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# Development of smart leathers: Incorporating scent through infusion of encapsulated Lemongrass oil

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**ABSTRACT**

Fragrant nanospheres were formed by emulsion polymerization technique using chitosan and acylic acid as a wall material. Several parameters such as ratio of lemongrass oil:surfactant, chitosan:acrylic acid, and relative lemongrass oil content in the nanospheres were analysed. Subsequently, the physico-chemical characteristics of nanospheres were characterized using FT-IR, dynamic light scattering, zeta sizer, and SEM. Results showed that, nanospheres were formed using ratios of lemongrass oil:Triton X-100 of 1:1, chitosan:acrylic acid of 1:2 and showed high oil loading (~300%) and encapsulation efficiency (~33%) with the average size of 117±11 nm. In addition to that, the antimicrobial activity of the nanosphere was tested against bacteria (*Bacillus subtilis, Bacillus cereus*) *and* fungi (*Rhizoctonia solani, Macrophomina phaseolina, Aspergillus fumigatus*). Further, the application of nanospheres loaded with lemongrass oil in leather processing was optimized (stage of addition and percentage offer). Physico-chemical characteristics such as physical strength, morphology, washability, perception evaluation and water diffusion and organoleptic properties were investigated. Results reveal that, nanospheres were penetrated in the leather matrix and its fragrance persisted after water and solvent wash. These nanospheres acts as a good delivery vehicle for the manufacturing of fragrance leather and will add economic value to the leather.

Keywords: Nanospheres; fragrance; leather; lemongrass oil

## INTRODUCTION

Leather when used as a material for apparels, and upholstery has an edge over synthetic materials owing to its uniqueness. In today's world, people expect materials to exhibit more than one property. In order to meet these changing demands, attempts are being made to develop "smart leather"[1-2]. The smart leather is one that responds to an external stimuli apart from performing its regular functions. In this present work, encapsulated oils have been incorporated into leathers such that controlled release of scent oils is achieved in order to make the scented leather. With the new trend of imparting pleasant smells, one can hope to satisfy psychological nodes of perception feel and smell whereby leather garments, leatherwear, upholstery and fancy leather etc., will find new dimensions of value addition. This scent infused leather will nevertheless increase the economic value of the product.

The essential oil extracted from the plant sources, consists of terpenes, sesquiterpenes and terpenoid hydrocarbons. They have potential applications in various fields as fragrances in cosmetic industries, as aromas in food industry and as antimicrobial agents in medicinal field to name a few[3-4]. The essential oils in these scent oils are highly volatile in nature due to the presence of the aromatic groups present in their hydrocarbon chain[3]. The application of scent oil in leather as such is impossible as they would evaporate in rapid phase due to their high volatility. In order to control their release, scent oils are microencapsulated using a biopolymer such that they can be retained for prolonged time. Lemongrass oil, which is one of the essential oils is extracted from the plant *Cymbopogan citrus*. This oil has excellent antimicrobial activity against bacillus species[5], which is widely used as preservatives and medicinal application. Lemongrass oil has predominant action on *Candida albicans,* which can be used to treat the infections caused by the *Candida albicans*[6].

Microencapsulates are tiny particles surrounded by a coating to give a small capsule. The material inside is referred to as core/active material and the material outside is wall or shell or membrane. Microcapsules are defined as particles, spherical or irregular, in the size range of about 50 nm to 2000 μm or larger, and are composed of an excipient polymer matrix (shell or well) and an incipient active component (core substance), which aids in controlled release of active components under specific conditions[7]. Micro capsular materials embrace a wide variety of products including paper coating, pressure sensitive adhesives, detergent components, detoxifying agents, implantable hormonal cells, sprayable agricultural aids,

magnetic contrast agents, and controlled release medication[8]. The chitosan, a copolymer of n-glucosamine and N-acetyl glucosamine has one primary amino and free hydroxyl group in its carbon chain. This composition induces a cationic charge to the chitosan. This makes the chitosan a suitable biopolymer for the interaction with anionically charged surfaces. With the above said parameters, the degree of deacetylation and molecular weight range determines its application for the controlled release of the encapsulated materials or solid forms[9-10].

This study aims at the incorporation of the lemongrass oil into the leather and enabling controlled release of the lemongrass oil through microencapsulation using chitosan. The chitosan and collagen interaction establishes electrostatic and hydrogen bonding, which retains the encapsulated scent oils strongly adhering to the collagen. In addition to this, this improves the mechanical properties of the leather by imparting softness to the leather[11]. Chitosan has a potent application in leather as a retanning agent[12] and antimicrobial coating[13], and its ability to act as an efficient shell material for the encapsulation of the scent oils are the reasons for it to be chosen in this study.

## MATERIALS AND METHODS

### MATERIALS

Lemongrass oil was purchased from Aromax trading corporation, Chennai, India. The chemicals such as chitosan, non-ionic surfactant, acrylic acid, potassium per sulphate were purchased from Sigma. Except the leather chemicals, all other chemicals used were of analytical grade. Leather chemicals were purchased from BASF, Colourtex, Clarient and Stahl Pvt Ltd, India.

