Preparation and Characterization of Polymeric Nanoparticles: An Interdisciplinary Experiment

Maria J. Ramalho[†] and M. Carmo Pereira*[,†]

[†]LEPABE, Department of Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

S *Supporting Information*

ABSTRACT: In this work, a laboratory experiment to introduce graduate students to nanotechnology is described. Students prepared poly(lactic-*co*-glycolic acid) (PLGA) nanoparticles using two different synthesis procedures, a single and a double emulsion−solvent evaporation method. The students also performed a physicochemical characterization of the prepared nanoparticles (NPs) by determining the hydrodynamic size and zeta potential values through Dynamic and Electrophoretic Light Scattering, respectively. The experiments were designed to be accomplished in two 180 min sessions and have been successfully conducted by 24 graduate students.

Single/double emulsion | Organic solvent evaporation | 200 nm filter | NPs collection

KEYWORDS: *Graduate Education/Research, Nanotechnology, Colloids, Chemical Engineering, Interdisciplinary/Multidisciplinary, Laboratory Instruction, Hands-On Learning/Manipulatives, Inquiry-Based/Discovery Learning*
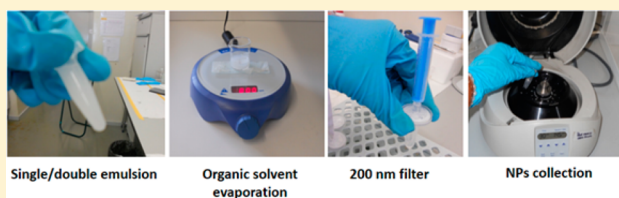
■ INTRODUCTION

The term colloids or colloidal dispersions refers to heterogeneous systems composed of microscopic or submicroscopic particles of a dispersed material in a continuous phase. Many colloids are dispersions of nanoparticles (NPs). Nanoparticles are assemblies of atoms or molecules at nanometer scale with unique and useful technical properties, different from those of the bulk material, attributable to their small size. With the size reduction, the surface/volume ratio is enlarged providing a larger reactive area of interface. The properties of this interface define the colloidal dispersions features.

These unique physicochemical characteristics justify the growing popularity of NPs in several applications as health, textile and food industries.[1,2] Their use in the health sector has proven to be extremely advantageous in creating breakthroughs in diagnosis, treatment and monitoring of several diseases, enabling early diagnosis and the controlled delivery of drugs.[3] Several materials have been widely studied and, in particular, poly(lactic-*co*-glycolic acid) (PLGA) proved to be an excellent candidate due to its well established clinical safety and advantageous physicochemical properties.[4]

The aim of this laboratory experiment is to provide graduate students with an introduction to the concepts of nanomaterials preparation using nanoparticle synthesis by the botton-up approach, by proposing an experiment of nanoencapsulation for upper-level undergraduate and graduate students in materials science, biotechnology, biomedical sciences, interfacial sciences, nanosciences; nanotechnology, pharmacy and supramolecular chemistry. This involves the preparation and characterization of PLGA NPs with the encapsulation of hydrophobic and hydrophilic molecules by the emulsion−solvent evaporation method. Pedagogical goals for this laboratory experiment

include: the practice of new laboratory techniques for the encapsulation of different molecules in nanocarriers, and the contact with the classical laboratory techniques used for the characterization of nanocarriers.

Despite the increasing use of nanomaterials in several industrial applications, not many laboratory experiments introducing different colloidal nanosytems in chemistry education curriculum are reported in the literature. A few experiments concerning metallic NPs such as gold, silver, and zinc oxide NPs were already described in this *Journal*.[5−8] Zinc oxide application in nanotechnology presents several advantages due to its characteristic properties. Hale and colleagues (2005) introduced a technique for the synthesis of zinc oxide NPs, in a third-year laboratory program for nanotechnology. The aim of this work was to provide a safer technique for the ones previously described. In this laboratory projects, students were presented with several theoretical concepts such as growth kinetics and nanoparticles physicochemical properties.[5] Guedens and co-workers (2014) introduced a microemulsion synthesis method for zinc hydroxide NPs to high school and undergraduate students. This experiment demonstrated that these NPs could be used in the sunscreens composition due to their ability to absorb UV radiation.[6] Sharma and co-workers (2012) introduced students with an experimental procedure to prepare gold NPs using tea leaves. One of the major goals of this work was, beyond present concepts related to nanotechnology, to present the concept of green chemistry to chemistry undergraduate students.[7] Orbaek and colleagues

