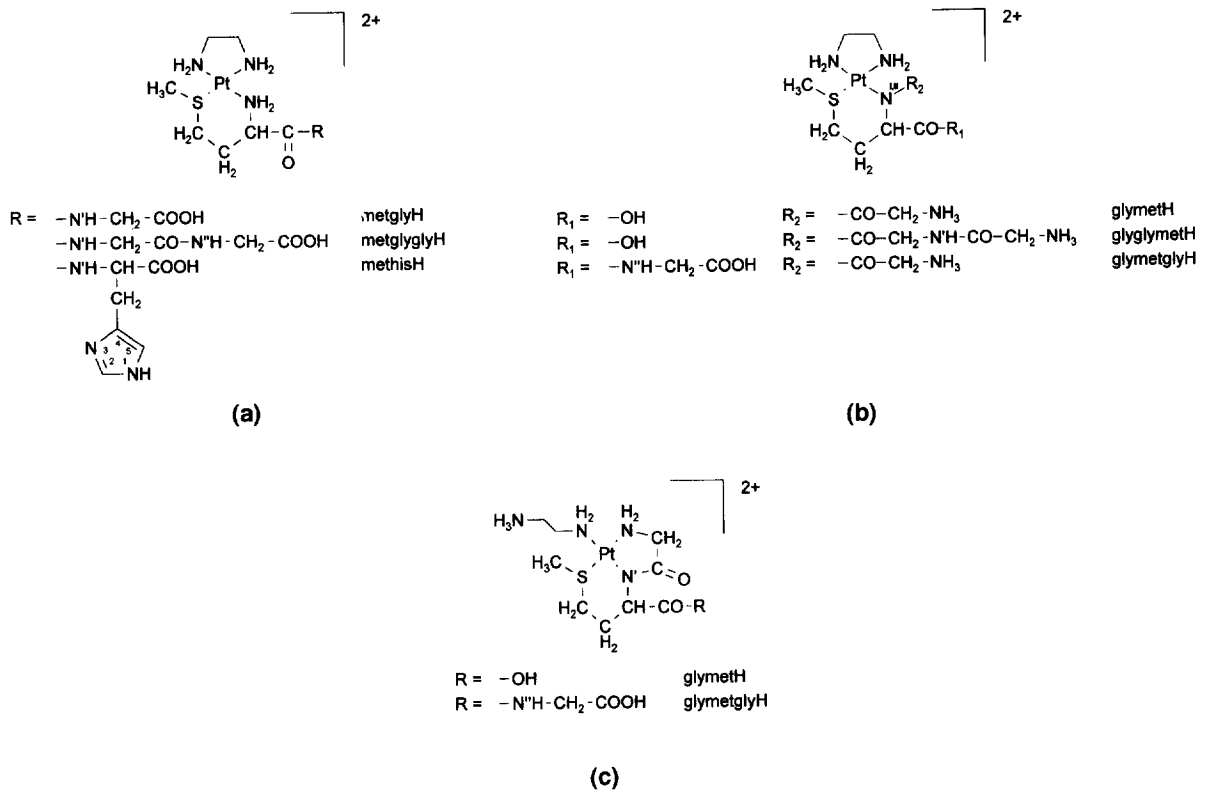

Fig. 1. 1:1 complexes with (a) N(amino), S(thioether) coordination for met*x*H peptides, (b) N′,N″(peptide), S(thioether) and (c) N(amino), N′(peptide), S(thioether) coordination for *x*metH peptides.

employed for the semi-preparative reversed-phase HPLC. After separation the solvent was removed from the fractions and the viscous oily residues treated with diethyl ether to afford powdered products for spectroscopic characterisation.

2.3. Chromatography

Analytical HPLC was performed with a Merck L-6200A high-performance liquid chromatograph equipped with a Rheodyne Model 7125 injector, a 20-μl injection loop and a variable-wavelength Merck L-4250 UV–Vis detector. A Knauer Model 64 HPLC pump equipped with a Knauer A0258 injector (20-μl injection loop) and a Merck L-4000A UV detector was also employed. Preparative pump heads, detector cells (2 mm instead of 5 mm) and injection loops (2 ml) were used for the semi-preparative separations. Chromatograms were registered and handled with the Knauer Eurochrom 2000 package. Analytical (25×0.4 cm I.D. or 12.5×0.4 cm I.D.; d_p=5 μm) and semi-preparative (25×2.0 cm I.D.; d_p=10 μm) columns were packed with Nucleosil 100-C_{18} (Macherey–Nagel) by a modified viscosity method. Merck precolumns (0.4×0.4 cm I.D.; d_p=5 μm) packed with LiChrospher 100 RP-18 were additionally employed for some separations. Column performance was controlled with two standard reversed-phase test mixtures [17,22]; void times were determined either with an $NaNO_3$ sample [eluent: CH_3OH–0.1 *M* NaH_2PO_4/Na_2HPO_4 buffer (50:50), pH 6.8] [23,24] or from the peaks of CH_3OH or water injected into an eluent of CH_3OH–water (66:34) [25]. Analytical HPLC was performed at a flow-rate of 1 ml min^{-1}, semi-preparative HPLC at a flow-rate of 15–20 ml min^{-1} for columns at ambient temperature (20°C). Mobile phases were made by combining known volumes of the organic modifier,

