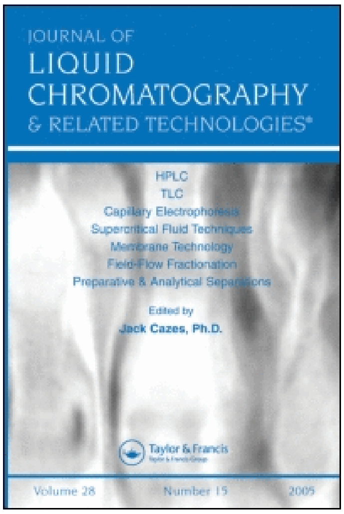

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EXPERIMENTAL ANALYSIS OF SECOND-ORDER EFFECTS ON GRAVITATIONAL FIELD-FLOW FRACTIONATION RETENTION OF SILICA PARTICLES

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ABSTRACT

One of the most attractive features of GrFFF is the possibility to perform particle size distribution analysis of supermicron particles dispersions by means of a very simple and inexpensive experimental apparatus, easily obtained by implementing a basic HPLC configuration. Optimization of the method requires the knowledge of the effects (second-order effects) capable of influencing sample retention and, thus, the accuracy of the scaling from the retention times axis to the particle size axis. In the framework of such an optimization, the role played by hydrodynamic effects in modulating retention is here first considered and the experimental verification of the empirical equations describing such effects is presented. The effects exerted on retention by changing different GrFFF systems is also studied by means of such an experimental approach.

INTRODUCTION

Gravitational field-flow fractionation (GrFFF) has been demonstrated to be able to characterize supermicron particles of either inorganic[1-6] or biological origin.[7-12] In particular, accurate particle size distribution analysis (PSD) by GrFFF of silica particles for HPLC column packing has been shown to be possible in a very simple, quick and inexpensive way.[4] PSD analysis requires the conversion of a fractogram, that is the signal *vs.* time response, into a size distribution curve, that is the mass frequency function *vs.* diameter curve. For a proper approach to GrFFF-based PSD optimization, however, it is required to focus on all factors which limit the direct application of the method.

First, the direct conversion from retention time to particle diameter values, which is required to obtain PSD profiles by GrFFF, is based on the assumption that fractionation is obtained in steric mode and that the relative steric correction factor γ is known or experimentally estimated. It has been reported, however, that in steric elution mode, that is, with γ values lower than two,[13] γ can be influenced by various effects such as the hydrodynamic effects and the mobile phase composition, both in GrFFF and sedimentation field-flow fractionation (SdFFF).[6,14-16] All these effects, here generally indicated as second-order effects, can, thus, significantly affect retention and even modify the elution mode in GrFFF. For instance, in a recent paper[6] it has been demonstrated that, with silica particles eluted by GrFFF, a reduction in ionic strength brings about a strong increase of the retention ratio and a significant reduction of the fractogram broadening in the case of spherical particles. Conversely, irregular particles do not show any significant change in peak retention parameters. Even the salt composition of the mobile phase has shown to significantly alter GrFFF retention. A reduction in retention was gradually attained by adding sodium chloride instead of sodium azide to the carrier solution. The effect of a retention modification ascribable to the presence of chloride ions suggests some kind of particle-wall interactions.

Second, quantitative PSD analysis requires the accurate control of the sample recovery:[17] second-order effects may play an important role also with respect to quantitative analysis. For instance, the role played by the mobile phase composition on retention and recovery was reported to be so critical to onset a hybrid elution mode which has been defined as Potential-Barrier FFF (PB-FFF).[18-20] The possibility to assess the presence of PB effects also in GrFFF has been recently described.[6,21]

In this paper a preliminary experimental approach to the evaluation of the second-order effects is presented. Among such effects, the extent of hydrodynamic forces, relative to the GrFFF systems here employed, has been first studied. In particular, the semi-empirical model given by Williams *et al.*[14-16] has been applied. Such a model is based on the existence of two main hydrodynamic contributions to SdFFF retention in steric mode: the retardation effect and the lift forces contribution, the latter being separately considered as due to near-wall lift forces and inertial lift forces. Williams and coworkers proposed, and experimentally verified in SdFFF, some empirical equations which relate particles' elevation during steric elution to various experimental parameters. These are particles' size, density, and flow rate, density and viscosity of the mobile phase. By applying the model described it is possible to determine the predicted and the experimental particles' elevations as a function of the above experimental parameters.

The validation of Williams' model is fundamental for the calculation of particles' elevations. In fact, it is shown in MATERIALS AND METHODS that particles' elevation values are required for the accurate determination of particles' size (or density) when the experimental value of the steric correction factor (γ) is not known. In other words, if particles' elevation can be accurately predicted, the conversion from the retention time to particles' diameter can be performed in a GrFFF experiment with no need of previous knowledge of the γ value.

In this work, the agreement between measured and predicted particles' elevations is verified with respect to the elution of silica particles by GrFFF. It is also shown that second-order effects other than hydrodynamic forces can influence retention of such particles. The chemical composition of the channel walls and of the carrier fluid have shown effects on particles' elevation which are comparable to those given by hydrodynamic effects alone. The aim of such preliminary work is to single out the experimental conditions for which the extent of the above second-order effects can be predicted or controlled in the framework of an optimized method of absolute PSD analysis of silica particles by GrFFF.

