Determination of nanoparticles concentration in solution based on Pickering emulsion destabilization analyses

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Abstract
The dynamic development of nanotechnology research has contributed to the fact that various types of nanoparticles are increasingly used on a large scale both for medical and biological purposes, but above all in many industrial fields. Such a wide application of nanoparticles is often connected with the need to estimate their characteristic parameters, such as size, size distribution or concentration. Existing instruments are usually quite expensive and not always available. Therefore, other cheaper and simpler methods based on analytical techniques are sought. In this paper, we have proposed a method to estimate the concentration of nanoparticles in solutions based on destabilization analyses of Pickering emulsions produced with their use. The fact of mutual relationship between emulsion concentration, nanoparticle concentration and emulsion stability was used here. The study was carried out using silica nanoparticles. It was presented how to apply the method and what are its limitations. Moreover, an example of its application for the determination of nanoparticle concentration in an unknown sample, obtained after analysis of the permeability of membranes in diffusion chambers, has been presented. The method can become a useful alternative for the determination of nanoparticle concentration in solution in places where no specialized equipment is available.

Keywords Nanoparticles · Nanoparticle concentration · Pickering emulsion · Emulsion destabilization

Introduction
The tremendous development of nanotechnology in the last decade has led to the widespread use of various types of nanoparticles in medicine, biology and industry. According to the European Commission, nanoparticles are small particles ranging in size from 1 to 100 nm (Commission Recommendation 2011/696/EU). They are most commonly used for medical purposes as disinfectants (Sudagidan et al. 2021). Continuous scientific progress shows their use as fluorescent biological labels (Yu et al. 2021a), drug carriers (Jin et al. 2020), they are also used for protein detection (Shahbazi et al. 2021), separation and purification of biological molecules and cells (Eivazzadeh-Keihan et al. 2021), in tissue engineering (Wang et al. 2021) and many others (Khalegh and Qaderi 2019). However, on a large scale, nanoparticles are used in industry. Carbon nanotubes are commonly added to various construction materials to improve their endurance properties (Al-Thobity and Gad 2021). Nano-fuels are used in environmental processes (Kegl et al. 2021). Applications of metal oxides in flexible solar cells are being developed (Kumar et al. 2020; Sheikholeslami and Ehteshami 2020), as well as for purification of polluted water (Yu et al. 2021b). In the cosmetics industry, mineral nanoparticles—such as titanium oxide—are used in sunscreens because of the low long-term stability offered by conventional chemical UV protection (Kaur et al. 2021). One very modern technology is the Sol–Gel process, which produces a solid material from nanoparticles (Zheng and Boccaccini 2017). Nanoparticles are nowadays widely used as components of processing slurries. Operation with traditional machining slurries introduces contamination to the environment, because they usually consist of toxic or polluted ingredients (Liao et al. 2021). The silica nanoparticles are employed to
overcome this challenge, by development of novel slurries to polish copper (Zhang et al. 2019), sapphire (Zhang et al. 2021), titanium (Zhang et al. 2018) and nickel alloys (Zhang et al. 2020). Nanoparticles and nanotechnology have also been used for the development of innovative machining approaches and diamond wheels (Zhang et al. 2015, 2017). With these widely used unique materials, high-performance devices are fabricated (semiconductors, microelectronic and optoelectronic equipment) with the use of developed slurries, approaches and diamond wheels (Zhang et al. 2012). Which is extremely difficult to be manufactured by traditional technologies and production processes. The key feature is that the developed novel methods and slurries eliminate the pollution to the environment, which is a significant contribution of the conventional manufacturing (Wang et al. 2018; Xie et al. 2020).