water and the perfluorinated carboxylic acids TFA, PFP and HFB; the pH value of these eluents was 2.1±0.1. Solvent composition is expressed in % (v/v); 0.124% HFB, 0.1% PFP and 0.073% TFA amounting to a concentration of 9.52 mmol l^{-1}. All HPLC solvents and solvent mixtures were degassed for at least 10 min by ultrasonic vibration prior to use. Analytical columns were conditioned by passing at least 30 ml of the mobile phase before separations were performed. After use of the IPRs the analytical columns were washed with at least 45 ml CH_3OH (~20 column void volumes). Correspondingly larger liquid volumes were employed for the equilibration and washing of the semi-preparative columns. Retention time, retention factor and other column performance data reported in this work are the average of three or more measurements. Absorbance detection was usually at 220 nm except for cases in which it was desirable to selectively remove unreacted peptide peaks from the chromatogram. In such cases 254 nm was employed. Peak identification was performed by comparison of the retention times with those of the pure substances or by inspection of the UV spectra obtained under stopped-flow conditions with the Merck L-4250 UV–Vis detector.

3. Results and discussion

Their strong ion-pairing tendency, low UV cut-off and excellent volatility have led to the frequent use of perfluorinated carboxylic acids as IPRs in reversed-phase liquid chromatographic separations [26–32]. Further advantages for the present work are provided by their solubility in diethyl ether [26], which enables their separation from insoluble Pt(II) complexes in preparative fractions, and by their lack of proton resonances, which simplifies the ^1H NMR characterisation of the peptide-containing products. A prerequisite is fulfilled by the effective absence of on-column coordination reactions between such IPRs and Pt(II) complexes [18]. The perfluorinated carboxylic acids exhibit pK_a values of less than 1, which means that they are strongly dissociated in aqueous solution. Their maximum concentration is limited to a value of about 0.1% (v/v) by the lower pH limit of approximately 2.0 for the stability of the stationary phase. Four separation parameters were systematically varied in the course of our studies on Pt(II) peptide complexes: (a) the concentration of the organic modifier; (b) the nature of the organic modifier; (c) the chain length of the IPR and (d) the concentration of the IPR.

3.1. Concentration of the organic modifier

Increase in concentration of the organic modifier leads to reduction in the retention factors k for the individual components of a reaction mixture as exemplified in Fig. 2 for the $[Pt(en)(H_2O)_2]^{2+}$/metglyH system with acetonitrile as organic modifier and PFP (0.1%) as IPR. The k values exhibit an approximately linear dependence on the logarithmic organic modifier concentration as demonstrated in Fig. 3 for 1:1 and 2:1 complexes of the $[Pt(en)(H_2O)_2]^{2+}$/methisH system. Line slopes, determined in this way, provide a means of comparing the influence of organic modifier concentration on the retention times for individual products of the $[Pt(en)(H_2O)_2]^{2+}$/peptide systems. The 1:1 complexes exhibit an N(amino), S(thioether) coordination mode and are typical for peptides of the type met$x$H (Fig. 1a).

3.2. Nature of the organic modifier

Specific interactions between individual organic modifiers and Pt(II) peptide complexes may be studied by comparing eluents with identical total polarity, as calculated using tabulated polarity parameters [33]. For example retention factors for 1:1 and 1:2 complexes of cyclo-alahis are compared in Fig. 4. The individual complexes exhibit respectively N^3(imidazole), N′(peptide), N^1(imidazole), N^3 and N^1, N^1 coordination modes. All three organic modifiers methanol, acetonitrile and THF provide the same elution order for the observed reaction products. Use of acetonitrile produces a relatively small reduction in retention times in comparison to methanol at intermediate total polarity. The difference increases, however, on going to lower polarities. In contrast, employment of THF leads to rapid elution of the Pt(II) complexes in comparison to both CH_3OH and CH_3CN.

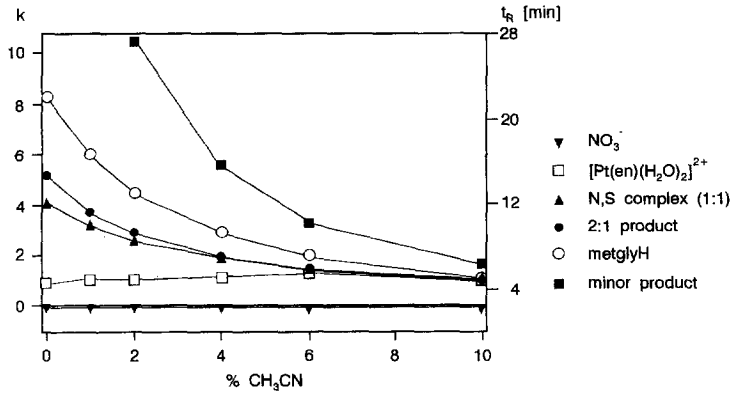

Fig. 2. Dependence of retention factors k for components of the $[Pt(en)(H_2O)_2]^{2+}$/metglyH system on the CH_3CN concentration using PFP (0.1%) as IPR (column 25×0.4 cm I.D, mobile phase pH=2.1). Reaction solutions at molar ratios of 2:1 (pH 3.3), 1:1 (pH 3.4) and 1:2 (pH 3.6) were considered. The complex with 2:1 stoichiometry could not be fully characterised.

