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Moringa Based Nanoparticles Coated Face-mask to Manage the Patients Suffering from Respiratory Disorder

Aditya Arora[1], Megha Verma[2], Vandana Gupta[1]*

[1] *Department of Pharmaceutical Sciences, Shalom Institute of Health and Allied Sciences Sam Higginbottom University of Agriculture, Technology & Sciences (SHUATS), Naini, Prayagraj, Uttar Pradesh, 211007, INDIA*

[2] *Department of Pharmacy Gyan Ganga Institute of Technology & Sciences, Tilwara Ghat, Jabalpur, Madhya Pradesh, 482003, INDIA*

*Corresponding Author: Vandana.gupta@shiats.edu.in
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Abstract

Human exposure to various air pollutants and contagious agents mainly occurs through two ways, namely, inhalation and skin contact. COVID-19 compelled the extensive use of respiratory face-mask by public world-wide. The people suffering from respiratory disorder like asthma were claimed to face lots of complications during wearing of mask such as physical distress, dizziness, perceived shortness of air, and headache. But masking has become a standard and as an effective barrier to curb the aerosol spreads. However, excessive use of single-use polymer-based facemasks can pose a significant challenge to the environment. The aim of present work is to develop medicinal plant (Moringa) based; eco-friendly nanoparticles coated face-mask fabric to manage the complications of the person while wearing face-mask. Moringa loaded chitosan nanoparticles were developed and used in the coating of face-mask fabrics. Further, developed nanoparticles and coated fabrics characterized for various parameters. The percentage yield of the various nanoparticle formulations was found to be in the range of (31.71±2.36%–96.36±2.29%). The particle size, Zeta Potential and polydispersity index (PI) of different formulations were in the range of (217.2±4.2nm- 5712.5±12.2nm), (2.8±0.9mV- 28.1±0.3mV) and (0.266±0.02mV- 2.315±0.10mV) respectively. Surface morphology have shown spherical, uniform and well dispersed particles as observed by optical microscope and scanning electron microscope and coated facemask has shown nanoparticles embedded in the matrix of fabric. The best optimized Moringa loaded chitosan nanoparticles (F1) coated face-mask has shown zone of inhibition about (25±1.5 mm) for antimicrobial effect against *Streptococcus pneumoniae* which indicates therapeutic efficacy of the respiratory face-mask.

1. Introduction

Respiratory disorder is affecting a major part of population globally. COVID-19 compelled the substantial use of respiratory face-mask by public world-wide. It was reported that patients with respiratory conditions like asthma and chronic obstructive pulmonary disease (COPD) experienced numerous side effects from wearing the mask, including headache, dizziness, physical discomfort, and a feeling of shortness of breath [1,2]. The currently used drugs for the treatment of respiratory disorders in modern medicine are away from appeasing as they only

come up with symptomatic relief, results in several adverse effects i.e. muscle tremor, hypokalemia, fluid retention, increased cell mass, increased appetite, weight gain, osteoporosis, capillary fragility, hypertension, peptic ulceration, diabetes, cataract, psychosis [3,4].

Monitoring drug levels is necessary for the bronchodilators like theophyline, which has a narrow therapeutic index [5]. Hence, herbal traditional medicines have been recommended from indigenous plants sources for the management of patients suffering from respiratory disorders and have been successful in controlling the disease as well [6-8]. Broad spectrum of medicinal plant preparations has been reported to possess therapeutic actions in respiratory disorders [9]. *Moringa oleifera (M. oleifera)* is a small or medium sized tree, cultivated throughout India. Seeds are used as purgative, antipyretic and anti-inflammatory [10, 11]. While, the plant exhibits antimicrobial properties [12]. It has been reported that alkaloid from the plant nearly features ephedrine in action and applicable in treatment of bronchial diseases. Alkaloid moringine unwind bronchioles [13].

The nanonization of phytoceuticals forefront to a high surface area to volume ratio, improvement in solubility and bioavailability, reticuloendothelial system (RES) uptake, an enhanced permeability and retention (EPR) effect, enhancement in tissue distribution of macrophages, sustained release, enhanced physicochemical stability, and so on [14]. Nanoparticles (NPs) contain the therapeutics embedded in the matrix or absorbed onto the surface [15]. The present study aimed at the unique approach i.e. to find out the effectiveness of *M. olifera* based nanoparticles coated respiratory face-mask in the management of the patient suffering from respiratory disorders. It is important to fabricate patient-friendly medicated respiratory face-masks that are safe upon disposal without causing secondary infections [16-20]. Therefore, focusing on the use of *moringa* nanoparticles coated biodegradable, breathable, and anti-microbial face-mask, especially for the patients suffering from respiratory complications. This work represents an unprecedented application in the field of face masks because, as of yet, no report has documented the use of face mask fabric coated with moringa nanoparticles to manage the complications of person while wearing one.

