A REVIEW ON NOVEL ANTIFUNGAL THERAPIES BY THE METHOD OF PREPARATIONS OF NANOBIOCOMPOSITES

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ABSTRACT

The growing threat of fungal resistance to conventional antifungal drugs necessitates the exploration of novel antifungal therapies. Antifungal nanobiocomposites, constructed by integrating nanoparticles with biocompatible polymers, have emerged as a promising strategy in this fight. This article delves into the design and development of these innovative materials. In this articlethe discussion on the various types of nanobiocomposite systems employed for antifungal applications, including polymer-based, ceramic-based, and carbon-based nanobiocomposites. The article explores the different classifications of antifungal drugs and their potential for incorporation into these nanobiocomposites. Current article elaborate on the methods used for preparing nanobiocomposites, encompassing both nanoparticle synthesis and integration with the polymeric matrix. Furthermore, we exporeThis article examines the methods used to characterize the resulting nanobiocomposites' chemical, biological, and physical characteristics.Finally, this address the challenges and future directions of research in this field, highlighting the potential of antifungal nanobiocomposites to revolutionize antifungal therapy.

KEYWORDS: Nanobiocomposites, Antifungal, Biopolymer, Nanotechnology, Microscopy

INTRODUCTION

Fungal infections come in many different forms, ranging from skin-related superficial infections to systemic infections that invade interior organs.Approximately 1.5 million of them require hospitalization and intensive care due to invasive fungal infections. Most of these common diseases are caused by many species of Candida, Cryptococcus, Aspergillus, and Pneumocystis; they are also the cause of aspergilosis, cryptococcosis.In that order. Although seldom fatal, superficial fungal infections are rather prevalent and have the potential to spread to other areas of the skin and possibly become widespread.Moreover, they have the potential to spread to other individuals and result in further bacterial skin infections, which can negatively impact an individual's quality of life. Skin mycoses are separated into three categories based on the fungi that cause them: mould, yeast, and dermatophytosis. Since invasive fungal infections have high rates of morbidity and mortality, they pose a serious threat to healthcare systems. These rates pertain specifically to immunocompromised patients, who are at a higher risk of contracting opportunistic infections. These include those living with HIV/AIDS, organ transplant recipients whose immune systems are weakened to avoid organ rejection, cancer patients receiving immunosuppressive chemotherapy, and patients with autoimmune diseases receiving immunosuppressive therapy[1].

The four main classes of medications now on the market for the treatment of invasive fungal infections are polyenes, azoles, allylamines, and echinocandins, based on their method of action. They are all troublesome in terms of their range of activity, drug-drug interactions, pharmacokinetics and pharmacodynamics, resistance mechanisms, and individual chemical toxicity. Furthermore, there are limitations regarding the clinical effectiveness and efficiency, mostly due to their physical-chemical characteristics, such as their hydrophobic nature whichresults in poor water solubility and selection issues[2].

With nanoscale systems, biodegradable polymers have a great deal of promise for drug delivery. In contrast, natural polymers are highly desirable for pharmaceutical use because of their minimal toxicity, compatibility with living organisms, degradability, and modifiability, allowing them to be customized to meet the specific requirements of the pharmaceutical industry. Many polysaccharide polymers, such as many types of gums.[3]

Conversely, biocomposite materials are made up of a minimum of two parts: a continuous reinforcement material that serves as the biopolymer's backbone and a discontinuous matrix phase that is typically a natural polymer. Commonly employed polymers include cellulose and chitosan, which have counter-ionic characteristics, and tripolyphosphate (TPP), which is used as a crosslinker. Physical crosslinking techniques, including as hydrophobic contacts, π–π stacking interactions, and electrostatic/ionic interactions, can be used for reinforcement. Chemical crosslinking techniques,For instance, tripolyphosphate crosslinking is used to generate chitosan nanoparticles, and coupling chemistry is used to create covalent crosslinks. Because of this, the NBCs' dimensions and characteristics make them a great option for drug delivery. All jet flow techniques are utilized form Micro to nano Jet tubes[4].

