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Formulation development of chitosan-based nanoparticles for delivery of a hydrophilic hexapeptide

Chen Y, Chan P and Benson HAE*

School of Pharmacy, Curtin University of Technology, GPO Box U1987, Perth, WA 6845, Australia
E-mail: h.benson@curtin.edu.au

Introduction

Coacervation as a technique for producing nanoparticles has many advantages. It involves only aqueous solvents and mild processing conditions and is therefore ideal for maintaining the stability of proteins and peptides.

In this study, we examined the formulation of novel nanoparticles using chitosan (CS) in combination with sulfobutyl ether-7- β -cyclodextrin sodium (SB-CD) for delivery of hydrophilic peptides. The hexapeptide dalargin, a Leu-enkephalin analogue with opioid activity, was chosen as a model peptide.

Methods

Formulation of solvent-free nanoparticles was achieved by a coacervation process in water using CS with SB-CD at room temperature, dispersed by a homogeniser. The hexapeptide was dissolved in SB-CD solution prior to addition to the CS solution which induced the coacervation process.

The physicochemical properties of the nanoparticles such as the mean particle size, zeta-potential and peptide entrapment efficiency of resultant nanoparticles were characterized. Particle size and zeta potential were analyzed using a Malvern Zetasizer 3000HS. Dalargin loading was calculated by the difference between the total amount of peptide added into the polymer solution initially and the amount remaining in the supernatant after centrifugation of nanoparticle suspension.

Release characteristics of dalargin-loaded nanoparticles were studied by membrane diffusion using Franz-cells with phosphate buffered saline pH 7.4 with sodium azide (1%) as the dissolution medium, under the controlled agitation at 37°C (Figure 1). Dalargin concentration was determined by a HPLC assay method involving a gradient system. The stability of dalargin in nanoparticles was characterised by HPLC analysis.

Results and Discussion

Nanoparticles were formed only within a narrow weight ratio range of the two polymers (CS:SB-CD) from 5:5 to 5:8. The smallest nanoparticle size of 254.2 nm was obtained when CS:SB-CD was 5: 6.5 with CS MW 100 kDa. The use of high MW CS (400 kDa) led to the formation of larger particle size nanoparticles ($\geq$ 329.9 nm). The zeta potential of CS/SB-CD nanoparticles was found to be very different from that of CS/dextran sulfate nanoparticles reported previously[1], showing positive zeta potential from 15.2 mV to 35.6 mV depending upon the ratio. The higher the CS:SB-CD ratio, the greater the magnitude of zeta potential (Table 1).

The entrapment efficiency of CS/SB-CD was 11.6%. The stability study revealed that dalargin tends to undergo rapid degradation in the absence of a preservative.

Table 1 Characteristics of CS/SB-CD nanoparticles

Weight ratio of CS: SB-CD	Molecular weight of CS (Da)	Mean particle size (nm)	Average zeta potential (mV)
5 : 5	100,000	392.4	35.6
	400,000	837.6	38.2
5 : 6	100,000	318.2	32.2
	400,00	579.5	33.7
5 : 6.5	100,000	254.2	23.3
	400,000	478.9	31.0
5 : 7	100,000	263.2	25.1
	400,000	329.9	27.7
5 : 8	100,000	395.5	15.2
	400,000	>1000	Not done

SB-CD nanoparticles released dalargin in a sustained fashion with the release rate half of that of a control (dalargin solution) (Figure 1).

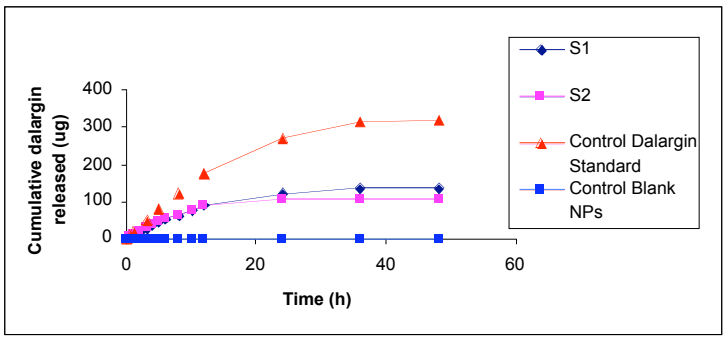

Figure 1 Dalargin release from CS/SB-CD nanoparticles

Conclusions

This study demonstrated that anionic cyclodextrin can be used in complex coacervation with chitosan to form nanoparticles. The presence of SB-CD in the nanoparticle formulation, the weight ratio of CS to SB-CD and the molecular weight of CS influenced the properties of the

nanoparticles and their dalargin carrying and release characteristics. However, further study is required to understand the factors controlling dalargin incorporation into CS/SB-CD nanoparticles to increase the peptide entrapment efficiency.

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References

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