Considering the use of nanoparticles on such a large scale in various fields of life, it is necessary to develop methods and techniques to accurately determine their parameters such as their size, shape, size distribution and concentration (Park et al. 2020; Pellequer et al. 2021). Modern devices based on advanced laser induced breakdown spectroscopy techniques have been designed (Menneveux et al. 2015). The well-known techniques are DLS (Dynamic Light Scattering) (Brar and Verma 2011; Yaraki and Tan 2020) or NTA (Nanoparticle Tracking Analysis) (Gallego-Urrea et al. 2011) and their numerous modifications (Alasonati et al. 2021) based on which it is possible to determine precisely the size of even very small particles. However, it is quite problematic to determine the concentration of nanoparticles in solution, especially when the concentration is low and the sample is characterized by high clarity. The most well-known techniques for determining the concentration of nanoparticles are gravimetric analysis, bicinchoninic acid assay, Bradford assay and alkaline hydrolysis combined with UV spectroscopy (Khramtsov et al. 2021). Single particle-inductively coupled plasma-mass spectrometry is also used (Chao et al. 2020). Less common, but also encountered methods used to determine the concentration of nanoparticles are based on turbidimetric techniques (Tchami et al. 2019).

Despite the relatively high accuracy of the mentioned methods, they are all characterized by a high degree of complicatedness and the use of complex components. This makes the devices, in which these techniques are used, expensive and difficult to operate. Considering the scale on which various types of nanoparticles are currently used, simpler and cheaper methods for estimating the concentration of nanoparticles in solution are sought. Such methods could be based on certain standards, which, when compared with the analyzed sample, would give satisfactory accuracy. Of course, it is not possible to create one method and one standard for all types of nanoparticles, so each type must be considered separately.

An important feature of a rather large group of nanoparticles is their ability to form so-called Pickering emulsions (Ramsden 1903; Pickering 1907; Metin et al. 2014). Emulsions are systems composed of two phases—oil and water, where one phase is dispersed in the other, while the stability of such a system is due to the presence of a third component—a stabilizer (emulsifier) (Becher 1957). In the case of Pickering emulsions, the role of stabilizer is fulfilled by nanoparticles. Among nanoparticles having the ability to stabilize emulsions, there are numerous types of inorganic particles including silica, clay, and hydroxyapatite (Hap), as well as some organic particles, can effectively serve as Pickering emulsifiers (Yang et al. 2017). The nanoparticles have the ability to absorb on the interfacial surface of the inner phase droplets, forming a coating on the surface that counteracts the droplet coalescence process (Low et al. 2020; du Sorbier et al. 2015). The process of coalescence involves the merging of individual droplets into one larger droplet, which then, due to density differences, leads to creaming or sedimentation, which are the clustering of droplets near the surface or at the bottom of the vessel. As a result, phase separation occurs and the emulsion loses its properties (Simovic and Prestidge 2004).

The stability of Pickering emulsions is dependent on the emulsion formulation, ambient conditions but most importantly on the concentration of nanoparticle addition (Chevalier and Bolzinger 2013). There are many papers in the literature on the stability of Pickering emulsions, where different factors affecting the phase separation rate are studied (Ashby and Binks 2000; Binks and Lumsdon 2001; Cunha et al. 2014; Binks and Yin 2016; Calabrese et al. 2018; Dammak and Sobral 2018; Lebdioua et al. 2018; Bao et al. 2019). All these works prove a direct relationship between emulsion concentration, nanoparticle concentration and destabilization rate. In this work, this reciprocal relationship was used to develop an indirect method for determining the concentration of dilute nanoparticle solutions. This method was based on data obtained for silica nanoparticles; however, similar estimates can be made for other nanoparticles having the ability to form Pickering emulsions. This method can be used to determine the concentration of nanoparticles in aqueous solutions obtained during particle permeability tests through filters in diffusion chambers, without the need for specialized equipment.

