DEVELOPMENT OF POLYMER-LIPID HYBRID NANOPARTICLES: CONVENTIONAL AND MICROFLUIDIC METHODS

Mirela M. Bortoli[1], Bruna G. Carvalho[1], Caroline C. Sipoli[2] & Lucimara G. de la Torre[1]*

[1] *Chemical Engineering / Department of Materials and Bioprocess Engineering/ Post Graduation, Universidade Estadual de Campinas/ School of Chemical Engineering, Campinas, Brazil.*
[2] *Chemical Engineering/ Department of Materials and Bioprocess Engineering, Federal University of Technology, Apucarana, Brazil.*
** Corresponding author's email address: ltorre@unicamp.br*

ABSTRACT

Among the numerous areas that nanotechnology encompasses, the medical field stands out due to the different applications of nanostructures for health treatments. Nanoparticles (NPs) are drug-delivery systems developed for transporting various active compounds. Intending to improve the incorporation and delivery of active compounds, hybrid nanostructures formed by polymers and lipids have stood out as promising systems. The present work proposes to develop hybrid NPs formed by lipids and zein, a natural biopolymer from corn. As controls, the zein NPs and liposomes were initially produced at different concentrations. Then, hybrid lipid/zein NPs were produced and evaluated using the bulk method, and microfluidic routes were explored in detail.

Keywords: Nanoparticle 1. Polymers 2. Lipids 3. Microfluidics 4.

1 INTRODUCTION

Nanotechnology is a field that explores the formation of different nanostructures, which can have applications in medical, environmental, and cosmetic fields[1]. Among these nanostructures, nanoparticles (NPs) are effective nanocarriers for transporting drugs and bioactive compounds for different disease treatments[2]. Additionally, the nanometric scale provides these materials with unique physicochemical properties that favor their use in biological areas[2]. In nanotechnology, hybrid NPs are examples of carriers that have been gaining attention in recent research due to the versatility and advantageous properties provided by the association of different structures, such as proteins and lipids[3].

NPs derived from natural sources are promising due to numerous functional groups facilitating self-organization and interaction with different components[3]. Zein, a natural protein found in corn, is an explored source for NP formation, primarily due to its hydrophobic characteristics that enable the encapsulation of poorly water-soluble components[3]. However, some limitations, such as aggregation sensitivity and degradability, reduce the drug delivery potential[4]. Thus, to improve the carrier properties, one of the strategies approached is to associate a new structure with these NPs. Liposomes can be used to form hybrid systems due to their versatility in incorporating both hydrophobic and hydrophilic compounds and their similarity to the cell membrane[4]. The proposal of forming core/shell hybrid systems can promote the combination of both structures' advantages and reduce each's disadvantages, thereby forming more effective drug delivery systems and improving mechanical stability, structural integrity, and biocompatibility[3]. The main preparation routes (bulk methodologies) for hybrid NPs formation are nanoprecipitation and high-pressure homogenization[3]. However, promising technological alternatives for hybrid formation are still being investigated, including microfluidics[8]. Microfluidics is a field that deals with the flow of fluids and devices on micrometric scales, promoting more refined mixing, which may generate the formation of more homogeneous NPs[7,8]. In this context, the work aims to study the formation of hybrid NPs using the conventional (bulk) methodology and microfluidics as an alternative route.

2 MATERIAL & METHODS

The experiments conducted aimed to evaluate the interaction of the zein (Sigma-Aldrich) with natural egg phosphatidylcholine lipids (EPC) (Lipoid) and cholesterol (CHOL) (Sigma-Aldrich) for the formation of hybrid NPs. As the first step, zein NPs and liposomes were produced separately, and then hybrid NPs formation was investigated using conventional and microfluidic methods.

Synthesis of zein NPs: The synthesis of zein NPs was carried out using the conventional nanoprecipitation method[5]. Zein was dissolved in an 85:15 (v/v) ethanol solution and stirred on a magnetic stirrer for one hour, resulting in a final solution of 20 mg. mL^{-1}. The solution was filtered using a vacuum pump and a 0.45 µm filter. Subsequently, the zein solution was dripped with a syringe pump (Harvard model PHD ULTRA) at a flow rate of 1 $mL.min^{-1}$ into 45 mL of ultrapure water and kept under constant stirring to promote the formation of NP.

