Development of casein micellar pediatric formulations

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INTRODUCTION

The current need for medicines specifically designed for children has driven the development of pediatric drug formulations. Important issues to consider are the ease of administration, dose flexibility, palatability, safety of excipients, stability and therapeutic equivalency of pediatric dosage forms (1).

In the present work casein (CN) based micellar formulations are considered attractive vehicles for the oral delivery of pediatric drugs, since caseins are nontoxic, biodegradable, GRAS (generally regarded as safe) materials and nanoencapsulation of drugs can improve their bioavailability. Chemical crosslinking of casein by carbodiimide (EDC) has been studied as an approach to improve the stability of the CN micelles and to tailor drug release. EDC was chosen because it induces cross-linking of biomaterials, without incorporation in the linkages, simply changing to water-soluble urea derivatives, which exhibit very low cytotoxicity (2).

This work aimed to study casein micelles as potential pediatric drug vehicles and, also, evaluate the effect of crosslinking and processing conditions (freeze-drying) on the casein micellar formulation.

MATERIALS AND METHODS

Casein dispersions (5mg/ml) were produced by casein solubilization with NaOH (1M) at room temperature under stirring (500 rpm) and then the pH was adjusted with HCl (1M) to 6.5, the natural pH of milk. Paracetamol (PC), one of the most widely used drugs in pediatrics, was the model drug. PC and/or crosslinker (EDC, 0.03M, during 24h) were added to casein dispersions. The dispersions obtained were freeze (-80ºC/24h)-dried (-50ºC, 0.035 mbar for 12h) and stored at room temperature over silicagel until further use. The characterization of casein micellar formulations, before and after freeze-drying (FD), was done in triplicate. Micelles were characterized for shape by transmission electron microscopy, particle size and zeta potential. FTIR spectra (4000–400cm^{-1}) and their second derivatives were collected and thermograms from DSC analysis (15-200ºC) obtained. PC was quantified by HPLC using C18 5 µm column and phosphate buffer (0.01M NaH_2PO_4 pH 5.8) to acetonitrile gradient. Free (non-micellar) PC was determined in the filtrate, after removal of micelles by filtration (Vivaspin 500, 10 kDa cut off) and centrifugation at 8000 rpm. Bound (micellar) PC (i.e. encapsulation efficiency, EE) was calculated according to the formula:

$$EE\ (Bound\ PC, \%) = \frac{Total\ PC - Free\ PC}{Total\ PC} \times 100$$

The release of PC from uncrosslinked and EDC-crosslinked micelles was compared in phosphate buffered saline (PBS) pH 7.4. Pure PC (control) and PC loaded casein micelles were placed into a dialysis membrane (cut off 12kDa) suspended into 100mL of PBS pH 7.4, at 37ºC, under stirring (100 rpm). Samples were taken at time intervals.

RESULTS AND DISCUSSION

Microscopic images showed particles (Fig. 1) with round shape.

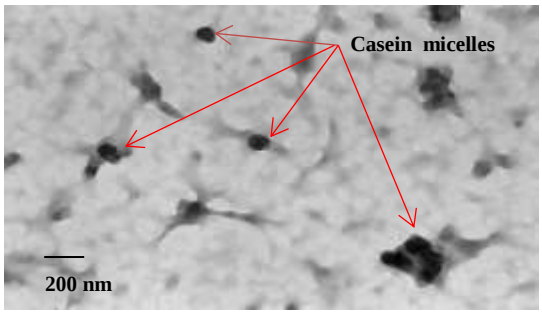

Figure 1. Transmission electron microscopy of casein micelles.

Size analysis showed that the micellar population in the control casein samples, accounted for approximately 60% of the casein molecules, (mean size = 178±22 nm). The rest of the casein molecules were found either in the monomeric form or assembled in larger agglomerates (data not shown). When PC was added to casein, a bimodal distribution was observed (d = 55±9 nm [60%] and d= 330±70 nm [30%]). The addition of the crosslinker resulted in the formation of small micelles (80±13 nm) which increased to 106±21 nm in the presence of PC. Drug loading may result in some micellar size increase due to expansion of the micelle core (3).

