Design of Leather Footwear for Diabetics Containing Chlorhexidine Digluconate Microparticles

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Abstract

ChlorHexidine Digluconate [CHD] was encapsulated inside an ethylcellulose shell material [Aquacoat ECD], and then spray dried to produce mixed microparticles (MPs). The validity and functional quality of the resultant [CHD-MPs] were analyzed on vamp and lining leather which are used to manufacture shoes for diabetics. The morphology, efficiency of encapsulation and *in vitro* release characteristics of the [CHD-MPs] were optimized in order to impregnate [CHD-MPs] onto leather footwear for diabetics. Scanning electron microscopy (SEM) was used to characterize the [CHD-MPs] and the leathers treated with it. SEM images illustrated that the [CHD-MPs] were spherical, smooth in shape and adhered well to leather. *In vitro* CHD-release studies from its MPs, and for leather treated with it were performed in phosphate buffer saline at pH =7.2. There was an inherently controlled release behaviour of CHD for all the formulations on leather. Finally, microbiologic studies on leather treated with [CHD-MPs] were done. This study suggested that footwear containing [CHD-MPs] is/will improve the quality of daily life for diabetics.

Introduction

The number of people with diabetes and foot disease is on the rise. About 15% of the world population [~150 million people] are diabetic. Previous reports highlight that the approximately 15% of people with diabetes worldwide will at some stage develop the health condition of diabetic foot ulceration which could lead to amputation.[1] The estimated prevalence of diabetes for adults between the ages of 20 and 70 worldwide for 2015 was 415 million. Diabetes is expected to affect one person in 10 by 2040 or 642 million people.[2]

Microencapsulation is widely used to provide controlled release of the desired active substance. This technology enables the shell material of the microcapsules to protect the core active substance from adverse reactions, loss or contact with light, heat, and air for a long time with a wall material sensitive to heat, temperature or pH. In addition, impractical core materials can be used effectively with this method.[3,4] In this way, it gives some advantages such as improved efficiency and reduced toxicity.[5] Spray-drying is useful for the microencapsulation technique that has a number of advantages such as continuous particle processing, low-cost encapsulation and the ability to handle labile materials because of the short contact time.[6,7] This technique is most commonly used in many fields such as pharmaceutical, agriculture, biotechnology, cosmetic, carbonless copying paper, footwear, and the food and flavor industry due to low cost and available equipment.[8]

Application of the microencapsulation method in the shoe industry and application of encapsulated core materials to shoe materials will improve the users' welfare and meet their expectations.[9] For example, the fragrance which is applied to the shoe by a microencapsulation method meets one of the basic consumer demands for the removal/masking of bad odors during the use of shoes.[10] In addition, microencapsulation treatments for shoes allow active or ongoing problems to be solved for different purposes: the removal of unwanted odors or the inclusion of antimicrobial agents, increasing the useful life of the microencapsulated shoe or using it in a controlled manner, preventing odors and releasing fragrance to increase the durability of its aroma.[4,11]

Ethylcellulose (EC) is a water-insoluble polymer. It is widely used for microencapsulation of pharmaceuticals to stabilize them against active interactions, hydrolysis and oxidation.[5,12] It has been used particularly as a coating material for sustained release systems.

Aquacoat ECD is an eco-friendly aqueous ethylcellulose dispersion, which matters especially for the preparation of the microparticles (MPs) and coating applications, resulting in environment-friendly technology. It has a solid content of 30% (26% ethylcellulose, 2.4% cetyl alcohol and 1.3% sodium dodecyl sulfate). It requires a plasticizer to decrease the minimum film forming temperature (81°C) and thus improve the film mechanical properties. For most applications, it is recommended that plasticizers are added at a loading of 24% of the latex solids level.[13]

Chlorhexidine digluconate (CHD) was chosen as the model drug in this study because it is an antiseptic agent with topical antibacterial activity. It is a fast-acting biocide that prevents the growth of different bacteria, fungi and yeasts.[14,15] CHD is known to be less toxic to human tissues than other antiseptics, has a high

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bactericidal activity against a wide spectrum of both gram-positive and gram-negative bacteria and is resistant to inhibition by blood and organic materials.[16,17,18] It is only available as an aqueous solution as the substance cannot be isolated as a solid. CHD is also used as a preservative or disinfectant in a wide range of water-based cosmetics and personal care products, in textiles and in food factories.