of their high surface-to-volume ratio, nanocomposites have extremely high matrix-reinforcement because interaction.The enhanced properties of composites are influenced by the traits of each component, their relative proportions, and the overall structure of the nanocomposites. Enhanced features such as NPs size and surface charge, mucoadhesiveness, and adsorptiveness all play a role in this etc., can be obtained when natural polymers are used in the creation of NBCs. These qualities can be tailored to work well for a variety of applications in non-traditional delivery methods, such as buccal, nasal, or rectal.

NANOBIOCOMPOSITES

By combining nanofillers with biopolymers, nano-biocomposites are created, which are very promising materials with enhanced characteristics while maintaining the material's biodegradabilityand lack of environmental toxicity[5].

Nanocomposites

Nanocomposites (NCs) are materials formed by incorporating nanoscale additions into one of the phases.It is anticipated that the combination of all the components would result in their displaying unexpected traits. Based on their matrix, there are three dissimilar types of nanocomposites: metal matrix nanocomposites (MMNC), polymer matrix nanocomposites (PMNC), and ceramic matrix nanocomposites (CMNC). The exceptional thermal, mechanical, fire-retardant, and solvent-resistant qualities of NC have drawn attention recently when compared to pure or conventional composite materials. It is widely acknowledged that the distribution and size of the particles, surface properties, geometric form and dispersion state can all significantly affect a composite's characteristics.The availability of nanoparticles has led to the increasing popularity of polymer nanocomposites. These materials offer enhanced mechanical properties such as modulus and strength, as well as improved permeability to water, gases, and hydrocarbons. They also demonstrate superior dimensional and thermal stability, chemical resistance, flame retardancy, and optical, dielectric, magnetic, and electrical properties.

Nanocomposites Classification

Nanocomposite materials, much like micro composites, can be categorized into three groups depending on the materials of their matrix: MMNC, PMNC, and CMNC.

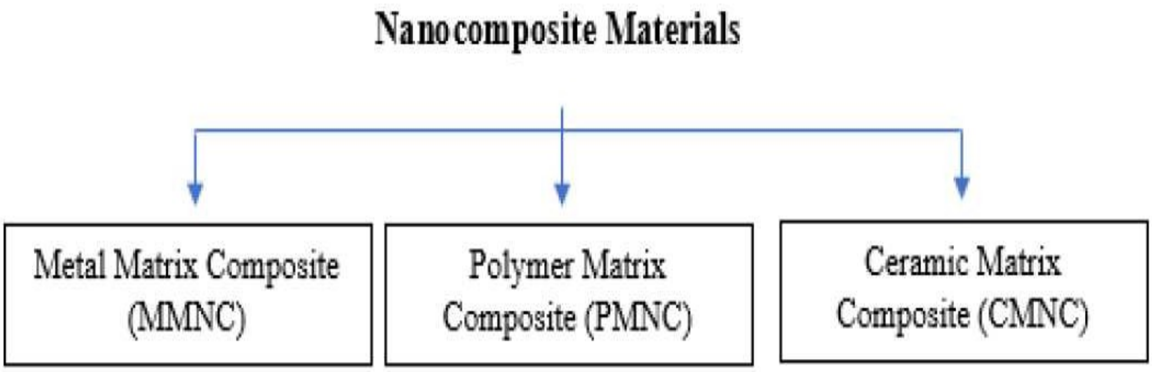

Figure 1: Diagram of NPs Matrix types

1. Metal Matrix Nanocomposites MMNC

LikeMetal matrix composites (MMCs), similar to other composites, are made up of at least two phases that are physically and chemically distinct and dispersed to impart unique properties that cannot be provided by either of the individual phases.A metallic matrix typically consists of two phases: a fibrous or granular phase. Some examples of composite materials in power transmission lines include an aluminum matrix strengthened with continuous A12O3 fibers, cutting tools made of cobalt (Co) particles.These nano composite MMNC are composed of nanoscale reinforcing material mixed with a ductile alloy or metal matrix.The high modulus and strength of these materials, combined with characteristics of both metal and ceramic, such as ductility and toughness, make them ideal for producing materials with high shear/compression strengths and high service temperatures. They have a great deal of promise for use in many other fields, such as the manufacturing of structural materials and the automotive and aerospace sectors[7].