2. Materials and Methods

2.1 Materials

Chitosan polymer (deacetylation degree 85%) was procured from Loba Chemie Pvt. Ltd., Colaba, Mumbai, India. Seed powder of high-quality Moringa *oleifera* was procured from Genius Herbs Pvt. Ltd., Teethipalayam, Coimbatore, India. Tween-80/Span-80 and Liquid Paraffin were procured from CDH Pvt. Ltd. New Delhi, India. Solvents and other reagents were of analytical grade and purchased from Thermo Fisher Scientific India, Pvt. Ltd., Powai, Mumbai, India.

2.2 Preparation of M. Oleifera Seeds Loaded Chitosan Nanoparticles

M. oleifera seeds loaded chitosan nanoparticles (M-Ch-NPs) has been prepared by solvent-evaporation method [48].0.1g Chitosan was added into 10 ml of 1% acetic acid solution with 1 ml acetone and 1 ml liquid paraffin followed by the addition of 1g *M. Oleifera* seeds powder. 0.1ml Tween-80 was added as it enhances the formation of nanoparticles and the mixture was stirred at 300rpm for 25-30 minutes or until complete evaporation of the solvent.

In general, entrapment efficiency and particle size of prepared nanoparticles are influenced by processing and formulation parameters [49]. such as chitosan concentration, chitosan molecular weight, type of chitosan derivative, nature of the drug, initial drug concentration used, drug-polymer ratio, nature of the cross-linking agent, type and concentration of the surfactant, and stirring speed.

2.3 Optimization of M-Ch-NPs

2.3.1 Process Variables

There were various process variables which can affect the preparation of nanoparticles and properties of entrapped moiety and formulation. The preparation procedure was accordingly optimized and validated (Table 1).

Table 1 *Optimization studies of Moringa loaded chitosan nanoparticles (M-CH-NPS)*

S. No.	Component	Formulation Code									
		Blank	F1	F2	F3	F11	F12	F13	F21	F22	F23
1	1% Acetic Acid (ml)	10	10	10	10	10	10	10	10	10	10
2	Acetone / Ethanol (ml)	1 Acetone	1 Acetone	2 Acetone	3 Acetone	1 Acetone	2 Acetone	3 Acetone	1 Ethanol	2 Ethanol	3 Ethanol
3	Chitosan (g)	0.1	0.1	0.2	0.3	0.1	0.2	0.3	0.1	0.2	0.3
4	Stirring Speed (RPM)	300	300	300	300	300	300	300	300	300	300
5	Rotation Time (Min)	30	30	30	30	30	30	30	30	30	30
6	Tween-80 / Span-80 (ml)	1.1 Tween-80	1.1 Tween-80	1.2 Tween-80	1.3 Tween-80	1.1 Tween-80	1.2 Tween-80	1.3 Tween-80	1.1 Span-80	1.2 Span-80	1.3 Span-80
7	Liquid Paraffin (ml)	1	1	2	3	1	2	3	1	2	3
8	Moringa Seed Powder (g)	-	0.1	0.2	0.3	0.1	0.2	0.3	0.1	0.2	0.3

2.3.2 Protocol Design

The preparation of M-Ch-NPs involves various process variables, but optimization was done by selecting three of them.

The three factors selected were:

- Effect of Chitosan: Surfactant ratio (Tween-80),
- Effect of various solvents (Acetone, Ethanol),
- Effect of various surfactants (Tween-80, Span-80).

During the preparation of a particular system, the other variables were kept constant. The effect of above three factors was studied on the following responses:

- ParticleSize
- Polydispersity Index (PI),
- Zeta Potential
- pH value
- Percentage Yield

2.4 Characterization of Prepared M-Ch-NPs

Prepared M-Ch-NPs were characterized on the basis of following parameters i.e. percentage yield, surfacemorphology, particle size analysis, Zeta potential & polydispersity index (PDI) and determination of pH.

2.4.1 Percentage Yield

The percentage yield (%) of nanoparticles produced by a reported method [50] is a significant factor in the pharmaceutical industry since it can predict industrial scale-up and commercial feasibility. The technique with the highest yield is preferred. The percentage yield was calculated to evaluate the effectiveness of the product operations. The % yield of M-Ch-NPs was calculated by gravimetric method. An appropriate volume of M-Ch-NPs suspensions were centrifuged at 16000 rpm for 30 minutes at 15°C using Eppendorf centrifuge (Eppendorf centrifuge 5810R, Germany) and sediment were dried. The percentage yield was calculated by using the following Eq.1. Each experiment was performed in triplicate (n=3)

$$\text{Percentage Yield} = \frac{\textit{Quantity of Ch-NPs Obtained}}{\textit{Total mass of all ingredients}} X100 \quad (1)$$

2.4.2 Surface Morphology of Prepared M-Ch-NPs

Light microscopy was applied for the structural evaluation of the particle, according to the reported method. A drop of diluted formulations i.e. blank (Ch-NPs) and drug loaded (M-Ch-NPs) were placed on a microscopic slide without a cover slip, and the morphological structure of the particles were monitored optically through light microscope (Olympus CX21i Biological microscope with Lafco India Optik Camera) at 200X and the microphotographs were captured.