A

(2014) prepared a protocol for the production of silver NPs for an undergraduate chemical laboratory. The authors intended to provide students with basic concepts of nanoparticles synthesis and physicochemical characterization.[8]

Also, only a few laboratory experiments to introduce polymer nanomaterials in the undergraduate and graduate chemistry curriculum are reported in the literature.[9−12] One experiment involves the encapsulation of lipophilic compounds into polymeric micro and nanoparticles with the goal to introduce the emulsion−solvent evaporation method.[9] In another work, a protocol for the design of a polymeric drug delivery system is described, focusing on the study of alginate polymerization reactions and its degradation and consequent release of the encapsulated compounds.[10] A similar laboratory experiment was also described using gelatin.[11] Other work describes the preparation methodology for PLGA films using a different synthesis technique from the one described in our work, but the main goal was to study the release kinetics of the prepared polymeric films.[12] Unlike these studies, our work not only focuses on the nanoencapsulation of molecules with different chemical characteristics and the characterization techniques but also introduces the concepts of colloidal stability. Several concepts related to the physical-chemical characterization of nanoparticles were also presented. For these reasons, in this work a protocol for the preparation of PLGA NPs to be implemented for educational purposes is presented.

■ EXPERIMENT OVERVIEW

Background

PLGA NPs can be produced using several techniques. The characteristics of the obtained NPs, as size and structural organization, depend upon the chosen method and solvents.[13−15] One of the most common methods, emulsion−solvent evaporation, is based on the shaping of the bulk material into nanostructures. Two types of the emulsion−solvent evaporation technique can be used: the single and double emulsion. The choice of each method depends upon the hydrophilicity of the drug to be encapsulated.[15]

In the single emulsion−solvent evaporation method, both drug (hydrophobic) and polymer are first dissolved in a water immiscible organic solvent, to form a single-phase solution. Then, this organic oil phase is emulsified in water containing an appropriate emulsifier. If the molecule to encapsulate is hydrophilic, it is necessary to prepare a double emulsion. This method is very similar to the single emulsion; however, the main difference is the formation of two emulsions instead of a single one. Here, the drug is dissolved in the aqueous phase. To induce size reduction to the nanoscale, sonication is used. The size reduction is induced through a high-energy process, breaking up the primary droplets of dispersed oil into nanosized droplets.[16,17] The subsequent removal of the organic solvent transforms droplets of dispersed phase into solid particles.[18−20]

To obtain a high efficiency, the organic solvent and the surfactant agent must be chosen carefully. A suitable solvent should be able to dissolve the polymer and to be poorly soluble in the continuous phase, and exhibit high volatility and low toxicity.[19] As for the emulsifier, the most suitable surfactant is the one that promotes a greater reduction of the interfacial free energy, thus, avoiding the coalescence and agglomeration of drops, leading to a stabilized emulsion.[19] Ethyl acetate and

Pluronic F127 met the aforementioned requirements and were chosen for this experimental procedure.

Reagents

PLGA Resomer RG503H (50:50; MW 24 000−38 000), ethyl acetate, Pluronic F127, and coumarin-6 (MW 350.43) were purchased from Sigma-Aldrich (St. Louis, MO). Trypan blue (MW 872.88) was acquired from Biochem Chemopharma (Loire, France). For TEM sample preparation, uranyl acetate (dehydrate, 424.146 g/mol) was purchased from Electron Microscopy Sciences (Hatfield, U.K.). All aqueous solutions were prepared in deionized and filtered ultrapure Milli-Q water (Milli-Q Academic, Millipore, France).