### METHODS

### MICROENCAPSULATION OF LEMONGRASS OIL

Chitosan was suspended in acrylic acid in various ratios 1:0.5, 1:1, 1:2 and vortexed in order to dissolve it. The optimized ratio in which chitosan is completely dissolved in acrylic acid was taken for the encapsulation of the lemongrass oil (Supporting information-Table S1). Lemongrass oil was dispersed as fine droplets in water under constant stirring. The lemon

grass oil to non-ionic surfactant Triton X-100 ratio was varied from 10 to 1 for the formation of stabilized emulsions. The light barrier properties or light transmittance of the emulsions were measured by exposing the emulsion to light at wavelength of 600 nm using a spectrophotometer (Shimadzu). Emulsions were transferred to a glass cuvette with a 1 cm path length and transmittance of the emulsions were measured relative to that of a reference cell containing distilled water at 25ºC and measured at different time intervals[14]. To avoid the effect of differences in the initial transmittance of samples, the results are presented as the ratio between values measured at different times with the initial transmittance (normalized values).

In order to encapsulate the emulsion the optimized 1:2 chitosan - acrylic acid mixture was added to the emulsion (oil:surfactant 1:1) and ammonium per sulphate was added to it to initiate the polymerization process. The entire reaction was carried out at 55±1 ˚C under continuous stirring for 8 hours in order to achieve the effective encapsulation of lemongrass oil. After the completion of reaction, the internal temperature of the round bottom flask was lowered to 25±1 ˚C and the encapsulated product was transferred to falcon tubes, further freeze dried at -40 ˚C for 24 hour after which lyophilisation was done and stored at 4 ˚C for further analysis.

## CHARACTERIZATION OF EMULSION AND ENCAPSULATED LEMONGRASS OIL

### Determination of the oil load, oil content and encapsulation efficiency

A known amount of lyophilized sample was crushed and dissolved in 1% Triton X-100 in distilled water. The solution was stirred and vortexed to ensure complete extraction of oil in Triton X-100 solution. Percentage oil load, oil content and encapsulation efficiency was determined using a calibration curve through extrapolating the absorbance values of the unknown concentration of the product. The preparation of calibration curve for lemongrass oil was described below: A known and sequential concentrations of lemongrass oil was dissolved in 1% Triton X-100 in distilled water and scanned in the ultraviolet range using UV-Vis spectrophotometer. The calibration curve was plotted by absorbance versus concentration at the $\lambda_{max}$ = 245 nm. Percentage oil load, oil content and encapsulation efficiency was calculated using the following equation[15-16]. All the experiments were carried out in triplicates.

$$Oil\ load(\%) = \frac{W_2}{W_3} \times 100$$

$$Oil\ content(\%) = \frac{W_1}{W} \times 100$$

$$Encapsulation\ efficiency(\%) = \frac{W_1}{W_2} \times 100$$

where, W is weight of nanospheres; $W_1$ is actual amount of lemongrass oil encapsulated in a known amount of nanospheres; $W_2$ is amount of lemongrass oil introduced in the same amount of nanospheres; $W_3$ is total amount of polymer used including cross linker.

**Surface tension and interfacial tension measurements using tensiometry**

Surface tension and interfacial tension measurements for oil and product were performed on a GBX 3S tensiometer from France, employing a platinum Du Nuoy ring probe with an accuracy of 0.1 mN/m and standardised with milli-Q water. All measurements were performed at 25±0.1 ˚C in triplicates. Surface tension measurements and the interfacial tension measurements at the hexane/water interface were performed on lemongrass oil and also on formulated emulsions.

**Dynamic light scattering (DLS) measurements**

The hydrodynamic size and charge of the emulsion and nanospheres were determined using dynamic light scattering (Zetasizer nano, Malvern instruments U.K) at 25$^{o}$C. Initially, the prepared encapsulated products (in solution form) were diluted in Milli-Q water before the experiments. All the experiments were performed in triplicate and average was taken.

**FT-IR spectral analysis**

Fourier transform infrared (FT-IR) spectra of lyophilized encapsulated lemongrass oil in nanospheres were recorded using an ABB MB 3000 FT-IR spectrometer at room temperature. The spectra were taken at 4 $cm^{-1}$ resolution, averaged over 31 scans in the range of 4000-650 $cm^{-1}$. Before taking the spectrum, the samples were mixed with potassium bromide (IR grade KBr was used as scanning matrix) to make nearly transparent and homogeneous pellets. The final spectra were collected after subtracting background spectra.

**Optical, scanning electron microscopic (SEM) and transmission electron microscopic (TEM) analysis**

The emulsion and encapsulated product was mounted on the glass slide and viewed under an optical microscope. The optical micrographs of the emulsified and encapsulated lemongrass oil were obtained with the help of a camera attached to microscope. The surface morphology of microencapsulated product was investigated by scanning electron microscopy (Quanta series 200). The microencapsulated product was coated onto a glass plate for the formation of film under controlled drying. The sample coated glass plate was then mounted on the metal stubs using an adhesive. After being vacuum-coated with a thin layer (100-150 Å) of gold, formulated micro emulsion products were analysed by SEM at 12 kV. Encapsulation of lemongrass oil in chitosan and acrylic acid wall material has been confirmed using TEM. Aliquot sample has been placed on a carbon coated copper grid and allowed to dry at room temperature for two hours prior to imaging. TEM images of encapsulated product were obtained using Hitachi H-7650 at an acceleration voltage of 80 kV at room temperature.

