Clotrimazole-loaded Silver Nano - cellulose fibre preparation and characterization

Nikhil Chaudhari[1]*, Vivekanand Chatap[1], Prashant Jain[2], Mahesh Bhat[2]

[1]Department of Pharmaceutics, H.R. Institute of Pharmaceutical Education & Research, Shirpur, Dhule-425405 M.S. India

[2]Nuper Therapeutics, A division of Jain Pharmaceuticals, Off. No. 106, Nyati Emporious, Near Balewadi Stadium, Baner, Pune-411045. M.S. India

Address for Correspondence:
Dr. V. K. Chatap, Associate .Professor
Department of Pharmaceutics,
H.R. Patel Institute of Pharmaceutical Education & Research, Shirpur, Dhule-425 405, Maharashtra, India. E-mail: vchatap@gmail.com

ABSTRACT:

In this study, a brand-new, simple procedure for environmentally friendly silver nanoparticle and Clotrimazole production on wheat fibre was reported. The Polyethylene glycol-400 (PEG 400) was used in the liquid phase chemical technique to produce silver nanoparticles. PEG 400 was used in the manufacture of silver nanoparticles as a stabilizer and reducing agent. The typical silver nanoparticle is 150 nm in size. The produced silver nanoparticles may be distributed in water, ethanol, and other polar solvents, and they have promising uses in the electrical and biological sciences. Silver nanoparticle aggregation was reduced by using ethanol as a solvent. Clotrimazole was physically loaded onto cellulose fibre using a physical loading technique. The Wheat fibre received an effective antifungal property from the combination of Clotrimazole and silver nanoparticles. After washing, there was hardly any loss in the antifungal effectiveness of the cotton fabrics treated with nano silver and clotrimazole. As more silver nanoparticles were loaded onto the outer layers of the white Wheat fabrics, their colour altered to a yellowish brown. Additionally, the antifungal effectiveness of wheat fibre loaded with drugs and AgNP was assessed against the common fungus Candida albicans. The presence of Clotrimazole and silver

nanoparticles on the wheat fibre was confirmed by the energy dispersive spectroscopy (EDS), scanning electron microscope (SEM), X-ray diffraction (XRD), and ultraviolet (UV) studies. The particle size analyzer determined the size of the silver nanoparticle.

Keywords: Silver nanoparticle, Wheat fibre, clotrimazole and antifungal Property.

INTRODUCTION:

The nanoparticles are referred to as particulate dispersion or solid particles having a size between 10 and 1000 nm [1]. A nanoparticle matrix is used to either dissolve, trap, encapsulate, or attach to nano particle matrix. upon the preparation technique, one can produce nanoparticles, nanospheres, or nanocapsules. Nanoparticles are mostly divided into organic and inorganic categories. Dendrimers [1], Solid- Lipid nanoparticles [2], Liposomes [3], Nanocrystals [1], Nanotube [4], and Polymeric nanoparticles [5]. The sol-gel method technique, Solvothermal synthesis, Chemical reduction, Laser ablation, Inert gas condensation, Biosynthesis of nanoparticles, and Polymeric nanoparticle are some of the preparation methods that have been developed for the synthesis of nanoparticles [6]. The size of silver nanoparticles is between 1 and 1000 nm [7]. It is widely acknowledged as silver ions and compounds with silver as an ingredient kill fungus. Due to this characteristic, silver is an attractive choice for many medical applications. When silver nanoparticles enter a bacterial cell wall and then break through it, the cell membrane changes structurally, becoming more permeable, and the cell eventually dies. The cell surface develops 'pits' and accumulates nanoparticles [8]. Ag-NPs are widely recognized for their antibacterial properties; however, their antifungal properties have not yet received enough attention [9]. Reducing method [10] Microemulsion method, UV-initiated photoreduction method, Photo-induced reduction method, Irradiation method, Microwave assisted synthesis [11], and Tollen’s method are some of the techniques utilized to produce silver nanoparticles. The most predominant kind of living terrestrial biomass is cellulose, which has several uses in contemporary industry [12] (Dorée, 1947). Long molecular chains can be created by covalently linking together the glucose molecules that are present. According to Williams and Wool (2000), sources of cellulose include leaf, seed, fruit, grass, and stalk. Cellulose may also be produced by certain microorganisms. Bacterial cellulose may be produced by Acetobacter xylinum [13].

Among other fungal infections, Candida albicans is treated with the broad-spectrum antifungal agent clotrimazole. Tinea pedis, or athlete's foot, is a fungal infection that is typically brought on by Candida albicans, and can be treated with clotrimazole [14]. Clotrimazole disrupt the formation of ergosterol, a crucial component of the fungal cytoplasmic membrane [15]. Imidazoles, such as Clotrimazole, essentially prevent the

microsomal cytochrome P450 (CYP450)-dependent action 14-a-lanosterol demethylation, a critical step in the synthesis of ergosterol by fungus. Ergosterol is consequently depleted and replaced with the abnormal sterol species 14-a-methylsterol, which impairs both the fluidity and permeability of the membrane. Reduced activity of membrane-bound enzymes, such as those involved in cell wall formation, increased cell wall repture, and cell content leakage are examples of downstream consequences [14].

Silver nanoparticle size and form may occasionally be controlled by using polyethylene glycols. There are very few studies on the reducing abilities of PEG to create metal nanoparticles when ethyleneglycol is used as the reducing agent to create silver particles [16]

MATERIAL:

Clotrimazole procured from Glenmark Pharmaceuticals Ltd. R & D Centre, Sinnar, Malegaon India and Silver nitrate from Merk, Life sciences, Mumbai India, polyethylene glycol-400 from Rankem, Bhiwandi, India, Ethanol, (CSS, China), Wheat fibres, (VITACEL), Sabaroud agar broth (Hi Media Pvt. Ltd. Thane India),

EXPERIMENTAL:

Silver Nanoparticle Preparation: At 800^{o} C, 100 mg of silver nitrate was dissolved in 4 ml of PEG 400. The resulting mixture was agitated for 1 hour at 800^{o} C. The once transparent and clean solution changed to a distinctive brown to dark brown shade, indicating the formation of silver nanoparticles. The fact that the silver nanoparticles in PEG remain stable for several months at room temperature without losing their characteristics indicates that the PEG matrix is an effective stabilizer for the nanoparticles.

Clotrimazole loaded AgNP-cellulose fibre Preparation: Clotrimazole 20mg was added in the 50ml of Ethanol. Stirred the mixture until the clotrimazole and ethanol were completely dissolved. The AgNP and PEG 400 matrix was filled with the prepared solution. The solution received 200mg of cellulose addition. 100 RPM stirring for the entire night at room temperature. Beaker coated with aluminium foil to prevent sample evaporation. The solution involved centrifuging at 12000 RPM at room temperature. After being washed twice with ethanol, the loaded fibre was gathered and dried.