Experimental Details

The experimental work was designed to be accomplished in two lab sessions. The synthesis protocol was conducted in a 180 min session and the characterization in another session. Twenty-four students worked in groups of three. In the first lab session, all the student groups prepared NPs for each methodology protocol here described. In the second session, the student groups characterized the NPS in parallel by rotating the techniques.

Synthesis of PLGA NPs: Encapsulation of Hydrophobic Molecules

The single-emulsion solvent evaporation technique was used for the encapsulation of coumarin-6 (C6), a fluorescent probe used as a model molecule for hydrophobic drugs. This method is a suitable choice for encapsulating hydrophobic drugs. PLGA (10 mg) was first dissolved in ethyl acetate (0.1 mL), and for encapsulation purpose, 1 mg of C6 was added. Then, 0.2 mL of an aqueous phase of 1% (w/v) Pluronic F127 was added dropwise to the prepared organic phase, and the moisture was vortexed. As a result, an oil-in-water emulsion was formed. To induce the nanosized droplets, a sonication process was carried out in an ultrasonic cleaner water bath (45 kHz, 5 min). During the sonication, the Eppendorf tube containing the emulsion should be immersed in a beaker with ice to avoid the water heating. The emulsion was then poured very fast into 2.5 mL of 0.1% (w/v) Pluronic F127, and the mixture was stirred (800 rpm) at room temperature for 30 min with a nitrogen stream in order to completely evaporate the organic solvent. It is important to note that this transfer step must be completed very quickly, in order to avoid PLGA NPs aggregation before being stabilized by the surfactant. The resulting suspension was filtered with Millex-GP Filter Units (0.2 μm, poly(ether sulfone), Millipore Express, Ireland) and incubated at 4 °C overnight to avoid NPs aggregation and increase their stability.

Synthesis of PLGA NPs: Encapsulation of Hydrophilic Molecules

Double-emulsion solvent evaporation technique is a suitable choice for encapsulating hydrophilic drugs. This method is very similar to the previous one; however, the main difference is the formation of two emulsions instead of a single one. In this method, trypan blue (TB) was used as a model for hydrophilic drugs. First, 0.02 mL of an aqueous solution of 100 mg/mL TB was added dropwise to 0.1 mL of organic phase containing PLGA polymer dissolved in ethyl acetate (100 mg/mL). Subsequent vortex and sonication yielded the first emulsion, a water-in-oil emulsion. Then, 0.2 mL of the 1% (w/v) Pluronic F127 aqueous solution was added dropwise to the prepared emulsion, and the remaining protocol was performed as already

described. As a result, a water-in-oil-in-water emulsion is formed.

Characterization of PLGA NPs

The size, polydispersity index, zeta potential, and morphology were the parameters used to characterize the produced nanoparticles. The prepared NPs were collected by centrifugation (14 000 rcf, 30 min), and the obtained pellet was resuspended in ultrapure water. Both pellet and supernatant were saved for analysis.

Dynamic Light Scattering (DLS) is a well-known technique to determine the size of nanoparticles in dispersion or solution. In addition to the size, DLS also allows the determination of the polydispersity index (PdI), an indicative of the heterogeneity of NPs sizes in a suspension.[21] For a near-monodisperse sample, a PdI of 0.1 or lower is expected.[22] The presence of aggregates can distort the results, since the equipment analyzes them as a large size single particle, resulting in higher PdIs.[23] In this work, DLS measurements were performed using a ZetaSizer Nano ZS (Malvern Instruments, Worcestershire, U.K.). All the determinations were performed in disposable cells (Sarstedt, Germany) and using water as dispersant medium. Mean values for each preparation were obtained by triplicate measurements. Each measurement was performed with 12 runs.

