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RESEARCH ARTICLE
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Preparation and Characterization of Levofloxacin Hemihydrate Laden Spray Dried Nanoparticles through Micro fluidization for Drug Delivery

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ABSTRACT

Aim To prepare and characterize Nanoparticles of Antibacterial Agent for Drug Delivery.

Method Levofloxacin Hemihydrate laden Nanoparticles were prepared using the Ionic gelation method followed by the micro fluidization technique using 400 bar pressure for 5 cycles. The F1 Nanoparticles suspension obtained was subjected to spray drying at a 1400 aspirator rate. The selected F1 Nanoparticles having an entrapment efficiency of 63.46% were characterised by a particle size analyzer, Zeta Analyser, SEM, AFM, FTIR, XRD, EDS, and In-*vitro* Cytotoxicity. The present research work was carried out during the year 2019-2020.

Result The particle size of F1 was found to be 159.0 nm. The F1 Nanoparticles showed a PI of 0.255, exhibiting the Nanoparticles as homogeneously dispersed colloids. The zeta potential of F1 was found to be 48.7 mV. Predicting the stability of nanoparticles SEM and AFM elucidate the surface morphology of F1. FTIR fingerprints show 1793.80 cm1 of carbonyl C=O, 2881.65 cm1 of aromatic C-H and 3535.52 cm1 of the O-H group of the carboxyl group of Levofloxacin. EDS detected oxygen, fluorine, and nitrogen as the elements present in Levofloxacin Hemihydrate and XRD confirmed an amorphous material with a few crystalline phases as diposite. There were no signs of eukaryotic cell disruption, showing non-toxicity and no bio reaction by F1Nanoparticles at 0.01g Inhibitory Concentration ($IC_{50\%}$) when characterised by an *In-Vitro* Cytotoxicity Study.

Conclusion Levofloxacin Hemihydrate laden Nanoparticles were lucratively prepared using Ionic gelation technique followed by the Micro fluidization further successful Characterization of Nanoparticles directs its potential in drug delivery for Treatment of Bacterial Infections.

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INTRODUCTION

Infection is caused by microorganisms invading the epithelial tissues and spreading through the circulating blood. "Macrophages remove these microorganisms from the blood. However, some microorganisms can evade macrophage absorption by escaping from phagosomes, which inhibit phagosome-lysosome fusion, resist lysosomal enzymes, or resist oxidative and non-oxidative cidal mechanisms".[1] The microbial defense mechanism makes it very difficult to remove cellular infections. An antimicrobial agent has the ability to kill or inhibit microbial growth. Despite the fact that antimicrobial development is well underway, intracellular infections such as renal infections and prostatitis inflammation are extremely difficult to treat.[2].Only a few antimicrobial agents can penetrate through cell membranes. Furthermore, antimicrobial toxicity to healthy tissues limits their use significantly. Another significant issue with antimicrobial agents is the acquired resistance of infectious microbes. Nanoparticles have high cellular penetration.

Nanotechnology applications have been studied for several decades.[3]Nanotechnology is concerned with the control and conception of materials in the range of 1–1000 nm.[4] These particles have been observed with distinct physicochemical properties, which include high reactivity, very small size range, ultra-small size, new interactions with biological systems, and a high surface to mass ratio.[5]

"Micro fluidization techniques have recently been used in the development of nanodelivery systems with improved drug stability and bioavailability. New, improved nanodelivery systems have been created. With enhanced stability, micro-fluidization techniques enable compounds to be encapsulated. The

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primary benefit of higher particle stability with smaller particle size is one of the techniques used in large scale fabrication of nanodelivery".[6] These have greater reproducibility and no developed aggregation in nanodelivery systems, as well as high reduced fusibility and larger entrapment efficiency with less use of solvents.[7] Micro fluidization-based nanodelivery systems were developed further to fulfill the objective of targeting the drugs .[8] This can be accomplished effectively with smaller particle size and greater bio accessibility by combining non-toxic, biodegradable, and food-grade biopolymers, which can broaden its use in the nanocojugate creation of antibacterial compounds for treatments for acute diseases like prostatitis.[9]

METHODS

Materials and Reagents

Chitosan, Sodium Alginate, Polyvinyl Chloride, Acetic acid were procured from Abrigo Chemicals, Mumbai. Levofloxacin Hemihydrate (LEV) was obtained from AG Traders Pune, Mumbai. All the reagents and solvent used are of analytical grade. For sample preparation, deionised water was used; the present work was carried out between 2019-2021.

