doi:10.20396/revpibic.v0i0.id

XXVI Congresso de Iniciação Científica Unicamp
17 a 19 de outubro Campinas | Brasil

PHARMACOTECHNICAL DEVELOPMENT OF BIOPOLYMER NANOSTRUCTURES OF CHITOSAN TO RELEASE BIOACTIVES

RANIERY WALADARES RICARDO*; JULIANA SOUZA RIBEIRO COSTA; VERÔNICA MUNIZ COUTO; LAURA DE OLIVEIRA NASCIMENTO

Abstract

Nanostructured drug delivery systems are formulations containing particles usually fabricated with polymers. They can be administered by all drug routes (depending on their characteristics) and in various pharmaceutical forms, including nanoemulsions, nanosuspensions, nanofilms and lyophilized powders. The size, shape and surface load influence the functionality of nanoparticles, as well as their stability.

Key words: *C-phycocyanin, chitosan, bioactive.*

Introduction

C-Phycocyanin (C-FC) is a water-soluble blue protein pigment that, together with Chlorophyll, captures light for photosynthesis in cyanobacteria. C-FC has several applications in the pharmaceutical area as natural food coloring, fluorescent marker applied to medical diagnosis and immunological assays.[1] In addition to commercial uses, the protein has widely described antitumor potential. However, this potential demands more robust formulations for protein stability and release. Nanoparticles of chitosan and alginate, for example, potentiate the action of the antimicrobial protein nisin for food preservation. The nanoparticles of chitosan crosslinked with tripolyphosphate are reproducible, scalable and with great capacity of encapsulation of active principles, reason why was chosen for this project.

In view of this, this study consists of the pharmacotechnical development of chitosan nanostructured formulations, cross-linked with sodium tripolyphosphate (TPP), for incorporation and subsequent sustained release of C-FC.[2]

Results and Discussion

In order to identify the best formulation incorporated with C-FC, a complete factorial design of three factors and two levels with center point was carried out and formulations were analyzed in relation to size and zeta potential by Dinamic Light Scattering (DLS), Zetasizer Nano ZS, Malvern Instruments Ltd, Malvern, England and by Nanoparticle Tracking Analysis (NTA), Nanosight, Malvern Instruments Ltd, Malvern, England).

The critical parameters studied were chitosan concentration, TPP concentration and rotation (rpm).

The DLS calculates the Brownian motion for the resulting fluctuations of diffraction intensity of the laser at an angle and for a set of particles while the NTA records this motion by image and for each particle. The chitosan nanoparticles are formed by ionic gelation, based on the electrostatic interaction between chitosan and TPP.

In Table 1 below, it can be observed that formulation 6 (F6) was the one that obtained the best results, in both Average size (212 ± 6,5nm), PDI (0,252) and Zeta potential (27,9 ± 1,11mV) . But even so, in a period of 7 days there was also precipitation of the formulation 6.

Table 1. Nanopaticles with C-phycocyanin: 2^3 factorial design with central point.

Formulations	Chitosan (mg/mL)	TPP (mg/mL)	Rotation (rpm)	Average size (nm)	SD	PDI	D10 (nm)	D50 (nm)	D90 (nm)	Zeta (mV)	SD
1	2,5	0,25	600	838,0	17,4	0,420	420	1130	3380	40,87	1,68
2	2,5	0,75	600	841,0	83,1	0,676	329	1270	4520	34,93	4,99
3	2,5	0,75	1000	543,8	122,3	0,479	235	699	3240	40,90	2,80
4	2,0	0,50	800	337,6	19,1	0,336	175	431	1070	38,30	3,30
5	1,5	0,25	1000	365,2	20,3	0,378	172	490	1320	31,80	3,21
6*	1,5	0,75	1000	212,0	6,5	0,252	114	248	560	27,9	1,11
7	2,0	0,50	800	658,8	28,4	0,443	315	912	2540	33,90	7,44
8	2,5	0,25	1000	526,8	41,6	0,409	301	708	1590	41,40	11,30
9	1,5	0,25	600	X	X	X	X	X	X	X	X
10	1,5	0,75	600	X	X	X	X	X	X	X	X
11	2,0	0,50	800	X	X	X	X	X	X	X	X

Table 2. Results by NTA of formulation 6.

Mean size (nm)	210.8 ± 4
D10 (nm)	124.3 ± 2.6
D50 (nm)	184 ± 3.9
D90 (nm)	296.7 ± 12.8
Concentration (particles/mL)	$1.92e^9 \pm 4.47e^6$

In Table 2, when analyzed the results of the NTA, we can verify that through this technique the average sizes of the nanoparticles are well below compared to the DLS.

Conclusions

Although some formulations precipitated, there was no direct correlation with one of the factors evaluated. The best performance formulation was 1,5 mg/mL chitosan, 0,75 mg / mL TPP and 1000 rpm agitation, with a mean size of 212,0 ± 6.5 nm, PDI of 0.252 (desirable = the lowest) and Zeta potential of 27.9 ± 1.1mV (desirable ~ 30).

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