行政院國家科學委員會補助專題研究計畫成果報告

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※ 異相錯合反應之分析應用-[1] 配位基交換柱層分析 ※

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※ [2] 毛細管電層析, [3] 鉗合離子層析 ※

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計畫主持人：劉春櫻

共同主持人：

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□赴國外出差或研習心得報告一份

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行政院國家科學委員會專題研究計畫成果報告

計畫編號：NSC 89-2113-M-002-072

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主持人：劉春櫻 國立台灣大學化學系

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一、 中文摘要

第一部份

本研究利用含十二烷基月桂酸及十八烷基硬脂酸銅碟型液晶單體為液晶原，並以十一烷基希酸或十五烷基希酸為間距連結聚矽氧烷高分子，以重疊的方式合成不同臂長之混合配位基之熱向性金屬液晶聚合物．上述所有液晶單體及含金屬錯合物側鏈型液晶聚合物除以元素分析,紅外線光譜及核磁共振光譜等確定其性質外，並以偏光顯微鏡及微差掃描卡計測定其液晶性質．而由層析最適當條件變數之探討，如管柱長度，注入器溫度，載流氣體種類，載流氣體流速，烘箱溫度，移動相流速等，將此毛細管固定相利用於高分子或環境樣品中鄰苯二甲酸酯類化合物之分析．

關鍵詞：金屬液晶聚合物，毛細管氣相層析固定相，鄰苯二甲酸酯類．

第二部份

本研究係先將 histidine 官能基以化學鍵結的方式鍵結於矽膠表面，所得產品官能基化情形分別以元素分析，紅外線光譜及電滲透流測定以鑑定之．再將此官能基化矽膠填充於具有自製 frit 之毛細管中，由電滲透流測定及最適電壓，背景電解質種類，及背景電解質濃度等變數之探討，將其利用於有基酸及光學異構物之分析．本研究並且探討所合成矽膠之鉗合性質，繼而將所製備官能基化矽膠與適當金屬錯合後作為配位基交換毛細電泳填充管柱，利用於具有電子給與者化合物之分析．

關鍵詞：官能基化矽膠，毛細管電層析，填充管柱，配位基交換，固定相

第三部份

本研究合成含 2-mercaptoethylamine 官能基及γ-aminobutyrohydroxamate 之樹脂，探討並比較此二種樹脂與釩、鉬、鎢等金屬離子之鉗合行為．由感應耦合電漿質譜儀最佳操作參數條件之探討,測定樹脂於不同酸度溶液中的金屬離子收容量，金屬離子在樹脂相和溶液相中的分佈比例等．再以單槽平衡法研究此樹脂於最適條件下對所探討金屬的濃縮回收率，進而將所合成樹脂分別作為濃縮管和分離管之管柱填充物質，以ICPMS作線上偵測,利用於不僅氧化態和立體化學有多樣變化，其化學性質也可說是過渡元素中最為複雜的釩、鉬、鎢等混合物之分析．

關鍵詞：鉗合樹脂， 鉗合層析，固定相．

Abstract

Part I: Two metallomesogenic polymers, P-$C_{11}CuC_{18}$ and P-$C_{11}CuC_{12}$ consisting of flexible aliphatic side chains with copper carboxylate complexes have been synthesized on the basis of addition reactions with polysiloxane. The applicability of the prepared columns to the analysis of phthalate esters that are of environmental concern was assessed. For preparation of the capillary column with its wall coated with the metallomesogenic polymer, both static and dynamic methods were employed. Factors affecting the retention and the sample selectivity on both columns were examined. A better separation of phthalates was achieved with P-$C_{11}CuC_{18}$ than P-$C_{11}CuC_{12}$. The former phase showed a higher solute-solvent interaction than the latter. With static coating, the wall-coated P-$C_{11}CuC_{18}$ capillary column (15 m × 250 μm I. D.) showed that the base-line separation of all 14 phthalates could be achieved within 38 min with high reproducibility. The calibration graphs for phthalate ester determination were linear over the range of 10 – 625 μg ml^{-1}. The mass detection limits were lower than the ng range based on three times standard deviation of seven measurements of the lowest peak that could be detected.

Key words: Metallomesogens, Siloxane polymer, Stationary phase, Phthalate esters.

Part II: A histidine-functionalized silica was prepared by covalent bonding of the functional groups to silane-treated silica gel. Conversion of functional groups was confirmed by IR spectra, elemental analysis, and potentiometry. The functionality of the silica gel is 0.293 mmol g^{-1}. The coordination behavior of the histidine-functionalaized silica was investigated by metal capacity and electron paramagnetic resonance (EPR). EPR measurements at different copper loadings were made. The results showed that the copper histidine complex might be distorted tetragonal. Both histidine- functionalized silica and its copper complex were employed as stationary phases for packed capillary electrochromatography (CEC). Electrical current was found helpful for evaluating the properties of frit construction and the stationary phase packing. Test samples include neutral compounds, inorganic anions and organic anions. Factors influencing the separation behavior have been studied. With copper-histidine functionalized silica under the condition of citrate buffer (10 mM, pH 4.0) and applied voltage of –20 kV, the separation of benzoic acid, D- & L-mandelic acid, phthalic acid and salicylic acid could be achieved within 12 min. The column efficiency for these acids was more than 1.2 × 10^5 plates m^{-1}, except salicylic acid.

Key words: Histidine-functionalized silica; Copper-histidine functionalized silica complex; Capillary electrochro- matography; Stationary phase.

Part III: In this work, two kinds of chelating resin, bis (2-aminoethylthio) methyllated resin (BAETM) and γ-amino-butyrohydroxamate resin (γ-ABHX) were

synthesized. To which the former was with a hydrophobic skeleton, and the latter was with a hydrophilic skeleton. The functionality for each one was 0.91 mmol g^{-1} and 2.21 mmol g^{-1}, respectively. The chelating behavior towards vanadium, molybdenum and tungsten as a function of pH were studied. To perform trace metals analysis in complex matrices, we have developed a hyphenated method: chelation ion chromatography coupled on line detection with ICP-MS. Both BAETM and γ-ABHX were employed in this investigation. With BAETM resin column (5 × 0.4 cm I.D.) as the separator, sample volume of 20 μL, nitric acid (pH 1.5) as the eluent and flow rate of 1 mL min^{-1}, the detection limits for the determination of vanadium, molybdenum and tungsten were 0.01 ppb and the linear ranges were up to 100 ppb for each element. For the determination of these elements (5 ppb for each) spiked in artificial sea-water samples (10^4 fold dilution with pure water), γ-ABHX resin column (3 ×0.6 mm I.D.; particle size, 100-140 mesh) demonstrated well resolved peak separation between the analytes and the matrix elements-calcium and magnesium, by using only sodium nitrate (10 mL, 10^{-4} M) as the eliminator.