MATERIALS AND METHODS

GrFFF Channel Systems

The GrFFF systems were built as described elsewhere:[4,6,22] two mirror polished plates were clamped together over a polymeric sheet (Mylar) a few tens of μm thick from which the channel volume had been removed.

Table 1

GrFFF Channel Systems

System	Length x Width (cm)	Walls	Ionic Modifier in the Mobile Phase	V_0 (cm^3)	w (cm)	C^a
1	50x2	glass, unmodified	NaN_3	1.92	0.0202	0.0797
2	50x2	glass, unmodified	NaN_3	2.01	0.0211	0.0932
3	50x2	glass, Cl-modified (2 weeks treatment)	NaCl	1.75	0.0184	0.0556
4	50x2	glass, Cl-modified (2 weeks treatment)	NaN_3	2.01	0.0211	0.0932
5	50x2	polycarbonate	NaN_3	1.81	0.0190	0.0632

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[a] C evaluated by interpolation of data reported in literature (see Figure 2).

The effective void volume V_0 was experimentally determined by eluting an unretained spectroscopic standard: K_2CrO_4 in Na_2HPO_4 0.05 M. The system and the channel dead volumes were measured and the effective V_0 was then calculated. After channel assembling the effective channel thickness w must result very close in value to the spacer thickness which was accurately determined with a micrometer. Actual w is often somewhat thicker, particularly when sealant is used to prevent leaks between the spacer and the channel walls. Since channel thickness is really critical for the evaluation of the coefficient C (Eq. 6) and, hence, of particles' elevations, it was accurately calculated from the exact value of the channel void volume divided by the geometrical base surface of the channel. The channel systems used in the experiments are listed in Table 1.

Silica Samples

Samples were porous, spherical silica particles of 5 μm diameter for HPLC column packing (LiChrospher SI-60) (Merck, Darmstadt, Germany),

Table 2

Specifications of Used Silica Samples

Sample	Name	Surface type	Shape	Diameter [cm]	ρ_{dry} [g cm^{-3}]	V_P [cm^3 g^{-1}]	$\Delta\rho^a$ [g cm^{-3}]
1	LiChrospher SI 60	polar	spherical	5.0 10^{-4}	2.3	0.85	0.441
2	LiChrospher SI 60 RP18e	unpolar	spherical	5.0 10^{-4}	1.8	0.85	0.317

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[a] $\Delta\rho$: see Eq. 9.

either non-derivatized or octadecil-sylanized. The first sample (Si-60) is polar, while the second (RP-18e) has a highly hydrophobic surface. Sample specifications are given in Table 2. Non-derivatized samples were dispersed at 0.5% (w/v) in Milli-Q water while RP18e samples were dispersed at the same concentration in TRITON X-100 5% (v/v). Injected amounts were always 100 μg.

GrFFF Experimental Conditions

Carrier flow was generated by a Model 2510 HPLC pump (Varian, Walnut Creek, CA). Flow rates spanned from 0.2 to 6.0 cm^3 min^{-1}.

Mobile phase was Milli-Q (Millipore, Bedford, Germany) water added with TRITON X-100 0.1 % v/v and with NaN_3 or NaCl 3.1 10^{-3} M. The carrier viscosity η was assumed to be 0.890 g cm^{-1} s^{-1} as reported in Ref. 16.

The detector was the Model 2550 UV-VIS flow-through spectrophotometer (Varian, Walnut Creek, CA) operating at 330 nm. Detector output was recorded on an X-Y chart strip integrator Model Mega 2 (Carlo Erba Strumentazione, Milan, Italy) and captured through a 12 bit I/O board Model Lab PC+ (National Instruments, Austin, TX) plugged into an AT-compatible 386-DX PC. Stop-flow injection procedure with 3 minutes of relaxation time was followed in all the experiments.

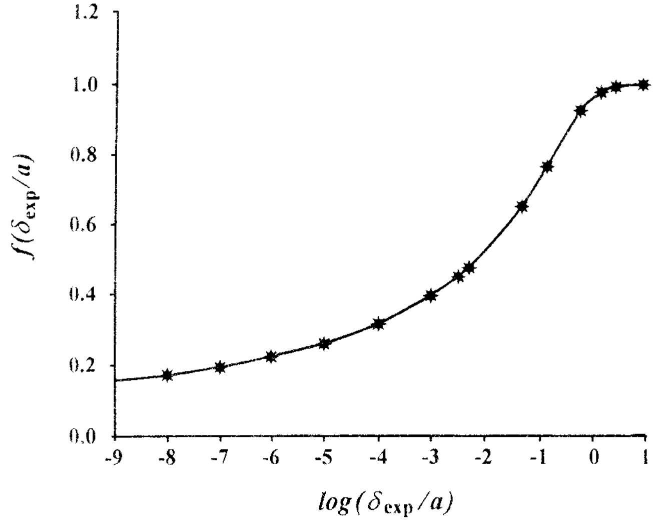

Figure 1. Interpolated retardation function $f(\delta_{exp}/a)$ from Ref. 14. (*): experimental data reported in Ref. 24.

