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Sustainable approach for development of antimicrobial textile material using nanoemulsion for wound care applications

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Abstract

Development of nanostructured textile material using sustainable finishing route and to evaluate its performance in the areas like wound management is challenging task. Stable and eco-friendly o/w nanoemulsion of tetrahydroxy curcumin derivative was developed by using high pressure homogenization method. The stable nanoemulsion for the selected drug was developed and evaluated for appearance, particle size distribution (100–300 nm), zeta potential (− 30.1 to − 31.1 mV) and drug content (80–83.3%). Nanoemulsion containing antimicrobial drug were impregnated on the textile material by exhaust methods using β-cyclodextrin and polycarboxylic acid as a crosslinking agent. Treated textile material is evaluated for its antimicrobial activity against standard antimicrobial strip using both AATCC 147 and AATCC 100, treated samples shows positive results against both positive and negative bacteria. Current eco-friendly technique would be innovative step towards development of medical textile for wound care applications.

Keywords: Nanoemulsion, Inclusion complex, Antimicrobial textiles

Introduction

In the last few years, health care professionals have faced with an increasing number of patients suffering from various skin related diseases such as wound, burn injuries and which are really difficult to treat and heal. Consumer's attitude towards hygiene and active lifestyle has created a rapidly increasing market for a wide range of medical textiles, which in turn has stimulated intensive research and development (Paterson 2009). As a result, the number of functional textiles with an antimicrobial activity incorporating both synthetic and biopolymers has increased considerably over the last few years. The synthetic antimicrobial agents are very effective against a range of microbes and give a durable effect on textiles; they are a cause of concern due to the associated side effects, such as action on non-target and microorganisms and water pollution. Hence, there is a great demand for antimicrobial textiles based on eco-friendly agents which not only help to reduce effectively the ill effects associated due to microbial growth on textile material but also comply with statutory requirements imposed by regulating agencies (Gotmare and Kole 2017). In a near future, textile materials treated with natural products will perhaps be the largest application in the area of medical textiles

due to their unique properties like biodegradability and excellent stability against bacterias. Many attempts were made to develop medicated textile products using natural materials like neem, chitosan, alginate etc. but, very limited work has been reported the use of curcumin and it's derivatives on the textile material even though it is showing excellent antimicrobial property against both positive and negative bacterias. The curcumin is main constituent which is responsible for providing medicinal value to turmeric. It contains (1,7-bis(4-hydroxy-3 methoxyphenyl)-1,6-heptadiene-3,5-dione) and other curcuminoids constitute the main phytochemicals of *Curcuma longa* L. (Zingiberaceae family) rhizome with the common name of turmeric (Ammon and Wahl 1991). The presence polyphenolic and methylated compound (Fig. 1) in the curcumin shows of antimicrobial activity has been gained significant attention of researchers in the pharma and textile all over the world (Lai and Roy 2004; Maheshwari and Singh 2006). Turmeric, an ancient colouring spice of Asia, as the main source of curcumin is traditionally used for many remedies (Hayakawa et al. 2011). Despite having multiple medicinal benefits and extremely superior safety profile, curcumin is having poor aqueous solubility due to presence of non-polar group (Anand et al. 2010). It is very difficult to apply curcumin on textile due to poor insolubility in water, The present work is aim to develop the nanostructured textile material impregnated with formulated stable o/w nanoemulsion of modified curcumin i.e. tetrahydroxy curcumin for healthcare applications.

Methods

Materials

The selected drug tetrahydroxy curcumin, surfactant co-surfactant and oil were purchased from M/S Fine chemicals Mumbai whereas auxiliary chemicals such as β-cyclodextrin and BTCA and sodium hypophosphate catalysts were purchased from M/S Pallav chemicals PVT.LTD Mumbai. The scoured and bleached 100% cotton fabric of 80 ends/in., 70 picks/in. and 100 gm/m^2 areal density (GSM) was purchased from local market. For antibacterial testing, Gram-positive bacteria *Bacillus subtilis* (*B. subtilis.*) and Gram-negative bacteria *Escherichia coli* (*E. coli*) were used.