Key Words: Chelating resin; Chelation ion chromatography;On-line detection; ICP-MS.

二、緣由與目的

Part I: The vast majority of liquid crystalline substances are organic compounds with a rod-like (calamitic) or flat disc-like molecular shape. In recent years, an increasing number of research groups have turned their attention to metal-containing liquid-crystals or metallomesogens. In 1984, Giroud-Godquin et al. suggested that the long-chain homologues of copper acetate might exhibit columnar mesophases owing to the adequate symmetry of their central binuclear cores [1]. Since then, Abied et al. began a systematic study on carboxylate complexes which are known to exist as binuclear molecules with two metal atoms bridged by four bidentate carboxylate groups [2]. The introduction of metal atoms provides liquid crystal chemistry and physics with different possibilities. The interesting properties have given rise to expectations of new applications of liquid crystals based on metallomesogens [3].

To meet particular requirements in liquid crystal display industries and other applications, many new liquid crystal polymers have been synthesized [4-8].Many metallomesogenic structures have also been introduced into polymeric systems. Metallomesogenic polymers can combine the promising properties of metallomesogens with the advantageous properties of polymers [9].

In our lab, both rod-like and disk-like metallomesogens were found to be highly promising as the stationary phases of gas chromatography for the separation of environmental pollutants [10-13]. The factors responsible for the elution order of most compounds analyzed with these metallomesogens were considered to be vapor pressure of the samples, polarity interaction, ligand exchange and molecular

geometry. The separation behaves somewhat like that of the complexation gas chromatography shown in the literature [14-17]. For further insight into the metallomesogens useful in the field of analytical chemistry, a systematic investigation of the molecular structure on the effect of the phase transition was studied.

We report here homologues of the metallomesogenic polymer with P-L_1CuL_2 where L_1 is C_{11} and L_2 are C_{12} and C_{18}. Study of the mesophases of these homologues can give information about the changes of properties of the mesophases with variation of the alkyl chain length. A comparison of similar phases of different homologues can help us gain insight into the orientation of molecules and molecular motions in these phases. Phthalate esters are used as plasticizers in polymeric materials such as polyvinyl chloride used for the production of consumer products and building materials [18-21]. Since there is considerably concern about the widespread occurrence of low levels of phthalate esters as plasticizers and the potential adverse impact of these upon the environment and human health [22, 23], the analytical application of these metallomesogenic polymers in this field will also be described.

Part II: Capillary electrochromato- graphy (CEC) can be viewed as a hybrid between capillary electrophoresis (CE) and microcolumn (capillary) liquid chromatography (μLC): its separation mechanism is largely chromatographic, while the mobile-phase transport is mediated by the electroosmotic action [1]. Despite the many advantages of CEC that have been demonstrated, several technical problems have slowed the development and general acceptance of CEC [2]. These include making suitable frits and packing stationary phases into a column. Likewise, packing very long columns would be a difficult task. Therefore the main focus of future research will be on solving current problems and improving the column technology [3-7].

The driving force in CEC is the electroosmotic flow (EOF) and this is highly dependent on pH, the buffer concentration, the organic modifier and the type of stationary phase. The chemistry used to prepare the stationary phase can have a dramatic effect not only on the separation but also on the speed of analysis. The vast majority of examples of CEC to date have been performed on either C_8 or C_{18} stationary phases and this is not surprising considering the prolific amount of data available on these phases from the HPLC literature, while the other modes are much farther behind. The possibility of using different HPLC stationary phases is often viewed as "higher selectivity" for CEC.

Several ion-exchange stationary phases have been introduced into CEC for the analysis of charged organic solutes [8-11], inorganic ions [12] or chiral analytes [13, 14]. Voltage- induced sample release from anion exchange supports in CEC was studied by Kitagawa and Tsuda [15]. Specially developed packings for CEC in which silica particles coated with a mixture of sulfonic acid groups or amino groups and alkyl moieties have been reported [16-18].

The alkyl chains act as stationary phases to retain solutes while the charged groups result in high EOF at low pH. The retention behavior of glycosphingolipids on the octadecyl sulfonated silica was also investigated by Zhang *et al.* [19]. Spikmans et al. [20] used a Hypersil Duet C_{18}/SCX (strong cation exchange) mixed mode column for the separation of corticosteroids. Ye et al. [21] used a strong cation exchange packing based on silica as the stationary phase and dynamically modified by CTAB for the performance of CEC. The separation of neutral, acidic and basic compounds was investigated. Mix-mode stationary phase (C_6 /SAX) has also been employed as a means for the simultaneous separation of acidic, basic and neutral compounds in a single CEC run by Haddad and coworkers [22]. Several mechanisms including electrophoretic migration, ion-exchange and hydrophobic interaction are involved in the separation of the test solutes.

Histidine (isoelectric point, p*I* 7.6) as functional grouping of chelating ion exchangers has been used for soft metal ion extraction [23], metal ion elution from a chelating stationary phase [24], a dipolar eluent component in cation chromatography [25], and mobile phase of electrostatic ion chromatography [26]. In this paper, we describe the preparation of a histidine-functionalized silica and its copper complex as the stationary phases for CEC. At pH values below the isoelectric point, histidine would behave with cationic properties and act as an anion exchanger. Its metal complex could be employed as the ligand exchange property for the separation of electron-donating compounds. Meanwhile, the correlation between the construction of column packing, frit formation and electrical current has also been discussed.

Part III: Within the last decade, ICPMS has emerged as one of the most powerful techniques in inorganic trace analysis. The favorable combination of low detection limits, relatively simple spectra and versatility with respect to sample introduction devices has made it becoming the method of choice for many trace and ultra-trace elemental analyses. However, a number of difficulties may still be encountered in the analysis of complex matrices samples, such as biological samples or seawater. Many works relating ICPMS interfaced with ion chromatography have been applied to simultaneous and multi-element analytical techniques for the determination of trace elements [1-7]. The concentrated salt solutions tend to influence the nebulization efficiency due to the high viscosity. In addition, the interferences due to polyatomic ions cause a serious problem [8].

