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High-performance liquid magneto-chromatography

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Abstract

High-performance liquid magneto-chromatography (HPLMC) is a new chromatography technique with two distinctive features: (a) a high surface area stationary phase with paramagnetic properties composed of magnetite embedded in a silica gel, and (b) a magnetic field (variable intensity 0–5.5 mT) that selectively retains paramagnetic substances in the stationary phase depending on their magnetic susceptibility. The system can also be used to separate diamagnetic compounds such as biologically active organic molecules, but these first need to be complexed with Fe and Cu compounds to render them paramagnetic. Herein, we describe the experimental setup and the relationship between the retention factor and the magnetic field intensity, i.e., the force interaction of the complexes in relation to the magnetized magnetite. The expression derived also provides the effective magnetic susceptibility ($\Delta\chi$) of the components separated.

Keywords: Separations; Magnetic-chromatography; Paramagnetic complexes; Amino acids

1. Introduction

The separation of different compounds according to their magnetic properties is common practice in the manufacturing and mining industries [1,2]. However, in recent years the use of this technique has expanded to other areas such as biotechnology and analytical chemistry [3]. Given its benefits including speed, simplicity and selectivity, magnetic separation has promising applications in the field of separation techniques.

Industrial magnetic separators [4,5] are used to separate ferromagnetic or paramagnetic particles, that is, substances with a positive magnetic susceptibility value (χ). Nevertheless, most biological molecules such as amino acids and proteins have diamagnetic properties (i.e., negative susceptibility) or are weakly paramagnetic (they are not affected by a magnetic field). Thus, magnetic supports need to be used to promote their retention. These magnetic supports are usually polymer particles doped with magnetite or with colloidal suspensions of paramagnetic compounds.

The development of new methods of separating magnetic particles has been the goal of several research teams. These efforts have resulted in the emergence of a series of analytical separation techniques, designated magnetophoresis, used to characterize particulate and polymeric materials [6–8]. Thus, the field flow fractionation (FFF) system developed by Giddings in the mid 1960s and marketed in the late 1980s was initially based on gravitational, thermal and electrical forces. However, the incorporation at the start of 1980 of a magnetic field [9] turned this system into a high resolution and reliable analytical method for particles and polymers from a few nm to 1 μm in the normal mode and from 0.5 to 100 μm in the steric mode [8]. The split-flow thin fractionation (SPLITT) method based on differences in transport rates can be applied to gravitational [10] or magnetic [11] fields among others, and is useful for the separation of macromolecules, colloids and particles. The SPLITT procedure is particularly useful for the preparative separation of large molecules (FW > 10^6). The technique known as magnetapheresis [12] evolved from ferrography, an analytical method based on the magnetic deposition of particles in a free-flowing, open stream closed to a magnetic field, and generates a deposition pattern of magnetically susceptible particles from the suspending medium under carefully controlled flow and magnetic field conditions. In electromagnetophoresis (EMP), whose theory was developed by Kolin [13], particles migrate through an electrolyte solution in a direction perpendicular to a magnetic field and an electric current, when the electric current is applied through the

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conductive fluid and the homogeneous magnetic field is perpendicular to the current [14]. Finally, high-gradient magnetic separation (HGMS) is a powerful separation process with a great potential for industrial wastewater treatment. HGMS relies primarily on a magnetic force as the mechanism for the capture of particles on collectors [15,16].

In this paper, we advance one more step to develop a chromatography system in which the stationary phase is made up of particles with paramagnetic properties. These particles, along with the analytes eluted by the mobile phase, are subjected to a low intensity external magnetic field, which plays a key role in the separation process. We, therefore, consider that the term high-performance liquid magneto-chromatography (HPLMC) fittingly describes the technique developed. Herein, we derive a theoretical expression describing the effect of the magnetic field on the analyte retention time, and illustrate its use by determining the magnetic susceptibility of copper-labelled amino acids.

2. Theory

The model proposed is based on the presence of a stationary phase of paramagnetic particles subjected to an external magnetic field. In these conditions, lines of induction occur around these particles (Fig. 1). The paramagnetic analytes would drive into the randomly distributed high gradients around the magnetized packing particles. The paramagnetic analytes could be attracted then into the slower moving fluid close to the surface of the paramagnetic stationary phase rather than being carried by the faster moving fluid in the larger spaces between particles. The separation takes place due to the difference in the attraction force exerted by the high gradients.

