FORMULATION, BIOFILM AND ANTI-BACTERIAL ACTIVITY OF CEPHALEXIN DOPED CARBON NANOPARTICLE INCORPORATED TOPICAL GEL

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ABSTRACT

The rise in production of Carbon out of pollution, medical sciences have started re-using the Carbon for treating various diseases because of it's versatile character as a molecule. Because of the use of Carbon as a carrier molecule, the characterization of the Carbon Doped Cephalexin nanoparticles is also conducted in this work. The appearance of the gel is ash-black in colour depending on the concentration of Carbon used in formulation. The aim of the present work is to formulate Carbon doped Cephalexin Nanoparticles as topical gel for testing its anti-microbial activity and also to check it's mechanism of action with the help of bio-film formation. In this article, the anti-microbial activity of the Carbon doped Cephalexin nanoparticle gel is tested against organisms like Protease, E.coli, and Staphylococcus sp. and Bacillus substilis as well as it's mechanism of action is tested using anti-biofilm production. The phase 0 of the clinical trials is conducted in this work in order to understand its anti-microbial characteristics. After formulating various batches of the Carbon doped Cephalexin topical gel based on varying concentration of Carbon, CC5 has been able to show the highest property of gel characteristic out of all the batches. Followed by several evaluation tests being conducted including the microbial assay, it was found that CC2 and CC5 showed the highest zone of inhibition, that is, highest anti-microbial activity out of all batches. The formulations has hence proven to show their respective anti-microbial activity against broad-spectrum of microbes with their respective mechanism of action being studied using the bio-film preparation and its ability to rupture the bio-film produced by the microbes.

KEYWORDS: Cephalexin, Carbon nanoparticles, Zero phase, Antibacterial activity.

INTRODUCTION

Nanoparticle is new path towards the novel drug delivery system which ranges between 1 to 100 nm in size. Though invisible to naked eyes of human beings, yet nanoparticles can exhibit different physical and chemical properties significantly as compared to other conventional drug delivery counterparts. The European Commission has stated that the particle size as per the number size distribution must be less than 100nm[1]. The nanoparticles have been categorized which includes fullerenes, metal nanoparticles, ceramic, and polymeric.

Because of possessing these characteristics, the nanoparticles have started to serve for various commercial as well as domestic applications, including catalytic reaction, various types of imaging, medicinal applications, research based on energy consumption, as well as for environmental applications like treatment of pollution[2]. Carbon has been found to be extremely essential in life because of it stability to form variety number, types of chains of different lengths. Carbon nanotubes (CNTs) or carbon nanoparticles are allotropes of carbon. These particles possess certain impressive such as structural flexibity , strong mechanical support, and electronic properties as these particles are small size and in mass[3]. Carbon-Nanotubes and nanoparticles have competently being used in Pharmaceutical field and medicinal field because of having high surface area which is proficient enough of adsorbing and conjugating with a wide variety of therapeutic as well as diagnostic agents such as in drug preparations, gene therapy, vaccine production and storage, antibodies, biosensors, etc. These particles has now established to be an excellent vehicle for delivery of the drug straightway into cells without getting metabolized in the body[4].

Drug Delivery System over Topical Region

Topical drug administration is a type of drug delivery or administration system which is localized at ophthalmic, rectal, vaginal and skin as some of the topical routes[5]. Novel topical dosage forms are a type of topical drug delivery systems which advances in using the carrier molecule as well as the main active pharmaceutical ingredient in minute sizes, ranging between micrometres to nanometres.

Features of Gel type of Dosage form

Gels are found to be highly stable and also have shown to avoid first pass metabolism, high retention time over the skin when applied, gives a cooling effect over the skin. Due to the increase in number of geriatric population and rising incidences of chronic wounds are some of the key factors which will drive the demand for gels in the upcoming years. However, the gel based topical dosage form market has been estimated to reach about 129.8 billion USD by the year 2025 at a CAGR of 6.4% in the midst of the forecast period itself[6]. Cephalexin has shown the ability to act towards certain type of bacterial infections, which includes the ear , bone and joint regions, skin, and urinary tract region.