2. NPS of Polymer Matrix Types-

The most widely used type of nanocomposite is PMNC, comprising of dispersed, evenly sized nanoparticles distributed within a polymer under ideal circumstances. In actuality, the polymer matrix is used to disperse agglomerated nanoparticles. Enhanced physical characteristics of functional nanocomposites present new potential in energy conversion, storage, micro-optics, and electronicsThe filler load often correlates with the desired change in characteristics. The composite flow characteristics limit large solid loading due to shape or molding constraints, which results in a modification of properties. To determine the right shear rate and temperature prior to the composites' forming process, the rheological properties, also known as flow properties, should be assessed using a variety of experiments[8].Their flow qualities are determined by the area of their surface and the huge polymer-filler interface layer that they form. Polymer nanocomposites have created new opportunities for a promising class of mixed materials by combining chemically bound nanoparticle fillers to enhance the characteristics of pure polymers.Over the past ten years, polymer nanocomposites have attracted curiosity and interest from all over the world. The sol-gel method appears to hold the most promise among all the methods utilized to create polymer nanocomposites. Following their dispersion at the molecular or adjacent molecular level, the nanoparticles were combined with the polymer gel[9].

3. NPS of Ceramic type-

This type is utilise system of Silicon carbile and al oxide. High strength Nanofibers are used sol-gel process, colloidal and precipitation procedures—are the most often utilized ways for creating micro composites. The structure and chemical characteristics of the final material can be controlled by a wide variety of parameters that can influence the many procedures and precursors like time, type and many more things[11].

Composite Nanoparticles

This idea enables the creation of the newest functional materials with unique biological, optical, magnetic, and electrical capabilities. Core/shell nanoparticles have recently attracted attention as a potential anode material for lithium-ion batteries. Carbon in the shape of graphite, amorphous carbon, or graphene makes up the majority of the organic molecule[13].

Current antifungal drugs

The development of antifungal medications that specifically target fungus and do not adversely affect mammalian cells is hampered by the similarity in cellular mechanism between fungi and mammalian cells. Antifungal agents are few in number compared to antibiotics, and many of them are harmful to eukaryotic host cells. Since bacteria and fungi differ significantly in their structures and constituent parts, concepts and methods that have proven successful in the development of antibacterial medications have not translated to the development of antifungal drugs[14]. Currently, over 80 distinct types of antifungal drugs are being used in clinical settings. They can be classified into five classes. The antifungal activities of these drugs can be further divided into four common targeted methods. Ergosterol is the first regular target of antifungal medications[15]. Because ergosterol is essential to the plasma membrane's biosynthesis and function, fungi may die if their synthesis is inhibited, bound to, or damaged.Moreover, the cell envelope (membrane- and cell wall-active medicines are effective here); preventing the synthesis of proteins, chitin, nucleic acids, and mannan; and other pathways such as producing reactive oxygen species (ROS) or lowering ATP are typical targets for antifungal treatments. Recent reports have identified sulphur metabolism as a possible new target for antifungals since sulfur-related processes are essential to the physiology of some fungal species, including Aspergillus fumigatus[16].

Table 1 Antifungal mechanisms of current antifungal drugs.