Preparation of Nanoparticles

LEV standard curve was prepared by using 0.1 N NaOH to estimate maximum wavelength of the drug by reading absorbance at 200nm to 400nm.The λ-max was further confirmed by UV spectra of Drug at varied Concentration. Chitosan solution was prepared in acetic acid which was stirred overnight to obtain a single phase solution. The aqueous solution of alginate was prepared, with 0.5 % PVA as stabilizer then this solution is added into calcium chloride solution containing Levofloxacin to form calcium alginate pre-gel. The Chitosan solution was added into the pre-gel and the mixture is allowed to stir for 24hrs. The prepared Colloidal suspension was stored at 4°C± 0.5°C for size reduction.

Micro fluidization of Colloidal Particles

The size reductions of colloidal particles were carried out by passing these colloidal particles through high-speed homogenizer (Panda High-Speed Homogenizer) at 400 bar pressure for 5 cycles. Then it is centrifuged for 1000x for 10 min. The Drug Laden Nanoparticles Solution F1 was obtained and subjected for Spray Drying process.

Spray drying of Colloidal Particles

Spray drying is the method in which feed is converted from fluid state to dried state. Spray drying gives dried particles with desired parameters. F1 processed in spray drying by maintain following parameters as Inlet air temperature (T)130°C, Outlet air temperature (T) 133°C, Feed Inlet ml/min 6, Aspirator rate 1400, Solvent Acetic acid (BP°C 118), water (BP°C 100), Spray Gas flow (mm) 40 , these desired particles F1, were used for characterization.[10]

Entrapment efficiency

Spray dried Nanoparticles F1 was Rehydrated with water in ratio of 1:10 The Percent Entrapment Efficiency (%EE) of F1 was determined by separating the Nanoparticles from solution containing free Levofloxacin by using cold centrifugation (REMI) at 12000 x rpm for 30 minutes. The amount of free Levofloxacin in the supernatant was measured by UV (Elico) at 287 nm.[11]

The Levofloxacin EE was calculated by following equation

$$\text{Drug Entrapment Efficiency}(\%) = \frac{\text{Amount of Drug in suspension}}{\text{Amount of Drug used in formulation}} \times 100 \quad (1)$$

Particle Size and Poly Dispersability Index

The measurement of random changes in intensity of light scattered from F1 Nano suspension was carried out [12] . The distribution of size population within particles is determined by Polydispersability Index (PI).

The F1 Colloidal suspension was further diluted phosphate buffer of pH 7.4 which was placed in electrophoretic cell. Particle size Distribution and Poly dispersability Index was measured at 25°c ± 1°c, in Horiba SZ 100, Dynamic Light Scattering (DLS).

Zeta-potential measurements

The zeta-potentials of F1.was determined for stability purposes at 25°C with Electrode Voltage of 3.3V. Samples were diluted with double distilled water before the measurement[13]. The measurements were carried out with the Horiba SZ100, Zeta Analyzer.

Surface Morphology

The F1 Nanoparticles prepared were subjected to SEM, AFM analysis .In sample preparation of F1 Nanoparticles for Scanning electron microscopy SEM analysis the Nanoparticles were first coated with gold in order to analyze surface morphology. Topography of F1 Nanoparticles was studied by Tapping mode by Bruker Multimode Atomic force Microscopy (AFM).

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR recognizes Functional groups in a molecule by generating an infrared Spectroscopy. From the above prepared Nanoparticles the optimized F1 Nanoparticle was studied for FTIR analysis by ATR using IRAffinity-1S, MIRacle10.

