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The Use of Ultrasounds in the Preparation of Chemosensory Microstructures †

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Abstract: In many fields, the goal is to obtain structures with small dimensions in the order of micro/nanometers. Small-sized systems can have countless applications in various industries such as cosmetology, medicine, and nutrition technology. Many techniques are used to obtain the most miniature possible spheres, such as interference with the composition, use of surfactants, or mechanical interference: rapid mixing, increased pressure, ultrasound. The use of ultrasound in the development of colloidal systems can be an effective method of reducing size of particles of dispersed phase and influencing the functions they represent. An important aspect here is the time during which the ultrasound is used. In this work, the influence of ultrasound on the chemosensory properties and size of produced ion-sensitive microspheres was investigated and compared. The chemosensory response of the developed microspheres was studied using spectrophotometry and spectrofluorimetry, while the size of the microsphere optodes was estimated by confocal microscopy.

Keywords: ultrasound; microstructures; chemosensors; optode; ion-selective sensors

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1. Introduction

Nowadays, the aim is to produce systems of the smallest size. The small size ensures a wide range of applications. Micro/nano-structures are used in a wide variety of industries, in pharmacy, drug delivery, cosmetics, as well as food technology [1]. Many techniques are used to obtain the most miniature possible layouts, including interference in the composition, surfactants, or mechanical interference: quick mixing, increased pressure, ultrasounds. For the first time, ultrasound was used to create an emulsion in the 1920s by Wood and Loomis. Since then, many scientists have used ultrasounds in their research [2].

Microemulsions are thermodynamically stable systems [3]. They consist of two immiscible liquids, water and oil [4], and the addition of a surfactant [3]. The process of preparing a microemulsion does not require a large amount of energy. For their preparation, a small amount of external energy is sufficient, for example, in the form of mixing. Many high-energy methods are used to obtain spheres of the smallest possible size, e.g., ultrasound [3]. Compared to other methods (mixers, homogenizers), ultrasonic waves give greater control over the properties of prepared emulsions and are more effective [5,6].

This work aimed to create two types of microemulsions in which the dispersed phase were chemosensitive microspheres and to investigate whether the different time of exposure to ultrasound affects their size and sensitivity to selected lipophilic ions. Each of these systems contains a chromoionophore, an ion exchanger, a surfactant, and a plasticizer. Changes in chemosensory properties were observed in the absorbance and fluorescence

modes, while the confocal microscopy observations were used to verify the size of the microspheres.

2. Experimental

2.1. Chemicals

HEPES, Tris-HCl, Pluronic F-127 were supplied by Sigma-Merck (Poznań, Poland). Milli-Q water was used for preparation of all aqueous solutions, including HEPES pH 7.4 and Tris-HCl buffer pH 9.0. Plasticizer ((2-ethylhexyl) sebacate, DOS), lipophilic salts (Tridodecylmethylammonium chloride, TDMAC, Potassium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate, KTFPB,), chromoionophores I and XI were obtained from Fluka (Selectophore). Freshly distilled tetrahydrofuran, THF (Fluka) was used as a solvent for the microspheres' components. All chemicals were used as received.

2.2. Preparation of Microspheres Suspensions

Two types of optical microspheres were prepared: anion-selective (AS) and cation-selective (CS), whose sensory properties were tested by means of model analytes, i.e., lipophilic ions (perchlorate anions and ammonium cations, respectively). The spheres' composition is as follows: AS contained chromoionophore XI, TDMAC, DOS, and Pluronic (F-127), while CS included chromoionophore I, KTFPB, DOS, and Pluronic (F-127). The components that were part of a given sphere type were weighed and then dissolved in 1.5 mL of THF. To thoroughly dissolve all ingredients, the vial was placed in an ultrasonic bath for 5 min. The next step was to pipette a 0.5 mL portion of the THF solution into 4.5 mL deionized water on a Vortex. The last step was to remove the solvent by passing compressed air through the solution (the process was carried out for 1 h). The resulting solutions were then dispensed into four vials. Three of them were sonicated for 5 min, 15 min, and 30 min, respectively, in an ultrasonic scrubber (Sonic-0.5, 80 W, 40 kHz, POLSONIC Palczyński Sp. J., Poland). Clear particle suspensions were obtained and used for further measurements using microtiter plates. In the case of AS, 50 μL of the prepared microsphere suspension was introduced into each well with 50 μL of H_2O for dilution and 100 μL portions of $NaClO_4$ in appropriate concentration were added. In the case of CS, 100 μL portions of prepared suspension of microspheres were pippeted to each well and 100 μL of the analyte was added (solution of NH_4NO_3 in respective concentration). Concluding, we tested the chemosensory response of both microsphere optodes in the presence of 0.1 M NaOH, 0.1 M HCl (for further calculations of protonation degree of chromoionophore), and calibration solutions suitable for each type of microsphere (1 μM-0.1 M $NaClO_4$ and 1 μM-0.1 M NH_4NO_3 for AS and CS optodes, respectively).

