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Characterization of a hydroxyapatite suspension by capillary zone electrophoresis after fractionation in a rotating coiled column

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A new comprehensive approach based on the field-flow fractionation of particles in a rotating coiled column and their characterization by capillary zone electrophoresis and scanning electron microscopy has been applied to the investigation of irregularly shaped hydroxyapatite sibmicron clusters in an aqueous suspension.

Calcium in bone and tooth tissues mainly occurs as hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$ (HAP). Therefore, ceramics and cements based on artificial HAP are increasingly applied in medicine as bone implants.[1,2] The morphology of crystals, purity, size and structure of HAP determine their key parameters. HAP nanoparticles can be used for the transport of substances, such as amino acids and proteins, through a cell lipid membrane.[3,4] They also exert cytotoxicity and apoptosis induction in some cancer cells, the anti-tumor activity and HAP-induced apoptosis being strongly dependent on the size of HAP particles.[5] Hence, sizing and characterization of phosphate-based materials is of particular importance since the size distribution and surface properties of submicron and micron particles may govern their physicochemical and biochemical properties.

The aim of this work was to study the applicability of field-flow fractionation in a rotating coiled column[6] (RCC) and capillary zone electrophoresis[7,8] (CZE) to the fractionation and characterization of HAP particles and their agglomerates in an aqueous suspension.† Both methods have been extensively applied to particulate matter.[6–8] Particles in the initial sample and separated fractions were visualized by scanning electron microscopy (SEM).

The size distribution pattern for the initial HAP sample, as calculated from the SEM image, is shown in Figure 1(*a*). Particles in the size range between 300 nm and 5 μm were present in the suspension, which contained clusters having different shape that consisted mainly of plates of about 200×40×10 nm [Figure 1(*b*)]. In addition, large aggregates were observed. The electrokinetic behavior of HAP particles was studied by CZE. Phosphate buffer solutions (pH 8.3) were used as carrier electrolytes. The optimal phosphate concentration was 10 mmol dm^{-3}. The electropherogram of the original suspension is presented in Figure 2(*a*). A group of narrow peaks following the peak of the EOF marker were recorded. These peaks were attributed to nanoparticle clusters.

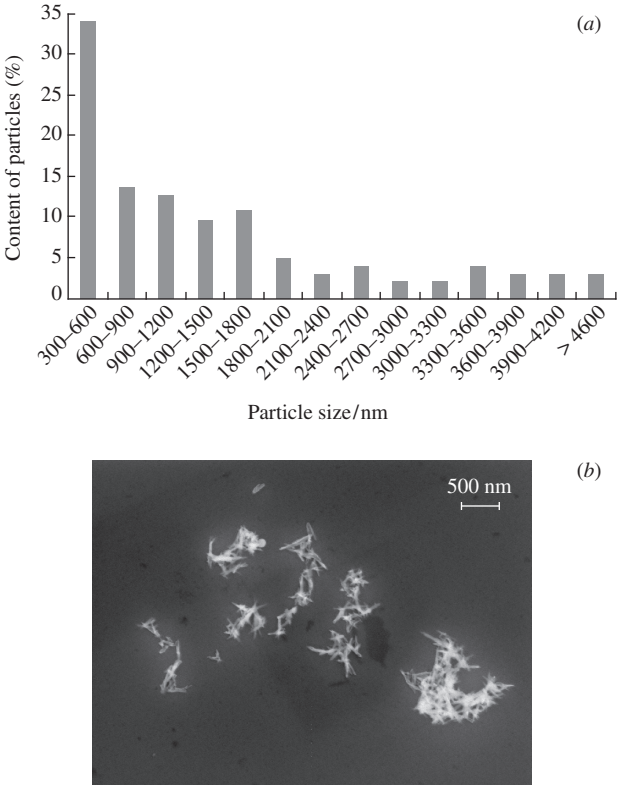

Figure 1 (*a*) Particle size distribution of HAP in the initial suspension (5×10^{-4}%), as calculated from SEM data. (*b*) Typical HAP cluster (SEM image).

† HAP was synthesized at the Moscow State University according to a reported procedure.[9] Suspensions for experiments were prepared by diluting the original suspension with phosphate buffer solutions (pH 8.5). A planetary centrifuge with a vertical cylindrical drum (Institute of Analytical Instrumentation, St. Petersburg) was used for the fractionation of particles. The separation column (total volume, 15 ml) was a Teflon tube with an inner diameter of 1.5 mm coiled onto a cylindrical drum. The fractionation of particles and their aggregates was achieved by a stepwise increase in the mobile phase flow rate from 0.5 to 16 ml min^{-1} at a constant column rotation speed of 300 rpm. The particles in the column effluent were detected spectrophotometrically.