Category of antifungal	Antifungal mechanism	Representive

Mechanism		drugs
Targeting ergosterol[17]	Perturbing membrane function through binding to ergosterol.	Amphotericin B
	Inhibiting ergosterol biosynthesis.	Imidazole
Targeting the cell envelope[18]	Disrupting membrane via pore formation	Antimicrobial Peptide
Targeting biosynthesis[19]	Inhibits DNA synthesis	Flucytosine
	Interfere with microtubule assembly	Griseofulvin.

Preparation of nanobiocomposite

Typically, 100 millilitres of Millipore water were used to dissolve one gramme of gelatin and one gramme of glycogen separately. After the solutions had completely dissolved, 100 mL of each was combined and stirred continuously for three hours at 1500 rpm while the stirring temperature was lowered to 70 C in 500 mL round flasks. After stirring the mixture for the predetermined length of time, it was approved to cool to room temperature. A 1 mg/mL antifungal drug was dissolved in Millipore water while it was being stirred magnetically[20]. After that, 10 mL of the dissolved drug solution was gradually extra to the glycogen-gelatin admixture solution and vigorously agitated for three hours at 40 °C and 1500 rpm. The resultant drug-loaded nanobiocomposite was lyophilized at 80 °C to preserve it for later evaluation[21].

ANTIFUNGAL NANOCOMPOSITES

Materials that include biocides or antimicrobials at the nanoscale to enhance their antimicrobial activity are known as antibacterial nanocompositesNanocomposites have a wide range of uses since they may be created using a variety of materials and biocides. The structural stability and constant thermal stability of the nanocomposite are sometimes enhanced by the adding of these nanocompounds[22].

Ag+ Based Antifungal Nanocomposites

Since antibiotic resistance can be prevented, antimicrobial metal nanoparticles (NPs) are being employed extranormally as an alternative to antimicrobial medications. They are connected to toxicity, though. One method of enhancing biocompatibility is to add them to biomaterials, like sugars, peptides, and proteins. Ag-Au alloy nanoparticles (Ag-AuNPs) among potential wide-ranging applications, such as coating materials for medical devices or medication administration, were produced using a green synthesis technique. The Ag content of these NPs determined their size; higher Ag led to larger NPs. Because of the silver ions, these nanocomposites exhibited strong antifungal movement against Candida albicans. by

MICs as low as 8 mg/ml, the Ag-Au alloy nanoparticles, however, demonstrated better antifungal activity than the silver nanoparticles. The Ag-Au alloy nanoparticles completely suppressed also cleared the growth and biofilm formation of fungus at concentrations greater than 16 mg/ml in a dose-dependent manner. Even at doses as high as 128 mg/ml, these nanocomposites did not significantly harm RAW264.7, Hela, or LO2 cells in MTT tests[23].

Even yet, it's unknown what antifungal mechanism(s) AgNPs have. Ag-Chit-NCs, or chitosan-silver nanocomposites, were created as a defence against P. expansum, a fungal disease that is frequently linked to animal infections, including dairy cattle, as well as the spoiling and storage of agricultural crops. 5.9 weight percent of silver was used in the development of the nanocomposite[24]. 72% of the nanoparticles were integrated and addicted to the chitosan matrix, according to a TEM investigation.

Antifungal Nanocomposites with Smooth Surfaces for Reduced Microbial Attachment

Increasing the surface roughness of the material can also have an antibacterial effect by reducing microbial growth sites. Some added nanodiamonds (ND) to the resin's surface to smooth it out and give PMMA antifungal properties for use as a dental filler. This technique bears similarities to that of adding biocompatible NDs to PMMA at varying weight concentrations (0, 0.5, 1, 1.5% by weight) in order to track changes in C. albicans adhesion[26]. Improved surface roughness decreases C. albicans adhesion, according to the results. Surface roughness was measured with a profilometer, and contact angle was measured with a goniometer. The material's surface exhibited fewer peaks and valleys and was smoother when NDs were added to PMMA. This resulted in a decrease in Candida albicans cell attachment to the PMMA surface relative to the control group, with the lowest adherence observed at 1% NDs. This was appropriate given the loss of settling sites.