Result and Discussion:

UV-Analysis: In the peak's appearance the production of Ag NPs is demonstrated by UV-visible spectra in the 400–450 nm region. At 430 nm, the spectra of AgNP have been found.

The stabilizer polyethylene glycol-400 is utilized. This finding suggests that PEG 400 has the ability to convert Ag ions into AgNP. The PEG-AgNO3's colour changing to a darker colour could be due to the incorporation of more and larger Ag NPs [17].

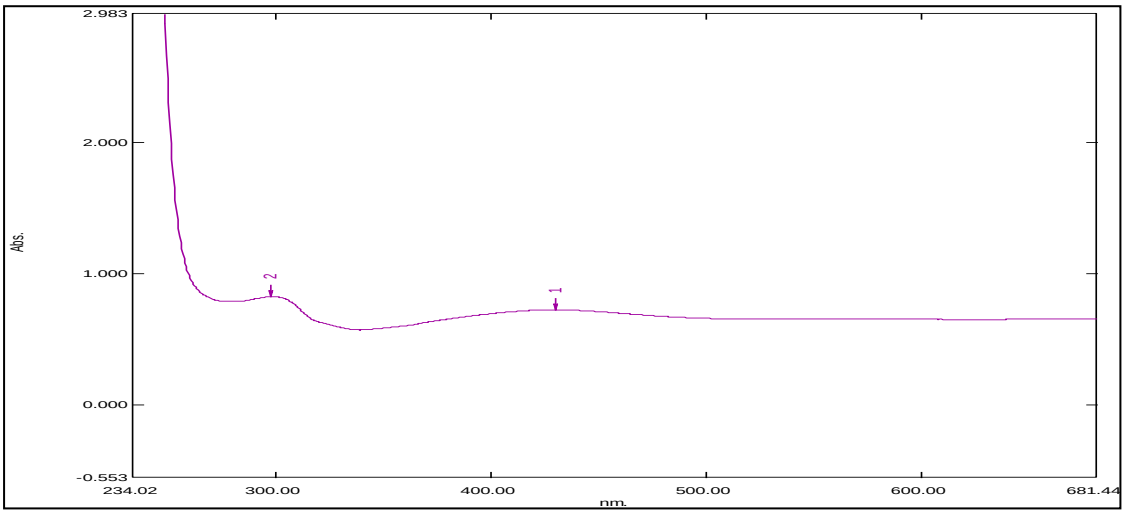
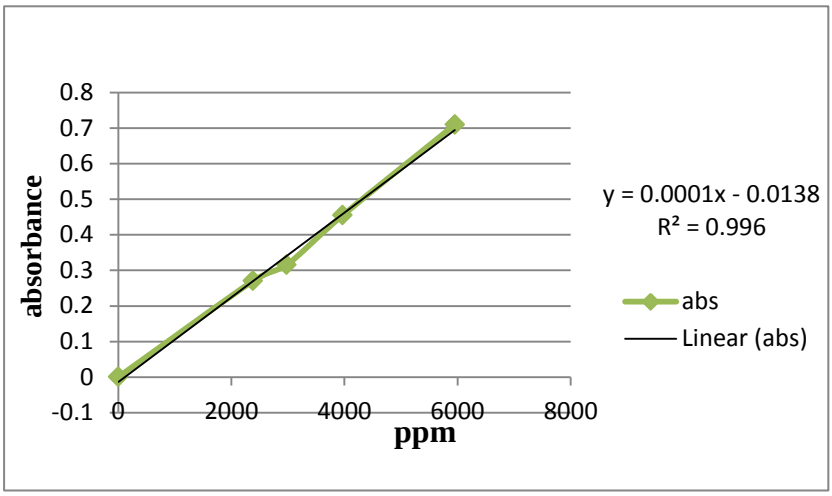
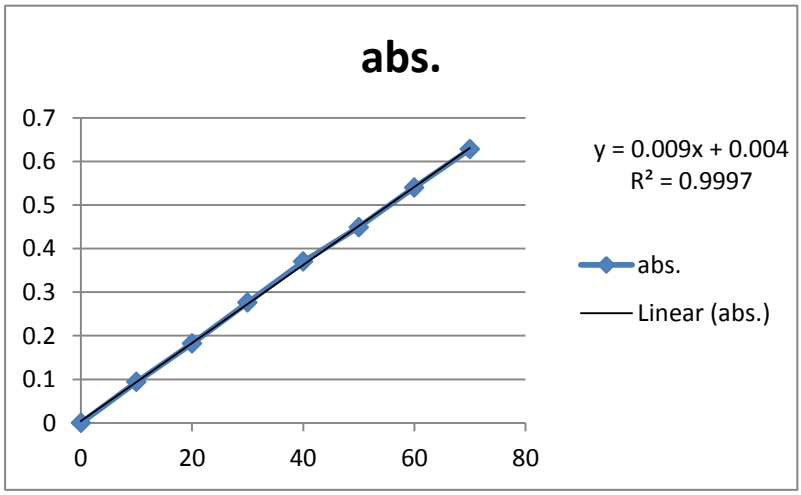


Figure No. 1: UV–visible spectra of Siver nanoparticles



Conc (ppm)	abs
0	0
2380	0.27
2976	0.315
3968	0.455
5952	0.71

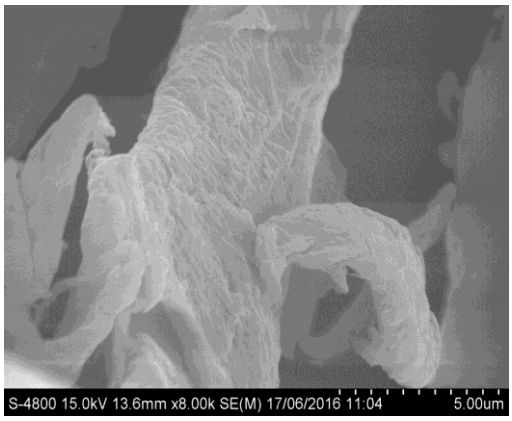
Figure No. 2: Calibration curve of Silver.



