Available online at www.sciencedirect.com

SCIENCE DIRECT

Talanta 66 (2005) 605–610

Talanta

www.elsevier.com/locate/talanta

Investigation of iron oxide nanoparticles by capillary zone electrophoresis

Natalia G. Vanifatova[a], Boris Ya. Spivakov[a],
Juergen Mattusch[b,*], Ulrich Franck[b], Rainer Wennrich[b]

[a] *Vernadsky Institute of Geochemistry and Analytical Chemistry, Russian Academy of Sciences, Kosygin Street 19, 117975 Moscow, Russia*
[b] *UFZ - Centre for Environmental Research, Analytical Chemistry, Leipzig/Halle, Permoserstr. 15, D-04318 Leipzig, Germany*

Received 2 April 2004; received in revised form 6 October 2004; accepted 1 December 2004
Available online 6 January 2005

Abstract

The electrophoretic behavior of γ-Fe_2O_3 nanoparticles was studied in aqueous solutions of Na_2SO_4–NaOH (pH 10.8) and of Na_2SO_4–Na_3cit (pH 7.1) as running electrolytes. Two electrophoretic zones (smooth and with spikes) due to colloidal and suspended particles of approximately the same size range were formed during the runs. The suspension stability and size distribution were shown to depend on the composition of electrolyte used for dispersing the solids. The effects of electric field strength, injection time, injection pressure as well as sodium citrate concentration were studied and particle electrophoretic mobilities were calculated. Electron micrographs of particles studied were obtained. Preparation of reference samples based on the colloidal γ-Fe_2O_3 has been discussed.

Keywords: Capillary electrophoresis; Iron oxide nanoparticles

---

1. Introduction

It is known that colloidal and suspended solid materials can often affect the migration, distribution and biological uptake of pollutants including toxic trace metals, organic compounds, and radionuclides. Therefore, it is important to study such particles, especially their chemical nature, structure, size and shape distributions, and the conditions controlling the sorption and desorption of various compounds on particle surfaces. Dominating inorganic components of particulate matter in natural waters are usually oxides and hydroxides of Al, Fe, Mn and Si, clay minerals as well as Ca and Ca–Mg carbonates. In recent years capillary zone electrophoresis (CZE) has drawn attention as a method for the separation of aqueous suspensions containing inorganic oxide nanoparticles differing in type and size. Successful separations of silica [1–3] and some metal oxide particles [4–6] have been described. Ballou and coworkers [4–6] have demonstrated the feasibility of using CE in the estimation of surface characteristics (isoelectric point, surface dissociation constants) of several metal oxide particles (titania, hematite and two polymorphic forms of alumina) on the basis of their electrophoretic behavior and on optimization of separation conditions for mixtures of chemically different metal oxide particles. Suspensions of Al_2O_3–Fe_3O_4–TiO_2 and Al_2O_3–Fe_3O_4–Fe_2O_3 including iron oxide particles were tested. The authors investigated the influence of soluble components of particle-suspending solutions (phosphate, pyrophosphate, carbonate, borate, nitrate and sodium), ionic strength and buffer pH. Suspensions of iron oxide particles such as Fe_2O_3 (hematite) (0.3–0.8 μm, Polysciences Inc.) [4–6], Fe_3O_4 (1.0 μm, Polysciences Inc.) [4] and Fe_3O_4 (0.2 μm, Polysciences Inc.) [6] were prepared from the oxide powders. Sedimentation studies and visual observations of the oxide particle suspensions performed by the authors indicated that the oxide particles were not fully dispersed as singles, but rather were present as aggregates of varying and undetermined size. The suspensions of Fe_3O_4 used had aggregates that varied in size from those remaining dispersed for many hours to large visible aggregates that settled-out in just a few minutes. The experiments with 0.2 μm Fe_3O_4 suspensions have shown that large aggregates

---

[*] Corresponding author. Tel.: +49 341 235 2319; fax: +49 341 235 2625.
E-mail address: juergen.mattusch@ufz.de (J. Mattusch).