2.3. Examination of the Optical Properties of Microspheres

To study the chemosensory properties of the obtained spheres, spectrophotometric and spectrofluorimetric measurements were used. Parameters used: absorbance for both systems was measured in the wavelength range from 300 to 700 nm, while the fluorescence successively—λex = 463 nm, λem from 483 nm to 700 nm for AS microspheres and λex = 614 nm, λem from 636 nm to 700 nm for CS microspheres. They were tested using a Synergy 2 multimode reader (BioTek Instruments, Inc., USA).

The size of the obtained microspheres was examined with a Fluoview FV10i confocal microscope (Olympus, Japan). The following parameters were used to observe the samples: λ_{ex} = 473 nm λ_{em} in range 490–590 nm for AS microspheres and λ_{ex} = 635 nm, λ_{em} in range 660–760 nm for CS microspheres. Microsphere measurements were made with the FV10i-SW software. The samples were observed using a Cellview™ Cell Culture Dish (Greiner Bio-One, Germany) with a glass bottom and four compartments. 300 μL of microsphere suspension were placed in each compartment.

3. Results and Discussion

3.1. Spectrophotometric and Spectrofluorimetric Measurements of Chemosensory Properties

The sensory response of the four independent replicates of the AS and CS microsphere suspensions was examined by recording the spectra in the absorbance and fluorescence modes against perchlorate and ammonium ions, respectively. Test solutions were prepared in appropriate buffers, 0.01 M HEPES pH 7.4 and 0.01 M Tris-HCl pH 9, to make the obtained changes of the chromoionophore spectrum independent of pH. The buffering is necessary so that only the target ion concentration changes the spectral evolution. Four batches of each of the optodes were prepared, which differed from each other in the time of exposure to the ultrasound. The signals were determined as a change in the protonation degree of the chromoionophore (1-α), which means the normalization of the obtained absorbance or fluorescence intensity towards signals recorded for totally protonated and unprotonated chromoionophore (in the presence of 0.1 M HCl and 0.1 M NaOH, respectively). To make the obtained results easier for comparison, the calibration graphs show Δ(1-α), the difference between the values of protonation degrees obtained for respective concentration and the lowest analyte concentration. The obtained results, Figure 1a, clearly show that in the AS optical system, ultrasounds used for 5 and 30 min caused a slight loss of sensitivity towards the tested ions, while the linear range did not change and it spans wide range from 10 µM to 0.1 M. Sonication lasting 15 min considerably worsens the sensitivity of the tested system. The spectrofluorimetric signal Figure 1b gives a similar picture to the data obtained with UV-Vis, and apart from the loss of sensitivity, the perchlorates are determined over the entire concentration range.

