NEW TRENDS IN SEPARATION OF SMALL COLLOIDS/ MACROMOLECULES

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TÓM TẮT

Báo cáo giới thiệu xu hướng mới trong kỹ thuật tách keo kích thước nhỏ và các cao phân tử bằng kỹ thuật phân đoạn dòng trường thuỷ lưu (Flow Field Flow Fractionation - FFFF) kết nối trực tiếp với đầu dò plasma kích hoạt phổ khối (ICP-MS) và đầu dò laser phân huỷ hạt keo (Laser induced breakdown detection - LIBD) mới được phát triển. Những hạn chế của thiết bị phân đoạn dòng trường thuỷ lưu đối xứng đã được xác định và khắc phục bởi qui trình tối ưu hoá phù hợp, hoặc bởi thiết bị phân đoạn dòng trường thuỷ lưu bất đối xứng. Với các điều kiện vận hành thích hợp, cả hai thiết bị, đối xứng và bất đối xứng, đã tách được hỗn hợp 4 polystyrolsulphonate có khối lượng phân tử trung bình 1,37 - 3,8 - 16,9 - 30,9 kDalton, cũng như hỗn hợp 3 keo chuẩn polystyrene có đường kính 19 - 50 - 102 nm. Cả hai thiết bị này có thể ứng dụng để nghiên cứu các mẫu môi trường, các tương tác giữa chất ô nhiễm với hệ keo trong nước thiên nhiên. Cả hai đầu dò LIBD và ICP-MS tạo điều kiện nâng cao độ nhạy và khả năng phân tích đồng thời đa cấu tử các hệ keo, cao phân tử.

ABSTRACT

Flow Field Flow Fractionation (FFFF) connected on-line to the Inductively Coupled Plasma Mass Spectrometry (ICP-MS) and/or to the new developed Laser Induced Breakdown Detection (LIBD) is presented as new trends in separation of colloids and macromolecules. Some inherent limitations of the symmetrical FFFF has been identified and overcomed by an optimization procedure, or by use of an asymmetrical FFF-Fractionator. Under suitable conditions, both the symmetrical and asymmetrical FFF-Fractionator successfully separated reference polystyrolsulphonates of mol mass 1,37 – 3,8 – 16,9 – 30,9 kDalton, as well as 3 polystyrene reference colloids of diameters 19 – 50 – 102 nm. Both the symmetrical & asymmetrical FFFF can be applied for studying environmental samples, interactions of pollutants with natural water-born colloids. The LIBD and ICP-MS as detectors increased the method's sensitivity and enabled multicomponent analyses of colloids/macromolecules on-line.

1. INTRODUCTION:

Water quality is one of the most serious problems in life sciences as well as in technology. As water pollutants, colloids/ macromolecules have been identified and quantified by optical methods, such as light-absorption and/or scattering detectors. However, the resulting spectra are dependent on sizes and shapes of colloids/ macromolecules in samples. Therefore, samples containing polydispersed colloids/ macromolecules should be pretreated by suitable fractionation methods, such as Gel Permeation Chromatography (GPC), Sequential Ultrafiltration (UF), etc... Each of these methods has inherent limitations, and the results obtained should be carefully discussed. Moreover, the conventional light scattering detectors suffer insufficient detection limits for colloids/macromolecules of small sizes (≤ ~ 100 nm). In order to increase the sensitivity and selectivity of an equipment set up, a combination of powerful fractionation techniques and innovative colloid detectors is highly required [1, 2].

In this work, results obtained by symmetrical and asymmetrical FFF-Fractionators connected on-line with the conventional UV-detector, the ICP-MS, the light scattering detectors, and the LIBD show that such combinations could present new trends in separation of colloids and macromolecules. For better understanding, the principles and problems of the FFFF are briefly outlined.

2. SYMMETRICAL FFFF (SYM-FFFF):

The sym-FFFF was developed by Giddings et al and described in many papers [e.g. 3-5]. A carrier solvent is pumped together with an injected sample through a rectangular channel. Passing in the flow through that channel via an outlet at its another end, colloids/macromolecules will be separated and afterthat detected by suitable methods. This formally reminds a GPC-run. However, the FFFF channel doesn't contain any gels as in the GPC columns. Therefore, interactions between the colloid/macromolecules to be separated and the stationary phase is minimized. Thus, the FFFF channel with a laminar flow profile inside reminds us the HDC capillary [6]. But, instead of an ~ 100m long HDC capillary, a typical FFFF channel is about 27cm long, 3cm wide, and 0.0254cm thick. The same solvent as the carrrier solvent is flowing through both the channel walls, perpendicular to the laminar flow, thus creating a "crossflow field". This field enabled different colloids/macro-molecules to be accumulated at different distances to the "accumulation wall" according to their sizes. The laminar flow then transported them with different velocities through the channel length and out to the detectors (Fig. 1).