For the determination of trace metals in complex sample matrices with chelating resins termed as chelation ion chromatography (CIC) has received considerable attention in recent years [9-17]. The selectivity of a chelating resin is attributed mainly to the nature of the immobilized ligand on the base matrix.

In the previous work, we found that the introduction of mercaptoethylamine in the polymeric aldehyde could lead to the

ΔH of 25.0 and 64.0 J g^{-1}, respectively were found. We found two exothermic peaks once more at 83.0 and 37.7 °C with ΔH of 53.6 and 29.8 J g^{-1}, respectively on the second cooling process. For P-$C_{11}CuC_{12}$, only one endothermic peak at 81.0 °C with ΔH of 61.3 J g^{-1} was shown at first heating and one exothermic peak was found at 51.5 °C with ΔH of 63.9 J g^{-1} on the cooling process. There were two endothermic peaks at 81.6 and 98.4 °C with ΔH of 67.0 and 5.3 J g^{-1}, respectively on the second heating. The results obtained, together with those of the metallomesogenic monomers from DSC measurements are summarized in Table 1. All DSC profiles display at 10 °C/min. The DSC data showed a larger enthalpy change for the polymer than for the monomer from the crystalline state to the discotic lamellar phase, except for P-$C_{11}CuC_{18}$. The lower phase transition temperature of C_{18} analogues compared with C_{12} analogues indicates an easier rearrangement of the molecule into an ordered structure. Among the polymers studied, P-$C_{11}CuC_{18}$ appeared to cover a widest range of the D_L liquid crystal phase (60 °C), P-$C_{15}CuC_{18}$ is next (45 °C) and P-$C_{11}CuC_{12}$ is narrowest, only 17 °C. In other words, the temperature range seems dependent on the different chain lengths of the mix-ligand. A possible explanation is that some chain segments are able to associate relatively quickly but do not contribute significantly to network formation. When the metallomesogen is cooled to room temperature after first heating, all exhibited the temperature range for the D_{ho} phase. But both CuC_{12} and P-$C_{11}CuC_{12}$ got only one liquid crystal phase and no D_L phase was indicated. Thermal gravimetric analysis (TGA) showed that the temperature for the significant weight loss was higher than 260 °C. The morphological observations under polarized light microscopy at different temperatures were consistent with those described in the thermal analysis.

3.2. Column performance evaluation

In this study, the prepared columns were evaluated for their performance in the GC separation of phthalate esters. Due to the widest range of the liquid crystal phase among the polymer prepared, P-$C_{11}CuC_{18}$ was therefore chosen as the first priority stationary phase for the separation of phthalates.

3.2.1. Optimization of capillary GC procedure

In a preliminary test, at the conditions of injection temperature of 350 °C, split ratio of 1/40 and inlet pressure of 10 kPa, namely linear velocity being 5.9 cm s^{-1} were found the best conditions for the separation of phthalate esters. Because of the wide range of boiling points for the compounds tested (220 – 413 °C), it is not easy to separate all of them isothermally within a reasonable time.For investigating the column efficiency, isothermal conditions from 95-110 °C were studied for the five faster eluted phthalates. The conditions from 150–180 °C were studied for the other seven more slowly eluting phthalates. The chromatograms demonstrate good selectivity of the new stationary phase toward the phthalate substitutents, even for the positional isomers.

The separation efficiencies at 110 °C and 170 °C are summarized as Table 2. Most results are satisfactory. However, the results suggest that the temperature program was needed for the separation of all phthalates in a single run.

The dependence of log k' of phthalate esters against reciprocal absolute temperature of the polymeric metallomesogens was studied. Changes in the slope around 104 °C would be indicative of phase changes in the column but no distinct inflection point was indicated. The closely parallel lines indicate a rather similar solubility among the tested solute in the mesogenic phases. Meanwhile no significant variation of the solute solubility between D_L and D_{ho} phases for each solute exhibited. The mentioned properties resulted in no strong break in this plot. Additionally only one liquid crystalline phase (D_{ho}) existed over the temperature range studied, so linear line was indicated.

Under the conditions previously selected, several assays were performed to find a temperature program that provided the separation of the phthalate esters. One of the best options was the following program: 110 °C for 7 min, to 160 °C at 5 °C min^{-1} and held 10 min, then to 200 °C at 7 °C min^{-1}. To which a complete separation of all compounds is demonstrated.

3.3. Mechanism for the separation

The factors responsible for the elution order of the phthalate esters were considered to be as follows.

3.3.1. Polarity interaction

In this work, the separation order was dimethyl phthalate > diethyl phthalate > diisopropyl phthalate > diisobutyl phthalate > di-n-butyl phthalate > dimethoxyethyl phthalate > di-n-amyl phthalate > butyl-benzyl phthalate (1) > di-(2-ethylhexyl) phthalate (2) > dicyclohexyl phthalate (3) > dibutoxyethyl phthalate (4) > di-n-octyl phthalate > dinonyl phthalate > diphenyl phthalate. With EPA method 8060 for the separation of phthalate esters using Rtx-5 (5% diphenyl-95% dimethyl polysiloxane from Restek, Bellefonte, PA, USA) [24], a significant difference for the separation order was 1 > 4 > 3 > 2 for the noted compounds. While using a more polar stationary phase, Rtx-50 (50% phenyl-50% methyl polysiloxane), not only a nonresolved peak for 4 and 3 but also a significant reverse order for the noted compounds, 4 ≈ 3 > 2 > 1 was found. It was obvious that the polarity of the new stationary phase might be intermediate among them.

3.3.2. Ligand exchange

Phthalates with π electrons in the benzene-ring could form complexes with the stationary phase having an empty orbital of the central metal atom. Diphenyl phthalate has most π electrons among the compounds tested, thus the retention is strongest. The presence of longer alkyl groups might lead one to expect a greater electron density on the oxygen atom. Two extra oxygen atoms were present for the dibutoxyethyl phthalate and dimethoxyethyl phthalate. The coordinating bond with the copper atom of the stationary phase for the mentioned compounds would be stronger than any other compounds. Thus they were retained longer than other substituents with similar alkyl

length. We note also that dibutoxyethyl phthalate (bp 210 °C) is farther from the predicted time than dimethoxyethyl phthalate (bp 230 °C).