**APPLICATION OF ENCAPSULATED LEMONGRASS OIL INTO LEATHER**

Wet blue sheep skins of 4 sq.ft. with 1 mm thickness were taken for application studies. Product was applied into leather based on the shaved weight. The leathers were shaved, washed and neutralised to a pH of 5.5-6. In order to optimize the stage of offer in leather processing, the encapsulated product was applied during various post-tanning operation such as after neutralization (in fresh bath), re-tanning, fat -liquoring and before fixing. In order to optimize the percentage offer of the encapsulated product in post-tanning operation after neutralization using various percentages from 1 to 6% (Table S2-Suporting information). The control (without scent infusion) and experimental (lemongrass oil infused leathers) garment leathers were produced. The leather was rinsed, piled overnight. Next day it was set, hooked for drying, staked, buffed and trimmed and the final leathers were subjected for evaluation. The detailed post-tanning process recipe for control and experimental leathers were tabulated in Table S3 and S4 (supporting information). The final leathers were evaluated for its colour, organoleptic and strength properties.

**CHARACTERIZATION OF LEMON INFUSED LEATHERS**

**Perception evaluation**

Ultimate test for the product is performance in the hands of the user. It is necessary, often utilizing a panel of people, to take a properly dried fragrance sampler and let them test it. Intensity of scent was carried out through a perception method using valuators, where the evaluation scale was carried out according to the following: The evaluators were asked to sense the smell and were asked to give a rating based on the Table 1.The intensity of scent has been indicated with the degree of intensity. Since such degrees have been provided with an arithmetic value, variants of the test are rounded to the closest value to average.

**Physical testing and evaluation of organoleptic properties**

This test was carried out using INSTRON Series IX to check whether the microencapsulated lemongrass oil have induced any changes to the physical properties like tensile strength, stitch tear strength in the leather. Samples for various physical tests from experimental and control crust leathers were obtained as per IUP method[17]. Specimens were conditioned at 80±40F and 65±2% R.H. over a period of 48 hours. Physical properties such as tensile strength and tear strength were examined as per the standard procedures[18-19]). Each value reported is an average of four (2 along and 2 across the backbone) measurements. Further, leathers were assessed for fullness, softness, grain tightness, grain smoothness, and colour uniformity by standard hand and visual evaluation technique and ratings were given on a scale of 1-10.

**Softness**

The softness of the control and experimental leather was measured using a MSA ST 300 digital leather softness tester supplied by MSA Engineering system limited. The method permits measurements of softness of leather without defacing the sample. The method of measurements were made as per IUP 36[20]. The leather prior to softness measurements were conditioned and measurements performed on locations specified under IUP 2[17]. The measurements were performed on locations using a 35 mm ring at 20±2 °C and with a

relative humidity of 65±2% and thickness of leather being 0.8mm. Higher value indicates higher softness.

**Washability test in water and solvent**

A known weight of leather is taken and put in a beaker containing 10 mL of water and dry cleaning agent (Perchloroethylene). It is allowed to remain in water for a period of 5minutes. It is then checked for the retention of fragrance after drying. This is repeated for about 3 times.

**Determination of contact angle and water diffusion coefficient**

To evaluate the surface properties of control and experimental leather samples, contact angle measurements were carried out using home built Contact angle equipment. In this experiment 5 µL of water and hexadecane were placed on the surfaces of samples and measurements were made and contact angle and surface energies were calculated.

Here the assumption is decrease in diffusion coefficient would indicate the coverage of pores by the microencapsulated particle. Based on this the sample that has maximum penetration and blockage of pores was determined. Samples with similar length, thickness & shape leather as well as control were taken. Their dry weights were recorded accurately. Then they were placed in beakers containing water. Samples of leather were weighed at periodic intervals between the wet and dry sample as a function of time for different time intervals. At each time before taking weights, the surface water is removed by gently using tissue paper. From this the % water uptake can be calculated using the following formula.

$$Water\ uptake(\%) = \frac{W_t - W_0}{W_\infty} \times 100$$

where, $W_t$ is the weight of the sample at any time; $W_o$ is the dry weight of the sample; $W_\infty$ is the weight of the sample at infinity. Time versus percentage of water uptake graph was plotted for the samples.

Diffusion coefficient for each samples were calculated using the formula,

$$D = \frac{\rho \times \%\ rate\ of uptake}{4 \times W_\infty}$$

where, ρ is the density

**Scanning electron microscopy**

Samples from the control (C) and experimental (E) leathers were cut from the official sampling position[17]. The leathers from both experimental and control process with uniform thickness were directly taken for analysis without any pre-treatment using Quanta 200 series. Scanning electron microscope for the grain surface and cross section were obtained by operating the SEM at low vacuum at an accelerating voltage of 12 KV with different magnification levels.

**Antimicrobial activity of the encapsulated lemongrass oil**

**Antibacterial activity**

The antibacterial activity was done by standard well disc diffusion method on MHA. 100 µL of fresh bacterial cultures were inoculated along with nutrient agar wells created using a sterile cork borer. Different concentrations of the encapsulated oils were loaded into the wells and the plates were incubated at 37 ºC for 24 h. Inhibition zones in diameters were measured in mm.

**Antifungal activity**

Three mycelial fungi such as *Rhizoctonia solani*, *Macrophomina phaseolina*, *Aspergillus fumigates* were used for *in vitro* antifungal activity. The agar plug of fungal inoculumwas placed onto the potato dextrose agar individually. The wells were made using a sterile cork borer (5 mm). The given sample was loaded as 25, 50, 75 and 100 µL per well concentration. The plates were incubated at 35°C and observed after 48 and 72 h. The inhibition zones were read at the point of complete inhibition of growth (ZOI).