Determination of Predicted Particles' Elevation Values

The basic equations for steric GrFFF retention are reported elsewhere [Ref. 23 and references therein]. For GrFFF in steric elution mode, the expression for retention is given by

$$R = 6\gamma \frac{a}{w} \tag{1}$$

where R is the retention ratio, γ is the steric correction factor, a (cm) is the particles' radius and w (cm) is the channel thickness. The quantity γ can be experimentally determined by calibration, that is, by plotting R *vs.* a for various standard samples of different radius.

The steric correction factor is related to particles' mean elevation. The theoretical approach to the evaluation of particles' mean elevation in a SdFFF channel was given by P. S. Williams *et al.* through the analysis of the hydrodynamic effects able to influence retention, *i.e.* the retardation effect and the lift forces contribution.[14-16]

The first contribution is usually considered as given by a perturbation of the mobile phase stream by the presence of finite particles within the fluid. Retardation effects result in particles' velocities which are smaller than that of the surrounding fluid. This effect is expressed by the equation

$$f\left(\frac{\delta}{a}\right) = \frac{v_p}{v(x)} \tag{2}$$

where x (cm) is the distance of the center of the particles from the accumulation wall, $\delta = x - a$ (cm) is called the particles' elevation with respect to the accumulation wall, v_p (cm s^{-1}) is the velocity of the center of the particle, $v(x)$ (cm s^{-1}) is the velocity of the surrounding fluid, and $f(\delta/a)$ is the retardation function, which was evaluated by P. S. Williams *et al.*[14] by fitting numerical results previously given by A. J. Goldman *et al.*[24] The derived $f(\delta/a)$ function, in numerical form, was also employed in the present paper to calculate the experimental particles elevation values (δ_{exp}) through the expression reported below (Eq. 4). The retardation function profile is reported in Figure 1.

Williams and coworkers also reported that, with the parabolic profile of the mobile phase flow given by

$$v(x) = 6\langle v\rangle \frac{x}{w}\left(1 - \frac{x}{w}\right) \tag{3}$$

where $\langle v \rangle$ (cm s^{-1}) is the average flow velocity, by combining Eq. (1), Eq. (2) and Eq. (3), one gets the expression for the experimental retention ratio R_{exp} as a function of particles' elevation δ as

$$R_{exp} = 6f\left(\frac{\delta}{a}\right)\frac{x_{exp}}{w}\left(1 - \frac{x_{exp}}{w}\right) \tag{4}$$

The above expression makes it possible to calculate the experimental values of x (x_{exp}) and, hence, particles' mean elevation values ($\delta = x - a$). It must be pointed out that Eq. (4) is strictly valid only if the R values correspond to the equilibrium values (R_{eq}), that is, when the steady-state distribution of the particles across the channel is rapidly obtained and maintained once elution starts. However, particles take a finite time for bringing themselves to the equilibrium distance (secondary relaxation) and, hence, the x value related to R_{exp} is a mean value. The direct consequence is that a correction should be applied to obtain R_{eq} from R_{exp} values. However, in a GrFFF system, if flow rates are not too high and particles of sufficiently high density are used, the secondary relaxation time can be considered as negligible with respect to the retention time and correction for R_{exp} values can be avoided. Such limiting condition has been taken into account in the choice of the experimental conditions employed in this work, and, thus, δ_{exp} values have been directly calculated by means of eq. (4).

When particles' mean elevation values (δ_{exp}) are higher than zero, then these particles are lifted away from the accumulation wall and elute at higher velocity than that of the fluid lamina at a distance from the accumulation wall which corresponds to the particle radius (*i.e.*, particles eluting in physical contact to the accumulation wall). The presence of forces capable of lifting away particles from the accumulation wall during elution must be, thus, postulated. A quantitative approach to lift forces was given by P. S. Williams *et al.*[14-16] who described lift effects due to two additive lift forces: inertial lift forces (F_{in}) and near-wall lift forces (F_{rw}). The relative expressions were given as

$$F_{in} = 13.5\pi \frac{\langle v\rangle^2 a^4 \rho_{mp}}{w^2} 19.85\left(0.19 - \frac{x}{w}\right)\left(0.5 - \frac{x}{w}\right)\left(0.81 - \frac{x}{w}\right)\left[1 + \frac{16}{25}\frac{x}{w}\left(1 - \frac{x}{w}\right)\right] \qquad (5)$$

$$F_{nw} = 6C\frac{a^3 \eta \langle v\rangle}{\delta w} \qquad (6)$$

where ρ_{mp} (g cm^{-3}) is the density, η (g cm^{-1} s^{-1}) is the viscosity of the mobile phase and C is an experimental coefficient which resulted strongly dependent on w.

