Short Communication

Interfacial effects in size-exclusion chromatography of latex

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ABSTRACT

The retention volume in size-exclusion chromatography (SEC) depends on both the size of the particle proper and the interfacial mutual repulsion of the surfaces, *i.e.*, between the pore wall and the surface of the particles. The presence of some interfacial repulsion is crucial to obtain a pure SEC mode of elution. It is therefore always present and should not be neglected as is usually done. Analysis of published data shows that the interfacial repulsion is well described by the Dejaguin–Landau–Verwey–Overbeek (DLVO) theory of colloidal stability, and its properties can therefore be studied in detail via chromatography. It is shown that interfacial repulsion depends on the size of the latex beads ans attains large values for larger beads. For a given size of the bead it depends strongly on its charge up to a limit of saturation, as predicted by DLVO theory.

INTRODUCTION

In size-exclusion chromatography (SEC), macromolecules or particles flow through a porous matrix and permeate all volumes accessible to their centres of gravity. For solutes comparable in size to the pore size, this accessible volume depends strongly on size and shape and hence larger particles elute first. In SEC proper, particles do not adhere to the matrix at all.

It was previously established [1–3] that universal calibration in SEC needs to account for both the effective hydrodynamic shape of the solute, which freely permeates the pores, and an interfacial wall effect. Whenever the interaction energy between the pore surface and the particles surface is attractive at some distance and the approach to this steric position is not kinetically inhibited by a high activation barrier, non-SEC modes determine elution. Electrostatic repulsion is a very effective means of preventing this adsorption and thus of establishing pure SEC conditions. A low ionic strength then is superior to high salt concentrations. However, the wall effect then becomes large at low ionic strength and must be explicitly accounted for, whereas it is usually neglected at high ionic strength or with neutral solutes. A universal calibration is therefore characterized by a total radius R:

$$R = R_{SEC} + R_{IF} = R_{SEC} + \kappa^{-1}\bar{x} \tag{1}$$

where $\bar{x}$ is the average electrostatic repulsion distance at equilibrium in multiples of Debye length κ, R is the total solute radius, R_{IF} is the interfacial contribution to R and R_{SEC} is the rotationally averaged mean radius of the solute, similar to or even identical with R_η, the equivalent body radius defined by intrinsic viscosity. For a detailed discussion of eqn. 1, see ref. 3. For spheres R_{SEC} is simply the radius of the bead and may also be determined by electron microscopy, even though

hydrodynamic radii may slightly differ from the microscopic dimensions of dried beads.

Styring et al. [4] presented the same rationale but did not analyse their data further for latex spheres on porous glass packings along these lines. The analysis of the wall effect based on their data is presented in this paper.

EXPERIMENTAL AND RESULTS

All data reported in the following are based on elution volumes measured and tabulated by Styring et al. [4], who also prepared the materials. A polystyrene seed latex (L1) was prepared in Aerosol-MA80 detergent solution similar to the method of Woods as modified by Styring et al. [4]. This latex was then either grown stepwise to larger sizes in Aerosol-MA80 solutions according to Dodge [7] (L-series) or was overcoated stepwise with poly(methyl methacrylate) in detergent-free solution according to Chainey and Hearn [8] (M-series), as detailed by Styring et al. [4]. Optionally latices were exhaustively dialysed against pure water to remove all detergent. When later run on the SEC columns in the presence of sodium dodecyl sulphate (SDS) or Ultrawet-K detergent, dialysed latices gave identical results to non-dialysed latices. This suggests that. Aerosol-MA80 has little influence on the different properties of the two types of latices. However, higher concentrations of initiator present during polystyrene seed growth might have incorporated more charged residues into the polystyrene latices than into the methacrylate latices. The sizes of the latices were determined by electron microscopy and are given in Tables 1 and 6 in ref. 4. The differently sized latices were run on a porous glass column at different ionic strength in the presence of detergent. Elution volumes are tabulated in Table 8 in ref. 4.

To apply the rationale of analysis devised by the present author [1–3], these primary data need to be converted into effective radii R via a universal calibration of their column set-up. The best choice is data obtained at 111 mM ionic strength in the presence of Ultrawet-K as surfactant (Table 10 in ref. 4), even though measurements made in SDS will be analysed below. There may be small differences in the viscosity radius R_η in these different solvents. However, an even larger error is introduced by using the electron microscopically determined latex radii

R_{EM} (Tables 1 and 6 in ref. 4) instead of R_η. By later taking only differences in R for the salient conclusions, however, this reduces to a minor order correction. Fig. 1 shows the universal calibration obtained for their particular mixed bed CPG–Fractosil porous glass column used in these experiments.

