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Determination of citrate released from stabilized gold nanoparticles by capillary zone electrophoresis

Jana Horská[1] · Juraj Ševčík[1] · Jan Petr[1] 🔘

Abstract In this article, we report a pilot study on release of citrate ions from citrate-stabilized gold nanoparticles by capillary zone electrophoresis. First, a method for determination of citrate released from nanoparticles was developed using 5 mM phthalate buffer pH 6.5 with polybrene capillary inner wall coating. Within these conditions, citrate migrated in 1.8 min and it can be determined with LOD of 7 µmol/L using molybdate as the internal standard. The release of citrate was initiated by addition of common MOPS buffer ions used in capillary electrophoresis to nanoparticles samples. Non-linear behavior was found that proves release of citrate from stabilized gold nanoparticles. The release is initiated when 5 mM MOPS is added to nanoparticles' solution. This behavior can partially explain zeta potential change of the nanoparticles from –34 mV in bulk solution to –28 mV in 50 mM MOPS.

Keywords Capillary zone electrophoresis · Citrate release · Gold nanoparticles · MOPS · Stabilization

Introduction

Nanoparticles (NPs) used to be stabilized during synthesis to prevent their fast aggregation. The most used stabilization agent is citrate (citric acid), mainly for silver and gold NPs. It forms negatively charged layer on the NPs surface that causes electrostatic repealing of NPs. The same principle is used in other NPs where the stabilization is, e.g., due to covering the surface with silica shell containing NH_2 groups. Here, the electrostatic forces between ionized NH_2 groups (NH_3^+) causing the stabilization, too (Butt and Kappl 2010). However, citrate, in the case of silver and gold NPs is not covalently bonded to the NPs surface. This leads to the very important effect of those NPs; if they are inserted into a background solution containing different co-ions, they are changing their zeta potential and overall behavior in< solutions (Illés and Tombácz 2006). This effect is highly pronounced in the zetametry, dynamic light scattering and also in capillary electrophoresis (CE).

CE is well known tool for characterization of NPs (Radko and Chrambach 2002; Trapiella-Alfonso et al. 2016a, b). CE was already employed for analysis of silver and gold NPs (Pyell et al. 2015a, b; Liu et al. 2005; Liu 2007, 2011), maghemite core–shell NPs (d'Orlyé et al. 2009; Petr et al. 2010; Baron et al. 2017a, b), or quantum dots (Vicente and Colón 2008; Hu et al. 2013; Voracova et al. 2015). Interestingly, Cacho et al. (Cacho et al. 2014) studied electrophoretic behavior of carboxylic core–shell NPs. Here, different electrophoretic behavior of NPs was observed when they were presented in acetate buffer pH 4.5 and in borate buffer pH 9.5. Different mobilities of NPs were reported when using also citrate buffer pH 4.5 and acetate buffer pH 4.5. It seemed that the change of "ionic corona" of NPs due to the presence of different ions in buffer led to different mobilities of NPs (zeta potentials of NPs). This can hypothetically answer why different zeta potential values used to be obtained for the same NPs dispersed in different buffers.

Generally, the change of NPs' background solution composition can have also the effect on CE

✉ Jan Petr
   secjpetr@gmail.com

[1] Regional Centre of Advanced Technologies and Materials, Department of Analytical Chemistry, Faculty of Science, Palacký University in Olomouc, 17. listopadu 12, 77146 Olomouc, Czech Republic

characterization of NPs. CE reflects not only the ionic behavior of analytes (NPs), but also it reflects background electrolyte composition in the separation mechanism (e.g., in the electrophoretic dispersion, etc.). This can be very problematic for physical–chemical characterization of NPs or when analyzing NPs with unknown structure. Hence, we report here a study of release of citrate ions from citrate-stabilized gold NPs as model NPs by capillary zone electrophoresis. The release was initiated by addition of common buffer ions used in capillary electrophoresis—3-(N-morpholino)propanesulfonic acid (MOPS).

