INVESTIGATION METHODS

Conductometric and Potentiometric Titration of Carboxyl Groups in Polymer Microspheres

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Abstract—A laboratory procedure has been suggested for determining the concentration of carboxyl groups at the surface of polystyrene microspheres at the latex concentration of a few hundredths of weight percentage (the COOH groups concentration below 4×10^{-4} mol/L). The procedure is based on conductometric and potentiometric titration. The influence of polymer particles concentration in the system, ionic strength of the solution, titration duration, and the system inertness on the obtained values of the carboxyl groups concentration and the determination accuracy have been investigated.

INTRODUCTION

Procedures for the synthesis of polymer particles differing in size (several nanometers to hundreds of micrometers [1]), shape [2] (from absolutely smooth spheres to heart-shaped rough particles [3]), morphology (macroporous, mesoporous, microporous [4], hollow [5], Janus particles [6]), and composition (polystyrene [7], poly(methyl methacrylate) [8], poly(N-isopropylacrylamide) [9], poly(divinylbenzene) [10], etc.) have been actively developed over the past three decades. The wide variability in the properties of polymer particles determines the variety of their applications: from paint and varnish industry to drug delivery systems [11, 12]. The use of polymer microspheres as carriers in express diagnostics has become more prevalent in clinical diagnostic laboratories. This advancement has enabled the rapid diagnostics of infectious diseases, diabetes, cardiovascular diseases, inflammations of various nature, oncology, etc. [13–15]. Application of polymer particles is particularly attractive due to the ease of immobilization of antibodies, antigens, DNA and RNA fragments on them via the interaction of the latter with functional groups ($-COOH$, $-NH_2$, and $-CH_2Cl$) at the polymer particles surface. The binding efficiency is determined by the functional groups nature, their surface density, and availability. In the present study, the focus will be on microspheres bearing carboxyl groups, due to their universality and the widest spread among test systems.

Not all functional groups on the surface of the particle can actively bind to peptides, antibodies, proteins, and other large molecules, due to steric hindrance. Fluorescent labels have been widely used to determine the concentration of the functional groups available in a particular method [16]. In turn, a variety of fast and accurate methods have been suggested to determine the total concentration of surface groups [17]. J. Dai et al. have developed a complexometric method based on the interaction of Fe^{2+} with carboxyl groups [18]. A similar method utilizing a colorimetric approach has been developed by A. Hennig et al. [19]. This method involves determining the concentration of free (not bound to the target functional groups) ions of divalent transition metals (Ni^{2+}, Co^{2+}, and Cd^{2+}) using Pyrocatechol Violet. In addition, direct colorimetric method involving the use of dyes (for example, Toluidine Blue O) has been described [20]. S. Zhu et al. have demonstrated the possibility of rapid determination of the surface groups concentration from the ζ-potential [21].

Among the variety of methods available, conductometric [22–27] and potentiometric [28–31] titrations are widely utilized for determining the concentration of carboxyl groups, since these procedures do not require sophisticated equipment or expensive chemicals. These methods serve as a benchmark for evaluating the results obtained from other techniques [18, 21, 32]. When conducted under the necessary conditions, they demonstrate accuracy comparable to high-tech techniques like solid-state NMR spectroscopy [32].

However, when researchers are faced with the task of determining the concentration of surface groups for the first time, they will encounter various experimental challenges and conflicting information in the literature. This becomes particularly apparent in systems where the latex concentration is a few hundredths of

mass percent, which usually corresponds to the concentration of carboxyl groups of $c_{COOH} < 4 \times 10^{-4}$ mol/L. Therefore, in this study, we present our own development of a laboratory technique for determining the concentration of carboxyl groups in polystyrene latex.

EXPERIMENTAL

Chemicals

Monomers: styrene (pure grade, 1% of hydroquinone, Reakhim or Soyuzkhimprom) was washed thrice with 0.1 M aqueous solution of NaOH, distilled under reduced pressure, and stored in a refrigerator; acrylic (99.5%, Vekton) and methacrylic (99.5%, Vetakhim) acids were purified via sequential freezing; divinylbenzene (80%, mixture of isomers, Aldrich) was used without additional purification.

Initiators: ammonium persulfate $(NH_4)_2S_2O_8$, (chemical pure grade, Reakhim) and AIBN (98%, Kemikal Lain) were stored in a refrigerator and used as received; benzoyl peroxide (98%, Chem Polimer) was purified of benzoic acid admixture via recrystallization from ethanol and stored in a refrigerator.

Stabilizers: sodium lauryl sulfate (chemical pure grade, Reakhim); poly(N-vinylpyrrolidone) with $M_w = 4.0 \times 10^4$ (Sigma-Aldrich) (PVP-40); poly(vinyl alcohol) with degree of hydrolysis 86.7−88.7 mol %, $M_w = 6.7 \times 10^4$ (Mowiol 8-88, Aldrich) were used without additional purification.

Solvents: ethanol (95%, Kemerovo pharmaceutical factory), 2-ethoxyethanol (99.9%, Reakhim), toluene (≥98.0%, Ekos-1), acetonitrile (≥99.9%, Koch-Light LTD).

Swelling agent: dibutyl phthalate (≥98.0%, Komponent-reaktiv).

Inhibitor: sodium nitrite (NaNO$_2$, ≥98.0%, KhlorenKhima).

Sodium hydroxide NaOH (analytical pure grade, Reakhim), hydrochloric acid HCl (fixanal solution) and potassium chloride KCl (fixanal) (both from Uralkhiminvest) were used for the titration.

Synthesis of 80 nm Polymer Particles

The 80 nm polymer particles bearing carboxyl groups were obtained via emulsion copolymerization of styrene and acrylic acid. 2900 mL of water was charged into a 3 L reactor, heated to 60°C, a stirring rate 300 rpm (Teflon two-blade stirrer, blade size 3 × 4 cm), bubbling with nitrogen was continued throughout the synthesis; 58 mL of styrene, 10.8 mL of acrylic acid, and 1.56 g of sodium lauryl sulfate were then added. The obtained emulsion was heated to 90°C during 30 min, and then 1.5 g of $(NH_4)_2S_2O_8$ dissolved in 10 mL of water was introduced in the reaction mixture. The polymerization started 2−5 min

upon the initiator addition, as judged visually (opalescence of the reaction mixture). The polymerization was performed during 24 h to reach the conversion of at least 90%. The particles were purified via tangential flow filtration using the filtering elements with the nominal molecular mass threshold $(300−750) \times 10^3$. The purification was considered complete when the system pH reached 5 (the starting pH being 2.2−2.3), which usually demanded seven changes of the buffer (deionized water).

Synthesis of 900 nm Polymer Particles

The 900 nm polymer particles bearing carboxyl groups were obtained via precipitation polymerization of divinylbenzene and methacrylic acid. 0.11 g of AIBN was dissolved in a mixture of acetonitrile (45 mL) and toluene (15 mL) in a 100 mL reactor. 1.98 mL of divinylbenzene and 0.96 mL of methacrylic acid were added to the obtained solution at bubbling with nitrogen. The reactor was then sealed and stirred with a shaker (150 rpm) in a water bath (70°C) during 24 h. The obtained particles were washed with ethanol (repeated centrifugation and decantation) to remove the solvents, monomers, and oligomers and then dispersed in deionized water.

Synthesis of 5200 nm Polymer Particles

The 5200 nm polymer particles bearing carboxyl groups were obtained via two-stage seed polymerization.