MATERIALS AND METHOD

Preparation of Carbon Soot

The carbon soot (CS) was prepared by combustion of candles and by using aluminum foil as obstruction for the collection of Carbon soot. For the above procedure, white candles about 400g were burnt under aseptic condition the soot was collected from the candles by keeping aluminum foil closely in contact with the flame of the candle. A thick coat of the soot was collected on the aluminum foil and was cooled. The aluminum foil was scrapped and the power was collected on another aluminum foil was stored in desiccator(anhydrous Calcium chloride)[7]. It took about 12 hours for complete combustion of candles**.**

Purification of Carbon Nanoparticle

For purification, Acetone: water in the ratio of 3:1 was used for the dissolution of the crude collected soot. After centrifugation, the undisclosed residue was discarded. After that clear solution was collected and heated in water bath was employed for evaporation of the solvent from the solution. Furthermore, powder form of Cephalexin doped carbon nanoparticles was collected. The solubility of nanoparticles was tested and was found to be soluble in acetone, methanol, and chloroforms[8].

Preparation of Cephalexin Doped Carbon Nanoparticle Gel

1 gm of cephalexin dissolved in 5ml of methanol to this prepared carbon nano particle was added and mixed well to obtain a grey colored suspension. The solution was transferred to China dish and solvent was made to evaporate to leave behind the drug doped carbon nanoparticle went for SEM analysis picture obtained. [9]

0.2g drug doped carbon nanoparticle was dissolved in sufficient amount of 0.1ml chloroform and 0.1 ml of Tween 80 was added continuously with stirring and it was stirred until odour of chloroform was removed and a dry agglomerate was obtained[10]. In a beaker 0.28g of disodium hydrogen phosphate and 0.11g of potassium dihydrogen phosphate was mixed in 10ml of water for the formation of phosphate buffer(10ml) at pH 6.8 . The phosphate buffer was added to the nano dispersion(agglomerate) and a viscous formulation was obtained. Varying amount of Carbopol 934 (as show in table) was added and using magnetic stirrer for 2 hours until the gel consistency was obtained. Triethanolamine maleate was added of about 0.5ml in order to neutralize the mixture and provide appropriate consistency [11]. All the formulations prepared were tested for FT-IR analysis & UV[12].

Drug Content: 1 gm of a Cephalexin doped carbon antimicrobial gel formulation was taken and the vesicles were introduced with 25ml of methanol by sonication for 15min. The solution was then placed in a centrifugation tube and was centrifuged at 10,000 rpm for 30 mins. Solutions were then diluted with 100ml with phosphate buffer of pH 7.4. Drug content was calculated for Cephalexin by using UV spectrophotometer at 254 [13].

Table 1: Cephalexin Doped Carbon Nanoparticle Gel Formulation

		CONCENTRATION			
S.NO	**FORMULATION**	**TWEEN 80**	**CEPHALEXIN**	**CARBOPOL 934**	**CARBON**
1	CC 1	0.1ml	0.1gm	0.5gm	0.1gm
2	CC2	0.1ml	0.1gm	0.5gm	0.15gm
3	CC3	0.1ml	0.1gm	0.5gm	0.2gm
4	CC4	0.1ml	0.1gm	0.75gm	0.1gm
5	CC5	0.1ml	0.1gm	0.75gm	0.15gm
6	CC6	0.1ml	0.1gm	0.75gm	0.20gm
7	STD	0.1ml	0.2gm	0.00	0.2gm

***In-vitro* Release Study**

An exact amount of formulation is spread out on membrane the donor and receptor available over the diffusion area (cathode). The receptor compartment (anode) was then filled with phosphate buffer at pH 6.8 and was continuously blended with a small magnetic bar at a speed of 50 rpm. The sample were withdrawn at different time intervals and replaced with same volume of phosphate buffer solution. The sample were analyzed in spectrophotometer [14].