The downstream motion of component bands in separation using external fields is characterized by the retention ratio R, a dimensionless parameter defined as [17], where U is the analyte velocity and v the fluid flow velocity

$$R = \frac{U}{v} \tag{1}$$

The particles in this separation system were mainly subjected to magnetic force, drag force, gravitational force, and Brownian motion. The gravitational force was parallel to the channel flow and was negligible, and Brownian motion is negligible for the micron-sized particles used in this study. Thus, we focused on the magnetic and drag forces in this separation system.

Magnetically induced particle velocity is derived from the balance of magnetic force and drag force. The magnetic force (F_m) acting on the particles can be expressed as a function of their effective magnetic susceptibility. The term "effective" refers to the medium in which the separation is performed. Hence, the movement of particles in a magnetic field is a function of their effective magnetic susceptibility $\Delta\chi$, and this magnetic force can be expressed as [14,18,19]:

$$F_m = \frac{V\Delta\chi}{2\mu_0}\nabla B^2 \tag{2}$$

where V is the volume of the particles, μ_o the vacuum magnetic permeability, ∇ the gradient operator, and B is the magnetic field intensity.

The drag force (F_d) can be expressed as [20]:

$$F_d = 3\pi\eta dU \tag{3}$$

where η is the carrier viscosity, d the particle diameter, and U is the particle migration velocity. Therefore, the magnetically induced particle velocity (U_m) can be obtained by balancing the magnetic force and drag force in Eqs. (2) and (3):

$$U_m = \frac{d^2\Delta\chi\nabla B^2}{288\eta\mu_0} \tag{4}$$

Eq. (4) indicates that the magnetically induced velocity is directly proportional to the effective magnetic susceptibility ($\Delta\chi$) and the intensity of the magnetic field around the particles ∇B^2. The gradient around the particles is related to the magnetization of the stationay phase particles. Since the magnetization of the particles is proportional to the applied field, we assume a proportionality between the magnetic field intensity of the particles and the ∇B^2 applied.

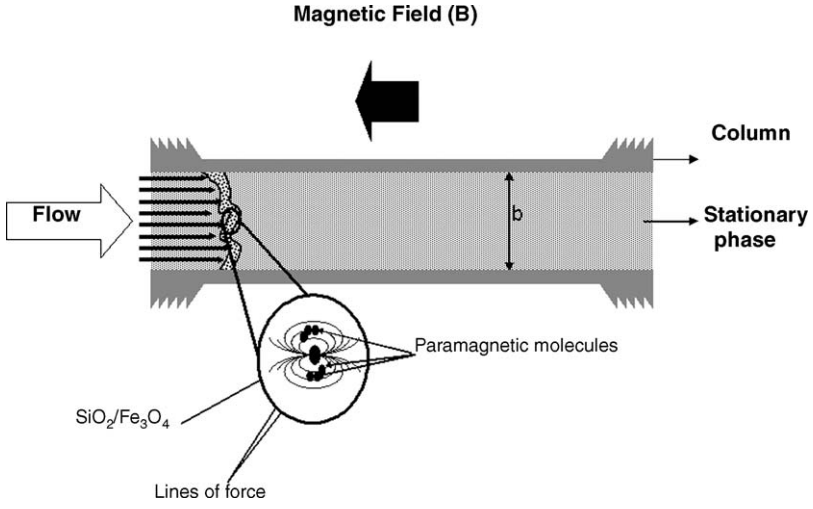

Fig. 1. Features of the chromatography column.

According to Eq. (4), an increment in the magnetic field intensity applied (B) on the column will produce the increase of the U_m value, then a lower motion of the analyte must be observed in the chromatogram (higher retention time).

3. Experimental

3.1. Standards, reagents and samples

All solutions were prepared by dissolving the corresponding analytical grade reagent in filtered, deionised water with a resistivity of 18.3 M Ω cm, and used without further purification. Phosphate buffer solution (PBS) was prepared by mixing equimolar amounts of $NaH_2PO_4{\cdot}2H_2O$ and $Na_2HPO_4{\cdot}H_2O$ (Fluka) and then adjusting the pH to the desired value with HNO_3 or NaOH.

A stock solution of 1 g l^{-1} was prepared by dissolving the appropriate amount of each amino acid: glycine, alanine, β-aminobutyric acid, leucine, methionine, histidine and asparagine (all from Aldrich) in PBS (0.1 M, pH 7). Standard 30 mg l^{-1} solutions were prepared daily by dilution of the corresponding stock solution. These solutions were stored at 4 °C.