Electrophoretic light scattering (ELS) was used to evaluate the zeta potential, a key parameter for the NPs suspension stability. Particles with very positive/negative zeta potential values will repel each other and resist to aggregation. On the contrary, low absolute zeta potential values results in aggregation and flocculation.[2,24] A physically stable nano-suspension will have a minimum zeta potential of absolute value of 30 mV.[13] Usually, the laboratory equipment incorporates several techniques in a single compact unit. In fact, in this work ZetaSizer Nano ZS also provided measurements of zeta potential by ELS. It used folded capillary cells from Malvern (Worcestershire, U.K.), and the dispersant medium was water. Mean values for each preparation were obtained by triplicate measurements. Each measurement was performed with 12 runs.

Transmission electron microscopy (TEM) is one of the most efficient and versatile tools for the characterization of nanoparticles morphology. The morphological examination of the NPs was performed by TEM Yeol Yem 1400 at an accelerating voltage of 80 kV (Tokyo, Japan). For that purpose, the samples were prepared with negative staining to improve their visualization. Ten microliters of NPs suspension in ultrapure water was stained with 2% (v/v) uranyl acetate for 45 s, immobilized on copper grids (Formvar/Carbon on 400 mesh Cu (50) from Agar Scientific), and air-dried for TEM visualization.[25] Uranyl acetate is a heavy metal salt capable of scattering electrons, and therefore enhancing the image contrast.[26]

Fluorescence microscopy was used for the morphologic studies of the prepared NPs using an Eclipse Ti−S inverted microscope (Nikon, Carnaxide, Portugal). The fluorescent dyes entrapped in the NPs' matrix allow a fluorescent visualization of the PLGA NPs.

Effect of pH on NPs Surface Charge

The effect of the suspension pH on the NPs surface charge was assessed by zeta potential measurements. After preparation, blank PLGA NPs were resuspended in aqueous buffers with different pH values (pH = 4, 7, and 10) and their zeta potential values were determined.

■ HAZARDS

All reagents should be handled with students wearing gloves and lab coats. Ethyl acetate is toxic, highly flammable and slightly hazardous in case of skin or eye contact. Owing to the presence of its organic vapors, students should perform this experiment in a fume hood. Uranyl acetate is a highly toxic and radioactive material and therefore should be handled with extreme care. Trypan blue is hazardous in case of ingestion. The other chemicals used do not have specific hazard recommendation.

■ RESULTS AND DISCUSSION

Students prepared and characterized PLGA nanoparticles obtained by a single or double emulsion−solvent evaporation method depending on the molecule's hydrophobicity. Figure 1 shows the picture of an obtained emulsion.

Figure 1. Colloidal suspension of PLGA NPs prepared by the single emulsion−solvent evaporation method.

The NPs obtained by both preparation methods exhibited mean diameters smaller than 200 nm as shown in Figure 2 with a narrow size distribution (PdI ≤ 0.1).

The emulsion technique yielded a well-stabilized nano-suspension, exhibiting high negative zeta potential values (<−30 mV). This parameter plays a crucial role in maintaining the nanosuspension stabilization. Therefore, the colloidal stability results from the electrostatic stabilization provided by the surface charge due to the PLGA carboxylic end-groups, and the steric stabilization due to the Pluronic F127 layer adsorbed on the NPs surface. During particle formation, the stabilizer adsorbs onto the NP surface enhancing the colloidal stability acting as a mechanical barrier inhibiting the aggregation of the NPs.[27] In Figure 3, a morphologic examination performed by TEM shows this Pluronic layer surrounding PLGA NPs. Additionally, TEM analysis revealed spherical and uniform shaped nanoparticles.