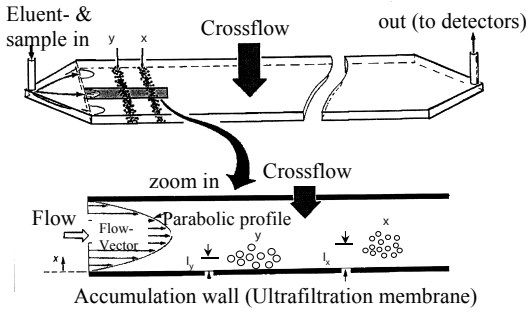

Fig.1: Principles of the Flow FFF

Under suitable experimental conditions which fit some theoretical approximations, the folowing simple equations relate the diffusion coefficients D and the hydrodynamic diameters d_H of the colloids in question with their retention volumes V_r :

$$\mathbf{D} = \frac{\mathbf{V_{cross}}}{\mathbf{V_r}} \cdot \frac{\cdot \mathbf{w^2}}{\mathbf{6}} \qquad \text{(eq. 1)}$$

$$\mathbf{d_h} = \frac{\mathbf{2kT \cdot V_r}}{\pi \cdot \eta \cdot \mathbf{V_{cross}} \cdot \mathbf{w^2}} \qquad \text{(eq. 2)}$$

3. ASYMMETRICAL FLOW FFF (asym-FFFF)

The first experiment with an Asym-FFFF was reported by Wahlund and Giddings in 1987 [7]. Unlike the sym-FFF channel which has both its walls permeable, the As- FFFF channel has only one wall permeable. Therefore, the crossflow cannot be circulated as in sym-FFFF. In fact, the inlet flow will leave the channel through the flow outlet as well as through the only permeable accumulation wall, resulting the crossflow. In this case, the crossflow is directed out to waste [7 - 11]. In order to maintain the crossflow constant at the membrane surface throughout the channel length and width, the asymmetrical FFF channel has a trapezoidal shape [10, 11].
In commercially available asym-FFF Fractionators, an experiment run generally contains 3 phases: Relaxation/focusing, elution, backflushing.

(1) During the first phase, the sample together with the carrier solvent is pumped into the channel through its inlet. The pure carrier solvent is also pumped into the channel through its outlet. Therefore, these two axial liquid flows (from the inlet and outlet) are opposite, and the carrier solvent can leave the channel only through the membrane. The total axial flow of the solvent should be zero at a point near to the channel inlet, named the focusing point. Colloids/macromolecules in the injected sample are focused at this point and the supposed exponential profile of their concentration distribution is established.

(2) During the second, elution phase, the carrier solvent enters the channel through the inlet and leaves it both through the outlet and through the membrane. The balance between the crossflow- and the channel outlet flow rates can be adjusted by needle valves. The outlet volume velocity

$V_{out.}$ is measured by a flow meter. The crossflow is measured either directly by a second flowmeter or simply calculated from the known V_{in} and V_{out}.

(3) During the third, backflushing phase, the pure solvent flow enters the channel from the outlet and flushs the retained materials out of the channel.

By simple approximations, again simple equations (eq.1) and (eq. 2) were derived. i.e., similarly as analyse with a sym-FFFF, the diffusion coefficients and the hydrodynamic diameters of colloids / macromolecules in question can be determined directly and in absolute fashion by measurement of their retention volumes.

4. INSTRUMENTATIONS:

Details of the experimental procedures are described elsewhere [12-17]. It's worth to mention here the instruments used:

The sym-FFFF system consists of a fractionator F-1000 (FFFractionation, Inc., Salt Lake City, USA), a 1100 Series Vacuum Degasser model G 1322A and a 1100 HPLC Iso - pump model G 1310A (Hewllet Packard, Waldbronn, Germany), a double piston precision pump P-500 (Pharmacia Biotech AB, Sweden).

