SYNTHESIS OF PEPTIDE-BASED NANOPARTICLES VIA MICROFLUIDIC PROCESS

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Abstract: *This work investigates the technological development of microfluidic systems for the formation of peptide-based nanoparticles using amino acids through polymerization, aiming applications in the pharmaceutical area. Peptides are molecules that have significant activities in various biological systems. The aim of this research is to investigate microfluidic processes for the production of peptide-based nanoparticles. The nanoparticles synthesis was carried out using a polydimethylsiloxane (PDMS)/glass hydrodynamic flow focusing microfluidic device, applying a central stream of coupling agent N,N′-Diisopropylcarbodiimide (DIC) and two lateral streams composed by Arginine (ARG). The effect of molar charge ratio $R_{AA/DIC}$ was evaluated in terms of hydrodynamic diameter and polydispersity index. Peptide-based nanoparticles were produced by microfluidic process with good physicochemical properties. In this way, this work aims to contribute to the nanotechnology field, especially towards new advances for drug delivery applications.*

Key-Words*: nanoparticles, peptides, microfluidics, nanothecnology*

Introduction: Researches in the field of nanotechnology has allowed potential applications in nanomedicine, mainly in the treatment of diseases and the development of techniques that promote the sustained release of biomolecules or to direct the delivery to specific sites minimizing side effects [1]. Thus, the encapsulation of drugs in nanoparticles is a promising alternative for the sustained release of different bioactive molecules. Amino acids and peptides are a class of materials that stand out for the production of these nanoparticles, since they have capacity for self-organization, structural variability, biocompatibility and facilitate the recognition by biological receptors. Their characteristics allow the formation of myriad nanostructures [2]. In this context, microfluidics emerges as a technological alternative for the production of nanoparticles, since it involves the flow of fluids in devices that have channels with micrometric sizes. Some of the advantages of using such devices are reduced sample volume, reduced reagent cost, and the ability to acquire large numbers of information from samples [3]. Therefore, the nanoparticles obtained in microchannels present a promising method when compared to conventional, allowing the production of nanoparticles with low polydispersity index and particle size. In this way, the work aims to evaluate the potential production of peptide-based nanoparticle via microfluidic platform, for future investigations in the pharmaceutical area.

Experimental: The production of polydimethylsiloxane (PDMS)/glass microfluidic devices was based on soft lithography. Peptide-based nanoparticles were produced using the amino acid Arginine (ARG) in HEPES that were crosslinked with N,N′-Diisopropylcarbodiimide (DIC) coupling reagent. Basically, DIC (30μL/min) in isopropanol was pumped in the central stream and hydrodynamicaly focused by two adjacent streams of aqueous solution of ARG (75μL/min) (Figure 1). For the production of peptide nanoparticles, was evaluated the effect of the molar charge ratio $R_{AA/DIC}$ ranged between 25, 10, 5, 3.33, 2.5, 0.5, 0.25 (mM), in this case, ARG was prepared at a concentration of 0,5 mM in HEPES (Figure 2). The buffer HEPES was prepared at 100 mM in pH (7). The peptide-based nanoparticles were characterized in terms of average hydrodynamic diameter and polydispersity index, using Malvern Zetasizer equipment.

Results and discussion: The production of peptide-based nanoparticles via the microfluidic route was hypothesized considering mixing principles involved in the hydrodynamic focusing technique as shown in Figure 1. This technique allows the contact of two phases of soluble fluids, being one an organic core stream containing the crosslinking agent (DIC) is compressed hydrodynamically by two side chains containing amino acids. As the flow occurs, the diffusive process (in the direction transverse to the flow) will contribute to the alcohol and water counter diffusion and also allows the gradual cross linking to occur, generating the nanoparticles.