To confer the amino acids paramagnetic properties, they were reacted with a complexing solution prepared by dissolving $CuCl_2{\cdot}2H_2O$ and 1,10-phenanthroline in stoichiometric amounts [21] in PBS (0.1 mol l^{-1}) according to Fig. 2.

The standards used were prepared by mixing 1 ml of Cu(*o*-phen)$^{2+}$ (20 mM) with 300 μl of amino acid stock solution and making the total volume up to 10 ml with PBS. The complexes formed are stable at the working pH and ionic strength.

3.2. Synthesis of the stationary phase and preparation of the chromatography column

Magnetite synthesized hydrochemically according to the method of Barrado et al. [22] is added to the reactor containing 20.0 ml of tetraethoxysilane (TEOS), 21.5 ml of water and 16.7 ml of ethanol. After the mixture has been stirred, the pH is adjusted to 10 using NH_3. Once the gel has formed, it is stirred for 24 h to complete the condensation process. The gel is then filtered, washed and dried at 50 °C for 48 h [23]. The solid synthesized is ferrimagnetic, it posses magnetic properties in presence of an external magnetic field and its magnetization is proportional to the magnetic field intensity applied.

Stationary phase particle size distribution was determined using a Horiba Model LA-900 laser light scattering particle size distribution analyzer with a helium–neon laser of wave length 633 nm and Horiba Data Systems software. The xerogel synthesized was dispersed in distilled water at 25 °C. The sample was continuously agitated in order to disperse the solid particles within the mixture. A few minutes later, laser light scattering measurements were made.

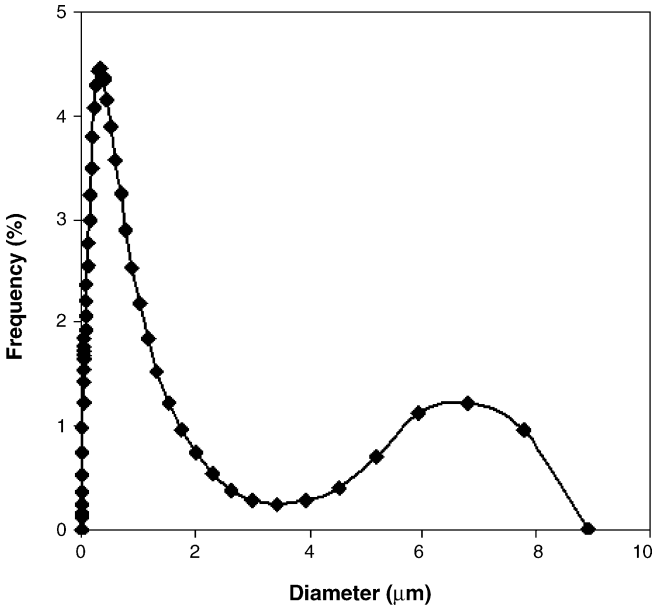

Fig. 3. Diameter distribution of the stationary phase.

The particle size frequency distributions for the stationary phase (Fe_3O_4–SiO_2) are shown in Fig. 3. There are two distributions clearly defined in the figure. Mean particle diameters were estimated as 0.5 and 6.8 μm for each distribution. The smaller particle size is most likely due to the silica gel, while the other size corresponds to silica gel embedded with magnetite.

A steel column (4.6 mm × 10 cm) was filled with a SiO_2/Fe_3O_4 suspension in phosphate buffer solution (0.1 mol l^{-1}, pH 7), which was then suctioned using a vacuum pump. The column was conditioned by passing PBS through the column at a constant flow rate of 1.0 ml min^{-1}.

3.3. Equipment and experimental conditions

The setup used included the basic components used for liquid chromatography (Fig. 4): a container for the mobile phase, a Gilson model 302 pressure pump, a Rheodyne mod. 7525 injection valve, a column as specified above and a UV–vis diode-array HP8453 spectrophotometer as detector. The column was wrapped with a copper coil (300 turns) such that the external magnetic field intensity (B) could be adjusted (from 0 to 5.5 mT) by varying the current applied to the coil by a power supply (SCIE-PLAS, mod. PSU 400/200). The magnetic field intensity

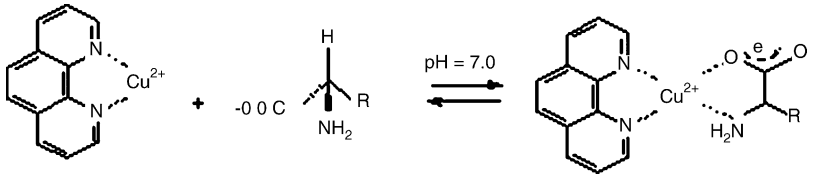

Fig. 2. Complexation reaction to confer the amino acids paramagnetic properties.