**RESULTS AND DISCUSSION**

**Transmittance measurements to standardise the proportions of oil: surfactant**

Oil and water are immiscible due to high interfacial energy, which results in phase separation. Hence, it becomes essential to reduce the interfacial energy between water and oil so as to form dispersion. Coverage of oil by non-ionic surfactant can decrease the interfacial energy to as low as 5 mN/m. The particles could be stopped from coagulating with each other by surrounding them with surfactant. The charge on the surfactant repels other particles electrostatically. The main function of the surfactant in micro emulsion polymerization was isolation of nano droplets of oil by micelles formation thus preventing phase separation. Micelles only form when the concentration of surfactant is greater than the critical micelle concentration (CMC). Hence, it is essential to optimize the amount of surfactant used. Different ratios of surfactant: oil ranging from 0.2:1 to 1:1 with increasing order of 0.2, and in higher range 2:1 was taken to identify the critical concentration required to produce a homogenized dispersion (i.e. complete coverage of droplets lowering the interfacial energy). Transmittance measurements were taken for the series of solution containing various proportions of non-ionic surfactant ranging from 0.2, 0.4, 0.6, 0.8 to 1:1. The results are tabulated in Table 2. The higher the transmittance percentage, the greater will be the transparency of the emulsion, which implies that the particle sizes are smaller. From the table it is observed that 1:1 ratio has a higher transmittance value compared to the other compositions indicating that the solution is less cloudy than the other ratio and provides better dispersion. Also concentrations of non ionic surfactants greater than 1:1 gave good dispersion. 1:1 has been taken as optimized concentration, so as to lower the surfactant concentration because excess surfactant can result in foaming and hindrance but the above formulation cannot be used as such (lemongrass oil+water+surfactant), because it will result in the migration of oil without encapsulation.

**Encapsulation of lemongrass oil through emulsion polymerization**

Emulsion polymerization technique has been adopted to encapsulate the emulsified lemongrass oil because it is one of the fastest methods and readily scalable. Chitosan and acrylic acid act as a wall material for the emulsified oil droplets and the reaction completion has been confirmed by the formation of clear solution (from emulsion to clear solution) in water. Encapsulation using chitosan can provide a shell, which would reduce the volatility of oil and may help in sustained release. The oil loading (%), oil content (%), and encapsulation efficiency (%) of lemongrass oil in nanospheres are 299.39±2.31, 91.77±1.16 and 32.66±1.13%, respectively.

**Determination of interfacial energy of lemongrass oil**

Density of oils are almost equal to 1, which indicates that the fluidity of oils are similar to water (ρ=1). Miscibility of oils in water will not be influenced by density. Interfacial energy is an indication of the hydrophobic or hydrophilic nature of any material. If the material has low interfacial energy it indicates the presence of hydrophilic groups. The interfacial energy for the lemongrass oil has been measured and it was observed that lemongrass oil has an interfacial energy of 11.15 mN/m. The interfacial energy is reduced by the wall material, which indicates that lemongrass oil may have high hydrophobic interaction with chitosan and stability of the encapsulation increases with increase in interaction between the oil and wall material.

**Determination of surface tension and particle size**

Surface tension analysis was done to analyse whether the oil has been encapsulated or not and measurements are tabulated in Table 3. Surface tension value of the product is found to be lesser than that of the lemongrass oil. The decrease in the surface tension is due to the presence of surfactant. The surfactant, Triton X-100 reduces the surface tension between the water and oil. Thereby molecules of water are no longer in the state of cohesion and leads to the formation of the tiny droplets.

A particle size measurement was carried out to analyse the size of the particles and also to know the homogeneity of the emulsion product. The mean particle size of nanospheres was measured and the results showed that the diameter in the range of 100-270 nm. The average diameter (intensity based harmonic mean) of the nanospheres is 117±11 nm. These nanospheres may block the pores of the fibril level, which has a pore size of 50-500 nm. Interaction of these nanospheres with leather matrix could be through hydrogen bonding with hydroxyl groups of chitosan. Particles are distributed between 5-10 nm and 100-500 nm (Figure given in supporting information, Figure S1). This indicates nanosphere formation is not in a homogenized manner, but small spheres are preferred because it can fit into the interspaces present in the leather matrix and it can easily adsorbed on the leather matrix. Variation in particle size can accommodate various levels of pore size presented in collagen matrix/skin. Diameter between 5-10 nm nanospheres can penetrate up to micro fibril level but

intensity of the particle presence is comparatively low. Diameter of particles between 100-500 nm can penetrate up to sub fibril and facile level pores.

**Oxidation index measurements**

Oxidation of oils is expected to be accelerated through a free radical mechanism[22]. However, in product, oil was encapsulated within the solid matrix and the oxygen could reach close proximal of the oil only by permeating through the solid wall matrix. Oxygen permeability was through the solid wall matrix. Oxygen permeability depends both on solubility and diffusion. Therefore, surfactant content is an important factor affecting both solubility and diffusion[22]. The concentration of lemongrass oil in essential oil and oil in formulated product has been listed in Table 3, which signifies the high oil content of the lemongrass oil.