The asym-FFFF system HRFFF 10.000 AF4 consist of a trapezoidal shaped channel, two pumps, an injector, etc. is provided as a whole (Postnova Analytics, Munich, Germany).

In both the sym- and asym-FFF channels, the accumulation wall was covered by a regenerated cellulose membrane with a nominal cut-off (C.O.) of 5 kDalton (Schleicher & Schuell, Dassel; resp. Postnova, Munich; Germany).

From the FFFF channel, the effluent is directed through an UV/VIS detector (Waters, USA; or Postnova Analytics, Germany), a DAWN – DSP –F light scattering detector (Wyatt Technology Corp., Santa Barbara, USA), an ICP-MS ELAN 6000 (Perkin-Elmer) or a prototype LIBD (developed in the INE, Research Center Karlsruhe, Germany).

5. RESULTS AND DISCUSSIONS:

After suitable optimization procedures, good separations of polystyrene reference colloids as well as polystyrolsulphonates were obtained (Fig. 2 & 3), demonstrating the separation efficiency of the sym-FFFF system [12, 13]. It was confirmed a linear relationship between the nominal diameters of the tested reference colloids as eq. 2 predicted. Also, calibration lines $lg(d_H)$ vs. lg(M) could be constructed for the reference polystyrolsulphonates, as well as for the reference proteins. The slopes obtained were 0.59 and 0.39 , which confirmed their random coiled and sphere shape, respectively.

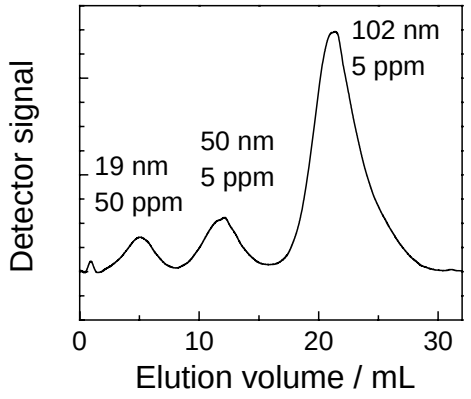

Fig.2: Separation of a mixture of three polystyrene reference colloids with the sym-FFFF / LLS system.

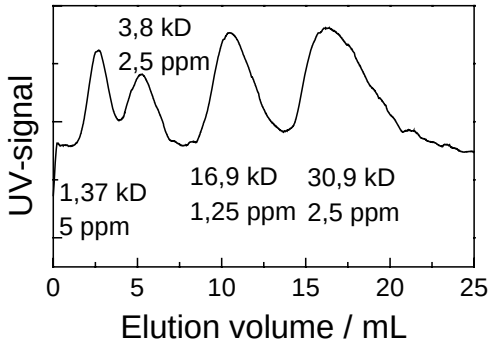

Fig.3 : Separation of a mixture of four reference polystyrolsulphonates with the sym-FFFF / UV system.

Fig. 4 shows fractograms of two reference proteins and Ferrihydride -a synthetic iron oxi/hydroxide colloid. Symmetric Gausian shapes of the fractograms confirmed their monomodal character. A value of ~ 10 nm, calculated for the hydrodynamic diameter of ferrihydrid colloids at the peak maximum, is

comparable with the value < 6 nm, obtained by TEM.

Similar results were obtained with the asymmetrical one, though, naturally with different operating conditions [16, 17].

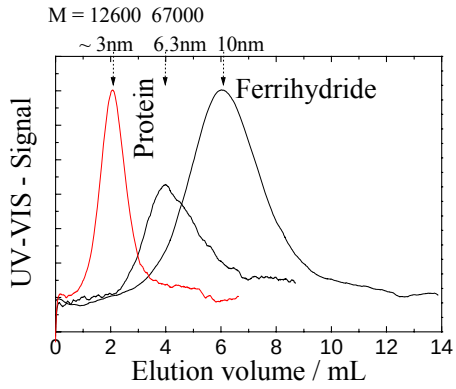

Fig.4 : Fractograms of 2 reference proteins and iron oxy/hydroxide colloids, obtained with the sym-FFFF / UV

When connected on-line to an ICP-MS, the FFFF system enabled to investigate interactions between heavy metal ions and colloids/macromolecules in a variety of situations. e.g. Fig.5 showed that under laboratory conditions, U(VI) and Eu (III) were attached to the colloidal humic acids quickly and almost completely. But the size distribution of such associations changed only very slowly with time [14].