3.3.3. Molecular shape

Solutes most similar to the disk-type ordered environment would interact more strongly with the stationary phase. Therefore dicyclohexyl phthalate was eluted after di-(2-ethylhexyl) phthalate. This might be due to the fact that the former is inserted more easily into the ordered liquid crystal structure.

3.4. Linear calibration range

Detector response was linearly correlated with the sample concentration injected over a range of 10-625 μg ml^{-1} for all the compounds studied. The calibration graphs of peak area against the quantity of each analyte were listed in Table 3. The correlation coefficients for all the calibration graphs were between 0.9998 and 0.9979. The three times standard deviation of seven measurements of the lowest peak that could be detected is less than 30 pg for 1 μl injection except for dimethoxyethyl phthalate. The established system with high reproducibility and low detection limit (Table 3) indicate highly promising applied to the real sample.

3.5. Comparison of P-$C_{11}CuC_{12}$ and P-$C_{11}CuC_{18}$ columns

The effect of lauric acid and stearic acid respectively as the ligands of the metallomesogenic polymer on the separation of phthalate esters was also investigated. In this work, a dynamic coating procedure [13] was used for the preparation of both capillary columns (10 m × 0.25 μm I.D.). Meanwhile a shorter column (10 m) than the above mentioned column (15 m) was employed. With the column temperature of 110 °C (hold 2 min) to 195 °C (hold 10 min) then to 215 °C at the rate of 3 °C min^{-1} and inlet pressure of 15 kPa (hold 30 min) to 10 kPa at –3 kPa min^{-1}, a complete separation of 16 phthalate esters was shown, except di-(2-ethylhextl) phthalate and dibutoxyethyl phthalate was non-baseline resolved. While at the column temperature of 110 °C (hold 2 min) to 190 °C (hold 10 min) at the rate of 5 °C min^{-1} then to 215 °C at the rate of 3 °C min^{-1} for $PC_{11}CuC_{12}$, only 12 peaks were observed for these compounds. As expectation, a better separation was demonstrated for P-$C_{11}CuC_{18}$ than P-$C_{11}CuC_{12}$. Meanwhile a stronger interaction was indicated for most phthalates toward P-$C_{11}CuC_{18}$.

Part II. The histidine-functionalized silica was characterized by elemental analysis, IR spectrum, hydrogen capacity and metal capacity. The functionality was 0.293 mmol g^{-1}. This is a heterogeneous system; the equilibrium cannot be attained as fast as in a homogeneous system. Hence the titration could not be carried out continuously in a single vessel. Accordingly a small amount and higher concentration of NaOH (2 μL, 0.2 M) each time was added to a large volume of aqueous solution (50 mL) containing the histidine-functionalized silica (0.1 g). From the titration curve the estimated pK_a value is 2.8 for the carboxyl group and 8.0 for the protonated imidazole nitrogen. By comparison with the pK_a values

of the histidine monomer (1.9 for the carboxyl group, 6.1 for the imidazole nitrogen), the results are reasonable since the silica gel support has a steric hindrance for the bonded functional groups.

The copper capacities of the histidine-functionalized silica at various pH values were studied (Table 1). The results show that the metal capacity at pH 5.0 is 0.15 mmol g^{-1}. Compared with the functionality of the prepared silica, a one to two metal ligand ratio was indicated. Electron paramagnetic resonance (EPR) studies of copper complexes yield valuable information regarding the nature of bonding between the metal ion and the donor atom. In this work, EPR spectra of the histidine-functionalized silica as a function of pH prepared in the presence of excess copper ion were measured at room temperature. Table 1 depicts EPR parameters for its copper complexes. The *g* values calculated from spectral data show that $g_{//} > g_{\perp}$ which is characteristic of tetragonal or square planar geometry [29] and the value of $g_{//} > 2.3$, indicates the ionic character of the metal-ligand bond [30]. If G value > 4.0, then the local tetragonal axes are aligned parallel or only slightly misaligned; If G < 4.0, significant exchange coupling is present and the misalignment is appreciable [31]. In the histidine- functionalized silica the EPR spectra of copper ions adsorbed in media of pH greater than 5 revealed distinct differences from those in media of pH less than 5. The copper-histidine functionalized silica complexes showed $g_{//}$ components in their EPR spectra. This was to be expected, since most of the imidazole groups in the histidine-functionalized silica exist in the free base form under these conditions. A greater extent of coordination would occur and a smaller motion of copper ions might be. Comparison of the EPR parameters with those of the copper-histidine complex covalently bonded on a silica matrix prepared by the sol-gel method [32], a ratio of copper to histidine residue equal to 2 was suggested. In other words the CuN_4 model can be deduced from the complexing behavior of the histidine-functionalized silica.

The IR spectra of the histidine-functionalized silica and silica gel matrix were also measured. The functionalized silica exhibited peaks centered between 2800 and 3000 (C-H) and at 1644 (C=N), 1380 cm^{-1} (C=O). Neither of these features is observed on the silica support. Other potential features of the histidine spectrum such as -C-O, -C=O and $-NH_2$ bands are either too weak to be observed or are masked by the residual signals from the silica matrix and/or water adsorbed on the silica surface.

3.2 **Analytical application of the histidine-functionalized silica**

3.2.1 Packing procedure

Three kinds of homemade packed column were tested, as shown in Fig. 1. Basically a retaining frit is made from silica and sodium silicate, and slurry of the stationary phase is pumped into the capillary at high pressure. Once packed, both retaining frits are burnt in place. The packed column coupled with an empty column having the detection window preinstalled in the instrument is then ready for use. Data presented typically represent

an average of the results obtained on two separate columns. In this way, the column lifetime will be longer. Preliminary tests showed that the migration time of the first type of partially packed column exhibited serious variation. Both the first and the second type of packed columns required high voltage and a longer time for the injection (Fig. 1a and 1b). This might be due to a frit existing just adjacent the sample inlet end. Bubble formation easily arose while changing a new packed column. Meanwhile it was followed immediately by a breakdown in the current. For solving this problem, the third kind of packed column was adopted in all further experimental work (Fig. 1c). Due to the limitation of the capillary installation in Spectra PHORESIS 100, a long open inlet length of capillary (at least 8 cm) has to be used. Of course this will make the assessment of the EOF more difficult. Therefore, the retention behavior was demonstrated with linear velocity in most cases.