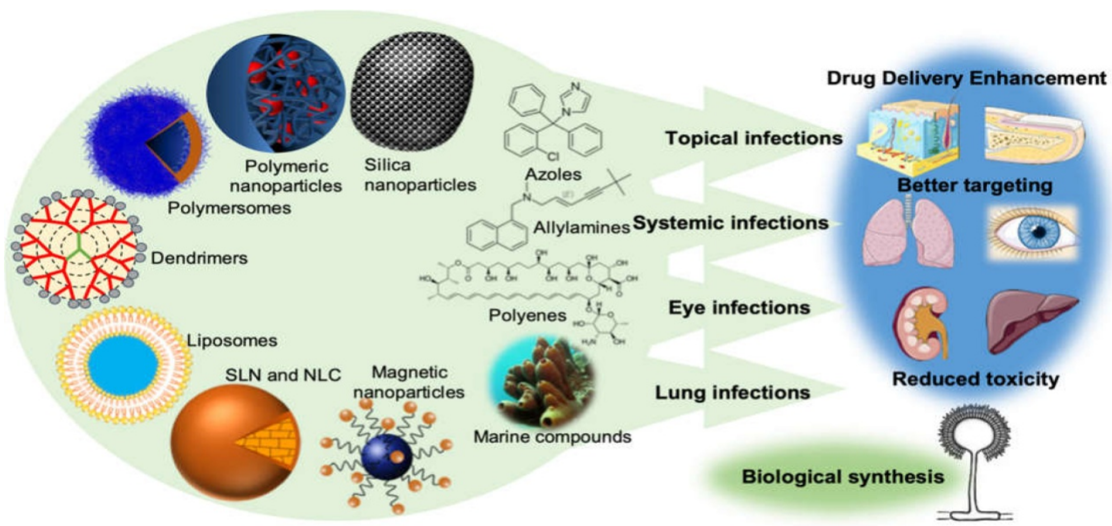

Figure 2. **Recent advancements in drug delivery systems utilizing nanotechnology aim to improve the effectiveness of drug administration, enhance targeting precision, and minimize the adverse effects associated with traditional antifungal medications.**

Characterization of Nanocomposites Structure

Optical microscopy

When characterizing the macroscopic composite structure, OM can be useful when the matrix is visible or the sample is very thin. However, it is not an appropriate tool for characterising nanosize components, such as CN, inside nanocomposites. For instance, the crystallisation behaviour of CN composites can be studied using polarised OM. The impact of CNC adds on the PLA polymer's crystallisation nucleation rate. It was found that the unmodified CNCs aggregated and were not as effective nucleation agents as the silylated CNCs after evaluating both silylated functionalized and unmodified CNCs. the polarized OM images demonstrating how the crystallites nucleate while cooling in the pure PLA, PLA with 1% CNC, and PLA with 1% silylated crystal melts at 210°C melts after0,5, and 10 minutes. Comparing the PLA with silylated crystals (0 min) to the PLA with unaltered CNC, it is evident that the former has some aggregates[27].

Electron microscopy

Scanning electron microscopy

SEM is one of the most widely used forms of microscopy to analyze the structure and surface of materials. These microscopes have a resolution of 1 to 5 nm. In addition to their great resolution, the photos' large depth of focus gives the impression that they are three-dimensional. SEM works on the basis of an electron cannon that produces and accelerates electrons via lenses to focus a beam of light with an extremely small spot size. To a depth of around 1 µ, these electrons interact with the object to produce signals that are used to create the image. X-rays, secondary electrons, and backscattered electrons are the three main signals. Depending on the specimen's atomic number, the elastically scattered back scattered electrons provide compositional contrast. These electrons originate from the specimen's depth (1 µm or more), and they have a high energy. Secondary electrons are low energy electrons that originate from the specimen's top surface and are mostly utilised for topographical imaging of the material (a few nm)[28].