Formulation method used for nanoemulsion

Initially low energy spontaneous method was used to prepare the blank nanoemulsion followed by high pressure homogenization method to make a stable o/w nanoemulsion of tetrahydroxy curcumin derivative. The tetrahydroxy curcumin showed maximum solubility in Labrafac PG, Tween 10 and Lauroglycol 90. The Numbers of

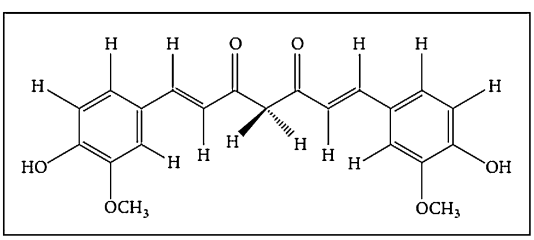

Fig. 1 Chemical structure of curcumin

Table 1 Optimized nanoemulsion batch

Sr. no.	Ingredients	Concentration
1	Oil (Labrafac PG)	15%
2	S_{mix} (Tween 20:Lauroglycol 90)	15%
3	Drug	1%
4	Water	Q. S. to 100 ml

batches were performed by taking oil surfactant ratio as 1:9 and 9:1 to get optimized and stable nanoemulsion. To formulate the nanoemulsion 1:1 ratio of oil:surfactant was found to be most suitable. The optimized formulation contain Oil (Labrafac PG) 15%, S_{mix} (Tween 10:Lauroglycol 90). The optimised batch (Table 1) was characterised for its particle size, zeta potential and drug content using standard method.

Particle size analysis of nanoemulsion

The average particle size of the both blank and optimized nanoemulsion was measured by nanoparticle size analyser.

Stability analysis

Stability of optimized batch was determined by measuring the zeta potential using zeta potential analyser. For determination of zeta potential values of the selected dispersions, a dilute suspension of the dispersion was subjected to a weak electric field, and the mobility of the particles was observed. Instrument used was Malvern Zetasizer (Nano ZS 90, Malvern Instruments, and UK).

Drug content

To confirm the active drug content in the nanoemulsion loaded with drug, analytical method was designed to determine the drug content; 2 mg of active drug was dissolved in a mixture of methanol and water (1:1), the solution was diluted to 100 ml in a volumetric flask and sonicated to facilitate complete dissolution of the drug. 1 ml of this solution was diluted to 10 ml using methanol. Sample was filtered through 0.22 μm membrane filter and analyzed by HPLC method for estimation of active content.

TEM (transmission electron microscopy) analysis

TEM (Philips, CM200) was employed to study the structure and shape and particle morphology of dispersion of nanoemulsion. TEM images provide valuable information on particle size, shape, and presence of various structures in the dispersion.

Preparation of standard curve

The standard curve were prepared to decide the concentration of nanoemulsion before the application against the both gram positive and gram negative bacteria. The standard curve was plotted against the zone of inhibition as a function of concentration of nanoemulsion. 10 mg of curcumin was dissolved in 10 ml of ethanol and 0.1, 0.2, 0.3, 0.4 and 0.5% solution were prepared for determination of antimicrobial activity.

Finishing process

For finishing, ternary aqueous solution containing the 10% polycarboxylic acid, 15% β-cyclodextrin and Sodium hypophosphate (SHP) 15% along with stable nanoemulsion were prepared. All fabric samples before and after treatment, were oven dried at 104 °C for 1 h, placed into a desiccators for period of 30 min, and immediately weighted. The fabric was impregnated with aforementioned solution using exhaustion method at 0.1, 0.3 and 0.5% concentrations of nanoemulsion keeping auxiliary concentration and processing temperature and residence time constant. Samples were then washed with hot water, spin dried, oven dried at 120 °C and weighted. The treated samples were evaluated for antimicrobial property.

Antibacterial activity

Antibacterial susceptibility testing was carried out using the well diffusion assay, as prescribed by the National Committee for Clinical Laboratory Standards. The antibacterial activity was qualitatively evaluated by measuring the size of the zone of inhibition of bacterial growth around the well by AATCC 147 test method. The antibacterial activity was quantitatively evaluated against *Bacillus subtilis* (*B. subtilis.*) and Gram-negative bacteria *Escherichia coli* (*E. coli*), according to the AATCC 100–2004 test method. Colonies of bacteria recovered on the agar plate were counted, and the percentage reduction of bacteria (R) was calculated by the following equation:

$$\mathrm{R}\ (\%) = \frac{(\mathrm{B} - \mathrm{A})}{\mathrm{B}} \times 100$$

where, A is the number of bacterial colonies from treated specimen after inoculation over 24 h of contact period and B is the number of bacterial colonies from untreated specimen after inoculation at zero contact time.