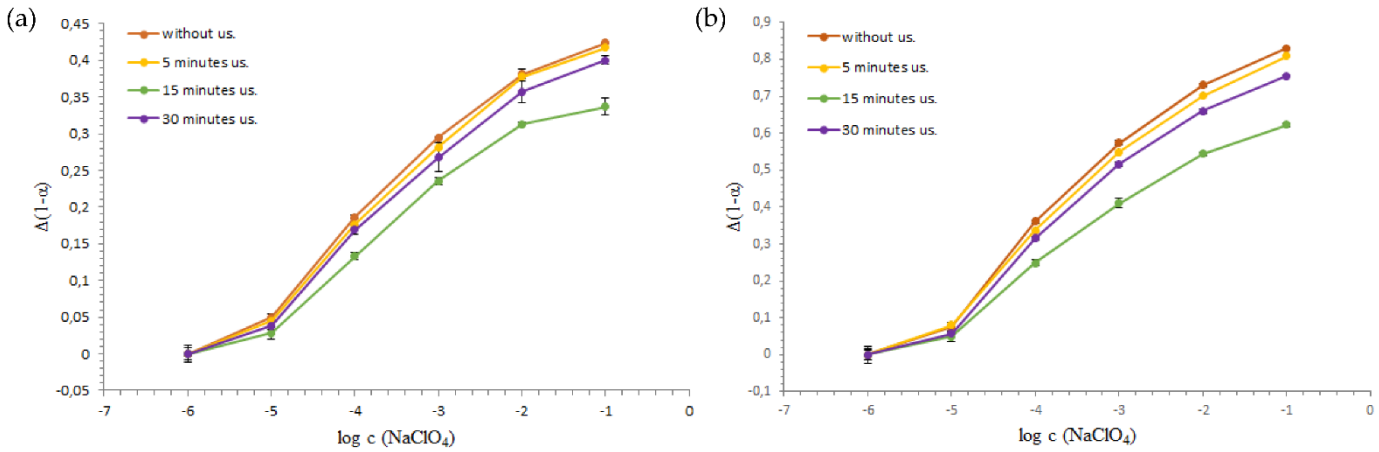

Figure 1. Influence of ultrasound treatment on sensory properties of AS microspheres: (**a**) spectrophotometric signal expressed as a change in protonation degree of chromoionophore for the microspheres without and with ultrasound exposure for varying time, (**b**) the spectrofluorimetric signal expressed as a change in the protonation degree of chromoionophore for the microspheres without and with ultrasound exposure for varying time. All calibration solutions were buffered with 0.01 M HEPES at pH 7.4. Calibration curve points were defined as the mean ± SD; n = 4.

In the case of CS microspheres—Figure 2c, the results obtained for UV-Vis are similar to those for AS—Figure 1a. The use of ultrasound did not contribute to the improvement of the chemosensory properties of the system and could even cause the narrowing of the obtained linear range. However, in the case of fluorescence, opposite effect can be observed, Figure 2d, where the sensitivity of the tested system significantly improves with increasing exposure to ultrasound.

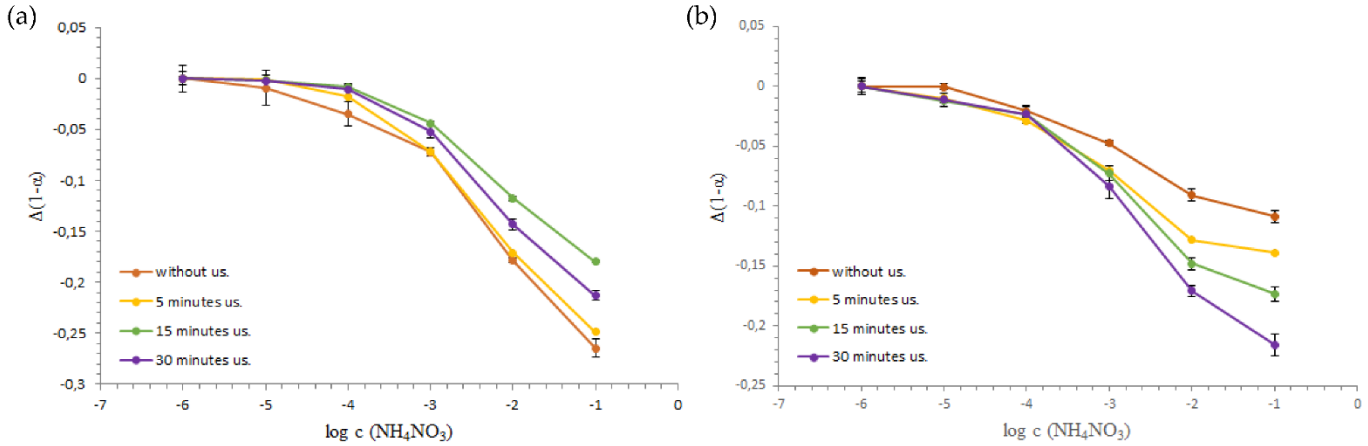

Figure 2. Influence of ultrasound treatment on sensory properties of CS microspheres: (**a**) spectrophotometric signal expressed as a change in protonation degree of chromoionophore for the microspheres without and with ultrasound exposure for varying time; (**b**) the spectrofluorimetric signal expressed as a change in the protonation degree of chromoionophore for the microspheres without and with ultrasound exposure for varying time. All calibration solutions were buffered with 0.01 M Tris-HCl buffer at pH 9. Calibration curve points were defined as the mean ± SD; n = 4.