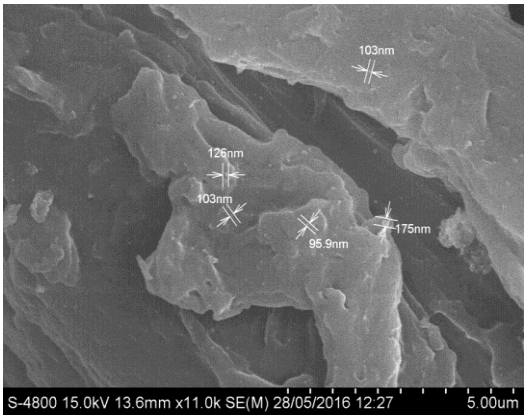
conc.(µg/ml)	abs.
0	0
10	0.094
20	0.182
30	0.276
40	0.37
50	0.449
60	0.54
70	0.628

Figure No. 3: Calibration curve of Clotrimazole

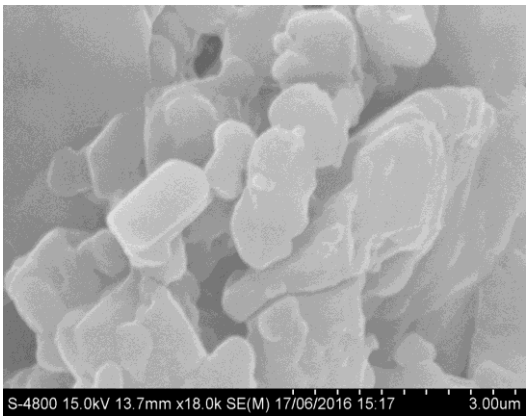
Scanning Electron Microscopy (SEM): Figure No. 4 (A) clearly shows SEM images of cellulose fibre, which are made entirely of cellulose fibres and free of any visible contaminants. An image with higher magnification demonstrates that the surface of the fibre is rough and has holes and wrinkles with a nanometer scale. In this study, Polyethylene glycol 400 was used as a stabilizer to create almost spherical, homogenous Ag NPs with a mean diameter of around 120 nm. SEM was utilized to monitor the morphological changes in cellulose fibres brought on by the deposition of Ag NPs [17]. Figure No. 4 (B). The morphological structure of the drug Clotrimazole was shown in Figure No. 4(C). Images of Clotrimazole powder at higher magnification show that the drug's particles have a rod-like crystal structure. In Figure No. 4 (D), silver nanoparticles with an average diameter of 113 nm and crystalline Clotrimazole particles are loaded onto cellulose fibres.



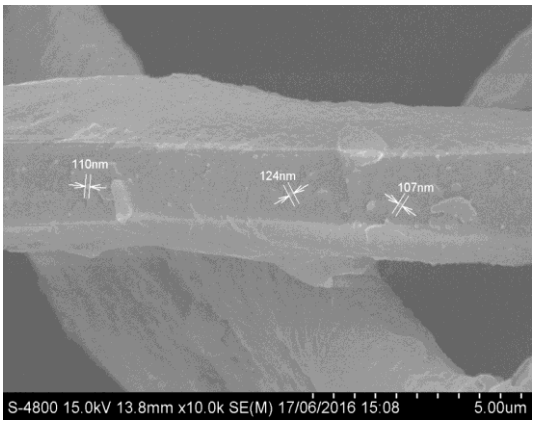
(A) Cellulose Fibre



(B) Fibre + AgNP



(C) Clotrimazole



(D) Fibre + AgNP+ Clotrimazole

Figure No.7 SEM Images

FTIR Spectroscopy: FTIR spectrum information for cellulose fibre, cellulose fibre + AgNP, fibre + AgNP + Clotrimazole, and Clotrimazole are as shown in figure No.5 and Table No.1. The two primary functional groups of cellulose linkage that are clearly seen here are the O-H group and the glycosidic linkage of the ether.

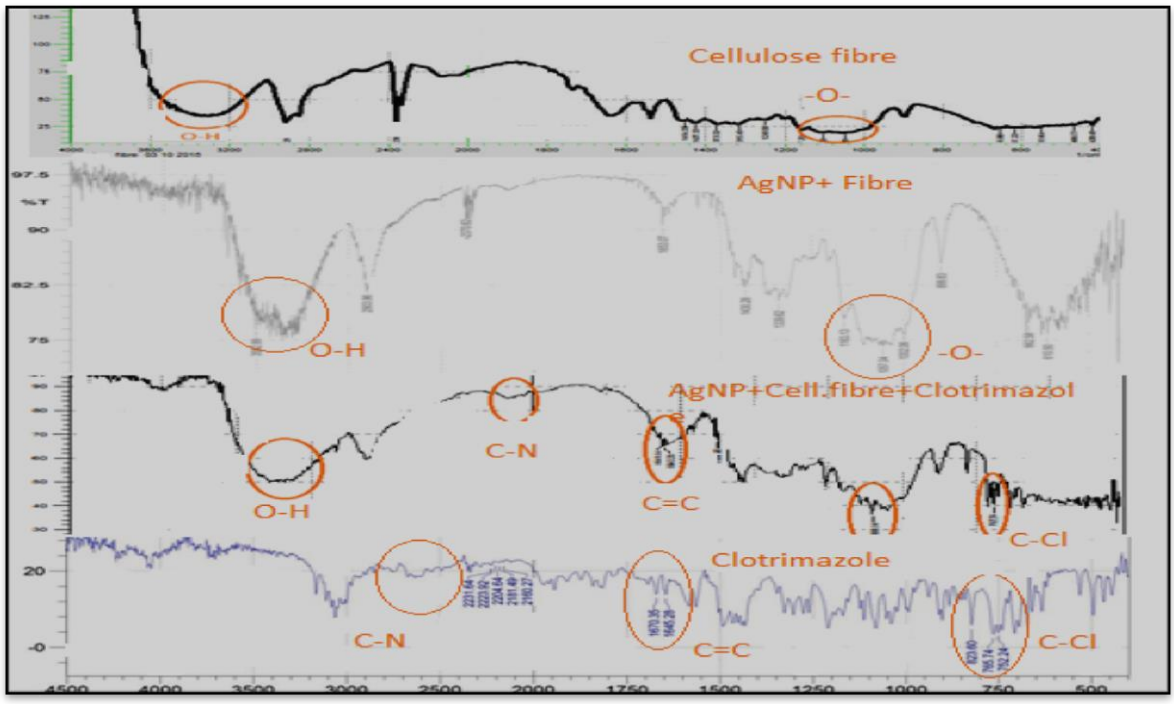


Figure No.5: IR spectra of Cellulose fibre, cellulose fibre + AgNP, Cellulose fibre + AgNP + Clotrimazole, Clotrimazole

The bands at 3300-3400 cm^{-1} are related to the interaction of Ag nanoparticles with the cellulose chain and hydrogen-bonded hydroxyl groups of the cellulose chain (peaks are due to higher wavenumbers). As a result, we can affirm that the cellulose fibre contains silver nanoparticles. The behavior of varied PEG molecular weights suggests that longer polymer chain length PEG will quickly reduce Ag+ to silver particles. AgNPs/Cellulose's IR spectra underwent another modification for the range at 1157 cm^{-1} as a result of the C-O-C Glycoside ether linkages. which, following the loading of Ag NP on the fibre, shifted at 1080 cm^{-1}.

The Clotrimazole's spectral analysis revealed the presence of C-Cl at 752 cm^{-1}. Stretching of the C=C bond is visible at 1643 cm^{-1}. As a result, C=N happens at 2220 cm^{-1}. Spectral data of cellulose fibre + AgNP plus Clotrimazole demonstrating the drug's obvious loading.