2.4.3 Determination of Particle Size, Zeta(ξ) Potential & Polydispersity Index (PI)

The particle size and ξ-potential of M-Ch-NPs were determined by Horiba Nanoparticle Analyzer SZ-100 (Horiba Scientific, HORIBA Instruments, Inc., Bunsen Drive Irvine, USA). A polydispersity index (PI) is used to describe the particle size distribution of nanoparticles. The average diameter, size distribution and ξ-potential studies were done by appropriate dilution of the formulation using distilled water with manual shaking for 5 min. Prior to measurements. The size distribution profile of M-Ch-NPs formulations was determined by employing the disposable polystyrene cuvette. For the determination of surface charge, diluted formulation was dropped into the clear polystyrene electrophoretic cell and the ξ-potential was determined.

2.4.4 Determination of pH

The pH of prepared M-Ch-NPs was observed using pH meter (Mettler Toledo, Polaris Pkwy, OH, USA). The pH electrode was calibrated first with standard buffer solution having established pH values spanning the measurement range. To measure the pH, the electrode was immersed in the formulation at room temperature until a stable reading was achieved. Each experiment was performed in triplicate (n=3).

2.5 Coating of Face-Mask Fabric with Optimized M-Ch-NPs

M-Ch-NPs formulation was applied to a sample of cotton fabric with the help of simple dip-coating technique which was used to apply the coating solution i.e. M-Ch-NPs nanoparticle formulation to the fabric. At room temperature, the fabric was dipped in to the formulation for 30 minutes with ultrasonication then the coated material was left to airdried completely [51].

2.6 Characterization of M-Ch-NPs Coated Face-Mask Fabric

2.6.1 Surface Morphology

Surface morphology of M-Ch-NPs coated face mask fabric was carried out using light microscopy (Olympus CX21i Biological microscope with Lafco India Optik Camera) at 200 ×and Scanning Electron Microscopy (SEM, Leo 430, England) at 100X magnifications. The samples of coated and non-coated fabrics were placed under the microscope and the microphotographs were captured.

2.6.2 Anti-microbial Activity

Anti-microbial activity of M-Ch-NPs coated fabric was carried out using spreading technique. In this process, 7 g of nutrient agar was dissolved in 250 ml of distilled water. Then the mixture was autoclaved at 121°C for 15 minutes. After autoclaving, the agar solution was left to cool down at room temperature. Then the solution was poured into the clean and dried petri-dish and kept in there frigerated until the solution got solidified. A single population of test strain *Streptococcus pneumoniae* was raised overnight in Mueller Hinton Broth (MHB) on a rotary shaker at 37°C revolving with 200 rpm followed by diluting the cultures with 0.9% NaCl. Inoculum was applied to the agar petri-dish. Then 3 samples of face-mask fabrics i.e. fresh uncoated virgin fabric (control), blank nanoparticle (Ch-NPs) coated fabric and M-Ch-NPs coated fabric respectively were cut in to small disk shapes and were placed on the surface of agar plate and was kept in the incubator (Harrison's Incubator; Harrison's Pharma Machinery, Pvt., Ltd., New Delhi) for 24 hours to observe the zone of inhibition of all the fabric samples. In the final step, the diameter of the zone of inhibition was measured [52, 53] Each experiment was performed in triplicate (n=3).

2.7 Statistical Analysis

Data are presented as the mean ± standard deviations (SD) using excels software. All the experiments were performed in triplicate (n=3).

3. Results and Discussion

3.1 Preparation and Optimization of M-Ch-NPs

The method used in the preparation of M-Ch-NPs involves formation of emulsion followed by solvent evaporation. *Moringa* loaded chitosan nanoparticles were successfully prepared using solvent evaporation method. The obtained nanoparticles (M-Ch-NPs) are made up of a central spherical core, surrounded by the surfactant Tween-80 which acts as stabilizer. Several studies have demonstrated an improved bioavailability of drugs encapsulated in nanoparticles [21-30]. Optimization of formulation with regard to various attributes has been a subject of importance in the area of formulation development [31]. Various formulations of M-Ch-NPs were prepared by varying the process and formulation variables. The most satisfactory M-Ch-NPs nanoparticles in terms of particle size, Zeta potential, poly dispersity index (PI), pH and % yield was obtained with chitosan amount of 0.1 g, 10 ml of 1% acetic acid solution, 1 ml of acetone, 1.1 ml of Tween-80, 1ml of liquid paraffin and *moringa* seed powder 0.1g respectively (Table 2). Further, with 300 rpm stirring speed of magnetic stirrer for 30 min gave best optimized chitosan nanoparticle formulation M-Ch-NPs (F1) as formulation F1 remained satisfactory in terms of size i.e. 695.5± 3.8nm, with good stability indicated by Zeta potential and expressed biocompatible pH. Most importantly the % yield of F1 formulation was found to be greatest i.e. 95.66± 4.23.