The seed polystyrene particles were prepared via dispersion polymerization [33]. To do so, 4.25 g of PVP-40 was dissolved in a mixture of 150 mL of ethanol and 50 mL of 2-ethoxyethanol in a three-neck reactor. The initiator (1.3 g of AIBN) was dissolved in 59 mL of styrene, and the obtained mixture was charged into the reactor. The polymerization was performed during 24 h at continuous stirring (200 rpm, Teflon two-blade stirrer, blade size 3 × 4 cm) and temperature 70°C. The obtained particles were washed with ethanol (repeated centrifugation and decantation) to remove the solvents, monomers, and oligomers and then dispersed in deionized water.

At the next stage, the seed particles were swollen in the presence of dibutyl phthalate. To do so, emulsion of dibutyl phthalate in water was obtained via the treatment of a mixture of 12.6 mL of dibutyl phthalate and 400 mL of 0.25% aqueous solution of sodium lauryl sulfate in an ultrasound bath (Sapfir 2.81) during 30 min. A suspension of the seed particles in 0.25% aqueous solution of sodium lauryl sulfate (3.15 g of the particles in 40 mL of the solution) was added to the emulsion. The swelling was performed during 24 h at 300 rpm. Then, emulsion of the monomers in water was prepared via dispersion of a mixture of monomers (divinylbenzene, styrene, and methacrylic acid, 14.4 mL each) with the initiator (2.16 g of benzoyl per-
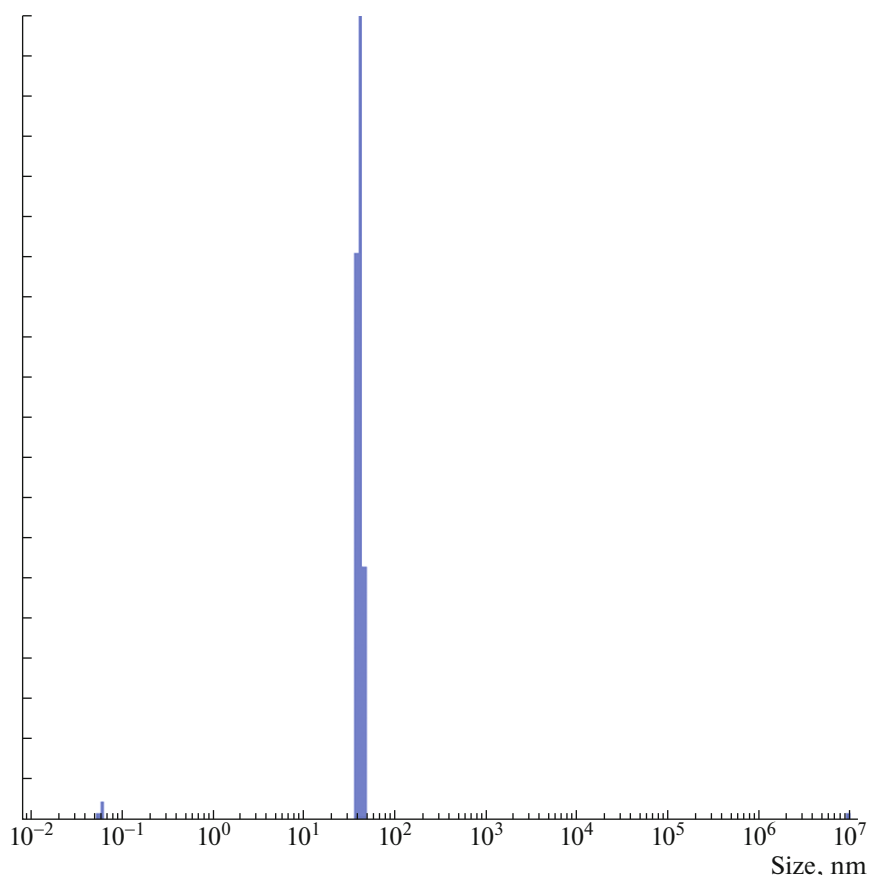
Fig. 1. Dynamic light scattering results of latex particles obtained via emulsion polymerization. Color illustrations are available in the online version.

oxide) in 400 mL of 0.25% aqueous solution of sodium lauryl sulfate with the ultrasound bath during 10 min and added to the obtained system. The swelling process was performed during 24 h at 300 rpm. 0.198 g of sodium nitrite and 5.14 g of poly(vinyl alcohol) dissolved in 75 mL of water were then added to the mixture. The polymerization was performed at continuous bubbling with nitrogen during 24 h at temperature 80°C and stirring rate 300 rpm. The obtained particles were washed with ethanol (repeated centrifugation and decantation) to remove the solvents, monomers, and oligomers and then dispersed in deionized water.

Methods

Size of the particles obtained via emulsion polymerization was determined by means of dynamic light scattering using a PhotocorCompact Z particles size analyzer (laser: $\lambda = 638$ nm, $P = 25$ mW). The measurements were performed in a square cell using two modes differing in the scattering angle (90° or 160°). The particles size was 80 nm (Fig. 1).

Size of the particles obtained via precipitation and two-stage polymerization was determined by means of optical microscopy using a Biomed-5 microscope

equipped with a Levenhuk M1000 Plus camera as well as scanning electron microscopy (SEM) using a Hitachi TM-1000 microscope. The images were analyzed using a free cloud service ParticlesNN (https://t.me/nanoparticles_nsk) [34−36]. Size of the particles obtained via precipitation polymerization was 900 nm, the particles prepared via two-stage polymerization were 5200 nm (Fig. 2).

The latex concentration was determined by gravimetry assuming that the stabilizer was quantitatively retained in the product.

Concentration of the carboxyl groups at the surface of the polymer particles was determined via acid-base conductometric and potentiometric titrations. Concentration of the carboxyl groups determined via conductometric titration using the optimized procedure equaled 660 ± 40 µmol/g; that value was used for theoretical assessment of the carboxyl groups concentration in the systems with different mass fractions of the polymer particles.

Conductometric and potentiometric titrations were performed simultaneously in the same cell, using an Anion 4120 conductometer and a Hanna Instruments HI 2221 potentiometer (electrode: Hanna HI
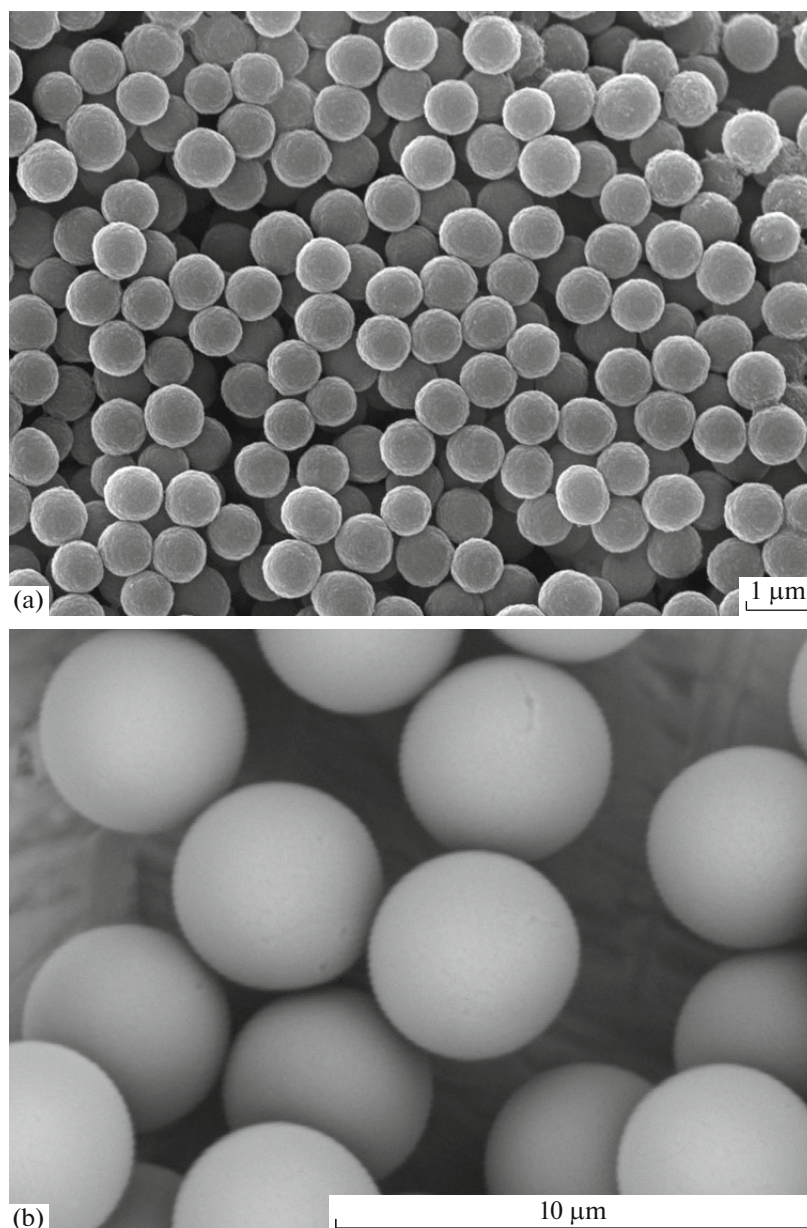
Fig. 2. SEM images of the samples prepared via precipitation polymerization (a) and two-stage polymerization (b).

