

Influence of polymeric coating on capillary electrophoresis of iron oxide nanoparticles

Hadi Baharifar · Ali Reza Fakhari ·
Hakimeh Ziyadi · Mohammad Ali Oghabian ·
Amir Amani · Reza Faridi-Majidi

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Abstract In this work, electrophoresis was successfully used to separate three different polymer-coated magnetic iron oxide nanoparticles with similar sizes (nominally 50 nm) using high-pH borate buffer system. The coating polymers were dextran, polyethylene glycol, or carboxymethyl dextran. The results showed that the migration time of carboxymethyl dextran coated nanoparticles is the longest due to relatively more negative surface charges. Investigation of the effects of buffer concentration, pH, electric field strength and the capillary temperature, on electrophoretic properties of samples was also carried out. The results showed that pH, electric field strength and the capillary temperature had indirect relations with both of the migration time and the separation resolution of three different polymer-coated nanoparticles while the buffer concentration had a direct relation.

Keywords Capillary electrophoresis · Iron oxide nanoparticles · Polymer coated nanoparticles · Separation

H. Baharifar · A. Amani · R. Faridi-Majidi (✉)
Department of Medical Nanotechnology, School of Advanced
Medical Technologies, Tehran University of Medical Sciences,
Tehran, Iran
e-mail: r.faridimajidi@gmail.com

A. R. Fakhari
Department of Chemistry, Faculty of Sciences, Shahid Beheshti
University, Tehran, Iran

H. Ziyadi
Pharmaceutical Sciences Branch, Islamic Azad University,
Tehran, Iran

M. A. Oghabian
Research Center of Science and Technology in Medicine
(RCSTIM), Imam Khomeini Hospital Complex, Keshavarz
Blvd, Tehran, Iran

Introduction

Magnetic nanoparticles are inorganic materials usually iron oxide nanoparticles as a core coated by a layer of a synthetic polymer or a carbohydrate as a shell for enhancing their biocompatibility and circulation times in body [1, 2]. They can be also used in other applications such as medicine, sensors, etc. Various methods such as Co-precipitation [3], thermal decomposition [4], microemulsion [5] and hydrothermal synthesis [6] have been suggested to synthesize polymer-coated iron oxide nanoparticles. Because of the various applications of magnetic nanoparticles, separation, sizing, characterization and analysis of these nanoparticles are very important especially for medical applications [e.g. magnetic resonance imaging (MRI)]. There are some methods based on magnetophoresis for the separation of magnetic particles, generally microparticles, including high-gradient magnetic separation (HGMS) [7], quadrupole magnetic field flow fraction (FFF) [8] and on-chip free-flow magnetophoresis [9, 10]. All of these methods work based on the mobility of magnetic particles in an external magnetic field. These methods are affected by magnetic behaviors and sizes of particles so they are not effective for separation and analysis of small particles (i.e. nanoparticles) especially with the same sizes [11]. On the other hand, in capillary electrophoresis (CE), other parameters such as surface interactions (between surface of capillary and nanoparticles), surface charges and morphology of particles also affect the migration time of particles through the capillary. These conditions introduce CE as a powerful separation and analysis method for nanoparticles in wide area of conditions.

During recent years, a range of reports have used CE as a novel, rapid and effective separation method for organic, inorganic, metallic, polymeric nanoparticles like polystyrene

nanospheres [12] and gold nanoparticles [13]. This technique can also be used to separate nanostructured microorganisms like viruses and subviral particles [14]. As a result, CE has several advantages including obtaining rapid results, high efficiency and resolution, a very small amount of the required samples and simplicity of instrumentation [15] which are because of high number of theoretical plates [16]. Surugau and Urban [17] reviewed the progress in the application of electrophoretic techniques for the separation of various nanoparticles and compared this technique with other techniques like gel electrophoresis. They showed that CE is an accepted analytical technique and suitable for quality control and clinical analysis and may be readily used by nanotechnology professionals.