X ray Diffraction (XRD)

The F1 Nanoparticles were also subjected to XRD analysis. "As X rays are produced in a cathode ray tube by heating a filament which generates electrons. X-rays are produced in a cathode ray tube by heating a filament which produces electrons, and accelerates the electrons toward a target by applying a voltage, and bombarding the target material with electrons. When electrons have sufficient energy to dislodge inner shell electrons of the target material, characteristic X-ray spectra are produced".[14]

Energy Dispersive X-ray Spectroscopy (EDS)

Energy Dispersive X-ray Spectroscopy estimate elements of inner core share of Nanoparticles. The Elemental Data Analysis of the F1 Nanoparticles had been performed in order to confirm the encapsulation of drug in the Polymer.[15]

Cytotoxicity Study

The toxic metabolites if produced by drugs by enzymatic reaction leads to toxicity. The cellular organelles including mitochondria responsible for bio activation in toxicity. *Saccharomyces cerevisiae* is used as eukaryotic cell model to study *In-vitro* cytotoxicity [16]. *Saccharomyces cerevisiae* cells were cultivated in Yeast Extract Medium P^H 7 ±0.2 for 27 Hrs at 27 °C. In 0.5 ml of Harvested cell suspension 0.01g as Inhibitory Concentration ($IC_{50\%}$) of Nanoparticles were added. The cells were stained with Crystal violet. The cell divisions were observed at 0 Hrs and 24 Hrs in Motic Optical Microscope. The cells were observed to asses *In -vitro* Cytotoxicity of Nanoparticles.

RESULTS

Preparation of Nanoparticles

The λ max of the Levofloxacin Hemihydrate was found to be 287 nm (fig.1). The Levofloxacin Hemihydrate Laden Chitosan Alginate Nanoparticles were prepared by within cell entrapment Technique. The drug containing suspension was subjected to five cycles of micro fluidization to decrease the size of formed colloidal Particles. The micro fluidized suspension was subjected to spray drying to obtain dried Nanoparticles (F1). These Nanoparticles were used for characterization by various methods.

Entrapment Efficiency

The entrapment efficiency of F1 Was found to be 63.46%which was Optimum drug loading . F1 Nanoparticles were selected for further investigation.

Characterization of F1 Nanoparticles

Particle Size and Poly Dispersability Index

The particle size of F1 Nanoparticles was found to be 159.0 nm (Figure.3). "The values of the Poly dispersability Index (PI) interpreted as PI 0.0 - perfectly uniform sample, PI 1 - highly Polydisperse sample PI ≤ 0.2 - appropriate in exercise for polymer-based Nanoparticle. A PI ≥0.3- to be interpreted as homogenous population of Nano sized Particles" [17]. The F1 Nanoparticles showed PI of 0.255 exhibiting the Nanoparticles as homogeneously dispersed colloids (fig.2).

Zeta-potential measurements

The zeta-potential of F1 was found to be 48.7mv. The F1 Nanoparticles were observed Highly Stable. As Zeta potential of ± 40 to ± 60 predicts Good Stability Behavior of the colloids (fig. 3).

Surface Morphology

The surface nature of F1 was analyzed by SEM at 20000X magnification by using 80 Pa Pressure on 5 µm scale. It was observed that F1 Nanoparticles have level surface with spherical shape. There was no tortuosity on surface of Nanoparticles observed (fig. 4).The Probe sensor Atomic force Microscopy AFM shows F1 Nanoparticles have height Range from -141.8 nm to137.3 nm (fig. 5).

Fourier Transform Infrared Spectroscopy (FTIR)

There are Three characteristics peaks of Levofloxacin at 1793.80 cm−1 of carbonyl C=O, 2881.65 cm−1 of aromatic C-H and 3535.52 cm−1 of O-H group of carboxyl group. The comparison of the spectra of F1 with the spectra of Drug reveals No drug–excipients interaction and entrapment of drug in polymer matrix (fig. 6).

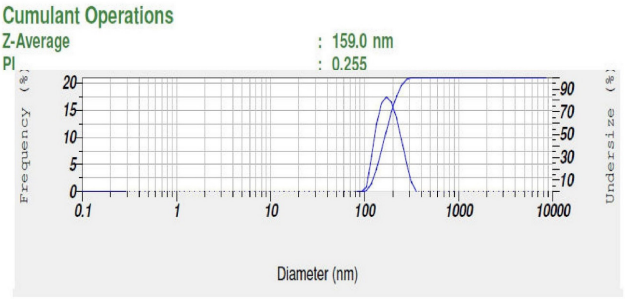

Fig. 2: Particle Size of F1 Nanoparticles.

