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Experimental study on the retention of silica particles in gravitational field-flow fractionation Effects of the mobile phase composition¹

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Abstract

Effects of mobile phase composition can play an effective role in modulating the retention of particles in gravitational field-flow fractionation (GFFF), the simplest and cheapest among field-flow fractionation (FFF) techniques. In the framework of an optimized procedure for the GFFF characterization of particulate systems, an experimental approach to the effects of the mobile phase composition on the retention of silica particles retention is presented. The role of the ionic strength and the presence of surfactant are emphasized, with special regards to the shape of the particles. Moreover, the first experimental evidence of potential-barrier GFFF is reported.

Keywords: Mobile phase composition; Silica particles; Particle interactions; Potential-barrier field-flow fractionation; Field-flow fractionation; Gravitational field-flow fractionation

1. Introduction

Gravitational field-flow fractionation (GFFF) is a technique suitable for the separation and further characterisation of particles in a size range of 1–100 μm [1]. For over two decades, GFFF has been used for the separation of various particulate systems, either inorganic [2–5] or of biological origin [6–10]. As with other field-flow fractionation (FFF) techniques, GFFF is an elution separation method that requires the application of a field or gradient. Here the field is just the earth's gravity applied perpen-

dicular to a very thin, empty channel with rectangular cross-section.

In a recent paper, GFFF is described as being capable of giving accurate particle size distributions (PSD) of silica particles used as chromatographic supports [11]. The relative simplicity with which PSDs can be obtained through a numerical transformation of the elution profile is due to the simple relationship existing between retention and particle size when GFFF is operating in steric mode: that is when larger particles elute earlier than smaller ones [3]. However, the direct application of the standard relation for the steric elution mode is obscured by the presence of other forces that compete with the primary field. Among these second-order forces, lift forces which depend on experimental conditions, act on particles' motion by opposing the gravity field. In

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this case, sample focusing can occur across the channel width and it is possible to operate GFFF in the elution mode referred to as hyperlayer mode (Hyp) [12]. In Hyp/GFFF, however, retention theory has not, as yet, been fully developed and simple relationships between retention and dimension are not available for PSD analysis by Hyp/GFFF.

In the framework of an optimized approach to PSD analysis of dispersed particulate systems by GFFF, all the factors determining second-order effects should be studied since they influence retention in a way that is not directly predictable from the standard theory. In any GFFF elution mode, as particles are swept down the channel by the mobile phase flow under the action of earth's gravity, other effects, such as adsorption, interface effects or chemical affinity could play an important role in modulating retention. The effects of sample overloading as well as of sample preparation and injection procedure have been recently described in the characterization of silica particles by GFFF [13]. The role of second-order effects such as particle–particle and particle–wall interactions has been widely studied in the literature in the case of submicrometer colloidal particles [14–17], while the effect of particle–wall interactions in modulating retention of supermicrometer particles has, as yet, been reported only on a qualitative basis [5,18,19]. On the other hand, the field due to second-order interactions not only can deviate retention from the ideal theory but it could also play a specific role by increasing the resulting selectivity of the separation system. The combined method could thus be employed to characterize samples in a more specific way than primary forces alone can do. The potential-barrier (PB) mode is a newly developed elution mode for FFF in which affinity of the sample for the channel walls is a result of a secondary, electrostatic field able to enhance the fractionation level [20,21].

In this work, an experimental approach to the analysis of the effects of mobile phase composition (i.e. ionic strength and presence of surfactant) on silica particles' retention in GFFF using a glass channel is presented. In order to better understand the specific role of short-distance forces such as the electrostatic interactions, experimental conditions were chosen such that the contribution of hydrodynamic forces to particles' elevation from the wall

was limited. The role of the ionic strength of the mobile phase is studied by measuring the effect of changes in ionic strength on retention of particles of a given diameter but different shape. The effect of surfactant in the mobile phase is studied by measuring the difference in retention obtained by surfactant-added and surfactant-free carriers at a given ionic strength. This preliminary study of the influence of the mobile phase composition in modulating retention suggested the experimental conditions for the assessment of PB in GFFF. Under proper experimental conditions total adhesion and further release of the sample particles has been obtained; this is, as far as we know, the first experimental evidence of PB/GFFF.

