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The Conductivity and pH Values of Dispersions of Nanospheres for Targeted Drug Delivery in the Course of Forced Equilibrium Dialysis*

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A – research concept and design; **B** – collection and/or assembly of data; **C** – data analysis and interpretation; **D** – writing the article; **E** – critical revision of the article; **F** – final approval of article; **G** – other

Abstract

Background. In the available literature, the problem of pH and conductivity in FED is evaluated separately, and limited mainly to the final purity of the synthesized polymer. In this study data from conductivity and pH measurements were evaluated in the context of the structure of the macromolecule.

Objectives. The aim of the study was to evaluate the conductivity and pH of dispersions of nanospheres synthesized with the use of N-isopropyl acrylamide (NIPA) as the main monomer, N,N'-methylenebisacrylamide (MBA) as the cross-linker and acrylic acid (AcA) as the anionic comonomer during the purification of dispersions *via* forced equilibrium dialysis (FED).

Material and Methods. Six batches of nanospheres were obtained in the process of surfactant free precipitation polymerization (SFPP) under inert nitrogen. The conductivity and pH of the dispersions of nanospheres were measured at the beginning of FED and after finishing that process. The conductivity in the systems being studied decreased significantly in the process of FED. The initial values of conductivity ranged from 736.85 ± 8.13 $\mu S \times cm^{-1}$ to 1048.90 ± 67.53 $\mu S \times cm^{-1}$ After 10 days, when the systems being assessed gained stability in terms of conductivity level, the values of conductivity were between 4.29 ± 0.01 $\mu S \times cm^{-1}$ and 33.56 ± 0.04 $\mu S \times cm^{-1}$. The pH values inreased significantly after FED. The resulting pH was between 6.92 ± 0.07 and 8.21 ± 0.07, while the initial values were between 3.42 ± 0.23 $\mu S \times cm^{-1}$ and 4.30 ± 0.22 $\mu S \times cm^{-1}$.

Conclusions. Conductivity and pH measurements performed during purification *via* FED provide important information on the composition of the resulting nanospheres, including the functional groups embedded in the structure of the polymer in the course of the synthesis, as well as the purity of the structures. The presence of a cross-linker and acidic comonomer in the poly-N-isopropyl acrylamide (polyNIPA) macromolecule may be confirmed by both the pH and the conductivity measurements (**Adv Clin Exp Med 2015, 24, 2, 219–226**).

Key words: nanosphere, conductivity, pH, N-isopropyl acrylamide, polymer, surfactant free precipitation polymerization.

Nanospheres have numerous potential applications for medical and pharmaceutical purposes [1–3]. They are proposed as drug delivery carriers due to their small diameters, in the range below the size of blood capillaries. One of the main problems is the impurity of nanospheres that are synthesized in various sorts of emulsions or by precipitation polymerization. The purity of the nanospheres produced is of major importance in the case of per-oral or parenteral applications.

Nanogels synthesized using N-isopropyl acrylamide (NIPA) are enjoying a growing interest

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among specialists in drug formation technology, bioengineering and biocompatible polymers [4–6]. The wide interest in the polymers occurred because of their ability to secrete large amounts of water at a temperature referred to as the volume phase transition temperature (VPTT). Consequently, one can expect the release of the drug substance from the nanospheres of polyNIPA under thermal influence. Importantly, the VPTT is in the range of known physiological temperatures, e.g. in the area of the surface of human skin. By modifying the composition and structure of the NIPA derivatives it is possible to synthesize various batches of macromolecules with programmed VPTTs in aqueous solutions. The schematic synthesis of polyNIPA from NIPA is presented in Fig. 1.