3.2.2 Packing and frit stability

Observing the initial and average currents seems helpful for evaluating the properties of frit construction and stationary phase packing. If the initial current is zero, the following conditions might apply. 1. The column is not wholly occupied by the background electrolyte (BGE). 2. The liquid level is too low in the buffer reservoir and no BGE can flow into the capillary. 3. There is a blockage or a crack in the capillary. 4. The concentration of BGE is too low. If a stable current cannot be achieved for a long time, this might be due to the following: 1. Bubble formation, 2. Contaminated BGE. 3. A longer conditioning time is required.

For a given system the rate of heat generation is directly proportional to the molar conductivity of the solution (buffer type), the buffer concentration, the applied voltage squared and the column diameter squared and are inversely proportional to column length. Therefore the current related to these parameters was also investigated.

3.2.2.1 Column length

When a potential difference is applied across a buffer-filled capillary, the presence of stationary phases would increase the resistance of the system. Resistance is inversely proportional to the current. With similar field strength (total length of 78 cm and applied voltage of -25 kV) and acetate buffer (10 mM, pH 5.0), the average current decreased as the histidine-functionalized silica packed column length increased (over the range of 5 ~ 40 cm was tested). Bubble formation could be seen notably when a longer packed column was used. Twice the packed column length, from 20 cm to 40 cm, even a decrease of 70 ~ 80% current per unit length was observed. While under the above mentioned condition but with phosphate buffer (10 mM, pH 5.0) and copper-histidine silica complex column, the current was merely changes from 5 into 3 μA when the active bed length of 5cm increases to 10 cm.

The mentioned behavior of the packed column (35 cm) compared also with that of a bare fused silica capillary, having frits at both ends and similar column length. With the applied voltage of +20 kV and phosphate buffer (25 mM, pH 4.0), a stable current (35

μA) displayed by coupling a buffer-filled capillary, while coupling an empty capillary column first then filling the buffer could see an unstable current with only 17 μA initially then decreased gradually to 4 μA after 5 min. Therefore, it can be concluded that coupling a buffer-filled capillary would be beneficial to the work. Additionally long time (forty minutes) was necessary for the migration of thiourea. The phenomenon may indicate that the surface chemistry of the frit might involve the retention of the solute more or less.

3.2.2.2 Type of buffer

Several kinds of buffer including acetic acid-sodium acetate, acetic acid-ammonium acetate, phosphoric acid-sodium dihydrogen phosphate and citric acid-sodium citrate have been tested as the BGE in this study. The results showed that at pH 4.0, no significant difference in current was observed except with citric acid. Since the equivalent conductivity [33] and the effective charge of citric acid at pH 4.0 are greatest among them (Table 2), the results are reasonable.

3.2.2.3 Buffer concentration

With the active bed length (5 cm), effective length (40 cm), total length (78 cm) and applied voltage of –20 kV, increasing the citrate buffer concentration from 5 mM, 7.5 mM to 10 mM, the current increased from 3.5 μA, 4 μA to 4.5 μA. It was found that the concentration of BGE more than 10 mM can give rise to Joule heating followed by loss of current as a result of bubble formation.

3.2.2.4 Applied voltage

The relationship between the current observed and the applied voltage was carried out under the condition of active bed length (10 cm), effective length (40 cm), total length (78 cm) and citrate buffer (10 mM, pH 4.0). The results demonstrated that -5kV vs. ~ 0.1 μA, -10 kV ~ 1 μA, -15 kV ~ 2μA, -20 kV ~ 3 μA, -25 kV ~ 4 μA. With applied voltage greater than -25 kV, a deviation from the Ohm's law was indicated. Meanwhile this occurred along with bubble formation and was followed by the loss of current.

3.2.2.5 Organic modifier

Bubble formation may be easy to occur at low fraction of acetonitrile for CEC with hydrophobic stationary phase like ODS. Usually with the addition of acetonitrile a higher EOF velocity can be obtained because of its low viscosity. In this work, acetonitrile (5~10%, v/v) has been tested as the mobile phase additive. Moreover, a smaller current and less EOF was found compared with that without the modifier. This might be due to the histidine-functionalized silica being a more hydrophilic stationary phase compared with ODS. For changing the linear velocity of the solute, other additives such as sodium lauryl sulfate (SDS), cetyltrimethylammonium bromide (CTAB) and copper ion have been employed. As expectation, CTAB may be adsorbed to the packing surface and increase the density of positive charge on the packing surface which results in higher EOF velocity. With a 20 cm histidine-functionalized silica active bed, effective length (51 cm), total length (83 cm), a mixture of phosphate buffer (pH 5.0, 25 mM) and CTAB (1 mM) as BGE, and the separation voltage of -25 kV, the

migration time of mesityl oxide was 79 min. The calculated EOF is 3.57×10^{-5} cm^2/Vs. Not only CTAB, but also SDS and copper ion, all increased the current to some extent compared with that without the modifier.

3.3 Column performance studies

It is desirable when evaluating a new stationary phase to test both the EOF and the chromatographic performance. Several neutral markers were tested to give an indication of the EOF. Finally the resolving power of the chromatographic phase was determined by the organic anions and inorganic anions.

For the determination of EOF in CEC the choice of an appropriate neutral marker is not easy, since the neutral marker always has some interaction with the stationary phases [34]. Acetone, benzyl alcohol, dimethyl sulfoxide, mesityl oxide and thiourea were selected as neutral markers for the histidine-functionalized silica and its copper complex packed columns. But all of them appeared some interaction with the stationary phase. This resulted in a very small EOF and a rather long time for the migration.

With a copper-histidine functionalized silica complex active bed (10 cm), effective length (51 cm), total length (83 cm), phosphate buffer (pH 4.0, 25 mM) and the separation voltage of -20 kV, the migration time of thiourea was 190 min. From that, the calculated EOF was 1.86×10^{-5} cm^2/Vs (linear velocity, 0.27 cm/min). The linear velocity was measured using L_d/t, where L_d denotes the effective length of the capillary column and t is the elution time. With similar condition as that of the copper-histidine functionalized silica but with the applied voltage of +20 kV, the EOF for the bare fused silica having frits at both ends was 8.82×10^{-5} cm^2/Vs (migration time, 40 min and linear velocity, 1.28 cm/min). A distinct lower EOF was demonstrated for the copper-histidine functionalized silica column.

