BIOPOLYMER-LIPOSOME COMPOSITE FOR FATLIQUOR APPLICATIONS – A 'GREEN' APPROACH TO OPTIMAL TRANSPORT AND DELIVERY OF NATURAL OILS

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Abstract. Combining 2 or 3 steps in leather processing to optimize chemicals usage, time for process and lower pollution load has been the prime objective of this work. The wastewater after fatliquoring process in post tanning/finishing of leathers contains surfactants, neutral salts and unspent or unbound oil. This is mainly due to the manner in which fatliquors are prepared. Normally the oil in water emulsions (fatliquors) are prepared through chemical modification of oils along with surface active agents that would enhance the dispersion of oil in water. In the processing of leather, The discharged chemical compounds from the fat liquors post tanning process are likely to exist as persistent organics in soil. In this paper, an ambitious effort to take forward the successful lessons from other sectors such as healthcare to leather processing is presented. Here, the use of liposomes as oil carriers in design of fat liquors has been envisaged. Here, the lacunae associated with liposomal carriers such as stability, encapsulation efficiency, the release of payload under desired conditions, etc. have been addressed. The study focuses on stabilizing the liposomes and the triggered delivery under the drum pH conditions during leather processing. A liposome -biopolymer composite based on Egg Phosphatidyl Choline and Pectin encapsulating oil (EPCPEC-O), has been prepared. Pectin influences the stability and oil encapsulation efficiency of the composite vesicles. Results show that the particle size of the oil encapsulated liposomes was 1.8 µm for EPC-O (oil encapsulated liposomes) system and for the EPCPEC-O 2.6 µm is observed. These systems have been applied on the leather as a lubricating agent. The release profile of the liposome composite was modeled using a dye instead of oil and its release under a narrow pH range was observed, suggesting that the oil could be released to fibres by modulating the pH. Preliminary studies indicate the potential of this product as a possible fatliquor is encouraging.

1 Introduction

The conventional practices for making fatliquor formulations are chemical modification of oils including sulfation and sulfitation; adding emulsifiers to solubilize the oil component inside the aggregated structures and using synthetic fatliquors. Synthetic fatliquors include sulphochlorinated products of C10-C20 long chain hydrocarbons. The step of Solubilization of the oils in water is associated with usage of large amount of emulsifiers which are hazardous and also energy intensive preparatory methods. The conventional fatliquoring with commercial fatliquors offers 85-90% of exhaustion leaving 10-15% of unexhausted matter in effluent that are made up of emulsifiers, metallic soaps, alkyl phenyl ethoxylates, chlorinated paraffin oils and non-volatile hydrocarbons which are known to be toxic.[1] This makes the process not eco-friendly. In this regard, The leather chemical manufacturing industry has been attempting to produce chemicals which are safe to use and result in zero discharge pollutants from the process. Replacing such systems with environment benign materials is important to make the process sustainable.[2] The present paper describes design of vegetable oil encapsulated liposomes, stabilized by biopolymers as a lubrication agent. It is expected thatThese alternative systems may overcome the disadvantages associated with conventional fatliquors while retaining the leather properties as with conventional method.

The unique properties of liposomes favor the encapsulation of a variety of materials. The renowned biocompatibility of these materials has already acquired much significance in food and pharmaceutical industrial applications.[3] Besides being active delivery vehicles they protect the cargos, their functional properties and bioactivity. The drawbacks associated with these systems are from their physico-chemical instability towards various factors and subsequent leakage of the

contents at undesired site.[4]In leather processing, Stability of the fatliquor product out of the drum and in the drum conditions is imperative as it affects the penetration of the active material and does its job. Pectin is a bio-polymer contain homogalacturonan backbone, and involves hydrophobic interactions through ester methyl groups and hydrophilic interactions through hydroxyl groups. It is being used in many industrial applications because of its biocompatibility and gelling property.[5] In the present study, pectin has been used to stabilize the lipid bilayers and to promote oil encapsulation in hydrophobic bilayer region of liposomes.

