ENCAPSULATED CHLORHEXIDINE DIGLUCONATE USAGE ON THE DIABETIC FOOTWEAR LINING LEATHERS

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It is important for a therapeutic shoe to have a wearable quality and its comfortable use is also prominent in terms of providing an increase in user's quality of life. In long-term treatment of diseases, medical products which are to be manufactured with medical leather materials might be a prominent alternative via local effect application as an adjuvant to the treatment. It is aimed in this study to produce microparticles (MPs) which contain the active agent chlorhexidine digluconate and application of these MPs on lining leather for manufacturing diabetic shoes while providing them a functional quality. Within the scope of the project, MPs loaded with drugs were obtained via spraying chlorhexidine digluconate (CHD) active agent and through spraying with ethyl cellulose polymer and ustulation. *In vitro* characterisation studies were performed on the acquired MPs. Additionally, active agent quantitation and *in vitro* drug delivery studies were also performed. Following the studies, the determined optimum MP formulations were applied on the leather, then existence and efficiency of MPs within the leather was shown in the subsequent studies.

Keywords: Chlorhexidine digluconate, encapsulation, diabetic shoes, leather

INTRODUCTION

Diabetic foot disease is a serious problem, with a life-time prevalence of 15–25% in the diabetic population (Boulton, 2000). While in the UK up to 100 people/week have a limb amputated as a result of diabetes, it is indicated that up to 80% of these amputations could have been prevented with correct management (Anonymous, 2011). Foot ulcers in people with diabetes are multi-factorial and linked to a variety of clinical risk factors, like peripheral neuropathy and vascular insufficiency, as well as biomechanical risk factors, such as increased plantar pressure (Ledoux, 2013; Crawford, 2007). In shoe plantar pressure assessment is becoming increasingly popular in both research and clinical practice to evaluate the effects of prescribed footwear in diabetic patients who have a foot ulcer or who are at risk for ulceration (Singh, 2005). Diabetic footwear plays an important role for the reduction of plantar pressure in people with diabetes (Panagiotis, 2015).

Microencapsulation is one of the most important forms of controlled release of active ingredient. This technology allows heat, temperature or pH sensitive components to be physically enveloped in a protective matrix or wall material in order to protect these ingredients or core materials from adverse reactions, loss or against light, heat and prolonged contact with air. It is also one of the most important forms of controlled release of substances and allows the utilization of some that otherwise would be unfeasible (Nirmala, 2013; Sanchez-Navarro, 2015). These systems offer some advantages over conventional dosage forms, including improved efficiency, reduced toxicity and improved patient compliance (Grattard, 2002).

In the footwear industry, the incorporation of microencapsulated substances into materials or components allows the concept of active shoes to be realized, which contributes to improve the welfare of users, satisfying their needs and expectations (Morace, 2012). Fragrances applied to footwear, both directly and through packaging, cover one of the main consumer demands regarding the solution of bad odours

generated during footwear use (Misher, 2007). Along the same lines, in shoe packaging, microencapsulation allows the development of active issues with different purposes: trapping undesirable odours or incorporation of antimicrobial agents, to be released over time in order to improve the useful life of the packed shoe or the incorporation of controlled released scents to avoid their degradation and to improve the durability of the aroma (Sanchez-Navarro, 2012; Sanchez-Navarro, 2011).

It is aimed in this study to produce microparticles (MPs) which contain the active agent Chlorhexidine digluconate (CHD) and application of these MPs on lining leather for manufacturing diabetic shoes while providing them a functional quality.

MATERIAL AND METHOD

Material

CHD 20% solution was purchased from Sigma-Aldrich. Aquacoat ethyl cellulose dispersion (Aquacoat® ECD) was gift from FMC BioPolymer (Philadelphia, PA). All other materials were of analytical grade.

Method

Preparation of MPs

MPs were carried out in a spray dryer model SD-Basic (Lab-Plant, Huddersfield, U.K). Aquacoat® ECD was used as a polymeric system. CHD and Aquacoat® ECD were mixed in distilled water. The drug to polymer ratios in the microencapsulating compositions were maintained in 1:1, and 1:2, respectively (Table 1).

Table 1. The composition of the formulations

Formulation Code	CD: EC*	Pump Speed (mL/dk)	Inlet Temperature (˚C)	Outlet Temperature (˚C)
F1	1:1	10	120	80
F2	1:2	10	120	80

(*): Aquacoat® ethyl cellulose

Particle Morphology

The morphology of the MPs was examined by a scanning electron microscope (SEM, FEI Quanta 250 FEG). The sample was mounted onto an aluminum stub and sputter-coated with gold palladium (Au/Pd) using a vacuum evaporator.