In the view of separation of small ions, CE is well known which is documented by many articles which focus their attention on choice of suitable background electrolytes including electro-osmotic flow (EOF) modifiers and UV—absorbing probe for indirect UV detection. Usually, the EOF modifier includes long chain alkyltrimethylammonium salts like cetyltrimethylammonium bromide (CTAB) that is added to the electrolyte and adsorb dynamically on the inner wall of the capillary (Raber and Greschonig 2000; Wang et al. 2003; Negro et al. 2003). Another question is the indirect UV detection. Here, UV absorbing species (referred to the probe ions or co-ions) are added to the background electrolyte (BGE). Then, the detection is accomplished as a result of displacement of these UV probe ions by the analyte ions. The probe must have the same charge as the analytes and for the best results the mobility of the probe and the analytes must be equal, in other case the peaks of analytes have fronted or tailed profiles (Doble and Haddad 1999; Naidu and Chen 2001). A wide variety of UV absorbing ions can be used as the probe in the BGEs, e.g., sodium chromate (Doble et al. 1997), pyromellitic acid (Yang et al. 1999), benzoate, phthalate, or sulfobenzoate (Shamsi and Danielson 1994).

Experimental

Chemicals

Electrolyte components: phthalic acid, sodium hydroxide, cetyltrimethylammonium bromide (CTAB), dodecyldimethyl-ammonium bromide (DDAB) and polybrene (PB) were purchased from Sigma Aldrich (St. Louis, MO, USA). The analyte standards: ammonium molybdate, MOPS, and citric acid were bought also from Sigma Aldrich. All the chemicals were of analytical grade purity. Gold nanoparticles (20 nm) stabilized by citrate were purchased from Sigma Aldrich, too. Stock standard solutions of all analytes were prepared at a concentration of 1 mg/mL in deionized water (18 MΩcm, MilliQ, Millipore) and were appropriately diluted in deionized water for preparation working solutions. Buffer solutions were prepared by dissolving the appropriate amount of an acid in deionized water; the pH was adjusted to the desired value by 50% (v/v) sodium hydroxide in deionized water.

CE apparatus and conditions

Electrophoretic measurements were performed on HP 3DCE system equipped with a DAD detector (Agilent Technologies, Waldbronn, Germany) using 50 μm I.D. bare fused-silica capillaries (Polymicro Technologies, Phoenix, USA) with 33.5 cm total length and 25.0 cm effective length; hydrodynamic injection time of 5 s by 50 mbar and voltage of −15 kV were used in these experiments. The capillary cassette was thermostated at 25 °C.

The capillary was rinsed every day, before the initial experiment, with 0.1 mol/L NaOH (10 min), deionized water (20 min), polybrene (20 mg/mL in water; 30 min) and then with the running buffer (10 min). Polybrene was used for the coating of capillary inner wall. Before each analysis the capillary was rinsed with 0.1 mol/L NaOH (2 min), deionized water (3 min), and then with the buffer (5 min). All the rinsing was done under a pressure drop of 935 mbar. All of the measurements were performed three times unless stated otherwise.

Results and discussion

Determination of citrate by capillary zone electrophoresis

The task of determination of citrate released from NPs needs also to separate MOPS that will be used as a releasing agent. Moreover, it should migrate slower than citrate since MOPS will be added to NPs in big excess. Molybdate was chosen as an internal standard since its presence in NPs samples is not probable; also its spectral and electrophoretic properties are adequate for its use (Chovancek et al. 2004).