Antibacterial Activity

Antibacterial activity of Cephalexin doped carbon nanoparticles was tested against several species of gram positive as well as gram negative bacteria with the help of cup plate method. The zone incubation was measured around each cup and compared with Cephalexin gel with out carbon nanoparticles. The entire operation was carried out in a laminar air flow unit. Each formulation solution was tested in triplicate [15].

Anti-Biofilm Activity

For testing the procedure of how the Cephalexin doped carbon nanoparticle gel works, a 96-well microtiter plate (flat bottom made of polystyrene) was being used for determining the anti-biofilm activity of the cephalexin

doped carbon nanoparticles gel as described by Guru Natham *et al* (2014) [16] and Bharapet *et al* (2016)[17] . The percentage of inhibition of biofilm formation was calculated using following equation

%Biofilm Inhibition = 1 – [OD620 of cells treated by Carbon nanoparticle/OD620 of the non-treated particles (control)]×100

RESULTS AND DISCUSSION

Percentage Drug Content

From the given statistical graph, it can be observed that the highest drug content out of the 6 formulation was found for CC6 , that is, 95.14%and the lowest drug content was found for CC4,that is, 85.25%. Hence, from the results obtained, it can be said that the drug has not degraded in the 6 formulations prepared. Therefore, Cephalexin doped Carbon nanoparticles are compatible showing consistent drug content throughout.

pH value of tropical Cephalexin doped carbon nano particle

From the given statistical graph, it can be interpreted that the highest noted value of pH was seen for CC5, that is, 9.12 and the lowest was noted for CC2, that is, 8.42.From the results obtained, it can be concluded that all the formulations were in successful in showing the optimised pH required for the formulation of the topical dosage form.

Grittiness

All the 6 formulations were successful in showing the required degrees of freedom as we can observe how evenly, all the 6 formulations have spread over the glass slides and the same was observed using an electron microscope. Hence fulfilled the required degrees of freedom from the particulate matter, none of the formulations showed grittiness.

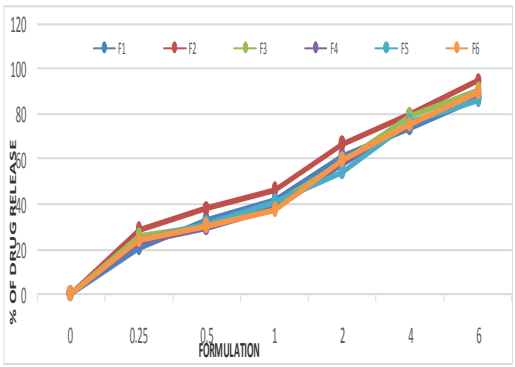

Figure 3: *In-vitro* drug release

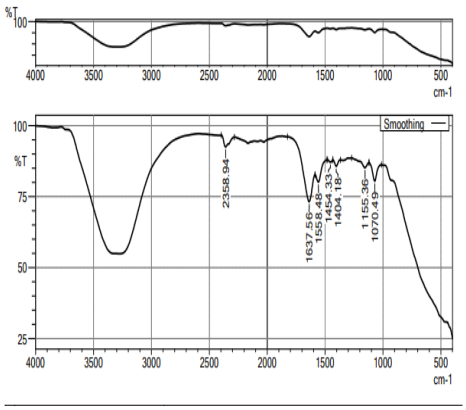

Figure 5: FT-IR of the formulated topical gel CC5

In-Vitro Drug Release

From the 6 formulations being studied for in-vitro drug release, it was observed that CC2 and CC5 had the highest cumulative amount of drug release of 95.67% and 95.59% respectively for upto 6 hours using cellophane membrane. The release rate of Cephalexin doped Carbon nanoparticles for the CC2 and for CC5 formulation was significantly higher then the other 4 formulations. Hence CC2 and CC5 can be considered as a good alternative formulation for decreasing the frequency of dose and also for proper maintenance of the concentration of the drug at the site of action. Given below shows the final report of the in-vitro drug release study.