Table 2 *Optimization studies of M-CH-NPS with their responses on particle size, Zeta potential, poly dispersity index (pi), pH and % yield*

S. No.	Formulation	Particle Size (nm)	Zeta Potential (mV)	Polydispersity Index (PI)	pH	Yield (%)
1	Blank	224.9 ± 3.4	3.7 ± 1.2	0.394 ± 0.08	6.5 ± 0.11	94.87 ± 2.22
2	F1	695.5 ± 3.8	28.1 ± 0.3	0.608 ± 0.01	6.5 ± 0.20	95.66 ± 4.23
3	F2	495.5 ± 4.6	2.8 ± 0.9	0.849 ± 0.02	7.0 ± 0.12	49.30 ± 4.11
4	F3	237.4 ± 4.0	8.2 ± 0.5	0.435 ± 0.03	6.0 ± 0.16	32.80 ± 3.28
5	F11	553.7 ± 5.5	6.9 ± 0.7	0.266 ± 0.02	7.0 ± 0.22	96.36 ± 2.29
6	F12	642.0 ± 6.4	3.7 ± 0.6	1.112 ± 0.09	6.5 ± 0.34	51.74 ± 5.45
7	F13	217.2 ± 4.2	9.3 ± 1.1	0.610 ± 0.02	6.0 ± 0.28	31.71 ± 2.36
8	F21	2663.2 ± 9.8	5.2 ± 0.8	2.315 ± 0.10	7.0 ± 0.31	88.12 ± 4.48
9	F22	3338.4 ± 9.4	12.4 ± 1.4	2.172 ± 0.20	7.5 ± 0.36	79.94 ± 5.34
10	F23	5712.5 ± 12.2	28.1 ± 1.3	1.088 ± 0.12	7.0 ± 0.29	39.28 ± 2.26

Results are represented as mean±SD (*n* =3)

3.2 In Vitro Characterization of Prepared M-Ch-NPs Formulation

3.2.1 Percentage Yield

The percentage yields of the prepared M-Ch-NPs formulations were observed in the range of 31.71±2.36 - 95.66±4.23. The best optimized formulation of M-Ch-NPs i.e. F1 has shown highest percentage yield which contained optimum concentration of chitosan, moringa seed powder, acetone, tween-80 and liquid paraffin that generate flocculation or particle packing, recovering maximum nanoparticles from the suspension.

3.2.2 Microscopic Study of Prepared M-Ch-NPs

The surface morphology of the prepared M-Ch-NPs formulations was observed by light microscope (Fig. 1). Light microscopic studies of developed M-Ch-NPs formulations have shown discrete, smooth surface spherical shape oval and elongate structures of nanoparticles. All the formulations have shown spherical shape particles except formulation F21 which shows elongate structure of nanoparticles.