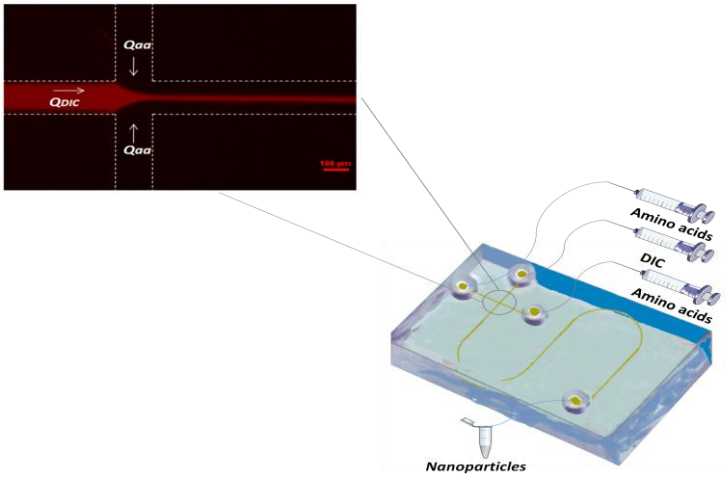

Figure 1: Synthesis of nanoparticles using microfluidic device based on hydrodynamic focusing.

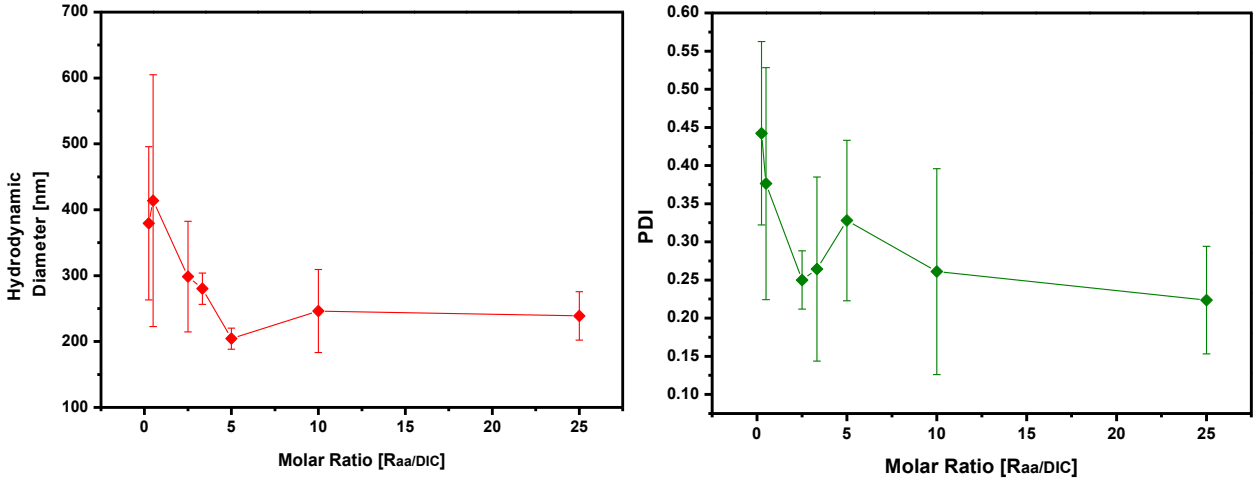

Figure 2: Characterization of peptide based nanoparticles in average hydrodynamic diameter and polydispersity index.

According to Figure 2, the average hydrodynamic diameter of the peptide-based nanoparticles increases when $R_{AA/DIC}$ decreases, so the higher the concentration (mM) of the DIC coupling agent, the larger the diameter of the nanoparticles. It is known that DIC is used to crosslink the peptide bond between amino acids (ARG), generating the peptides and thus, to reach the stable conformation of nanoparticle. In relation to the PDI, the same behavior was observed as seen in the mean diameter, $R_{AA/DIC}$ decreases and the PDI tends to increase, so the excess of DIC produces polydisperse nanoparticles. This may be associated with the fact that the higher the concentration (mM) of DIC, higher is effect of amino acids reticulation, leading for nanoparticles formation of biggest diameter. Therefore, the ARG was able of produced peptide-based nanoparticles with potential application in drug delivery, for example, due mainly the size being in nanometric scale.

Conclusion: Peptide-based nanoparticles were produced through the microfluidic process therefore the hydrodynamic focusing technique was efficient in favor of the diffusion process for the production of the nanoparticles. However, in relation, to variation of diameter, due mainly to the presence of DIC, new studies and characterization tests must be performed in order to find better conditions for the production of peptide-based nanoparticles, since these are fundamental to guarantee an ideal condition for application in nanomedicine.

References and acknowledgements:

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