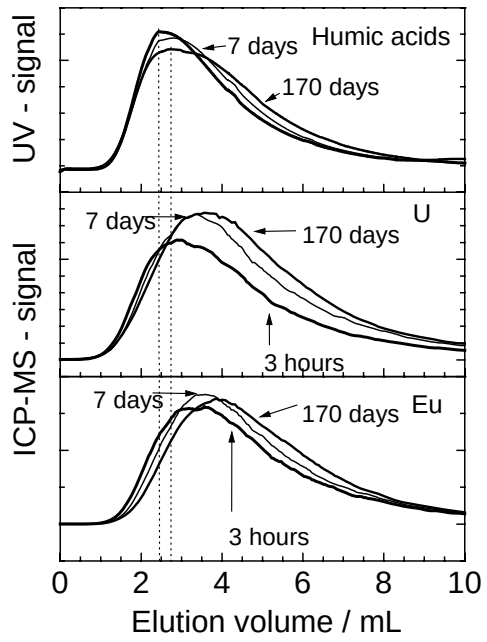

Fig.5: Application of the sym-FFFF / UV / ICP-MS system to investigate interactions colloids - metal ions.

Application of the system sym-FFFF / UV / ICP-MS on environmental samples was also performed. Fig.6 shows that trace amounts of heavy metal ions in a natural humic rich groundwater is preferentially attached to higher colloid size fractions comparing to humic colloids [14].

Fig.6 also demonstrates the advantageous possibility to program the crossflow to fit an wide spectrum of sample colloid sizes in a sample. While with the GPC, such samples necessitate more than one column. For the results shown in Fig.6, the crossflow was kept constant at 5 mL/min for the first 15 minut, enabling small humic colloids to be eluted. For colloids of higher size fractions which need small field intensity to be eluted, the crossflow was linearly decreased to 0.5 ml/min during 5 minut and then again kept constant at this value. Here, application of the ICP-MS as detector is meaningsful not only for the composition analyse, but also for speciation of colloidal particles.

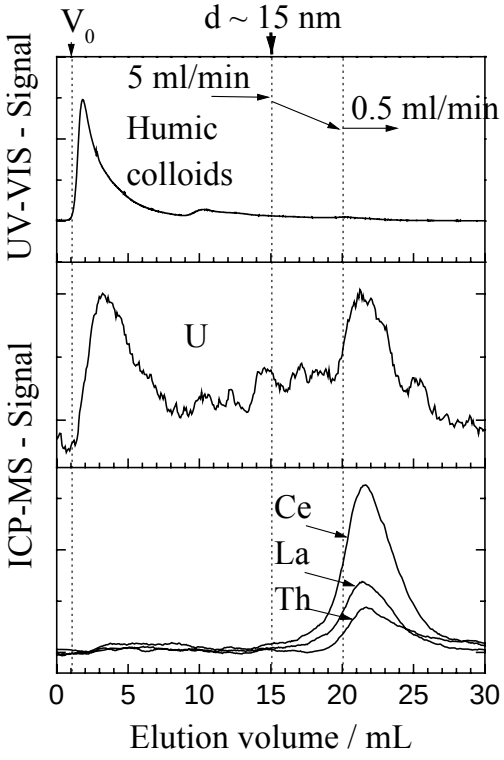

Fig. 6: Application of the sym-FFFF / UV / ICP-MS system to investigate size

distribution and composition of colloids in environmental samples.

Besides the advantage of sensitive and multicomponent analyse, the ICP-MS provides signal intensities independent on colloid sizes and shapes, while that of the light scattering is strongly affected by colloid sizes (Fig.7). Because of the higher sensitivity of light scattering detectors for bigger colloids, these detectors often producing overestimated mean values of colloid sizes. Therefore, an application of ICP-MS connected on-line with the FFFF is helpful in getting colloid size distribution free from artefacts [15].