The foot activity of diabetics is not adequate; because the veins are clogged and blood circulation has been reduced. This diabetic foot has a reduced sensitivity, is/feels cold and an open wound on it heals slowly because of decreased cell feed.[1,19] If healing is slower than the microorganism growing activity, the condition of the wound may deteriorate which can lead to amputating the foot.[20,21] The suggested footwear for diabetics containing the new [CHD MPs] will be a favorable solution for these patients to improve the quality of daily life. One of the most important problems facing diabetics is microorganism activity; even inside of footwear or an open wound on their feet. Thus, the mere presence of CHD on the footwear surface could reduce the incidence of infection for these patients.

We aimed in this study to produce MPs with Ethyl Cellulose [EC] as a shell material and CHD as active agent therein in order to apply the resultant [CHD-MPs] to vamp and lining leather for the manufacture of shoes for diabetics. Within this scope, MPs loaded with the CHD drug were obtained via co-spray-drying CHD and EC. *In vitro* characterization studies such as loading capacity, encapsulation efficiency and morphological studies of MPs were performed. Additionally, *in vitro* CHD delivery studies of its MPs were performed. Finally, [CHD-MPs] were applied on lining and vamp leather. The presence and efficiency of MPs loaded leather samples were determined in subsequent studies. By the end of the study the controlled release of CHD was enabled successfully on lining and vamp of diabetic footwear leather at the finishing stage of leather making. The controlled release of the CHD in footwear for diabetics will be investigated for comfort and hygiene metrics in a future study.

Experimental

Materials

CHD 20% solution was purchased from Sigma-Aldrich. Aquacoat ethylcellulose dispersion (Aquacoat ECD) was a gift from FMC BioPolymer (Philadelphia, PA). All other materials were of analytical grade. Leathers are prepared via tanned, dyed and all mechanically processed, ready for finish application via spray gun with microcapsule material.

Methods

Preparation of MPs

MPs were carried out in a spray dryer model SD-Basic (Lab-Plant, Huddersfield, U.K). Aqueous ethylcellulose dispersion (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 was maintained in 1:1, and 1:2, respectively. The compositions of the formulations in spray-drying are shown in Table I.

Table I

The composition of the formulations

Formulation Code	CHD: Aquacoat ECD
F1	1:1
F2	1:2

The main components of the system are the feed system of the microencapsulating formulation, constituted by a peristaltic pump, a two-fluid atomizer (nozzle diameter of 0.5 mm) and an air compressor; the feed system of the drying gas, constituted by a blower, an air filter, and a temperature control system. The resulting compositions were fed to the spray dryer at the following conditions: pump speed of microencapsulating composition 10 mL/min, inlet air temperature 120˚C and outlet air temperature 80˚C. The dried product was collected during the experiments.

Particle Morphology of the MPs

The MPs morphology via spray dried was determined 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.

Conditions of Ultra-Performance Liquid Chromatography (UPLC)

CHD content of the MPs was determined by UPLC (Thermo Scientific Accela). Separation was achieved on Thermo Hypersil Gold (100 x 2.1mL, 3 µm) C18 column at 25°C by using an isocratic elution method with 0.1% formic acid in water:10 mM buffer solution of sodium phosphate monobasic (pH=3): Acetonitrile (20:40:40) at a flow rate of 300 µL/min This method was validated according to the ICH guidelines.[22]

Encapsulation Efficiency of the MPs

1 mg of the drug-loaded MPs were dissolved in 1 ml of the phosphate buffer saline (PBS, pH 7.4). Then mixed 200 rpm at room temperature for 24 h. In order to determine the amount of active substance loaded in the MPs, the solution was filtered through a 0.22 µm syringe filter and the filtrate analyzed by validated UPLC at 258 nm. The amount of active substance was determined using a calibration curve. Each batch was evaluated five times. The encapsulation efficiency (% EE) was calculated using the following equations:[23]

$$\%\ EE = \frac{B\ (the\ weight\ of\ active\ substance\ found\ in\ MPs)}{A\ (total\ weight\ of\ active\ substance)} \times 100$$