**Experimental part**

The basis for the development of a method for determining nanoparticle concentrations in solution was a thorough analysis of the stability of Pickering emulsions, produced using the studied nanoparticles as a stabilizer. Therefore, series of oil-in-water emulsions stabilized with
silica nanoparticles were created and then subjected to detailed stability studies. The internal phase of the emulsion was the mineral oil (Orlen Platinum Classic Life +), the viscosity of which was 48.12 ± 0.05 and the density equaled 886.25 ± 0.15 kg/m³ at 22 °C. The aqueous phase was distilled water. Hydrophilic silica nanoparticles with an average particle diameter of 9 nm were used as a stabilizer. The procedure for creating the emulsion was following: the measured amount of silica was mixed with the aqueous phase and subjected to ultrasound (40 kHz) for 300 s. Then, this mixture was combined with the oil phase and submitted to emulsification, using a high-speed mixer with a rotational frequency of 20,000 s⁻¹ for 180 s. The emulsion prepared in this way was immediately subjected to nephelometric analysis and droplet size distribution analysis. Droplet size distribution was analyzed using Anton Paar Particle Size PSA Analyzer 1190. PSA equipment through laser diffraction technology can determine the particle size and size distribution. Laser diffraction measures particle size distributions by measuring the angular variation in intensity of light scattered as a laser beam passes through a dispersed particulate sample. The angle of the laser beam and particle size have an inversely proportional relationship, where the laser beam angle increases as particle size decreases and vice versa. The instrument allowed the determination of the size diameter distributions of all the systems studied and the determination of the mean diameter. Figure A1 (Appendix) presents the mean diameter values for all the systems studied. To determine the changes in the distribution of the oil droplets diameter, the samples were analyzed immediately after forming the emulsion (t = 0 s) and after 1 h (t = 3600 s). This made it possible to determine if and to what extent the droplet coalescence process occurs. The nephelometric analysis was performed with the use of Turbiscan LabExpert by Formulation. In this device, the measuring cell containing the emulsion sample was scanned with a light beam. Using detectors, the amount of reflected light (BS) and light transmitted (T) through the beam was measured. Scanning took place every 30 s for an hour, which allowed to observe changes in the emulsion structure over time along the sample. Emulsions with different concentrations of the oil phase \( \varphi_e \) and different concentrations of nanoparticles \( \varphi_z \) were analyzed. The concentration range of the internal phase was between 0.10 and 0.60 vol. The addition of silica nanoparticles was defined as the weight concentration in relation to the water phase. The addition of silica nanoparticles ranged from 0.1 to 4.5% by weight. Destabilization studies of the resulting systems were performed for each sample for 1 h. The temperature at which the measurements were carried out was 22 °C.

**Analysis of the destabilization process**

The proposed method for determining the concentration of nanoparticles in solution is based on relating the amount of nanoparticles, the concentration of the emulsion to the rate of the destabilization process. Therefore, in the first step, a detailed description of the disintegration process of the created systems will be presented. Prepared under strictly defined conditions oil-in-water emulsions, stabilized with silica nanoparticles, were analyzed during one hour from the time of their formulation. At this time, the kinetics of the creaming process were observed. An exemplary photo of the tested emulsions after preparation and after 1 h is shown in Fig. 1.

As can be seen visually, from the pictures (Fig. 1), after an hour the creaming process takes place, that is, under the influence of gravity, oil phase drops collect at the top of the sample, while the water phase remains at the bottom of the sample. The kinetics of the creaming process in time can be represented by Fig. 2, where the blue color corresponds to the clear zone (water) and the orange color to the reflecting light zone (oil drops). However, it can be observed that phase separation was finished after this time.

This process can be studied also over time by analyzing the light transmitted through the sample using a Turbiscan LabExpert device. The sample was scanned for an hour, at intervals of 30 s. As a result, diagrams of the transmitted light T and reflected light BS versus sample height for subsequent, successive scans were obtained, as shown in Fig. 3. The first scans were represented in blue color, the next scans moved towards red color.

![Fig. 1 Pictures of emulsion samples stabilized with silica nanoparticles](image-url)
As can be seen from Fig. 3 in the initial time (blue lines), the values of transmitted and reflected light were constant along the height of the sample. However, for subsequent scans, the reflected light values began to decrease at the bottom of the sample, while the transmitted light values increased. The curves of the relationship T and BS versus height determined two zones. The first represents the height at which low BS values and higher T are recorded, which refers to the separated aqueous phase. The second one is represented by high BS and zero T values, which refer to the layer of cream created by the oil droplets of the emulsion. As can be seen, the thicknesses of these zones change over time towards an increase in the water zone and a decrease in the emulsion zone. Changes in the height of the water zone over time for emulsions with an initial concentration of 0.25 vol. using different concentrations of silica nanoparticles are presented in Fig. 4.