**Optical, scanning and transmission electron microscopy of product**

The optical micrographs of product shows that the formation of the encapsulated products are polydispersed and uniformly distributed as the tiny droplets where surrounded by the wall material, which is distinctly observed in Figure 1.

Scanning electron micrographs were taken to analyze the surface morphology of the sample and to analyse the size and shape of the microsphere. Micrographs were taken at different magnification 500, 1000 and 4000X. The scanning electron micrographs of encapsulated lemongrass oil at different magnifications have been presented in Figure 2. The micrographs of lemongrass oil encapsulated product show that the surface morphology is smooth and most of the spheres are in circular shape with some of them in irregular shape with less than 5 μm size.

Figure 3 provides the TEM pictures of nanospheres. It can be observed from the micrographs that, the wall material and oil has been differentiated visually by lighter and darker colour. As well as nanospheres exhibited the definite shell (darker colour) around the oil. Particle sizes of the nanospheres are less than 50 nm as well as aggregated on the surface.

**FT-IR analysis**

FTIR spectrum of lemongrass oil containing chitosan-acrylic acid encapsulate is given in Figure 4. Lemongrass contains usual active contents such as citral (geranial and neral) and

essential oil (1-2% on a dry basis) as well as some additional unusual active components namely limonene, citronellal, ß-myrcene and geraniol. There are 65 compounds found in chemical composition of essential oil of lemongrass[23]. Lemongrass oil showed a strong absorption peak at a wave number of 1694 $cm^{-1}$,which is carbonyl stretching band of lemongrass oil and it is shifted to 1726 $cm^{-1}$ in the case of encapsulated product (Figure 4.). Peak at 1511 $cm^{-1}$ and broad band at 3415 $cm^{-1}$ can be assigned to NH bending of $NH_3^+$ functional group present in the chitosan. 1512 and 1457 $cm^{-1}$can be assigned to the asymmetric and symmetric stretching vibration peaks of the carboxyl groups of poly acrylic acid[24]. These results reveal that the encapsulated product contains lemongrass oil and the carboxylic groups of the acrylic acid and amino group of the chitosan interact electrostatically during the polymerization. Furthermore, there is a shift in the characteristic peak of lemongrass oil, which indicates that there is a interaction between lemongrass oil and wall material, this may help in retaining the fragrance in the encapsulated product.

## APPLICATION STUDIES AND LEATHER CHARACTERIZATION

These studies were primarily carried out to optimize the % offer and stage of incorporation of the synthesised product. Based on the exhaustion and release, it was found that 5% oil content offer on shaved weight after neutralization resulted in better fragrance. This might be due to the fact that after neutralization, the leather matrix will have lot of free spaces for the encapsulated oils to go in. The control and experimental leathers were produced as stated in experimental section.

### Perception evaluation

The evaluators were asked to sense the smell and give rating based on the Table 1. The leather sample with rating 3 to 4 is considered to be better as it indicates a nice fragrance without irritation as presumed in Table 1. It has been observed that leathers scented with lemongrass give a wet touch and feel while sensing leather, with the degree of intensity is 3.8.

### Contact Angle and Diffusion coefficient

For both control and the experiment leathers were showed good hydrophilicity and lipophilicity suggesting that the scented oil has penetrated the leathers and do not reside on the surface. Contact angle measurements did not show different surface properties for control and samples an indirect method to evaluate penetration of microencapsulated oils in to the samples need to be developed[21]. The calculated results of rate of % water uptake and diffusion coefficient are presented in Table 4 and its pictorial representation has been shown in Figure 5. Assuming the leather sample has similar pore size distribution; their diffusion coefficient can be compared. The results indicate that water diffusion is slower in lemongrass scented leather than in control. Though absolute values of D do not have much significance, a comparison suggests the diffusion coefficient value of lemongrass is due better blockage of pores by the microencapsulated particles for the other leather sample, water takes shorter time to penetrate, which means either the pores are not completely covered or these micro emulsion particles in the pores are not very stable and oils evaporate faster.

**Washability test in water and solvent**

Washability test with water has been carried out to analyse the perception of fragrance after washing. Three washings were made with an interval of five minutes. The fragrance was perceived in all the leathers except orange oil infused leather, because the intensity of orange is as such low. The fragrance perception in scented leathers after water washings is given in Table 5. When pH>6, chitosan is insoluble in water, which is a wall material[25], this insoluble nature may help to protect the lemongrass oil during water washing and storage. These results confirmed that the microencapsulated lemongrass oil has been released in a slow and sustained manner. Washability test with solvent (trichloro ethylene) has been carried out to analyse the perception of fragrance after dry cleaning because trichloro ethylene is the commonly used dry cleaning solvent. Three washings were carried out with five minute intervals. The fragrance intensity increased initially. After the second wash the fragrance was perceived in all the leathers just as before washing, except orange oil infused leather because orange oil intensity is as such low.

**Strength and organoleptic properties**

The leather treated with scented encapsulate has been tested for physical strength characteristics to assess whether scent infusion had any effect on the strength characteristics

of the leather and the results are tabulated in Table 6. The scent infused leather has been made for the purpose of leather goods and garments application. The strength properties are comparable with the standards or even better than the standard values.