Table II

Basic leather finishing recipe with drug-loaded MPs

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

FT-IR Analysis

FT-IR spectra of the CHD, Aquacoat ECD, and samples were obtained using a Perkin Elmer Spectrum 100 FT-IR spectrometer (Perkin Elmer Spectrum 100, Massachusetts, USA) at room temperature, with wavenumbers ranging from 4000 to 650 cm^{-1}, using four scans with a resolution of 4 cm^{-1}.

In Vitro Drug Release of the MPs

In vitro release studies were performed speed of 100 rpm in phosphate buffer saline (PBS) at 37°C. MPs were suspended in tubes containing 10 mL of PBS. At the appropriate time intervals, the medium in the corresponding tube was filtered through a 0.22 µm filter and the released amount of CHD determined by the validated UPLC method. Sink conditions were maintained in the receptor compartment during *in vitro* release studies. The experiment was carried out five times.

Application of the MPs on the Leathers

MPs were applied on the lining and vamp leathers for diabetic footwear in the finishing process by using spraying pistol. MPs that contained antimicrobial material were added into finishing recipe (Table II) as 20 g per m^2.[24]

Scanning Electron Microscopy (SEM) of the Leathers with the MPs

The morphology of the samples was examined by an SEM (HITACHI TM 1000). 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 Leathers with MPs

In vitro release studies were performed speed of 100 rpm in PBS at 37°C. Vamp and lining leathers with 10 cm^2 area were placed in a beaker containing 125 mL of PBS. The samples withdrawn directly at appropriate time intervals were analyzed by a validated UPLC method as previously described. The experiment was carried out five times.

Microbiologic Studies on Leathers with MPs

Agar disc diffusion method was used to examine the effect of drug, MPs, and leather with MPs against 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. Then, 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.

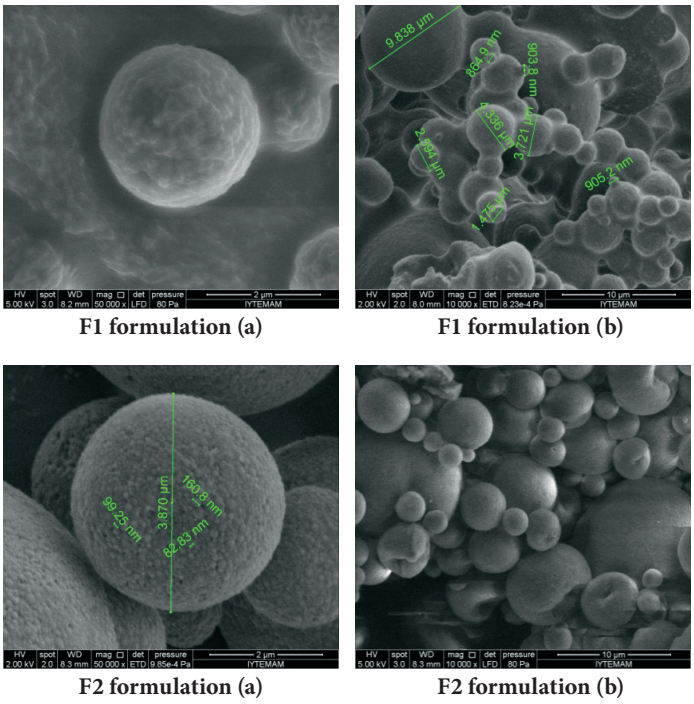


Figure 1. SEM images of MP formulations, F1 formulation at 50000× magnification (a), F1 formulation at 10000× magnification (b), F2 formulation at 50000× magnification (a), F2 formulation at 10000× magnification (b)

Results and Discussion

Particle Morphology

The morphology of the spray-dried MPs was examined by SEM. According to the SEM images, MP formulations had a spherical shape with rough surface morphology. The MPs exhibited irregular shapes also. They do not show the presence of the free drug on their surfaces. These morphological characteristics point out that the CHD is dispersed all over the MPs. Figure 1 showed the morphology of the spray-dried MP formulations. In other spray-drying studies performed by using aqueous polymeric dispersions, MPs with similar morphological characteristics were also obtained.[25,26]