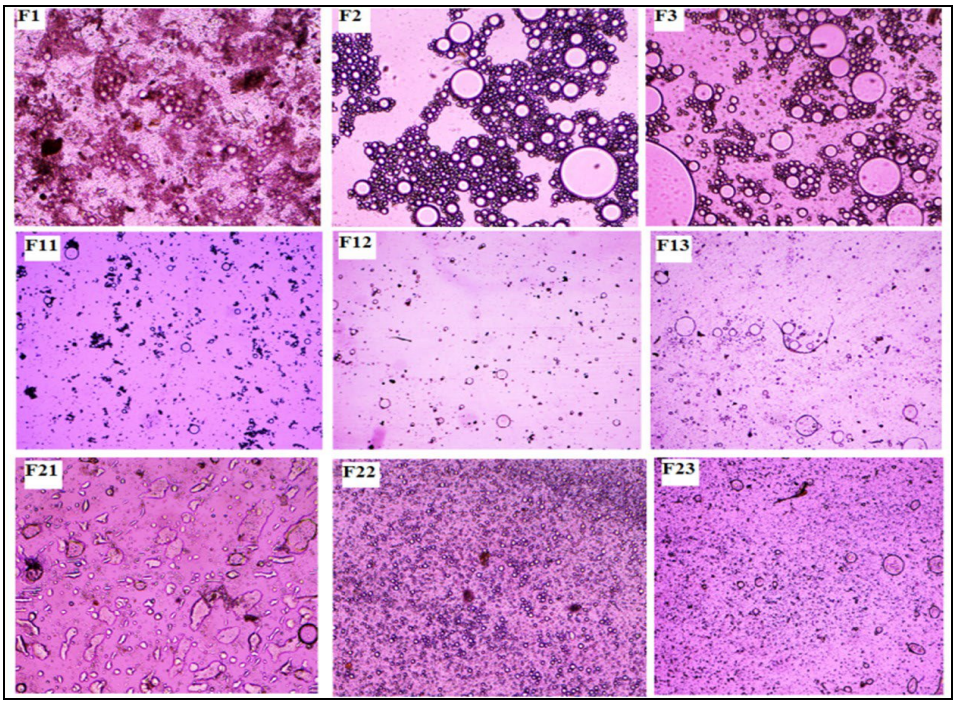

Fig. 1 *Visualization of all 9 prepared formulations of M-Ch-NPs from F1 to F23 by light microscope at 200X magnification*

3.2.3 Determination of Particle Size, Size, Zeta (ξ) Potential & Polydispersity Index (PI)

Fig. 2 and 3 show the results of particle size and PI of various M-Ch-NPs formulations including blank. It was observed that average particle size was increased from 217.2 ± 4.2nm to 5712.5 ± 12.2nm, at room temperature (RT) which indicates that particle size of the F23 formulation was quite high. This may be due to high content of chitosan and *moringa* seed powder. However, it was observed that the average particle size of optimized formulation i.e. F1 was remained satisfactory means 695.5 ± 3.8nm. In addition, the PI of the optimized was 0.608 ± 0.01 and it ranged from 0.266 ± 0.02 to 2.315 ± 0.10, that shows an adequate level of vesicle homogeneity, with the exception of a few formulations with high polymer to entrapped moiety ratios.

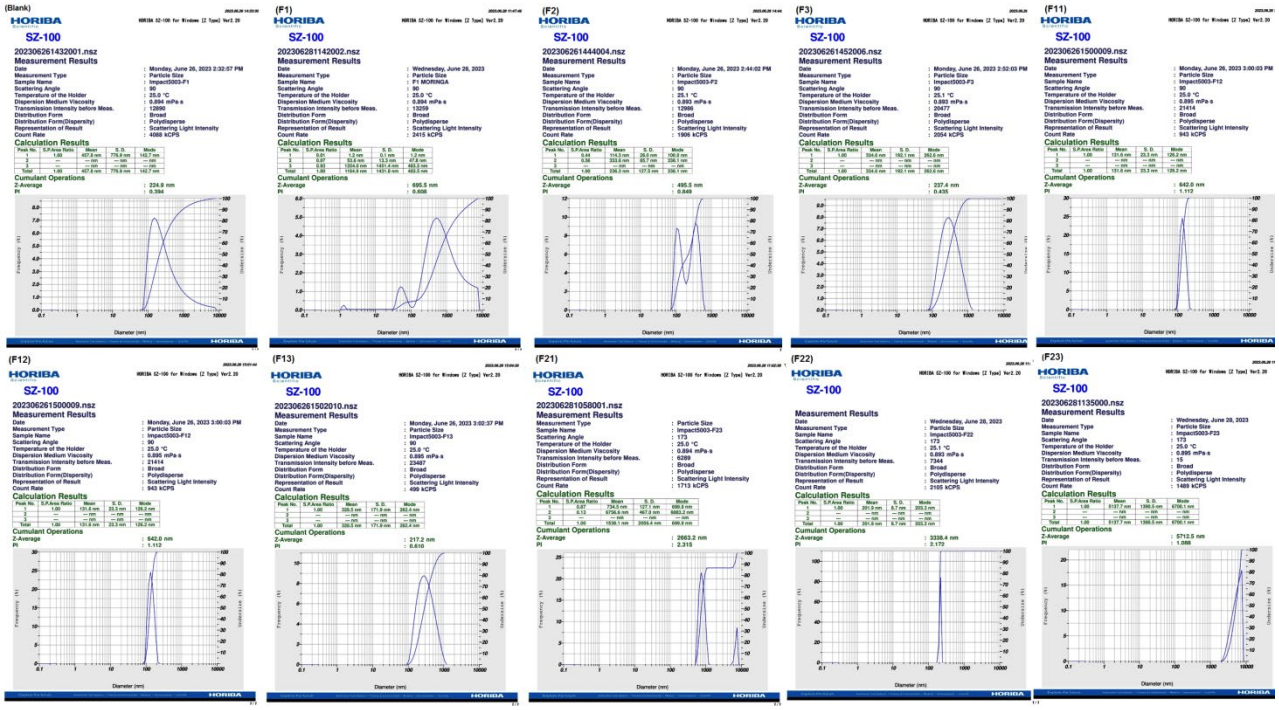

Fig. 2 *Average particle size and size distribution curve of all the formulations i.e. Blank to F23 moringa loaded chitosan nanoparticles (M-Ch-NPs)*