Toxicity test

To confirm non-toxicity of samples skin irritation study was performed using Draize patch test method. Acute skin irritation of samples was evaluated in vivo in six animals in a group for each sample after they have been shaved. The product is applied to the skin and the appearance of oedema and/or erythema was evaluated at 24, 48 and 72 h. after application. The scoring system enables products to be classified from non-irritant to very irritant. Finally the primary irritancy index (P.I.I.) was calculated by dividing the sum of the scored reaction by number of evaluation intervals. The degree of irritancy was categorized based on the P.I.I. shown in Table 2.

Table 2 Evaluation of primary irritation index (P.I.I.)

P.I.I.	Evaluation
0.00	No irritation
0.04–0.99	Irritation barely perceptible
1.00–1.99	Slight irritation
2.00–2.99	Mild irritation
3.00–5.99	Moderate irritation
6.00–8.00	Severe irritation

Bending length

Bending length of the treated sample was measured using ASTM D4032-08 as function of fabric hand value.

ATR-FTIR analysis

Attenuated total reflection (ATR) Fourier transform infrared (FTIR) spectra of the untreated and treated cotton fabrics were recorded to observe the functional group using Shimadzu FTIR-8400S machine with a scan rate of 32 scans per minute at a resolution of 1 cm^{-1} in between 800 and 4000 $cm^{-1.}$

Scanning electron microscope (SEM)

The SEM micrographs were taken from Philips electron microscopy to study the surface morphology of samples treated with different concentrations of nanoemulsion also to validate the uniform dispersion of nanoemulsion throughout the surface of fabric. SEM, Model—Philips-XL30 was used to study the morphology of treated and control samples.

Results and discussion

Particle size analysis

Particle size and polydispersity index of the dispersions were determined using Malvern Zetasizer (Nano ZS 90, Malvern Instruments, UK), to confirm that the desired size range has been achieved during preparation. Figure 2 shows that, the particle size analysis of *Tetra Hydroxy Curcumin*. It was observed that nanoemulsion droplets have a uniform distribution with a mean particle size of 199.5 nm for blank Nanoemulsion batch, 217 nm for drug loaded batch before homogenization and 101.9 nm for optimized drug loaded batch after homogenization respectively. This nanoemulsion was applied on cotton fabric to impart antimicrobial property as discussed in the later part of the paper.

Stability analysis

Zeta potential of optimized nanoemulsion was determined as a function of stability using zeta potential analyser. The zeta potential was found -30.1 ± 0.1 (Fig. 3), it confirms that the formulated nanoemulsion is stable at room temperature which helps to avoid separation of aqueous and organic phase when loaded tetra hydroxy curcumin.

Drug content

Antimicrobial activity of drug loaded nanoemulsion is related to the active content in the drug. The drug content in optimized and homogenised nanoemulsion batch was measured using analytical grade HPLC instrument. Table 3 shows that the drug content in the optimised batch is 80.98% and it further increases up to 82.85% with increase in homogenization pressure from 400 to 800 bars, this is due to the reduction in the particle size which enables more surface area for encapsulation the drug.