Using Fig. 1, one can now convert all elution volumes for different latices and ionic strength (Table 8 in ref. 4) into radii. Fig. 2 shows the data for the surfactant-coated polystyrene–latex spheres for each solute (L1–L6) as a function of total ionic strength. This includes the concentration of SDS monomer and yields values slightly higher than those listed in ref. 4, which are based on support electrolyte alone. The data are scattered and may even reveal some non-linearity; however, for simplicity a first analysis is done in terms of a linear function of $I^{-1/2}$. Most likely deviations at high ionic strength originate from the commencement of adsorption noted by Styring et al. [4]. Fig. 3 shows the same analysis for poly(methyl methacrylate)-coated latex spheres. Comparing Fig. 2 with Fig. 3, it is obvious that the charge effect of the methacrylate surface is less than that for pure polystyrene, indicating that less surfactant has been adsorbed on the methacrylate surface than on pure polystyrene. It may be, however, that the prime difference is not
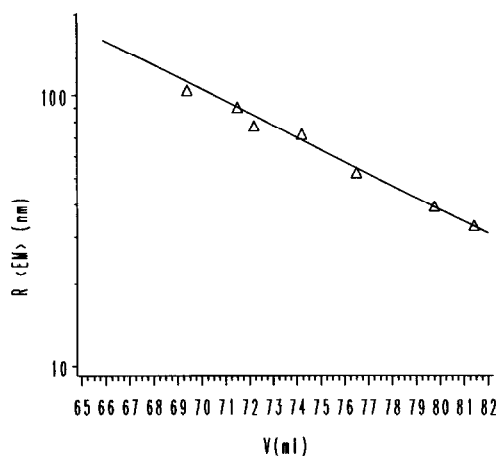

Fig. 1. Universal calibration of the mixed-bed CPG–Fractosil column used by Styring et al. [4] based on their data for $I = 111$ mM (Table 10 in ref. 4). The electron microscopically determined radii R_{EM} are presumably slightly smaller than the viscosity radii R_η, but errors will have little effect in the present context.
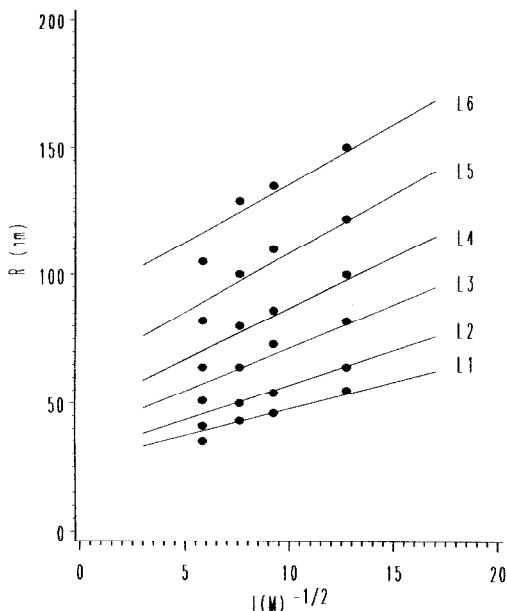
Fig. 2. Total size R of surfactant-coated polystyrene latex beads (L) as a function of ionic strength. Measurements were made at 0.0295, 0.0173, 0.0117 and 0.0062 M ionic strength with 0.0017 M SDS and various amounts of sodium nitrate. Elution volumes from Tables 8 in ref. 4 were converted into radii via universal calibration (Fig. 1).

Fig. 3. Total size R of methacrylate-coated polystyrene latex beads (M) as a function of ionic strength. Measurements were made at 0.0173, 0.0117 and 0.0062 M ionic strength with 0.0017 M SDS and various amounts of sodium nitrate. Elution volumes from Table 8 in ref. 4 were converted into radii via universal calibration (Fig. 1).
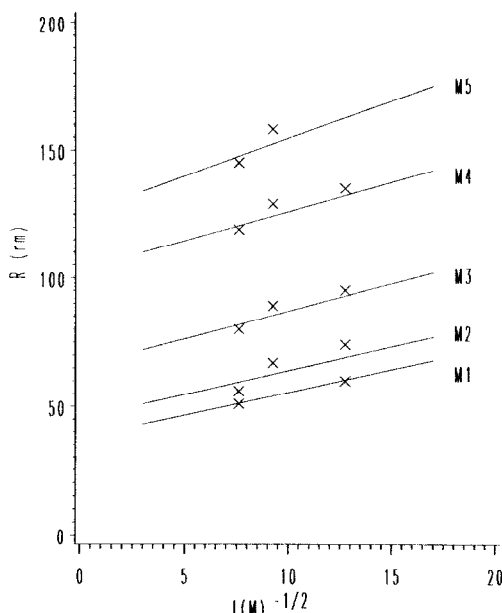
SDS adsorbed from the elution fluid but different amounts of ionic residues incorporated during manufacture (see above). Alternatively, some Aerosol-MA80, which is used during manufacturing of the polystyrene latex but not for the methacrylate coating step, may have become entangled in the particle structure and thus not able to dialyse.

The average electrostatic repulsion distance $\bar{x}$, which measures the wall effect, is obtained from the slopes in Figs. 2 and 3 according to eqn. 1 since κ depends on $I^{1/2}$ [3]:

$$\bar{x} = 3.3 \cdot \frac{\mathrm{d}R}{\mathrm{d}I^{-1/2}} \qquad (2)$$

where the numerical factor assumes an aqueous eluent at room temperature; $\bar{x}$ is a universal parameter as it no longer depends on ionic strength but only on matrix charge and the size and charge of the solute polyelectrolyte.