Reviewing the literature, most of the reports in the separation of nanoparticles by CE have focused on metallic nanoparticles such as gold and silver nanoparticles [13, 17–25] with only limited reports including the separation of polymeric nanoparticles like polystyrene and latexes [26–28]. Although there are few reports using CE in the analysis of iron oxide nanoparticles (γ -Fe₂O₃, hematite and magnetite) [29–32] as metal oxide inorganic nanoparticles, there are no remarkable studies describing the effect of polymeric coating on the separation of coated inorganic nanoparticles, based on literature review.

This paper, for the first time, describes the effects of four parameters of CE including the buffer concentration, pH, electric field strength and the capillary temperature, on the separation of iron oxide nanoparticles with different coating types including dextran (DEX), polyethylene glycol (PEG), or carboxymethyl dextran (CMDEX) having similar sizes.

Experimental

Materials and chemicals

Magnetic nanoparticles were gifted by Materials Science and Engineering Faculty of Sharif University in the aqueous suspension with the nominal particle diameter \approx 50 nm (Table 1) that had been measured by dynamic light scattering (DLS). The coating polymers of nanoparticles were DEX, PEG or CMDEX, Table 1.

All other chemicals were purchased from Merck Chemical Co. (Germany) and used without further

purification. Borate buffer was selected for analysis [33, 34] and prepared by adding 1.0 mM boric acid (H₃BO₃) solution to 1.0 mM sodium tetraborate (Na₂B₄O₇·10H₂O) solution to reach to a desired pH. It has been known that borate solution acts as a buffer among 7.4–9.2 pH values, however the used range in this study was among 8.0–10.0 pH values to investigate the pH effect on migration time even in the pH values out of the buffer range.

Instrumentation and procedure

All experiments were performed using Lumex Capel 105 automated CE instrument (Ohiolumex, Twinsburg, Russia) coupled with a UV detector (200–400 nm) equipped uncoated fused silica capillary (Ohiolumex) 75.0 μ m id.

For all runs, the capillary had an effective length of 50 cm and total length of 60 cm. Separations were performed under electric field strengths of 266.6–366.6 V/cm (16.0–22.0 kV).

To study the temperature effect, temperature was varied from 20.0 to 50.0 °C, in all other runs the temperature was maintained at 30.0 °C. Hydrodynamic injections were employed to inject the particle suspensions at the anode end of the capillary. Injection of the sample was performed throughout at a pressure of 30 mbar for 5 s (\sim 20 nL).

For all experiments the wavelength of the monochromator of the CE instrument was fixed at 390 nm and detected by the UV absorbance detector of the electrophoresis instrument.

At the beginning of each day, the capillary of the instrument was rinsed with 0.5 M of HCl (for 5 min) and deionized water (for 5 min), then it was conditioned by rinsing with 0.5 M of NaOH for 10 min and deionized water (for 5 min) and at the end the buffer was injected for 10 min to capillary. Each nanoparticles suspension was diluted by the buffer to reach the concentration of 500 ppm and injected in to the capillary after shaking for about 10 s. Between the runs, the capillary was rinsed with the buffer for 5 min. The volume levels in the anode and cathode vials were monitored and adjusted to prevent gravity flow during the sample run. At the end of each day the capillary was rinsed with deionized water and two ends of the capillary tube were kept on deionized water to prevent drying.

Results and discussion

Capillary electrophoresis of magnetic nanoparticles with different coatings

In this study, to show the effect of coatings on electrophoretic behavior, DEX, PEG and CMDEX coated magnetic nanoparticles were injected separately in to the

Table 1 Properties of nanoparticles used for separation studies

Possible phase	Coating polymer	Hydrodynamic size (nm)	Concentration (mg/ml)
Fe ₃ O ₄	DEX	58 \pm 11	2
Fe ₃ O ₄	PEG-6000	53 \pm 12	1
Fe ₃ O ₄	CMDEX	55 \pm 13	2

capillary under similar conditions (i.e. borate buffer with $\text{pH} = 9.0$, $C = 20.0 \text{ mM}$, $E = 16.0 \text{ kV}$, $t = 30.0 \text{ }^\circ\text{C}$). The electropherograms of these experiments are given in Fig. 1. The chemical structures of coating polymers used in this research are also shown in Fig. 1. As shown in this figure the migration times of these three samples are 5.3, 3.5 and 6.1 min, respectively. This shows that the migration time of nanoparticles with a relative more negative surface charges (i.e. CMDEX coated magnetic nanoparticles) is longer than those having less negative surface charges (i.e. DEX and PEG coated nanoparticles).