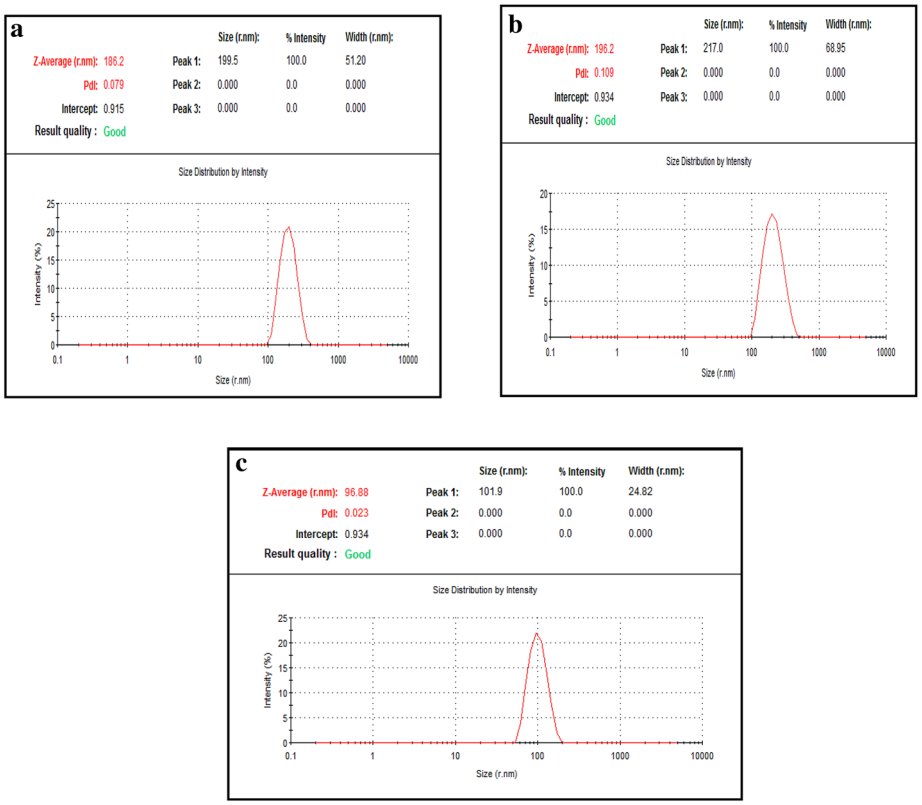

Fig. 2 Particle size analysis of *Tetra Hydroxy Curcumin Nanoemulsion*. **a** Blank nanoemulsion batch. **b** Drug loaded batch before homogenization. **c** Drug loaded batch after homogenization (optimized batch)

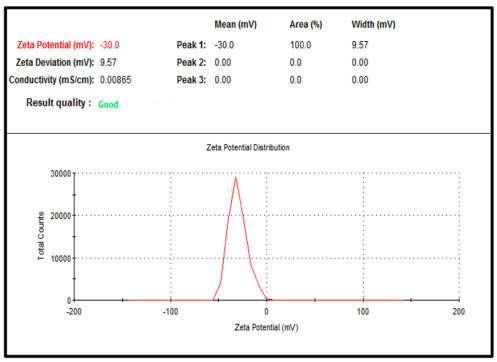

Fig. 3 Zeta potential of optimized nanoemulsion batch

Table 3 Drug content in nanoemulsion

Sr. no.	Stages	Drug content (%)
1	Before homogenization	80.98
2	After 400 bars	80.57
3	After 600 bars	81.32
4	After 800 bars	82.95
5	Before homogenization	80.98

TEM (transmission electron microscopy) analysis

The optimized and homogenized batch was evaluated for shape and structure using TEM at 20,000× (Fig. 4). Nanoemulsion was examined by negative staining with 1% phosphotungstic acid. TEM images revealed the presence of spherical structures in dispersion with the particle range of 200 nm for batch before homogenization and 114.96 nm for homogenized batch after homogenization. The uniform particle size distribution was achieved after homogenization with low polydispersity index 0.023 reported in Fig. 2c. It was also observed that from Figs. 2c and 3 the presence of single pick for optimized batch after homogenization revels that the particles are uniformly distributed.

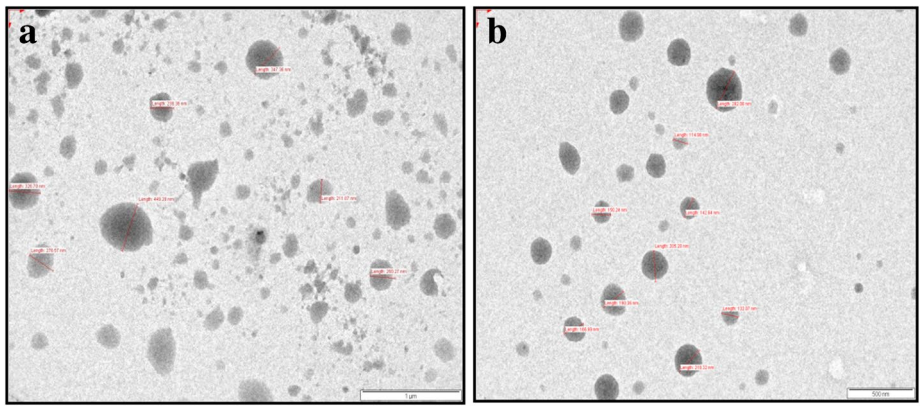

Fig. 4 TEM images of nanoemulsion. **a** Before homogenization batch. **b** After homogenised batch (optimized batch)

Standard curve for antimicrobial activity

The standard curve was plotted to decide the concentration of nanoemulsion for getting reliable results with respect to antimicrobial activity. Figure 5a, b shows that 0.5% concentration of nanoemulsion shows that maximum inhibition with mean zone of inhibition 22.3 mm and 96% reduction against gram positive bacteria and 21 mm and 93% reduction against gram negative bacteria.