0039-9140/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2004.12.016

co-migrated with small aggregates and any single particles that were present. The observed independence of migration time on particle size for 0.2 μm Fe_3O_4 is consistent with theoretical expectations for particles in the micrometer size range. Thus, the size separation of 0.2–1.0 μm iron oxide particles has not been achieved under the conditions studied.

The aim of our investigation is to estimate the capabilities of CZE in size separation of iron oxide using one of a commercial oxide powder with a particle size range of 20–30 nm, where the dependence of migration time on particle size should be observed. The second goal is to study the effect of electrolyte type on iron oxide size distribution. In addition, we wish to find long-time stable compositions with relatively narrow size distributions for preparing reference samples.

2. Experimental

2.1. Materials and chemicals

A commercial γ-Fe_2O_3 powder (99+%; gamma; Alfa, Karlsruhe, Germany) with the nominal particle diameters 20–30 nm specified by the manufacturer was used. Standard suspensions of iron oxide nanoparticles were prepared by suspending the powder in a solution of composition matching that of the corresponding running electrolyte. The iron oxide concentration was 0.01% (v/v). Prior to each run the particle suspensions were ultrasonicated for 5 min to improve the dispersion.

Na_2SO_4, Na_3cit, and NaOH utilised for preparing the electrolyte solutions were of analytical reagent grade (Merck, Darmstadt, Germany). Deionised water (Milli-Q, Millipore Corp.) was used throughout all the experiments.

2.2. Instrumentation and procedures

A P/ACE^{TM} System MDQ (Beckman Instruments, Inc., Fullerton, CA) equipped with a diode array detector was used. The P/ACE System MDQ software configured on a Pentium computer workstation managed the control, collection, and analysis of experimental data. Uncoated fused-silica capillaries of 75 μm i.d. and 360 μm o.d. with a total length of 40.2 cm and an effective length of 30.0 cm were used. All separations were performed with the power supply set for positive polarity and at a constant voltage. The temperature was maintained at $25 \pm 0.1\,^{\circ}$C during all runs. Hydrodynamic injections of samples were performed at the anode end of the capillary. Deionised water was used as an electroosmotic flow marker. Triple injection was performed by successive injections of water (5 s), a running electrolyte (5 s), and a suspension of iron oxide particles in an electrolyte having the composition of the running electrolyte. Preliminary experiments showed that water injection does not influence the peak position. This procedure allowed us to check the electroosmotic flow rate in the sample run. The wavelengths used for detection ranged from 190 to 400 nm. The data rate was 4 Hz. The optical spectra of particles studied were found to have no maxima, and a gradual increase in absorbance was observed with decreasing the wavelength. This means that the highest peaks can be obtained on the electropherograms recorded at the lowest wavelengths. However, noticeable noise can appear in this wavelength range. Therefore, two wavelengths of 200 and 254 nm were selected for the detection of particle zones in electrophoretic runs.

The capillary was rinsed with 0.1 M NaOH (5 min) and then with water (5 min) at a pressure of 20 psi (1 psi = 6894.76 Pa) before the first daily experiment and after the last one. It was conditioned by rinsing with the running electrolyte (5 min) before runs. Vials were refilled with a fresh portion of the electrolyte after each run.

Transmission electron micrographs were taken using a Leo EM 912 Omega system operating with an Omega filter and at 100 keV. For analysis initial magnifications were between 10,000× and 100,000×. In the figures the initial magnifications are between 50,000× and 100,000×. The final magnifications are given by the bars.

3. Results and discussion

3.1. Injection

The effect of injection time and injection pressure on the peak height of iron oxide particles was studied. The peak height was a linear function of the injection time in the range from 5 to 20 s. Similar plots were obtained at injection pressures of 0.2 and 0.5 psi. Thus, any experimental conditions studied can be used for the hydrodynamic injection of weighty iron oxide particles. In most of runs the lowest injection pressure of 0.2 psi and the lowest injection time of 5 s were applied. Under these injection conditions the injected sample volume was about 15 nl and occupied not more than 1% of the total capillary volume.

Previous studies including the comparison of electropherograms from the runs, performed at the same positive or negative anode polarity in electrokinetic injection and separation steps, indicated that in all suspensions iron oxide particles were negatively charged. These results were in agreement with those reported in [4] where the isoelectric point (pI) equal to 5.0 was calculated for Fe_2O_3 in an indifferent electrolyte such as sodium nitrate solution using the two-site dissociation model.