2. Experimental

The GFFF channel employed here was constructed as described elsewhere [1]: two mirror-polished glass plates were clamped together over a PTFE sheet from which the channel volume had been removed. Channel dimensions were chosen in order to reduce the effect of lift forces on the net field: the ribbon-like channel was 0.0200 cm thick, 2 cm wide and 90 cm (tip-to-tip) long. Flow-rate was set at 1.009 ± 0.004 ml/min ($n=35$). The void volume was determined with an unretained probe (K_2CrO_4) and was found to be 3.28 ± 0.02 ml ($n=35$). The channel just replaced the column of a standard HPLC system: the carrier flow was generated by a Model 2510 HPLC pump and the channel outlet was connected to a Model 2550 UV detector (Varian, Walnut Creek, CA, USA) operating at 330 nm. The signal was recorded on a strip-chart integrator Model Mega 2 (Carlo Erba, Milan, Italy) and captured through a 12 bit I/O DAQ board Model Lab PC+ (National Instruments, Austin, TX, USA) plugged into an AT-compatible 386-DX personal computer.

Samples were silica particles of known porosity for HPLC column packing, either spherical ($5 \mu\text{m}$; LiChrospher Si-60) or irregular ($5 \mu\text{m}$; LiChrosorb Si-60) (Merck, Darmstadt, Germany) dispersed at 1% (w/v) in Milli-Q (Millipore, Bedford, MA, USA) water. The injected amount was $10 \mu\text{l}$ ($100 \mu\text{g}$ of silica). Bulk density of such samples was given by the manufacturer as $\rho_{\text{He}} = 2.30 \pm 2\%$ (g ml^{-1}). Sam-

ples were sonicated for 1 min and then injected. The flow was stopped after injection to let particles settle on the accumulation wall. The mobile phase was Milli-Q water added with different quantities of NaN_3 , commonly used in FFF practice as bactericide. The amount of salt determined the ionic strength of the mobile phase: in these experiments the salt concentration ranged from $3.08 \cdot 10^{-3}$ to $2.80 \cdot 10^{-5}$ M. For surfactant-added mobile phases, the non-ionic surfactant Triton X-100 (Aldrich, Steinheim, Germany) was used at 0.1% (v/v) in the final solution.

3. Results and discussion

3.1. The role of mobile phase composition on GFFF retention

Preliminary analysis of the effects of mobile phase ionic strength and type of surfactant in GFFF on retention was already described for both polymer (polystyrene) [5] and silica particles [18]. In the first paper, the influence of the type and concentration of surfactant on polystyrene particles' retention with different surface-modified accumulation walls was studied. In the second paper, the influence of the mobile phase ionic strength on the retention of silica particles eluted in hyperlayer mode was reported. However, neither the separate effects of ionic strength and surfactant, nor a comparison between different ionic strength modifiers have been, thus far, reported. Moreover, it has not been clearly explained why short-distance forces such as particle-wall interactions could modulate retention of particles eluting in hyperlayer mode. In this mode, in fact, the equilibrium distance of particles from the wall is definitely much higher than the range within which electrostatic interactions act. In our approach, therefore, GFFF elution conditions were chosen to assess steric mode and to let particles move very close to the accumulation wall. Under these conditions, the fractionated sample could experience electrostatic interactions to a significant extent. In steric mode, the retention ratio can be approximated as [4]: $R \cong 6\gamma(a/w)$ where a is the particles radius, w the channel thickness and γ is called the steric correction factor which accounts for hydrodynamic effects