Leathers were also assessed by three experienced tanners for organoleptic properties through standard hand and visual evaluation technique and ratings were given on a scale of 1-10 for each property. Higher points indicates better property, which has been presented in Table 6. The organoleptic properties are comparable to the control leathers and even better than the control The softness of the leather measured as described in experimental section show that the experimental leather has more softness values when compared with the control. From this it can be inferred that lemongrass oil infusion has a significant role in altering the softness properties of leather.

**Scanning electron microscopic analysis of leathers**

The scanning electron micrographs of scent infused leather show that surface of the leather is clean and smooth even at a higher magnification (Figure 6). The cross sectional view indicates that microspheres are blocked in between the pores. The SEM photographs agree with product characterization results with respect to the compatibility of the product and also with water diffusion coefficient measurements (Table 4) of the leather characterization.

**In-vitro antibacterial activity of product**

The chitosan encapsulated lemongrass oil exhibited antimicrobial activity against bacteria and fungi (Table 7). The mechanism of inhibition may be due to the individualistic effect of chitosan and lemongrass oil or a combinational effect. Both chitosan and lemongrass oil possess excellent antimicrobial activity as chitosan blocks the ion channel mechanism of the microbes forming an impermeable thick layer around the cell and lemongrass oil has the ability to interact with microbial cell resulting in poly cation-anion interaction thereby leading to disruption of cell wall and leakage of cellular constituents[26-28]. Another possible mechanism is the low molecular chitosan invades the cell and inhibits the m-RNA synthesis thereby lysing the microbes[28-29]. Chitosan has the ability to inhibit the growth of microorganisms such as bacteria and fungi. Chitosan dissolved in acidic medium acts as an excellent antimicrobial agent as it forms a thick layer by precipitation around the cell whose charge is neutral. As a consequence of this, the ion channel is blocked by the thick layer of

chitosan, which is considered to be essential for the living cell[30]. Another possible mechanism is that the charge interaction between the low molecular weight chitosan and the microbial cell results in the inhibition of the DNA synthesis and leads to disruption of the cell wall and leakage of the intercellular matter[28]. The lemongrass oil, which consists of terpenes, has potent effect on mitochondria, thereby inhibiting the respiration of the microbes. The essential oil predominantly consists of polyphenols, which has ability to alter the metabolism, affecting normal cell growth and leading to death of the microorganisms[31]. Hence, the combinatorial action of chitosan and lemongrass oil exhibit great antimicrobial activity, which is added advantage to the end product.

**Conclusion**

Present study attempts to produce scented leather using encapsulation as a technique to arrest the volatility of scent oils. Lemongrass oil was encapsulated in chitosan and acrylic acid for the formation of nanospheres resulting in the smaller spheres with the diameter of 117 nm, which may have high preferential application in leather and textiles. This study demonstrated that the proper combination of surfactants and wall material for the encapsulation of lemongrass oil, which in turn produces nanospheres. These nanospheres were diffused well in the leather matrix and deposited on the collagen fibres. Triton X-100 was used as emulsifier, which produces stable emulsion with the ratio of 1:1 (v/v). The encapsulation has been made from stable emulsion formulations using emulsion polymerization technique. The lemongrass oil in the nanospheres were well retained in leather matrix after washing with water and solvent. The encapsulation process and scented products are low in cost, nontoxic, biocompatible, and biodegradable.

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**Figure Captions**

**Fig. 1.** Optical microscopy images of the (a) encapsulated and (b) emulsion of lemongrass oil

**Fig. 2.** Scanning electron Micrographs of encapsulated product with different magnifications 4000x, 1000x and 500X

**Fig. 3**. TEM images of the encapsulated product

**Fig. 4.** FT-IR spectrum of encapsulated product

**Fig. 5.** Pictorial representation of water diffusion inhibitions in fibrous packed porous matrix

**Fig. 6.** Scanning Electron Micrographs of (a) control and (b) lemongrass oil infused (experimental) leather, with different magnification for surface and cross section

**Fig. 1.**

**(a)** **(b)**

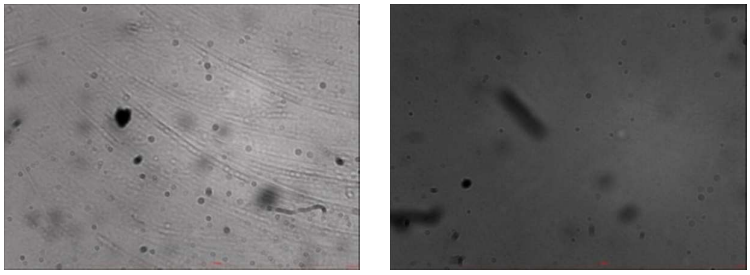
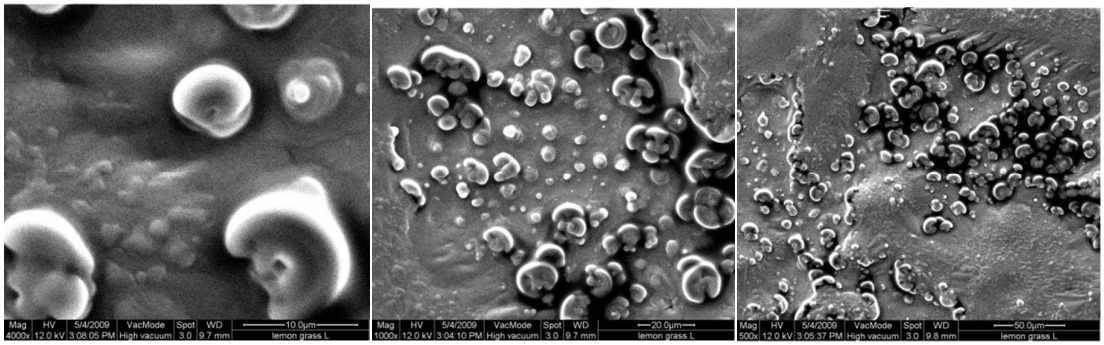