3.2. Effect of electric field strength

The effect of the electric field strength on the peak position, peak height, and peak shape of iron oxide colloids was studied. The mixture 1.67 mM Na_2SO_4 + 1.00 mM NaOH, pH 10.8, was used as a running electrolyte. The electric field strength ranged between 125 and 500 V/cm. The peak position displacement was found to be corresponding to the

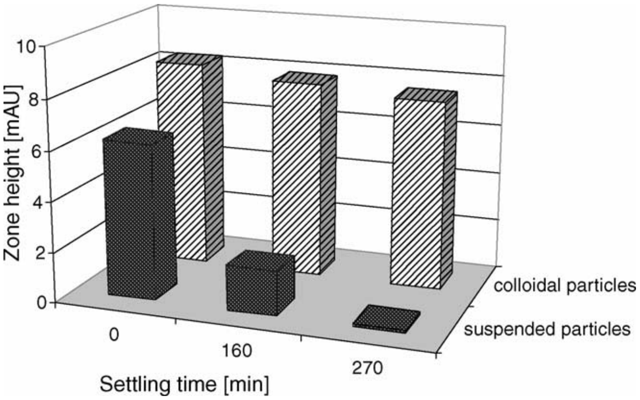

Fig. 1. The effect of settling time on the sample composition. Running electrolyte: 1.00 mM NaOH + 1.67 mM Na_2SO_4, pH 10.8; electric field strength 250 V/cm; current 8 μA; injection pressure 0.2 psi; injection time 5 s; wavelength 254 nm.

variation in the electric field strength. It means that the electrophoretic mobility of iron oxide particles was constant within the interval studied. Variations in peak heights and peak widths were in accordance with the migration time differences.

3.3. *Effect of electrolyte type*

3.3.1. *Na_2SO_4*

The 2.0 mM sodium sulfate concentration was selected. It is known that the lower ionic strengths provide more stable suspensions due to increasing the particle ζ potential. However, using the running electrolytes with sodium sulfate concentrations less then 2.0 mM leads to a noticeable irreproducibility of the electroosmotic flow rate which seems to be due to variations of the capillary wall ζ potential. Nevertheless, in contrast with water the suspension of iron oxide nanoparticles in 2.0 mM sodium sulfate solutions was unstable and fast particle sedimentation occurred in a few minutes.

3.3.2. *Na_2SO_4–NaOH*

Suspensions with a pH close to the pI value of iron oxide particles are expected to be unstable. With a pH increase, iron oxide particles become more negatively charged due to dissociation of surface groups and kinetically more stable suspensions should be formed. Therefore, an alkaline solution was used for suspending this kind of particles. The mixture 1.00 mM NaOH + 1.67 mM Na_2SO_4 at pH 10.8 was tested for electrophoretic runs. To compare the results the ionic strength of this electrolyte was kept the same as that of 2.0 mM Na_2SO_4 solution in the previous experiment and was equal to 6.0 mM. In the electropherogram of freshly prepared samples immediately after their ultrasonication, a broad peak with spikes appeared. During storage, partial particle sedimentation occurred (Fig. 1), and after 270 min only a single peak without any spikes was observed in the electropherogram (Fig. 2A). For these runs the sample injections were performed from the same level of the sample vial as that for the first one. For comparison, the electropherogram of particle sediment after its redispersal in the running electrolyte is also presented in Fig. 2B. In the counterelectroosmotic CZE mode used in our experiments iron oxide particles migrated to the detector due to the electroosmotic flow and their electrophoretic mobility was calculated as the difference between the electroosmotic mobility and the apparent particle mobility. The electroosmotic and electrophoretic mobility values were equal to $(96.13 \pm 0.7) \times 10^{-5}$ cm^2/V s ($n = 20$, $P = 0.95$) and $(-34.0 \pm 0.7) \times 10^{-5}$ cm^2/V s ($n = 14$, $P = 0.95$) respectively. For the spikes zone, the electrophoretic mobilities corresponding to the boundaries of intervals including the spikes were calculated. They are $(-21.6 \pm 0.3) \times 10^{-5}$ cm^2/V s ($n = 7$, $P = 0.95$) at the start position and $(-33.9 \pm 1.1) \times 10^{-5}$ cm^2/V s ($n = 7$, $P = 0.95$) at the end position. Thus, the mobility intervals calculated for two kinds of electrophoretic zones studied are wholly overlapped. It can be concluded, that the electrophoretic zone observed consists at least of two types of particles. One of them is thought to be the colloids being stable in the running electrolyte studied. The other one represents an unstable suspension of iron oxide nanoparticles in alkaline solution which can include both single particles and some aggregates.