As a result, the key functional groups C=N at 2220 cm^{-1}, C-Cl at 752 cm^{-1}, and C=C at 1643 cm^{-1} are present.Additionally, the presence of glycoside ether and hydroxyl linkages at 3300 cm-1 and 1080 cm^{-1}, respectively, may indicate the presence of a cellulose chain [18]

Table No.1: Different functional group present in IR

	Functional group	**Range(cm^{-1})**
Cellulose fibre	O-H	3300 (stretching)
	-O-	1157 (stretching)
Cellulose fibre + AgNP	O-H	3300 (stretching)
	-O-	1080 (stretching)
Cellulose fibre + AgNP+ Clotrimazole	O-H	3400
	C=C	1643 (stretching)
	C-O-C	1080 (stretching)
	C-Cl	752 (stretching)
	C=N	2220 (stretching)
Clotrimazole	C=C	1643
	C-Cl	752
	C=N	2220

Particle size analysis: The AgNP was stabilised using PEG 400. Ag NP's particle size was noticed. In order to minimise the agglomeration of silver nanoparticles, ethanol was utilised as a solvent. After solvent scanning, AgNP particle size was found to be between 159 and 416 nm, and percentage intensity was found to be 81.7% and 185, respectively. Furthermore, a poly dispersive index of 0.571 was noted. The Z average was 335.6 (the intensity weighted mean hydrodynamic size of the ensemble collection of particles) [19]. The oberves results as shown in figure No.6 & Table No. 2.

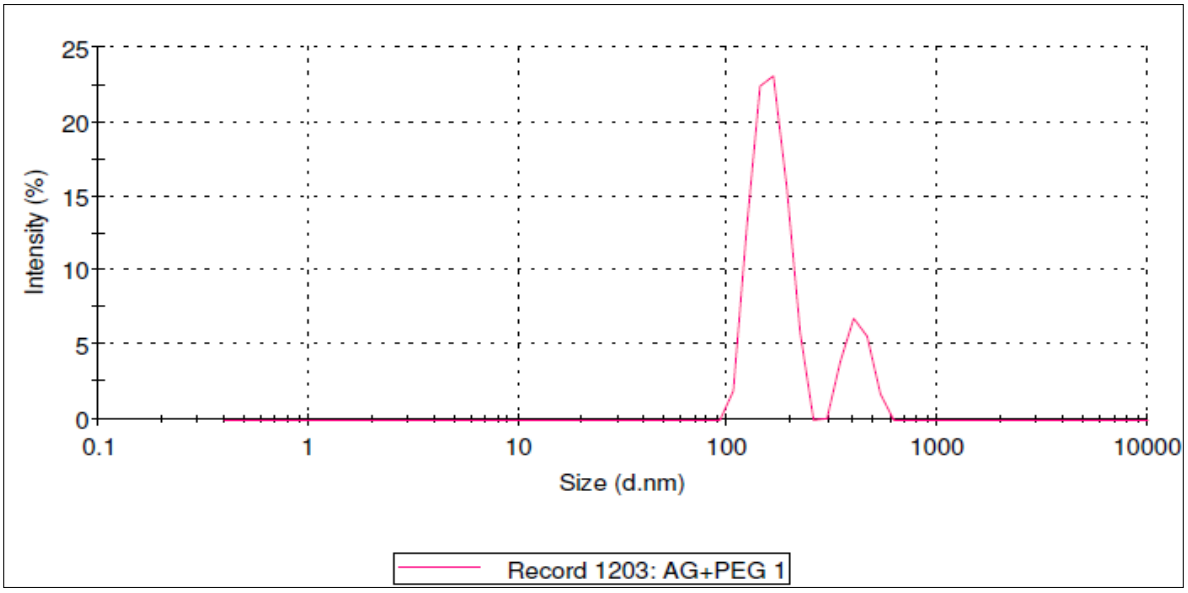


Figure No.6: Size analysis of silver nanoparticle

Table No.2: Size analysis of silver nanoparticle

			Size (d.nm)	%Intensity	Width (d.nm)
Z Average (d.nm)	**335.6**	**Peak-1**	159.1	81.7	28.65
Pdl	**0.571**	**Peak-2**	416.0	18.3	57.02
Intercept	1.08	**Peak-3**	0.000	0.0	0.000

Elemental detection X-ray spectroscopy

Cellulose Fibre + AgNP: Based on the illustration (Figure No. 7) The elemental analysis of a silver nanoparticle and cellulose fibres using SEM and EDS revealed that carbon, oxygen, and silver are all present in the majority of the cellulose fibre, confirming the existence of silver within the silver nanoparticle-cellulose fibres. For carbon, oxygen, and chloride, lower signals were seen. These signals point to the existence of biomolecules made of silver nanoparticles, which may have acted as stabilizing molecules [9].

A peak at 2.98 keV indicates that silver is present inside the silver nanoparticle. Carbon and oxygen found in the sample at 10.14% and 11.40%, respectively, whereas silver is detected at 0.24% [17].

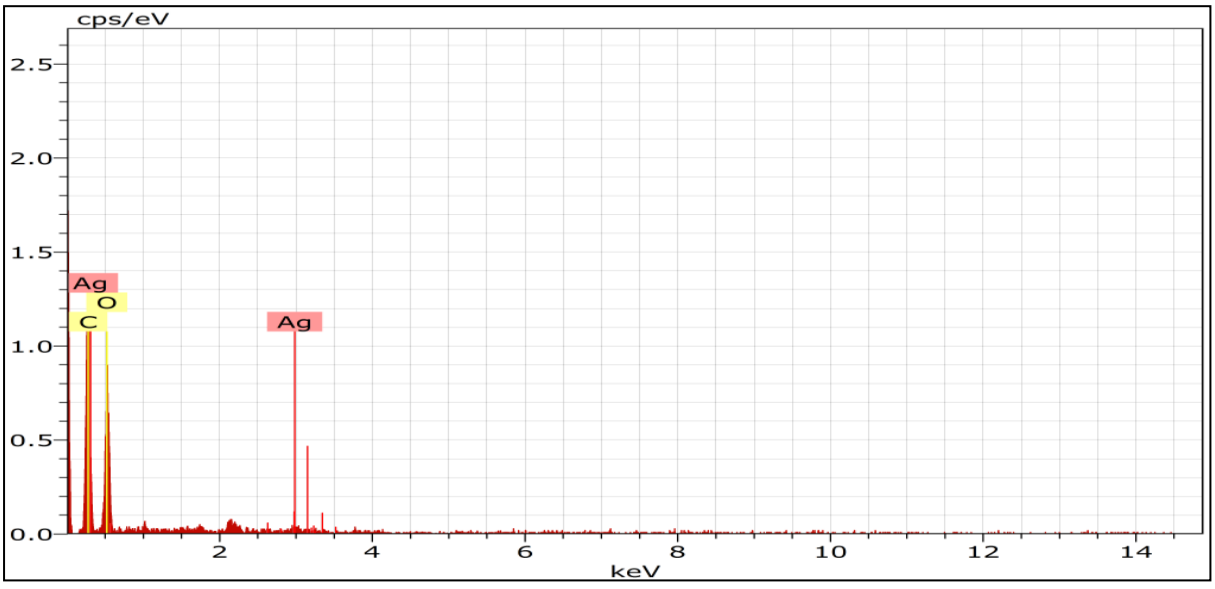


Figure No.7: EDX spectrum of silver nanoparticle loaded cellulose fibre

Clotrimazole: The presence of the elements Chlorine, Nitrogen, Carbon, and Nitrogen in figure No.8 may serve as confirmation that the material being examined is Clotrimazole. For carbon, oxygen, and chloride, lesser signals were seen; these indications point to the