on particle velocity [22]. In the ideal case γ is approximately one, while smaller values indicate that particles are somewhat retarded and forced to travel close to the wall. As long as particles are dragged so close to the accumulation wall, interactions of supermicrometer silica particles can be due to short-distance electrostatic forces generated by the different dissociation constants for the acidic groups of the glass walls and the particles as well as by the ionic exchange equilibria with the ion species in the mobile phase. In fact, the surface activity of silica particles is due to the presence of hydroxyl groups on the silica surface and to adsorbed water [23]; the same can be said of the glass surface of the channel walls. Moreover, surfactant and ion species in the mobile phase can be adsorbed on to the glass wall surface and, thereby, may change its electrical properties. In particular, as far as particle-wall interactions are concerned, the total energy of interaction can be expressed in terms of Van der Waals and double-layer potential between a particle and a surface at a given distance or in terms of electrostatic forces due to a charge on the particles and on the wall. A comprehensive description of these effects for submicrometer particles in sedimentation (Sd) FFF was presented by Hansen et al. [14,15], Mori et al. [16,17] and by Koliadima and Karaiskakis [21].

3.2. Effects of ionic strength

In Fig. 1a,b, the effect of mobile phase ionic strength on retention of silica particles is shown. The mobile phase was surfactant-free. The interaction potential changes with ionic strength and clearly modulates sample interactions with the wall. Moreover, the extent of such effects is influenced by sample morphology. Irregular particles are completely retained at the highest ionic strength ($I = 3.08 \cdot 10^{-2}$ M) (Fig. 1b, trace 1) while spherical particles already show no elution at ionic strengths ten times lower (Fig. 1a, trace 1). As shown in Table 1, for spherical particles the retention ratio increases as ionic strength (I) decreases, while the retention ratio of irregular particles shows only an increasing trend of its mean values. At the lowest ionic strength, the increase in retention ratio is significant for spherical particles (ca. 11%) compared to that of irregular ones (ca. 3%). These experimental findings are rather

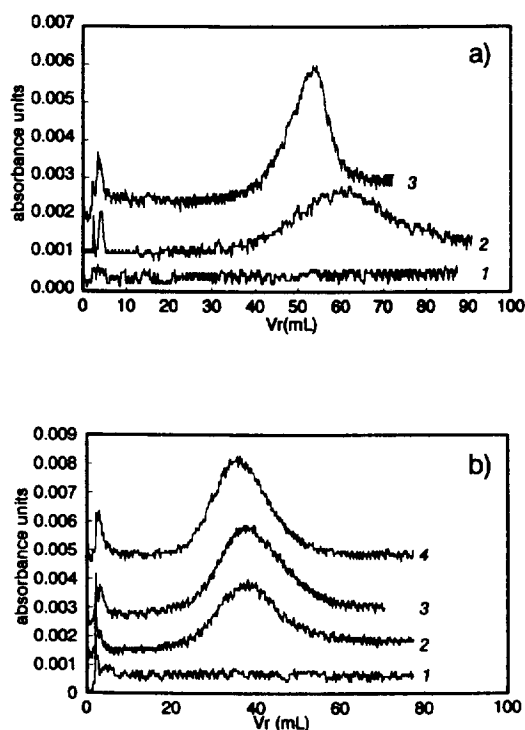


Fig. 1. Effect of the ionic strength (I) of the mobile phase on retention. Mobile phase: water. Added salt: NaN_3 . (a) Sample: $5 \mu\text{m}$; LiChrospher, sample load = $100 \mu\text{g}$. 1: $I = 3.08 \cdot 10^{-3} \text{ M}$; 2: $I = 3.08 \cdot 10^{-4} \text{ M}$; 3: $I = 2.80 \cdot 10^{-5} \text{ M}$. (b) Sample: $5 \mu\text{m}$; LiChrosorb, sample load = $100 \mu\text{g}$. 1: $I = 3.08 \cdot 10^{-2} \text{ M}$; 2: $I = 3.08 \cdot 10^{-3} \text{ M}$; 3: $I = 3.08 \cdot 10^{-4} \text{ M}$; 4: $I = 2.80 \cdot 10^{-5} \text{ M}$.