In general, satisfactory separation conditions may be achieved for each of the $[Pt(en)(H_2O)_2]^2$/peptide reaction mixtures with any of the three organic modifiers employed. However, optimisation is in each case an empirical process, as retention times cannot simply be predicted on the basis of elution data for the other organic modifiers. Acetonitrile exhibits the important advantage of smaller peak-widths at half height (w_h), which enables a better recognition of minor products than for the other organic modifiers. However, THF and methanol are more suitable for the separation of reaction mixtures containing significant concentrations of unreacted $[Pt(en)(H_2O)_2]^{2+}$. This is due to the fact that the diaqua complex can display an on-column reaction with acetonitrile leading to the formation of $[Pt(CH_3CN)(en)(H_2O)]^{2+}$ or $[Pt(CH_3CN)_2(en)]^{2+}$. These complex cations manifest themselves as shoulders or even partially separated peaks in the resulting chromatograms for lower acetonitrile concentrations (1–2%). At higher concentrations (e.g. Fig. 2) only one broad peak can be observed for the various cations. Independent of the concentration of the organic modifier, use of methanol or THF leads only to the single peak of the diaqua complex with a small w_h value. Similar findings have been reported in a study of the separation of hydrolysis products of cisplatin [9]. In contrast to $[Pt(en)(H_2O)_2]^{2+}$, the

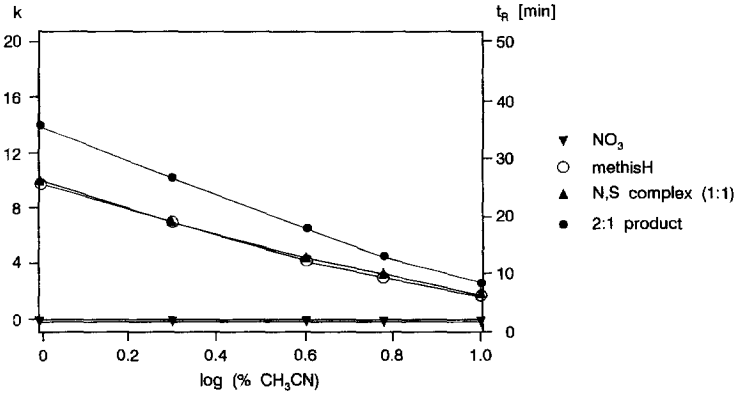

Fig. 3. Dependence of retention factors k for components of the $[Pt(en)(H_2O)_2]^{2+}$/methisH system on the logarithmic CH_3CN concentration using PFP (0.1%) as IPR (column 25×0.4 cm I.D, mobile phase pH=2.1). Reaction solutions at molar ratios of 2:1 (pH 3.5), 1:1 (pH 4.1) and 1:2 (pH 6.0) were considered. The complex with 2:1 stoichiometry could not be fully characterised.

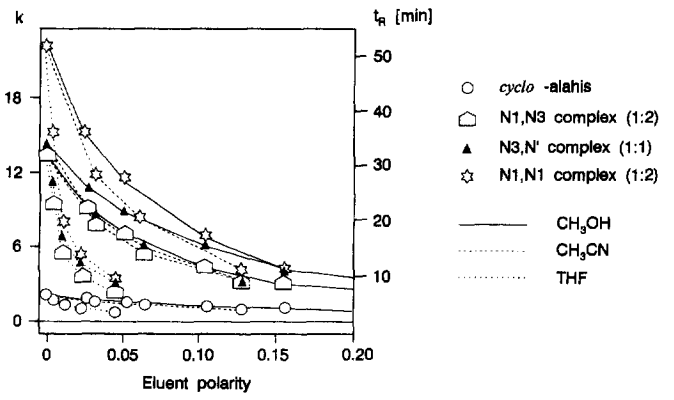

Fig. 4. Comparison of the retention factors k for 1:1 and 1:2 complexes of the $[Pt(en)(H_2O)_2]^{2+}$/cyclo-alahis system as a function of the total polarity of the eluent [column 25×0.4 cm I.D., PFP (0.1%) as IPR, mobile phase pH=2.1]. Reaction solutions at molar ratios of 2:1 (pH 3.5), 1:1 (pH 3.8) and 1:2 (pH 5.3) were considered.

product of an on-column reaction between $[Pt(dien)(H_2O)]^{2+}$ and acetonitrile could not be detected within the investigated concentration range (1–10%) of the organic modifier.

3.3. Chain length of the perfluorinated carboxylic acids

Variation of the chain length of the IPRs has a decisive influence on the retention behaviour of Pt(II)/peptide reaction mixtures. The importance of the chain length of perfluorinated carboxylic acids for the observed retention times of free peptides has been discussed previously [26,28–32]. Fig. 5 now depicts the dependency of the retention factors of major species of the $[Pt(en)(H_2O)_2]^{2+}$/glymetH system on the acetonitrile concentration for the IPRs TFA, PFP and HFB. The 1:1 complexes exhibit either the N′(peptide), S(thioether) or the N(amino), N′(peptide), S(thioether) coordination modes (Fig. 1b,c) with the en ligand being only monodentate in the latter case [18]. Both peptides display a monodentate S(thioether) coordination in the 1:2 complex. It is apparent from Fig. 5 that chain lengthening leads to an increase in the retention factors for all species considered. Interestingly the