UPLC Conditions

The UPLC method development was carried out according to Havlikova et.al. (2007) method.[22] The method gave a peak of CHD in 1.21 min and the chromatogram is shown in Figure 2. CHD was successfully separated from other compounds. The calibration curve of CHD was linear in the concentration range of 0.5-75 µg/mL (r^2=0.9988). This method was validated according to ICH guideline recommendation Q2 (R1).

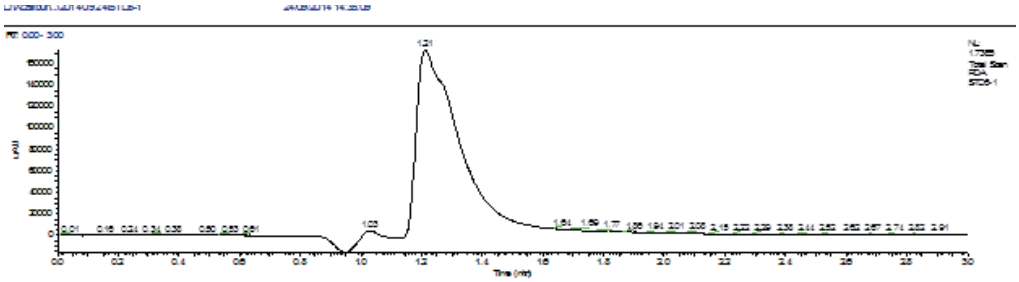


Figure 2. UPLC chromatogram at 258 nm

Encapsulation Efficiency

CHD loaded MPs were produced with a high drug encapsulation efficiency. The encapsulation efficiency of F1 and F2 formulation were found 92.725±5.303% and 88.969±3.250%, respectively. Considering the high encapsulation efficiency, it can be concluded that the spray dryer method is a simple and suitable technique for producing CHD loaded MPs. The results of this study demonstrated that the encapsulation efficiency of the MPs was affected by the drug:polymer ratio. A tendency of a decreased encapsulation efficiency was observed with increasing polymer ratio. This is similar to the effect of the drug:polymer ratio found in the study of Desai and Park (2005).[27]

Fourier Transform Infrared Spectroscopy (FT-IR)

Interaction between the drug and polymer is commonly brought about by identifiable changes in the FT-IR patterns. FT-IR patterns of CHD, Aquacoat ECD and samples are demonstrated in Figure 3-4. Characteristic peaks at 1644, 1530 and 1492 cm^{-1} were exhibited in the FT-IR spectra of CHD (Figure 3-4). The peak 1644 shown also 1643 at leather samples with a new band 1643 (vibration of N-H CHG).[28,29] Also fingerprint of CHG at 1492 peak respectively the aromatic chlorophenol of CHG.[29,30,31] These two bands are shown a bit less quantitatively, according to capsule formulation at 1:2. Moreover, the bands at 1058 (–C–O–C– stretching)[32] and 1376 (due to OH stretching of the intramolecular H-bridge between OH groups)[33]