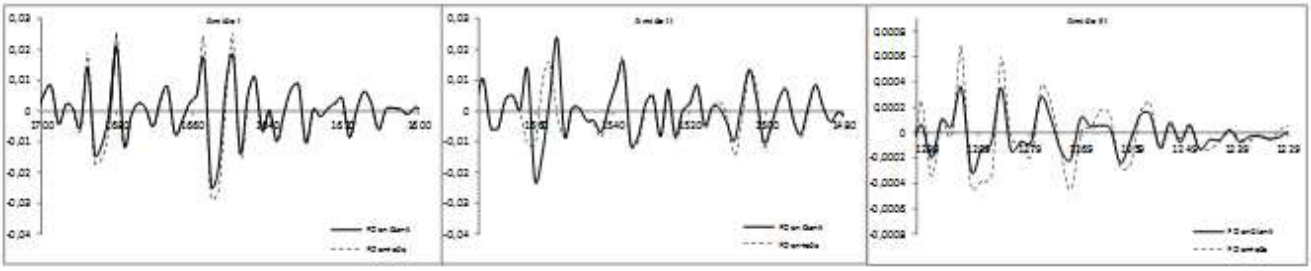

Figure 2. Second derivative amide I, II and III spectra of crosslinked and uncrosslinked casein.

The process of FD appeared to enhance the micellar size homogeneity in all casein formulations increasing the micellar populations to over 80-90% (data not shown). Negative surface charge (-5 to – 8 mV) was verified for all the formulations produced and did not change significantly during FD. The fact that micelles maintained their size and charge, with just minor changes, is an indicator for adequate production conditions. The encapsulation efficiency of PC in casein dispersions was approximately 30% and remained stable during FD in uncrosslinked casein, but dropped down to 20% in FD EDC-casein samples.

Casein (uncrosslinked and crosslinked) failed to show a thermal event in the temperature range considered (DSC study) (data not shown). Interestingly, PC was not detectable in the casein-micelles , as the typical melting endotherm of paracetamol (polymorph form I) at approximately 169ºC was absent, suggesting that PC existed in the micelles in the amorphous state or was dissolved. FTIR bands of PC in casein micelles (uncrosslinked and crosslinked) were predominantly invisible due to interference with the casein bands. The second derivative spectra of EDC-CN displayed altered stretching frequencies and peak shifts, compared to uncrosslinked CN, in the amide II region: 1564-1550 cm^{-1} and amide III region: 1284-1278 cm^{-1}, 1273-1255 cm^{-1}, which could be attributed to potential intramolecular cross-linking (Fig. 2).

The release of PC from casein micelles in PBS, pH 7.4, at 37ºC, is shown in Fig. 3. A burst effect was observed for the PC unbound into the casein micelles: the unbound PC was released at a similar rate as the control pure drug ($p>0.7$). However, significantly retarded release was found for the PC entrapped into the casein micelles as compared to the free drug control ($p<0.0001$). The use of crosslinker further retarded the release of the drug. The bound (approximately 20%) into the EDC-crosslinked micelles PC was released at a significantly lower rate compared to that bound into uncrosslinked casein micelles drug ($p<0.01$).

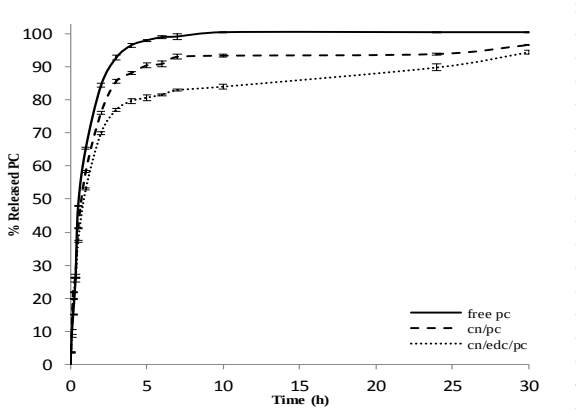

Figure 3. Release of paracetamol (%) in PBS, pH 7.4 at 37ºC, from FD casein micelles

CONCLUSION

The study suggests that casein could be a vehicle for the delivery of drugs in pediatrics, permitting a straightforward production process with minimal number of non-toxic excipients. The study also provides information on the use of carbodiimide as a crosslinker for the production of stabilized casein micelles with retarded release of the encapsulated drug that would reduce dosing frequency.

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