Antibacterial activity AATCC 147

Curcumin has a unique conjugated structure including two methoxylated phenols and enol form of β-diketone. It exists in a keto-enol tautomerism with the enol structure enables curcumin to form additional inter and intramolecular hydrogen bonds (Araujo and Leon 2001). The mechanism for the antimicrobial activity of curcumin has not been completely understood. However, the existence of methoxyl and hydroxyl groups is believed to be responsible for the antimicrobial activity of curcumin (Reddy et al. 2013).

In this regard Gram-positive bacteria (*B. subtilis*) and Gram-negative bacteria (*E. coli*) were susceptible to the both fabric samples treated with different concentration of nanoemulsion and Erythromycin standard antimicrobial strip, which showed the mean zone of inhibition 27.8 mm for *B. subtilis* and 27.3 for *E. coli* whereas the sample treated with 0.5% concentration had more antibacterial activity with a mean zone

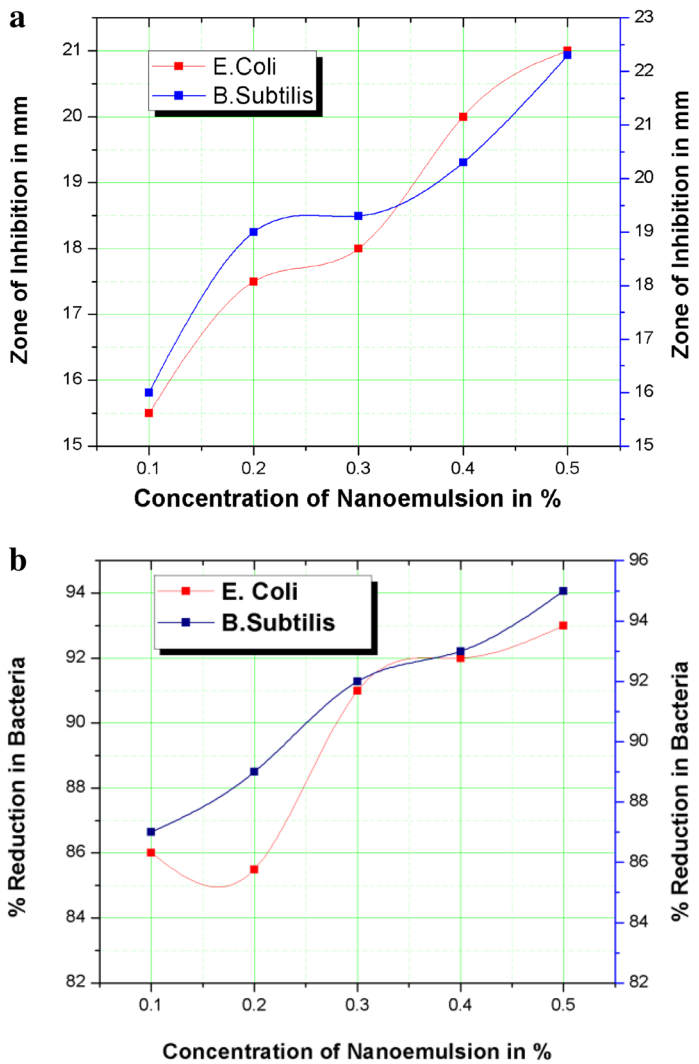

Fig. 5 Standard curve for antimicrobial activity. (**a**) Standard curve for zone of inhibition for gram positive bacteria (*B. subtilis*) and gram negative bacteria (*E. coli*). (**b**) Standard curve for % reduction in bacteria for gram positive bacteria (*B. subtilis*) and gram negative bacteria (*E. coli*)

of inhibition of 21.3 mm for *B. subtilis* and 19.8 for *E. Coli* followed by 18.3 mm for *B. subtilis* and 17.5 mm for *E. coli*, 16.6 mm for *B. subtilis* and 14.8 mm for *E. coli* sample treated with 0.3 and 0.1% concentration respectively. It was found that the nano emulsion loaded with tetrahydroxy curcumin drug shows more inhibitory effect for gram positive bacteria *B. subtilis* than the gram negative bacteria *E. coli* (Tables 4, 5 and Fig. 6).