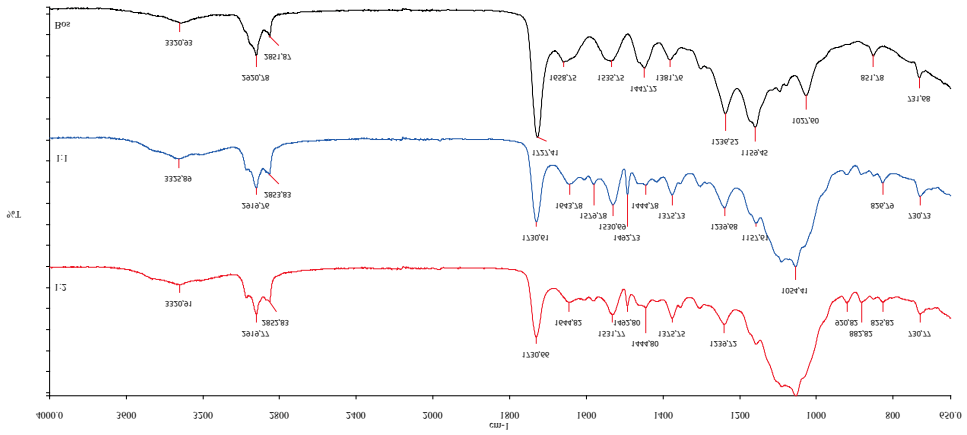


Figure 3. The FT-IR spectrum of the lining leather after without MPs and with two different formulations

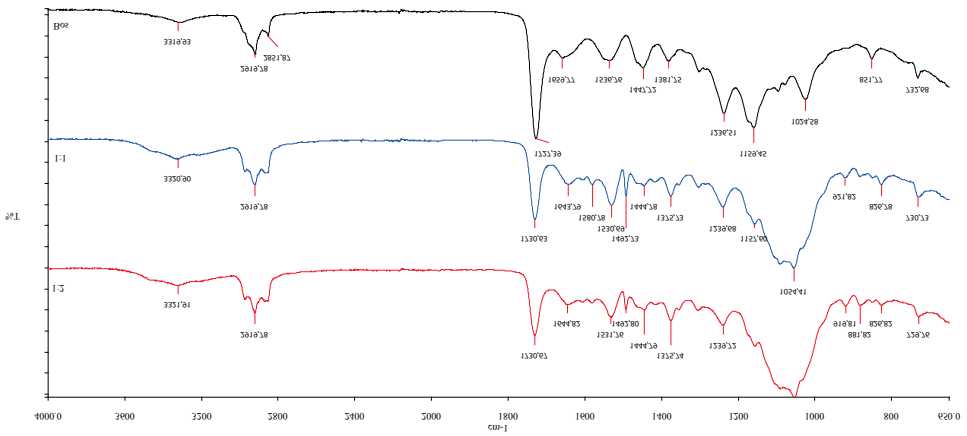


Figure 4. The FT-IR spectrum of the vamp leather after without MPs and with two different formulations

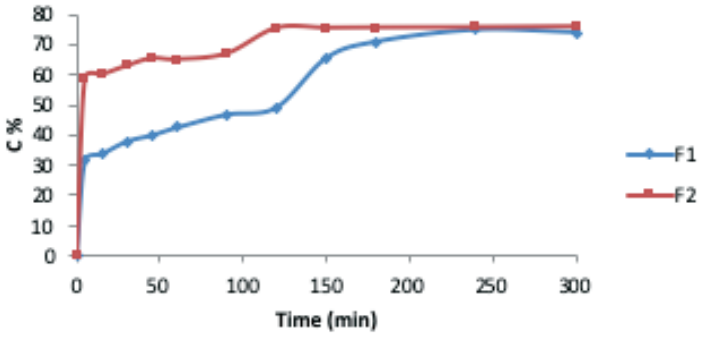


Figure 5. *In vitro* drug release of MPs

bands are characteristic peaks that present at capsules shell material which is ethyl cellulose. By the present band it can be observed that CHG microcapsules have been applied to the leather samples.

In Vitro Drug Release of the MPs

In vitro, drug release studies showed that in CHD release from MPs was very fast. This is probably due to well-done swelling or burst effects. Figure 5 shows *in vitro* release of the MPs. Usually, drug delivery systems are high in the first period due to the application accumulation on the surface of the applied sample.[34] Similarly, MPs also offer initial burst release behavior. MPs prepared by using the spray drying method generally have a matrix structure. Therefore, depending on the method and loading concentration, the drug substance may be present in the MPs as well as on the outer surface when the MPs are exposed to the dissolution media, the drug on the outer surface (non-encapsulated drug) causes a sudden drug release.[35,36]

SEM of the Leathers with the MPs

Electron microscopy images of MP-free and MP-treated with two different formulations vamp and lining leather samples were examined. As seen in Figures 6 & 7, there were more MPs on the F2 formulation samples surface because the F1 formulation's polymer quantity is half of the F2 formulation.