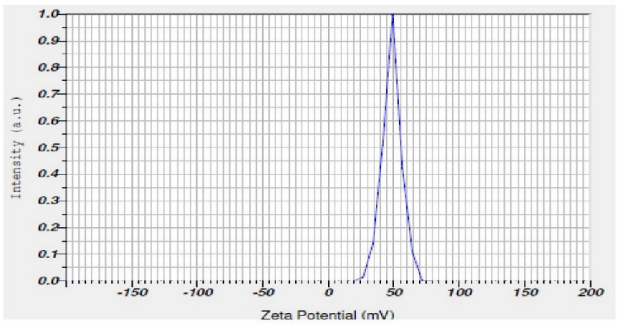

Fig 3: Zeta Potential of F1 Nanoparticles.

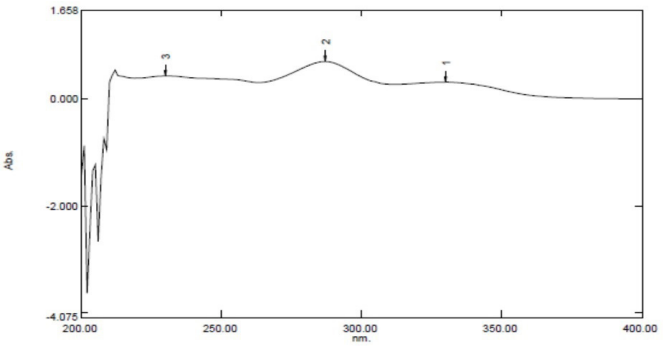

Fig. 1: Maximum wavelength of Levofloxacin.

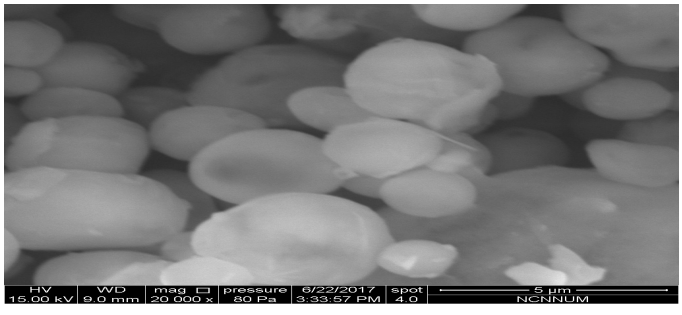

Fig. 4: SEM of F1 Nanoparticles.

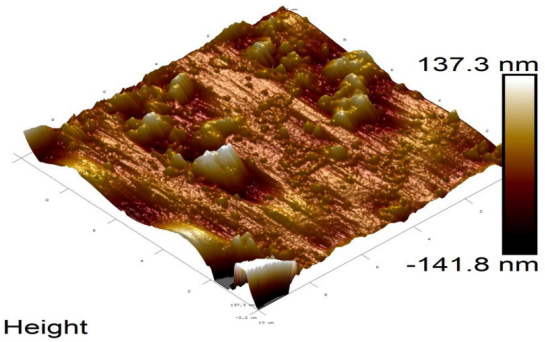

Fig. 5: AFM of F1 Nanoparticles.

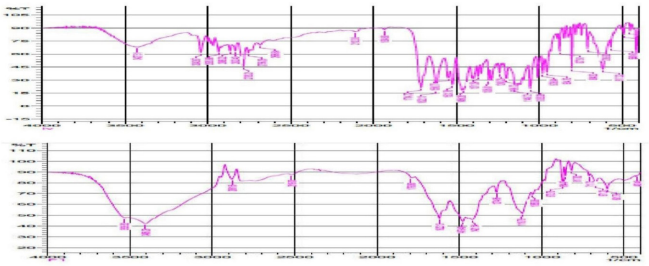

Fig. 6: Overlay FTIR Spectrum of F1 Nanoparticles and Levofloxacin Hemihydrate.