To understand the mechanism of this finding, one should consider the theory of CE. Based on this theory, in the pH

values above 3, silanol groups on the surface of the capillary (i.e. SiO^-) are created. In CE negative charge on the capillary wall creates double layer of charge. After that, the layer next to surface moves toward cathode which is called electro-osmotic velocity [35, 36]. Because of this condition, velocity of materials is affected by their charges, which means velocity order is $\mu^+ > \mu > \mu^-$. Thus, the migration time of nanoparticles having relative more negative charges (i.e. CMDEX coated nanoparticles) through the capillary is longer than others.

Then, a mixture of nanoparticles having equal portions of DEX, PEG and CMDEX coated magnetic nanoparticles was injected to the capillary. Figure 2 is the

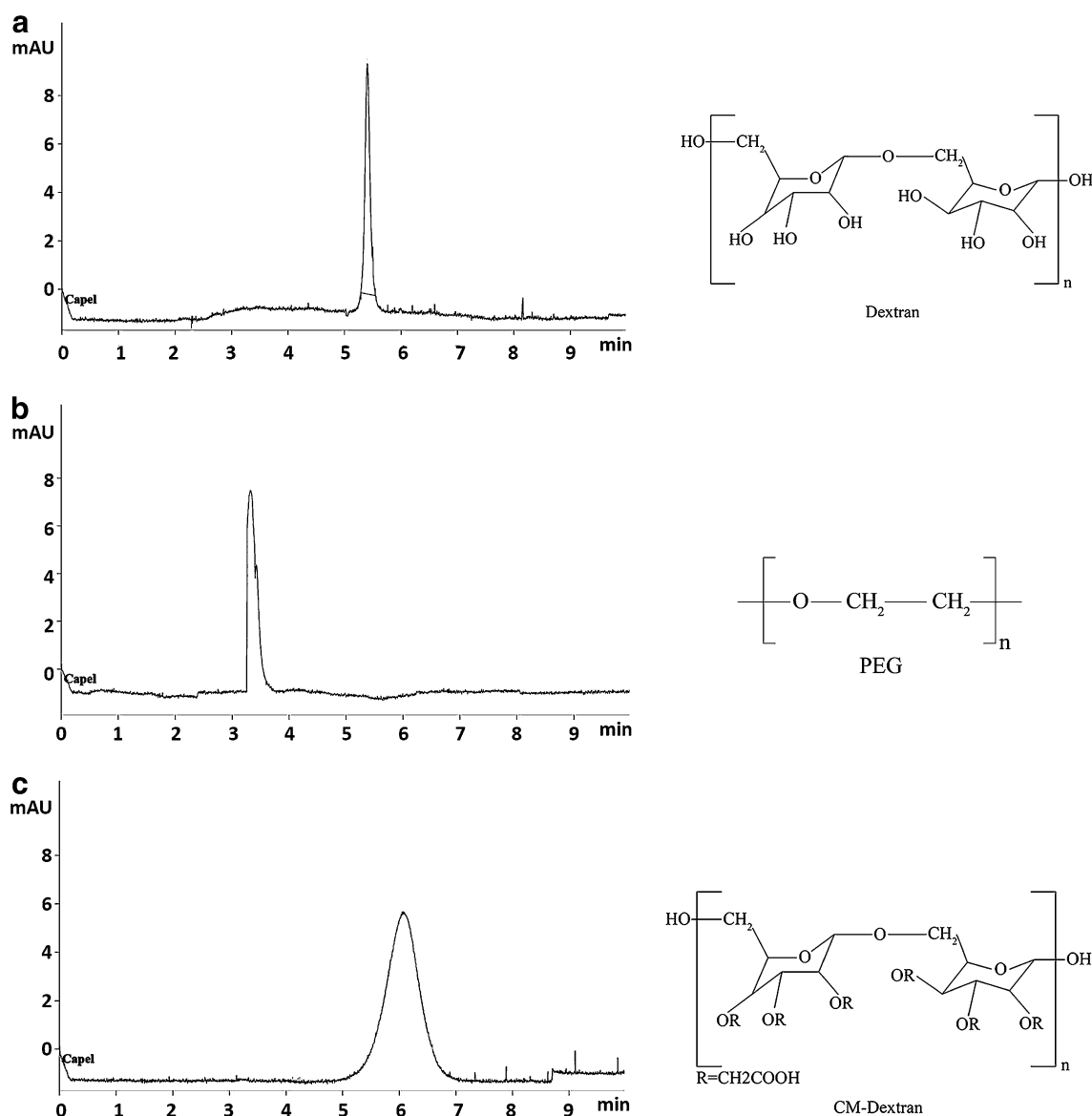
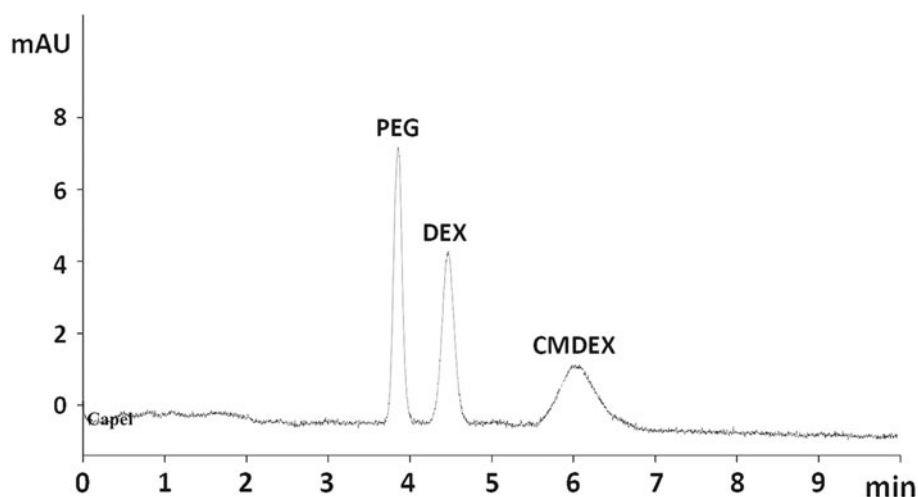


