# DEVELOPMENT OF CASEIN MICELLAR PEDIATRIC FORMULATIONS

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## INTRODUCTION

The current need for medicines specifically designed for children, which consider ease of administration, dose flexibility, palatability, safety of excipients, stability and therapeutic equivalency of pediatric dosage forms [1], has driven the development of pediatric drug formulations.

In the present work casein (CN) based micellar formulations are evaluated as vehicles for the oral delivery of pediatric drugs, since caseins are nontoxic, biodegradable, GRAS 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.

## MATERIALS AND METHODS

### Production of casein particles

Casein (CN) dispersions (5mg/ml) were produced by CN 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) was used as a model drug. PC and/or crosslinker (EDC, 0.03M, 24h) were added to CN dispersions. These dispersions were freeze (-80ºC/24h)-dried (-50ºC, 0.035 mbar/ 12h) and stored at room temperature over silica gel until further use.

### Characterisation of casein micelles

Micelles were characterized, before and after freeze-drying (FD; n=3) for shape (TEM), particle size and zeta potential. FTIR spectra (4000–400 $cm^{-1}$) and DSC thermograms (15-200ºC) obtained. PC was quantified by HPLC (C18, 5 µm column) in a 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 (8000 rpm). Bound (micellar) PC (*i.e.* encapsulation efficiency, EE) was calculated from:

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

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 CN micelles were placed into a dialysis membrane (cut off 12kDa) suspended into 100mL of PBS pH 7.4, at 37ºC, under stirring (100 rpm) and samples taken at time intervals.

## RESULTS AND DISCUSSION

Microscopic images showed particles (Fig. 1) with round shape. Size analysis revealed that the micellar population in the control CN samples, accounted for approximately 60% of the CN molecules (mean size=178±22 nm). The rest of the CN molecules were found either in the monomeric form or assembled in larger agglomerates (data not shown). When PC was added to CN, 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 [2].

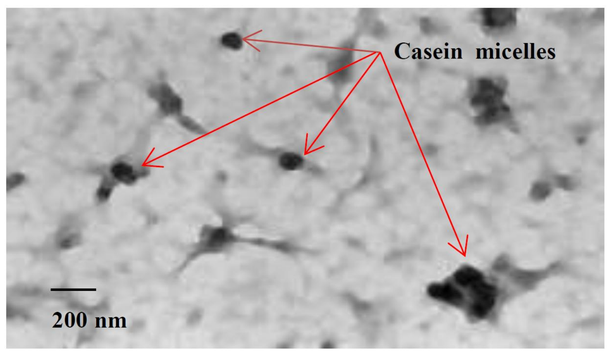


**Figure 1: Transmission electron microscopy of casein micelles.**

The process of FD appears to enhance the micellar size homogeneity in all CN 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 CN dispersions was approximately 30% and remained stable during FD in uncrosslinked CN, but dropped down to 20% in FD EDC-CN 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 CN-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 CN-micelles (uncrosslinked and crosslinked) were predominantly invisible due to interference with the CN 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 crosslinking (Fig. 2).