interesting since they can be regarded as a proof of the short-distance character of the electrostatic interactions affecting retention in GFFF. In terms of near-wall lift forces theory, the predicted equilibrium distance between spherical particles and the accumulation wall can be determined as $\delta_{\text{eq}} \approx 1.0 \mu\text{m}$ (calculated to be consistent with Eqs. 25 and 21 of Ref. [22]), while at the highest ionic strength the actual equilibrium distance determined from experimental retention measurements was $\delta_{\text{app}} \approx 0.1$

μm (calculated to be consistent with Eq. 17 of Ref. [22]). The difference between predicted and experimental particle elevation supports the role of consistent particle-wall attractions in modulating retention at high ionic strength. On the other hand, retention values obtained for irregular particles correspond to equilibrium distances from the accumulation wall which are significantly longer than those for spherical particles of the same diameter. This effect is reflected in the less marked retention dependence on ionic strength for irregular samples.

For elution techniques, the peak shape is also a valid marker of the actual retention mechanism: its deformation with respect to the gaussian reference shape was associated with the onset of mixed elution mechanisms in FFF [24]. If peak shapes here are compared at the lowest ionic strength, it is evident that the peak of spherical particles appears fronted (Fig. 1a, trace 3), while irregular particles maintain a nearly gaussian profile (Fig. 1b, trace 4).

3.3. Effects of the surfactant

The role of surfactant in modulating retention is shown in Fig. 2a,b and Table 2, where the dependence of retention on ionic strength is seen when a neutral surfactant was added to the carrier. Surfactants play an active role in modulating retention, probably due to their adsorption on both channel and particles surfaces [18,19]. This last finding was emphasized by Pazourek et al. who effectively modulated the retention of silica samples by adding different amounts of surfactants of different nature (cationic, anionic, non-ionic) to the mobile phase. In the present work the type of surfactant was chosen to verify the single effect of the adsorption of neutral surfactant molecules both on the particles and the channel surface. In this way, one can distinguish between the effects due mostly to changes in ionic

Table 1
Dependence of retention on ionic strength

Sample	Retention ratio (confidence limits at 95%)			
	$I = 3.08 \cdot 10^{-2} \text{ M}$	$I = 3.08 \cdot 10^{-3} \text{ M}$	$I = 3.08 \cdot 10^{-4} \text{ M}$	$I = 2.80 \cdot 10^{-5} \text{ M}$
$5 \mu\text{m}$; LiChrospher	–	0	0.054 ± 0.001	0.061 ± 0.002
$5 \mu\text{m}$; LiChrosorb	0	0.087 ± 0.001	0.089 ± 0.002	0.092 ± 0.001

Mobile phase: water– NaN_3 .