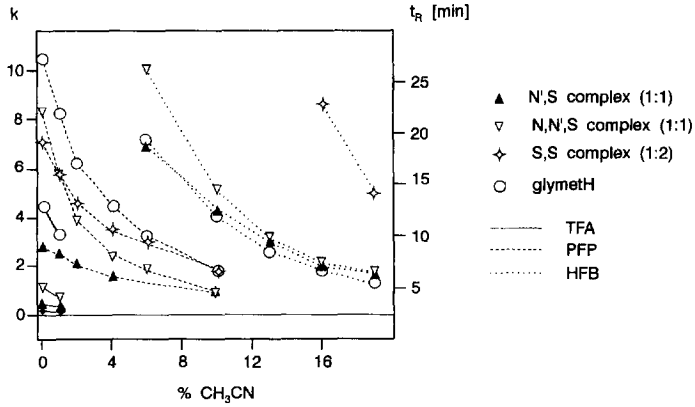

Fig. 5. Influence of the chain length of the IPR (9.52 mM) on the dependency of the retention factors k on the CH_3CN concentration for the major products of the $[Pt(en)(H_2O)_2]^{2+}$/glymetH system (column 25×0.4 cm I.D., mobile phase pH=2.1). Reaction solutions at molar ratios of 1:1 (pH 3.5), 1:2 (pH 3.6) and 1:3 (pH 3.9) were considered.

relative change in the k values increases dramatically on going from the free peptide to the 1:1 complexes and finally the 1:2 complex. This finding is in accordance with a general conclusion of our investigations, namely the observation that the hydrophobic influence of the IPRs on the retention factors is dependent on the charge of the species involved. This increases from +1 for glymetH at the pH value of 2.1, over +2 for the 1:1 complexes to +4 for the 1:2 complex. The concomitant increase in the number of interactions between the charged species and the IPRs leads to the observed delays in elution, with the effect being most pronounced for the 1:2 complexes.

The observation that higher charge always leads to a more pronounced increase in the relative retention time on going to a more hydrophobic IPR does not, of course, allow the general conclusion that the t_R value will necessarily be larger for a more highly charged cation when using a particular IPR. Indeed the reversed elution order is observed for the 1:2 complex, the 1:1 complexes and the free peptide glymetH for TFA (Fig. 5); a similar trend is also found for the other Pt(II)/$x$metH systems investigated in this work. This finding suggests that the short chain IPR TFA is not capable of fully neutralising higher charges to provide externally neutral conglomerates that can be effectively retained. In contrast, the elution order for the longer chain IPR HFB is always in accordance with the expectation that higher charge will lead to the electrostatic interaction of the component with more IPRs, leading to an increase in retention time due to hydrophobic contacts of these associated molecules with the stationary phase. Such a consistent trend cannot be established for the IPR PFP with its intermediate chain length.

In addition to cation charge, two further factors appear to play an important role in determining the retention behaviour of peptide complexes, namely the extent of their own hydrophobic interactions with the stationary phase and the topographical relationship of their charge centres (e.g. Pt atoms or protonated amino groups) and hydrophobic side chains. Indices have been presented for the hydrophobic contributions of individual amino acid building units to the total retention factors of free peptides separated by reversed-phase ion-pair liquid chromatography. Such contributions readily explain the marked increase in the retention time observed on lengthening a side chain (e.g. cyclo-glyhis vs. cyclo-alahis) or the peptide backbone (e.g. metglyH vs. metglyglyH). However, they cannot be employed to interpret the significant differences in the t_R values for isomeric peptides such as glymetH/metglyH (Figs. 2 and 5 for PFP) or glyglymetH/glymetglyH/metglyglyH. This topographical influence, which appears to have been neglected for peptides in the past [16], is particularly apparent for the Pt(II) complexes studied in this work. For instance the 1:1 complexes of metglyH and glymetH (Figs. 2 and 5 for PFP) with the same total charge (+2 at pH 2.1) and hydrophobicity exhibit strikingly different retention times. An explanation for this phenomenon may be sought in the differences in charge localisation within the respective complexes (Fig. 1), which will determine the stereochemistry of their interaction with IPR molecules. Our observations for these and analogous peptide complexes indicate that a simple correlation of structure and retention time is not possible. As a result of the complexity of the interactions with a particular IPR each reversed-phase separation must be optimised empirically. Typical chromatograms are depicted in Fig. 6 for the $[Pt(en)(H_2O)_2]^{2+}$/glymetH system.

3.4. Concentration of the perfluorinated carboxylic acids

At lower concentrations, increasing the quantity of IPR leads to a linear increase in the retention factors. The observed dependency curves generally flatten off to approach a maximum value at higher concentrations. However, as depicted in Fig. 7, the exact form of the curve is dependent on the particular peptide complex and IPR. For instance an increase in TFA concentration leads to a dramatic increase in the k values for $HglymetH^+$ and $HglyglymetH^+$ (pH = 2.1) but has relatively little effect on the retention behaviour of the more highly charged 1:1 and 1:2 peptide complexes. In contrast, Guo et al. [31] established an increased concentration dependency for the retention factors on raising the charge of the free peptides. This state of affairs is also observed for the longer chain IPR HFB (Fig. 7b). The reversal of the elution order for the 1:1 and 1:2 peptide