FTIR

FT-IR spectra for the active pharmaceutical ingredient, Cephalexin and for the prepared topical gel was obtained using FT-IR JASCO 460. The graphs were obtained as shown in Figure 5. From the graphs obtained, and the standard graph of the Cephalexin [18] where compared, there was not any adverse interaction noted between Carbon and Cephalexin.

SEM Analysis

The Carbon nanoparticle were observed to be clean, spherical shaped, fulfilling the required nanosize. The SEM picture shown as the surface micrographs at different magnification shows the irregular coagulation of large lumps over the surface of the soot sample along with major black pores and fissures in formulation. SEM image shows that there is no breakage and even distribution of the drug particles with the carbon carriers and the size of the particle are in the range of less than 90nm to 50nm, which results where matching with the previous study[819].

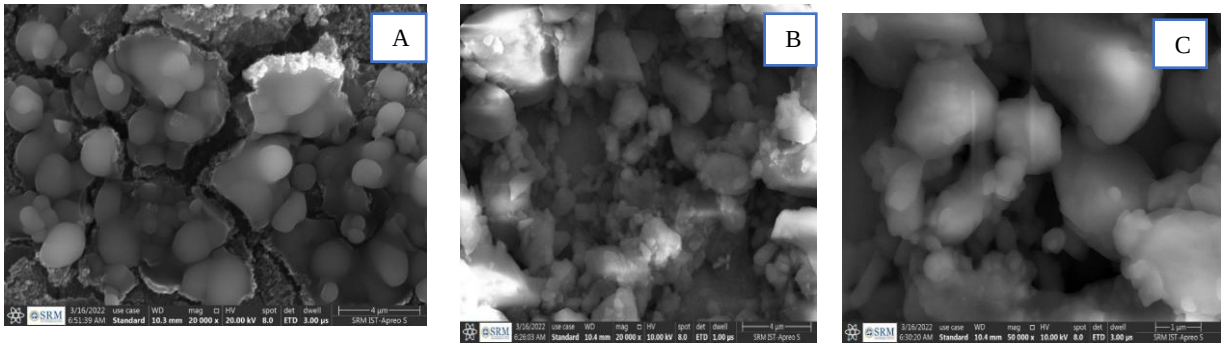

Figure 6: SEM Image. A: Cephalexin doped Carbon nanoparticles topical gel. B&C: Carbon nanoparticles

ANTI BACTERIAL ACTIVITY:

Anti Bacterial Activity

The table shown here gives us a clear evidence on the anti-bacterial activity with the formation of zone of inhibition of different concentration of Cephalexin doped Carbon nanoparticle gel over various concentration of pathogenic bacteria is displayed in the table using Dunnett's posttest. From the study conducted, we were revealed to that the Nanoparticles which were incorporated in the topical gel were very effective against both gram-positive (*Staphylococcus aureus and Bacillus substillis*) and gram negative (*E.coli* and *Proteus vulgaris*) bacteria by showing higher zone of inhibition than the standard gel formulation. The table given below shows the zone of inhibition for several formulations.