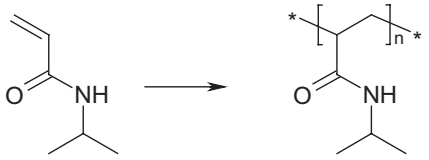

Fig. 1. NIPA and polyNIPA

Polymerization is initiated using the appropriate initiator, usually ammonium persulfate, potassium persulfate and sodium persulfate. In order to obtain dispersion of the nanospheres it is necessary to ensure the proper composition of the mixture of substrates, including the main monomer, comonomer, cross-linker (e.g., N,N'-methylenebisacrylamide – MBA), the initiator system, and in some cases the surfactant. Polymerization carried out in one reactor – so-called "batch-synthesis" – requires the selection of individual components that will react with each other with a similar rate. The rate of reaction of the individual comonomers influences the final composition of the resulting polymer, according to the Mayo-Lewis equation [7]. The compositions used for the preparation of the polymers are extremely diverse; they include NIPA, the aforementioned initiators, comonomers like acrylic acid (AcA) [8], methacrylic acid and fumaric acid [9], acrylamide [10], maleic acid [11], hydroxyethyl methacrylate, N-vinylpyrrolidone [12] or N-tert-butyl acrylamide [13, 14], as well as 4-pentenoic acid [15].

The very small size (between 10 and 1000 nm) results in severe complications during the purification process. Nanoparticles obtained in different conditions may contain various amounts of residual organic solvents, monomers, comonomers, surfactants, inorganic salts, initiators, accelerators, as well as non-cross-linked polymer aggregates. To avoid adverse reactions within the human organism, the level of these impurities should be very low. Semipermeable cellulose membranes are often applied during the equilibrium dialysis performed in the process of purifying nanoparticles. The membranes reduce the diffusion rate of impurities to minimal values, and a respective decrease in the concentration of impurities in the donor solution retentate, is observed. That procedure can be enhanced by various approaches, including diafiltration preceded by a concentration process, membrane filtration, crossflow membrane filtration, filtration by nonwoven electrospun polymer fibers, purification by spin centrifugation-dialysis and many other processes. Regular and frequent replacement of the solvent in the process of equilibrium dialysis can be used during forced equilibrium dialysis (FED) [16–23].

Normally the aggregates of the synthesized polymer can be eliminated by filtration through sintered glass filters. In the research field, dialysis using a concentration gradient is acceptable; however, the presence of an acceptable level of impurities should be confirmed, e.g. by conductometric measurements. The length of time of the dialysis depends on the conditions of the process, as well as on the purified material; a reasonable level of conductivity is obtained in various time periods ranging from days to months [24–26].

The aim of this study was to evaluate the conductivity and pH of dispersions of nanospheres synthesized by surfactant free precipitation polymerization (SFPP) using NIPA as the main monomer, MBA as the cross-linker and AcA as the anionic comonomer in the course of purification of the dispersions *via* FED.

Material and Methods

Material

In the course of SFPP the following reagents were used: N-isopropylacrylamide (NIPAM, Aldrich, USA, 97%), N,N'-methylenebisacrylamide, (MBA, Aldrich, USA, 99%), ammonium persulfate (APS, Aldrich, Germany, 98%), acrylic acid (AcA, Aldrich, Germany, 99%), purified water with a conductivity not exceeding 5 mS cm^{-1} (osmotic column ODOS-20 Preksim, Poland, with an Excelon PES filter, Germany).

Methods

A round-bottom, 4-neck glass reactor with a volume of 2 L was used to carry out the synthesis in surfactant free precipitation polymerization (SFPP) conditions, as performed earlier in the

authors' laboratories [13]. The 4-neck cover enabled the environment and the introduction of the reactants to be controlled. The temperature was monitored by a contact thermometer and a magnetic stirrer with a "feedback" heating element. The reactor was placed in a 5-liter water bath which was set on the magnetic stirrer. The compositions of the synthesized batches of nanospheres are summarized in Table 1.

The incorporation of the comonomers into the nanospheric structures was confirmed by Fourier-transformed infrared spectroscopy (FTIR). The assessments were performed using a Spectrum GX FT-IR spectrometer (PerkinElmer, USA) with a Golden Gate ATR (Specac, UK) and reproducible sample loads of about 300 MPa. The obtained data were analyzed by the PerkinElmer Life Spectrum software, version 5.0.2. The spectra of pure monomer NIPA were compared with the resulting purified and freeze-dried products of polymerization. During SFPP, unsaturated compounds are saturated and bands higher than 3000 cm^{-1} should not be observed [27]. The respective unsaturated bonds were saturated; the saturation was confirmed comparing the FTIR spectra of samples P1-P6 to the spectra of the vinyl components NIPA, MBA and AcA.