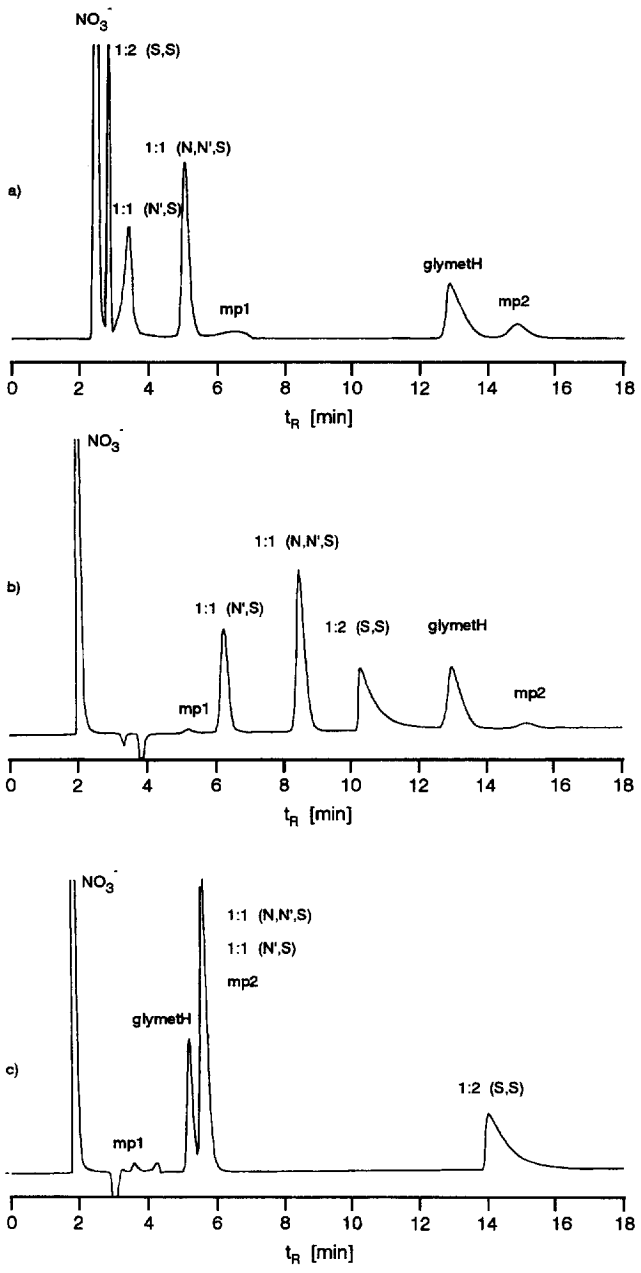

Fig. 6. Chromatograms for the $[Pt(en)(H_2O)_2]^{2+}$/glymetH system (mp = minor product) at a molar ratio of 1:3 (pH 3.9); (column 25×0.4 cm I.D., mobile phase pH = 2.1, wavelength = 220 nm). (a) 0.073% TFA, 100% water; (b) 0.1% PFP, 4% CH_3CN; (c) 0.124% HFB, 19% CH_3CN.

complexes on going from TFA to HFB is once again apparent from Fig. 7; an intermediate behaviour is exhibited by PFP. This enables an optimised separation of the components of the $[Pt(en)(H_2O)_2]^{2+}$/glymetglyH reaction system at an intermediate concentration of 4.76 m*M* PFP (Fig. 8).

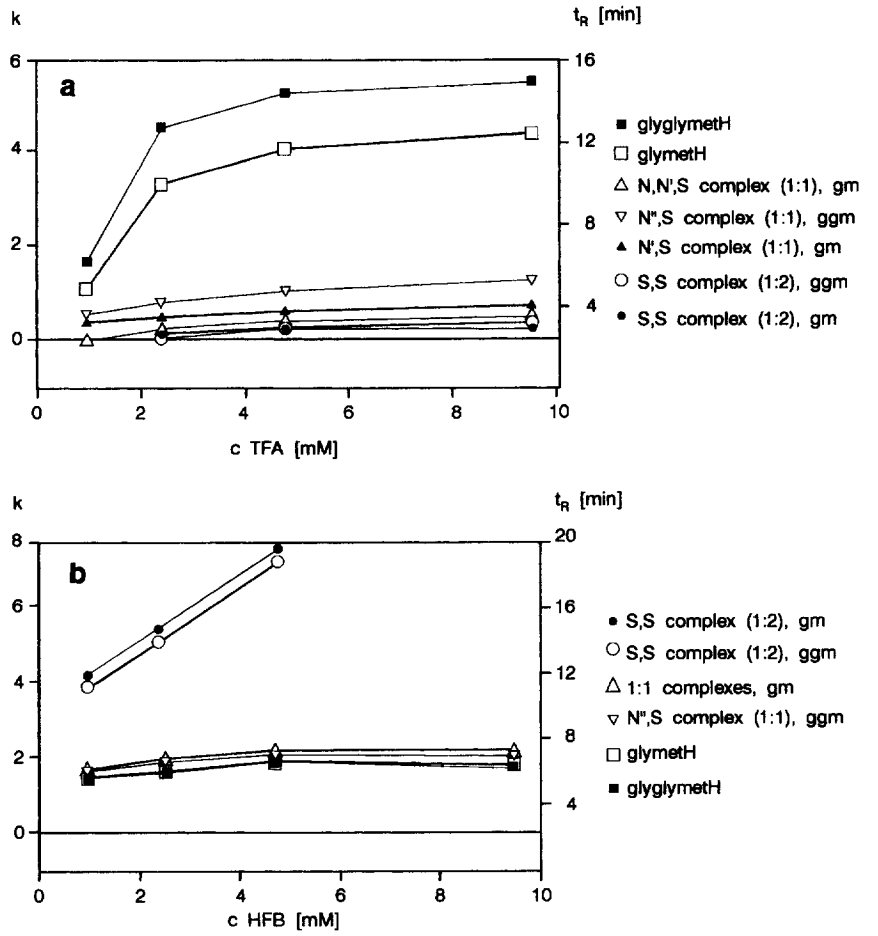

Fig. 7. Dependency of the retention factors k on IPR concentration for the $[Pt(en)(H_2O)_2]^{2+}$/glymetH (gm) and $[Pt(en)(H_2O)_2]^{2+}$/glyglymetH (ggm) systems (column 25×0.4 cm I.D., mobile phase pH=2.2±0.2). (a) TFA, 100% water; (b) HFB, 16% CH_3CN.