2 Materials and Methods

Egg phosphatidylcholine (EPC) 65% TLC, pectin from apple, chloroform (HPLC grade) were obtained from Sigma chemicals. Conventionally processed wet blue goat skins, Commercial synthetic, semi-synthetic vegetable based tanning materials have been used for the present study. Different syntans like naphthalene sulphonic acid, acrylic, phenol based, melamine based (commercial grade) obtained from local companies have been used.

Preparation of oil encapsulated liposomes

In a round bottom flask, 1:4 (vol.ratio)ratio of EPC and Castor oil was dissolved in Chloroform and Methanol mixture (9:1). This mixture was shaken well to get homogenous solution and placed on a Rota evaporator to remove the solvent, that results in the solubilization of EPC by the oil. This mixture was further kept in a desiccator overnight in order to remove solvent traces if any. Milli Q water(in the case of EPC-O) and Pectin solution (0.5%W/V) (in the case EPCPEC-O) has been added to this mixture to get a final 20%W/V oil. This solution was fixed to rota evaporator under 50 ^{o}C bath conditions. The resulting solution transferred to a separating funnel to remove free oil and pectin.

Determination of particle size and charge

The products EPCPEC-O and EPC-O were diluted 100 times by using Milli-Q water. These dispersions have been analyzed for their particle size by Dynamic Light Scattering technique. A high performance particle sizer (Zetasizer Nano series, Malvern), operating at 4 mW He-Ne laser power, scattering angle of 175˚C and wavelength of 633 nm was used to determine the particle size.

Optical microscopy

The products (EPC-O, EPCPEC-O) were analyzed for their droplet size and distribution by using an Optical microscope, Trinocular microscope with camera, Carl Zeiss(AxioscopeA2/Axiocam 105). All images were collected at 63X magnification.

Oil encapsulation efficiency

Both EPC-O and EPCPEC-O have been analyzed for oil encapsulation efficiency. These samples were centrifuged at 10000 RPM in a mini-centrifuge for 30 Minutes. The supernatant or the top layer was collected with the help of a syringe and placed in a pre-weighed crucible. This crucible was heated in hot air oven and change in mass readings was collected periodically, this was repeated till no further mass change of the residue was noticed. This final value was expressed as free oil or unbound oil.

Use of the oil encapsulated liposomes for leather lubrication

The efficacy of encapsulated composite products (EPC-O, EPCPEC-O) in leather lubrication was tested and compared with the conventional fatliquors. A Conventional leather processing for automotive upholstery leathers has been adopted for the control leather. Commercially available fatliquors of different class were offered as a total of 18% to the weight of the leather. In case of experimental leather trials, commercial fatliquors were replaced with the products designed in this study(EPC-O, EPCPEC-O).

3 Results and Discussion

The oil encapsulated liposomes (EPC-O) and oil encapsulated liposome-biopolymer composite (EPCPEC-O) have been analyzed for their physico-chemical characteristics by DLS and Optical microscopy.

Figure 1. Visual images of the EPC-O (Left) and EPCPEC-O (Right).

The visual images of the oil encapsulated liposomes are represented Figure1. From the image, it can be observed that free oil gets phase separated from the oil encapsulated liposomes in EPC-O system(Left). Whereas, in the case of EPCPEC-O system, phase separation is observed as oil encapsulated liposomes (organic layer) and aqueous layer containing unbound pectin. The organic layer of the EPCPEC-O system seems to be uniform and viscous. The visual stability of these products was observed over 6 months, and no further layer separation was observed. From Figure.1. it is clear that EPC-O system contains more amount of free oil compared to the EPCPEC-O. The oil encapsulation efficiency of EPC-O system found to be 20% and 95% for the EPCPEC-O system. The DSC results from our studies indicated pectin stabilizes the lipid bilayers (Through its hydrophobic interaction in the bilayer region) associated with an increase in the Enthalpy of the system(Data not shown here). These results suggest that pectin in the EPCPEC-O system stabilizes the lipid bilayers as well as encourages efficient oil encapsulation. The observed Zeta potential values for EPC-O system was -12mV and for the EPCPEC-O, -16mV.