A Capel 105 capillary electrophoresis system (Lumex, Russia) equipped with an on-column UV detector, a deuterium lamp and a UV monochromator operated in the wavelength range of 185–400 nm were used. Uncoated fused-silica capillaries of 75 μm i.d. and 360 μm o.d. with a total length of 37.5 cm and an effective length (the length to the detector) of 28.0 cm were used. The temperature during a run was 25±0.1 °C. The wavelength was 220 nm. The separation of particles in a carrier electrolyte was performed in a counter-electroosmotic mode with the power supply set for positive polarity at an applied voltage of 25 kV. Hydrodynamic injections were performed at the capillary anode end under a pressure of 30 mbar for 5–10 s. Dimethyl sulfoxide was an electroosmotic flow (EOF) marker. The electrophoretic mobility μ_{ep} was calculated as $\mu_{ep} = \mu_{eo} - \mu_{app}$, where μ_{eo} is the electroosmotic mobility and μ_{app} is the apparent mobility. The test sample and the separated fractions were also characterized using a JEOL JSM6700F cold cathode field emission scanning electron microscope (Japan) with high resolution (1 nm at 15 kV, 2.2 nm at 1 kV).

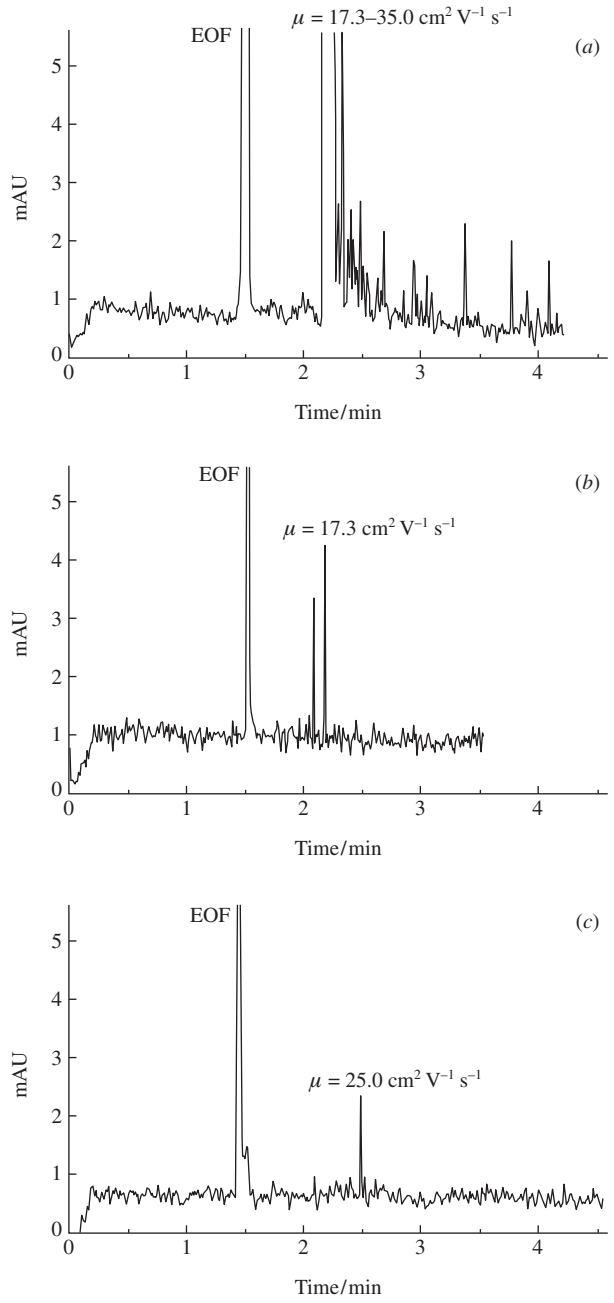

Figure 2 Electropherograms of (*a*) initial HAP suspension, (*b*) fraction *2* and (*c*) fraction *5* separated by field-flow fractionation in a rotating coiled column. Conditions: pH 8.5 (10 mM phosphate buffer solution); dimethyl sulfoxide as an electroosmotic flow marker; sample injection, 30x/10 mBarxs.