existence of biomolecules. Nitrogen (0.65%), Chlorine (0.51%), and Carbon (10.13%) were all present in the Clotrimazole under analysis.

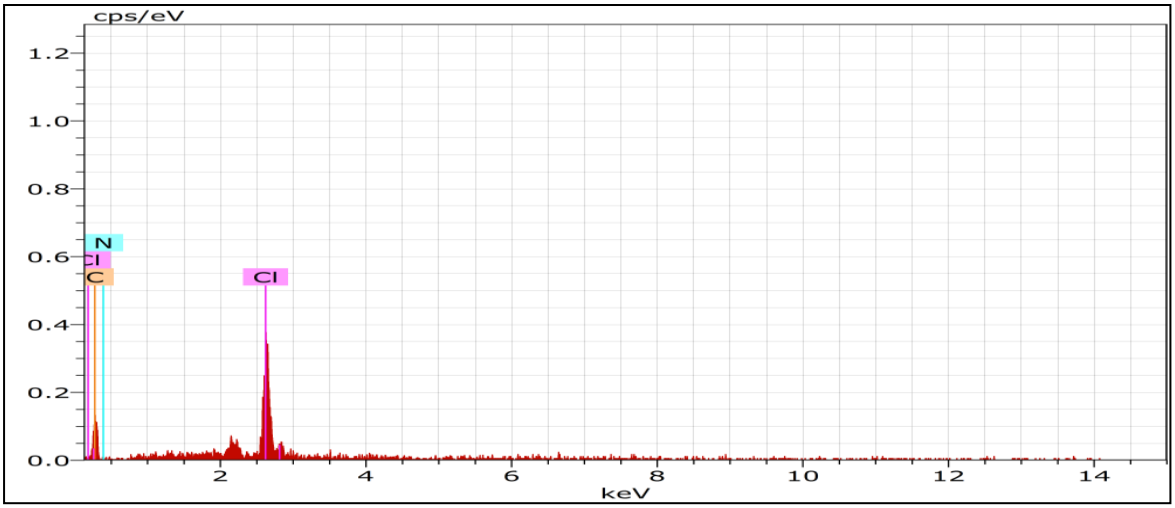


Figure No. 8: EDX spectrum of Clotrimazole

Fibre + AgNP + Clotrimazole: Figure No.9 confirms the existence of Carbon, Oxygen, Chlorine, and Nitrogen. Additionally, the presence of Silver confirms the production of silver nanoparticles. As seen in the image, the analysed sample included silver (0.15%) at 2.98 keV. Similarly, the presence of nitrogen (5%), chlorine (0.23%), and other elements can establish the existence of clotrimazole. Sample has (11.82%) carbon content.

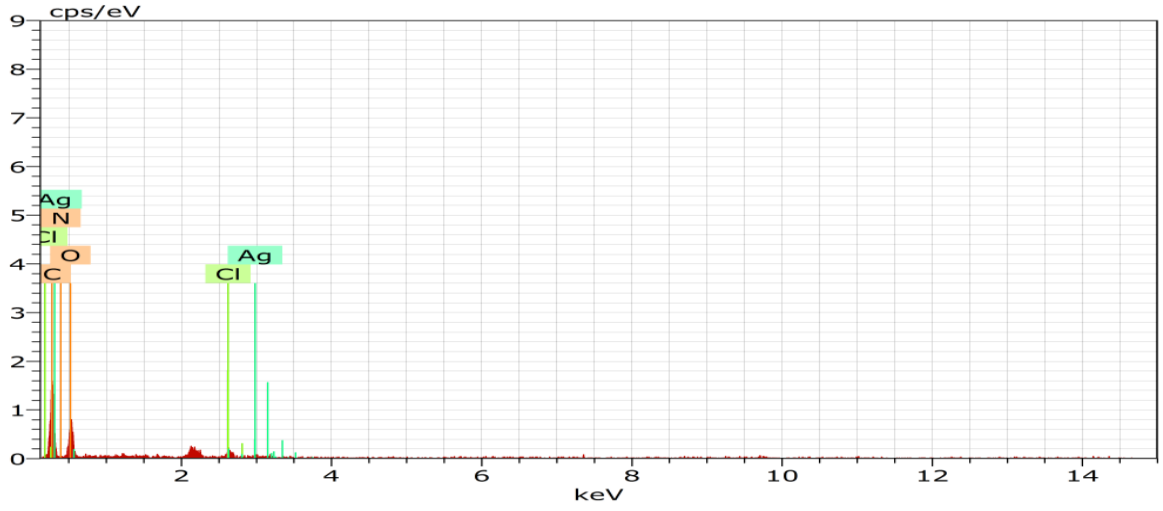


Figure No. 9. EDX spectrum of Fibre + AgNP + Clotrimazole

X-ray diffraction spectroscopy

X-Ray spectrum of plane wheat fibre: The diffraction maxima at 2θ given below can be attributed to the partial crystalline nature of natural polymers that resemble cellulose. The partial elimination of the amorphous areas during the acid hydrolysis treatment of cellulose

causes the stronger diffraction peaks, which indicate a high degree of crystallinity in the structure. To determine the phase structure of the samples, XRD was utilized. As seen in