Synthesis of liposomes: Liposomes were synthesized by preparing a lipid solution containing EPC and CHOL (66:34) at a concentration of 25 mM using the ethanol injection method[6]. A volume of 500 µL of the lipid solution was injected with the aid of a syringe pump at a flow rate of 1.48 $mL.min^{-1}$ into a beaker containing 5 mL of ultrapure water and kept under agitation for 4 minutes at 10,000 rpm using an ultra Turrax (IKA®T25 digital model). Six samples were prepared with different concentrations (0.1, 0.2, 0.5, 1.0, 1.5, and 2.27 mM). Finally, the hybrid NPs zein/lipid was prepared using the nanoprecipitation method.

Hybrid NPs zein/lipid formation (ethanol injection): Before forming the hybrid NPs, the EPC/CHOL (66:34) lipid solution at 25 mM and the main zein hydroalcoholic solution at 55.20 mg.mL^{-1} were prepared. Aliquots with different concentrations (2.40, 1.60, 1.20, 0.80, 0.40, 0.35, 0.25, 0.18, 0.16 mM) of zein were prepared by diluting the main zein solution. A solution of zein/lipid was formed by mixing 500 µL of the lipid solution (25 mM) and 500 µL of the zein hydroalcoholic solution with all different concentrations in 3 mL of 85:15 (v/v) hydroalcoholic solution. This mixture formed final solutions with the respective lipid/zein molar ratios (10, 17, 20, 33, 50, 70, 100, 130, 167). The solution previously produced was then dripped in a stirring tank at a flow rate of 1.48 mL.min^{-1} into 5 mL of ultrapure water and kept under agitation with an ultra-Turrax for 4 min at 10,000 rpm, as illustrated in Figure 1A.

Hybrid NPs zein/lipid formation (microfluidic method): A T-junction microfluidic device with channel dimensions of 140 µm and a depth of 50 µm was used for the microfluidic route. Using the 167 (lipid/zein) molar ratio, the zein and lipid solution was prepared for a total flow rate (TFR) of 200 µm.min^{-1}(Figure 1B) with different flow rate ratios (FRR) 10, 5, and 1.

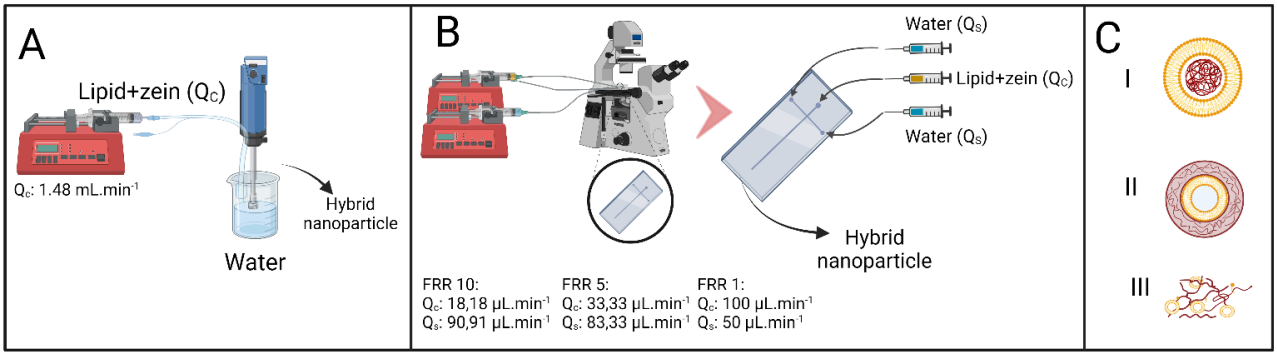

Figure 1 Representation of the formation of hybrid NPs by the conventional route (ethanol injection) (A). Representation of the formation of hybrid NPs by microfluidics (B). Values side flow (Q_s) and central flow (Q_c) for each FRR 10, 5, and 1. Type of Hybrid NPs (C). Created with Biorender.com.