1131B), respectively, at constant stirring (magnetic stirrer, 200 rpm).

A precisely measured volume of the latex was diluted with deionized water or KCl solution of the desired concentration (10^{-3}–10^{-2} mol/L) to total volume 80 mL. Solutions of KCl were obtained via dilution of its 0.1 M solution prepared from the fixanal. The titration was performed using a 2 mL microburette at stirring and (for most systems) with bubbling with nitrogen (flowrate ~30 mL/min). Since the volumes of the solution and the titrant differed significantly, we neglected the change in the sample volume during titration. The readings were stabilized within 1 min (conductometry) and about 30 s (potentiome-

try). A freshly prepared solution of NaOH (~0.05 M) was used as titrant; its concentration was determined under the same conditions to avoid the systematic error. To do so, 1 mL of 0.1 M solution of HCl prepared from the fixanal solution was diluted with deionized water to 80 mL and titrated with the alkali solution, the equivalence point was determined by means of conductometry and potentiometry.

Reverse potentiometric titration was performed using a Hanna Instruments HI 2221 potentiometer at continuous stirring and bubbling with nitrogen (flowrate ~30 mL/min). 5 mL of freshly prepared solution of NaOH (~0.05 M), standardized via potentiometric titration of the standard HCl solution, was added to

certain volume of latex with known concentration. The obtained solution was sealed to avoid the contact with carbon dioxide, kept for 1 day for equilibration, and then prepared for titration via dilution with deionized water to 80 mL. The excess of alkali was titrated with 0.1 M standard solution of HCl.

The data on the concentration of carboxyl groups can be expressed differently in the literature:

1. mol/L, amount (in moles) of carboxyl groups at the latex per a liter of the latex. Latex is an aqueous dispersion of polymer particles. This concentration depends on the amount of carboxyl groups at the microspheres as well as the microspheres concentration in water. The titration data is obtained in mol/L.

2. mol/g, amount (in moles) of carboxyl groups at the latex per a gram of dry polymer particles. This value reflects the amount of carboxyl groups at the microspheres surface. It is also often expressed in $\mu eq/g$ in the literature, where 1 eq/g = 1 mol of the COOH groups per 1 g of the polymer particles.

The concentration of carboxyl groups (mol/L) determined via titration was recalculated into the mol/g units via the following equation and expressed in $\mu mol/g$ for comparison with the reference data:

$$c_{COOH}\left(\frac{mol}{g}\right) = \frac{c_{COOH}(mol/L)}{c_{lat}}$$
$$= \frac{v_{COOH}}{V_{lat}} \cdot \frac{V_{lat}}{m_{microsph}} = \frac{v_{COOH}}{m_{microsph}},$$

v_{COOH} being the amount (in moles) of the carboxyl groups, V_{lat} being the latex volume, and $m_{microsph}$ being the polymer particles mass.

3. pcs/nm^2, the amount (in pieces) of carboxyl groups per a unit area of the polymer microspheres. It also reflects the amount and density of carboxyl groups at the microsphere. The following equation was used for the recalculation

$$c_{COOH}\left(\frac{pcs}{nm^2}\right) = \frac{c_{COOH}\left(\frac{mol}{g}\right) N_A m_{microsph}}{S_{microsph}}$$
$$= \frac{c_{COOH}\left(\frac{mol}{g}\right) N_A \rho_{microsph} \frac{1}{6}\pi d^3}{\pi d^2}$$
$$= \frac{1}{6} c_{COOH}\left(\frac{mol}{g}\right) N_A \rho_{microsph} d,$$

with N_A being the Avogadro's number, $S_{microsph}$ being the surface area of the microspheres, $\rho_{microsph}$ being the microspheres density (in the present study, the density of polystyrene equal to 1.06 g/cm^3 was used), and d being the polymer microsphere diameter.
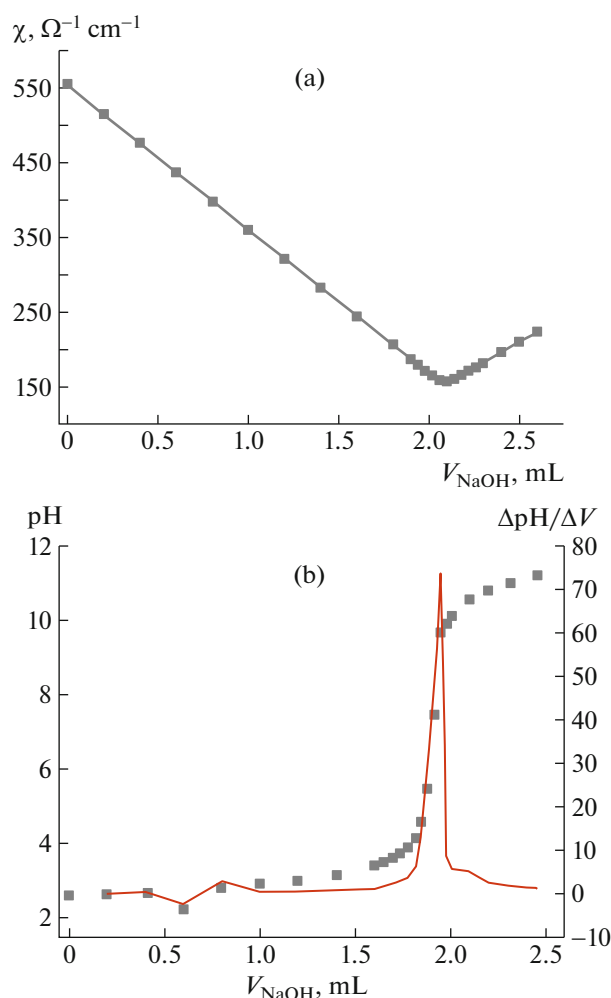

Fig. 3. Curves of conductometric (a) and potentiometric (b) titration of strong acid (HCl) with alkali (NaOH).