The zeta potential values of a colloidal nanosuspension depend on the NP surface charge and on the free salt ion concentration. As Figure 4 shows, zeta potential of the PLGA
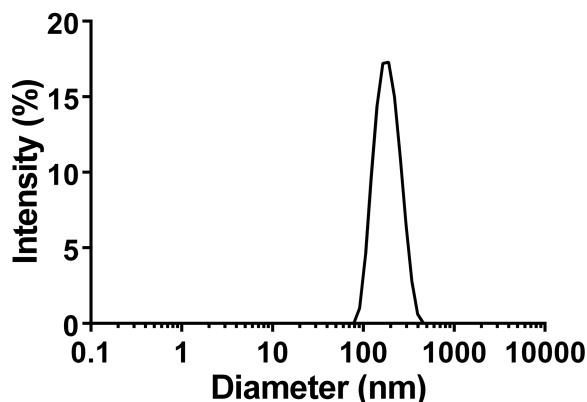
Size distribution by intensity

Figure 2. Hydrodynamic diameter of the PLGA NPs prepared by the single emulsion solvent evaporation method measured by DLS technique.
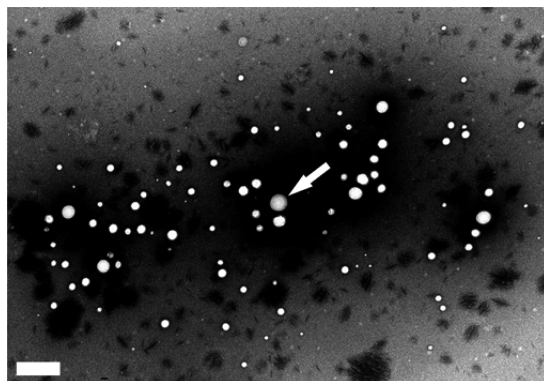

Figure 3. Transmission electron microscopy images of the PLGA nanoparticles prepared by the single emulsion solvent evaporation method; the scale bar corresponds to 500 nm. The PLGA NPs appear as white spheres, and the white arrow indicates the Pluronic layer surrounding the PLGA NP.
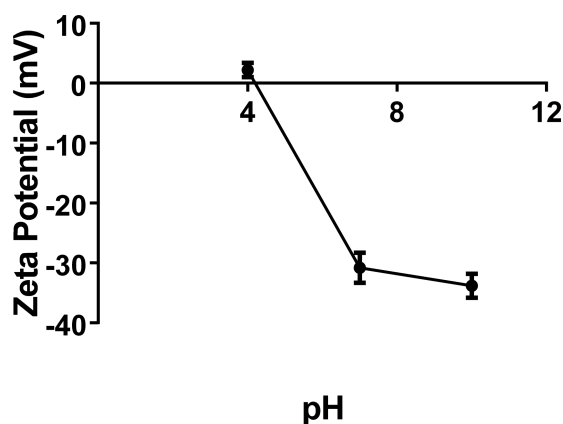

Figure 4. Variation of zeta potential of PLGA NPs with the pH values of the medium. Zeta potential was measured in 0.01 M PBS buffer adjusted to different pH values with either 0.1 M sodium hydroxide or 0.1 M hydrochloric acid.

NPs prepared using the single-emulsion method decreased with the decrease of pH value of the medium. The PLGA NPs acquired a positive charge at acidic pH due to the protonation of the carboxylic groups in PLGA. Oppositely, at neutral and basic pH most of surface charges of PLGA nanoparticles become negative. A similar behavior was observed in the PLGA NPs synthesized using the double-emulsion method.

At acidic pH, a second population of NPs with sizes at the micrometer range appeared, suggesting that the NPs aggregation had occurred. Due to the protonation of the carboxylic groups in PLGA at acidic pH values, charge repulsion between the NPs/electrostatic stabilization is reduced.