In GrFFF, when the sample band approaches its steady-state distribution across the channel, particles' mean elevation predicted by lift forces theory can be determined by equating lift forces (F_L) to gravitational force (F_G), that is

$$F_G = F_L = F_{in} + F_{nw} \qquad (7)$$

$$F_G = \frac{4}{3}\pi a^3 \Delta\rho g \qquad (8)$$

where $\Delta\rho$ (g cm^{-3}) is the difference between particles' density and carrier's density and g is the earth gravity acceleration (cm s^{-2}). The exact value of $\Delta\rho$

can be calculated by the method proposed by J. C. Giddings *et al.*:[25]

$$\Delta\rho = \frac{\rho_{dry} - \rho_{mp}}{\rho_{dry} V_P + 1} \tag{9}$$

where ρ_{dry} (g cm^{-3}) is the density of bulk dry samples and V_P (cm^3 g^{-1}) is the sample porosity.

By comparing experimental particles' elevations (by Eq. 4) and predicted elevations computed through Eqs. 5, 6, 7, 8, it is possible to verify whether particles' elevations can be considered as only due to the lift forces described accordingly to Williams' model. A significant difference between experimental and predicted particles' elevation may suggest the presence of other forces which can act together with lift forces by either enhancing or reducing the extent of particles' lift.

Determination of the Experimental Coefficient for Near-Wall Lift Forces

As far as the evaluation of the coefficient C is concerned, Williams *et al.* determined its values for three different SdFFF systems.[14-16] Such an experimental determination of C was based on R measurements in a very large combination of particles' diameters and SdFFF systems with mobile phases of different viscosity. In this work, a sufficient number of channel systems and samples to follow the same systematic experimental approach for the determination of the coefficient C was not available.

An interpolation from data reported by Williams *et al.* was, thus, used. Such an interpolation method was thought to be reasonably acceptable for the following reasons: first, the reported measurements were based on SdFFF channels and the GrFFF systems are sedimentation systems in which the field is just Earth's gravity; second, the data were obtained through a very large number of measurements and hence can be considered representative and sufficiently accurate to be extended to other systems and, third, Williams and coworkers found that channel thickness w is the parameter that strongly influences C values.

Graphical results for the performed interpolation are shown in Figure 2. It must be pointed out that the plotted curve does not conform to any theoretical expression but it only corresponds to the best fitting polynomial for the reported data. The numerical values of interpolated C are reported in Table 1.

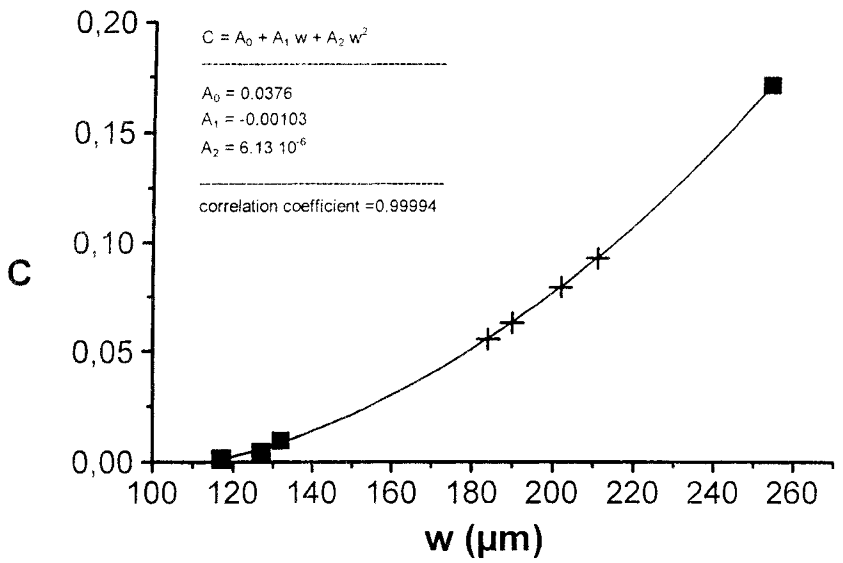

Figure 2. Interpolation of the C values relevant to the systems used. (■): experimental data;[14-16] (+): interpolated data.

RESULTS AND DISCUSSION

The main goal of this work was to check whether the model for hydrodynamic effects above described could be applied also in GrFFF. If experimental conditions for which Williams' approach holds true in GrFFF can be found, then it can be assumed that mean distances of particles from the accumulation wall (δ) during their elution in a GrFFF system can be predicted. In this case, then, the equation for retention (Eq. 4) can be used if it is known the retardation function $f(\delta/a)$ (Eq. 2).

Once verified, the applicability of Williams' model for the determination of particles' mean elevation, the experimental approach here presented through the comparison between experimental and predicted δ values, allows also to evaluate the presence of other second-order effects which can significantly act on retention. In this work, just some preliminary data obtained for two silica samples of a given diameter (5 μm) but different surface and density are presented. Further work on particles of different size and surface is currently on progress.