Antibacterial activity AATCC 100

The quantitative analysis of the percent reduction in Gram-positive (G+, *B. subtilis*) and Gram-negative (G−, *E. coli*) bacteria was after application of nanoemulsion. From the Table 5, it can be seen that the highest colonies reduction (%) was observed in the case of fabric treated with 0.5% nanoemulsion against both *B. subtilis* and *E. coli* followed by samples treated with 0.3 and 0.1% when compared to standard antibiotic

Table 4 Zone of inhibition of untreated, standard and treated sample

Sample	Mean zone of inhibition (mm)	
	B. subtilis	***E. coli***
Untreated sample	0	0
Standard sample (erythromycin 10 mg)	27.8	27.3
Test sample at 0.5% (w/w)	21.3	19.8
Test sample at 0.3% (w/w)	18.3	17.5
Test sample at 0.1% (w/w)	16.6	14.8

Table 5 Percent reduction in bacteria of untreated, standard and treated sample

Sample	Reduction in bacteria (%)	
	B. subtilis	***E. coli***
Untreated sample	0	0
Standard sample (erythromycin 10 mg)	99	95
Test sample at 0.5% (w/w)	93.5	92
Test sample at 0.3% (w/w)	91.5	90
Test sample at 0.1% (w/w)	87.5	85.5

strip, Moreover, the reduction percentage in colonies against *B. subtilis* and *E. coli* is more than 90% at 0.5% concentration.

The observed antibacterial property of the nanoemulsion treated fabric might be due to the presence of polyphenol and hydroxyl compounds jointly may rupture the negatively charged bacterial cell wall, finally killing the bacteria and providing antibacterial property.

Toxicity test

The skin irritation study of samples confirms that the treated sample is non-irritant. From Table 6 and Fig. 7 it clearly confirms the primary irritation index of the sample is zero. This also confirms that the chemicals used in the formulation and application of nanoemulsion is non-irritant when compared to positive control.

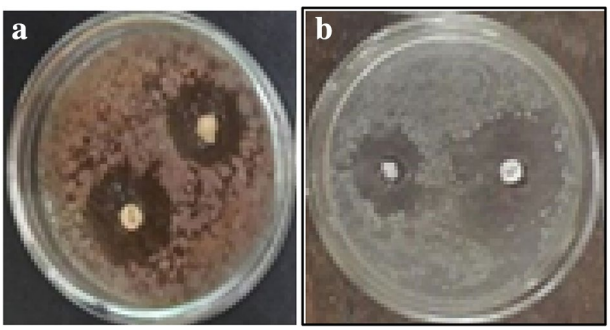

Fig. 6 Images for zone of inhibition of fabric treated with curcumin nanoemulsion and erythromycin standard antibiotic strip against **a** *B. subtilis* (Gram positive bacteria) and **b** *E. coli* (Gram negative bacteria)

Table 6 Skin irritation test of the sample on rat skin

Sample details	Score of the rat skin					
	24 h		48 h		72 h	
	Erythema	Edema	Erythema	Edema	Erythema	Erythema
Positive control	4	4	4	4	4	4
Negative control	0	0	0	0	0	0
Test sample	0	0	0	0	0	0