Physicochemical properties analysis: All NPs (zein NPs, liposomes, and hybrid NPs) were investigated in terms of mean diameter and polydispersity (PDI) using Dynamic Light Scattering (DLS) and zeta potential (ZP) measurements performed using a Malvern Zetasizer. All samples were measured at 25ºC and a final lipid concentration of 0.2 mM in triplicate.

3 RESULTS & DISCUSSION

Synthesis of zein NPs and liposomes: The aim of the experiments was to evaluate the interaction of zein with EPC and CHOL for the formation of hybrid NPs. The first step was to analyze the formation of zein NP and liposomes separately as a control process. The formation of zein NPs studied at different concentrations (0.5; 1.0; 2.0; 3.0; 4.0; 5.0 mg.mL^{-1}) resulted in average mean diameters between 83.18 nm and 130.63 nm, PDI between 0.14 and 0.16 indicating good homogeneity, and ZP values ranging from 28.52 to 43.23 mV, characterizing them as cationic NPs. The liposomes, on the other hand, showed average mean diameter ranging from 69.35 nm to 85.56 nm, a PDI of 0.21±0.01 and a ZP with an anionic character with values in the range of-9.68 to -19.10 mV. The difference in zein NP and liposome charges can contribute to the formation of hybrid NPs by electrostatic interaction. Therefore, after understanding the behavior of the obtained structures (zein NP and liposomes), the ideal composition of zein and lipids was studied.

Hybrid NPs zein/lipid formation (ethanol injection): Different proportions of zein were investigated, keeping the lipid concentration fixed at 1.38 mM. From Table 1, it can be observed that the mean diameter of the hybrid NPs varied as the amount of zein increased. The same behavior can be seen for ZP values, which oscillated from anionic (-10.64 mV) to cationic (24.32 mV). In other words, the amount of zein present for formation influences the physicochemical properties of the NPs. The precipitate formation was observed for ratios 17, 20, and 33, which presented ZP close to isoneutrality (ZP close to zero). This behavior may be associated with strong electrostatic interactions between zein (cationic charge) and lipids (negative charge), leading to destabilization. However, in 50, 70, 100, 130, and 167 molar ratios, the ZP presented an anionic character and average mean diameter greater than 191 nm and PDI between 0.55 and 0.29. So, it suggests a possible formation of hybrid structures, with zein being incorporated by the liposomes (Figure 1C-I), as the charge presented in these ratios approximates the charges of the liposomes. Finally, for ratio 10, the ZP is positive, and the average mean diameter of the hybrid NP is close to 389 nm. This fact may indicate that the NP formation occurred differently from what was intended with the liposome being incorporated by zein NP (Figure 1C-II).

Table 1 Physicochemical properties of hybrid NPs obtained by ethanol injection method.

Molar ratio	Mean diameter (nm ± SD)	PDI ± SD	ZP (mV±SD)
10	389.63±0.29	0.30±0.02	24.32±0.25
17	-	-	11.87±0.09
20	-	-	6.92±0.02
33	-	-	-4.24±0.53
50	530.87±6.64	0.32±0.02	-9.22±0.41
70	235.97±2.52	0.47±0.01	-10.87±0.24
100	200.11±2.87	0.55±0.03	-8.26±0.44
130	190.90±1.71	0.44±0.02	-9.26±0.34
167	191.87±2.69	0.29±0.002	-10.64±0.07

*Data referring to triplicates of independent experiments.

In addition to the ZP, analysis of the average mean diameter of NPs suggests the formation of hybrid NPs. Comparing the mean diameter of zein NPs and liposomes with hybrid NPs, a "sum" of structures indicates incorporation to form hybrids since all values found for the hybrid structure are greater than those found for the control.