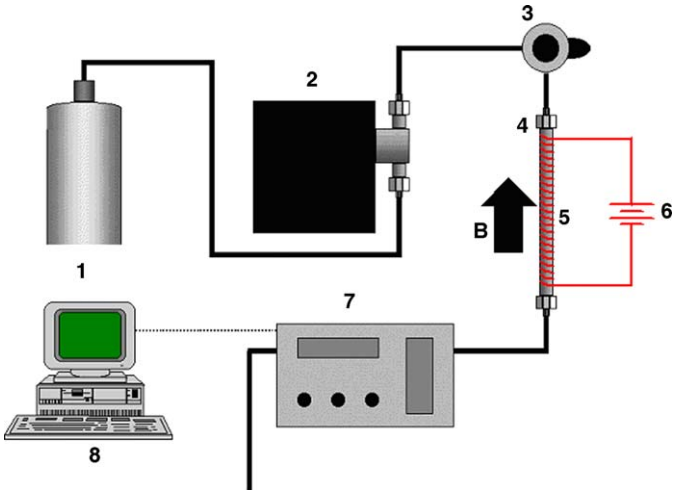

Fig. 4. Basic components of the HPLMC setup: (1) mobile phase, (2) high pressure pump, (3) injection valve, (4) column, (5) copper coil, (6) power supply (to adjust the magnetic field intensity, *B*), (7) detector and (8) computer.

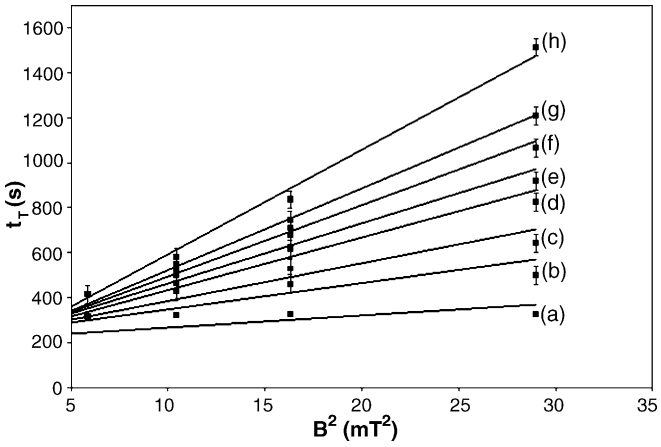

Fig. 6. Plot of t_r (s) against the square magnetic field intensity (mT^2). (a) Cu(*o*-phen)$^{2+}$, (b) glycine, (c) alanine, (d) β-aminobutyric acid, (e) leucine, (f) methionine, (g) histidine, (h) asparagine. Test conditions as described for Fig. 5.

was calculated using the expression $H = nI/l_c$, where H is the magnetic field strength (A m^{-1}), n is the number of turns in the coil, I the current applied (A) and l_c is the coil length (m).

The paramagnetic complexes prepared were detected at a wavelength of 266 nm. The mobile phase used was methanol–PBS (25:75), pH 7, and the injection volume was 25 μl.

4. Results and discussion

To establish how the method worked, we evaluated the effect of the magnetic field intensity on the retention time of several ternary Cu^{2+}-*o*-phenanthroline-amino acid complexes when injected into the mobile phase (methanol–PBS 25:75, pH 7) under the conditions indicated previously: mobile phase flow rate 1 ml min^{-1}, detection wavelength 266 nm and injection volume 25 μl. As an example, Fig. 5 shows the results obtained using L-leucine. It may be observed that the retention time increases with the magnetic field intensity.

When a magnetic field intensity of magnitude different to zero is applied on the column, the Fe_3O_4 particles supported on the SiO_2 surface (stationary phase) are magnetically induced, creating a field that contributes to the net field sensed by the paramagnetic analytes. In absence of convection and when the magnetic field intensity is sufficiently greater than the drag force, the magnetic force created by the external field can be attractive and in some cases large enough to allow the magnetite to retain the paramagnetic analytes such as in the case of high-gradient magnetic separation [15,16].