Fluorescence microscopy analysis revealed spherical shaped nanoparticles as shown in Figure 5. Although not having the
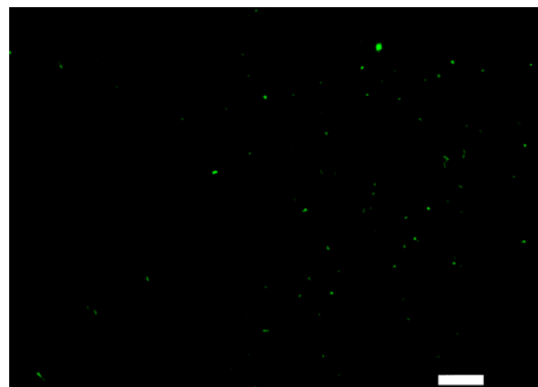

Figure 5. Fluorescence microscopy image of the prepared PLGA nanoparticles. The PLGA NPs were labeled with a fluorescent dye, coumarin-6. The scale bar corresponds to 10 μm.

necessary resolution for the NPs morphological characterization, this microscopy technique allowed the students to confirm the entrapment of the fluorescent marker into the NPs polymeric matrix.

As shown in the results presented above, both methods of synthesis, single and double-emulsion, yielded PLGA NPs with similar physicochemical characteristics. This allow us to conclude that both techniques are suitable methodologies for the preparation of PLGA NPs, and the choice of the most appropriated method to use depends only upon the hydrophilicity of the molecule to be encapsulated.

■ PEDAGOGICAL ASSESSMENT

This experiment was designed to be introduced to a nanotechnology course for graduate students. The theoretical concepts related with nanoparticles synthesis and characterization were presented to students in the corresponding lecture course, and for that reason, students were expected to understand this laboratory work. However, some theoretical concepts were still provided to the students in the Background section in Notes for students (see Supporting Information file). Before the beginning of the experimental session, the instructor presented a brief introduction to the work and asked a few questions to the students about the experiment. At the end of the laboratory work, the students prepared a report with the obtained results and their discussion. The students were encouraged to compare their results to others described in the literature.

Summative assessment was achieved through the elaboration of the final report. The report showed that the students understood the theoretical concepts related to the preparation and characterization of nanoparticles. The percent of students answering correctly on report questions is presented in Table 1.

Table 1. Comparison of Students' Correct Responses on Report Questions Probing NP Conceptual Understanding

Questions and Statements for Student Response	Correct Answers, % ($N = 24$)
1. What is the function of the Pluronic F127?	90
2. Are both of the prepared NPs colloidal suspensions stable? Justify.	80
3. Discuss about the surface charge of the PLGA nanoparticles.	90
4. Explain the changes in the surface charge of NPs with the variation of pH values.	85

Formative assessment was achieved by accompanying the students in the lab, carefully observing if students were able to successfully conduct all the tasks and understanding the theoretical fundaments of the characterization techniques. Comparison of the results obtained by the students with the ones reported in the literature for PLGA NPs shows that students successfully conducted the laboratory techniques yielding nanoparticles with the expected physicochemical properties. The results from the formative assessment also showed that all the students were able to describe the conducted experiment, and successfully relate it to the analysis of the obtained results. The student's gain of a set of laboratory skills was also assessed. Table 2 lists these skills and the respective percentage of the students conducting the tasks successfully.

Table 2. Comparison of Laboratory Steps Students Completed Correctly

Laboratory Skills	Students Conducting the Step Correctly, % ($N = 24$)[a]
Calculations for solutions preparation	80
Balance use	90
Micropipette use	80
Emulsion preparation	75
Sonicator use	100
Filtration	100

[a]These results indicate the gain of the intended laboratory skills.

Although the authors' priority in formative assessment was the students learned laboratory skills, student's perspective on the experiment was also assessed. Students did not exhibit unexpected difficulties during the experimental protocol execution, and seemed to be very interested in understanding the theoretical explanation for each protocol step. Ninety percent of the students evaluated the experimental work as "stimulating", over 80% of the students indicated that the difficulty level of this activity was appropriate for their grade, and finally approximately 90% of students stated that this laboratory experiment increased their enthusiasm for nanotechnology.

CONCLUSION

A preparation method for PLGA NPs was described where students were able to have a first contact with nanomaterials and some important concepts related with other subjects like surface forces, emulsion techniques, and characterization techniques. Preparation and characterization of PLGA NPs, for the encapsulation of both hydrophilic and hydrophobic molecules, has been the subject of many scientific articles,[18,20]

but none of them have an educational purpose targeting graduate students.