Transmission electron microscopy

The fundamental idea of the TEM is that high energy electrons are transported through an extremely narrow region of the material. The image is created by electron scattering when the beam hits the specimen.The electrons originate from an electron cannon, A filament generates an electron beam, which is then demagnified and controlled by one or more condenser lenses positioned below the gun, adjusting the beam's diameter as it reaches the specimen encased within an objective lens located just beneath the condenser lenses. Following this, the projector and intermediate lenses further magnify the image, which is subsequently projected onto a fluorescent screen or film. The contrast in Transmission Electron Microscopy (TEM) images results from electron scattering. In bright field (BF) imaging mode, an objective aperture allows only electrons that pass through unaffected to form the image. Denser or thicker areas of the specimen scatter electrons more and thus appear darker because the aperture blocks the scattered electrons. Consequently, regions of the sample without any material will appear bright in BF imaging.

Wide-Angle X-ray Diffraction (WAXD) is employed to assess the alignment of cellulose nanocrystals (CNCs) within composites by measuring variations in X-ray diffraction intensities related to specific planes within the cellulose crystal structure according to the sample's orientation. WAXD analyzes how diffracted X-rays from a sample vary with respect to the diffraction angle relative to the primary X-ray beam direction, using monochromatic X-rays with a wavelength of 0.1541 nm generated under specific voltage and current settings. X-ray diffraction occurs only under particular conditions, depending on the solid's crystalline structure. For cellulose I, diffraction peaks are observed at angles of 28°, 14.5°, 16.6°, 20.4°, 22.7°, and 34.4°, corresponding to the Miller indices for the crystallographic planes (110), (102), (200), (004), (100), (010), and (110), respectively.

CONCLUSION

In this article, This article explored the exciting field of antifungal nanobiocomposite design and development. In this article discussion on various types of nanocomposites employed for this purpose, including polymeric, ceramic, and metallic composites. This also reviewed the different classifications of antifungal drugs that can be incorporated into these nanobiocomposites. The article then delved into the preparation methods for these nanobiocomposites, highlighting techniques like solvent casting, sonication, and electrospinning. Finally, this article addressed for the crucial characterization techniques used to evaluate the properties and efficacy of the resulting nanobiocomposites. These characterization methods ensure the successful development of novel antifungal nanobiocomposite materials with tailored properties for combating fungal infections.To improve the antifungal activity, biocompatibility, and targeted distribution of these nanobiocomposites, future research in this field should concentrate on optimizing their design and development.By addressing these challenges, antifungal nanobiocomposites hold immense potential for revolutionizing the treatment of fungal diseases.

REFERENCES

1) Aguilar-Sánchez, R., Munguía-Pérez, R., Reyes-Jurado, F., Navarro-Cruz, A. R., Cid-Pérez, T. S., Hernández-Carranza, P., et al. (2019). Structural, Physical, and Antifungal Characterization of Starch Edible Films Added with Nanocomposites and Mexican Oregano (LippiaBerlandieri Schauer) Essential Oil. Molecules 24 (12), 2340. doi:10.3390/molecules24122340

2) Alghuthaymi, M. A., Abd-Elsalam, K. A., Shami, A., Said-Galive, E., Shtykova, E. V., and Naumkin, A. V. (2020). Silver/Chitosan Nanocomposites: Preparation and Characterization and Their Fungicidal Activity against Dairy Cattle Toxicosis Penicillium expansum. JoF 6 (2), 51. doi:10.3390/jof6020051

3) Almeida, L. d. F. D. d., Paula, J. F. d., Almeida, R. V. D. d., Williams, D. W., Hebling, J., and Cavalcanti, Y. W. (2016). Efficacy of Citronella and Cinnamon Essential Oils on Candida Albicans Biofilms. Acta Odontologica Scand. 74 (5), 393–398. doi:10.3109/00016357.2016.1166261.