3.3.1 Inorganic and organic ions

The most commonly used columns in CEC have been ODS, but in view of the need to maintain a high enough EOF and the hydrophobic surface of the stationary phase, ODS can only work well in a mobile phase with relatively high pH and the presence of an organic modifier. Therefore there is an urgent need to develop new kinds of stationary phase suitable for CEC separation of strongly polar acidic compounds [35].

With histidine-functionalized silica and its copper complex respectively as the stationary phase, the effect of buffer pH on the linear velocity of potassium hydrogen phthalate (KHP) was studied. The linear velocity in the histidine-functionalized silica decreased dramatically over the pH range from 7 to 5. When KHP was run on the copper complex, no significant increase in analysis time on going from low to high pH was indicated. Results of this experiment may be ration- alized that a smaller fraction of the protonated histidine-functionalized silica at higher pH values results in a less anion exchange reaction and this leads to a greater linear velocity. Besides, a larger electropho- retic mobility of the solute would be due to the greater fraction of the dissociated form. While for the copper-

histidine complex, increasing the pH results only in a slight decrease of linear velocity. This may reflect a more stable analyte-copper complex in the ligand exchange system. Moreover at pH 4.0, there seems to be no significant difference in the linear velocity between these two stationary phases. In other words, the differences for the interaction force of the anion exchange and ligand exchange reactions might not be distinct.

With 10 cm active bed length of the copper-histidine complex; 40 cm effective length and 78 cm total length (100 μm I.D.) as well as phosphate buffer (pH 7.0, 10 mM) and a separation voltage of –20 kV, the linear velocities (cm/min) of $NaNO_2$, $NaNO_3$, Na_2SO_4 and KHP were 1.36, 5.33, 7.43 and 0.49 respectively. Clearly the prepared CEC column is useful for the separation of all the mentioned compounds, especially for nitrite and nitrate mixture, which is not easy in CE separation [36] due to the rather similar equivalent conductance (Table 2). In this work, the differences in linear velocity might be attributed to the greater coordinating property of nitrite ion toward the central copper ion than nitrate ion.

3.3.2 Separation of the test mixture

Phosphate and ammonium acetate buffer have been employed as the BGE for the separation of organic acid mixtures. However, retention times longer than 2h were indicated. To shorten the analysis time, a shorter active bed (5 cm) and a stronger coordination buffer, which would interact with the central copper ion were used. As expectation, sharp and symmetrical peaks were obtained in less than 12 min using citrate buffer (10 mM, pH 4.0). The elution order is benzoic acid > D-mandelic acid > L-mandelic acid > KHP > salicylic acid. According to the effective charge and the stability constants of these compounds toward copper ion (Table 2), this fact strongly indicates that the separation mechanism is predominantly based on a ligand exchange reaction besides the electrophoretic mobility. Of course several other retention mechanisms might also be involved.

With lower concentration of citrate buffer (7.5 mM, pH 4.0), five peaks can be observed but the elution strength decreased obviously. The elution time of the late-eluted salicyclic acid is even more than 100 min with the citrate buffer (5 mM, pH 4.0) (data not shown). It can be seen that the retention decreased with increase of buffer concentration. The phenomena further prove a displacement reaction occurred. In other words, ligand exchange reaction indeed involved in the separation mechanism. Additionally the direction of EOF in bare fused silica is opposite to that of the sample inlet. At lower ionic strength, the EOF may be more rapid resulting in slower migration of the solute to the packed column. Therefore longer time was needed for the elution of these solutes. Besides, the influence of the buffer concentration on the distinguishing ability of the enantiomer was found not so much.

In order to understand the effect on retention behavior of a stronger chelating

agent than citrate buffer, we chose EDTA (5 mM, pH 4.0) as the model. Not only less retention but also more complete elution, namely a greater peak area was observed. Meanwhile the coelution of D-& L- mandelic acid was indicated. The predomi- nant form of EDTA at pH 4.0 is H_2Y^{2-}. A greater effective charge and stronger coordinating ability (Table 2) results in the mentioned properties.

In separation of organic acids, retention parameters and column performance are listed in Table 3. The column efficiency for most of the analytes was more than 1.2×10^5 plates/m, except salicylic acid. However, columns were considered successfully made only if they were uniform and free of void after conditioning, use and storage. The relative standard deviations of the migration time are over the range of 3~7% for five consecutive injections. Of course reproducibility cannot be better as that of the open tubular column. But improvements are expected as pressure applied to both ends of the capillary and frit more homogeneous.

Part III: In this work, bis (2-aminoethylthio) methylated resin (BAETM) and γ-amino-butyrohydroxamate resin (γ-ABHX) columns were investigated for the separation of molybdenum, tungsten and vanadium. The matrix for the former was styrene-divinylbenzene copolymer, while the latter was acrylonitrile-divinyl-benzene copolymer. The pH dependence of the sorption behavior of these resins toward molybdenum, tungsten and vanadium was tested by the batch equilibrium method. Samples were run in excess resin-ligand concentration. After equilibration, the amount of metal ion in the solution or in the resin phase was determined to calculate the distribution coefficient (D). To perform this study, acetate buffer (0.1 M) or nitric acid was used for adjusting the pH. For BAETM, molybdenum, tungsten and vanadium were retained most strongly at pH 4. The affinity increased in the order of vanadium < molybdenum < tungsten. As expectation, the greater the ionic size, the more stronger affinity for the soft ligand was. Positive molybdenyl ion, MoO_2^{2+}, existed in the high acidity medium (pH~1), isopoly molyb-denum, $Mo_xO_y^{n-}$ existed in the pH around 1 to 2, while negative charge molybdate ion, MoO_4^{2-}, existed in the higher pH range [24]. Therefore a greater affinity toward the resin at higher pH range was rational. While at pH above 4, lower affinity for Mo might be due to the highly stable of molybdate ion. For γ-ABHX which contains hard ligand, a different selectivity order: molybdenum > vanadium > tungsten was shown. The order reversal gave the evidence for the preference of soft ligand toward the soft metal ion. By further comparison the adsorption behavior of Mo, W, and V in these two resins, a very similar tendency for Mo and W was indicated at most conditions. Vanadium exhibited a different phenomenon. A favorable retention was under the condition of pH above 4. Since at lower pH, the predominant form of vanadium is VO^{2+}, negative charge form of polyvanadate or metavanadate existed at weak acidity or neutral medium, the mentioned results are reasonable [21].