3.5. Retention behaviour of the perfluorinated carboxylic acids

The investigation of the behaviour of the IPRs themselves provides an insight into the retention mechanism of the employed reversed-phase separation system. Injection of an IPR into an eluent containing the same IPR always leads to a positive peak in the chromatogram. When the eluent contains a different IPR both positive and negative peaks are registered, with those of the eluent IPR being always positive. This is in accordance with a dynamic exchange process at the stationary phase involving partial desorption of the eluent IPR and partial adsorption of the injected IPR as described in the ion interaction model of Bidlingmeyer [34,35]. In accordance with expectation, the retention factors of the IPRs increase with chain length, as depicted for an eluent containing PFP in Fig. 9. In general, molecules displaying the same charge as the IPR would be expected to exhibit increased k values at lower IPR concentrations as a result of reduced electrostatic repulsion due to incomplete covering of the stationary phase by the IPR. Surprisingly this effect cannot be established for the perfluorinated carboxylic acids (Fig. 9), suggesting that the covering of the reversed-phase is already optimal at the lowest concentration studied (1 m*M* PFP). Compara-

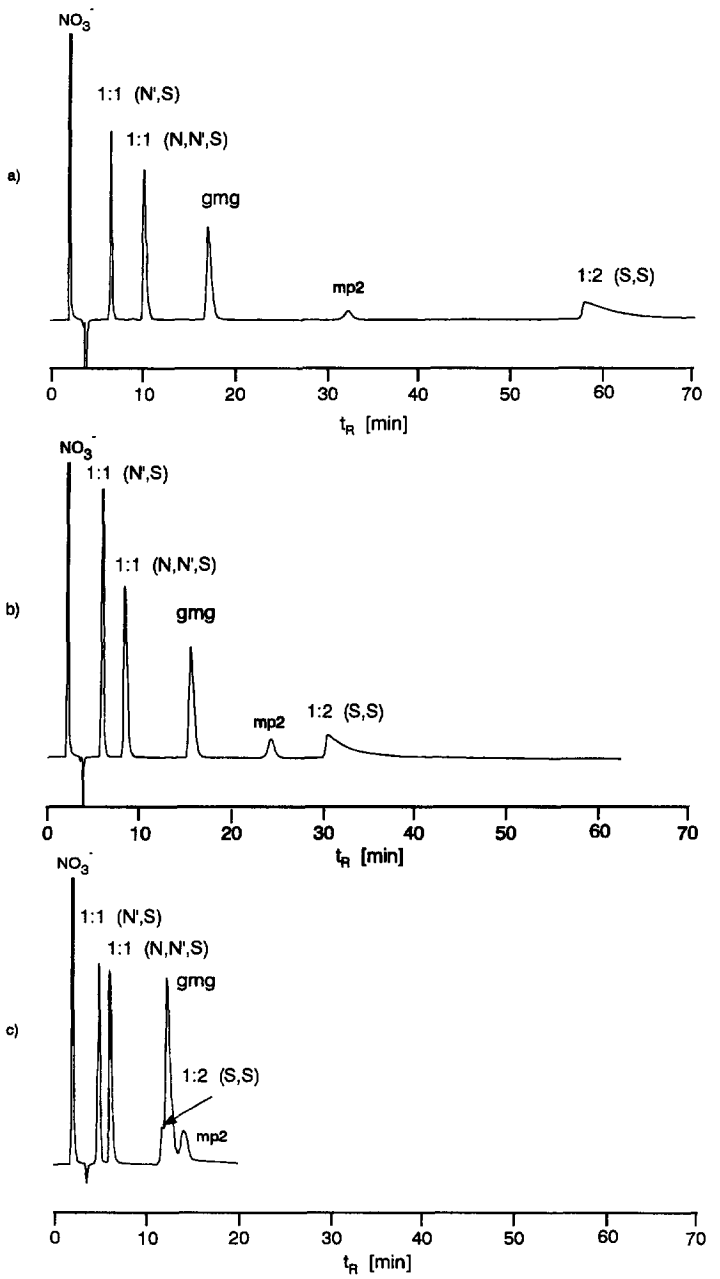

Fig. 8. Chromatograms for the $[Pt(en)(H_2O)_2]^{2+}$/glymetglyH (gmg) system (mp = minor product) at a molar ratio of 1:3 (pH 4.2); [column 25×0.4 cm I.D., CH_3CN–water (2:98), mobile phase pH = 2.2±0.2, wavelength = 220 nm] for varying PFP concentrations. (a) 9.52 m*M*; (b) 4.76 m*M*; (c) 0.95 m*M*.

tive investigations at lower concentrations were not possible as these lead to a significant increase in the pH value of the eluent.