Table 2: Anti-Bacterial activity of formulation of Cephalexin doped Carbon nanoparticles topical gel

FORMULATION	**Zone of inhibition in mm**			
	Escherichia coli (ATCC 2592)	***Bacillus substilis (ATCC 2717)***	***Protease vulgaris (ATCC 2612)***	***Streptococcus aureuses (ATCC 2923)***
STD	14±0.03mm	15±0.12mm	20±0.45mm	18±0.21mm
CC1	16±0.04mm	27±0.32mm	23±0.27mm	20±0.35mm
CC2	24±0.05mm	26±0.23mm	25±0.62mm	23±0.56mm
CC3	15±0.06mm	28±0.34mm	24±0.28mm	25±0.23mm
CC4	20±0.05mm	27±0.25mm	23±0.43mm	26±0.67mm
CC5	24±0.08mm	26±0.65mm	22±0.36mm	27±0.12mm

CC6	29±0.15mm	28±0.34mm	24±0.34mm	24±0.23mm

Figure 7: Zone of inhibition of the cephalexin doped carbon nanoparticles against pathogenic bacteria. a)Staphylococcus aureuses *(ATCC 25923)* b)Bacillus substilis *(ATCC 2717)* c) *Escherichia coli (ATCC 2592)* d) *Protease vulgaris (ATCC 2612)*

Anti-Biofilm activity

In vitro anti-biofilm activity of Cephalexin doped carbon nanoparticle gel of several formulations were being estimated in a dose dependent manner against the biofilm forming microbes, that is, *E.coli*, *Bacillus substillis*, *Proteus vulgaris* and *Staphylococcus aureus* species. All the species were grown in 96 well plate microtier plates for 24 hours and the observations were noted as shown in the table. The MICs of Anti-biofilm activity for several formulations were being expressed in terms of percentage biofilm activity and it was revealed that the biosynthesized carbon doped Cephalexin doped carbon nanoparticles inhibited the biofilm formed by the bacterial species, relative to the negative control being used in the experiment. The MICs of anti-biofilm activity was expressed in percentage biofilm activity and all Cephalexin doped Carbon nanoparticles , irrespective of which concentration of carbon were used in their synthesis, exhibited an excellent MIC value against bio-film formation [3].

Table 3: Percentage reduction in biofilm activity of the Cephalexin doped Carbon nanoparticles topical gel on the bacteria

		Cephalexin doped carbon nanoparticle topical gel						
S.NO	**ORGANISM**	**STD**	**CC1**	**CC2**	**CC3**	**CC4**	**CC5**	**CC6**
1	***Escherichia coli (ATCC 2592)***	**60**	**80**	**82**	**85**	**88**	**95**	**92**
2	***Bacilius substilis (ATCC 2717)***	**50**	**65**	**79**	**82**	**83**	**89**	**91**
3	***Proteasevulgaris (ATCC2612)***	**67**	**82**	**72**	**85**	**88**	**94**	**90**
4	***Streptococcus aureuses (ATCC 2923)***	**48**	**71**	**88**	**79**	**68**	**93**	**89**

CONCLUSION

From the above evaluation , it was found that the Cephalexin doped Carbon Nano-particle incorporated gel has proven to have all the properties required to be present in a Novel Topical Dosage Form like optimized size, shape required for better penetration through the skin and also for treating various bacterial infection. Out of the 6 formulations prepared of varying concentration of Carbon, CC5 has proven to show the highest anti-microbial activity and highest level of drug content. For upcoming studies, the CC5 formulation can be submitted for the in-vivo studies.