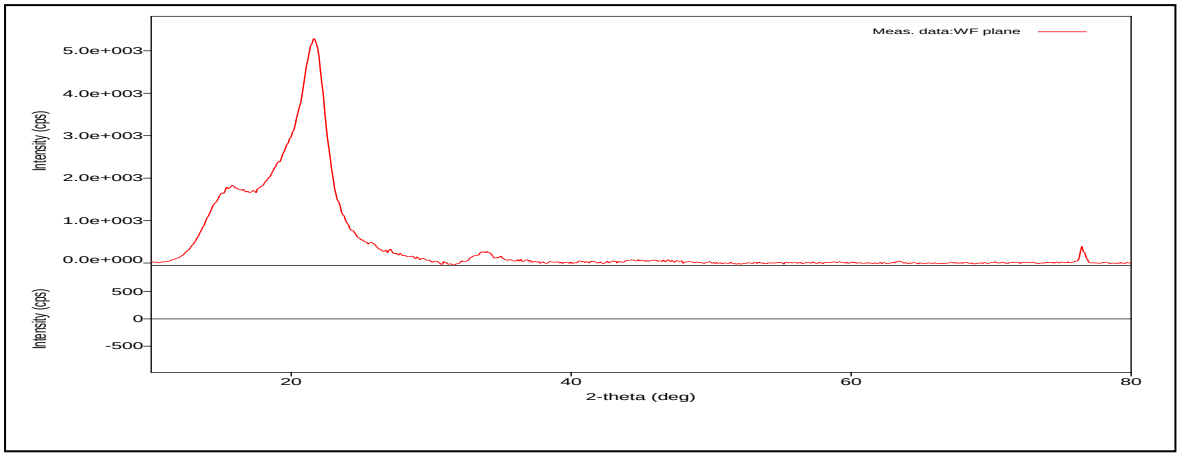


Figure No. 10, the typical cellulose II structure has three diffraction peaks that are assigned to native cellulose and represent the typical structure of cellulose. These peaks are located at 2θ = 15.75^0, 21.74^0, 34.06^0, 45.3^0, and 76.44^0, respectively.

Figure No. 10: X-Ray spectrum of plane wheat fibre

X-Ray spectrum of Fibre + AgNP + Clotrimazole: The partly crystalline nature of natural polymers that resemble cellulose can be attributed to the diffraction maxima at 2 that are shown below. The higher degree of crystallinity in the structure is shown by the sharper diffraction peaks. To determine the phase structure of the samples, XRD was utilized. Numerous peaks that appeared in the graph indicated that both cellulose and clotrimazole have crystalline structures. Typical cellulose and Clotrimazole diffraction peaks were shown in Figure No.11, with the peaks occurring at 2θ = 14.72^0, 21.80^0, 31.41^0, 33.99^0, 37.36^0, 43.38^0, 45.39^0, and 76.455^0.

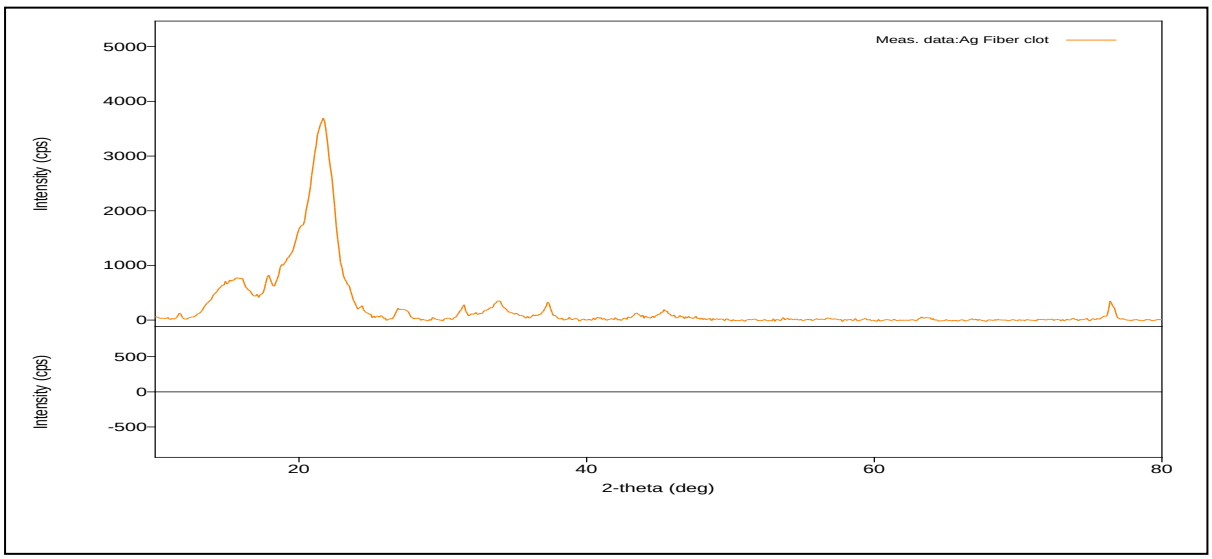


Figure No.11: X-Ray spectrum of Fibre + AgNP + Clotrimazole

Drug release:

Loading of the Drug to the cellulose fibre: Drug loading to the fibre is accomplished using the passive loading technique, which goes as follows: 200 mg of AgNP-cellulose fibres + 10 mg of Clotrimazole were taken, along with 50 ml of ethanol, which was maintained for stirring overnight and covered to prevent ethanol evaporation at the magnetic stirrer. The mixture was then centrifuged for 30 minutes at 15,000 rpm. Product that contained drugs was gathered.

Entrapment Efficiency and Drug Release Study:

Entrapment Efficiency: Centrifugation and UV analysis were used to calculate encapsulation efficiencies. The drug-loaded AgNP fibres were cleaned with enough ethanol and then redissolved in new ethanol. The mixture was then centrifuged for 30 minutes at 15,000 rpm. The unentrapped drug-retaining supernatant was collected separately, and its absorbance at 268 nm was measured. The absorbance value, which was based on the common Clotrimazole calibration curve, was used to compute the concentration of free drug.

$$\text{Entrapment Efficiency (\%EF)} = \frac{\text{Clotri.}T - \text{Clotri.}S}{Clotri.T} \times 100$$

The Drug Loading Ability of MCFNCs was calculated using the following Formula:

$$\text{Drug Loading Ability (\%DL)} = \frac{Clotri.AgNP.fibres}{W.fibre} \times 100$$

Where, Clotri.T = total amount of Clotrimazole taken; Clotrimazole = amount Clotrimazole present in supernatant; Clotri. AgNP.Fibre = amount of Clotrimazole on loaded AgNP-fibre; W.fibres = total amount of Cellulose fibre taken [20]. Similarly, by entering the result of the linear equation in the following formula, the amount of silver nanoparticle present on the fibre may be determined.

$$\text{Entrapment Efficiency (\%EF)} = \frac{\text{AgNP.}T - \text{AgNP..}S}{AgNP.T} \times 100$$

$$\text{Loading Ability (\%DL)} = \frac{Clotri.AgNP.fibres}{W.fibre} \times 100$$

Table No.3: % content of Clotrimazole and Silver nanoparticles

	E. Efficiency	**% Loading**
AgNP	61.55%	19.38%
Clotrimazole	62.49%	3.12%

In-Vitro Drug Release Study: It is obvious that different drug release fibres have different mechanisms. An in vitro drug release investigation revealed that the first burst release phase was followed by a plateau or steady release during the rest interval. The release pattern initially follows the Zero order Kinetic before providing the Control release Pattern over time. The potential justification for the variations in the releasing mechanisms the pH of the testing media contained nanocomposites with medicines integrated, which may have had a little impact on the AgNP-fibre-Clotrimazole structure.