RESULTS AND DISCUSSION

Direct Potentiometric and Conductometric Titration

Conductometric titration is based on the measurement of the solution electroconductivity χ. The solution conductivity is changed during the titration, the abrupt change is observed at the equivalence point (Fig. 3a), which allows determination of the analyte concentration in the system even in the case of colored or turbid solutions. The change in the electroconductivity is due to the change in the ions concentration; hence, the increase in the total system volume upon addition of the titrant should be taken into account or the change in the total volume should be negligibly small.

Potentiometric titration is based on the change in the potential of the indicator electrode depending on the amount of the added titrant. The inflection of the titration curve observed at the equivalence point (Fig. 3b, points) corresponds to complete neutraliza-
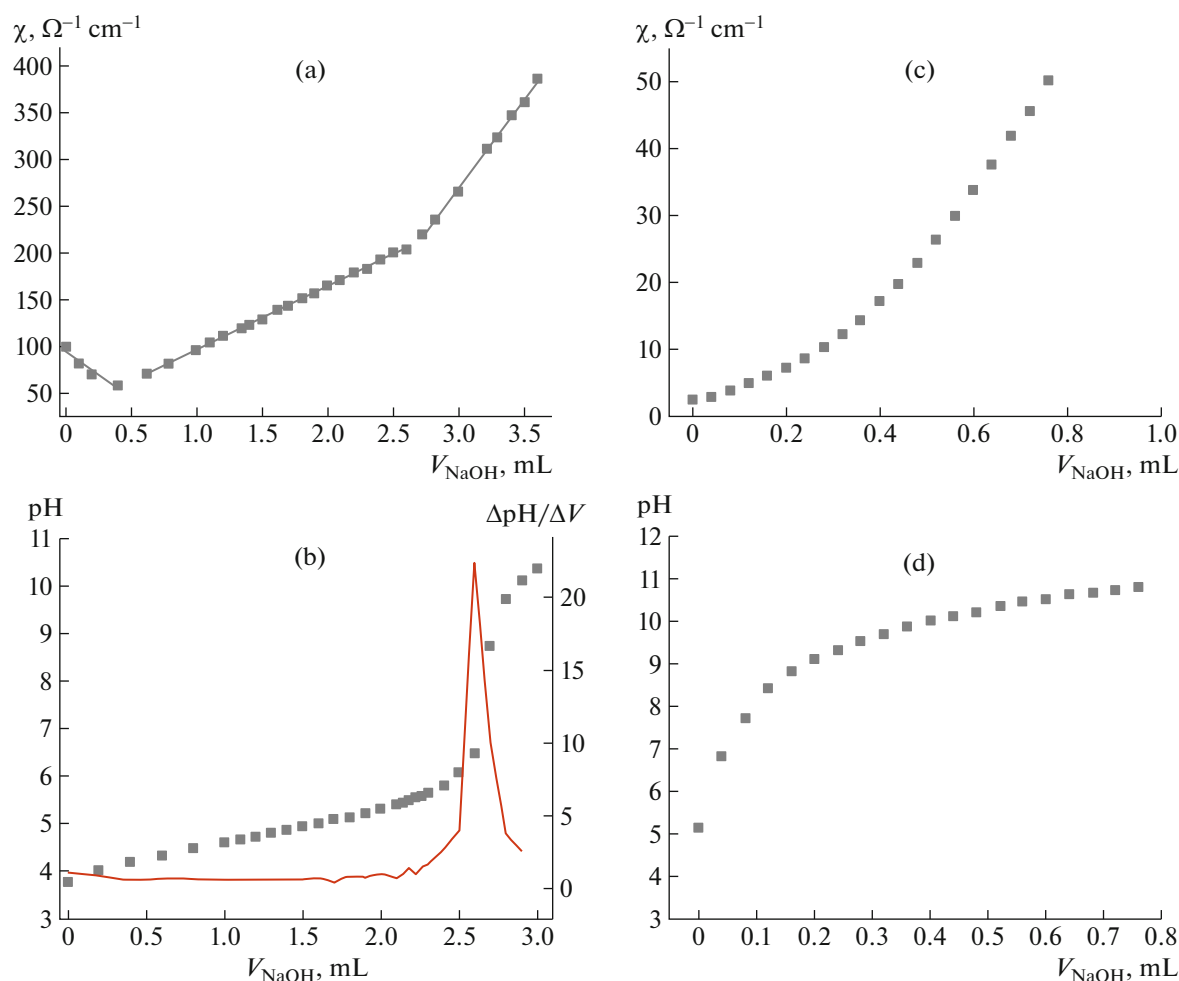
Fig. 4. Curves of conductometric (a, c) and potentiometric (b, d) titration of weak (propionic) acid (a, b) and polymer particles (c, d) with alkali (NaOH).

tion of the protons in the system. The equivalence point can be the most conveniently determined via plotting the dpH/dV value as a function of the volume of the added alkali (Fig. 3b, curve). To do so, hereafter we used the numerical differentiation formula

$$f'(x) \approx \frac{f(x + h) - f(x)}{h}.$$

Carboxyl groups at the polymer particles surface can be generally regarded as a weak acid. During the conductometric titration, an inflection corresponding to the equivalence point as well as a minimum due to disappearance of free protons in the system can be observed (Fig. 4a). At the same time, potentiometric titration curve for such system differs little from the case of a strong acid titration (Fig. 4b).

In practice, certain difference is observed between a weak acid solution and the latex, due to immobilization of the carboxyl groups at the particle surface and,

hence, decrease in their mobility [37]. Therefore, the shape of both conductometric and potentiometric titration curves can be significantly different. In the conductometric curve, nonlinearity in the acidic range and less distinct transition at the equivalence point can be observed (Fig. 4c). This can lead to significant error in determination of the equivalence point coordinates. The potentiometric curve can either show no inflection or the inflection can be hardly noticeable [38], resembling a curve with saturation (Fig. 4d) giving no information on the functional groups concentration. Such changes in the shape of the curves are most likely due to significantly slower diffusion of the protons from the depth of the electrical double layer surrounding the charged polymer particle demanding longer time for equilibration: from several hours to several days [39]. The equilibration can be ensured by the use of reverse titration, when the sample is prepared several hours/days before the titration, adding an excess of alkali to the latex.

Reverse Potentiometric Titration

A classical curve of reverse titration of alkali excess with an acid displayed in Fig. 5a contains two inflection points, the first one corresponding to the excess alkali and the second one corresponding to protonation of the carboxyl groups. The position and size of the inflections can be different depending on the nature and concentration of the present groups.

The concentration determination can be problematic if the observed inflections overlap each other or are not completely separated, which can make determination of the equivalence points ambiguous. Such situation was observed during titration of the poly(styrene-*co*-acrylic acid) particles, when the inflections in the titration curves were close (Fig. 5b). The change in the latex concentration did not significantly affect the curve shape (Fig. 5c). Although this method can be used to characterize the polymer particles, the closeness of the inflections can make the obtained results less accurate. Therefore, we elaborated an alternative approach to determine the concentration of carboxyl groups at the latex surface, which could be used either independently or in combination with reverse potentiometric titration.

Effects of the System Parameters on Determined Concentration of Carboxyl Groups

In order to accurately determine the concentration of carboxyl groups by means of direct conductometric and potentiometric titration, it is essential to meet several conditions: the absence of any impurities affecting the titration result accuracy (primarily carbon dioxide) and well-defined titration curves that allow unambiguous interpretation of the results. The error related to CO_2 dissolved in solution or adsorbed at the polymer particles can be leveled by preliminary bubbling of the mixture with an inert gas or by titration of the latex with high concentration of the polymer particles. The shape of the titration curves can be improved by increasing the concentration of carboxyl groups in the system or by introducing a neutral salt to the solution. This can sharpen the inflections in the conductometric titration curve and make them more pronounced in the potentiometric titration curve [40]. The influence of these parameters on the shape of the titration curve and the determined value of the carboxyl groups concentration will be considered below.