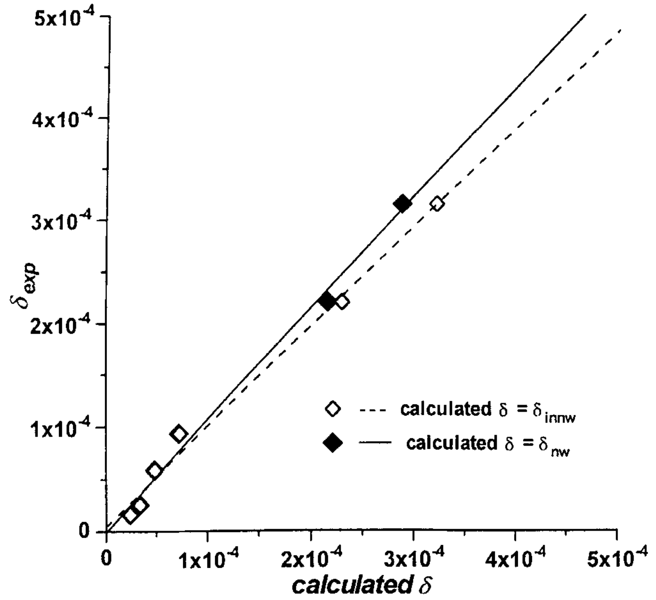

Figure 3. Comparison between experimental (δ_{exp}) and calculated elevations. *System 1, sample 1.* (dashed line): *calculated δ* values by Eqs. 5÷7; (solid line): *calculated δ* values by Eq. 6. See Table 3, rows 1, 2.

For each of the employed samples, different sets of retention measurements were performed for each channel system (Table 1) by varying the flow rate. The experimental approach here followed was always based on the comparison between predicted and experimental particles' elevation at various flow rate.

Experimental mean elevations (δ_{exp}) determined by Eq. 4 were reported against predicted mean elevations (*calculated δ*). These latter values were obtained for both samples by equating Eq. 8 to Eq. 7 and solving for δ the expression for lift forces in Eq. 5 and/or Eq. 6.

The prediction of δ values with changing channel systems and the analysis of the experimental deviations from Williams' model will be described below. All the discussed data are summarized in Table 3.

Table 3

Comparison Between Experimental and Calculated δ Values[a]

	System	Sample	Calc.δ	Intercept	Slope	Corr. Coeff.	Data Pts.	Range of Flow Rate (cm^3 min^{-1})
1	1	1	δ_{innw}	$4\ 10^{-6}\pm2\ 10^{-5}$	1.0±0.1	0.9951	5	0.5÷6.0
2	1	1	δ_{nw}	$-9\ 10^{-7}\pm2\ 10^{-5}$	1.1±0.1	0.9954	5	0.5÷6.0
3	1	2	δ_{innw}	$-5\ 10^{-6}\pm3\ 10^{-3}$	0.9±0.2	0.9952	5	0.5÷4.5
4	1	2	δ_{nw}	$-1\ 10^{-5}\pm4\ 10^{-5}$	1.0±0.2	0.9915	5	0.5÷4.5
5	2	1	δ_{nw}	$4\ 10^{-6}\pm1\ 10^{-5}$	0.93±0.09	0.9969	8	0.5÷6.0
6	2	2	δ_{nw}	$3\ 10^{-5}\pm2\ 10^{-5}$	1.1±0.1	0.9933	8	0.5÷6.0
7	3	1	δ_{nw}	$1\ 10^{-6}\pm5\ 10^{-5}$	3±1	0.9765	5	0.4÷1.8
8	3	2	δ_{nw}	$4\ 10^{-5}\pm5\ 10^{-5}$	2.9±0.8	0.9820	5	0.4÷1.8
9	4	1	δ_{nw}	$4\ 10^{-5}\pm5\ 10^{-5}$	1.6±0.3	0.9877	8	0.5÷4.0
10	4	2	δ_{nw}	$1\ 10^{-4}\pm1\ 10^{-4}$	1.4±0.4	0.9552	8	0.5÷4.0
11	5	1	δ_{nw}	$2\ 10^{-5}\pm2\ 10^{-5}$	0.5±0.1	0.9700	9	0.5÷6.0
12	5	2	δ_{nw}	$2\ 10^{-5}\pm2\ 10^{-5}$	0.5±0.1	0.9242	9	0.5÷6.0

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[a] Regression analysis for the δ_{exp} *vs. calculated* δ. Confidence level: 95%.

Prediction of δ Values

The results for a GrFFF system with bare, unmodified floating glass walls and mobile phase containing Triton X-100 0.1% (v/v) and NaN_3 3.1 mM (*System 1*) are first considered with respect to *sample 1* (LiChrospher 5 μm). To predict particles' elevation (*calculated* δ) both the contributions due to inertial and near-wall lift forces (Eq. 5÷Eq. 7) have been first considered; in this case the calculated values of δ are indicated in Table 3 as δ_{innw}. The comparison between experimental and predicted mean elevations is reported in Figure 3 (symbol ◊, dashed line) with the results of regression analysis reported in Table 3. One can first observe that retention ratio values increase with increasing flow rate. This is the first proof of hydrodynamic effects able to lift particles away from the accumulation wall: in fact, by Eq. 4, one calculates experimental particles' elevation values which increase with increasing flow rate. Linear regression analysis performed for experimental against predicted δ values is able to verify whether the applied model for the description of hydrodynamic effects on retention is valid. In our experiments, a straight line with null intercept and unit slope shows that such a model based on Eqs. 5÷9 is able to predict δ values for the given combination of *sample* and GrFFF *system*. From Figure 3, it is evident that there is no significant difference between experimental elevations (δ_{exp}) and the elevations predicted by taking into account both inertial and near-wall lift forces (δ_{innw}) in Williams' model. Such experimental finding is, to the authors' knowledge, the first experimental verification of Williams' model for lift forces in GrFFF. It is, therefore, demonstrated that δ values can be predicted in an optimized GrFFF system and, as a consequence, that accurate characterization of silica particles may be performed with no previous knowledge of the value of the steric correction factor. The use of GrFFF for size characterization without calibration is noteworthy for the low cost of this technique when compared to other FFF systems. Moreover, it is also possible to foresee that, if the model is followed, the experimental mean elevation values can be used also to determine particles' density through the use of Eq. 7 and Eq. 8.