In Vitro Drug Release of the MPs

In vitro release studies were performed speed of 100 rpm in PBS at 37ºC. MPs were suspended in tubes containing 10 ml of PBS. At the appropriate time intervals, the medium in the corresponding tube was filtered through 0.22 μm filter and released CHD amount determined by validated UPLC method. Sink conditions were maintained in the receptor compartment during *in vitro* release studies (n=5).

Application of the MPs on Lining Leathers

MPs were applied on the lining leathers for diabetic footwear in the finishing process by using spraying pistol. MPs that are containing antimicrobial material were added into finishing recipe (Table 2) as 20 g per m^2 (Kleban, 2002).

Table 2. Basic finishing recipe with MPs

Material	Quantity	Application
Water	100 part	3x Spray
Anionic wax	50 part	
Nonionic aliphatic polyurethane binder	25 part	
MPs	12 part	

SEM of the Lining Leathers with the MPs

The morphology of the samples was examined by a SEM (HITACHI TM 1000).

In Vitro Drug Release of the Lining Leathers with MPs

In vitro release studies were performed speed of 100 rpm in PBS at 37ºC. Lining leathers with 10 cm^2 area were placed in beaker containing 125 ml of PBS. The samples withdrawn directly at appropriate time intervals were analyzed by a validated UPLC method as previously described (n=5).

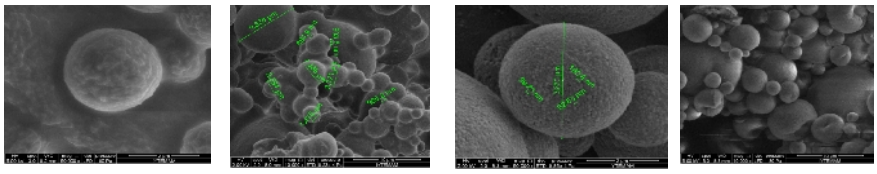
Microbiologic Studies on Lining Leathers with MPs

Agar disc diffusion method was used to examine the effect of drug, MPs and lining leather with MPs against to the test microorganisms. Test microorganisms were incubated at 37°C for 18 hours in the Muller Hinton Broth (MHB) medium. After incubation, microorganisms were inoculated into petri dishes containing Muller Hinton Agar (MHA) medium as 10^5 CFU/mL. Lining leather samples with 12.7 mm diameter were placed into the petri dishes. All petri dishes were incubated at 37°C for 24 hours. Finally, inhibition zones were measured for determining the antibacterial activity.

RESULTS AND DISCUSSION

Particle Morphology

According to the SEM images, MPs had a spherical shape with a rough surface morphology. The MPs exhibited irregular shape also. They do not show the presence of free drug on their surfaces. These morphological characteristics point out that the CHD is dispersed all over the MPs (Figure 1). In other spray-drying studies performed by using aqueous polymeric dispersions, MPs with similar morphological characteristics were also obtained (Rattes, 2007; Arici, 2014).



F1 formulation (a) F1 formulation (b) F2 formulation (a) F2 formulation (b)

Figure 1. SEM images of MPs at 50000x magnification (a) and at 10000x magnification (b)

In Vitro Drug Release of the MPs

In vitro drug release studies showed that in CHD release from MPs was very fast which is probably the consequence of very good swelling properties or it could be related

with burst effect (Figure 2). In general, the initial rates of release for many drug delivery systems are high during the first period, most likely due to the release of drug enriched on the sample surfaces (Kenawy, 2002). The same behaviour, defined as the initial burst release, is presented in the MPs. MPs prepared by using spray drying method generally have a matrix structure. For this reason, besides drug substance being in the particles, depending on the loading concentration, it can also be on the outer surface of the particles. When the MPs are exposed to the dissolution media, the drug on the outer surface (non-encapsulated drug) causes a sudden drug release (Ghorab, 1990; Saravanan, 2003).