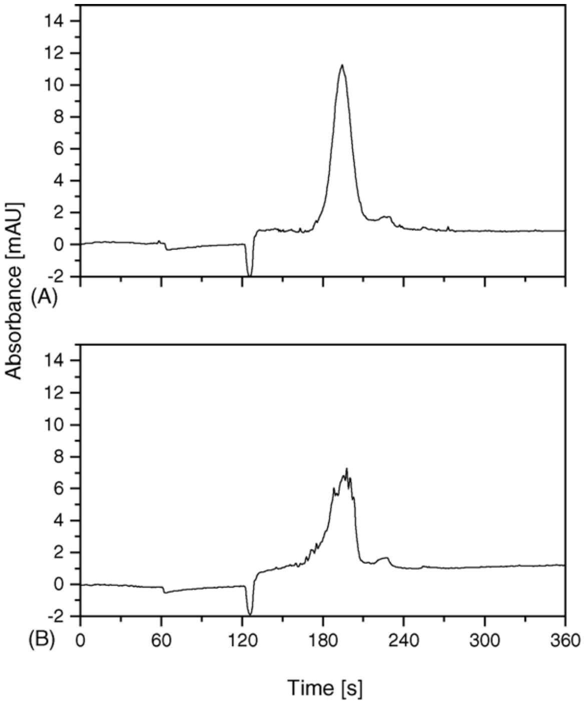

Fig. 2. The shapes of γ-Fe_2O_3 electrophoretic zones for different sample compositions. (A) Initial suspension after settling time of 270 min; (B) particle sediment after its redispersal in the running electrolyte wavelength 200 nm; other conditions as in captions for Fig. 1.

3.3.3. *Na_2SO_4–Na_3cit*

The addition of citrate anions to sodium sulfate solution was assumed to stabilize the iron oxide suspensions to a more or less extent and allowed us to perform electrophoretic runs