Fig. 1 Left **a** the electropherogram for DEX coated nanoparticles, **b** the electropherogram for PEG coated nanoparticles and **c** the electropherogram for CMDEX coated nanoparticles (analysis

condition: borate buffer, $\text{pH} = 9.0$, $C = 20.0 \text{ mM}$, $E = 16.0 \text{ kV}$, $t = 30.0 \text{ }^\circ\text{C}$. Right the chemical structure of DEX, PEG and CMDEX

Fig. 2 Separation of different coated nanoparticles injected simultaneously in borate buffer, analysis condition: pH = 9.0, C = 20.0 mM, E = 16.0 kV, t = 30.0 °C



electropherogram of this experiment and shows that the migration times of DEX, PEG and CMDEX coated magnetic nanoparticles are 4.6, 3.9 and 5.9 min, respectively. A comparison of Fig. 2 with Fig. 1 concludes that the migration time of each different coated by nanoparticle is slightly affected in the presence of the other coated nanoparticles.

Effect of pH

One of the most important factors on electrophoretic mobility and the electropherogram shape is pH. Based on the literature review, the considerable variation on electrophoretic properties occurs via variations of pH especially out of the buffer range and an increase in pH value increases the electro-osmotic mobility and so decrease in migration time [35, 36]. In this work, the effect of pH of borate buffer (in the pH range from 8 to 10) on CE of a mixture of nanoparticles coated with DEX, PEG and CMDEX was studied. Five CE experiments were performed to investigate the effect of pH on the migration times. As Fig. 3 shows, both migration time and resolution of separation decrease in higher pH values and a value of pH in the range 7.4–9.2 shows an acceptable separation which is in agreement with other studies [27, 28].