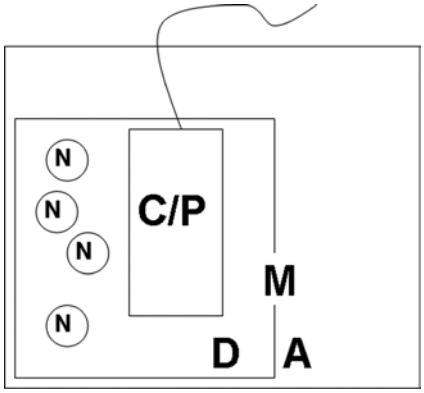

Fig. 2. The scheme of dialysis purification of the synthesized nanospheres and the measurements performed. N – nanospheres; D – the donor compartment with purified nanospheres; A – the acceptor compartment with water; M – a semipermeable membrane with a MWCO of 12,000–14,000 Da; C/P – the conductivity sensor/pH electrode

Forced Equilibrium Dialysis

After completion of the SFPP in an inert nitrogen atmosphere, the nanospheres were subjected to purification *via* equilibrium dialysis in the system presented in Fig. 2. A 30 mL quantity of each dispersion of nanospheres (P1–P6) was closed in a container prepared from semipermeable membrane with a molecular weight cutoff (MWCO) of about 14,000 Daltons (the donor compartment), and placed in glass cuvettes filled with 300 mL of deionized water (the acceptor compartment). The water was replaced at regular time intervals over 10 days. After 10 days the conductivity in the acceptor compartment decreased to stable low values ranging from 2.01 to 2.78 $\mu S \times cm^{-1}$, and the process was considered finished.

Table 1. Composition of reactants and stirring speed in surfactant free precipitation polymerization

Nanosphere (type)	Acronym	Stirring [rpm]	Substrates [%]				
			NIPA	MBA	AcA	APS	H_2O
polyNIPA (500)	P1	500	0.250	–	–	0.050	99.700
polyNIPA-co-MBA (500)	P2	500	0.250	0.050	–	0.050	99.650
polyNIPA-co-MBA-co-Ac (500)	P3	500	0.250	0.050	0.025	0.050	99.625
polyNIPA (1000)	P4	1000	0.250	–	–	0.050	99.700
polyNIPA-co-MBA (1000)	P5	1000	0.250	0.050	–	0.050	99.650
polyNIPA-co-MBA-co-Ac (1000)	P6	1000	0.250	0.050	0.025	0.050	99.625

NIPA – N-isopropylacrylamide, MBA – N,N'-methylenebisacrylamide, AcA – acrylic acid, APS – ammonium persulphate.

Evaluating the Conductivity and pH

The conductivity and pH were measured in the donor compartment before and after the purification of the nanospheres *via* forced equilibrium dialysis. A membrane with a MWCO of about 14,000 Daltons was used in the process. The measurements evaluated in this study included the initial values and the final stable values of conductivity and pH from the 10-day dialysis. A multifunction CX 741 compute rmeter (Elmetron, Poland) was used with a glass KSAgP-301 W 887 electrode (Eurosensor, Poland) for the pH measurements; and with an EPS-2ZN conductivity sensor (Eurosensor, Poland) with a cell constant of 0.85 cm^{-1} for the conductivity assessments. The measurements were taken in the solution as shown in the diagram in Fig. 2.

Evaluation of the Hydrodynamic Diameter of the Nanospheres

In order to determine the hydrodynamic diameter of the synthesized nanospheres a Zetasizer (Malvern, USA) was used in dynamic light scattering (DLS) mode. The measurements were performed at 25°C, repeated 5 times and the averages were calculated.

Results

Conductivity of the Evaluated Nanospheres

Nanosphere conductivity evaluated in the donor compartment directly after the reaction ranged from 736.85 ± 8.13 μS × cm^{-1} to 1048.90 ± 67.53 Ms × cm^{-1}. The conductivity of the dispersions of nanospheres P1–P3, obtained by stirring at a speed of 500 rpm, was slightly lower than that of dispersions of nanospheres P4–P6, synthesized in the same conditions but with a higher stirring speed of 1000 rpm (Fig. 3A and 3B). The values of conductivity were arranged in the same pattern for batches P1–P3 and P4–P6, i.e. P2 > P3 > P1, and P5 > P6 > P4.