Although it is a simple experimental procedure, this protocol involves many concepts of colloidal science and chemistry, allowing the students to explore the concept of hydrophobic/hydrophilic molecules, as well as to explore the physical and chemical conditions that control the stability and the aggregation of the NPs suspension.

Synthesis and characterization of nanocarriers and comparing results to literature were important research skills gained in this experimental work.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available on the ACS Publications website at DOI: 10.1021/acs.jchemed.5b00837.

Instructions for the students and notes for the instructor (PDF, DOCX)

AUTHOR INFORMATION

Corresponding Author

*E-mail: mcsp@fe.up.pt.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

REFERENCES

(1) Barnes, G.; Gentle, I. Chapter 1 - Introduction. In *Interfacial Science: An Introduction*; Oxford University Press Inc.: New York, NY, 2011; pp 1−9.

(2) Barnes, G.; Gentle, I. Chapter 9 - The liquid-solid interface: adsorption; colloids. In *Interfacial Science: An Introduction*; Oxford University Press Inc.: New York, NY, 2011; pp 242−287.

(3) Kawasaki, E. S.; Player, A. Nanotechnology, nanomedicine, and the development of new, effective therapies for cancer. *Nanomedicine* **2005**, *1* (2), 101−9.

(4) Danhier, F.; Ansorena, E.; Silva, J. M.; Coco, R.; Le Breton, A.; Préat, V. PLGA-based nanoparticles: An overview of biomedical applications. *J. Controlled Release* **2012**, *161* (2), 505−522.

(5) Hale, P. S.; Maddox, L. M.; Shapter, J. G.; Voelcker, N. H.; Ford, M. J.; Waclawik, E. R. Growth Kinetics and Modeling of ZnO Nanoparticles. *J. Chem. Educ.* **2005**, *82* (5), 775.

(6) Guedens, W. J.; Reynders, M.; Van den Rul, H.; Elen, K.; Hardy, A.; Van Bael, M. K. ZnO-Based Sunscreen: The Perfect Example To Introduce Nanoparticles in an Undergraduate or High School Chemistry Lab. *J. Chem. Educ.* **2014**, *91* (2), 259−263.

(7) Sharma, R. K.; Gulati, S.; Mehta, S. Preparation of Gold Nanoparticles Using Tea: A Green Chemistry Experiment. *J. Chem. Educ.* **2012**, *89* (10), 1316−1318.

(8) Orbaek, A. W.; McHale, M. M.; Barron, A. R. Synthesis and Characterization of Silver Nanoparticles for an Undergraduate Laboratory. *J. Chem. Educ.* **2015**, *92* (2), 339−344.

(9) Teixeira, Z.; Durán, N.; Guterres, S. S. Annatto Polymeric Microparticles: Natural Product Encapsulation by the Emulsion−Solvent Evaporation Method. *J. Chem. Educ.* **2008**, *85* (7), 946.

(10) Friedli, A. C.; Schlager, I. R.; Stephen, W. W. Demonstrating Encapsulation and Release: A New Take on Alginate Complexation and the Nylon Rope Trick. *J. Chem. Educ.* **2005**, *82* (7), 1017.

(11) Sylman, J. L.; Neeves, K. B. An Inquiry-Based Investigation of Controlled-Release Drug Delivery from Hydrogels: An Experiment for High School Chemistry and Biology. *J. Chem. Educ.* **2013**, *90* (7), 918−921.

(12) Stevens, E. S.; Baumstein, K.; Leahy, J.-M.; Doetschman, D. C. Polymer−Plastics Experiments for the Chemistry Curriculum. *J. Chem. Educ.* **2006**, *83* (10), 1531.

(13) Hans, M. L.; Lowman, A. M. Biodegradable nanoparticles for drug delivery and targeting. *Curr. Opin. Solid State Mater. Sci.* **2002**, *6*, 319−327.