Effect of the electric field strength

The effect of electric field strength on migration times and separation quality was studied for a mixture consisting iron oxide nanoparticles coated with DEX, PEG and CMDEX. For this purpose, four CE experiments were done at four electric fields 16.0, 18.0, 20.0 and 22.0 kV (266.6, 300.0, 333.3 and 366.7 V/cm, respectively) using a mixture of the three types of the nanoparticles of which representative data are shown in Fig. 4. As the figure shows, the migration

times can be varied by changing the strength of the electric field and an increase in electric field strength causes a decrease in migration times of three types of nanoparticles. These results are based on the theory of electrophoresis [35, 36]. As it can be observed from Fig. 4 separation resolution of the mixture of nanoparticles in lower electric field strength is better than in higher ones. So, the electric field strength of 16.0 kV can be suitable for separation.

Effect of the buffer concentration

Based on the theory of CE, an increase in buffer concentration yields ion traffic in the capillary and therefore increases the migration time. So to get an acceptable electropherogram (and/or separation), one needs to find the best buffer concentration [35, 36]. To achieve the suitable range of buffer concentration, a few experiments were performed in this work and separation of a mixture of DEX, PEG and CMDEX coated nanoparticles were carried out at 20.0, 40.0, 60.0 or 80.0 mM borate buffer as a series of experiments. Results are shown in Fig. 5. It is shown that increasing the buffer concentration from 20.0 to 80.0 mM considerably increases the migration times (i.e. decreases mobilities) of the three different types of nanoparticles and also enhances the resolution of separations. The reason for this phenomenon is decreasing electro-osmotic mobility consistently by increasing buffer concentration [29]. Among the used buffer concentrations, the highest quality of separation appears at the highest buffer concentration. However, it needs more time to separate nanoparticles because of higher migration time than others. Although the resolution was the highest in 80.0 mM of buffer concentration, 20.0 mM of concentration buffer may be a better system for rapid separations of the nanoparticles as shown in Fig. 5 while an acceptable resolution is observed for this system.

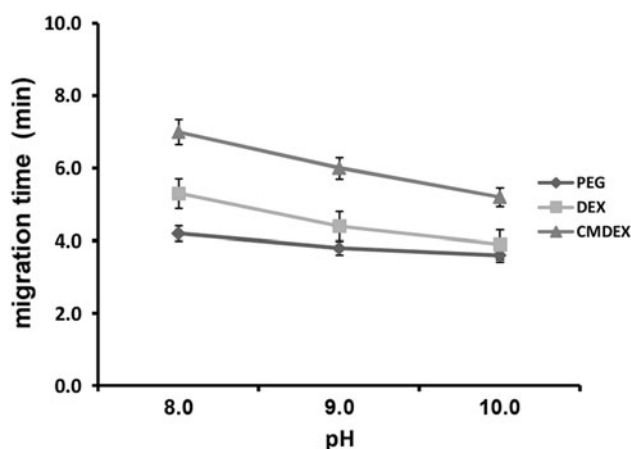


Fig. 3 Effect of pH on electrophoretic separation of mixture of DEX, PEG and CMDEX in borate buffer, analysis condition: $C = 20.0$ mM, $E = 16.0$ kV, $t = 30.0$ °C

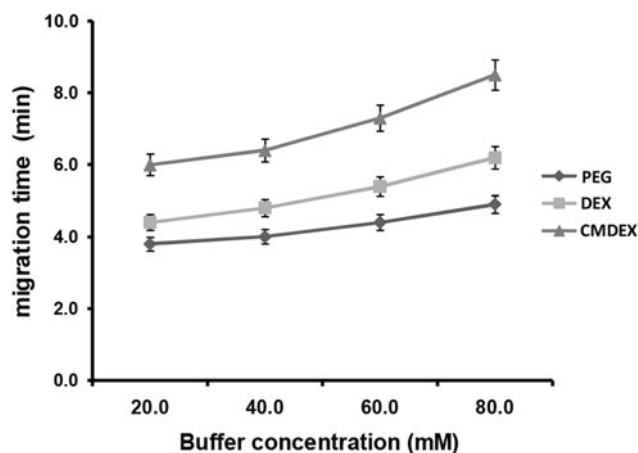


Fig. 5 Effect of buffer concentration on electrophoretic separation of mixture of DEX, PEG, and CMDEX in borate buffer, analysis condition: pH = 9.0, $E = 16.0$ kV, $t = 30.0$ °C