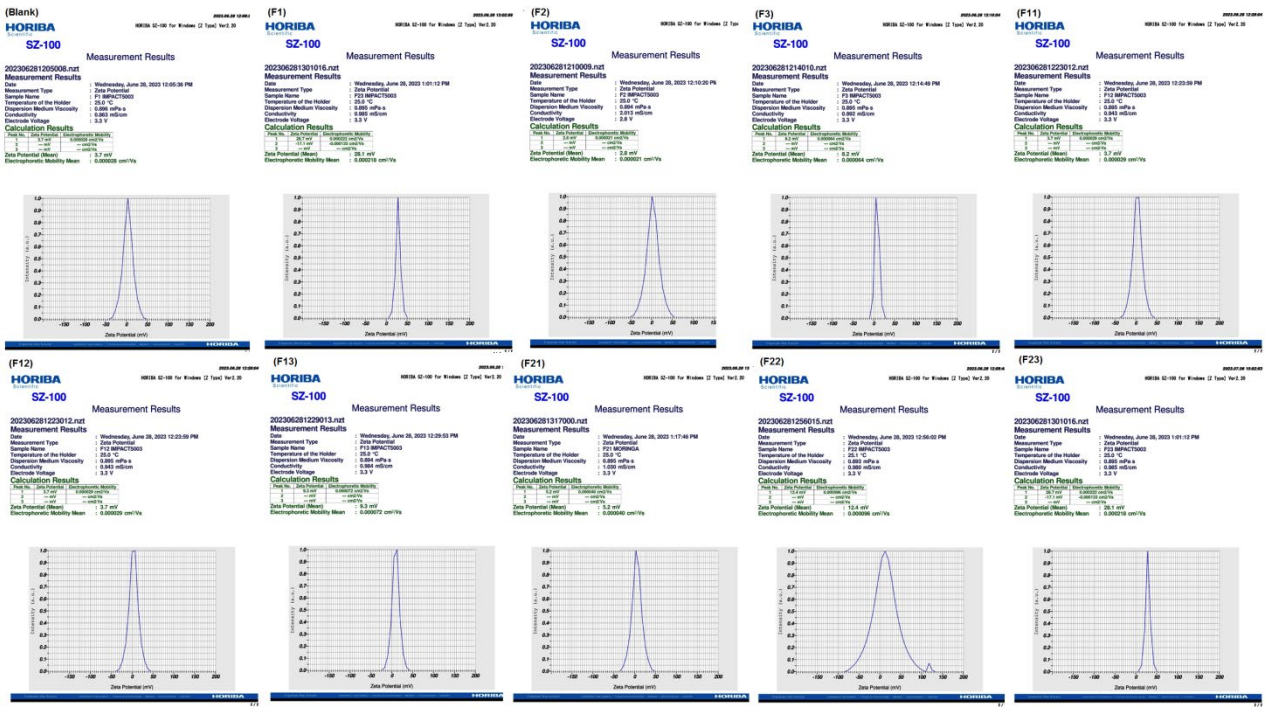

Fig. 3 *Mean Zeta potential results and curve of all the formulations i.e. Blank to F23 moringa loaded chitosan nanoparticles (M-Ch-NPs)*

Results of PI stipulate homogeneous populations of particles, suggesting that the homogeneity of vesicles is influenced by the concentration of polymer. The results of ξ-potential value (Fig. 3) of various M-Ch-NPs formulations were remarkably altered from 2.8 ± 0.9 mV to 28.1 ± 1.3. ξ-potential is thus indicative of probable physical stability of the formulation. Particles with ξ-potentials larger than ±60 mV have excellent stability, where particles with Zeta values between -10 mV and +10 mV, will experience rapid agglomeration unless they are sterically protected [32,33]. It is predicated on the idea that particles with similar charges will not clump together if two nearby particles have high enough ξ-potentials of the same sign rather than opposing each other. [34,35]. Optimized M-Ch-NPs formulation (F1) possessed a satisfactory ξ-potential of 28.1 ± 0.3. F1 nanoparticles (M-Ch-NPs) perfectly covered by a non-ionic surfactant i.e. Tween-80 tend to remain stable despite having a lower ξ-potential. Such behaviour is caused by greater steric stabilization and decreased electrostatic stabilization [36].

3.2.4 Determination of pH

The pH of the various M-Ch-NPs formulations were in the range of 6.0 ± 0.16 to 7.5 ± 0.36 (Table 2), which revealed that the developed formulation is compatible physiologically reflecting no risk of discomfort [37].