3.6. Semi-preparative scale up

As discussed in the Section 1 the reversed-phase

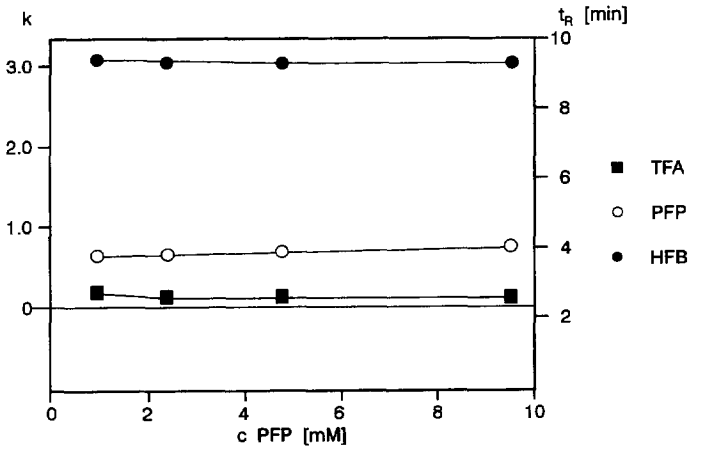

Fig. 9. Dependency of the retention factors k for TFA, PFP and HFB on the PFP concentration of the eluent [column 25×0.4 cm I.D., CH_3CN–water (2:98), mobile phase pH = 2.2±0.2].

chromatographic system for Pt(II) peptide complexes should allow semi-preparative separations of high selectivity and reasonable analysis times. Criteria for a scale-up are baseline separations of at least 3–5 min between neighbouring peaks and a total analytical separation time of not more than 40 min. The major difficulty is provided by the "overloading" of the IPR which leads to a reduction in retention time for all peaks in the chromatogram. In contrast to the volume increase of the stationary phase on going from a column I.D. of 0.4 to 2.0 cm, it is necessary to retain the maximum analytical concentration of the IPR, as an increase would lead to a pH value below 2.0 with accompanying destruction of the reversed-phase. The required 4- to 12-fold increase in the concentration of the peptide complexes leads to a reduction in the average number of interactions between component and IPR molecules, resulting in peak broadening and tailing associated with shorter t_R times. This restricts the semi-preparative separation to a typical value of 20 mg for a product mixture. Baseline peak separation is not always possible. The peptide complexes can be characterised by FAB-MS, ^1H and ^{13}C NMR spectroscopy [18], but effective use of IR spectroscopy is prevented by the characteristic bands of the carboxylate groups of the IPRs, which function as counterions. A comparison of typical analytical and semi-preparative chromatograms is provided in Fig. 10 for the $[Pt(en)(H_2O)_2]^{2+}$/glymetH system using PFP as the IPR. The peak shifts enable in this case a simpler semi-preparative separation than would be expected on the basis of the previously established analytical criteria.

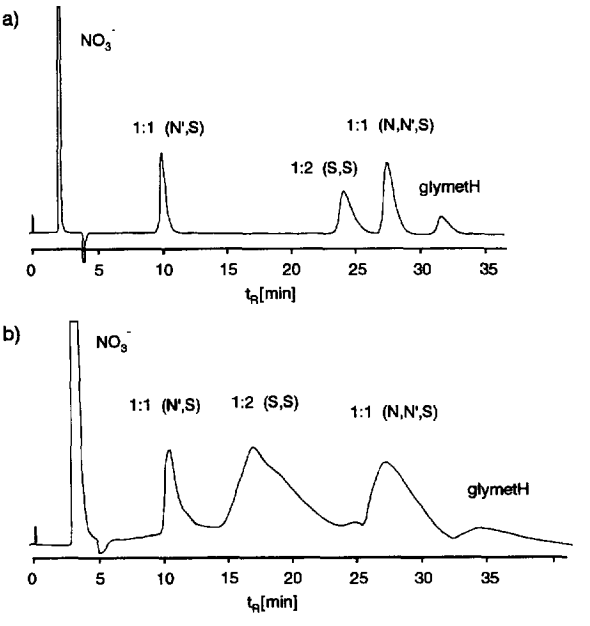

Fig. 10. Chromatograms for the $[Pt(en)(H_2O)_2]^{2+}$/glymetH system at a molar ratio of 1:2 (pH 3.6); (0.1% PFP, 100% water, mobile phase pH = 2.1, wavelength = 220 nm). (a) Analytical, $F = 1$ ml min^{-1}, column 25×0.4 cm I.D., $d_p = 5$ mm; (b) semi-preparative, $F = 16.8$ ml min^{-1}, column 25×2.0 cm I.D., $d_p = 10$ mm.

4. Conclusions

Reversed-phase liquid chromatography with the perfluorinated carboxylic acids TFA, PFP and HFB as IPRs provides a flexible analytical and semi-preparative separation system for the $[Pt(en)(H_2O)_2]^{2+}$/peptide systems considered in this investigation. The retention behaviour of the peptide complexes is influenced not only by their charge and hydrophobicity but also by their coordination mode and structure. In general, employment of 0.1% PFP and a concentration of organic modifier in the range 0–10% is found to provide the highest selectivity, as for this IPR both charge and structure of the components influence retention behaviour to a similar extent. The dominant influence of charge leads to a reduced selectivity for the shorter and longer chain IPRs TFA and HFB. A major advantage of the investigated chromatographic system is provided by the high volatility and lack of proton resonances for the IPRs, which greatly simplify the spectroscopic characterisation of the fractions of semi-preparative separations.