From previous literature,[14] it has been deduced that, because of the low δ_{exp} values obtained in all the performed experiments, the contribution due to inertial lift forces could have been negligible and, therefore, the simple expression only for near-wall lift forces (Eq. 6) can be used to predict particles' mean elevation. That inertial lift forces could be actually neglected under the experimental conditions used with *system 1* and *sample 1*, it is demonstrated in Figure 3 where it is also reported the straight line obtained by plotting δ_{exp} values against the *calculated* δ values obtained by considering only near-wall lift forces (δ_{nw}) (symbol ◆, full line). It is found that a new straight line is obtained with intercept and slope values which are, still, not significantly different from zero and unity, respectively. Since in all data (Table 3) the experimental particles' elevation values were comparable in magnitude to those obtained for the case of *system 1* and *sample 1* above discussed, it was deduced that inertial lift forces could be considered negligible for all the experimental cases here discussed and, thus, Eq. 6 was always employed in determining predicted particles' elevation (δ_{nw}).

All the data up to now reported are referred to bare, polar silica particles. Similar results were obtained when the nature of particles' surface was changed to a highly hydrophobic one (RP18e). A set of experiments similar to those reported in Figure 3 was performed on *system 1* for LiChrospher RP18e (*sample 2*). The results are reported in Figure 4 and Table 3. It can be clearly observed that, also with this sample, the channel *system 1* behaved ideally with respect to predicted hydrodynamic effects. In Figure 4, the regression lines obtained by taking into account, for the prediction of particles' elevation, either inertial and near-wall lift forces (symbol O, dashed line) or near-wall lift forces alone (symbol ●, full line) are again compared. As in the cases discussed above (Figure 3), both straight lines in Figure 4 do not show significant differences in intercept and slope values which are, respectively, zero and one. The very similar retention behavior for highly hydrophilic and hydrophobic particles could be surprising. However, it can be explained by the fact that the

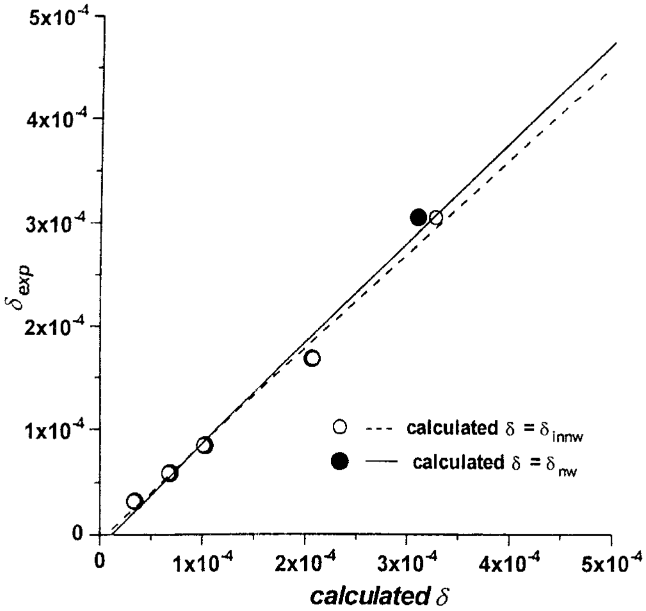

Figure 4. Comparison between experimental (δ_{exp}) and calculated elevations. *System 1*, *sample 2*. (dashed line): *calculated δ* values by Eqs. 5 ÷7; (solid line): *calculated δ* values by Eq. 6. See Table 3, rows 3, 4.

high concentration of surfactant (5%) used to disperse *sample 2* (LiChrospher RP18e) in the batch solution is able to drastically modify the surface polarity of the particles. It must be noted that, if the surface of RP18e particles were not modified by the adsorption of polar groups, they could not even be fractionated in a GrFFF channel.