At zero field strength ($U_m = 0$), the paramagnetic analytes take a similar time to elute. Then, motion is associated with the drag force and the retention ratio value is close to 1. This suggest some non selective retention of the complexes at zero field strength. The results obtained show a increase in the retention times with increasing magnetic filed intensity suggesting that retention is likely to be due to the magnetic interaction with the magnetized Fe_3O_4 separation.

According to the model proposed, the retention time is related to the square of the magnetic field intensity applied. Fig. 6 shows the results for all the amino acids tested including L-leucine. In effect, a linear relationship between 5 and 30 mT^2 may be noted in each case and this relationship always showed a correlation coefficient close to unity.

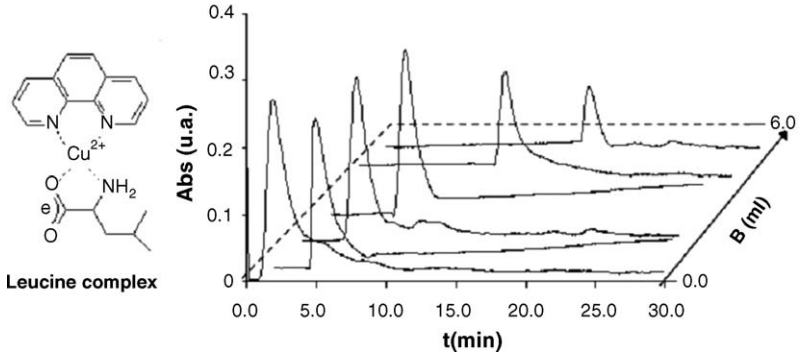

Fig. 5. Effect of magnetic field intensity (*B*) on the retention time of a L-leucine paramagnetic complex. Complex concentration, 7.6×10^{-5} mol l^{-1}; mobile phase, methanol–phosphate buffer (0.1 mol l^{-1}, pH 7) 25:75 (v/v), flow rate 1 ml min^{-1}. Column Fe_3O_4/SiO_2 (100 mm × 4.6 mm I.D.); detection wavelength, 266 nm; injection volume, 25 μl.

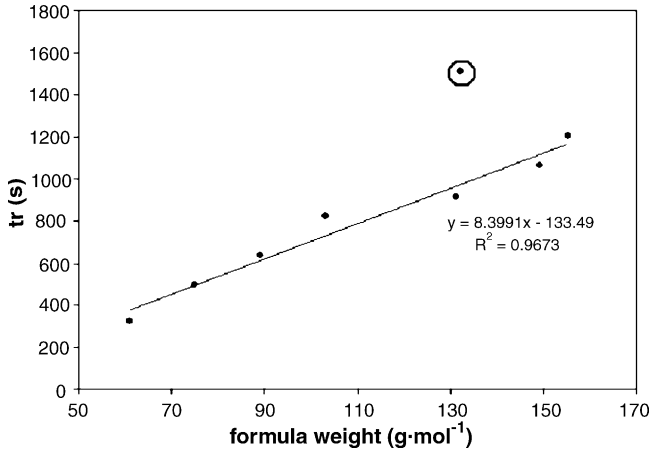

Fig. 7. Changes in the retention time of the different amino acid complexes according to their formula weight (g mol^{-1}).

Fig. 7 also indicates a linear relationship between the retention time t_r and formula weight of the compound such that $t_r = 8.399\ FW - 133.5$. Using this expression to calculate the formula weight of asparagine gives a value of 196.15 g mol^{-1}, which differs from its real value of 132.1 g mol^{-1} by 64.05 g mol^{-1}. This difference is twice the formula weight of methanol (32.04 g mol^{-1}), one of the mobile phase components. It, therefore, seems that the solvent actively contributes to the structure of the metal/asparagine complex, a phenomenon previously observed when working with dissolved metal complexes [24].

5. Conclusions

We present a new chromatography technique denoted HPLMC. This analytical tool is the first to achieve the chromatographic separation of weakly paramagnetic molecules in solution, based on the application of a magnetic field. Among its advantages is that the external magnetic field required is much less intense than that used in techniques such as FFF, SPLITT and magnetapheresis, also minimizing the equipment needed. Paramagnetic analytes with high magnetic susceptibility in a sample can be separated using a magnetic field gradient.

This technique could be used as a qualitative and quantitative selective tool, by tagging diamagnetic molecules with a paramagnetic centre (forming metal complexes) at specific ionic strength and pH values. The method is also useful for separating and detecting many different analytes, such as amino acids, peptides and proteins, along with some metalloproteins.

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