3.3 Coating of Face-Mask Fabric with Optimized M-Ch-NPs

The ability of face-masks to protect against infection and pollution can be potentially improved by incorporating or coating with the components that provide comfortability and compatibility with efficacy without reducing the filtration efficiency. Many phytochemicals, such as variety of flavonoids, which may aid in the treatment or prevention of bronchial constrictions and airway inflammation, are abundant in Moringa oleifera [38]. Post coating of face-mask fabric with optimized M-Ch-NPs have shown some visual changes in the surface texture, indicating M-Ch-NPs buildup in some areas.

3.3.1 Surface Morphology of M-Ch-NPs Coated Face-Mask Fabric

The appearance of fiber surfaces of a nanoparticles (M-Ch-NPs) coated mask is presented in Fig. 4 and 5. It was found that the nanoparticles were distributed evenly on the fiber surface. The untreated face-mask fabrics (Fig. 4a and 5a) appear as smooth fibers whereas treated (Fig. 4b and 5b) clearly shows the deposition of M-Ch-NPs on the surface of the fabric. The dip-coating ministrations with ultrasonication assists in the dispersion of particles during the coating. The coating may aid to the physical adsorption of M-Ch-NPs on the fabric finish due to ultrasonication process [39].

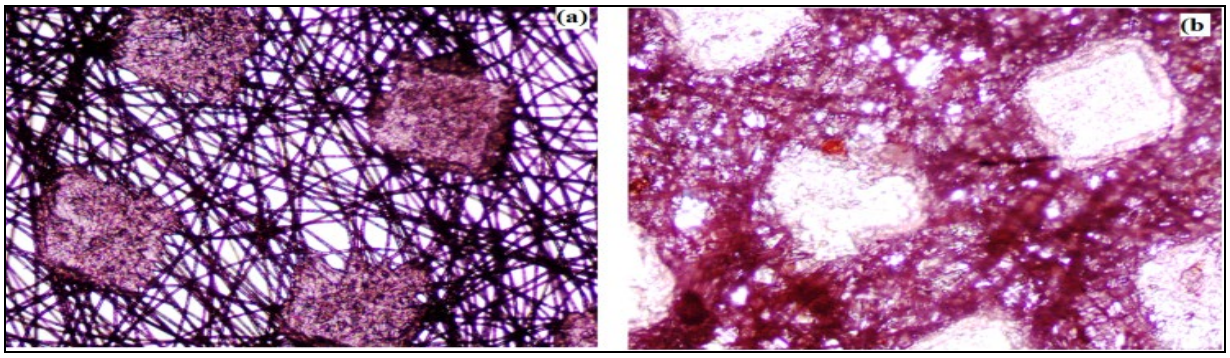

Fig. 4 *Light microscopic images of (a) uncoated virgin face-mask fabric; and (b) M-Ch-NPs coated face-mask fabric at 200X magnification*

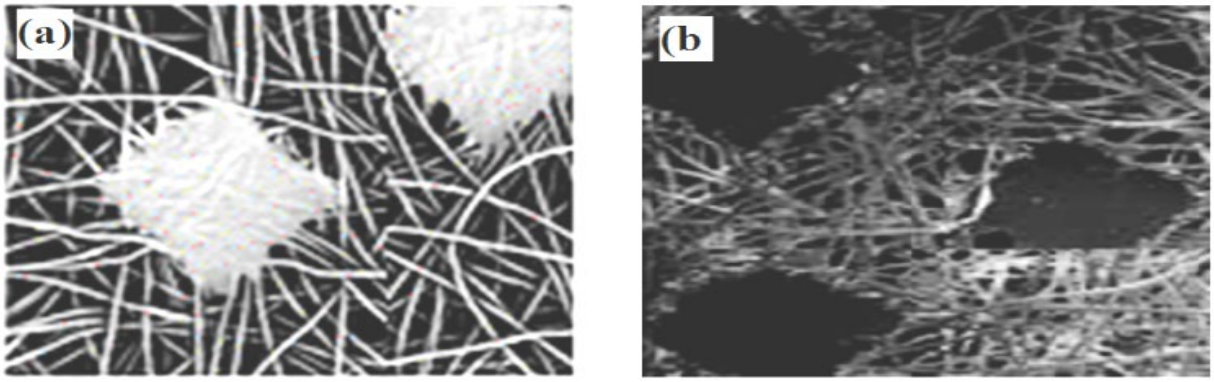

Fig. 5 *Scanning electron microscope (SEM) images of (a) uncoated virgin face-mask fabric; and (b) M-Ch-NPs coated face-mask fabric at 100X magnification*