In order to test whether the ideal behavior shown by a GrFFF system like that up to now discussed was reproducible, another GrFFF channel (*system 2*) made by other two bare, floating glass walls and another strip of Mylar of the same thickness as that used for *system 1* has been tested. The effective channel thickness *w* resulted slightly different from that of *system 1*, probably because of the effect of the somewhat different amount of silicone sealant used to prevent carrier leaks in the glass-made channels. *System 2* was assembled exactly in the same way as *system 1* and the same experimental procedures employed with *system 1* were repeated with *system 2* for both *sample 1* and *sample 2*. In both cases, regression analysis did not show any significant difference with respect to *system 1*. The results did not change, as it can be seen from the data relative

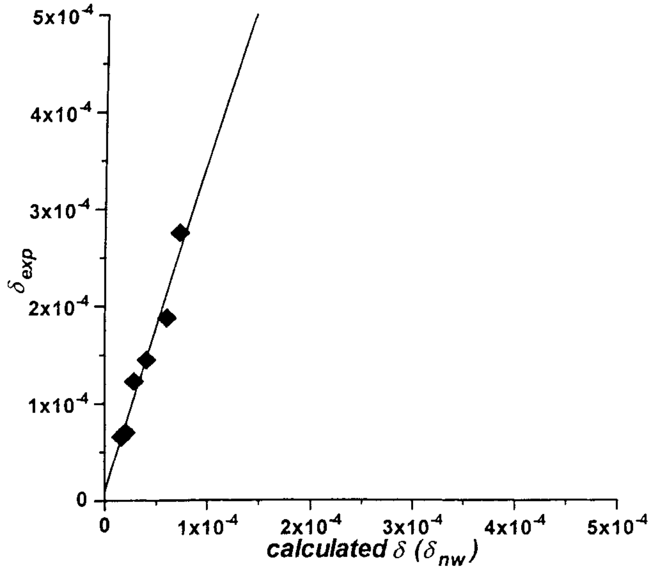

Figure 5. Comparison between experimental (δ_{exp}) and calculated elevations. *System 3, sample 1.* See Table 3, row 7.

to *system 2* in Table 3. Even though much more experimental work must be done for a systematic study of the second-order effects in GrFFF retention, from such preliminary results it can be observed that, under well-defined experimental conditions, the elution in a GrFFF channel can be described by using the model for hydrodynamic effects that was already verified for the elution of particles in Steric/SdFFF systems. These promising results can justify further efforts towards a wider applicability of GrFFF for the characterization of supermicron particles.

Deviations From Predicted δ Values

The possibility to predict particles' elevation of silica particles in a GrFFF channel by means of Williams' model for hydrodynamic forces in steric SdFFF would suggest that no second-order effects other than hydrodynamic ones exerted any influence on particles' elevation in the GrFFF experiments discussed above. However, it is known that particle-wall interaction, likely of electrostatic nature, can influence retention in steric FFF mode when particles elute very close to the accumulation wall. At least in the case of *sample 1*, the effect of such particle-wall interactions could be offset in the channel systems discussed above by the similar nature and polarity of channel walls (glass) and particles' surface (silica).

In order to test whether modifications in the channel systems could change particles' retention from that one predicted by hydrodynamic effects alone, channel walls of different nature were employed. Since, in a previous work,[6] it was reported that the use of sodium chloride in the mobile phase can give adsorption of such an anion (Cl^-) on channel walls and modify GrFFF retention of silica particles, a set of retention measurements at different flow rates was performed in a channel in which NaCl in place of NaN_3 had been passed for two weeks before the experiments (*system 3*). The results for *sample 1* are reported in Figure 5 and Table 3. It must be observed that, in the case of *system 3*, although the range of the obtained δ_{exp} values was comparable to that obtained for *sample 1* with *systems 1* and *2*, here similar particles' retention values were obtained at slower flow rates. This is a first indication that other forces than hydrodynamic ones act on retention when chloride ions are present in the GrFFF system. In fact, a comparison between the data obtained for *sample 1* and *system 3* (Figure 5) and those for *sample 1* and *system 1* (Figure 3) indicates a large increase above unity of the slope value in the linear regression relative to the former case. Similar results were obtained with *sample 2*. Such an increase of the slopes indicates that experimental mean elevations steadily increase, at any flow rate, with respect to the calculated values. This effect must be ascribed to the presence of chloride ions on the accumulation wall, even though the mechanism of interaction has not, as yet, been fully understood. It is possible that adsorption of chloride ions on channel walls gives rise to repulsive effects with silica particles that are also negatively charged. Chloride ions, in fact, are known to display high chemical affinity for glass surfaces. Since silica particles have already shown to experience other type of interactions able to alter retention ratio values,[6] it is again demonstrated that mobile phase composition must be carefully chosen in the framework of an optimized approach to GrFFF. It is here shown that other effects, likely of electrostatic nature, can affect retention in GrFFF comparably to that exerted by hydrodynamic forces.

That repulsive effects can be partially due to a given amount of chloride ions permanently adsorbed on the accumulation wall can be deduced by analyzing the data obtained with *system 4* which is physically different from *system 3*, even if prepared and assembled in the same way. The main difference lies in the fact that while with *system 3* NaCl was also used as the ionic modifier of the mobile phase, with *system 4* after two weeks of treatment with NaCl in the mobile phase, the standard NaN_3-added mobile phase was used for the retention experiments. From the comparison of the data obtained by these two systems (Table 3), it can be observed a repulsive effect also when chloride has been removed from the mobile phase. Such a repulsion is less intense than that observed with NaCl in the mobile phase (*system 3*), that is lower values of particles' elevation (lower slopes from the linear regressions) were obtained. It is possible that repulsive effects are determined by chloride ions adsorbed on glass walls. A desorption of chloride ions from channel walls when NaCl is no longer present in the carrier solution can explain the different behavior observed with *system 3* and *system 4*. In fact, some preliminary studies indicated that further changes in GrFFF retention ratio values of silica particles when NaCl was present in the mobile phase decrease with increasing the time of treatment with such a NaCl-added carrier solution.[6] In order to better explain the mechanism of the glass wall modification in presence of chloride ions, it would be interesting to study the kinetic of absorption of Cl^- on the glass walls. By this way, it could be possible to establish whether the system evolves towards a saturation state, with the final effect of giving constant and reproducible values of retention ratio.