Primary Irritation Index (P.I.I.) for developed sample = 0/24 = 0

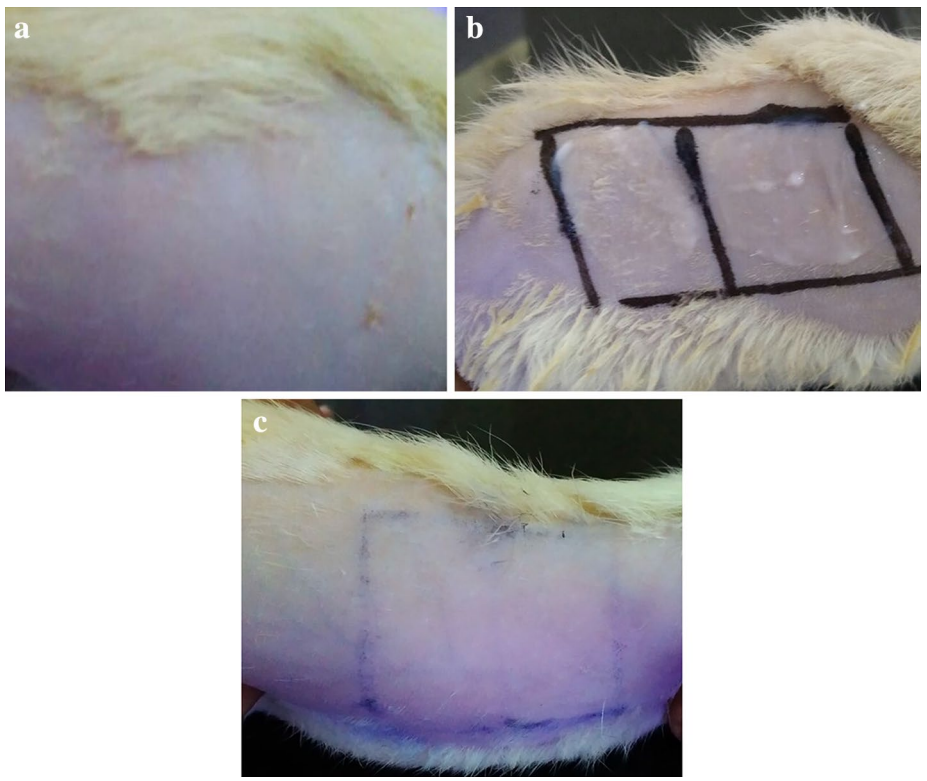

Fig. 7 Skin irritation study **a** skin without application of sample, **b** skin after application of sample at day 0, **c** skin after application of sample at 72 h

Bending length

Bending length of treated samples was measured to understand the hand value of fabric, from Table 7. It was observed that the bending length of the samples treated with nanoemulsion increases linearly with concentration. This might be due to the effect of high concentration of nanoemulsion and presence of cross linking agents β-cyclodextrin and polycarboxylic acid.

ATR-FTIR analysis

FTIR analysis was carried out for understanding the functional groups present in the control and without treated fabric as shown in Fig. 8a. As far as the FTIR analysis of

Table 7 Bending length of treated and untreated samples

Test sample (%)	Bending length (cm)[a]	
	Before finish	After finish
0.5	4.3	4.8
0.3	3.8	4.2
0.1	3.6	4.0

[a] Bending length of control sample: 3.4

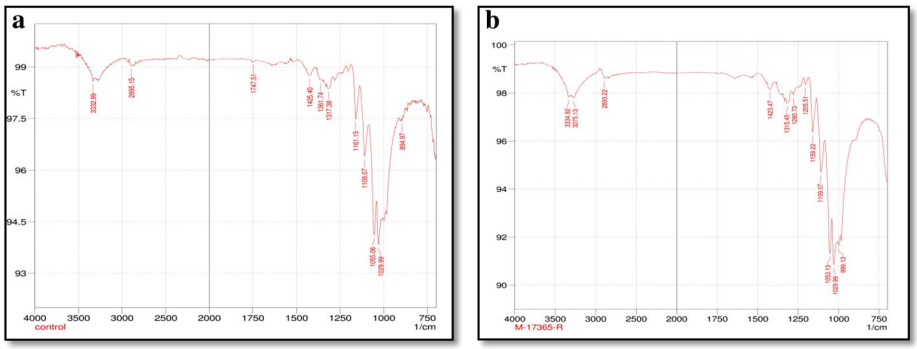

Fig. 8 ATR-FTIR images **a** control sample, **b** treated fabric with 0.5 nanoemulsion

the control sample is concerned, it showed abroad strong picks in the range of 1200–1030 cm^{-1} confirms the presence of C–O bond. Moreover, the interesting finding was observed for the treated sample (b) where it shows the peak at 1024 cm^{-1} which may be due to C–O stretching of the phenolic compound because the absorption has taken place slightly at the higher side of energy due to the partial double bond character between C and O.

Figure 8b shows that the valence vibrations of the C–H bonds in the phenolic with maxima at 3375 and 3334, cm^{-1}, respectively, are registered which confirms the presence of curcumin which contains polyphenolic group in the core structure. The band of valence vibrations of the C=O bond in the carboxyl group is observed at 1423 and 1315 cm^{-1}. In the interval 1200–1030 cm^{-1} the absorption bands of the valence vibrations of the C–O bonds in the ether and hydroxyl groups of β-cyclodextrin picks at 1080 and 1027 cm^{-1} are registered.