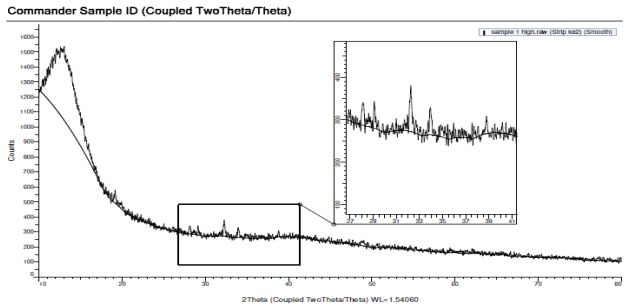

Fig. 7: XRD of F1 Nanoparticles

X ray Diffraction (XRD)

The physical nature of Levofloxacin laden Nanoparticles was determined by using X-ray Diffraction study XRD. The intensities produced against 2 theta degree were analyzed. When the sample F1 was exposed from 0 to 80 2 theta degrees the obtained pattern showed a mostly amorphous material with few crystalline phases as Diposite (Fig.7)

Energy-Dispersive X-ray spectrometry (EDS)

The Elemental Data Analysis of the F1 Nanoparticles had been performed in order to confirm the encapsulation of Levofloxacin Hemihydrate in the Polymer. The elements like Oxygen, Fluorine and Nitrogen are present. Fluorine being the element present in Levofloxacin Hemihydrate it can confirm that the drug had been encapsulated well into the polymer (fig. 8).

Cytotoxicity Study

In-Vitro Cytotoxicity study by using Saccharomyces cerevisiae Eukaryotic cells refined in Yeast Extract Medium PH 7 ±0.2

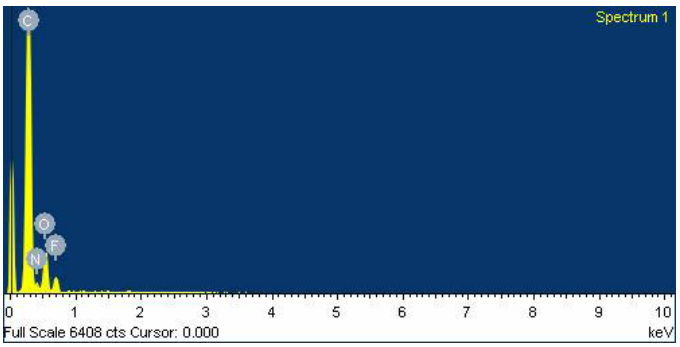

Fig. 8: Different element present in the selected area of F1 Nanoparticles

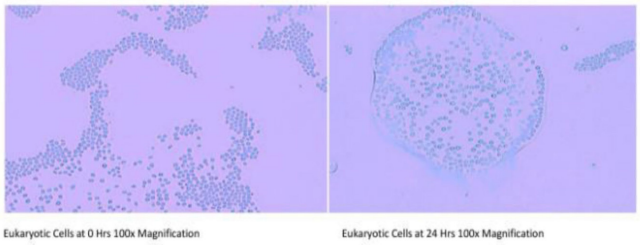

Fig. 9: *In-Vitro* cytotoxicity study of F1 Nanoparticles on Saccharomyces cerevisiae as a Eukaryotic Cell model stained with Crystal violet

for 27 Hrs at 27 °C. On addition of 0.01g as 50% Inhibitory Concentration (IC50) F1 Nanoparticles. The Bioactivation Reactions and cellular disruption were not observed at 0 Hrs and at 24 Hrs .the eukaryotic cells were shown no sign of any toxicity under optical microscopy at 100 x Magnification (Fig 9).

DISCUSSION

The spray dried F1 Nanoparticles were obtained .F1 Nanoparticles with an effective % Entrapment of 63.46% was analyzed by Particle Size Analyzer, Zeta Analyzer, SEM, AFM, FTIR, XRD, EDS, and In-vitro Cytotoxicity. The particle size of F1 was found to be 159.0 nm, F1 Nanoparticles exhibited a PI of 0.255 indicating Nanoparticles as colloids dispersed in the same manner. Zeta power of F1 received 48.7mv. Predicting Nanoparticles Stability.SEM and AFM elucidate the spherical shape and Flat Texture of F1. FTIR fingerprints functional Groups of the Levofloxacin. XRD authenticate amorphous substances with a few crystalline phases such as Diposite. Energy-Dispersive X-ray spectrometry identifies Oxygen, Fluorine and Nitrogen is a component of Levofloxacin Hemihydrate. No cytotoxicity was observed for F1.Levofloxacin Hemihydrate Nanoparticles can be used for treatment of varied inflammations as renal infections, Prostatitis infection.

CONCLUSIONS

Levofloxacin Hemihydrate laden Nanoparticles were successfully prepared using Ionic gelation technique followed by the Micro fluidization .The Nano drug delivery gained importance in therapeutics because of high penetration across cellular linings and very small size.