5. Abbreviations

en	ethylenediamine
dien	diethylenetriamine
metH	L-methionine
glyH	glycine
hisH	L-histidine
alaH	L-alanine
gm	glymetH
gmg	glymetglyH
ggm	glyglymetH
mp	minor product
d_p	particle size of stationary phase
F	flow-rate

References

[1] S.E. Sherman and S.J. Lippard, Chem. Rev., 87 (1987) 1153.
[2] R.F. Borch and M.E. Pleasants, Proc. Natl. Acad. Sci. USA, 76 (1979) 6611.
[3] R.F. Borch, J.C. Katz, P.H. Lieder and M.E. Pleasants, Proc. Natl. Acad. Sci. USA, 77 (1980) 5441.
[4] W.W. Alden and A.J. Repta, Chem. Biol. Interact., 48 (1984) 121.
[5] C.M. Riley, L.A. Sternson, A.J. Repta and S.A. Slyter, Anal. Biochem., 130 (1983) 203.
[6] L.A. Sternson, A.J. Repta, H. Shih, K.J. Himmelstein and T.F. Patton, in M.P. Hacker, E.B. Douple and I.H. Krakhoff (Editors), Platinum Coordination Complexes in Cancer Chemotherapy, Martinus Nijhoff, Boston, MA, 1984, p. 126.
[7] P.T. Daley-Yates and D.C.H. McBrien, Biochem. Pharmacol., 32 (1983) 181.
[8] P.T. Daley-Yates and D.C.H. McBrien, Biochem. Pharmacol., 33 (1984) 3063.
[9] W.A.J. De Waal, F.J.M.J. Maessen and J.C. Kraak, J. Chromatogr., 407 (1987) 253.
[10] C.M. Riley, L.A. Sternson and A.J. Repta, Anal. Biochem., 124 (1982) 167.
[11] P.C. Dedon and R.F. Borch, Biochem. Pharmacol., 36 (1987) 1955.
[12] S.K. Mauldin, F.A. Richard, M. Plescia, S.D. Wyrick, A. Sancar and S.G. Chaney, Anal. Biochem., 157 (1986) 129.
[13] S.K. Mauldin, M. Plescia, F.A. Richard, S.D. Wyrick, R.D. Voyksner and S.G. Chaney, Biochem. Pharmacol., 37 (1988) 3321.
[14] P. del Socorro Murdoch, J.D. Ranford, P.J. Sadler and S.J. Berners-Price, Inorg. Chem., 32 (1993) 2249.
[15] W.S. Hancock and R.L. Prestidge, in B.A. Bidlingmeyer (Editor), Preparative Liquid Chromatography (Journal of Chromatography Library, Vol. 38), Elsevier, Amsterdam, 1987.
[16] S.S. Isied, J. Lyon, A. Vassilian and G. Worosila, J. Liq. Chromatogr., 5 (1982) 537.
[17] H.J. Götze, W.S. Sheldrick and A.F.M. Siebert, Fresenius' J. Anal. Chem., 346 (1993) 634.
[18] A.F.M. Siebert and W.S. Sheldrick, J. Chem. Soc., Dalton Trans., in press.
[19] L.F. Heneghan and J.C. Bailar Jr., J. Am. Chem. Soc., 75 (1953) 1840.
[20] F. Basolo, J.C. Bailar Jr. and B.R. Tarr, J. Am. Chem. Soc., 72 (1950) 2433.
[21] G.W. Watt and W.A. Cude, Inorg. Chem., 7 (1968) 335.
[22] P.A. Bristow and J.H. Knox, Chromatographia, 10 (1977) 279.
[23] O. Fini, F. Brusa and L. Chiesa, J. Chromatogr., 210 (1981) 326.
[24] M.J.M. Wells and C.R. Clark, Anal. Chem., 53 (1981) 1341.
[25] G.E. Berendsen, P.J. Schoenmakers and L. de Galan, J. Liq. Chromatogr., 3 (1980) 1669.
[26] D.R.K. Harding, C.A. Bishop, M.F. Tarttelin and W.S. Hancock, Int. J. Peptide Protein Res., 18 (1981) 214.
[27] G. Winkler, P. Briza and C. Kunz, J. Chromatogr., 361 (1986) 191.
[28] W.M.M. Schaaper, D. Voskamp and C. Olieman, J. Chromatogr., 195 (1980) 181.
[29] H.P.J. Bennett, C.A. Browne and S. Solomon, J. Liq. Chromatogr., 3 (1980) 1353.
[30] A.N. Starratt and M.E. Stevens, J. Chromatogr., 194 (1980) 421.

[31] D. Guo, C.T. Mant and R.S. Hodges, J. Chromatogr., 386 (1987) 205.
[32] C. Olieman and D. Voskamp, in W.S. Hancock (Editor), CRC Handbook of HPLC for the Separation of Amino Acids, Peptides and Proteins, Vol. 1, CRC Press, Boca Raton, Ann Arbor, 7th ed., 1990.
[33] V.R. Meyer, Praxis der Hochleistungs-Flüssigchromatographie, Salle Sauerländer, Frankfurt, Aarau, 1990.
[34] B.A. Bidlingmeyer, S.N. Deming, W.P. Price Jr., B. Sachok and M. Petrusek, J. Chromatogr., 186 (1979) 419.
[35] B.A. Bidlingmeyer, J. Chromatogr. Sci., 18 (1980) 525.