SEM (scanning electron microscopy) analysis

The SEM micrographs were taken from Philips electron microscopy to study the surface morphology of treated samples also to validate the uniform dispersion of nanoemulsion throughout the surface of fabric. As shown in Fig. 9a, the control s fabric had a smooth surface, with no surface deposition. The treated fabric with 0.1, 0.3 and 0.5% concentration as indicate in Fig. 9b–d reveals that the surface of the fabric is coated with the solution of drug loaded nanoemulsion. This natural surface coating might have supported in increasing the antibacterial properties of the treated cotton fabric.

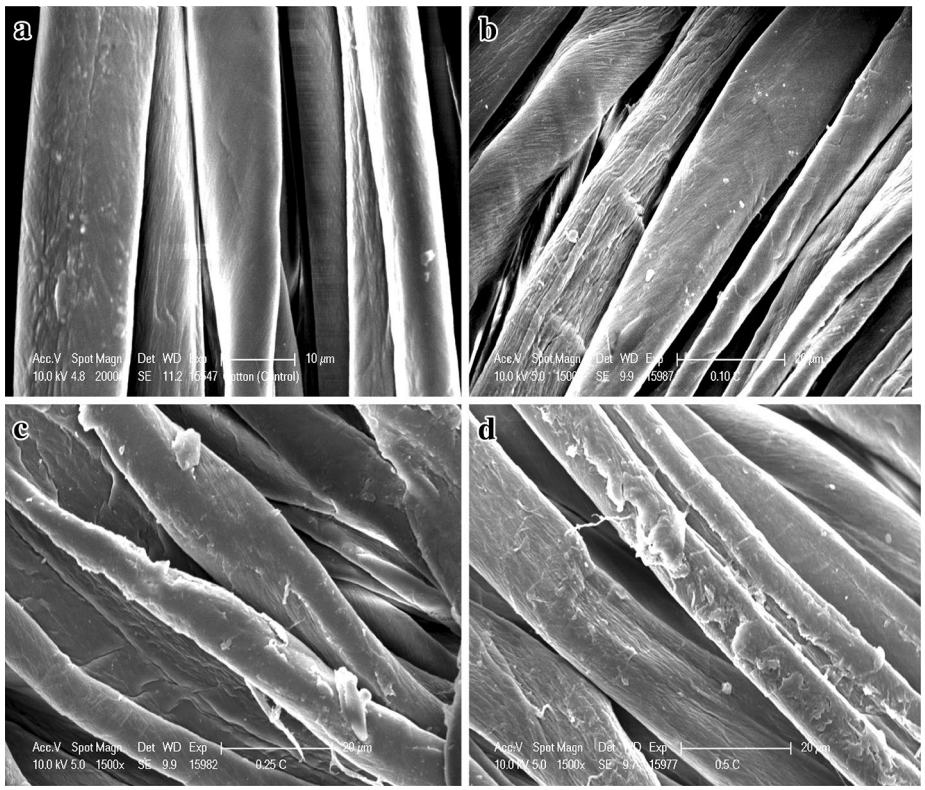

Fig. 9 **a** SEM analysis of control sample, **b** treated fabric with 0.1% concentration, **c** treated fabric with 0.3% concentration and **d** treated fabric with 0.5% concentration

Conclusions

The present work shows, stable o/w nanoemulsion loaded with *Tetra Hydroxy Curcumin* drug was formulated using high pressure homogenization technique with mean particle size 101 nm, 0.30 mv stability and 82.95% drug content. The observed zone of inhibition for *B. subtilis* and *E. coli* suggests that fabric treated with nanoemulsion loaded with active drug shows antibacterial properties (as per AATCC 147) that can effectively inhibit the growth microbes when compared to standard sample. The finished fabric showed excellent antibacterial activity with respect to reduction in bacteria for both against *B. subtilis* and *E. coli* bacteria as per AATCC 100 test method. It was found that the fabric treated with 0.5% nanoemulsion shows maximum reduction in bacteria about 93.5% against *B. subtilis* and 92% against *E. coli* than treaded with 0.3 and 0.1% when compared to standard sample, this is due to the presence of β cyclodextrin and polycarboxylic acid which forms the inclusion complex and acts like a cross linking agent for fixation of nanoemulsion in textile material.

Authors' contributions

All the authors contributed to carry out the entire research work and drafting of manuscript. All authors read and approved the final manuscript.

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Competing interests
The authors declare that they have no competing interests.

Ethics approval and consent to participate
Not applicable.

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