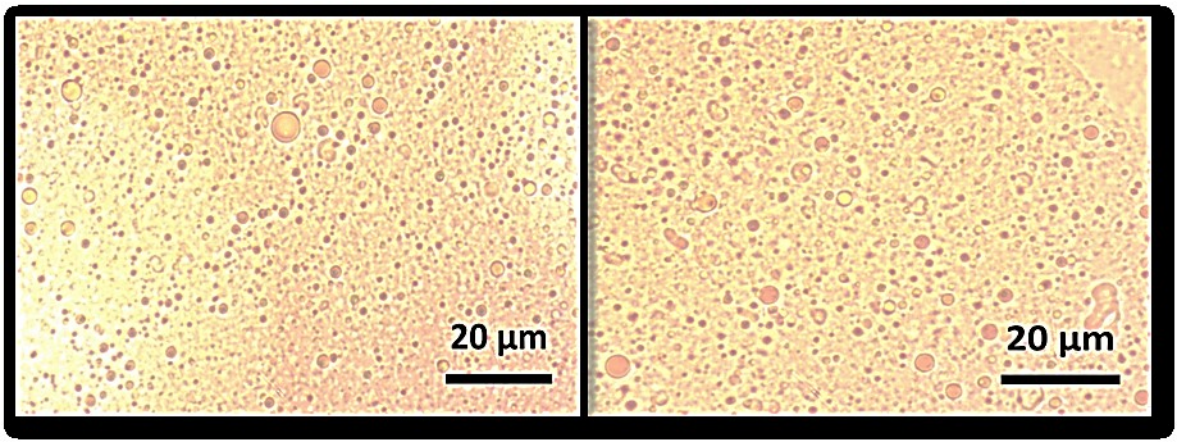

Figure 2. Optical Microscopic images of the EPC-O(Left) and EPCPEC-O(Right), Scale bar represents 63X magnification.

The optical microscopic images of the EPC-O and EPCPEC-O, Figure.2. the average droplet size was found to be around 2.2-2.5µm for both systems. From the DLS results it is confirmed that particle size of the EPC-O system found to be 1804nm and for the EPCPEC-O 2623nm which is in line agreement with the result observed from the optical microscopy. Stability of the leather auxiliaries and subsequent diffusion into the leather matrix is crucial in manufacturing of leather. Both of these systems was found to form stable droplets in the water medium. In order to understand the triggering factors for the release of the encapsulated materials, a model dye Carboxy Fluorescene was encapsulated and its release pattern on change in the pH and temperature was studied. The results suggest that a rapid release of dye at pH 3 and a temperature above 55 ^{o}C was observed. It is well known that the beating action during the leather processing increases the bath temperature, at this conditions the release of the contents were anticipated. The EPC-O and EPCPEC-O systems applied on the leather to evaluate their performance in the leather lubrication. Visual assessment of the leathers carried out independently by 3 experts in a scale of 1-10, indicated that leathers had good roundness and softness. The EPC-O system has surface deposition of oil, possibly due to presence of large unbound oil in it. With the current understanding for softy uppers, the EPCPEC-O system provides good lubrication

4 Conclusion

A stabilized liposomal systems encapsulating oil as a delivery vehicle to deliver its contents under the triggered pH conditions and temperature is described. Biopolymer induced stability and ensures the oil encapsulation in the bilayer region for the composite vesicles. The oil encapsulation efficiency was high for the EPCPEC-O system when compared to EPC-O system. The leathers treated with EPCPEC-O system are soft and round in nature and are comparable with control leather. The leather treated with EPC-O system found to show surface deposition of the oil. The work demonstrates a process towards developing fatliquors based on biodegradable materials, avoiding the emulsifiers and conventional route to make oil in water emulsions, thus helping in optimal use of chemicals, better diffusion and lower pollution load. It is expected that this design and improvement in the process would help in cost and time effective leather process.

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