The conductivity in the studied systems decreased significantly in the process of purification. After 10 days, when the assessed systems had gained stability in terms of the conductivity level, the values of conductivity were between 4.29 ± 0.01 μS × cm^{-1} and 33.56 ± 0.04 μS × cm^{-1}. The values of conductivity of the samples assessed after purification were arranged in different way than the values of the samples before purification; after purification the pattern was P3 > P1 > P2 and P6 > P4 > P5. The relationships are presented in Fig. 3C and 3D.

The pH of the Evaluated Nanospheres

The pH of the post-reaction mixtures was between 3.42 ± 0.23 and 4.30 ± 0.22 (Fig. 4). The pH values were higher in the case of post-reaction mixtures prepared stirring at 500 rpm, namely from 3.91 ± 0.16 to 4.30 ± 0.22. When stirred at the higher speed of 1000 rpm, the mixtures' pH values ranged from 3.42 ± 0.23 to 3.62 ± 0.15. The dispersions of nanospheres P1 and P2 were characterized by a similar pH regardless of whether they were stirred at a speed of 500 rpm or 1000 rpm during SFPP, while the P3 nanospheres had a significantly lower pH (Fig. 4A, 4B).

The pH values increased significantly after FED was performed for 10 days. The resulting pH was between 6.92 ± 0.07 and 8.21 ± 0.07. In the group of dispersions stirred at 500 rpm, the pH values were higher than in the group of dispersions stirred at 1000 rpm, with exception of the P2

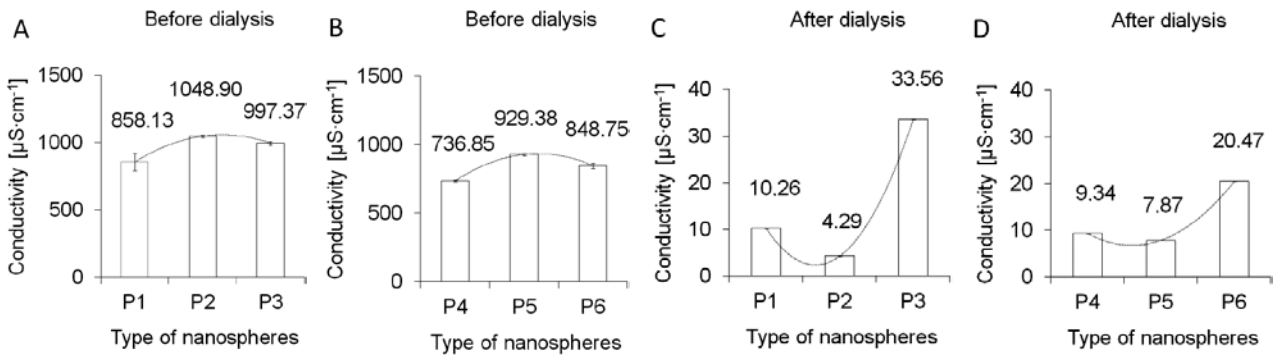

Fig. 3. The conductivity of the dispersions evaluated in the donor compartment directly after the reaction (A, B), and after 10 days of equilibrium dialysis (C, D)

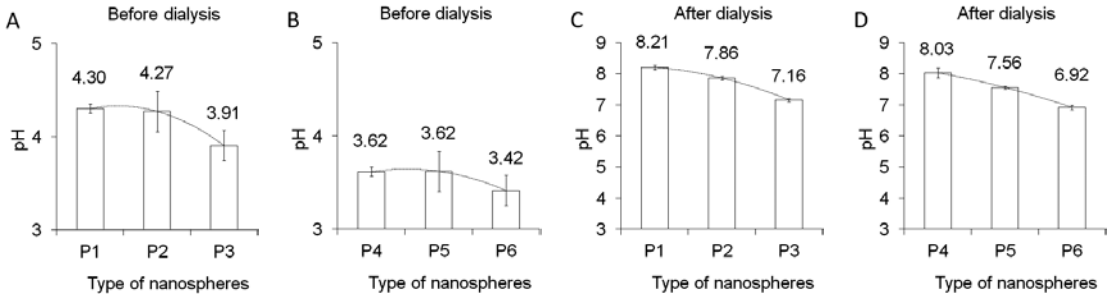

Fig. 4. The pH of the dispersions evaluated in the donor compartment directly after the reaction (A, B), and after 10 days of equilibrium dialysis (C, D)

nanospheres. The pH decrease in the group of purified nanospheres follows the pattern P1 > P2 > P3, as shown in Fig. 4C and 4D.