First, electrolyte systems for indirect UV detection were studied. Phthalate was chosen as the UV absorbing probe since it has the closest mobility to the main analyte—citrate [estimated by the Peakmaster software using the ionic strength correction (Jaroš et al. 2004, echmet.natur.cuni) for pH 5.5; phthalate has the mobility of -37.5×10^{-9} $m^2\ V^{-1}\ s^{-1}$ and citrate has the mobility of -46.2×10^{-9} $m^2\ V^{-1}\ s^{-1}$]. In the next experiments, we tested various conditions to obtain the best separation of target analytes. First, the effect of concentration of the probe co-ion, phthalic acid, (1–30 mM) titrated by NaOH at pH 5.5 was studied (polybrene was used for coating the capillary). The best separation of all the analytes was observed for

concentration of 5 mM. Higher concentrations did not provide any separation. Then, the effect of EOF modifier was studied. We chose three EOF modifiers: CTAB and DDAB (both 10 mg/mL), which were presented directly in the electrolyte, and PB which was used for coating of the capillary inner wall prior to the analysis. The best results (in the view of repeatability of migration times) were achieved with PB. Finally, the effect of pH was evaluated with sodium as the counter-ion. The pH of 4.5; 5.0; 5.5; 6.0; 6.5 and 7.0 was tested using NaOH solution for adjustment of the pH. The best separation according to the peaks' resolution and shape was observed at the pH 6.5. This pH is also favorable supposing that the addition of MOPS to the sample will increase its peak area and citrate should be still determined without any interference. Here, according to the Peakmaster software using the ionic strength correction, citrate has mobility of -53.0×10^{-9} m^2 V^{-1} s^{-1} while MOPS ions have lower mobility (-4.6×10^{-9} m^2 V^{-1} s^{-1}). Under optimal conditions, CZE was performed using 5 mM phthalate/NaOH at pH 6.5 with the same results, as predicted by the Peakmaster. Basic validation parameters according to the ICH guidelines of the method are displayed in Table 1. As can be seen, the

Table 1 Validation parameters of the method

Parameter	Value
Migration time	1.8 min
Intraday precision	
Migration time, RSD	0.6%
Peak area, RSD	2.8%
Interday precision	
Migration time, RSD	1.5%
Peak area, RSD	4.5%
Recovery	96.4%
Calibration equation	$y = 8470\ (27)\ x + 12\ (5)$
Correlation coefficient	0.9978
LOD	0.7×10^{-5} mol/L
LOQ	2.2×10^{-5} mol/L

Molybdate was used as an internal standard; intraday precision was measured from 5 successive measurements; interday precision was measured from 9 successive measurements done in 3 days (each day 3 measurements); precision was measured using citrate concentration of 1×10^{-4} mol/L; recovery was measured using addition of citrate at concentration level of 5×10^{-4} mol/L to gold NPs analyzed before; the increase of peak area was used to calculate the recovery; calibration was done using range of 1×10^{-5} mol/L to 1×10^{-3} mol/L; estimation of calibration equation coefficients is displayed with standard deviations in parentheses; LOD and LOQ was estimated as 3 and 10 S/N value, respectively, using QC.Expert statistical software. The signal-to-noise (S/N) value was calculated directly by the acquisition and data analysis software of the CE instrument (ChemStation software)

method can be used for the study of the amount of citrate released from gold nanoparticles.

Determination of citrate released from gold nanoparticles

Commercial gold nanoparticles (20 nm) stabilized by citrate were used as model nanoparticles. These NPs have different zeta potential in bulk solution (-34 ± 2 mV) and in electrolytes traditionally used for CE analysis, e.g., -11 ± 1 mV in 50 mM acetate pH 4.5, -28 ± 2 mV in 50 mM MOPS pH 7.5 or -77 ± 3 mV in 50 mM borate pH 9.5 (all the data measured by Malvern Zetasizer). The migration of these NPs in that electrolytes is related to their zeta potential. The NPs are adsorbed into the capillary when using acetate as the BGE; in MOPS, the NPs' peak is not reproducible (shape as well as migration time) similarly as that in the borate electrolyte. Traditionally, gold NPs are analyzed by CE with addition of SDS where the zeta potential increases dramatically (-61 ± 3 mV in 50 mM MOPS pH 7.5 with 50 mM SDS). The changes in the zeta potential described above are probably due to the ligand/ion-exchange equilibrium where the citrate ions are exchanged by other ions from the solution as noted also by recent publications (Park and Shumaker-Parry 2015; Dinkel et al. 2016).