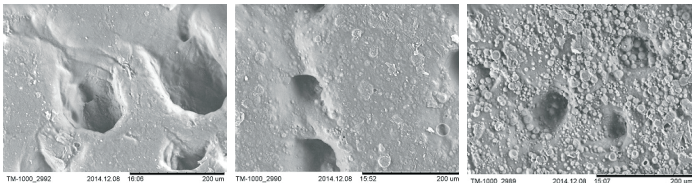


Figure 6. SEM images of the lining leather after finishing process

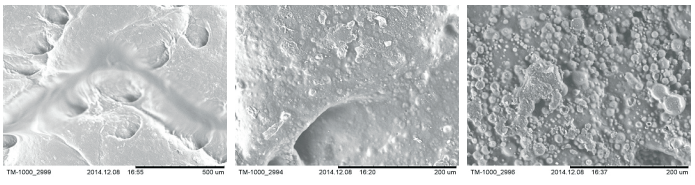


Figure 7. SEM images of the vamp leather after finishing process

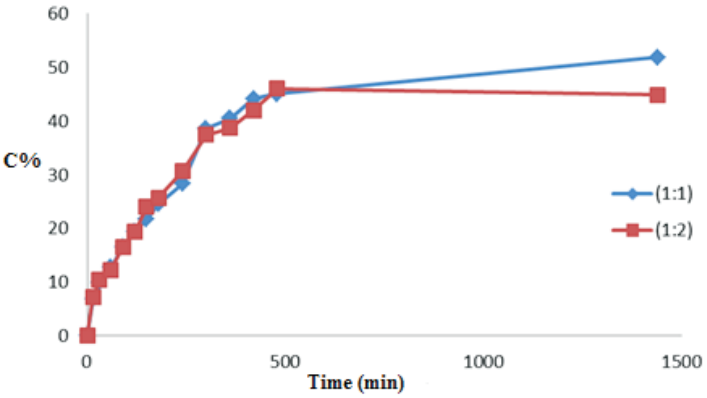


Figure 8. *In vitro* release of the lining leathers with MPs

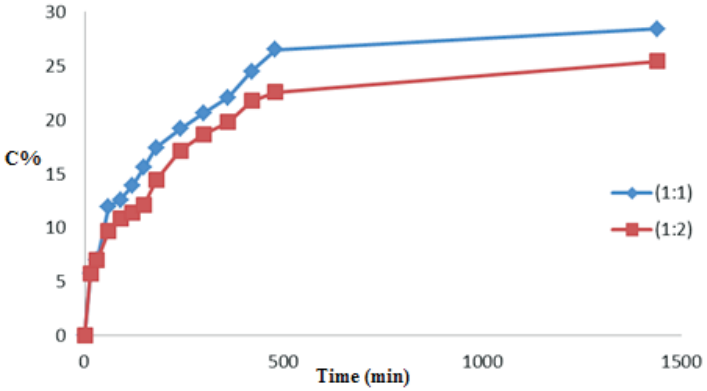


Figure 9. *In vitro* release of the vamp leathers with MPs

In Vitro Drug Release of the Leathers Impregnated with MPs

The *in vitro* release results of the leathers impregnated with MPs in pH 7.4 PBS at 37°C are presented in Figure 8 and Figure 9. As seen in figures, there was controlled release behavior for all formulations from leathers. CHD entrapped within the MPs caused sustained-release more than 24h. Comparing the formulations among each other, the drug release ratio of vamp leather was higher than the release of lining leather up to 72h.