4) Ammar, D. A., Noecker, R. J., and Kahook, M. Y. (2010). Effects of Benzalkonium Chloride-Preserved, Polyquad-Preserved, and sofZia-Preserved Topical Glaucoma Medications on Human Ocular Epithelial Cells. Adv. Ther. 27 (11), 837–845. doi:10.1007/s12325-010-0070-1

5) Anitha, A., Sowmya, S., Kumar, P. T. S., Deepthi, S., Chennazhi, K. P., Ehrlich, H., et al. (2014). Chitin and Chitosan in Selected Biomedical Applications. Prog. Polym. Sci. 39 (9), 1644–1667. doi:10.1016/ j.progpolymsci.2014.02.008

6) Azevedo, M. M., Ramalho, P., Silva, A. P., Teixeira-Santos, R., Pina-Vaz, C., and Rodrigues, A. G. (2014). Polyethyleneimine and Polyethyleneimine-Based Nanoparticles: Novel Bacterial and Yeast Biofilm Inhibitors. J. Med. Microbiol. 63 (9), 1167–1173. doi:10.1099/jmm.0.069609-0.

7) Baig, N., Kammakakam, I., Falath, W., Kammakakam, I., 2021. Nanomaterials: A review of synthesis methods, properties, recent progress, and challenges. Mater. Adv. 2, 1821–1871. https://doi.org/10.1039/d0ma00807a.

8) Beg, S., Rahman, M., Jain, A., Saini, S., Midoux, P., Pichon, C., Ahmad, F.J., Akhter, S., 2017. Nanoporous metal organic frameworks as hybrid polymer–metal composites for drug delivery and biomedical applications. Drug Discov. Today 22, 625–637. https://doi.org/10.1016/j.drudis.2016.10.001.

9) Bharathiraja, S., Bui, N.Q., Manivasagan, P., Moorthy, M.S., Mondal, S., Seo, H., Phuoc, N.T., Vy Phan, T.T., Kim, H., Lee, K.D., Oh, J., 2018. Multimodal tumor-homing chitosan oligosaccharide-coated biocompatible palladium nanoparticles for photo-based imaging and therapy. Sci. Rep. 8. https://doi.org/10.1038/s41598- 017-18966-8.

10) Bhatt, P., Lalani, R., Vhora, I., Patil, S., Amrutiya, J., Misra, A., Mashru, R., 2018. Liposomes encapsulating native and cyclodextrin enclosed paclitaxel: Enhanced loading efficiency and its pharmacokinetic evaluation. Int. J. Pharm. 536, 95– 107. https://doi.org/10.1016/J.IJPHARM.2017.11.048.

11) V. V. G. Schmid, H. Krug, R. Waser, Nanotechnology: volume 4: information technology II. John Wiley & Sons, 2008.

12) A. Nel et al., "Nanotechnology environmental, health, and safety issues," in Nanotechnology Research Directions for Societal Needs in 2020, Springer, 2011, pp. 159–220.

13) J. F. Mongillo, "Nanotechnology 101 Greenwood Press," Westport, CT, pp. 4–13, 2007.

14) I. Manas-Zloczower, Mixing and compounding of polymers: theory and practice. Carl Hanser Verlag GmbH Co KG, 2012.

15) T. V Kosmidou, A. S. Vatalis, C. G. Delides, E. Logakis, P. Pissis, and G. C. Papanicolaou, "Structural, mechanical and electrical characterization of epoxy-amine/carbon black nanocomposites," Express Polym. Lett., vol. 2, no. 5, pp. 364–372, 2008.

16) W. Gacitua, A. Ballerini, and J. Zhang, “Polymer nanocomposites: synthetic and natural fillers a review,” Maderas. Cienc. y Tecnol., vol. 7, no. 3, pp. 159–178, 2005.