The standard deviation calculated from the pH measurements decreased after purification, with exception of the pH of the P2 nanospheres, which was 0.05 in the pH measurements both before and after FED.

DLS studies confirmed that the obtained polymeric structures are in the range between 536.20 ± 23.38 nm and 605.00 ± 11.34 nm, and may be classified as nanospheres.

Discussion

During free radical polymerization all the components may influence the conductivity and pH, but the most important influence is exerted by the activity of the initiator and the ionized comonomer. The potential factors influencing the pH and conductivity in the systems in this study are presented in Table 2.

In the case of P2, the highest level of conductivity was observed at the initial stage of purification performed *via* FED. Since the same concentrations of initiator were used in the synthesis, the same number of molecules of initiator was involved in all the evaluated reactions: i.e., those performed without a cross-linker (P1 and P4), those with a cross-linker (P2 and P5), and those involving both a cross-linker and an acidic comonomer (P3 and P6).

Logically, the conductivity measured *via* the semipermeable membrane directly after the reaction should be higher in the case with the highest concentration of remaining initiator (not embedded into the macromolecule). The differences between P1 and P2, as well as between P4 and P5, were observed at the initial stage of purification. The resulting cross-linked structures (P2, P5) incorporated a lower number of molecules of initiator into the macromolecule, as compared to the non-cross-linked structures (P1, P4). The observed high conductivity results from the presence of non-incorporated molecules of APS. Curiously,

Table 2. The main factors influencing conductivity and pH measured in normalized conditions

Stage	Conductivity	pH
BF	non-reacted components (APS, AcA) functional groups in the macromolecule (–SO_3H, –COOH, amide groups) resistance of the environment (3D structures) polarization effects (NIPA, MBA)	non-reacted components (APS, AcA, NIPA, MBA) functional groups in the macromolecule (–SO_3H, –COOH, amide groups) resistance of the environment (3D structures) polarization effects (NIPA, MBA)
AF	functional groups in the macromolecule (mainly –SO_3H, –COOH, amide groups) resistance of the environment (3D structures) polarization effects (NIPA, MBA)	functional groups in the macromolecule (–SO_3H, –COOH, amide groups) resistance of the environment (3D structures) polarization effects (NIPA, MBA)

BF – before FED, AF – after FED.

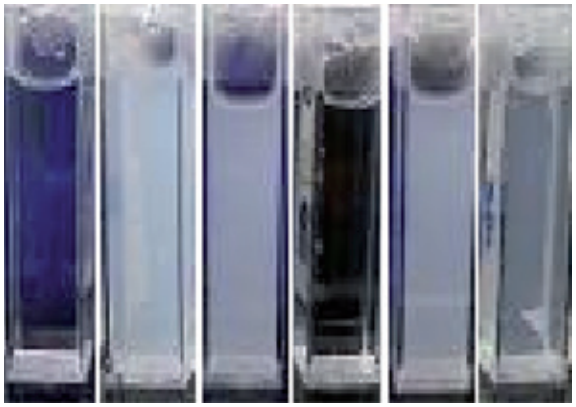

Fig. 5. Macrophotographs of the dispersions of synthesized nanospheres P1–P6, from transparent P1 on the left to semi-transparent P6 on the right

the conductivity in samples P3 and P6 is not higher, but rather lower than in samples P2 and P5. It is possible that the potential increase in conductivity resulting from the additional presence of non-reacted AcA may be balanced by increased resistance of the environment due to the increased concentration of a weak electrolyte – the remaining AcA.

Finally, by the end of the purification, the P1 and P4 samples were characterized by intermediate conductivity, which results from the ionogenic activity of terminal sulphonate groups in linear macromolecules. The formation of three-dimensional macromolecular structures in samples P2 and P5 results in a significant decrease of conductivity due to the decrease of mobility of the ions. This is confirmed by macrophotographs of the dispersions of synthesized polymers (Fig. 5).