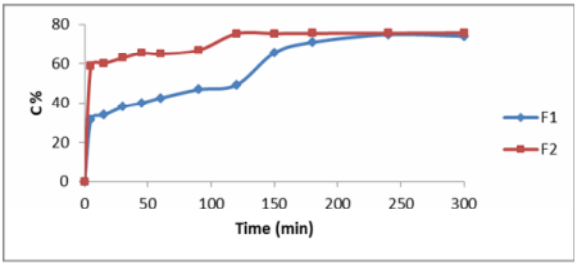
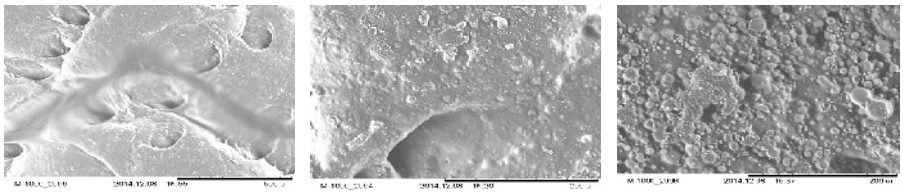


Figure 2. *In vitro* release of MPs

SEM of the Lining Leathers with the MPs

Lining leather samples that were applied finishing recipes with MPs containing antibacterial drug and without MPs was examined by SEM. As seen in Figure 3, there were fewer MPs on the lining leather surface because of the F1 formulation's polymer quantity is half of the F2 formulation.



Without MPs F1 formulation F2 formulation

Figure 3. SEM images of the lining leather after finishing process

In Vitro Drug Release of the Lining Leathers with MPs

The *in vitro* release results of the leathers in pH 7.4 PBS are presented in Figure 4. As seen in Figure 4, there was a steady release of the CHD into the PBS for all formulations. CHD entrapped deep within the MPs sustained the release to more than 24h. Comparing the formulations among each other, the drug release ratio of lining leather with F1 was higher than the release of lining leather with F2.

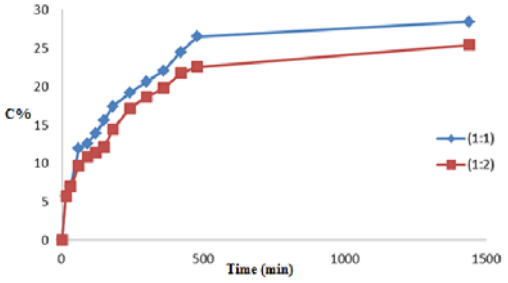
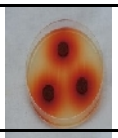
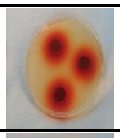
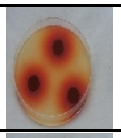
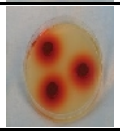


Figure 4. *In vitro* release of lining leathers with MPs

Microbiologic Stuies on Lining Leathers with MPs

It was not seen any clear inhibition zone around the lining leather samples with MPs in Table 3. On the other hand, slightly inhibition zone was seen on some leather samples examining by microscope. This situation could be interpreted that MPs don't show antimicrobial property comparing the non-capsulated drug. Effect of applying the MPs on the lining leather of the diabetic shoes was positive considering that encapsulation is used the permanence of the drug on the material and controlled release.

Table 3. Microbiologic test results of lining leathers with MPs

Formulation Code	*Staphylococcus aureus* ATCC 6538-P	*Escherichia coli* ATCC 12228	*Pseudomonas aeruginosa* ATCC 27853	*Candida albicans* ATCC 10239	*Klebsiella pneumoniae* CCM 2318	*Enterococcus faecalis* ATCC 29212	*Staphylococcus epidermidis* ATCC 12228
F1							
F2							

CHD diffusion was not occurred on the lining leather surface, because of this inhibition zone was not seen. However, antimicrobial effect can be evaluated with proliferation or without proliferation in the area under the leather samples. This effect is expressed as contact inhibition. It was not seen any proliferation on the contact surface of the lining leathers in Table 3. Also, there was not seen any proliferation surface or edge of the lining leathers.

CONCLUSION

In this study, CHD was microencapsulated by the spray drying method using an Aquacoat® ECD, followed by its application on lining leather for manufacturing diabetic shoes. Aquacoat® ECD has proved to be a useful polymer for formulating CHD MPs using spray drying technology in an aqueous system. Using MPs produced with an

Aquacoat® ECD have the main advantage of being ecofriendly, due to the fact that organic solvents can be avoided. Water was the only solvent used. SEM photographs showed smooth shaped MPs and good adhesion between the leathers and the MPs. In a conclusion, diabetic shoes with MPs will be an adjuvant therapy to the oral therapy by releasing drug on the applying area for a long time.

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