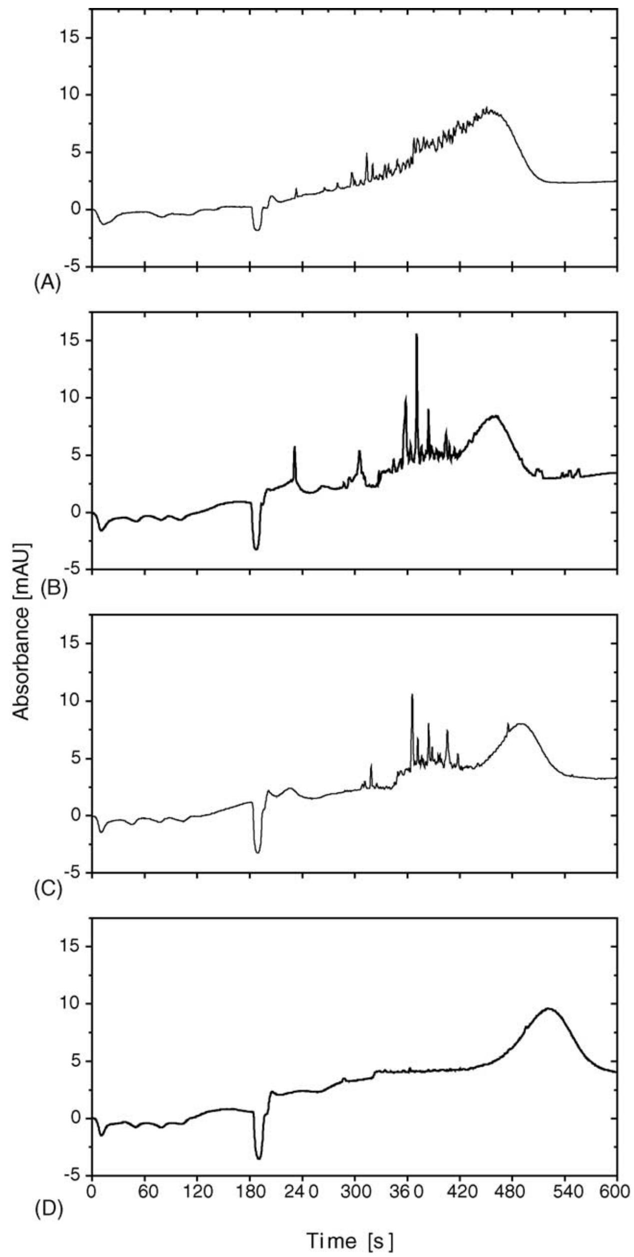

Fig. 3. The shapes of γ-Fe_2O_3 electrophoretic zones at different sample compositions. (A) Freshly prepared initial suspension; (B–D) settling times 50, 100, and 160 min, respectively. Running electrolyte: 1.0 mM Na_3cit + 2.0 mM Na_2SO_4, pH 7.1; current 11 μA; wavelength 200 nm; other conditions as in captions for Fig. 1.

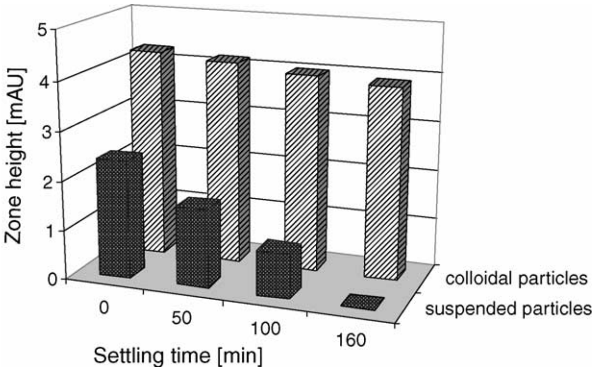

Fig. 4. The effect of settling time on the sample composition. Wavelength 254 nm; other conditions as in captions for Fig. 3.

in a neutral medium. The effect of sodium citrate concentration at pH 7.1 on the separation efficiency of iron oxide initial suspension was studied. In all the runs the ionic strength was maintained at 12.0 mM while changing the sodium citrate concentration between 0.5 and 2.0 mM. For freshly prepared samples after ultrasonication, a broad asymmetric peak independent of the citrate concentration used appeared in the electropherogram (Fig. 3A). As can be seen from Fig. 3A, there are numerous spikes on the left side of this peak contrary to the right one. During storage, when the particle settling occurred, the separation efficiency improved and the resolution of two electrophoretic zones was achieved. Some representative electropherograms are shown in Fig. 3B and C. For those runs the sample injections were performed from the same level of the sample vial. At last, after storage for 160 min only a single smooth peak without any spikes was observed in the electropherogram (Fig. 3D). The comparison of the

