O6-4 **Encapsulated chlorhexidine for sustained release antibacterial effects**

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INTRODUCTION

The aim of this work is to develop nanocapsules containing chlorhexidine, a well known biocide with a broad spectrum antibacterial activity, in order to obtain disinfecting solutions or hand washes with both immediate and long lasting antimicrobial activities. A new process was optimized to produce these nanocapsules.

MATERIALS AND METHODS

Nanocapsules are made by interfacial polymer deposition using poly-ε- caprolactone MW: 425 000 $gmol^{-1}$ (Aldrich France) and carboxymethyl cellulose sodium salt (MW 550 000 $gmol^{-1}$ (Cooper France). Polysorbate 80 (Seppic France) and phospholipids (Epikuron 200 - Lukas Meyer France) are used as surfactants

RESULTS

Nanocapsules description

Nanocapsules consist in a shell made of a blend of an hydrophobic polymer (poly-ε-caprolactone , PCL), an hydrophilic polymer (carboxyméthylcellulose) and surfactants surrounding a lipid core containing chlorhexidine. They are manufactured by an original solvent-free patented process (Pirot, 2009) which induces the formation of a solid shell when the oil phase containing PCL is in contact with an aqueous solution of the other excipients.

The mean size of the chlorhexidine loaded nanocapsules (CHX-NC) is 500 nm. The standard chlorhexidine concentration in the suspension is 2%, but other dilutions can be obtained upon request. The suspension is very fluid and can be sprayed. It is stable at room temperature and at 4°C for at least 6 months.

Mechanism of release

Chlorhexidine is released by diffusion from the fatty core of the capsules. *In vitro* experiments and *ex vivo* permeation kinetics through hairless rat skins demonstrate that sustained release can be observed over 10 to 20 hours (Lboutounne 2004). Encapsulation allows formulating chlorhexidine in aqueous solutions without solvent and precluding salts from co-precipitating with this active.

Antimicrobial properties

- ***Minimum Inhibitory Concentration (MIC)***

The MIC of a 0.15% CHX-NC is similar to a 0.15% aqueous solution of chlorhexidine digluconate, on 8 bacterial strains. The MIC values remain unchanged after 40 days of storage (Lboutounne 2004). See table 1.

Table 1: MIC of chlorhexidine nanocapsules

MIC (µg/ml)	CHX-NC		solution
	Day 1	Day 40	Day 1
S.epidermidis	1,2	1,2	1,2
S. aureus CIP53154	1,2	1,2	1,2
E. faecalis	9,4	nd	4,7
E. hirae CIP 5855	1,2	1,2	9,4
E.aerogenes	37,5	37,5	75
P. aeruginosa	75	75	75
P. aeruginosa CIP A22	75	75	75
E. coli CIP54127	1,2	1,2	1,2

- ***Long term efficiency***

Porcine ear skin was treated during 3 minutes by either a 0.6% CHX-NC, an unloaded suspension of nanocapsules or a 1% chlorhexidine digluconate solution. The skin was then contaminated by a 100 µl sample of Staphylococcus epidermidis inoculum (10^6 CFU/ml) 3 times: 0, 4 and 8 hours after treatment. Surviving bacteria were checked 4 hours after each contamination.

Curves on figure 1 show that CHX-NC are efficient at least 8 hours after treatment, whereas unloaded nanocapsules or chlorhexidine digluconate are less efficient and failed to maintain antibacterial protection after 4 hours.

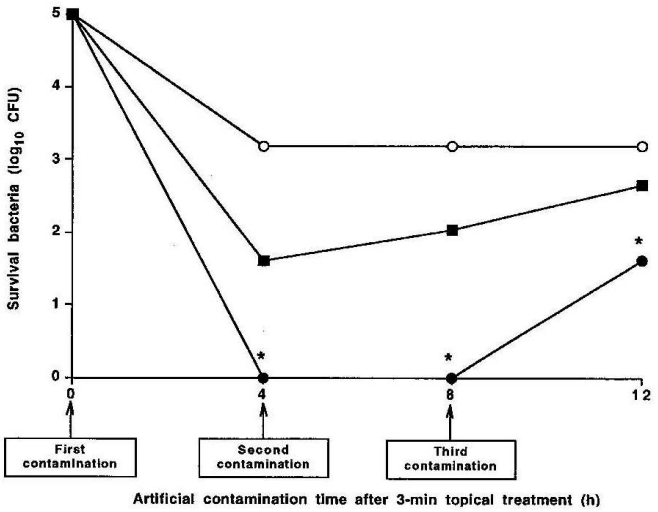

Figure 1: Effect of chlorhexidine nanocapsules on the survival of bacteria according to time

- ***Use of microcapsules in hand rub gels***

A hand rub gel containing CHX-NC was tested against the resident human skin flora in comparison with a 2-propanol solution and a commercial ethanol based gel. After 30s hand rub, the immediate bactericidal effect was found identical or better than the propanol solution. Sustained antibacterial effect was demonstrated in two sets of experiments through a 3h period, while ethanolic solution failed to reduce significantly the value of surviving bacteria (Nhung.2007)

Following to these results, hand gel containing CHX-NC was listed in WHO guidelines on Hand Hygiene in Health Care (World Health Organization 2009).

CONCLUSION

A new type of nanocapsules containing chlorhexidine was developed, using a solvent free process. Capsules are safe and can easily be incorporated into disinfecting formulations. They proved to have both an immediate and a long lasting effect, by opposition to conventional formulations which have only a short term efficacy.

Nanocapsules containing different concentrations of chlorhexidine can be manufactured using the process. It seems also possible and interesting to encapsulate other types of active ingredients using this technology.

REFERENCES

- Lboutounne H. et al (2004). Characterisation of Transport of chlorehidine loaded nanocapsules through hairless and wistar rat skin. Skin Pharmacology and Physiology, 17: 176-182
- Nhung D.T.T. et al (2007). Sustained antibacterial effect of a hand rub gel incorporating chlorhexidine - loaded nanocapsules. International Journal of Pharmaceutics , 334, 166-172
- Pirot F. et al. Patent WO 2009/138606: Novel method for producing nanocapsules in the absence of solvent and nanocapsules produced thereby
- World Health Organization 2009 - ISBN 978 92 4 159790 6 (NLM classification: WB 300)