**Fig. 2.**




**(a)** **(b)** **(c)**

**Fig. 3.**

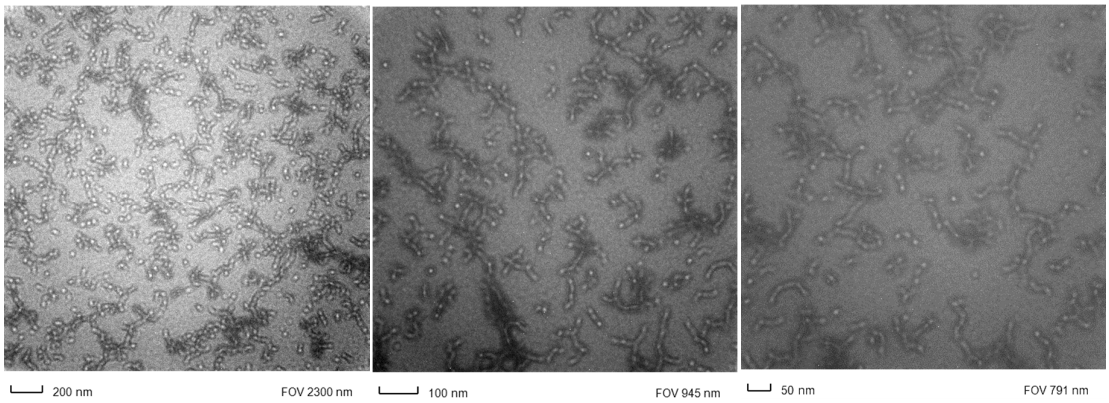


**Fig. 4.**

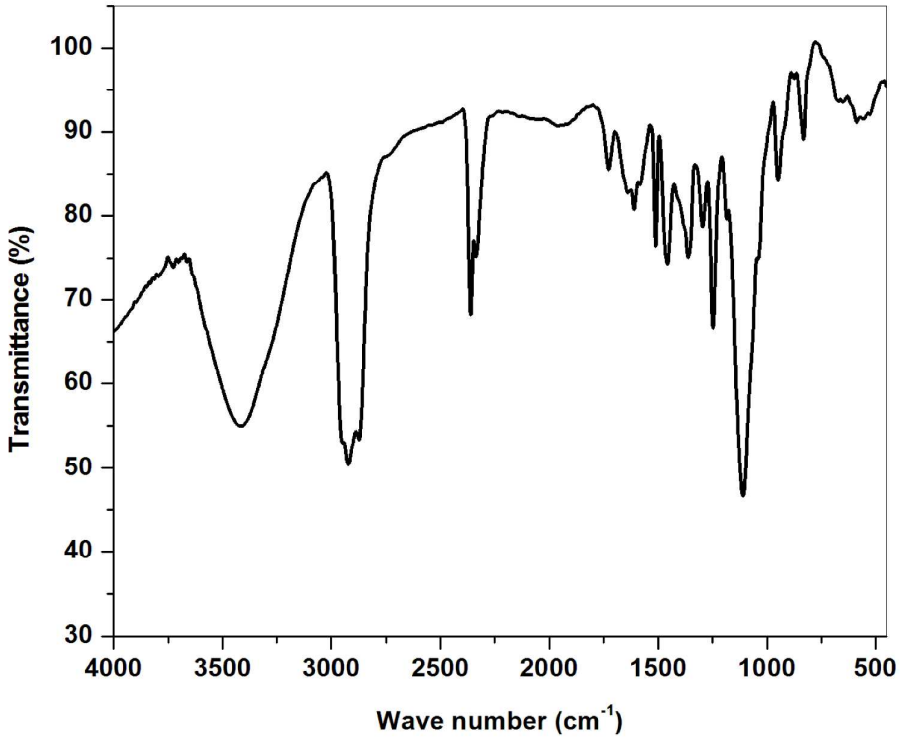


**Fig. 5.**

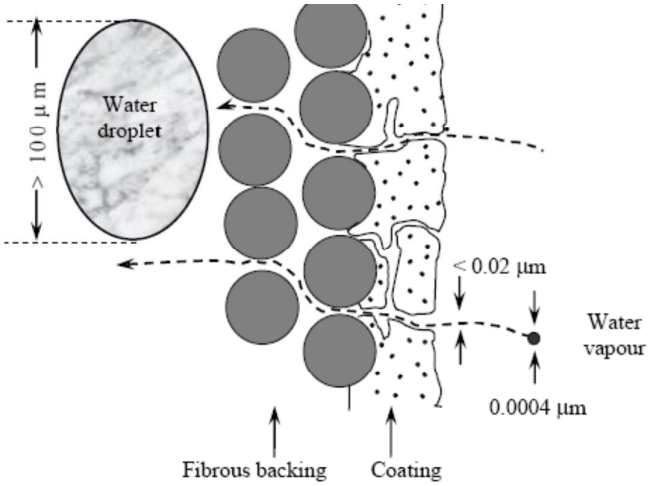
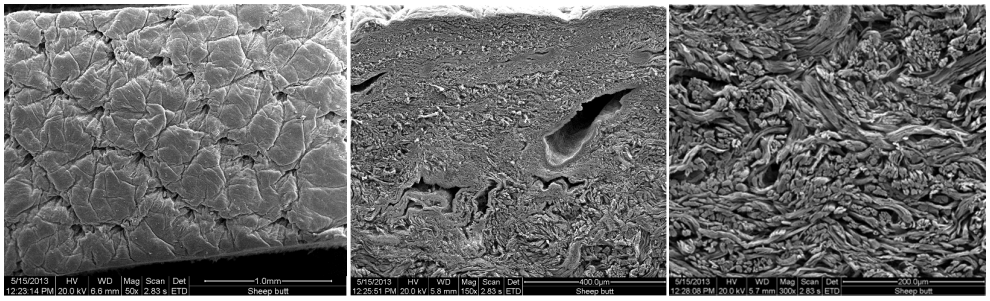


**Fig. 6.**

**(a)**



**(b)**

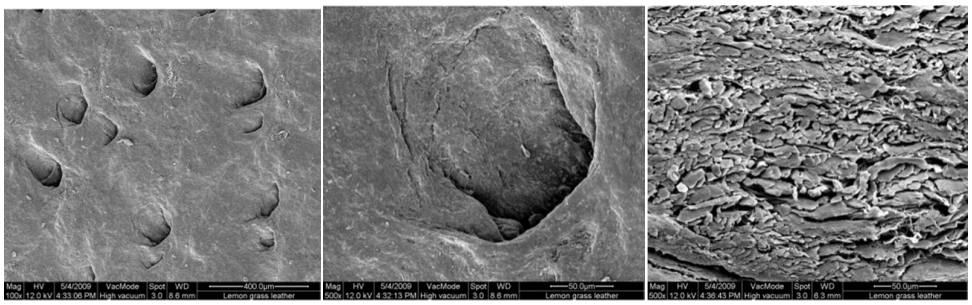


**Table 1. Perception evaluation for lemongrass oil infused leather**

| Intensity of fragrance emission by leather | Degree of Intensity |
|---|---|
| Unperceived | 1 |
| Perceived | 2 |
| Clear perception with no feeling of discomfort | 3 |
| Perception with feeling of discomfort | 4 |
| Perception with a strong feeling of discomfort | 5 |
| Unbearable | 6 |

**Table 2. Transmittance values of various proportions oil and surfactant**

| Oil: Non-Ionic Surfactant | Transmittance (%) |
|---|---|
| 1:0.2 | 0.02 |
| 1:0.4 | 0.02 |
| 1:0.6 | 0.02 |
| 1:0.8 | 0.03 |
| 1:1.0 | 71.93 |

**Table 3. Characteristics of lemongrass oil and encapsulated product**

| S.No. | Characteristic studies | Lemongrass oil | Encapsulated product |
|---|---|---|---|
| 1 | Density (g/mL) | 0.898 | - |
| 2 | Interfacial Energy (mN/m) | 11.15 | - |
| 3 | Surface Tension (mN/m) | 29.5 | 28.3 |
| 4 | Average Particle Size (nm) | - | 102.1 |
| 5 | Concentrations of Oils in Essential Oils and Products (μM/L) | 21.79 | 6.06 |