Investigations on channel walls of different material than glass were also performed by using polycarbonate walls (*system 5*). Results for *sample 1* are reported in Figure 6 and in Table 3, while data for *sample 2* are reported only in Table 3. Both the two samples (polar and non polar surfaces, respectively) have shown identical behavior, *i.e.*, the slope values were comparable even though their values were lower than unit. Polycarbonate walls showed, therefore, an opposite effect to that observed with chloride ions on glass, since experimental elevation values lower than predicted were observed. It can be deduced that, with polycarbonate walls, some type of particle-wall interactions of attractive nature can play on retention, which are comparable in magnitude to those explained in terms of hydrodynamic forces alone.

It is important to focus on the significant differences observed between experimental and predicted values of δ by changing only the mobile phase or channel wall material, since it is a first indication of the existence of second-order effects other than hydrodynamic forces. These forces modulate retention to an extent comparable to the hydrodynamic forces alone, and their effect can be reflected in a change in the coefficient C values. In fact, although in all cases the values of the slopes can be influenced by the uncertainty associated with the coefficient C value, it is worth to note, for instance, that the behavior observed for one of the Cl-modified channels (*system 4*) with respect to the unmodified glass walls channel (*system 2*) can be only explained in terms of such electrostatic forces. *System 4* was indeed obtained directly from *system 2* by just passing the NaCl-added mobile phase. Consequently, no differences in

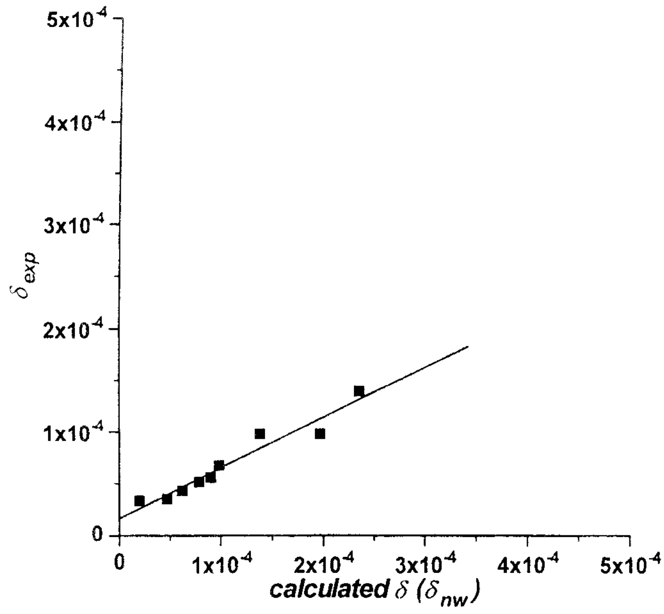

Figure 6. Comparison between experimental (δ_{exp}) and calculated elevations. *System 5, sample 1.* See Table 3, row 11.

the channel thickness and void volume values (see Table 1) can be ascribed in the measurements relatively to such channels. The value assumed for *C* for *system 4* can thus be thought of as being affected by the same level of uncertainty due to the channel thickness as it is the *C* value assumed for *system 2*.

As a consequence, it can be deduced that the different slope value obtained for channels of different walls composition, as *system 2* and *system 4*, can be due, at least partially, to electrostatic effects that are able to eventually affect the *C* coefficient value.

The method based on the validation of Williams' approach to hydrodynamic effects in steric FFF could thus be used as a tool for investigating and quantitatively evaluating the overall second-order effects acting on a GrFFF system.

CONCLUSIONS

A preliminary experimental study of the second order effects on GrFFF retention has demonstrated that, under well-defined experimental conditions for the elution of silica particles, a properly designed GrFFF system can follow the quantitative model reported in literature for hydrodynamic effects on SdFFF retention in steric mode.

It is also demonstrated that, for quite dense particulate matter used as injected sample and eluted at not too high flow rate, inertial lift forces can be negligible with respect to near-wall lift forces, thus simplifying retention prediction from lift forces equations.

By means of the experimental verification of the model for hydrodynamic effects, it has been also determined how other experimental variables as walls' surface nature and mobile phase composition can introduce significant changes on GrFFF retention. In particular, second-order effects induced by changing the GrFFF systems have been here demonstrated to be comparable, in magnitude, to hydrodynamic effects. Therefore, the approach here applied to validate lift forces equations in GrFFF has shown to be a promising tool also for the investigation of the nature of the overall second-order effects in the framework of a wider applicability of optimized GrFFF analysis.

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