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REFERENCES

1. Khan I, Saeed K, Khan I. Nanoparticles: Properties, applications and toxicities. *Arab J Chem*. 2019;12(7):908-931. doi:10.1016/j.arabjc.2017.05.011
2. Bhowmik D, Gopinath H, Kumar BP, Duraivel S, Kumar KPS. Recent Advances In Novel Topical Drug Delivery System. *Pharma Innov J*. 2012;1(9):12-31.
3. Debnath SK, Srivastava R. Drug Delivery With Carbon-Based Nanomaterials as Versatile Nanocarriers: Progress and Prospects. *Front Nanotechnol*. 2021;3(April):1-22. doi:10.3389/fnano.2021.644564
4. Habiba ., Din IU, Tasleem S, Mubashir M, Bakht MA. Antimicrobial Activity of Antibiotic Doped Carbon Nanoparticles Extracted from Kitchen Soot against Pathogenic Gram-negative Bacteria. *J Pharm Res Int*. Published online 2020:1-8. doi:10.9734/jpri/2020/v32i3430958
5. Lipsa FD, Ursu EL, Ursu C, Ulea E, Cazacu A. Evaluation of the antifungal activity of gold-chitosan and carbon nanoparticles on fusarium oxysporum. *Agronomy*. 2020;10(8):1-11. doi:10.3390/agronomy10081143
6. Ahmed S, Alhareth K, Mignet N. Advancement in nanogel formulations provides controlled drug release. *Int J Pharm*. 2020;584:119435. doi:10.1016/j.ijpharm.2020.119435
7. Aloysius C, Varghese AA, Ali SP, et al. Antibacterial activity of carbon nanoparticles isolated from chimney soot. *IET Nanobiotechnology*. 2019;13(3):316-319. doi:10.1049/iet-nbt.2018.5183
8. Singh JP, Nandi T, Ghosh SK, Srivastava J, Tripathi SK, Prasad NE. Carbon nanoparticle synthesis, separation, characterization, and tribological property evaluation. *Sep Sci Technol*. 2018;53(14):2314-2326. doi:10.1080/01496395.2018.1446982
9. Mawazi SM, Al-Mahmood SMA, Chatterjee B, Hadi HAB, Doolaanea AA. Carbamazepine gel formulation as a sustained release epilepsy medication for pediatric use. *Pharmaceutics*. 2019;11(10):1-13. doi:10.3390/pharmaceutics11100488
10. Chandra Pandey S. Preparation and Evaluation of Hydrogel Nanoparticles as Drug Delivery System. *J Nanomed Nanotechnol*. 2019;10(01):1-7. doi:10.4172/2157-7439.1000526
11. Elmowafy M, Samy A, Abdelaziz AE, et al. Polymeric nanoparticles based topical gel of poorly soluble drug: Formulation, ex-vivo and in vivo evaluation. *Beni-Suef Univ J Basic Appl Sci*. 2017;6(2):184-191. doi:10.1016/j.bjbas.2017.03.004
12. M. M. Otrokov, I. I. Klimovskikh, F. Calleja AMS, O. Vilkov, A. G. Rybkin, D. Estyunin, S. Mu JHD, A. L. Vázquez de Parga, R. Miranda HO, F. Guinea, J. I. Cerdá, E. V. Chulkov AA. Carbon Nanoparticles decorated with cupric oxide Nanoparticles prepared by laser ablation in liquid as an antibacterial therapeutic agent To. *Mater Res Express Accept*. 2018;(111):0-13.
13. Mujahid M, Ahmad L, Ahmad M. SYNTHESIS OF ZnO NANOGEL FOR THE TREATMENT OF SUPERFICIAL SKIN MICROBIAL INFECTIONS. *J Drug Deliv Ther*. 2017;7(2). doi:10.22270/jddt.v7i2.1391
14. He H, Pham-Huy LA, Dramou P, Xiao D, Zuo P, Pham-Huy C. Carbon nanotubes: Applications in pharmacy and medicine. *Biomed Res Int*. 2013;2013. doi:10.1155/2013/578290
15. PATIL A, KONTAMWAR P. Formulation and Evaluation of Antifungal Nanogel for Topical Drug Delivery System. *Asian J Pharm Clin Res*. 2021;14(10):127-134. doi:10.22159/ajpcr.2021.v14i10.42436
16. Prusty A, Parida P. Development and Evaluation of Gel Incorporated with Biogenically Synthesised Silver Nanoparticles. *J Appl Biopharm Pharmacokinet*. 2015;3(1):1-6. doi:10.14205/2309-4435.2015.03.01.1
17. Dwivedi C, Tripathi A, Pradhan DK, Yadav R. RESEARCH ARTICLE NANOEMULSION SYSTEMS : POTENTIAL APPROACH FOR DRUG DELIVERY Apollo College of Pharmacy , Anjora , Durg , Chhattisgarh 491001 , Royal College of Pharmacy Raipur CG 2. 2014;3(2):11-30.
18. Kim TH, Raiz A, Unni AD, et al. Combating Antibiotic-Resistant Gram-Negative Bacteria Strains with Tetracycline-Conjugated Carbon Nanoparticles. *Adv Biosyst*. 2020;4(9):1-9. doi:10.1002/adbi.202000074
19. Horvat S, Yu Y, Böjte S, et al. Engineering Nanogels for Drug Delivery to Pathogenic Fungi Aspergillus fumigatus by Tuning Polymer Amphiphilicity. *Biomacromolecules*. 2020;21(8):3112-3121. doi:10.1021/acs.biomac.0c00489
20. Patil T V., Patel DK, Dutta SD, Ganguly K, Randhawa A, Lim KT. Carbon nanotubes-based hydrogels for bacterial eradiation and wound-healing applications. *Appl Sci*. 2021;11(20). doi:10.3390/app11209550
21. Martínez-Higuera A, Rodríguez-Beas C, Villalobos-Noriega JMA, et al. Hydrogel with silver nanoparticles synthesized by Mimosa tenuiflora for second-degree burns treatment. *Sci Rep*. 2021;11(1):1-16. doi:10.1038/s41598-021-90763-w