As can be seen from the graph in Fig. 4, the thickness of the water phase changes with time depends on the concentration of silica nanoparticles. For emulsions produced at low $\phi_z$ concentrations, the thickness of the oil phase increases rapidly and reaches high values, when compared to emulsions prepared with a large addition of nanoparticles. At the final stage of measurements, the increase in thickness lowered and reached final height ($h_{max}$). In the case of an emulsion with an oil phase of 0.25 vol. at a...
nanoparticle concentration of $\phi_z$ equal 2.67%, the water phase was not observed, the thickness water equaled zero, which means that the emulsion remained stable for the duration of the measurements.

**Analysis of creaming process velocity changes**

By tracking changes of concentration in emulsion samples over time, it is important to know the process velocity changes over time. The velocity of the destabilization process was calculated from the dependence:

$$v = \frac{h_{wi}}{t_i},$$  \hspace{1cm} (1)

where $t_i$—measurement time from the beginning of the destabilization process to time $i$ (s), $h_{wi}$—thickness of water phase at any given time $i$ (m).

For emulsions with an oil phase share of 0.25 vol. at different concentrations of silica nanoparticles, the graph of average velocity versus time, is shown in Fig. 5.

As can be seen in Fig. 5, the average process velocity decreased over time. Finally, it reached the constant value. For the emulsion with the smallest addition of stabilizer, the velocity in the initial stage of the process took large values, but it stabilized over time. In the case of the emulsion at which no creaming process took place (addition of $\phi_z = 2.67\%$ silica/water), the velocity equaled zero. The smaller nanoparticles addition, the higher velocities noted. It should be remembered that the rate of destabilization of such emulsions does not depend only on the addition of nanoparticles (stabilizer) but also on the concentration of the internal phase of the emulsion itself. Therefore, destabilization studies were carried out at different volume fractions of the emulsion internal phase. Figure 6 presents graphs of changes in average velocity over time for different concentrations of emulsion inner phase ($\phi_e$) at the same concentration of stabilizer—$\phi_z = 0.67\%$—for Fig. 6a and $\phi_z = 1.33\%$—for Fig. 6b.

Based on the graphs presented in Fig. 6, it can be observed that the velocity of the process strongly depends on the proportion of the internal phase of the emulsion. The less concentrated the emulsion, the higher velocity recorded. At low concentrated emulsions, in the initial stage of the process, the velocities reached high average values, and as the process decreases, they achieve a constant value. For

![Fig. 5](image-url)  
**Fig. 5** The average velocity of creaming process versus time—at different concentrations of silica nanoparticles additions—emulsion 0.25 vol

![Fig. 6](image-url)  
**Fig. 6** The average velocity of creaming process versus time at different initial concentrations for a $\phi_z = 0.67\%$, b $\phi_z = 1.33\%$
emulsions with higher concentrations, changes in velocity over time were small, and for stable emulsions (φ_e = 0.45, ϕ_z = 1.33%) were zero. Based on the study, it can be concluded that the rate of destabilization is closely related to both the concentration of nanoparticles and the concentration of the emulsion itself, and it is possible to quantitatively capture this relationship.