To study this effect, we added different amounts of MOPS to NPs' samples; equilibrate them for 30 min (off-line) and then quantified the amount of citrate released from the NPs by the capillary zone electrophoresis method developed before. An example of CZE analysis of nanoparticles is given in Fig. 1.
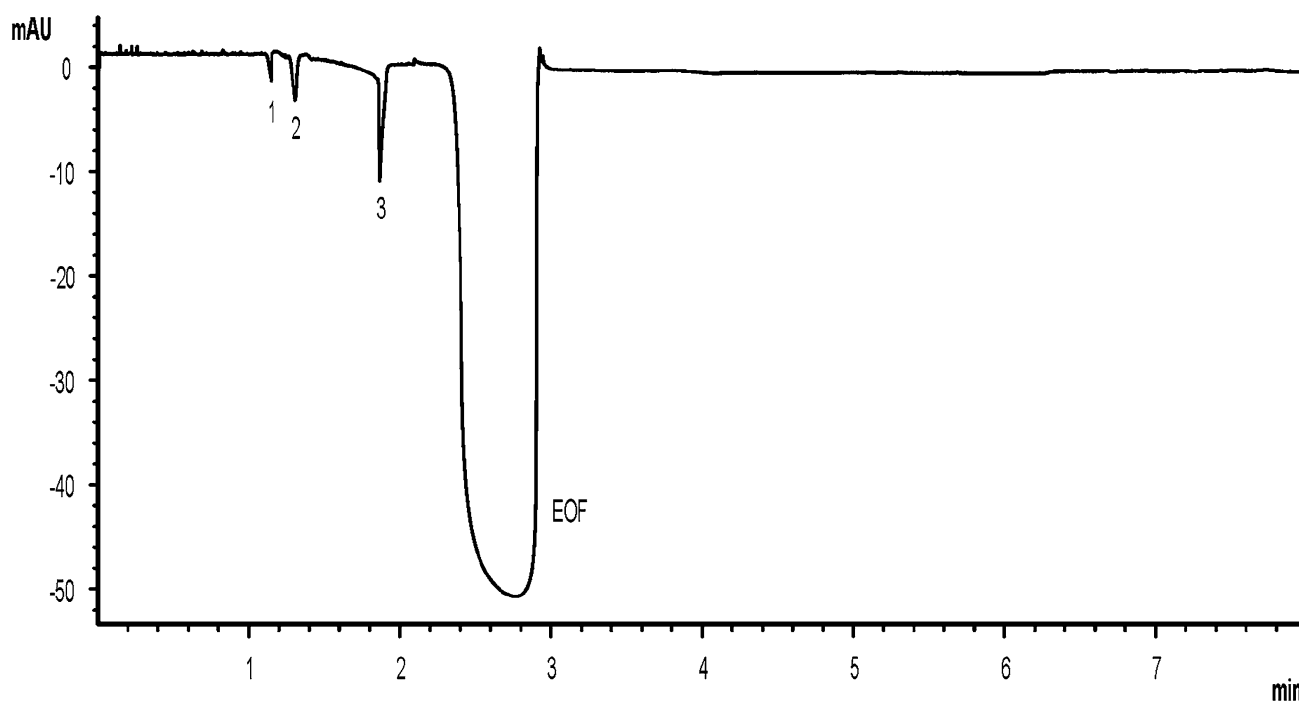
The dependency of citrate peak areas on concentration of MOPS is a non-linear curve (see an example in Fig. 2). The citrate peak area was constant till 5 mmol/L MOPS was added; then the peak area increased linearly (R^2 0.9983) up to addition of 0.3 mol/L MOPS where the NPs' surface was probably fully covered. It should be noted that the surface was probably not fully covered by the MOPS ions itself, but the coverage included MOPS as well as citrate ions which were not fully eliminated, as described by Park and Shumaker-Parry (2015).