According to the data, the molar ratio 167 exhibited the most satisfactory characteristics for forming hybrid NP systems, such as low PDI and smaller sizes. In this sense, Figure 2 presents a size distribution of the hybrid structure and the control zein NP at a concentration of 0.5 mg.mL^{-1} and liposomes at 1.5 mM. It is possible to observe that the sizes of the hybrid NPs slightly increased when compared to zein NPs and liposomes, which may characterize the incorporation of zein and liposomes. As the zeta potential is negative, it is also possible that the structure's core is composed of zein, and the lipids are being used as coating (shell).

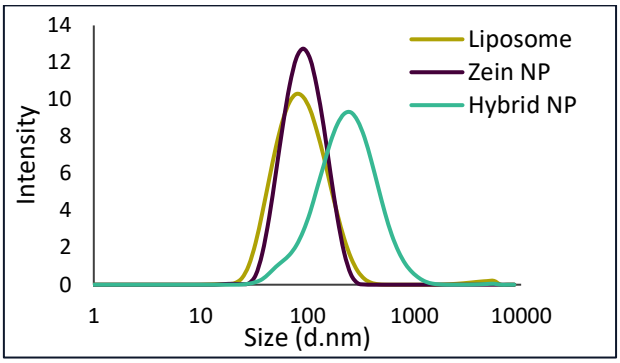

Figure 2 Intensity-weighted size distribution of the liposomes, zein NPs, and hybrid NPs. The lines distribution represent the profile of three independent experiments.

Hybrid NPs zein/lipid formation (microfluidic method): Following the input configuration employed in the bulk methodology, the formation of hybrid NPs was then investigated via microfluidics. Using a T-junction device and the zein-to-lipid ratios corresponding to the molar ratio 167, at a TFR of 200 µL.min^{-1} and different FRR (10, 5, and 1). The formation of aggregates at the inlets was noticed for all conditions, leading to accumulation and clogging of the device, as illustrated in Figure 3. It indicates that the pre-mixture of zein and lipid leads to an initial disorganized formation of structures; however, while agitation provided by ultra-Turrax promoted self-organization, the same was not observed via the microfluidic route.

Figure 3 Bright field image of macroaggregates formed in the microfluidic device channel under hybrid NPs synthesis in 5 sec (A) and 15 sec (B).

4 CONCLUSION

The results demonstrated the feasibility of producing hybrid NPs via a single-step conventional methodology. By studying the proportions between lipid and zein, it was possible to find the most suitable ratio for forming hybrid NPs with satisfactory physicochemical properties: the ratio 167 (1.38 mM of lipids to 0.006 mM of zein). However, when the process was transferred to the microfluidic route, employing the same input conditions and concentrations of reagents, it was observed that it did not yield satisfactory results. Upon executing the experiment, the formation of macroaggregates in the channel was observed, leading to their clogging. New experiments will be conducted to optimize the microfluidics process.

REFERENCES

1 CHAMUNDEESWARI, M. JESLIN, J. VERMA, M. L. 2019. Link Springer. 17. 849-865.
2 CRYER, A. M. THORLEY, A. J. 2019. Pharmacology & Therapeutics. 198. 189-205.
3 GAGLIARDI, A. VOCI, S. GIULIANO, E. et al. 2021. Materials Science and Engineering: C. 128. 112331.
4 TENCHOV, R. BIRD, R. CURTZE, A. E. ZHOU, Q. 2021. ACSNANO. 15. 16982-17015.
5 AGOSTINHO. M. S. P. 2022. Produção de nanopartículas de zeína para incorporação dos compostos bioativos presentes no extrato do açafrão-da-terra.
6 DE LA TORRE, L. M. PESSOA, A. C. S. et. al. 2021. DNA Vaccines. 2197. 253-269.
7 BALBINO, T. A. SERAFIN, J. M. JESUS, M. B. DE LA TORRE, L. G. 2017. Colloids and Surfaces B: Biointerfaces.152. 406-413.
8 ZHANG, L. CHEN, Q. MA, Y. SUN, J. 2020. ACS Applied Bio Materials. 3. 107-120.

ACKNOWLEDGEMENTS

The authors thank to the funding agencies CAPES (Coordination for the Improvement of Higher Education Personnel), CNPEM (National Center for Energy and Materials Research), and FAPESP (Research Support Foundation of the State of São Paulo) for all the support.