Bubbling with nitrogen. The authors of [41] have pointed out that for latex systems with latex consentration below 2 wt % and concentration of the surface carboxyl groups about $2-3$ $\mu mol/g$ ($c_{COOH} = 6 \times 10^{-5}$ mol/L) the results obtained are irreprodicible and often overestimated. Along with other researchers [42, 43], they have explained the observed phenomena by contamination of the latex with carbon dioxide and
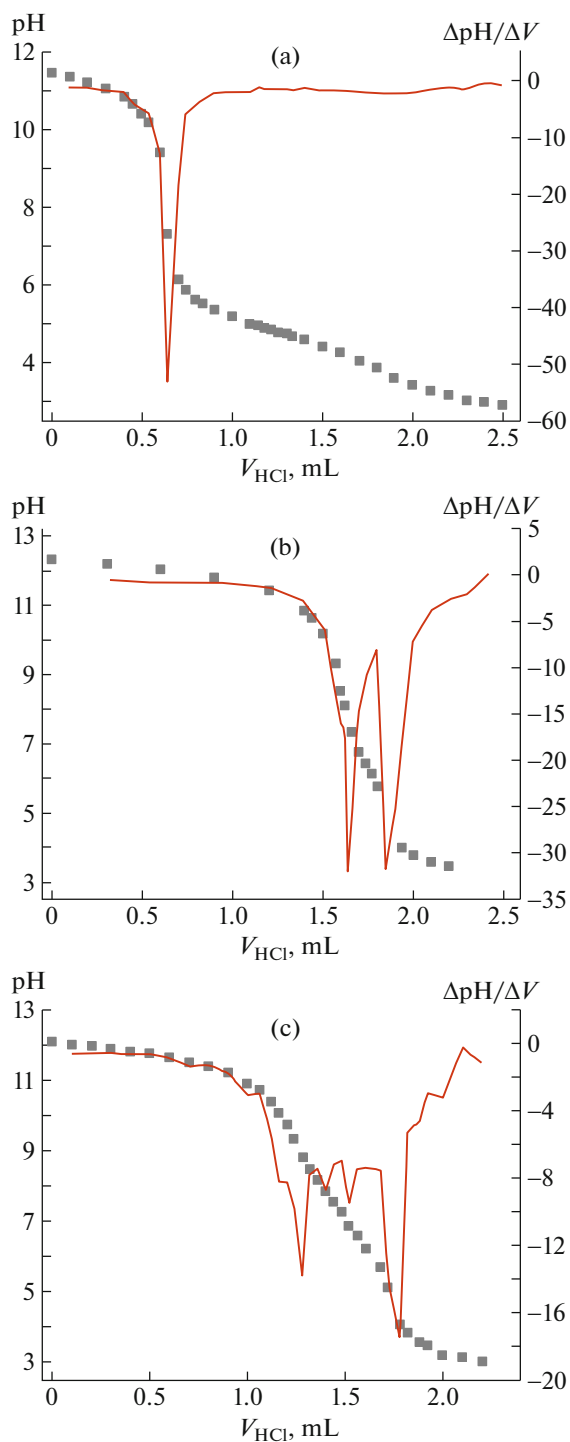

Fig. 5. Curves of reverse potentiometric titration of propionic acid (a), latex 0.04 wt % ($c_{COOH} = 2.8 \times 10^{-4}$ mol/L) (b), and latex 0.1 wt % ($c_{COOH} = 6.5 \times 10^{-4}$ mol/L) (c). In both cases, concentration of carboxyl groups was ~ 660 ± 40 $\mu mol/g$ (5.1 ± 0.3 pcs/nm^2).

have suggested bubbling with an inert gas for several minutes/hours prior to titration and during it to resolve the issue [41, 44].
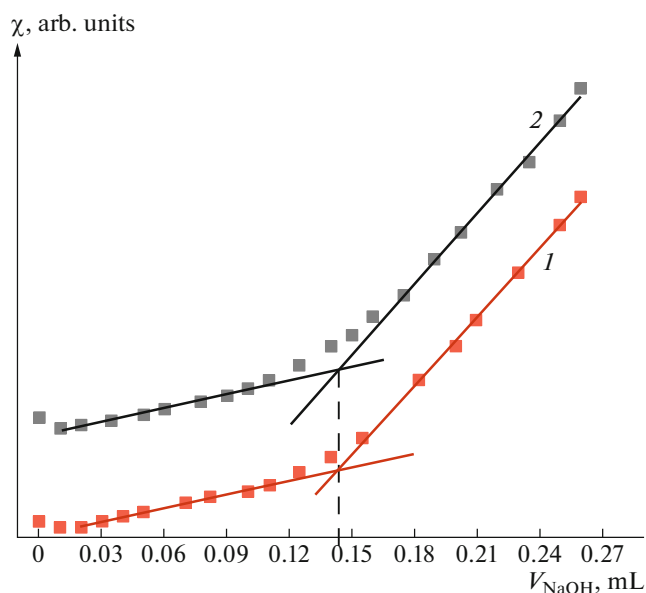
Fig. 6. Curves of conductometric titration in the presence (*1*) and absence (*2*) of bubbling with nitrogen of the latex (styrene/acrylic acid copolymer particles) with the particles mass fraction 0.013 wt % and surface concentration of carboxyl groups ~660 $\pm$ 40 µmol/g (5.1 $\pm$ 0.3 pcs/nm^2) (c_{COOH} = 8.6 $\times$ 10^{-5} mol/L), KCl concentration 10^{-3} mol/L.

In our practice, it is common to encounter the systems with carboxyl group concentrations within this range. Therefore, we performed conductometric titration of the system containing 0.013 wt % of the styrene/acrylic acid copolymer particles with surface concentration of carboxyl groups ~660 $\pm$ 40 µmol/g (c_{COOH} = 8.6 $\times$ 10^{-5} mol/L) with nitrogen bubbling (flowrate ~ 30 mL/min) during 30 min prior to titration and during it as well as without bubbling. The results presented in Fig. 6 evidenced no significant difference between the conductometric curves.

It has been stated in several studies that only vigorous bubbling can remove CO_2 [45] and the gas flowrate used in the present study was likely insufficient. However, it has also been demonstrated that bubbling with an inert gas cannot decontaminate the system at all [39].

Nevertheless, titration under inert atmosphere or with inert gas bubbling is a common practice [46, 47]; therefore, further experiments were performed under the same conditions.

Concentration of carboxyl groups. Concentration of CO_2 in water under standard conditions, assuming carbon dioxide content in air of 400 ppm, is of 1.4 $\times$ 10^{-5} mol/L (the Henry constant of CO_2 equals 0.034 mol/(kg$_{water}$ bar) [48]). Hence, if concentration of the carboxyl groups in the system exceeds the theoretical CO_2 concentration by 5−10 times [41−43], the

obtained results can be considered reliable. To verify that suggestion, we considered the solutions containing from 1.0 $\times$ 10^{-2} to 6.0 $\times$ 10^{-2} wt % of the styrene/acrylic acid copolymer particles with the carboxyl groups surface concentration of ~660 $\pm$ 40 µmol/g (c_{COOH} $\approx$ 6.4 $\times$ 10^{-5}−3.9 $\times$ 10^{-4} mol/L). Titration of the latex with lower concentration of the polystyrene particles was impossible due to the absence of visible inflection in the conductometric titration curve.