Mathematically, we can consider the ion-exchange equilibrium:

$$n \equiv / - \text{Cit}^{3-} + m\ \text{MOPS}^- \leftrightarrow m$$
$$\equiv / - \text{MOPS}^- + n\ \text{Cit}^{3-},$$
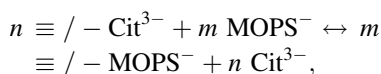
where the symbol " $\equiv/-$ " stands for the ion bounded onto the NPs; and the equilibrium constant that can be expressed from the equation:

$$K_c = \frac{[\equiv / - \text{MOPS}^-]^m [\text{Cit}^{3-}]^n}{[\equiv / - \text{Cit}^{3-}]^n [\text{MOPS}^-]^m}$$

Fig. 1 An example of CZE analysis of citrate in NPs sample. BGE: 5 mM phthalate/NaOH at pH 6.5; voltage: −15 kV; peak assignment: 1, molybdate; 2, citrate; 3, MOPS
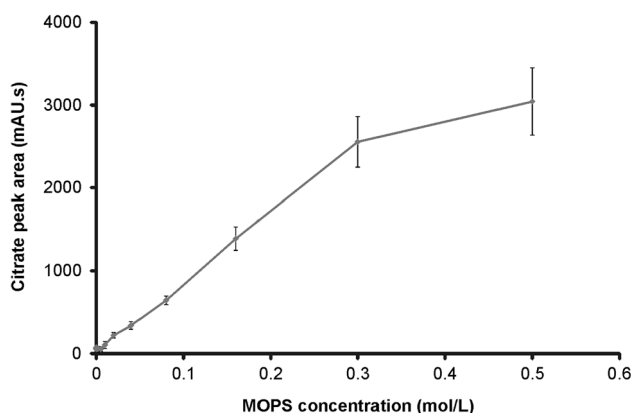
Fig. 2 An example of dependence of citrate peak areas on concentration of MOPS added

CE is well known as a tool that allows determination of equilibrium constants (Yokoyama et al. 2001; Michalcová and Glatz 2016; Neaga et al. 2016). However, there is a discrepancy with the basic theory of ion-exchange equilibrium where the number of charge exchanged during the ion-exchange reaction is keeping constant (three molecules of $MOPS^-$ should exchange one molecule of Cit^{3-}).

Within the NPs, the addition of NPs stabilized with citrate into the MOPS buffer leads to the change in zeta potential and therefore, it also leads to change of their surface charge (and therefore the number of $MOPS^-$ and Cit^{3-} molecules exchanged does not directly correlate with

the charge). It means that within this equilibrium, the ion-exchange equilibrium theory is not applicable. Moreover, there can be also steric effects because the ion-exchange takes place onto the surface of NPs and the sizes of citrate and MOPS ions are similar. Therefore, the data cannot be analyzed to provide rough comparison of nanoparticles and their release of citrate.

But, it is visible (Fig. 2) that citrate is released from NPs when added to the MOPS buffer. This is probably the reason why citrate-stabilized gold NPs changes its surface charge (zeta potential) from −34 ± 2 mV (bulk solution) to −28 ± 2 mV (50 mM MOPS), measured by Malvern Zetasizer. Moreover, it also reflects the effect that these NPs are not stabilized covalently and hence their behavior, e.g., in the environment or in the body can be very different than that in water.

Conclusion

In this article, we report a pilot study on release of citrate ions from citrate-stabilized gold NPs by capillary zone electrophoresis. The release was initiated by addition of common buffer ions used in capillary electrophoresis (MOPS) since we studied electrophoretic behavior of these NPs before and it seemed that the addition of the buffer ions changes ionic properties of these NPs. During our current study, we found that the NPs are releasing citrate

ions when presented in MOPS buffer. This is very important for general characterization of NPs by CE because only addition of NPs into BGE changes their "ion corona" and therefore, their behavior. This can be also very interesting for biological applications of NPs where "stabilized"-NPs are loaded into cells. It seems that the contact of NPs with proteins, small ions, etc., inside the cells can affect charge properties and overall behavior of NPs. This can also lead to the question if the targeted synthesis of NPs can be successful if the cellular environment totally changes behavior of NPs.

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