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EFFECT OF DIFFERENT EXCIPIENTS AND PROCESSING CONDITIONS ON CASEIN MICELLAR FORMULATIONS FOR CHILDREN

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PURPOSE

Investigation of the potential of casein micellar formulations as drug vehicles in pediatrics.

OBJECTIVES

- Study casein micelles as potential pediatric drug vehicles;
- Evaluation of the effect of different excipients (cryoprotectants and crosslinkers) and processing conditions (freeze-drying) on the casein micellar formulations.

METHODOLOGY

Production of casein particles

Casein (CN) dispersions (5mg/ml) were produced by casein solubilisation in NaOH (1M) and consequent pH adjustment with HCl (1M) to 6.5, the natural pH of milk. Several CN formulations were prepared by the addition of a cryoprotectant (mannitol or lactose, 1% w/v), a crosslinker [carbodiimide (EDC) or genipin (GP), 0.03M over during 24h] and paracetamol (PC), as a model drug. The dispersions obtained were freeze (-80°C/ 24h) -dried (-50°C, 0.035 mbar/ 12h) and stored at room temperature for 3 months.

Characterization of casein micelles

Micelles were characterized before and after freeze-drying (FD, n=3) for shape by microscopy (TEM), particle size and zeta potential (LD) and encapsulation efficiency (%). Potential interactions between PC and micelles excipients were monitored from FTIR spectrophotometry (4000–400cm^{-1}) and calorimetry (DSC, 15-200°C). PC was quantified by HPLC (reverse phase C_{18}-5 µm column) in a phosphate buffer / acetonitrile gradient. PC release from micelles into PBS (pH 7.4, 37°C) was studied when pure PC (control) and PC loaded casein micelles were placed into a dialysis membrane (cut off 10kDa) and samples taken at time intervals.

RESULTS AND DISCUSSION

Particle size, shape and zeta potential

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 (Fig 1).

Crosslinking promoted micellar formation and size homogeneity with more compact micellar structure for casein-EDC samples (80±13 nm) and larger micellar size (205±8 nm) for casein-GP samples. The addition of cryoprotectant (mannitol or lactose) to the casein dispersions, resulted in the formation of small micelles with average sizes of 89±24 and 77±7nm, respectively. Micellar loading with PC generally increased the average size of the CN micelles, which may be due to expansion of the micelle core. The process of FD was found to decrease polydispersity, however, causing usually, an increase in the average diameter, although the shape remained round (Fig. 2).

Negative surface charge (-5 to -8 mV) was verified for all the formulations produced and did not change significantly during FD (Fig. 1).

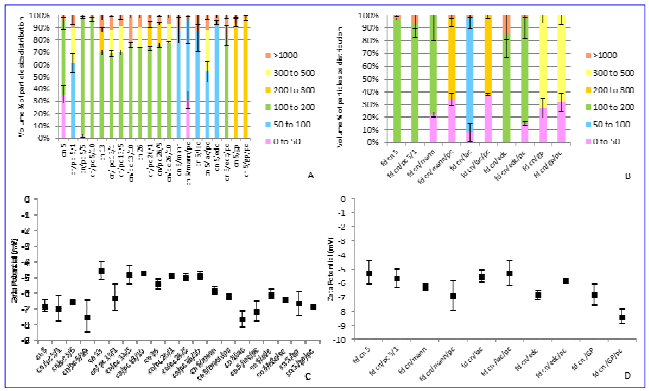

Fig. 1. Influence of the CN concentration and the addition of PC, cryoprotectants and crosslinkers on the casein micellar size (nm) distribution (A, B), and zeta potential (C, D), before (A, C) and after FD (B, D).

Calorimetric analysis (DSC) and FTIR

CN (uncrosslinked and crosslinked) failed to show thermal events in the temperature range. The presence of liquid crystals in eutectic mixture was observed for the CN-mannitol formulations. PC was not detectable in the CN micelles, as the typical melting endotherm of PC (polymorph form I) at approximately 169°C was absent, suggesting that PC exists in the micelles in the amorphous state or dissolved.

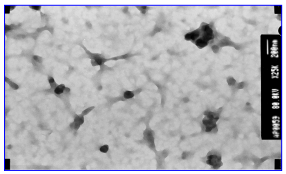

Fig. 2. Transmission electron microscopy of casein micelles.

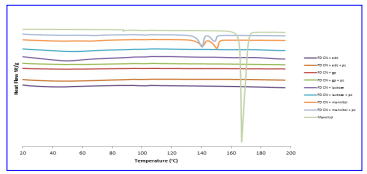

Fig. 3. Thermograms of FD casein (5mg/ml) formulations containing PC, cryoprotectants or crosslinkers.

The second derivative spectra of crosslinked CN displayed altered stretching frequencies and peak shifts, compared to uncrosslinked casein, in the amide II: 1564-1550 cm^{-1} and amide III region: 1284-1278 cm^{-1}, 1273-1255 cm^{-1}, which could be attributed to intramolecular crosslinking. (Fig. 4).

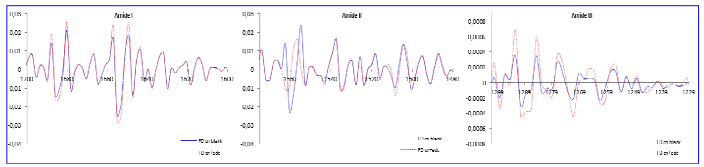

Fig. 4. Second derivative amide I, II and III spectra of crosslinked and uncrosslinked casein.

Encapsulation efficiency (EE) and drug release

The EE of CN micelles was approximately 30%, but dropped down to 14% in FD GP-CN samples (Table I).

Table I: EE (%) before and after FD in micellar casein formulations containing cryoprotectants or crosslinkers

Formulation CN:PC 5:1	Encapsulated PC (%) before freeze-drying	Encapsulated PC (%) after freeze-drying
cn/mann/pc	30.4±0.7	30.0±2.8
cn/lac/pc	28.0±0.9	21.6±2.0
cn/edc/pc	31.3±2.2	21.5±0.6
cn/gp/pc	29.4±0.7	14.0±1.5

The release of PC from CN micelles in PBS, pH 7.4, at 37°C showed a burst effect for the PC unbound into the CN micelles, which 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 CN micelles as compared to the control of free drug ($p<0.0001$). 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 as compared to that bound into uncrosslinked casein micelles drug ($p<0.01$). The addition of cryoprotectants, also promoted the delayed release of PC from the CN micelles as compared to the control CN micelles ($p<0,05$).(Fig. 1, left).

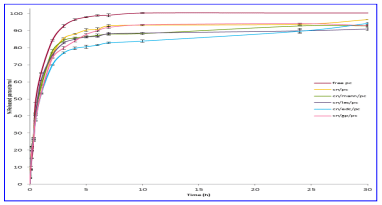

Fig. 5. PC release (%) from FD casein micelles.

The particle morphology, size, drug content, EE and release profiles were stable over 3 months of storage for all the formulations, except for GP crosslinked CN micelles, whose samples were not totally redispersible at the end of the first month.

CONCLUSIONS

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

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