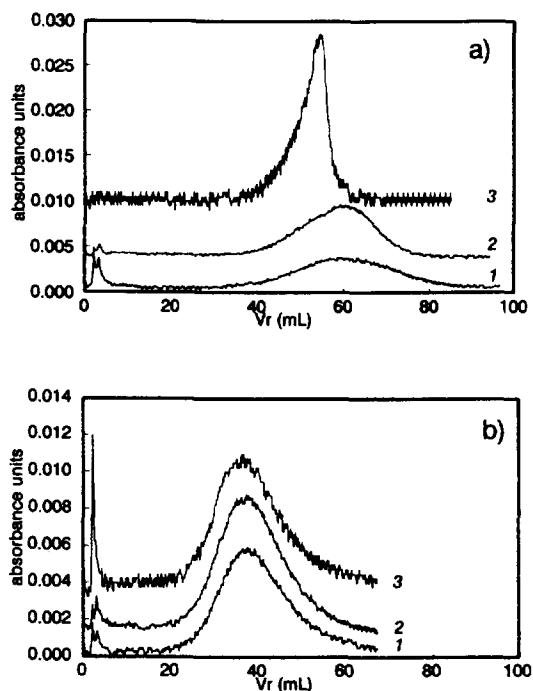


Fig. 2. Effect of the ionic strength (I) of the mobile phase on retention. Mobile phase: 0.1% (v/v) Triton X-100. Added salt: NaN_3 . (a) Sample: 5 μm ; LiChrospher, sample load=100 μg . 1: $I=3.08 \cdot 10^{-3} \text{ M}$; 2: $I=3.08 \cdot 10^{-4} \text{ M}$; 3: $I=2.8 \cdot 10^{-5} \text{ M}$. (b) Sample: 5 μm ; LiChrosorb, sample load=100 μg . 1: $I=3.08 \cdot 10^{-3} \text{ M}$; 2: $I=3.08 \cdot 10^{-4} \text{ M}$; 3: $I=2.8 \cdot 10^{-5} \text{ M}$.

strength and those related to the presence of surfactant molecules on the glass and silica surfaces. Fig. 2 and Table 2 show that retention of irregular particles is practically constant with decreasing ionic strength whereas, at the lowest I value, the increase in retention ratio for spherical particles is comparable to that observed for the surfactant-free mobile phase. However, total adhesion of samples on the channel wall (i.e. no elution) was never observed here, even at the highest ionic strengths. On the other hand, without surfactant, irregular and spherical particles

showed different limiting values of the ionic strength for their adhesion on the accumulation wall.

3.4. Optimization of the mobile phase composition in GFFF

The use of surfactant definitely proves that it reduces the risk of sample adhesion. The best ionic strength for optimizing fractionation is that where the particle diameters calculated from experimental retention values obtained at different ionic strengths (Table 2) correspond to nominal values. In the case of the spherical sample tested here ($d_{50}=5.1 \mu\text{m}$; nominal specification), the steric correction factor γ has previously been calculated to be 0.70 [11], thus confirming that these particles were considerably retarded with respect to the ideal case ($\gamma=1$). With this value of γ , the particle diameter was $d=5.12 \pm 0.28 \mu\text{m}$ ($I=3.08 \cdot 10^{-3} \text{ M}$, $I=3.08 \cdot 10^{-4} \text{ M}$), and $d=5.70 \pm 0.29 \mu\text{m}$ ($I=2.8 \cdot 10^{-5} \text{ M}$). It can be seen that the GFFF-derived dimensions perfectly match the nominal value as long as the ionic strength used is above $3.08 \cdot 10^{-4} \text{ M}$. At the lowest ionic strength ($2.8 \cdot 10^{-5} \text{ M}$), the difference between the GFFF-based and the nominal particle diameter increases to more than 11%. The observed effects on retention ratio and peak shape of spherical particles at the lowest ionic strength were also reproducible after repetitive changes of the ionic strength (see Table 2). At the lowest ionic strength the peak was again strongly fronted and sharper than at high ionic strength. However, peak shape effects were significantly evident for the surfactant-added mobile phase (compare Fig. 2a, trace 3) to Fig. 1a, trace 3) suggesting that repulsive interactions should dominate when particle-wall attractions are reduced by the presence of surfactant.

Although the injected amount chosen for all the experiments (100 μg) was moderately high in order

Table 2
Dependence of retention on ionic strength

Sample	Retention ratio (confidence limits at 95%)		
	$I=3.08 \cdot 10^{-3} \text{ M}$	$I=3.08 \cdot 10^{-4} \text{ M}$	$I=2.8 \cdot 10^{-5} \text{ M}$
5 μm ; LiChrospher	0.054 \pm 0.003	0.054 \pm 0.003	0.060 \pm 0.003
5 μm ; LiChrosorb	0.087 \pm 0.002	0.088 \pm 0.002	0.090 \pm 0.001