3.3.2 Anti-microbial Evaluation of M-Ch-NPs Coated Face-Mask Fabric

Microbial pathogens in stagnant water can include *Schistosoma* spp., *Burkholderia pseudomallei* [40], *Vibrio vulnificus* [41], *Mycobacterium* spp. [42], *B. Pseudomallei* [43], *P. aeruginosa, Mycobacterium* spp. [44], *Legionella* spp. [45] and those might be contributed to respiratory infection. The investigation of antibacterial properties of M-Ch-NPs coated face-mask fabric was performed against *Streptococcus pneumoniae* as microbial sample using the disc diffusion technique. The untreated virgin face-mask fabric was employed as a reference (control). Fig. 6 shows the anti-microbial activity of Ch-NPs and M-Ch-NPs coated fabric disc after incubation for 24h (Fig. 6b). The M-Ch-NPs coated showed a significant inhibition zone around the coated fabric disc. The zone of inhibition represents the anti-microbial activity of M-Ch-NPs coated face-mask fabric [46]. The uncoated face-mask fabric discussed as reference did not show any anti-microbial activity. The zone of inhibition diameter was measured to be 25 ± 1.5 mm and 12 ± 0.5 mm for M-Ch-NPs and Ch-NPs coated fabric discs respectively. It is commonly known that the cationic nature of chitosan gives it anti-microbial activity [47]. The antimicrobial activity of face mask fabric coated with Ch-NPs made solely of chitosan may be the reason for this whereas the high infiltration of the moringa component may contribute to the enhanced anti-microbial activity of the M-Ch-NPs coated fabric.

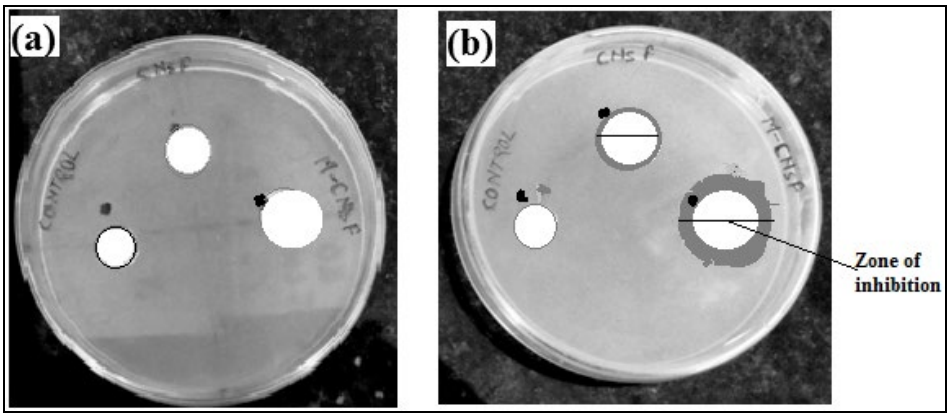

Fig. 6 *Anti-microbial activity of fresh uncoated virgin fabric (control), blank nanoparticle (Ch-NPs) coated fabric and M-Ch-NPs coated fabric before (a) and after (b) incubation for 24 h*

4. Conclusion

In present investigation, the moringa loaded chitosan nanoparticles (M-Ch-NPs) were impregnated on to the outermost layer of the face-masks fabric to reduce the complications while wearing the face-mask especially by the patients suffering from respiratory disorders. Although, further investigation is needed to verify the effects. The coating of M-Ch-NPs to face-mask fabric involved a simple ultrasonic dip coating process that assisted in the homogeneous dispersion of particles. M-Ch-NPs were well adsorbed on the surface of face-mask fabric due to the physical and chemical interactions.

The M-Ch-NPs coated face-mask fabrics exhibited good antimicrobial activity against infections caused by broad spectrum microbes which may cause respiratory complications. COVID-19 compelled us to wear the face-mask and PPE. In future also, use of face-mask could make a major contribution in reducing the impact of such kind of vulnerable outbreak. The present results are in line with the need for an efficient face-mask material (fabric coated with M-Ch-NPs) to handle the complications while wearing face-mask and to protect the personal from COVID-19 like outbreak. Moreover, M-Ch-NPs have shown appropriate and optimum physico-chemical properties in terms of percentage yield, surface morphology, size, size distribution, Zeta potential, pH which indicated biocompatible and environment friendly natural nanomaterials-based face-mask fabric. By employing furthermore investigations might help to expand the practical applicability of M-Ch-NPs, coated fabric as an effective and environmentally friendly alternative in the manufacture of face-mask fabrics and other safety products in the future.

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Conflict of Interest

Authors declare that there is no conflict of interests regarding the publication of the paper.

Author Contribution

Concept *– V.G., A.A.;* ***Design*** *– A.A.;* ***Supervision*** *– V.G.;* ***Resources*** *– V.G., M.V.;* ***Materials*** *– A.A.;* ***Data Collection and/or Processing*** *– A.A., V.G.;* ***Analysis and/or Interpretation*** *– M.V., V.G.;* ***Literature Search*** *– A.A.;* ***Writing*** *– V.G;* ***Critical Reviews*** *– A.A., M.V., V.G.*

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