(14) Danhier, F.; Ansorena, E.; Silva, J. M.; Coco, R.; Le Breton, A.; Preat, V. PLGA-based nanoparticles: an overview of biomedical applications. *J. Controlled Release* **2012**, *161* (2), 505−22.

(15) Makadia, H. K.; Siegel, S. J. Poly Lactic-co-Glycolic Acid (PLGA) as Biodegradable Controlled Drug Delivery Carrier. *Polymers (Basel, Switz.)* **2011**, *3* (3), 1377−1397.

(16) Kentish, S.; Wooster, T. J.; Ashokkumar, M.; Balachandran, S.; Mawson, R.; Simons, L. The use of ultrasonics for nanoemulsion preparation. *Innovative Food Sci. Emerging Technol.* **2008**, *9* (2), 170−175.

(17) Mahdi Jafari, S.; He, Y.; Bhandari, B. Nano-Emulsion Production by Sonication and Microfluidization—A Comparison. *Int. J. Food Prop.* **2006**, *9* (3), 475−485.

(18) Frasco, M. F.; Almeida, G. M.; Santos-Silva, F.; Pereira, M.d.C.; Coelho, M. A. N. Transferrin surface-modified PLGA nanoparticles-mediated delivery of a proteasome inhibitor to human pancreatic cancer cells. *J. Biomed. Mater. Res., Part A* **2015**, *103* (4), 1476−1484.

(19) Li, M.; Rouaud, O.; Poncelet, D. Microencapsulation by solvent evaporation: state of the art for process engineering approaches. *Int. J. Pharm.* **2008**, *363* (1−2), 26−39.

(20) Ramalho, M. J.; Loureiro, J. A.; Gomes, B.; Frasco, M. F.; Coelho, M. A. N.; Pereira, M. C. PLGA nanoparticles as a platform for vitamin D-based cancer therapy. *Beilstein J. Nanotechnol.* **2015**, *6*, 1306−1318.

(21) Tscharnuter, W. Photon Correlation Spectroscopy in Particle Sizing. In *Encyclopedia of Analytical Chemistry*; Meyers, R.A., Ed.; John Wiley & Sons, Ltd: Tarzana, CA, 2006; pp 5469−5485. DOI: 10.1002/9780470027318.a1512.

(22) Nobbmann, U.; Connah, M.; Fish, B.; Varley, P.; Gee, C.; Mulot, S.; Chen, J.; Zhou, L.; Lu, Y.; Shen, F.; Yi, J.; Harding, S. E. Dynamic light scattering as a relative tool for assessing the molecular integrity and stability of monoclonal antibodies. *Biotechnol. Genet. Eng. Rev.* **2007**, *24*, 117−128.

(23) Jiang, J.; Oberdörster, G.; Biswas, P. Characterization of size, surface charge, and agglomeration state of nanoparticle dispersions for toxicological studies. *J. Nanopart. Res.* **2009**, *11* (1), 77−89.

(24) Freire, J.; Domingues, M.; Matos, J.; Melo, M.; Veiga, A.; Santos, N.; Castano, M. R. B. Using zeta-potential measurements to quantify peptide partition to lipid membranes. *Eur. Biophys. J.* **2011**, *40* (4), 481−487.

(25) Parveen, S.; Sahoo, S. K. Long circulating chitosan/PEG blended PLGA nanoparticle for tumor drug delivery. *Eur. J. Pharmacol.* **2011**, *670* (2−3), 372−383.

(26) Haschemeyer, R. H. M.; Meyers, J. Negative staining. In *Principles and Techniques of Electron Microscopy: Biological Applications*; Hayat, M.A., Ed.; Van Nostrand Reinhold Company: New York, NY, 1972; pp 110−114.

(27) Vandervoort, J.; Ludwig, A. Biocompatible stabilizers in the preparation of PLGA nanoparticles: a factorial design study. *Int. J. Pharm.* **2002**, *238* (1−2), 77−92.