Peak positions show that, under experimental conditions, HAP particles were negatively charged. The absolute electrophoretic mobilities corresponding to these particles were in the range between 17×10^{-5} and 22×10^{-5} cm^2 V^{-1} s^{-1}. Note that the electrophoretic mobility values were the same for runs performed in different days. It allowed us to conclude that there are some relatively stable clusters in the original suspension and they can be separated from each other, as well as from large aggregates, by using a fractionation technique.

For the field-flow fractionation of HAP clusters and aggregates, the operation parameters of RCC were optimized according to a published procedure.[10] Overall, ten fractions were separated in RCC by a stepwise increase in the mobile phase (phosphate buffer) flow rate (Figure 3) and then studied by SEM and CZE. Size distributions for fractions *2* and *5* based on SEM measurements are shown in Figure 4 as an example. A comparison between

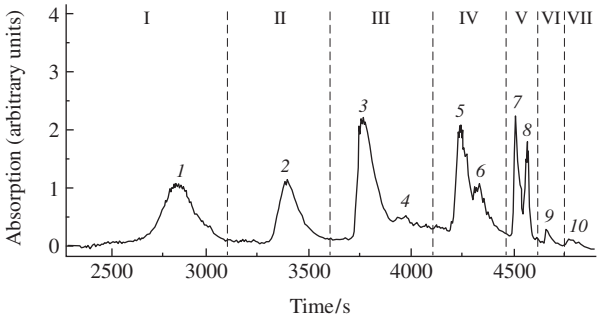

Figure 3 Fractogram of the initial HAP suspension as obtained by field-flow fractionation in a rotating coiled column. *1–10* are fraction numbers. Conditions: column rotation speed, 300 rpm; initial mobile phase flow rate, 0.5 ml min^{-1}; step increasing flow rate up to (I) 2.0, (II) 3.0, (III) 5.0, (IV) 7.0, (V) 10.0, (VI) 13.0 and (VII) 16.0 ml min^{-1}; detection at 254 nm.

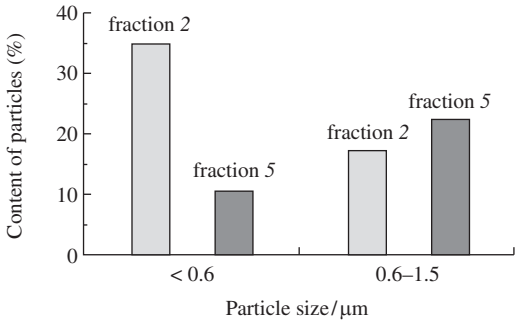

Figure 4 Characteristic size distribution of HAP particles in fractions *2* and *5* separated by field-flow fractionation in a rotating coiled column, as calculated from SEM images.

these distributions indicates that the average weighted size of particles in fraction *2* is smaller than that in fraction *5*.

A comparison between the electropherogram of fraction *2* [Figure 2(*b*)] and that of the original suspension [Figure 2(*a*)] revealed that the positions of the last peak differed significantly. The last peak in the electropherogram of fraction *2* corresponds to a smaller absolute electrophoretic mobility. Hence, the electropherogram presented in Figure 2(*b*) is a fragment of the electropherogram corresponding to the initial suspension [Figure 2(*a*)]. Thus, fraction *2* contains only some sorts of particles (clusters) belonging to the original suspension. In the next fractions, the highest absolute electrophoretic mobility of particles was found larger than that in previous ones [see Figure 2(*c*)]. The distributions of absolute electrophoretic mobilities calculated for several fractions illustrate this regularity. Therefore, correlations between the size and the absolute electrophoretic mobility of HAP clusters in the initial sample and separated fractions were found.

Note that a dynamic light scattering technique (Zetasizer Nano-ZS instrument, UK) was also tested in order to estimate particle size distributions in the suspensions. However, the results were somewhat incorrect. In all measurements, a single peak was obtained, whose position corresponded to an overestimated particle size. This can be due to aggregation processes.

We can conclude that a combination of CZE and field-flow fractionation in RCC is applicable to the characterization of complex HAP suspensions containing irregularly shaped particles in a wide-ranged size distribution. Submicron and micron HAP clusters and aggregates can be partially fractionated using RCC. CZE can be used for the characterization of surface electric properties of HAP particles. The electrophoretic mobility depends on particle (aggregate) size. Thus, the average particle size in HAP suspensions can be estimated though the particle shape is irregular.

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