Preconcentration

Effect of the flow rate on the breakthrough capacity

The effect of sample flow rate on the breakthrough capacity of BAETM resin column was investigated. With BAETM column (5 × 0.4 cm I.D.) as the separator, sample volume of 20 ml, nitric acid (pH 1.5) as the eluent and flow rate of 1 ml min^{-1}, calibration curves were constructed for all three metal ions. Typical regression parameters are presented in Table 1. The detection limits were 0.01 ppb and the linear range were up to 100 ppb for each element. In all cases, linearity was excellent within the concentration range examined.

Optimization of elution condition of analyte elements

Nitric acid is the first acid of choice because it has a background spectrum very similar to that of water. According to the adsorption behavior of these resins, only mineral acid seems not a promising eluent in the separation column. Some parameters such as ionic strength, flow rate and other chelating agents, such as nitic acid, sodium nitrate, ethylenediamine, nitrilotriacetic acid, oxalic acid, thiourea and EDTA were varied to be as the eluent. The results showed that sodium nitrate was the best among the eluents tested for bis(2-aminoethylthio) methylated resin. Ethylenediaminetetra-acetic acid (EDTA) was the best stripping agent for these metal ions on γ-amino-butyrohydroxamate resin, but no resolution for them was indicated. Ethylenediamine seems a better reagent for the resolution among the tested reagents.

Analytical application

The signal ratio for the determination of vanadium, molybdenum and tungsten (5 ng ml^{-1} for each) spiked into the sea water by ICPMS was 0.75 : 0.42: 1. While that for the pure water was 0.94: 0.41: 1. Matrix effect for the determination of vanadium in sea water was obvious.

Thus the chromatographic behavior of molybdenum, tungsten and vanadium in sea water with γ-aminobutyrohydroxamate resin was investigated. With the amount of 0.1 g resin packed into a PEEK column (1 × 0.6 cm I.D.), sodium acetate buffer (0.1 M, pH 3) as the condition solution (4 ml min^{-1} for 3 min), sodium nitrate (0.1 mM) as the eliminator (2 ml min^{-1} for 5 min) and EDTA (0.1 mM, pH 6.5) as the eluent (2 ml min^{-1}), the mixture of vanadium, molybdenum and tungsten (5 ng ml^{-1} spiked for each) could be resolved from the matrix elements of the sea water, magnesium and calcium. However, by comparison with that for the analysis of pure water, an obvious matrix effect for the determination of vanadium was still demonstrated.

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Part I:

Table 1. Phase transitions of the metallomesogenic polymers[a]

	Phase transitions (°C) and corresponding enthalpy changes (J/g)	
	Heating	Cooling
Polymer		
CuC_{18}[b, c]	k -- 54.0 (23.0) -- D_L -- 125.0 (37.4) -- D_{ho} -- 200 -- i	i -- 200 -- D_{ho} -- 107.6 (52.6) -- D_L -- 41.1(13.5) -- k
CuC_{12}[b]	k-- 94.0 (21.9) -- D_L -- 118.3 (83.9) -- D_{ho} -- 200 -- i	i -- 200 -- D_{ho} -- 71.3 (102.9) -- k
P-$C_{15}CuC_{18}$[c]	k-- 50.7 (36.4) -- D_L -- 95 (4.4) -- D_{ho} -- 200 -- i	i -- 200 -- D_{ho} -- 77.5 (5.8) -- D_L -- 33.2 (43.7) -- k
P-$C_{11}CuC_{18}$	k -- 44.4 (25.0) -- D_L -- 104 (64.0) -- D_{ho} -- 200 -- i	i -- 200 -- D_{ho} -- 84.5 (54.9) -- D_L -- 36.6 (25.0) -- k
P-$C_{11}CuC_{12}$	k -- 81.6 (67.0) -- D_L -- 98.4 (5.3) -- D_{ho} -- 200 -- i	i -- 200 -- D_{ho} -- 51.5 (64.0) -- k

[a]Data are from second heating and first cooling scans. k: crystalline, D_L: discotic lamellar phase, D_{ho}: ordered hexagonal columnar discotic mesophase, i: isotropic phase. [b]Metallomesogenic monomer. [c]Data from ref. 13.

Part I:

Table 2. Separation efficiency of the fused-silica capillary column with wall-coated metallomesogenic polymer[a]

Analytes	Retention time (min)	*N* (plates·m^{-1})	*HETP* (mm)
Dimethyl phthalate[b]	3.88	1251	0.79
Diethyl phthalate[b]	4.99	1137	0.88
Diisopropyl phthalate[b]	5.81	1335	0.75
Diisobutyl phthalate[b]	12.40	885	1.13
Di-n-butyl phthalate[b]	19.50	815	1.23
Butylbenzyl phthalate[c]	9.48	987	1.01
Di-(2-ethylhexyl) phthalate[c]	12.43	889	1.13
Dicyclohexyl phthalate[c]	12.74	1000	1.00
Dibutoxyethyl phthalate[c]	14.22	1353	0.74
Di-n-octyl phthalate[c]	16.26	620	1.61
Dinonyl phthalate[c]	18.72	1277	0.78
Diphenyl phthalate[c]	25.70	914	1.09

Stationary phase: wall coated with P-$C_{11}CuC_{18}$ (15 m × 250 μm I.D.); Injection volume: 1 μl; Injector temperature: 350 °C; Inlet pressure: 10 kPa; Split ratio: 1/40; Carrier gas: nitrogen; Sample concentration: 320 μg ml^{-1}. [b]Column temperature: 110 °C; [c]Column temperature: 170 °C. Abbreviation: *N*, number of theoretical plate; *HETP*, height equivalent to theoretical plate.