17) F. Wypych and K. G. Satyanarayana, “Functionalization of single layers and nanofibers: a new strategy to produce polymer nanocomposites with optimized properties,” J. Colloid Interface Sci., vol. 285, no. 2, pp. 532– 543, 2005.

18) Shahzadi, I.; Masood, M.I.; Chowdhary, F.; Anjum, A.A.; Nawaz, M.A.; Maqsood, I.; Zaman, M.Q. Microemulsion Formulation for Topical Delivery of Miconazole Nitrate. Int. J. Pharm. Sci. Rev. Res. 2014, 24, 30–36.

19) Elmoslemany, R.M.; Abdallah, O.Y.; El-Khordagui, L.K.; Khalafallah, N.M. Propylene glycol liposomes as a topical delivery system for miconazole nitrate: Comparison with conventional liposomes. AAPS Pharmscitech 2012, 13, 723–731. [CrossRef]

20) Maha, H.L.; Sinaga, K.R.; Masfria, M. Formulation and evaluation of miconazole nitrate nanoemulsion and cream. Asian J. Pharm. Clin. Res. 2018, 11, 319–321. [CrossRef]

21)Kumar, P.S.; Hematheerthani, N.; Ratna, J.V.; Saikishore, V. Design and characterization of miconazole nitrate loaded nanosponges containing vaginal gels. Int. J. Pharm. Anal. Res. 2016, 5, 410–417.

22) Qushawy, M.; Nasr, A.; Abd-Alhaseeb, M.; Swidan, S. Design, Optimization and Characterization of a Transfersomal Gel Using Miconazole Nitrate for the Treatment of Candida Skin Infections. Pharmaceutics 2018, 10, 26. [CrossRef]

23) Ge, S.; Lin, Y.; Lu, H.; Li, Q.; He, J.; Chen, B.; Wu, C.; Xu, Y. Percutaneous delivery of econazole using microemulsion as vehicle: Formulation, evaluation and vesicle-skin interaction. Int. J. Pharm. 2014, 465, 120–131. [CrossRef] [PubMed]

24) J.H.M. Willison and A. J. Rowe. Replica, shadowing and freeze-etching techniques. In Practical methods in electron microscopy, M. Audrey Glauert (Ed.) Elsevier/NorthHolland: Biomedical Press (1980).

25) A. Pei, Q. Zhou and L.A. Berglund, Functionalized cellulose nanocrystals as biobased nucleation agents in poly (L-lactide) (PLLA) – crystallization and mechanical property effects, Compos. Sci. Tech. 70 (2010) 815-821.

26) I. Kvien and K. Oksman, Orientation of cellulose nanowhiskers in polyvinyl alcohol, Appl. Phys. A. 87 (2007) 641-643.

27) J. Goldstein, D. Newbury, D. Joy, D. Lyman, P. Echlin, E. Lifshin, L. Sawyer and J. Michael, Scanning electron microscopy and X-ray microanalysis, 3rd edn. New York: Kluwer Academic/Plenum Publishers (2003).

28) P.J. Goodhew, J. Humphreys and R. Beanland, Electron microscopy and analysis, London: Taylor & Francis (2001).

29) P.C. Braga and D. Ricci, Atomic force microscopy: biomedical methods and applications, Springer 242 (2004).

30) P. Eaton and P. West, Atomic force microscopy, Cary, NC, USA: Oxford University Press, (2010).

31) S. Bandyopadhyay, S.K. Samudrala, A.K. Bhowmick and S.K. Gupta. Applications of atomic force microscope (AFM) in the field of nanomaterials and nanocomposites. In Functional nanostructures: processing, characterization, and applications, S. Seal, (Ed,) Springer, 2007. pp. 504-568. 32. E.E. Brown and M-P.G. Laborie, Bioengineering bacterial cellulose/poly(ethylene oxide) nanocomposites, Biomacromol. 8 (2007) 3074-3081.