In addition, it's likely that the drug, after dissolving from the drug particles, will bond to the cellulose fibres in a composite form. In the event of slow diffusion, this might result in desorption restricted kinetics, which may lead the release to follow zero-order kinetics [21]. Using the dialysis bag diffusion technique, the in-vitro release of Clotrimazole through drug-loaded Ag-Fibre was determined.

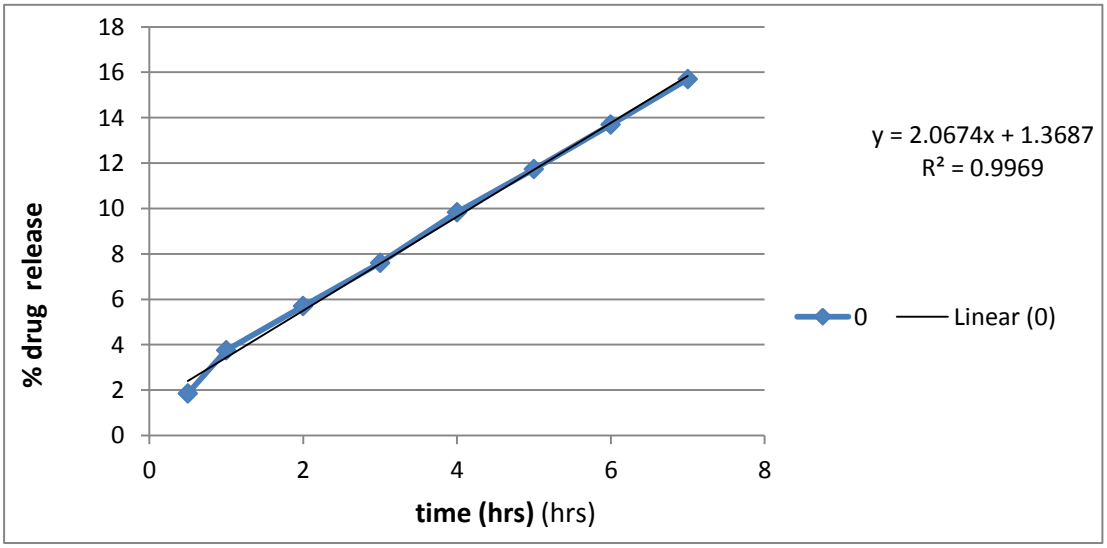


Figure No. 12: Drug release profile of drug Clotrimazole

Studies on the release of Clotrimazole from silver fibres were conducted in an ethanolic phosphate buffer with a pH of 6.8. The 500ml beaker of the dissolving media, which was agitated at 100 rpm and kept at room temperature, was filled with the dialysis bag. To stop the dissolving media from evaporating, the Beaker was covered. At certain intervals (0.5, 1,

2, 3, 4,5, 7, 8 hours), samples (3 ml) were removed off the beaker and the exact same volume of new dissolving media was added to maintain a consistent volume. At λ max at 268 nm, samples were spectrophotometrically measured.

Table No.4: Drug release profile of Clotrimazole

Time in hrs.	Abs.	mcg/ml	mg/ml	mg/1ml	Error	mg/500ml	% release
0	0	0	0	0	0	0	0
0.5	0.084	2.0540541	0.002054	0.0102703	0	1.848648649	1.84864865
1	0.162	4.1621622	0.004162	0.0208108	0.01027	3.745945946	3.75621622
2	0.241	6.2972973	0.006297	0.0314865	0.031081	5.667567568	5.69864865
3	0.318	8.3783784	0.008378	0.0418919	0.062568	7.540540541	7.60310811
4	0.408	10.810811	0.010811	0.0540541	0.104459	9.72972973	9.83418919
5	0.484	12.864865	0.012865	0.0643243	0.158514	11.57837838	11.7368919
6	0.562	14.972973	0.014973	0.0748649	0.222838	13.47567568	13.6985135
7	0.641	17.108108	0.017108	0.0855405	0.297703	15.3972973	15.695

Anti-fungal Investigation: Candida albicans was used as the test subject for the anti-fungal research using silver nanoparticles and clotrimazole. 1 g/ml or less of Clotrimazole inhibited Candida albicans species. The fungicidal activity of Candida albicans species was also demonstrated at 1–5 g/L.

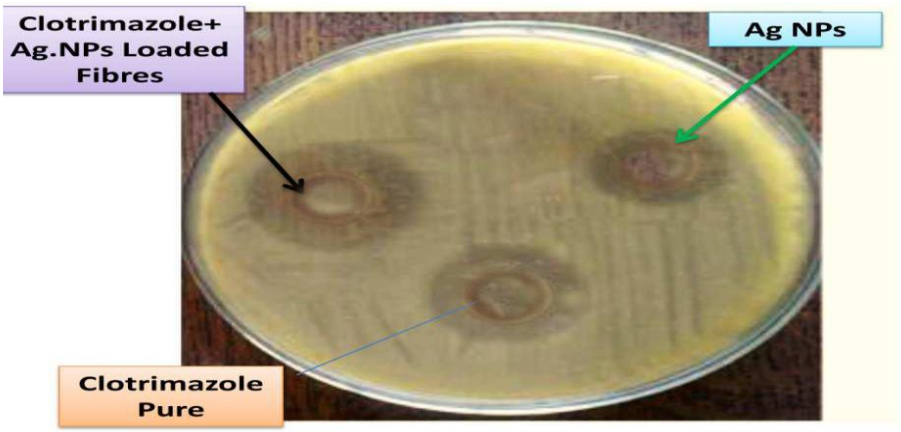


Figure No. 13: Anti-fungal activity of Clotrimazole, Silver nanoparticle, Clotrimazole-Silver nanoparticles loaded fibres

Minimum inhibitory concentration (MIC): The MIC inhibitory concentration was defined as the lowest concentration that, when compared to the growth in the control wells, inhibited 80% of the growth. In the wells, a suspension of silver nanoparticles was introduced at a concentration of 1 to 5 g/ml. [22].

Preperation of Sabaroud Dextrose Agar (SDA) medium: 9.75 gm 150 cc of the distilled water were used to suspend the Sabaroud dextrose agar. The solution was heated to boiling in order to thoroughly dissolve the medium. autoclaved for 15 minutes at 1210°C and 15 lbs of pressure to sterilize.

Determination of Antifungal activity: A 5μg/ml dispersion of silver nanoparticles demonstrated considerable antifungal efficacy against Candida albicans in fungi. The 5 mm-wide zone of inhibition for the suspension of silver nanoparticles. A 7 mm zone of inhibition was visible when clotrimazole (1μg/ml) was diluted. After shaking, silver nanoparticle suspensions in Clotrimazole solution were added to the well of plates, and when the plates were heated to between 24-280^{o}C, the AgNP suspension and Clotrimazole solution showed a 12 mm-wide zone of inhibition.