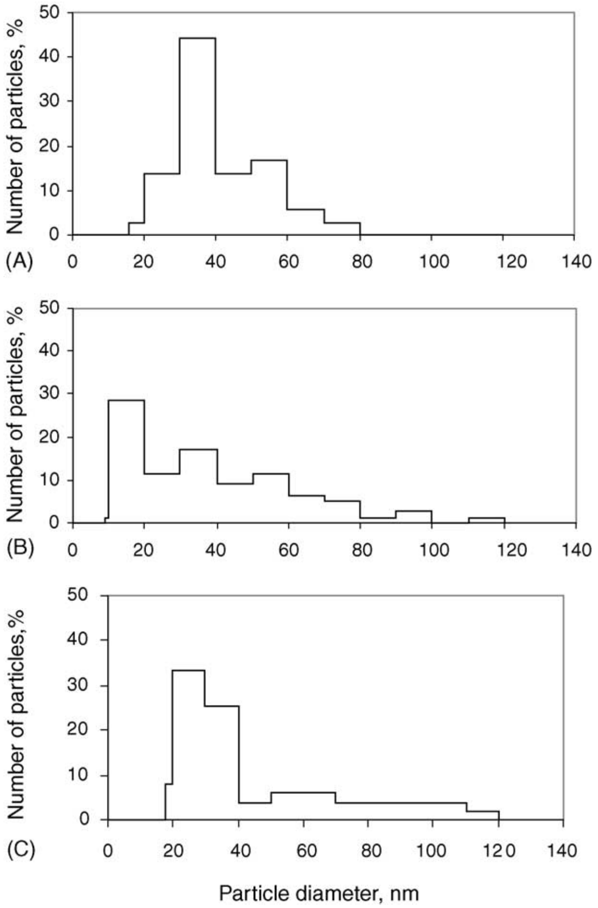

Fig. 5. The effect of dispersive medium on γ-Fe_2O_3 particle size distribution. (A) H_2O; (B) 1.00 mM NaOH + 1.67 mM Na_2SO_4, pH 10.8; (C) 1.0 mM Na_3cit + 2.0 mM Na_2SO_4, pH 7.1.

electropherograms presented allowed us to conclude that the height of the left electrophoretic zone gradually decreased in time down to its disappearance, while a peak appeared in the right part of the electropherogram the height of which was practically independent of the storage time. Its slight decreasing was probably due to partial overlapping with the neighbouring zone (Fig. 4). The calculated electrophoretic mobility of this peak as well as the electroosmotic mobility showed no noticeable dependence on the citrate concentration and were equal to $(-38.8 \pm 0.8) \times 10^{-5}\text{cm}^2/\text{V s}$ ($n = 10$, $P = 0.95$) and $(64.1 \pm 0.4) \times 10^{-5}\text{cm}^2/\text{V s}$ ($n = 17$, $P = 0.95$), respectively. The electrophoretic mobilities corresponding to the boundaries of intervals including spikes were calculated. They are $(-26.2 \pm 0.8) \times 10^{-5}\text{cm}^2/\text{V s}$ ($n = 10$, $P = 0.95$) at the start position and $(-36.9 \pm 0.6) \times 10^{-5}\text{cm}^2/\text{V s}$ ($n = 10$, $P = 0.95$) at the end position. Thus, the mobility intervals calculated for two kinds of electrophoretic zones studied are not overlapped. It can be concluded that the right electrophoretic zone arose from the colloids being stable in the running electrolytes studied. The left electrophoretic zone arose from the suspended iron oxide nanoparticles gradually settling in citrate solutions.