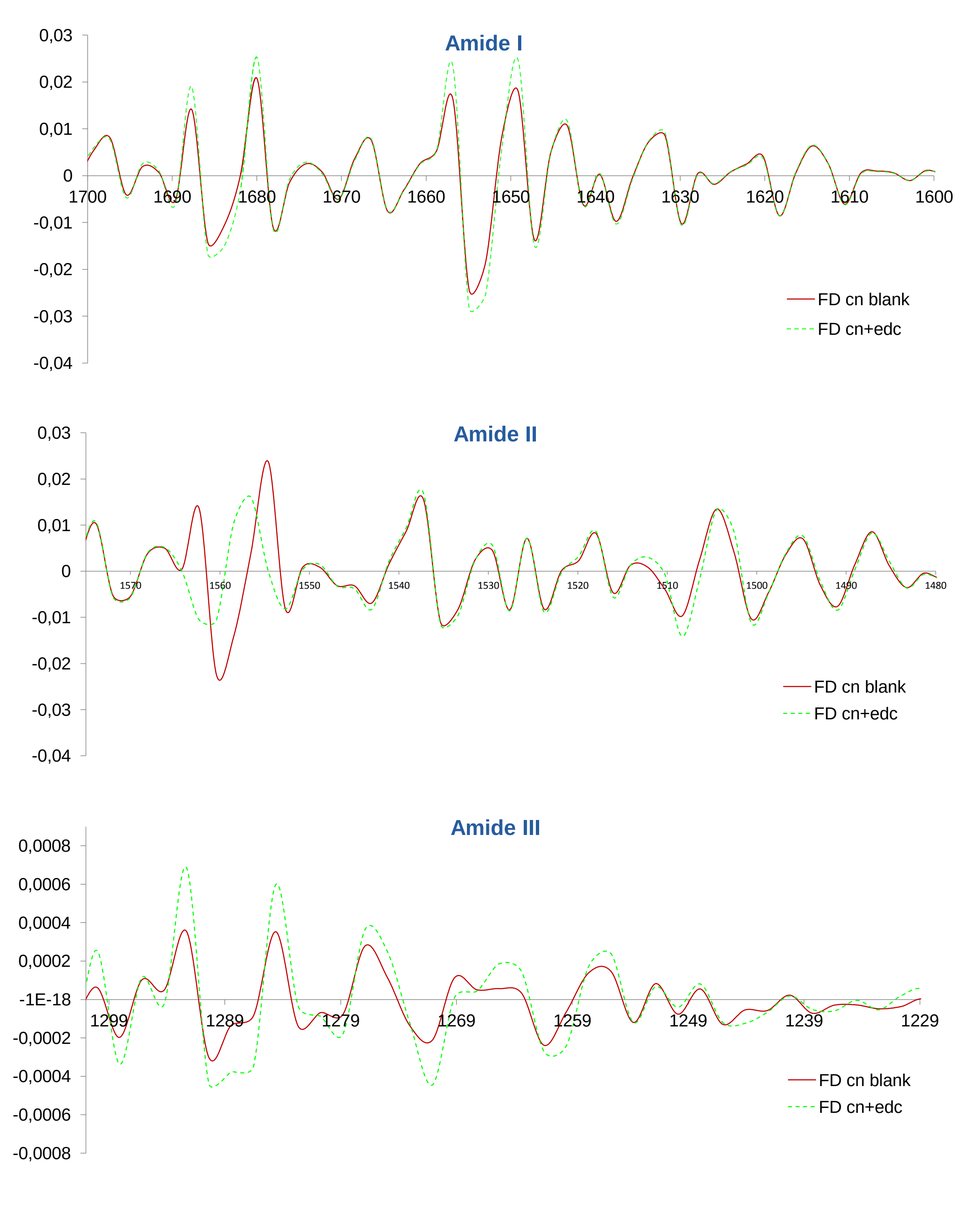


**Figure 2: Second derivative amide I-III spectra of crosslinked and non-crosslinked CN.**

The release of PC from CN-micelles in PBS, pH 7.4, at 37ºC, is shown in Fig. 3. A PC unbound burst effect was observed for the CN-micelles at a similar rate as the control pure drug (p>0.7). However, a significantly retarded release was found for the PC entrapped into the CN-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 CN-micelles (p<0.01).

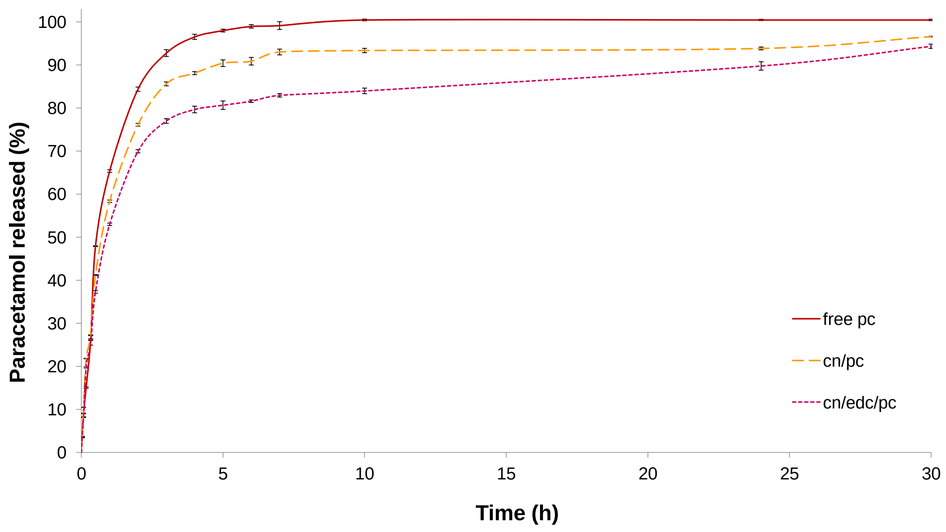


**Figure 3: Release of paracetamol from freeze dried casein micelles.**

## CONCLUSIONS

- CN can be used as a drug delivery vehicle in pediatrics, using a straightforward production process and minimal number of non-toxic excipients.
- The use of carbodiimide as a crosslinker for the production of stabilized CN micelles retards release of the encapsulated drug, thus reducing dosing frequency.

## REFERENCES

[1] Ali A.A., Charoo N.A., Abdallah D.B. (2014) Drug Dev. Ind. Pharm. 40(10):1283-99.
[2] Torchilin, V.P. (2007) Pharm. Res. 24(1):1-16.

## ACKNOWLEDGEMENTS

A.P. Alves de Matos is gratefully acknowledged for the TEM images.
This work was supported by Fundação para a Ciência e a Tecnologia (PTDC/DTP-FTO/1057/2012).