ABBREVIATIONS

LEV: Levofloxacin Hemihydrate; %EE: Percent Entrapment Efficiency; PI: Poly dispersability Index; SEM: Scanning Electron

Microscopy, AFM: Atomic Force Microscopy, FTIR: Fourier Transform Infrared Spectroscopy; XRD: X ray Diffraction; EDS: Energy-Dispersive X-ray spectrometry.

Author Contributions

Vaibhav K. designed the study, collected, analyzed the data and Prepared the initial manuscript; Lalit S. collected the data; Tukaram K , and Rajeshwar S. edited the initial version of the manuscript. All authors approved the final version of the manuscript.

Data Availability Statement

Data are not publicly available on legal or ethical grounds, but after this paper have published, these data can use as a reference.

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REFERENCES

1. Yamamoto T. Bacterial strategies for escaping the bactericidal mechanisms by macrophage. Yakugaku Zasshi. 2006.
2. Gokce HI, Ross G, Woldehiwet Z. Inhibition of phagosome-lysosome fusion in ovine polymorphonuclear leucocytes by Ehrlichia (Cytoecetes) phagocytophila. J Comp Pathol. 1999;
3. Zhang L, Pornpattananangkul D, Hu C-M, Huang C-M. Development of Nanoparticles for Antimicrobial Drug Delivery. Curr Med Chem [Internet]. 2010;17(6):585–94. Available from:http://www.eurekaselect.com/openurl/content.php?genre=article&issn=0929-8673&volume=17&issue=6&spage=585
4. Zhang L, Gu FX, Chan JM, Wang AZ, Langer RS, Farokhzad OC. Nanoparticles in medicine: Therapeutic applications and developments. Clin Pharmacol Ther. 2008;83(5):761–9.
5. Khanafer KM, Vafai K. The role of nanoparticle suspensions in thermo/fluid and biomedical applications. In: Nanoparticle Heat Transfer and Fluid Flow. 2016.
6. Ganesan P, Karthivashan G, Park SY, Kim J, Choi DK. Microfluidization trends in the development of nanodelivery systems and applications in chronic disease treatments. Int J Nanomedicine. 2018;13:6109–21.
7. Jaradat E, Weaver E, Meziane A, Lamprou DA. Microfluidics technology for the design and formulation of nanomedicines. Nanomaterials. 2021;11(12).
8. Tomeh MA, Zhao X. Recent Advances in Microfluidics for the Preparation of Drug and Gene Delivery Systems. Mol Pharm. 2020;17(12):4421–34.
9. Magri V, Boltri M, Cai T, Colombo R, Cuzzocrea S, De Visschere P, et al. Multidisciplinary approach to prostatitis. Arch Ital di Urol e Androl. 2018;
10. Mahdavi SA, Jafari SM, Ghorbani M, Assadpoor E. Spray-Drying Microencapsulation of Anthocyanins by Natural Biopolymers: A Review. Drying Technology. 2014.
11. López-López M, Fernández-Delgado A, Moyá ML, Blanco-Arévalo D, Carrera C, de la Haba RR, et al. Optimized preparation of levofloxacin loaded polymeric nanoparticles. Pharmaceutics. 2019;11(2):1–13.
12. Mezni A, Alghool S, Sellami B, Ben Saber N, Altalhi T. Titanium dioxide nanoparticles: synthesis, characterisations and aquatic ecotoxicity effects. Chem Ecol. 2018;
13. Zhao K, Chen G, Shi X ming, Gao T ting, Li W, Zhao Y, et al. Preparation and Efficacy of a Live Newcastle Disease Virus Vaccine Encapsulated in Chitosan Nanoparticles. PLoS One. 2012;7(12):1–11.
14. Harlow RL. Single-Crystal X-Ray Diffraction. In: Materials Characterization. 2018.
15. Hodoroaba VD. Energy-dispersive X-ray spectroscopy (EDS). In: Characterization of Nanoparticles: Measurement Processes for Nanoparticles. 2019.
16. Canoy JL, Bitacura JG. Cytotoxicity and antiangiogenic activity of turbinaria ornata agardh and padina australis hauck ethanolic extracts. Anal Cell Pathol. 2018;
17. Shashi K, Satinder K, Bharat P. a Complete Review on: Liposomes. Int Res J Pharm. 2012;3(7):10–6.