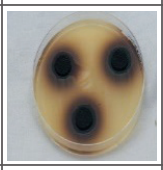
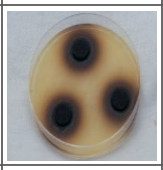
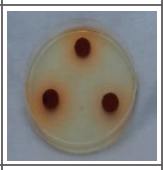
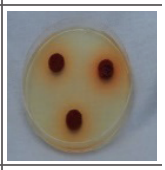
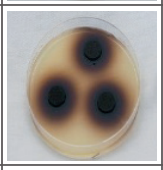
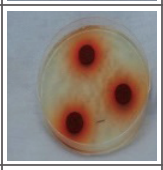
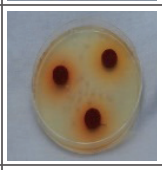
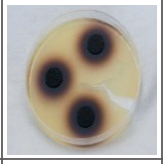
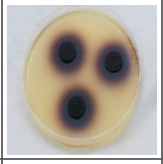
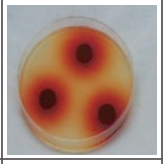
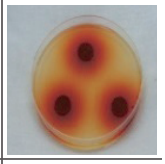
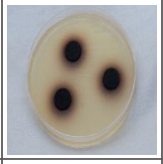
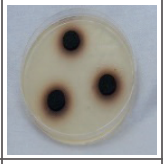
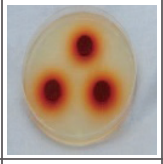
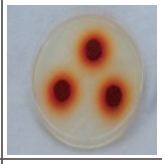
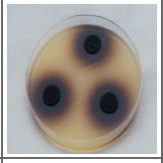
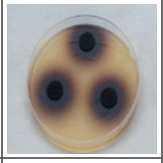
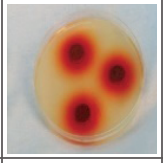
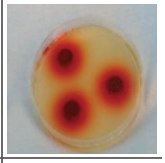
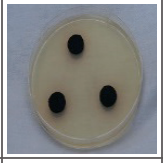
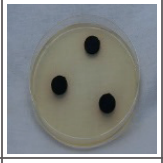
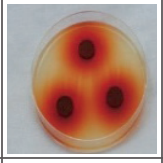
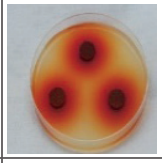
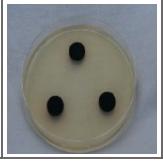
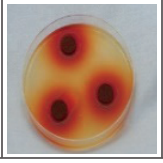
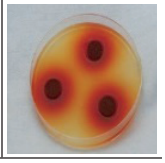
Microbiologic Studies on Leathers with MPs

It was not observed any clear inhibition zone around the vamp and lining leather samples with MPs in Table III. Despite this, on some leather samples, small inhibition zones were seen by microscope. This situation could be interpreted that MPs don't show antimicrobial property comparing the non-capsulated drug. Applying the MPs to the diabetic shoe leathers is affected positively and encapsulation application is used permanence of drug on samples and controlled release.

At all samples, the inhibition zone was not seen, which means that CHD diffusion did not occur. However, the 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 vamp and lining leathers in Table III. Also, there

Table III

Microbiologic test results of the leathers with MPs

	Vamp Leather Samples		Lining Leather Samples	
	F1	F2	F1	F2
Staphlylococcus aureus **ATCC 6538-P**				
Escherichia coli **ATCC 12228**				
Pseudomonas aeruginosa **ATCC 27853**				
Candida albicans **ATCC 10239**				
Klebsiella pneumoniae **CCM 2318**				
Enterococcus faecalis **ATCC 29212**				
Staphlylococcus epidermidis **ATCC 12228**				

was not seen any proliferation surface or edge of the vamp and lining leathers.

Conclusion

The active substance [CHD] was dispersed with ethyl cellulose [EC] wall material and then microencapsulated by spray drying. The resultant product [MPs] was applied to vamp and lining leathers to manufacture shoes for diabetics. Aquacoat ECD wall material has been proven to be a useful polymer in the microencapsulation of CHD active substance formulation by spray drying technology in an aqueous system. In this aqueous system, MP production using EC dispersion is an environmentally friendly method because organic solvents are not used. Particle morphology, FT-IR study, encapsulation efficiency and *in vitro* drug release studies on spray-dried MPs were assessed. SEM photographs showed smooth shaped MPs which adhered well to leather and the MPs. The *in vitro* drug release studies showed the release ratio of vamp leather was higher than that for lining leather. CHD drug release was controlled for all formulations onto the leather. The results suggest that leather footwear for diabetics containing CHD-Aquacoat ECD MPs might be a potential medical leather with topical antiseptic properties to support the treatment of diabetics.

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