CONCLUSION:

The best conditions for the production of size-controlled, evenly distributed silver nanoparticles in Polyethylene glycol) 400 were obtained by effectively fabricating Cellulose fiber/Ag NPs composite microfibrils in conjunction with Clotrimazole. Clotrimazole was physically put onto cellulose fibre. With potential uses in the dielectric and biological domains, produced silver nanoparticles dispersed in water, ethanol and other polar solvents.

In order to administer Clotrimazole in a regulated manner, wheat fibres were employed as a new carrier. When making Ag NPs, poly (ethylene glycol 400) was used as a reducing agent and a stabilizer in small reactors. The technique used suggests cellulose that is easily available, biodegradable, and easy and environmentally favorable to prepare. When combined with silver nanoparticles, the broad-spectrum antimycotic drug clotrimazole effectively killed the fungus Candida albicans. attaining the desired properties for a topical formulation to provide the best therapeutic result.

ACKNOWLEDGEMENT:

The study was performed by the support of H.R.P.I.P.E.R, Shirpur, Dhule Maharashtra (India and Nuper Therapeutics A Division of Jain Pharmaceuticals, Baner, Pune, Maharashtra (India)

REFERENCES:

[1] Mohanraj, V. & Chen, Y., Nanoparticles–a review, *Trop J Pharm Res*, 2006, 5, 561-573.

[2] Iravani, S., Korbekandi, H., Mirmohammadi, S. & Zolfaghari, B. Synthesis of silver nanoparticles: chemical, physical and biological methods, *Res. in Pharm. Sci.* 2014, 9, 385.

[3] Segal, L., Creely, J., Martin, A. & Conrad, C., An empirical method for estimating the degree of crystallinity of native cellulose using the X-ray diffractometer, *Textile Res Journal*, 1959, 29, 786-794.

[4] Nakatsubo, F., Kamitakahara, H. & Hori, M., Cationic ring-opening polymerization of 3, 6-di-O-benzyl-α-D-glucose 1, 2, 4-orthopivalate and the first chemical synthesis of cellulose, *J of the Ame Chem Soci*, 1996, 118, 1677-1681.

[5] Nagavarma, B., Yadav, H. K., Ayaz, A., Vasudha, L. & Shivakumar, H., Different techniques for preparation of polymeric nanoparticles—a review, *Asian J. Pharm. Clin. Res,2012*, 5, 16-23.

[6] Heera, P. & Shanmugam, S., Nanoparticle characterization and application: an overview, *Int J Curr Microbiol Appl Sci,2015*, 4, 379-86.

[7] Prabhu, S. & Poulose, E. K., Silver nanoparticles: mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects, *Int Nano Letters,2012*, 2, 1-10.

[8] Mukherjee, P., Ahmad, A., Mandal, D., Senapati, S., Sainkar, S. R., Khan, M. I., Parishcha, R., Ajaykumar, P., Alam, M. & Kumar, R. Fungus-mediated synthesis of silver nanoparticles and their immobilization in the mycelial matrix: a novel biological approach to nanoparticle synthesis. *Nano Letters*, 2001, 1, 515-519.

[9] Senthilkumar, P., Bhuvaneshwari, J., Prakash, L. P. & Kumar, R. S., Green Synthesis and Characterization of Silver Nanoparticles from Aqueous Extract Brown Seaweed of Padina Boergesenii and its antifungal activity, *world J of Pharm. Pharm. Sci.*, 2015, 4(10), 1858-1870.

[10] Zhang, G., Keita, B., Dolbecq, A., Mialane, P., Secheresse, F., Miserque, F. & Nadjo, L., Green chemistry-type one-step synthesis of silver nanostructures based on MoV–MoVI mixed-valence polyoxometalates, *Chemistry of Materials*, 2007, 19, 5821-5823.

[11] Cozzoli, P. D., Comparelli, R., Fanizza, E., Curri, M. L., Agostiano, A. & Laub, D., Photocatalytic synthesis of silver nanoparticles stabilized by TiO2 nanorods: A

semiconductor/metal nanocomposite in homogeneous nonpolar solution, *J of the Ame. Chem. Soci.*, 2004, 126, 3868-3879.

[12] Doree, C., The methods of cellulose chemistry including methods for the investigation of substances associated with cellulose in plant tissues. The methods of cellulose chemistry including methods for the investigation of substances associated with cellulose in plant tissues, 1947. *No. Edn-2 (Revised)* pp xii +543 pp

[13] Williams, G. I. & Wool, R. P., Composites from natural fibers and soy oil resins, *Applied Composite Materials*,2000, 7, 421-432.

[14] Cudmore, S. L., Delgaty, K. L., Hayward-Mcclelland, S. F., Petrin, D. P. & Garber, G. E., Treatment of infections caused by metronidazole-resistant Trichomonas vaginalis. *Clin microbiology reviews*, 2004, 17, 783-793.

[15] Warnock, D. W. & Richardson, M. D. 1991. Fungal infection in the compromised patient, John Wiley & Sons Ltd.

[16] Rowe, R. C., Sheskey, P. J. & Weller, P. J. 2006. Handbook of pharmaceutical excipients, pharmaceutical press London.

[17] Luong, N. D., Lee, Y. & Nam, J.-D., Highly-loaded silver nanoparticles in ultrafine cellulose acetate nanofibrillar aerogel, *Euro Poly Journal*, 2008, 44, 3116-3121.

[18] Basuny, M., Ali, I. O., EL-Gawad, A. A., Bakr, M. F. & Salama, T. M., A fast green synthesis of Ag nanoparticles in carboxymethyl cellulose (CMC) through UV irradiation technique for antibacterial applications, J of Sol- Gel Scie & Tech, 201, 75, 530-540.

[19] Zodrow, K., Brunet, L., Mahendra, S., Li, D., Zhang, A., Li, Q. & Alvarez, P. J., Polysulfone ultrafiltration membranes impregnated with silver nanoparticles show improved biofouling resistance and virus removal, *Water research*, 2009, 43, 715-723.

[20] Kontogiannopoulos, K. N., Assimopoulou, A. N., Tsivintzelis, I., Panayiotou, C. & Papageorgiou, V. P., Electrospun fiber mats containing shikonin and derivatives with potential biomedical applications, *Int J of Pharmaceutics*, 2011, 409, 216-228.

[21] Cui, W., Li, X., Zhu, X., Yu, G., Zhou, S. & Weng, J., Investigation of drug release and matrix degradation of electrospun poly (DL-lactide) fibers with paracetanol inoculation, Biomacromolecules,2006, 7, 1623-1629.

[22] Priscyla D Marcato & Nelson Duran, New aspects of nanopharmaceutical delivery systems, *J Nanosci Nanotechnol*, 2008, 8(5):2216-29.