Part I:

Table 3. Summary for the determination of phthalate esters with the wall coated P-$C_{11}CuC_{18}$ capillary column[a]

Analytes	Retention time (min)	Linear equation[b] (y: peak area, μV sec; x: concentration, μg ml^{-1})	Detection limit (pg)
Dimethyl phthalate	6.57 ± 0.04[c]	y = 14.37x + 60.43 (0.9994)[d]	18.24
Diethyl phthalate	9.07 ± 0.07	y = 15.34x + 92.40 (0.9993)	24.33
Diisopropyl phthalate	10.35 ± 0.08	y = 16.66x + 100.80 (0.9994)	16.56
Diisobutyl phthalate	15.19 ± 0.05	y = 16.52x + 194.87 (0.9994)	15.29
Di-n-butyl phthalate	17.32 ± 0.11	y = 15.87x + 188.70 (0.9993)	20.87
Dimethoxyethyl phthalate	19.21 ± 0.03	y = 11.02x – 126.96 (0.9979)	40.94
di-n-amyl phthalate	21.12 ± 0.03	y = 15.67x + 217.38 (0.9991)	10.17
butylbenzyl phthalate	27.00 ± 0.10	y = 14.19x + 119.76 (0.9987)	24.48
di-(2-ethylhexyl) phthalate	31.15 ± 0.03	y = 14.79x + 378.75 (0.9999)	16.02
dicyclohexyl phthalate	31.67 ± 0.03	y = 8.96x + 35.89 (0.9994)	28.16
dibutoxyethyl phthalate	32.76 ± 0.03	y = 15.37x + 665.67 (0.9998)	18.12
di-n-octyl phthalate	33.88 ± 0.06	y = 10.05x + 454.98 (0.9995)	14.38
dinonyl phthalate	34.86 ± 0.03	y = 13.50x + 341.65 (0.9992)	16.88
diphenyl phthalate	37.05 ± 0.04	y = 13.50x + 395.15 (0.9993)	17.63

[a]Conditions as Fig. 9. [b] Linear range studied: 10 – 625 μg ml^{-1}. [c]Average of eight measurements. [d]Correlation coefficient.

Part II:

Table 1. Parameters of the EPR spectra for histidine functionalized silica-copper complexes

pH	Capacity(mmol g^{-1})	$g_{\perp}$	$g_{/\!/}$	A(G)	G[a]	ΔH_{pp} (G)
1.72	0.02	2.069	---	---	---	215.4
3.58	0.05	2.076	---	---	---	214.9
4.34	0.06	2.061	---	---	---	103.4
5.41	0.18	2.066	2.449	143.2	6.80	95.5
6.23	0.30[b]	2.062	2.452	145.8	7.17	92.8
9.19	>0.32[b]	2.059	2.454	148.5	7.69	92.7
~7[c]		2.063	2.26	174		

a) $G = (g_{/\!/} - 2) / (g_{\perp} - 2)$

b) Precipitation occurs

c) Copper-histidine complex covalently bonded on a silica matrix prepared by sol-gel method (data from Ref.32)

Part II:

Table 2. Chemical and physical properties of the compounds related in this work

Compound	pK_a[a] (25°C)	log k_f[b]	Effective charge[c]	Equiv. conduct.[d]
Acetic acid (60)[e]	4.75	1.83[f]	-0.166	40.9
Ammonium ion (18)	9.25	4.12[g]		
Phosphoric acid (98)	2.15; 7.20; 12.35	3.2[f]	-0.387	33; 33; 69
Citric acid (192.1)	3.13; 4.76; 6.40	5.90[f]	-1.025	70.2
Chloride (35.5)		-0.06[g]		76.35
Nitrite (46.0)		1.19[g]		71.80
Nitrate (62.0)		-0.01[g]		71.40
Sulfate (96.1)		0.95[g]		80.00
Benzoic acid (122.1)	4.20	1.76[f]	-0.387	32.4
Phthalic acid (166.1)	2.95; 5.40	2.69[g]	-0.956	52.3
Mandelic acid (152.2)	3.40	2.70[g]	-0.799	
Salicylic acid (138.1)	2.97	10.6[f]	-0.914	36.0
EDTA (292)	2.00; 2.66; 6.16; 10.24	18.80	-1.962	
Histidine (155.2)	1.82; 6.0; 9.2	10.16[f]	+1.00	

a)Data from Ref. [37,38]. b)Formation constant of copper complex, data from Ref. [37-39].

c)Effective charge at pH 4.0. d) Limiting equivalent ionic conductance (S cm^2 $equivalent^{-1}$): data from Ref. [33].

e)Molecular mass. f)At 25°C, μ=0.1. g)At 25°C, μ=1.0.

Part II:

Table 3. Separation efficiency of the copper-histidine functionalized silica complex in packed CEC[a)]

Acid	Retention time (min)	Peak width (min)	Half peak width (min)	Plate height (μm)	$N^{b)}$ (plates m^{-1})	$R_s^{c)}$
Benzoic	5.828	0.057	0.035	0.322 (0.064)[d)]	153600	--
D-Mandelic	6.180	0.056	0.033	0.261 (0.052)	194290	6.23
L-Mandelic	6.816	0.072	0.046	0.404 (0.081)	121630	9.94
KHP	7.252	0.078	0.049	0.418 (0.083)	121350	5.81
Salicylic	10.103	0.189	0.117	1.200 (0.240)	41310	21.36

a) Capillary column: 100 μm i.d., packing length: 5 cm with 5 μm copper-histidine functionalized silica complex, effective length: 35 cm, total length: 73 cm; Background electrolyte: citrate (10 mM, pH 4.0); Sample concentration: 1 mM for each; Electrokinetic injection: -2 kV for 2 sec; Separation voltage: - 20 kV; Detection at 220 nm

b) Plate numbers

c) Resolution

d) Reduced plate height

Part III:

Table 1 Performance of the proposed methods[a,b]

Analyte	V	Mo	W
Linear range (ng ml^{-1})	up to 100	up to 100	up to 100
Linear equation	883 x + 2951	670 x + 1807	465 x + 812
R	0.9986	0.9999	0.9999
DL (ng ml^{-1})	0.01	0.01	0.01

[a]Column: bis (2-aminoethylthio)methylated resin (5 × 0.4 cm I.D.); Sample volume: 20 µl; eluent: nitric aid (pH 1.5); flow rate: 1ml min^{-1}; Detector: ICPMS pulse detector

[b] ICP-MS instrumental settings: Sampling and skimmer cones, Nickel;

Argon flow rate: Plasma gas, 15 l min^{-1}; Auxiliary, 1.2 l min^{-1}; Nebulization, 1.1 l min^{-1}; Sample uptake, 0.9 l min^{-1}.

RF power supply:1200 W. Lens voltage:7.5 V

Data acquisition: Scanning mode, Peak hopping; Dwell time, 100 ms;

Reading/sweep, 1; Sweeps/replicate, 1; Replicates, 5

Isotopes monitored, ^{51}V, ^{98}Mo, ^{184}W