3.2. Confocal Microscope Imaging

Figures 3 and 4 show pictures of microspheres that were taken with a confocal microscope. The performed analyzes confirmed the spherical shape of the optodes with uniform distribution of chromoionophore on the whole volume of AS microspheres, and capsule-like structure in the case of CS microspheres. It has been noticed that the spheres produced by the 30-min application of ultrasound are smaller in comparison to optodes exposed to ultrasound less intensively. The diameters of the created spheres oscillate between 2–5 μm in the case of both types of microspheres.

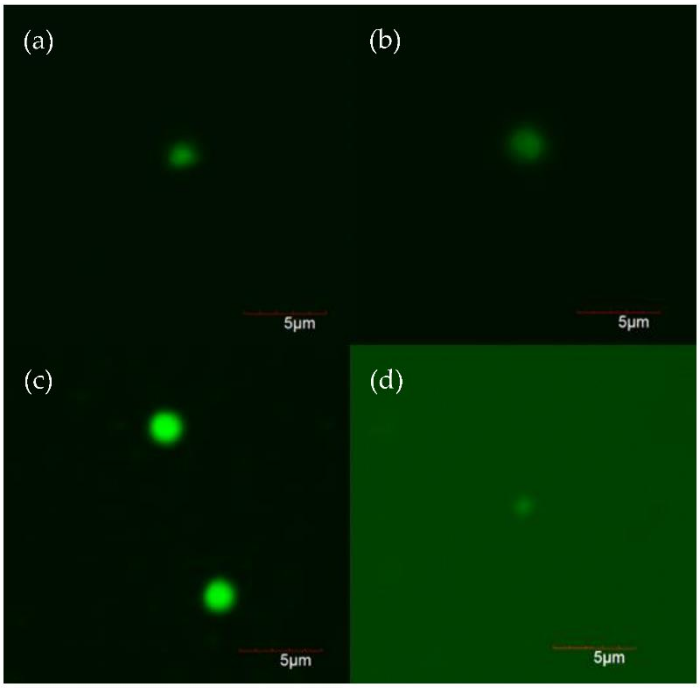

Figure 3. Confocal microscope images of AS microspheres: (**a**) fabricated without the use of ultrasound; (**b**) after 5 min under ultrasound treatment; (**c**) after 15 min under ultrasound treatment; (**d**) after 30 min under ultrasound treatment.