P1 and P4 are transparent, whereas the P2 and P5 are non-transparent, due to the presence of dense dispersions of cross-linked macromolecules. In P3 and P6 the high conductivity is connected to the excess of ions released from the carboxyl groups incorporated during the polymerization. Some other factors, like the polarization patterns within the macromolecules, may also enhance the ionization of the particle; however, evaluating those phenomena oversteps the bounds of this study.

Changes in the pH value may be useful for assessing the purity and composition of the obtained microspheres. As stated above, in the present experiment 6 batches of nanospheres were synthesized; batches P1 and P4 included only NIPA as the monomer, and the resulting product was translucent. Batches P2 and P5 incorporated the efficient cross-linker MBA; while the nanospheres P3 and P6 were cross-linked by MBA, and included AcA as an additional anionic comonomer. The concentrations of H^+ in each of the dispersions were calculated. The estimated differences in proton concentration ($[H^+]$) based on the assessed pH of dispersions P1–P6 are presented in Table 3.

In general, the slightly alkali pH of the dispersion of pure polyNIPA comes from the amide groups of NIPA; however, the presence of the acidic initiator and acidic comonomer influences the $[H^+]$ in the system.

The initial pH measurements, performed before the equilibrium dialysis, revealed that the estimated $[H^+]$ results from the impurities, i.e. from non-reacted monomer, comonomers and initiator, as well as from the incorporated anionic groups: the acidic initiator APS and the AcA; it is those two that should have the most impact on the pH. During the equilibrium dialysis the non-covalently bonded anionic entities – e.g. the initiator and AcA – are removed from the post-reactant mixture. This leads to a decrease in $[H^+]$, but part of the $[H^+]$ is connected to the structures incorporated into the nanospheres during SFPP. The comparison of $[H^+]$ in the evaluated preparations confirms that the addition of AcA to the composition results in a stable co-polymer of NIPA and AcA, stabilized by cross-links of MBA (P3 and P6).

Table 3. Estimated differences in proton concentration ([H+]) based on the assessed pH of dispersions P1–P6

Nanospheres				
	before dialysis		**after dialysis**	
	$[H^+]$ mol/L	**SD**	**$[H^+]$ mol/L**	**SD**
P1	5.46×10^{-05}	$\pm 2.07 \times 10^{-05}$	6.26×10^{-09}	$\pm 8.72 \times 10^{-10}$
P2	5.37×10^{-05}	$\pm 6.09 \times 10^{-06}$	1.38×10^{-08}	$\pm 1.64 \times 10^{-09}$
P3	1.32×10^{-04}	$\pm 5.14 \times 10^{-05}$	6.89×10^{-08}	$\pm 8.25 \times 10^{-09}$
P4	2.51×10^{-04}	$\pm 6.85 \times 10^{-05}$	9.77×10^{-09}	$\pm 3.43 \times 10^{-09}$
P5	2.40×10^{-04}	$\pm 2.94 \times 10^{-05}$	2.75×10^{-08}	$\pm 2.78 \times 10^{-09}$
P6	4.19×10^{-04}	$\pm 1.53 \times 10^{-04}$	1.21×10^{-07}	$\pm 2.06 \times 10^{-08}$

It is curious that the pH was higher and the $[H^+]$ was lower in the systems that were stirred more intensively. During the reaction the molecules are in motion. The possibility of creating an active complex should increase along with any increase in motion in the reactant mixture. In the starting conditions, the number of polymer molecules depends on the number of molecules of initiator present within the system. The intensive stirring may hinder the agglomeration of small polymeric macromolecules, so the anionic groups have better conditions for ionization. This concept is in agreement with the results of the theoretical and practical considerations published by Dušek [28–30].

Another question arises when P1 and P2 are compared, or P4 and P5: why P2 and P5 are slightly more acidic than P1 and P4, respectively. It seems that the addition of the cross-linker MBA may increase the dissociation of the acidic group of the incorporated terminal acid groups from the initiator. Further studies are need to evaluate the detailed effects outlined in Table 3.

The authors have concluded that the measurements of conductivity and pH performed during purification *via* FED provide important information on the composition of the resulting nanospheres. If there is a possibility to compare nanospheres prepared under controlled conditions, important information can be obtained. The presence of a cross-linker and acidic comonomer incorporated into polyNIPA macromolecules can be confirmed both by pH measurements and by conductivity measurements. The stirring speed has an impact on the resulting conductivity and pH of dispersions of nanospheres.

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