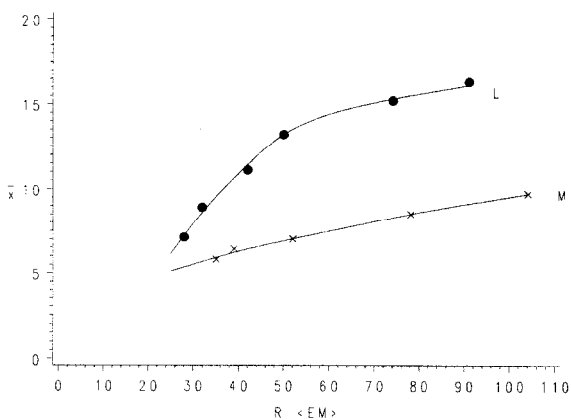The final result is shown in Fig. 4, which presents

Fig. 4. Dependence of repulsion distance $\bar{x}$ in units of Debye length, obtained from the slopes of Figs. 2 and 3 according to eqn. 2, on the size of the latex beads, R_{EM}. This electron microscopic radius is here taken to be similar to R_{SEC}. (●) Surfactant-coated polystyrene latex spheres (L) based on data in Fig. 2; (×) methacrylate-coated polystyrene latex spheres (M) based on data in Fig. 3.

$\bar{x}$ as a function of the microscopic particle dimensions. In addition to this size dependence, $\bar{x}$ critically depends on the surface charge of both the solute and pore wall. The latter is the same for both types of latices, hence the difference reflects the different surface charge on the latex. Presumably the methacrylate surface is nearly uncharged whereas the polystyrene-based surface contains a very high charge density due to surfactant molecules. It is possible that the surface potential of these polystyrene beads is saturated, which would yield the maximum wall effect for the given column, but one cannot exclude that the maximum effect is even larger.

DISCUSSION

The present analysis of the data of Styring *et al.* [4] according to the rationale developed previous [1–3] confirms that wall effects in SEC depend greatly on the size of the solute particle. Comparison of the present data with those previously obtained on TSK-6000 PW [3] reveals that the porous glass is more highly charged than the TSK-6000 PW, which does, however, contain significant residual charges. The TSK PW material was argued to contain a hairy surface of polymer fibres protruding from the nodules that form the pores [3]. The present data with porous glass thus establish the wall effect with guaranteed geometrically smooth pore surfaces. It further has the advantage of avoiding any complications in analysis originating from the asymmetric shape of the sample (DNA) in one of the previous studies [3]. The magnitude of the wall effect is in line with the theory of surface forces as discussed in detail previously [3].

The present analysis demonstrates that SEC may be used to characterize the polyelectrolyte properties of macromolecules and particles. In fact, the adsorption of methacrylate-coated polystyrene at high ionic strength [4] is a direct consequence of the lower charge density of these particles compared with surfactant-coated polystyrene and is probably not a chemical property of the methacrylate.

In previous studies [1–3], the linear eqn. 1 was found to be well suited to describe the data. Some of the present data, in contrast, show a definite curvature (Fig. 3), suggesting a more complicated dependence on ionic strength. This possibility was anticipated previously on theoretical grounds [3]. The scatter of the data is such, however, that it seems

inadvisable to propose a multi-parameter fit. Deviations from linearity are most significant above 15 mM ionic strength and might also originate from the commencement of adsorption. If so, this would suggest that the $I^{-1/2}$ law applies only at lower ionic strength. In this case the $\bar{x}$ values for methacrylate might be even lower whereas the polystyrene data remain as analysed.

Apparently more highly charged, the magnitude of interfacial effects is larger for polystyrene latex on controlled-pore glass than for similarly sized DNA and viruses on TSK-6000PW. A previous study of latex by hydrodynamic chromatography even reported interfacial effects equivalent to about 27 Debye length [5].

The repulsion distance $\bar{x}$ reflects a delicate balance between electrostatic repulsion and Van der Waals attraction in line with Dejaguin–Landau–Verwey–Overbeek theory. It may not be explained solely by electrostatic terms, thus confirming previous conclusions [3]. It must be emphasized that the wall effect, analysed here in terms of ionic strength-dependent elution of polyelectrolytes, is not limited to charge effects. As Van der Waals forces are generally attractive, the very existence of an SEC mode of elution for neutral molecules requires that solvation forces are repulsive, again establishing a repulsion distance, *i.e.*, a wall effect. This wall effect may differ for solutes of different chemical nature and for the same solutes in different solvents.

This study extends previous analyses of interfacial effects [2,3,5,6]. The salient conclusions are that interfacial effects are large contributors to the total observation and are large in absolute terms, they increase dramatically with increasing size of the solute particles and for a given size they increase with increasing charge density (up to a limit of saturation).

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