22. Nagpal M, Kaur M. Nanomaterials for skin antifungal therapy: An updated review. *J Appl Pharm Sci*. 2021;11(Supplement 1):15-25. doi:10.7324/JAPS.2021.11s102
23. Zou J, Lv L, Qi X. Design of chitosan-poloxamer nanogel and evaluation of its therapeutic effect on skin burn. *Mater Express*. 2021;11(11):1800-1807. doi:10.1166/mex.2021.2099
24. Sadozai SK, Khan SA, Karim N, et al. Ketoconazole-loaded PLGA nanoparticles and their synergism against Candida albicans when combined with silver nanoparticles. *J Drug Deliv Sci Technol*. 2020;56:101574. doi:10.1016/j.jddst.2020.101574
25. Kavitha R, Dhamodharan N, Dhivya C. Screening, isolation, and antibacterial activity of antibiotic producing bacteria obtained from saprophytic soil samples. *Asian J Pharm Clin Res*. 2017;10(3):92-96. doi:10.22159/ajpcr.2017.v10i3.14472
26. Arafa MG, El-Kased RF, Elmazar MM. Thermoresponsive gels containing gold nanoparticles as smart antibacterial and wound healing agents. *Sci Rep*. 2018;8(1):1-16. doi:10.1038/s41598-018-31895-4
27. KUSHAGRI SINGH[1] AM& AS. Preparation, Characterization and in Vitro Release Profile of Ketoconazole Loaded Chitosan Nanoparticle. *Int J Med Pharm Sci*. 2016;6(5):1-8. http://www.tjprc.org/view-archives.php
28. Prakash K, Satyanarayana VM, Nagiat HT, Fathi AH, Shanta AK, Prameela AR. Formulation development and evaluation of novel oral jellies of carbamazepine using pectin, guar gum, and gellan gum. *Asian J Pharm*. 2014;8(4):241-249. doi:10.4103/0973-8398.143937
29. Endo EH, Makimori RY, Companhoni MVP, Ueda-Nakamura T, Nakamura C V., Dias Filho BP. Ketoconazole-loaded poly-(lactic acid) nanoparticles: Characterization and improvement of antifungal efficacy in vitro against Candida and dermatophytes. *J Mycol Med*. 2020;30(3):101003. doi:10.1016/j.mycmed.2020.101003
30. Maiti D, Tong X, Mou X, Yang K. Carbon-Based Nanomaterials for Biomedical Applications: A Recent Study. *Front Pharmacol*. 2019;9(March):1-16. doi:10.3389/fphar.2018.01401