Mobile phase: 0.1% Triton X-100– NaN_3 .

that UV extinction signals were not too influenced by noise, the poor signal-to-noise ratio sometimes observed in the fractograms was due to two experimental artefacts: the low resolution of the acquisition software initially used to handle data and the noise entering the DAQ board from a common ground connection. A full 12-bit acquisition driver and rewired ground connections eventually improved the signal-to-noise ratio. The occurrence of sample overloading with comparable injected amounts was recently reported to affect peak shape and to reduce retention of silica particles in GFFF [13]. However, the effects on peak shape observed there were in terms of peak tailing rather than of peak fronting, as reported in the present paper. Since sample overloading in GFFF has been observed to depend on the extent of lift forces [13], it could not have been very effective under the present experimental conditions, because of the limited action of these forces. Otherwise, in SdFFF the mechanism of sample overloading has already been shown to closely resemble the effects of an increase of particle–particle repulsions with the ionic strength of the mobile phase [15]. A comprehensive analysis of the role of the ionic strength on sample overloading is beyond the scope of the present paper.

It is clear that for silica particles and glass-wall GFFF systems, retention can be influenced by a resulting electrostatic field modulated by the mobile

phase composition, if lift forces are kept constant and low. Therefore, care must be taken in the choice of the mobile phase whenever optimum conditions for accurate GFFF procedures are sought. Further proof on the very active role of the mobile phase in GFFF is provided by preliminary measurements with different salts. In fact, different types of ions can influence electrostatic interactions. A detailed analysis of the effects of various ionic modifiers as well as of the possible mechanism by which they could act on retention (e.g. adsorption on channel walls) is currently under study. For instance, the use of NaCl in the mobile phase, even after long conditioning with NaN_3 , shows long-term effects on retention. Sodium chloride has already been shown to influence retention of silica particles in GFFF [25]. As a preliminary approach, NaCl has been shown to give long-term effects of retention reduction, as reported in Fig. 3. It can be deduced that care should be taken also in the choice of the type of the electrolyte used.

3.5. Potential-barrier GFFF

From the compared analyses of retention perturbations due to variation of the ionic strength in the surfactant-added and surfactant-free mobile phases, one may focus on two points. First, under the experimental conditions chosen for surfactant-free

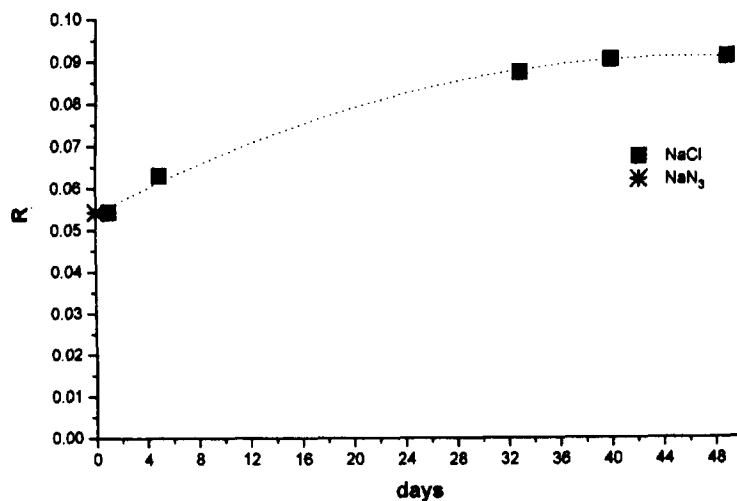


Fig. 3. Long-term effects of changing ionic strength modifier on retention of silica particles: NaN_3 (*), NaCl (■). Sample: $5 \mu\text{m}$; LiChrospher. Mobile phase: 0.1% (v/v) Triton X-100, $I=3.08 \cdot 10^{-3} M$.