The increase in the polymer particles concentration led to a smooth increase in the inflection depth in the conductometric curve, which was especially noticeable by comparing the samples with the mass fraction of particles 0.01 (Fig. 7a) and 0.06 wt % (Fig. 7b). The presence of distinct inflection simplified identification of the equivalence point and reduced the error in its determination (Fig. 7c) from 15% at 0.01 wt % to 2% at 0.06 wt %. The change in the concentration over the probed range did not result in any significant change of the shape of the potentiometric titration curve (Fig. 7d). At the same time, the concentration of the carboxyl groups was not overestimated in the case of the dilute latex, it remained approximately the same (557−591 µmol/g) for the considered latex concentration. It could be concluded that the influence of the dissolved carbon dioxide was insignificant for that system.

Hence, the increase in the latex concentration led to the enhancement of the inflection in the conductometric curve, which allowed reduction of the error in determination of the carboxyl groups concentration, whereas the effect of the CO_2 admixture was absent.

Electrolyte concentration. The increase in ionic strength of the solution (introduction of a neutral salt) favors salting out (shrinkage) of the electric double layer [37] around the polymer particles, which in turn accelerates the system equilibration. In the present study, we varied concentration of KCl in the system over the 10^{-3}−10^{-2} mol/L range, which is typical for conductometric titration of latexes [37]. It is assumed that the effect of salt at a lower concentration is insignificant, whereas the instrument inaccuracy is increased at higher salt concentration.

The increase in ionic strength of the solution changed the shape of the conductometric (Fig. 8a) and potentiometric (Fig. 8b) titration curves, making the inflections more pronounced in both cases. The values determined via conductometric titration were not significantly changed, but the measurement error was decreased (Fig. 8c). The latter fact was due to better manifestation of the equivalence point in the conductometric titration curves. The determination of equivalence point becomes possible for potentiometric titration curves at the KCl concentration 10^{-2} mol/L. The obtained value 620 $\pm$ 40 µmol/g was in good agreement with the values obtained by means of conductometry (640 $\pm$ 40 µmol/g).
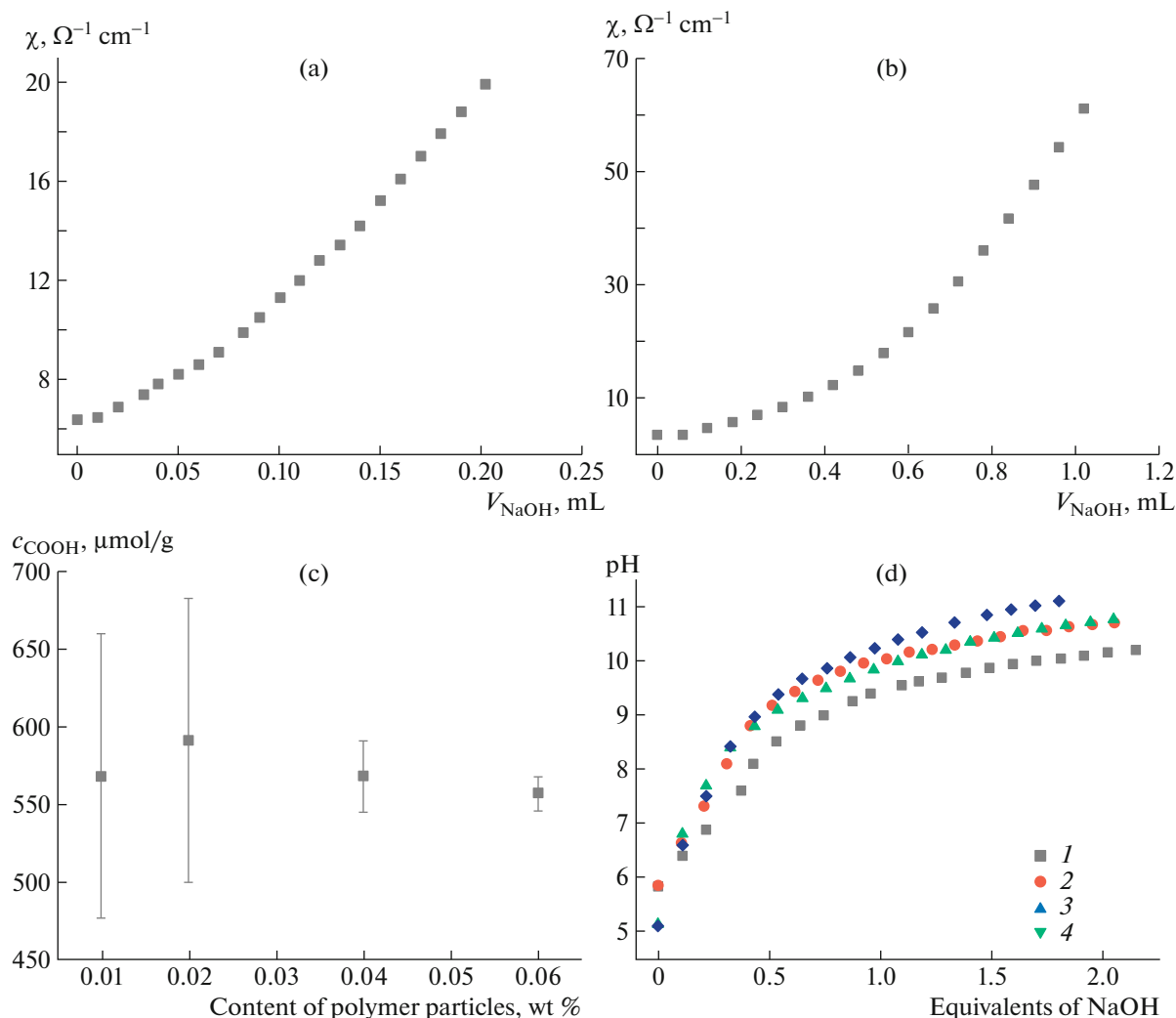
Fig. 7. Curves of conductometric titration at mass fraction of particles 0.01 (a) and 0.06 wt % (b), dependence of the determined concentration of carboxyl groups on mass fraction of the polymer particles in the system (c), and curves of potentiometric titration (mass fraction 0.01 (*1*), 0.02 (*2*), 0.04 (*3*), and 0.06 wt % (*4*)) (d). The data are for the styrene/acrylic acid copolymer particles with surface concentration of carboxyl groups ~660 ± 40 μmol/g (5.1 ± 0.3 pcs/nm^2) without addition of KCl.

The increase in ionic strength of the solution also enabled detection of the inflections in the conductometric curve at lower concentrations of the latex (Fig. 9a) in comparison with the electrolyte-free system (Fig. 7a). At the latex concentration below 0.01 wt %, the determined carboxyl groups concentration increases, which could be the evidence of the CO_2 admixture influence at so low amount of the polymer particles [39]. Hence, the optimal conditions for the titration were as follows: concentration of the carboxyl groups at least 6.6×10^{-5} mol/L (which corresponded to 0.01 wt % of the latex for the considered system) and KCl concentration ~10^{-3}–10^{-2} mol/L in the case of conductometric titration and KCl ≥ 10^{-2} mol/L in the case of potentiometric titration.

Rate of titration. The addition of neutral salts accelerates the system equilibration. However, there is another possible approach, the increase in the titration duration to achieve the equilibrium in the system [39]. Figure 10 displays two curves of conductometric titration obtained during 30 and 120 min, the values at each point being recorded 1−2 and 3−4 min upon the titrant addition, respectively. The plot in Fig. 10 unambiguously demonstrates that the obtained curves were identical. That fact could evidence either complete equilibration of the system in both cases or necessity in significantly longer times for the equilibration (maybe several days).