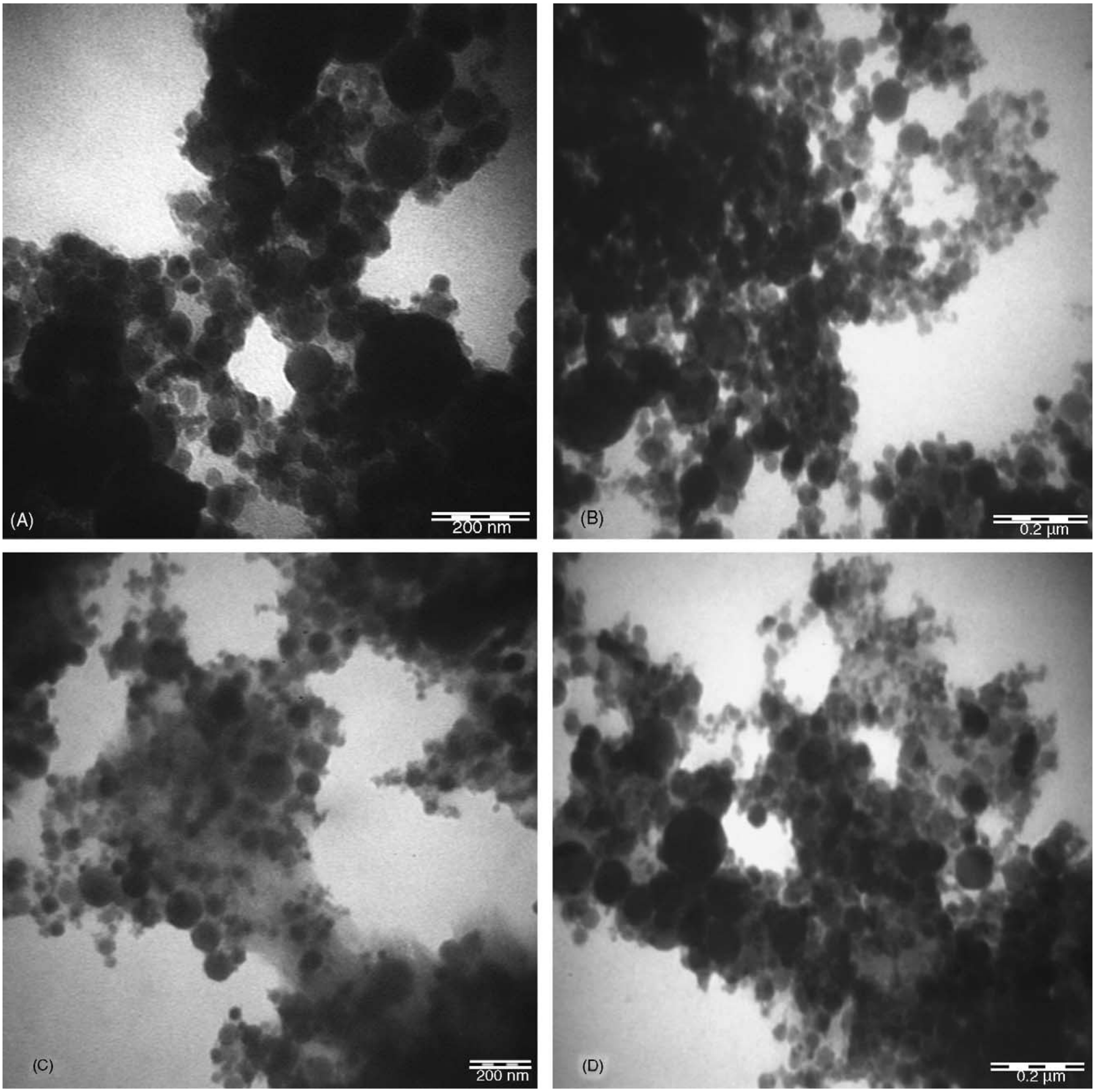

Fig. 6. Electron micrographs. Electrolyte: 1.00 mM NaOH + 1.67 mM Na_2SO_4, pH 10.8; (A) initial suspension; (B) colloidal solution; electrolyte: 1.0 mM Na_3cit + 2.0 mM Na_2SO_4, pH 7.1; (C) initial suspension; (D) colloidal solution.

3.4. Transmission electron spectroscopy (TEM) studies

The electron micrographs were obtained for initial suspensions in water and the electrolytes. The mixtures 1.00 mM NaOH + 1.67 mM Na_2SO_4 at pH 10.8 or 1.0 mM Na_3cit + 2.0 mM Na_2SO_4 at pH 7.1 were used. Size distributions based on the particle diameter measurements are presented in Fig. 5. The size distribution is shown to depend on the type of solution that served for dispersing the iron oxide powder. As can be seen, change of water for the electrolytes leads to differences in distributions. However, more than 90% of the particles are within the same size range as in the water suspension. Besides, iron oxide nanoparticles of largely spherical shape were observed (Fig. 6A and C). Consolidation and agglomeration of particles do not give noticeable contributions to size alteration. This fact allows us to think that the effects arising from the ionic strength increasing and changes in pH during the sample drying on grids before TEM measurements were insignificant. This conclusion is in agreement with the results published by Ledin and Karlsson for α-Fe_2O_3 [7].

The electron micrographs were also obtained for colloids in the electrolyte solutions. TEM images are presented in Fig. 6B and D. The corresponding size distributions are presented in Fig. 7. More than 95% of particle diameters ranged between 10 and 60 nm.