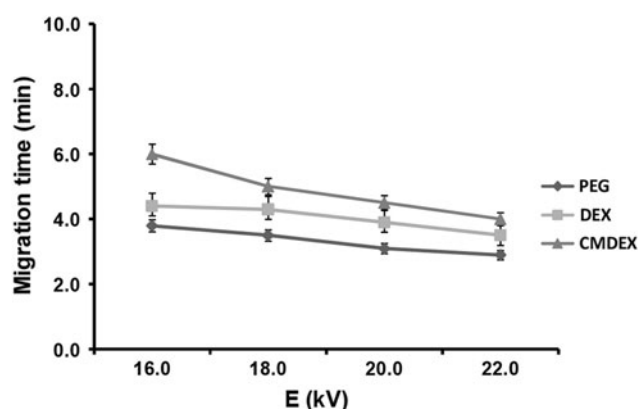


Fig. 4 Effect of electric field strength on electrophoretic separation of mixture of DEX, PEG, and CMDEX coated nanoparticles in borate buffer, analysis condition: pH = 9.0, $C = 20.0$ mM, $t = 30.0$ °C

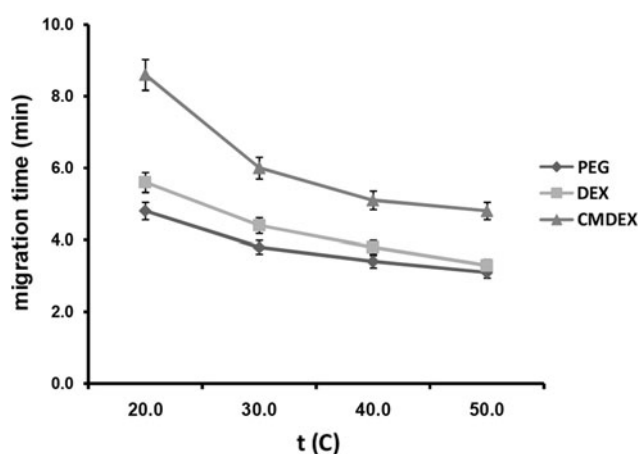


Fig. 6 Effect of temperature on electrophoretic separation of mixture of DEX, PEG, CMDEX in borate buffer, analysis condition: pH = 9.0, $C = 20.0$ mM, $E = 16.0$ kV, $t = 20.0$ – 50.0 °C

Effect of the capillary temperature

Influence of temperature variation has been rarely studied on CE separation [35]. The impact of temperature of capillary on the electrophoretic behavior of a mixture of iron oxide nanoparticles coated with DEX, PEG and CMDEX was investigated in this study. For this purpose, in a series of experiments, the temperature was changed from 20 to 50 °C.

The electropherograms of these experiments are given in Fig. 6. As the figure shows in higher temperatures, migration time of the nanoparticles decreases compared to lower temperatures. As reported previously, increasing temperature decreases buffer viscosity and increases particle mobilities which is the reason for decrease in migration time [35]. On the other hand, increasing temperature broadens the peaks of the electropherogram and also decreases the resolution of separation significantly, as

shown in Fig. 6. Based on these results, we can conclude that in lower temperatures, the resolution is better than that of higher temperatures but peak weight and migration time are high. Thus, the mid-range temperatures (i.e. 30 °C) seem to be more useful for the separation of used nanoparticles.

Conclusions

This study showed that CE can be used to separate and analyze the different polymer (i.e. DEX, PEG, or CMDEX) coated iron oxide nanoparticles with approximately similar sizes (nominally 50 nm). Among the coated nanoparticles, CMDEX coated nanoparticles showed the longest time of migration due to relatively more negative surface charges. The investigation of the effects of buffer concentration, pH,

electric field strength and the capillary temperature, on electrophoretic properties of samples also showed an indirect relation of pH, electric field strength and capillary temperature with both migration time and resolution of separation. In addition, a direct relation between the buffer concentration and the two responses was observed.

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