However, the earlier data on the change in the ionic strength and the latex concentration revealed that the system could be equilibrated within 1−2 min upon the
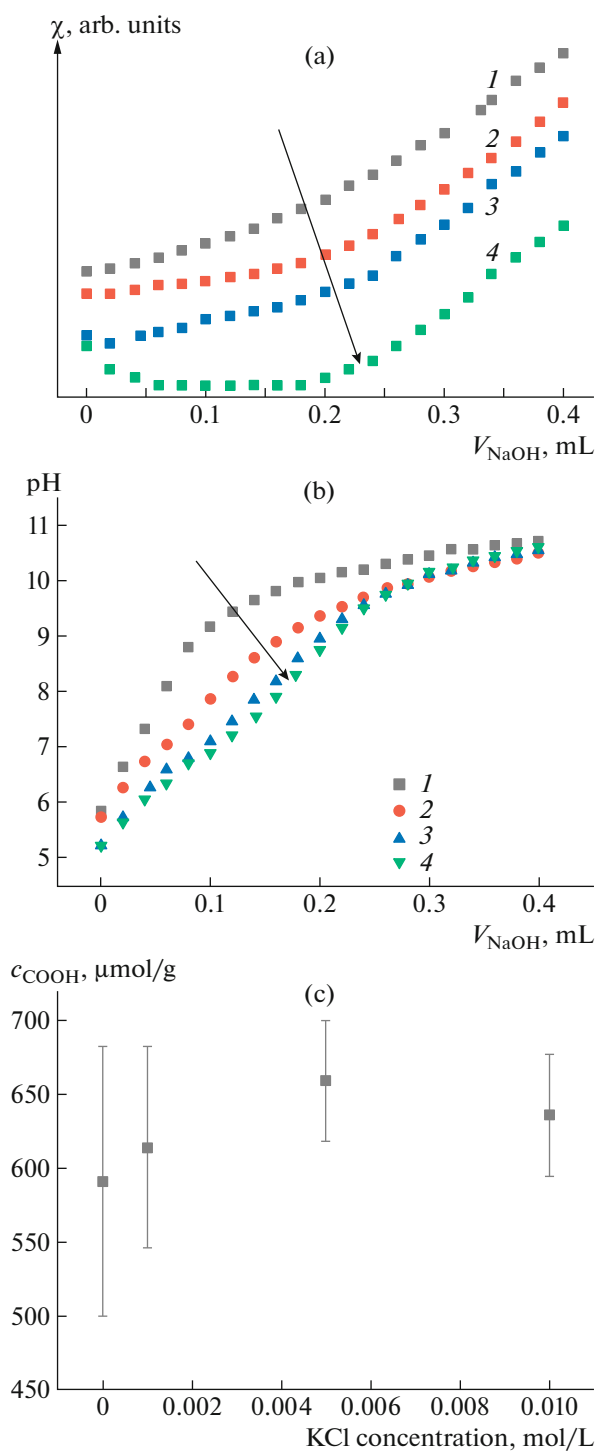
Fig. 8. Curves of conductometric (a) and potentiometric titration (b), dependence of the value of the carboxyl groups concentration determined by means of conductometric titration (c). Without KCl (*1*), in the presence of 10^{-3}, 5×10^{-3}, and 10^{-2} M of KCl, respectively (*2–4*). The data are for the latex of the styrene/acrylic acid copolymer particles with mass fraction of the particles 0.02 wt % and surface concentration of carboxyl groups ~$660 \pm 40 \,\mu mol/g$ ($5.1 \pm 0.3 \, pcs/nm^2$).
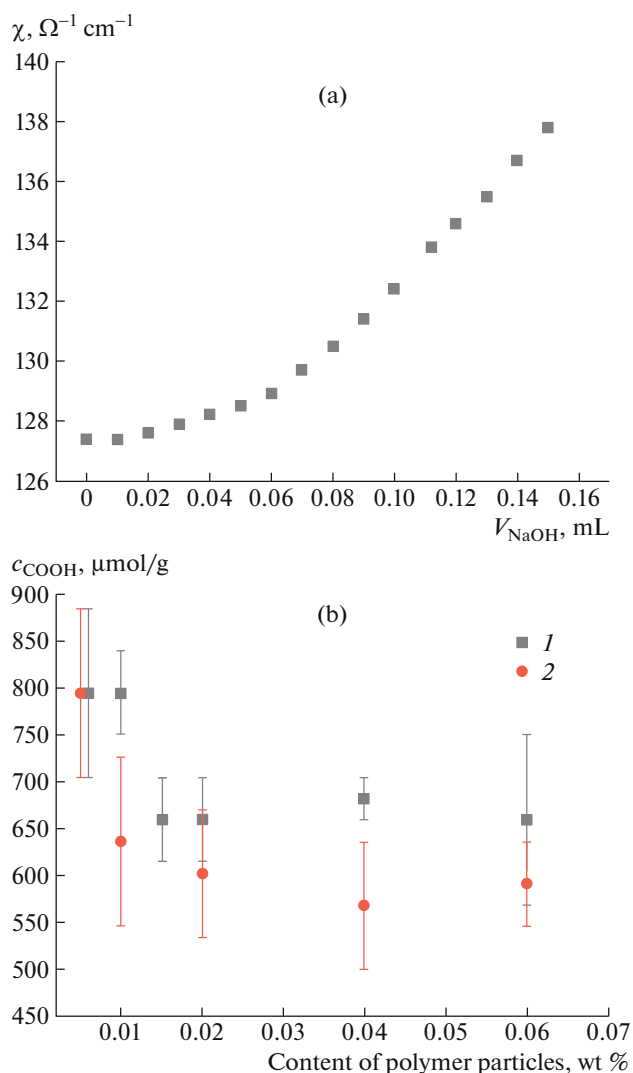

Fig. 9. Curve of conductometric titration (5×10^{-3} wt % of the latex, 10^{-3} mol/L of KCl) (a) and the value of the determined carboxyl groups concentration as function of the mass fraction of the polymer particles at KCl concentration 5×10^{-3} wt % (*1*) and 10^{-3} mol/L (*2*) (b). The data are for the latex of styrene/acrylic acid copolymer particles with the particles mass fraction 5×10^{-3} wt % and surface concentration of the carboxyl groups ~ $660 \pm 40 \,\mu mol/g$ ($5.1 \pm 0.3 \, pcs/nm^2$).

titrant addition. Moreover, the titrant itself (alkali solution) could be saturated with carbon dioxide during slow titration, which could impart significant measurement error. In view of the above, 1−2 min waiting time upon addition of each portion of the titrant and no longer than 60 min for the entire process were suggested as the optimal duration of the titration.