mobile phases, particle–wall interactions dominate the net potential balance to give the total sample adhesion at the highest ionic strengths. Second, spherical particles are more influenced by the secondary, electrostatic field than irregular particles at a given ionic strength. According to the observed effects of the surfactant and the ionic strength, such particle adhesion proved reversible, yielding the first experimental evidence of PB/GFFF. PB was first reported by Karaiskakis et al. for SdFFF [20,21,26]. In the present work it is proved that, with proper channel walls and mobile phase composition, particle–wall interactions can display a PB effective also in GFFF. As shown in Fig. 4, carrier A ($\text{NaN}_3\text{-H}_2\text{O}$; $I=3.08\cdot 10^{-2} M$) totally prevented elution of an irregular silica sample. As the mobile phase was changed to carrier B (0.1% Triton X-100 in water) the sample appeared with a retention ratio ($R=0.088$) very close to the value obtained for the same sample at the lowest ionic strength with surfactant-added mobile phase (see Table 2). After this first example of PB/GFFF, the combined method could be applied to separate bands with different limiting electrolyte concentrations for their adsorption on the accumulation wall: e.g. spherical and irregular silica particles of equal size. In fact, because of the different limiting values of ionic strength for the total adhesion of spherical or irregular particles, PB/GFFF can be used to fully separate samples on shape alone.

More extensive applications are currently under study for silica particles as well as for other particulate materials.

4. Conclusions

The role of the mobile phase composition must be stressed in the framework of a general optimization of GFFF. In fact, the mobile phase composition was shown to highly affect retention of supermicrometer silica particles in Steric/GFFF with glass walls. In particular, an experimental proof of the short-distance character of the second-order, electrostatic effects in GFFF is reported here. The dependence of retention on the ionic strength is affected by sample morphology: spherical particles are more influenced than irregular samples. The presence of surfactant in the mobile phase inhibits sample adhesion on channel walls. In fact, with surfactant-free mobile phases, total adhesion of particles (no elution) occurred at the highest ionic strength used. Such a total adhesion appeared at different ionic strength conditions for spherical and irregular particles of a given diameter, confirming again the dependence on morphology and the short-distance nature of such second-order interactions. Furthermore, sample adhesion was shown to be reversible, giving the first reported example of PB/GFFF. Because of the difference in the ionic

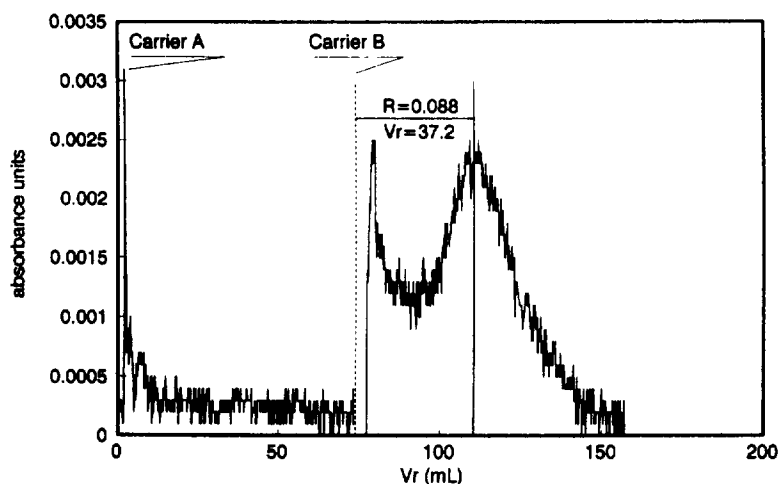


Fig. 4. Example of potential-barrier GFFF (PB/GFFF): Sample: 5 μm ; LiChrosorb, sample load 100 μg . Carrier A: $\text{NaN}_3\text{-H}_2\text{O}$, $I=3.08\cdot 10^{-2} M$; Carrier B: 0.1% Triton X-100–water.

strength values at which adhesion of spherical and irregular particles takes place, such a low-cost technique could be used to fractionate, at high resolution, samples of same size but different shape.

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