**Table 4. Diffusion Coefficient, % Water Uptake and Perception Evaluation of Leathers**

| Sample | Rate of % Water Uptake | | | Diffusion Coefficient ($m^2$/min) | Degree of Intensity |
|---|---|---|---|---|---|
| | T = 10 min | T = 25 min | T = 45 min | | |
| Control | 24.952 | 23.825 | 25.002 | 13.93 | |
| Lemongrass | 21.642 | 23.395 | 24.930 | 12.4 | 3.8 |

**Table 5. Fragrance Perception Test in Water and solvent Washing**

| Experimental Leathers | Water | Solvent |
|---|---|---|
| **Washing 1** | Perceived | high intensity Perceived |
| **Washing 2** | Perceived | Perceived |
| **Washing 3** | Perceived | Perceived |

**Table 6. Organoleptic and physical strength properties of leather**

| Sample | Control | Encapsulated product |
|---|---|---|
| **Tensile Strength (kg/Cm$^2$)** | 264.72 ± 2.5 | 251.52 ± 4 |
| **Tear Strengths (N/mm)** | 121.40 ± 3 | 115.80 ± 4 |
| **Fullness** | 7 | 8 |
| **Softness** | 9 | 8 |
| **Grain tightness** | 8 | 7.5 |
| **Grain smoothness** | 9 | 8 |
| **Colour uniformity** | 8 | 7 |

**Table 6.Antimicrobial activity of encapsulated product**

| Sample Name | Lemongrass oil product | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Bacillus cereus* | | | | *Bacillus subtilis* | | | | *Aspergillus fumigatus* | | | |
| Concentration (mg/mL) | 25 | 50 | 25 | 50 | 75 | 100 | 75 | 100 | 25 | 50 | 75 | 100 |
| zone of inhibition | 4 | 5 | 7 | 9 | 11 | 13 | 13 | 15.5 | 7 | 9 | 11 | 13 |