The method applicability. Polymer microspheres used in the diagnostic test systems can often be up to several micrometers in diameter, thus larger than those being used to elaborate the titration procedure.
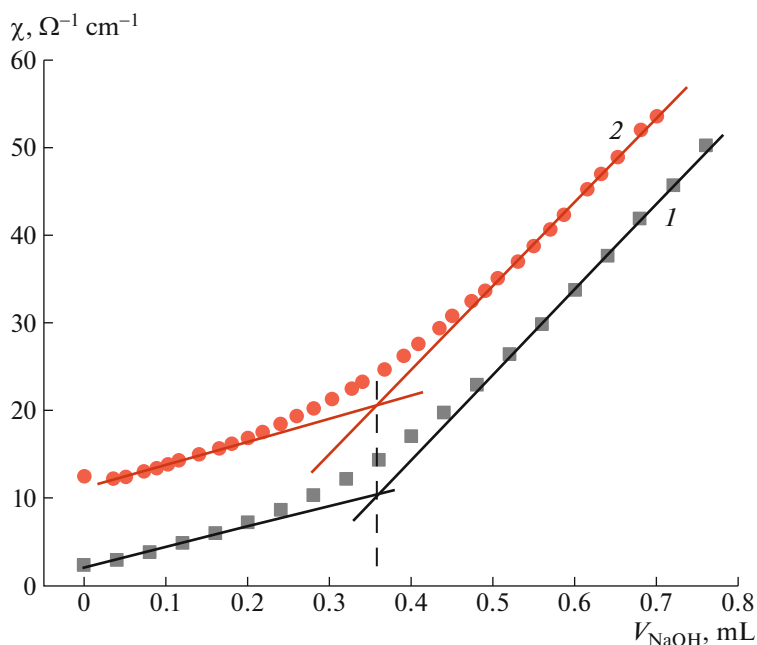
Fig. 10. Curves of conductometric titration of the latex of styrene/acrylic acid copolymer particles with mass fraction of the particles 0.02 wt % and surface concentration of the carboxyl groups ~ 660 ± 40 μmol/g (5.1 ± 0.3 pcs/nm^2) (c_{COOH} = 1.3 × 10^{-4} mol/L) in the absence of KCl. Titration duration: 30 min (*1*) and 2 h (*2*).
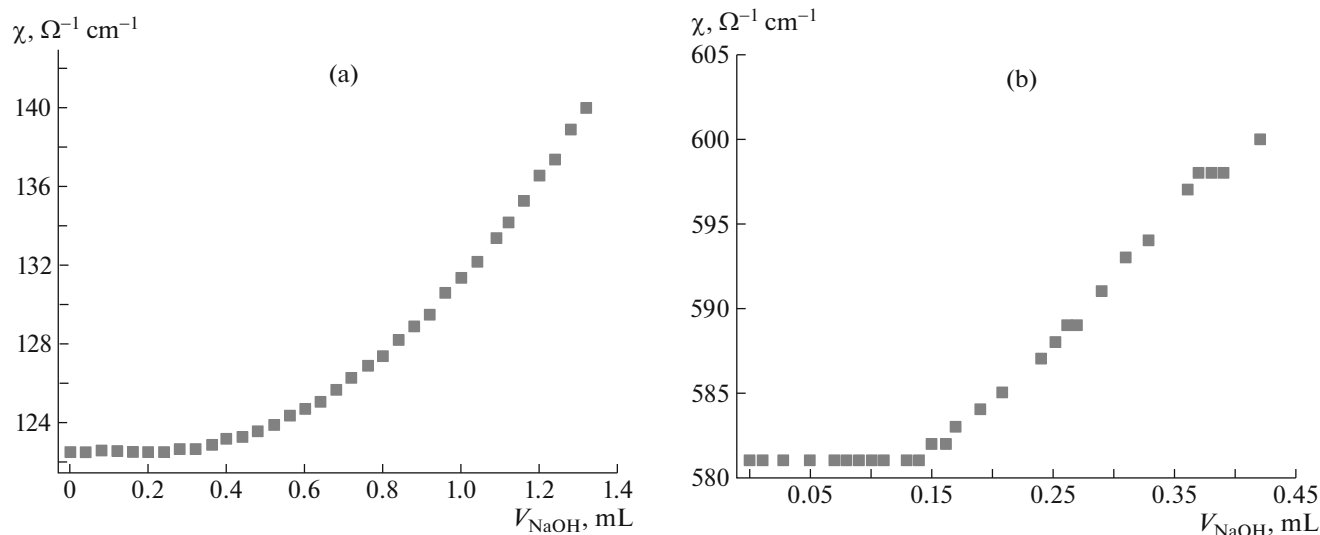

Fig. 11. Curves of conductometric titration of the 900 nm polymer particles at the particles mass fraction 0.12 wt % in the presence of 10^{-3} mol/L of KCl (determined concentration of the surface carboxyl groups 340 ± 20 μmol/g or 29 ± 2 pcs/nm^2) (a) and of the 5200 nm polymer particles at the particles mass fraction 0.07 wt % in the presence of 5 × 10^{-3} mol/L of KCl (determined concentration of the surface carboxyl groups 150 ± 10 μmol/g or 80 ± 5 pcs/nm^2) (b).

To validate the developed laboratory procedure in the case of larger particles, we synthesized and analyzed the microspheres with diameter of 900 and 5200 nm.

The obtained titration curves are shown in Fig. 11. They did not significantly differ from the curves obtained during the titration of the 80 nm latex at the

same concentration of KCl in the solution. The obtained data revealed good coincidence, with error of no more than 10%.

CONCLUSIONS

In the scope of this study, the procedures of direct conductometric and potentiometric as well as reverse potentiometric titration of the styrene/acrylic acid copolymer particles were considered. The influence of different parameters on the shape of conductometric and potentiometric curves as well as the determined concentration of the carboxyl groups was investigated. It was shown that the increase in the latex concentration as well as the increase in the ionic strength of the solution favored better pronounced inflection in the titration curves and reduced the measurement inaccuracy. At the same time, bubbling with nitrogen or increase in the titration duration did not significantly affect the determined values.

It was shown that the reverse potentiometric titration described in the present study could be used to analyze the concentration of carboxyl groups at the particles surface; however, it should be noted that the closeness of the inflections could distort the measurement results, and we therefore recommend to use this procedure with special care.

Direct conductometric and potentiometric titrations are more reliable methods. As a result of this study, the following recommendations for laboratory measurement of carboxyl groups concentration in polystyrene microspheres latex:

—Freshly prepared solution of alkali (sodium hydroxide) should be used as titrant. Experiments showed that solution with alkali concentration of 0.05 mol/L could be stored in a sealed flask for at least a weak, and the titration results obtained during that period were consistent with each other.

—Determination of the alkali concentration should be performed using the same method and equipment which will be further used for the latex titration.

—Dilution of the system during titration should be either taken into account or leveled by using the titrant volume much smaller in comparison with the system volume.

—Concentration of the carboxyl groups in the system should be at least 6.6×10^{-5} mol/L (at least 0.01 wt % for the 80 nm particles considered in this study) to avoid the influence of diluted carbon dioxide. The optimal concentration can be determined from the shape of the conductometric titration curve: the inflection should be unambiguously determined.

—To improve the clarity of the inflection in the conductometric as well as potentiometric curve, neutral salts with concentration of $\sim 10^{-3}-10^{-2}$ mol/L (in the case of single-charged ions) can be added.

—Bubbling of the titrated solutions with nitrogen at the flowrate of 30 mL/min does not affect the titration curves but is a common practice. If used, bubbling should be performed 15−30 min prior to the titration and during the entire titration process.

—During the titration, the system should be allowed to equilibrate. Therefore, in the case of analysis of an unknown system, it is recommended to perform at least two titrations differing in the duration and then choose the optimal duration or further optimize the system (increasing the amount of neutral salt).

—Simultaneous use of conductometric and potentiometric titrations affords more reliable results confirmed by two methods, which is convenient even in the absence of the inflection on the potentiometric curve. Potentiometric titration aids in determination of the titration endpoint (transformation into alkaline buffered solutions with small change in pH upon the titrant addition), which facilitates the titration.

The presented method to determine the concentration of carboxyl groups was elaborated using suspensions of the particles with size of 80 nm and tested for the 900 nm size particles obtained via copolymerization of divinylbenzene and methacrylic acid and for the 5 μm particles synthesized via copolymerization of styrene and methacrylic acid. In the considered cases, the obtained data were consisted within 10%.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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