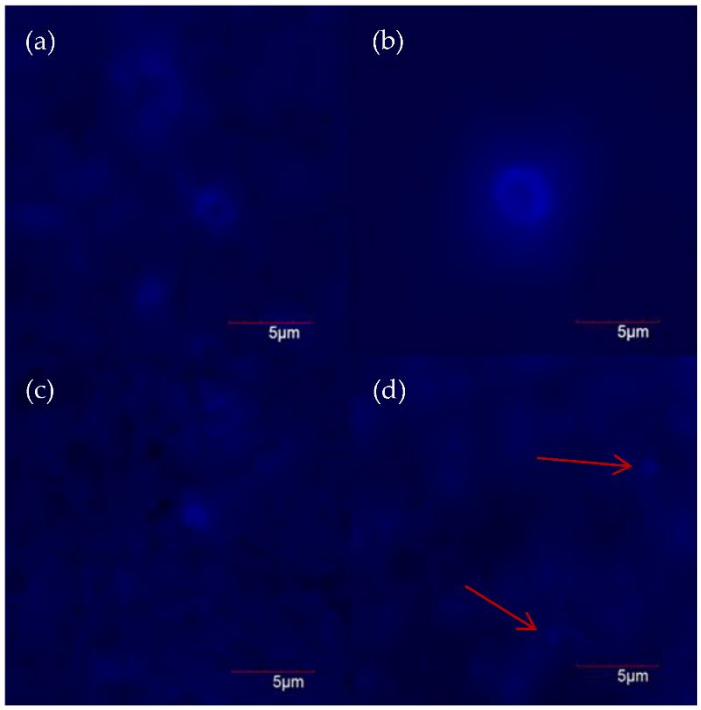

Figure 4. Confocal microscope images of CS microspheres: (**a**) fabricated without the use of ultrasound; (**b**) after 5 min under ultrasound treatment; (**c**) after 15 min under ultrasound treatment; (**d**) after 30 min under ultrasound treatment.

Based on the observation of the fluorescence intensity of the microspheres, it can be concluded that they differ to a small extent in the fluorescence intensity, thus, ultrasound treatment did not affect their size and morphology significantly. However, the fact that they emit a fluorescent signal is indicative of the incorporation of a chromoionophore into the microstructures.

4. Conclusions

The results presented in this paper show that in the case of anion-selective optode microspheres, the use of ultrasound in the preparation stage of the system did not improve their chemosensory properties. In the case of the cation-selective microspheres, no positive changes were noticed concerning the spectrophotometric measurements. On the other hand, in the case of fluorescence measurement, ultrasound caused the increase of the sensitivity of the produced CS microspheres. The best sensory response was obtained for the optodes that was subjected to ultrasound exposure for 30 min. The results obtained on the confocal microscope confirmed that the produced optodes are spherical and their size ranges between 2–5 µm. Moreover, it was observed that with the 30-min application of ultrasound, the obtained microspheres become smaller comparing to those less exposed to ultrasounds. Summing up, although the use of ultrasound can lead to size reduction of the fabricated microstructures, it has limited effect on chemosensory properties of microoptodes, allowing for improvement only in the case of fluorescence response of CS system. Research should be undertaken on further optimization of the tested systems by modifying the amount of surfactant and the duration of the ultrasound effect.

Institutional Review Board Statement:

Informed Consent Statement:

Data Availability Statement:

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Conflicts of Interest: The authors declare no conflict of interest.

References

1. Modarres-Gheisari SM, M.; Gavagsaz-Ghoachani, R.; Malaki, M.; Safarpour, P.; Zandi, M. Ultrasonic nano-emulsification—A review. *Ultrason.—Sonochem.* **2019**, *52*, 88–105, https://doi.org/10.1016/j.ultsonch.2018.11.005.
2. Kumar, R.; Kaur, K.; Pandey, S.K.; Kumar, R.; Uppal, S.; Mehta, S.K. Fabrication of benzylisothiocynate encapsulated nanoemulsion through ultrasonication: Augmentation of anticancer and antimicrobial attributes. *J. Mol. Liq.* **2018**, *263*, 324–333, https://doi.org/10.1016/j.molliq.2018.04.110.
3. McClements, D.J. Nanoemulsions versus microemulsions: Terminology, differences, and similarities. *Soft Matter* **2012**, *8*, 1719–1729, https://doi.org/10.1039/C2SM06903B.
4. Tartaro, G.; Mateos, H.; Schirone, D.; Angelico, R.; Palazzo, G. Microemulsion Microstructure(s): A Tutorial Review. *Nanomaterials* **2020**, *10*, 1657, https://doi.org/10.3390/nano10091657.
5. Saravana, P.S.; Shanmugapriya, K.; Gereniu CR, N.; Chae, S.J.; Kang, H.W.; Woo, H.C.; Chun, B.S. Ultrasound-mediated fucoxanthin rich oil nanoemulsions stabilized by κ-carrageenan: Process optimization, bio-accessibility and cytotoxicity. *Ultrason.—Sonochem.* **2019**, *55*, 105–116, https://doi.org/10.1016/j.ultsonch.2019.03.014.
6. Kaltsa, O.; Michon, C.; Yanniotis, S.; Mandala, I. Ultrasonic energy input influence on the production of sub-micron o/w emulsions containing whey protein and common stabilizers. *Ultrason.—Sonochem.* **2013**, *20*, 881–891, https://doi.org/10.1016/j.ultsonch.2012.11.011.