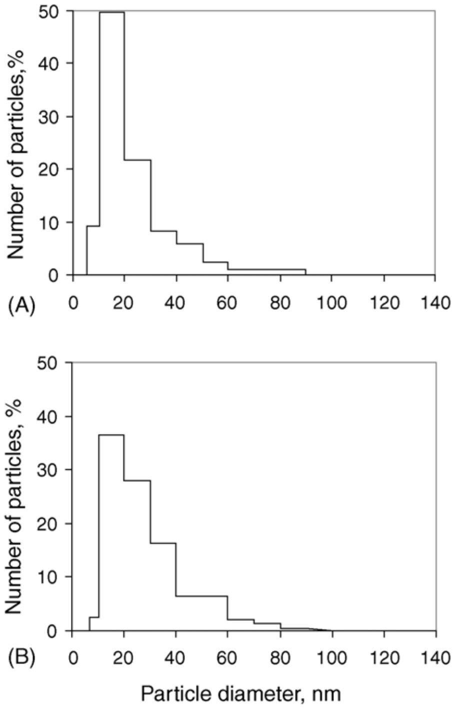

Fig. 7. Size distributions of colloidal solutions. Electrolyte: (A) 1.00 mM NaOH + 1.67 mM Na_2SO_4, pH 10.8; (B) 1.0 mM Na_3cit + 2.0 mM Na_2SO_4, pH 7.1.

4. Conclusions

The comparison of our results with those published by Quang et al. [4–6] showed that electrophoretic zones from iron oxide particles having spikes or without them can appear in electropherograms obtained in all electrolytes used. According to our results, the differences in the shape of these zones seem to arise because of the coexistence of suspended and colloidal iron oxide particles in the samples studied and are determined by the relative contributions from these two different kinds of particles. The comparison of two peaks in two different electropherograms from Fe_3O_4 presented by Petersen verifies this conclusion. The peak with spikes in one of the electropherograms was due to all Fe_3O_4 particles but the smooth peak in the other one was from only the particles that remained suspended for more than 60 min. Some amounts of small aggregates can co-migrate with single suspended particles in the electrophoretic zone, while large aggregates are too weighty and their fast settling occurs within a few minutes after sample preparation.

Up to now a positive electrophoretic mobility standard with the average particle size of 60 nm× 20 nm was developed by NIST. The standard reference material 1980 (SRM 198) contains 500 mg/L goethite (α-Fe OOH) suspension saturated with 100 μmol/g phosphate in 50 mM sodium perchlorate solution at pH 2.5 [8]. The specified mobility of the stock solution diluted 10-fold with water is $(25.3 \pm 1.2) \times 10^{-5}$ $cm^2/V\,s$ at 25 °C. In contrast to the standard described above iron oxide colloids which are stable in alkaline or neutral electrolytes have been obtained after sedimentation of the solids. They are negatively charged, form sharp electrophoretic zones and have particle size distributions ranging between 6 and 80 nm in citrate and alkaline solutions. Therefore, they could be used as a raw material for preparing reference samples.

Acknowledgements

We gratefully acknowledge the funding kindly received from the Saxon Ministry of Science and Art (SMWK), and the Russian Academy of Science.

References

[1] R.M. McCormick, J. Liq. Chromatogr. 14 (5) (1991) 939.
[2] J. Janca, S. Le Hen, M. Spirkova, J. Stejskal, J. Microcolumn Sep. 9 (1997) 303.
[3] N.G. Vanifatova, B.Ya. Spivakov, J. Mattusch, R. Wennrich, Talanta 59 (2003) 345.
[4] C. Quang, S.L. Petersen, G.R. Ducatte, N.E. Ballou, J. Chromatogr. A 732 (1996) 377.
[5] G.R. Ducatte, N.E. Ballou, C. Quang, S.L. Petersen, J. Microcolumn Sep. 8 (6) (1996) 403.
[6] S.L. Petersen, N.E. Ballou, J. Chromatogr. A 834 (1999) 445.
[7] A. Ledin, S. Karlsson, A. Duker, B. Allard, Analyt. Chim. Acta 281 (1993) 421–428.
[8] G.V.F. Seaman, R.J. Knox, Electrophoresis 22 (2001) 373–385.