Determination of a standard curve for estimating the concentration of nanoparticles in solution based on the average destabilization rate

Although changes in destabilization velocity during the process are important, it is more useful to determine the average velocity \( v_m \) at a given time. To determine this, the height \( h_w \) (Fig. 4) was read for the final time (i.e. the value of the aqueous phase thickness recorded for the time 3600 s) and divided by the total time of the process (that is, for 3600 s). This allowed for the average destabilization velocity to be compared with the concentration of the nanoparticles and the concentration of the emulsion itself. Figure 7 presents the dependence of the silica nanoparticle concentration \( \phi_z \) on the destabilization velocity \( v_m \) for three different concentrations of the internal emulsion phase \( \phi_e \). From this graph, it can be observed that this velocity decreases with increasing concentration \( \phi_z \), in other words, an increase in the concentration of silica nanoparticles causes a slower velocity of the destabilization process, noted after an assumed time (1 h). However, the trend is different for different concentrations of the inner phase of the emulsion. For an emulsion with a concentration of 0.1 vol., this decrease is milder than for an emulsion with a concentration of 0.45 vol. For each emulsion concentration, however, the dependence of creaming velocity on stabilizer concentration in each case can be described by a straight line.

The dependence of nanoparticle concentration on the average destabilization rate was also described by a linear relationship:

\[
\phi_z = A \cdot v_m + B, \tag{2}
\]

where symbols \( A \) and \( B \) are the parameters of the straight line, where \( A \) is the directional coefficient and \( B \) is the intersection point with the \( \phi_z \) axis. The values of these parameters are given in Table 1 for emulsion concentrations \( \phi_e \) 0.1; 0.25 and 0.45 vol.

The developed plot of the dependence of \( \phi_z \) on \( v_m \), presented in Fig. 7, can be treated as standard curves for determining the concentration of nanoparticles in an unknown sample. For this purpose, it is necessary to prepare a Pickering emulsion with this sample in exactly the same way as the emulsions were created when preparing the standard curves. Such emulsion should then be subjected to destabilization analysis to determine the average velocity \( v_m \). Knowing the value of \( v_m \), it can be plotted against the standard curve and the nanoparticle concentration \( \phi_z \) can be read off for a given emulsion concentration. Using a well-defined methodology for preparing emulsions and testing the destabilization process under the same conditions, it is possible to obtain results with an accuracy of ± 10%, which has been confirmed experimentally. Taking into account the precision of even specialized equipment, such accuracy is satisfactory for practical use.

Method limitations

The basis for using the method to determine the concentration of nanoparticles in solution is the preparation of a suitable standard curve, i.e., the dependence of the concentration of the nanoparticles on the destabilization rate at a given emulsion concentration. The first limitation is that the nanoparticles under test must have the ability to form Pickering emulsions. If they exhibit such an ability, an appropriate

| Table 1 | Summaries of \( A \) and \( B \) parameters of relation (2) for different emulsion concentrations |
|---------|-------------------|--------|--------|
| Emulsion concentration \( \phi_e \) | \( A \times 10^{-6} \) | \( B \) | \( R^2 \) |
| 0.10    | −0.5269           | 4.263  | 0.986  |
| 0.25    | −0.3895           | 2.514  | 0.968  |
| 0.45    | −0.2332           | 0.908  | 0.998  |
choice of oil phase is necessary. The research presented here relates to a solution of nanoparticles in water. Experimental studies are needed to determine whether solutions with a solvent other than water will be applicable here. An important aspect is choosing the right concentration of the internal phase of the emulsion at which the systems are formed. The concentration of the emulsion must be chosen so that the destabilization process occurs with a given addition of nanoparticles. It must be remembered that above a certain concentration of nanoparticles, the emulsion remains stable and then the application of this method will not give results. Figure 8 shows the dependence of the concentration of silica nanoparticles in the aqueous phase on the concentration of emulsions prepared in the study at which the emulsion remains stable.

The curve of the dependence of $\phi_z$ on $\phi_e$, presented in Fig. 8, defines what are the limitations of using the method at a given emulsion concentration. If in an unknown sample the concentration of nanoparticles in solution is above the presented curve, the method will not give results. Therefore, the emulsion concentration should be chosen so that the amount of nanoparticles in solution is not so high as to form stable emulsions. As can be seen in Fig. 8, the lower the concentration of the internal phase, the higher the concentration of nanoparticles is needed to maintain full stability. Therefore, if higher concentrations of nanoparticles are expected in an unknown sample, emulsions with lower concentrations should be selected. On the other hand, if small amounts of nanoparticles are expected in solution, it is better to create emulsions at higher concentrations, as this can provide better accuracy of results.