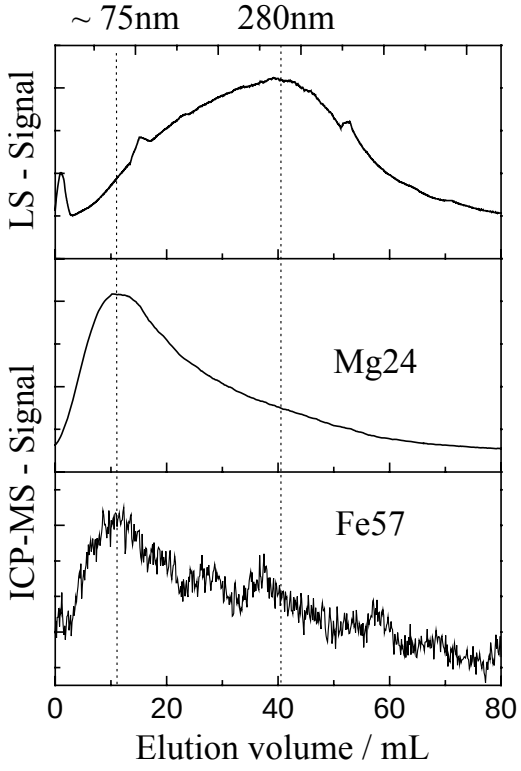

Fig. 7: Application of the sym-FFFF / LLS / ICP-MS system to investigate a sample of bentonite colloids.

A serious drawback of the FFFF is the band broadening and therefore the high degree of sample dilution during an analyse. For this reason, sensitive detectors are highly required. Therefore, a prototype of the LIBD was connected on-line to the sym-FFFF [12].The LIBD is an innovative colloid detector developed in the INE, Research Center Karlsruhe, Germany. It's principles and a variety of applications is published in many papers [e.g. 18, 19]. Fig. 8 shows fractograms of a dilute mixture of 3 polystyrene reference colloids with nominal diameters of 19, 50, and 102 nm, acquired by a light scattering detector and an LIBD. The exceptional sensitivity of the LIBD, especially for colloids < 100 nm, is clearly demonstrated. While the LS detector can show only background signal, the LIBD detector shows even higher peaks for colloids of diameters 19 and 50 nm.

Moreover, application of the ICP-MS and the LIBD revealed another serious problems with the commercial sym-FFF fractionator, i.e. corrosion of the channel ceramic frits might occur. Consequently, background levels of the LIBD-, and some of the ICP-MS signals were significantly increased. This affects the detection limits of the sym-FFFF / LIBD. Also, It makes the speciation of Si by the system sym-FFFF / ICP-MS impossible [14, 15].

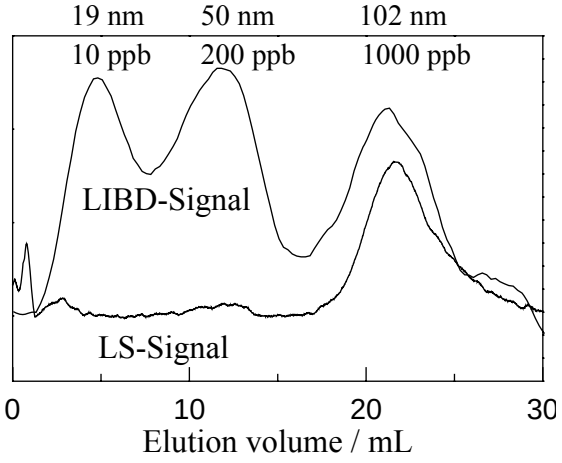

Fig. 8: Application of the sym-FFFF / LLS / LIBD system to separate a mixture of polystyrene reference colloids

While a sym-FFFF with permeable walls of another materials than ceramic was not available, the only choice to avoid these problems is an asym-FFF fractionator. For with this equipment, impurities resulting from a possible ceramic corrosion could not reenter the FFFF channel and affect the detector signals. All the investigation mentioned above have been done again with the asym-FFFF. The results obtained have confirmed our supposition in full extent [16, 17] .

Compared to the sym-FFFF, the asym-FFFF show higher sensitivities as well as separation resolutions due to its possibility to focuse sample particle components into a very sharp band in an on-line preconcentration step prior to

separation step. Furthermore, the possiblility of visual observation of coloured particle migration through the channel is impressive and valuable for equipment fine adjusting and troubleshooting.

6. CONCLUSION

Both the sym- and asym- FFFF are powerful separation techniques. In combination with the sensitive ICP-MS and the LIBD as detectors, the sym- and especially the asym-FFFF represents new trends in separation of colloids and macromolecules. Their abilities were proved with colloids of different compositions, in very dilute samples.

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