**Example of application of the method for determination of nanoparticle concentration based on emulsion destabilization analysis**

To demonstrate how the presented method can be used in practice, we will illustrate an example of its application to determine the concentration of nanoparticles in solution obtained after diffusion. The experiments were performed using diffusion chambers (Franz chambers) to determine the rate of diffusion of nanoparticles through various membranes. After the diffusion process, an aqueous solution of silica nanoparticles of unknown concentration was obtained. Figure 9 shows a scheme of procedure for determining the concentration of nanoparticles in a sample according to the proposed method.

The volume of the sample was 20 ml. The first step was to prepare a Pickering emulsion using the sample. A comparative emulsion concentration of 0.25 vol. was chosen. While preparing the standard curve under the given conditions, an emulsion sample of 100 ml was prepared in the same vessel with the same stirring rates and time. The emulsion sample with the unknown nanoparticle concentration also had to have the same volume at formation. Therefore, the unknown sample was first thoroughly mixed, using ultrasound, with 55 ml of water and then mixed with 25 ml of oil. The emulsification process was then carried out in the same manner.
as in the formation of the standard curve. The emulsions produced were subjected to destabilization analysis for 1 h using Turbiscan. As a result of these analyses, changes in transmitted and reflected light along the sample height were obtained (Fig. 10a). From this, it was possible to estimate the changes in the height of the aqueous phase $h_w$ (Fig. 10b). The final value (after 3600 s) of $h_w$, which was 14.2 mm, has been read from the graph. This value was then divided by the duration of the process and the average velocity $v_m$ was obtained, which equaled $3.94 \times 10^{-6}$ m/s. Having this value, it can be plotted on the calibration curve graph (Fig. 7) or substituted under formula (2) for a concentration of 0.25 vol. and the value $\phi_z$ was calculated, which in the given example was 0.98%. Note, however, that the read concentration $\phi_z$ refers to the concentration of particles in the aqueous phase of the emulsion (100 ml). That is, in this case, we know what the concentration is in the nanoparticles in 75 ml of water. However, we are interested in what the concentration was in the original 20 ml sample. This is easy to calculate because the same proportion as in 75 ml was found in 20 ml. So for our case, we find that the concentration of nanoparticles in the aqueous solution obtained after the diffusion process was 3.67%.

**Summary and conclusions**

An important aspect is the ability to quantify the concentration of nanoparticles present in the solution. In this work, an indirect method to make such determinations has been proposed. This method was based on destabilization analyses of nanoparticle-stabilized emulsions. The relationship between the destabilization rate of such emulsions and their internal phase concentration and nanoparticle content was used. After a thorough investigation of the destabilization mechanism, the relationship between the nanoparticle concentration and the destabilization rate of emulsions with a given internal phase concentration was described. This allowed the development of a standard curve from which the concentration of nanoparticle content in an unknown sample can be determined. An example of the application of such method is also presented, where the concentration of nanoparticles in an aqueous solution obtained after diffusion was determined. The presented method referred to silica nanoparticles, but a similar methodology can be used to determine the concentration of other nanoparticles having the ability to form Pickering emulsions. It should be added, that this method does not require a detailed ananlysis of the destabilization process. Only the average values of its velocity are necessary, which can be determined on the basis of simple optical techniques. The presented method gives satisfactory accuracy, especially considering that the results obtained with even expensive, high-tech equipment are associated with considerable error (Brown et al. 2013; Shang and Gao 2014; Merkus 2020). The paper also presents the limitations of the approach, but it should be emphasized that the advantages of this method are simplicity and lack of the need for specialized equipment. It is worth keeping in mind that despite the continuous development of measurement techniques, simple analytical methods are still in demand for economic reasons.

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**Declarations**

**Conflict of interest** None.
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