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Original Article

Antifungal activity of nanostructured polyaniline combined with fluconazole

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ABSTRACT

Conductive polymers are gaining much interest in field of pharmacy and biomedicine. Polyaniline (PANi) is one of such conducting polymer belonging to semi-flexible rod polymer family. There are some potential applications of PANi in biosensors, biomedical applications, drug delivery and tissue engineering because of its excellent stability, high conductivity and easy mode of synthesis. Nanofibres of polyaniline combined with fluconazole are prepared by simple and cost effective sol-gel method using D-10-camphorsulfonic acid (D-CSA) as a dopant and as a surfactant, and ammonium persulfate as the oxidant. The synthesized nanostructured material was dissolved in dimethylsulfoxide at different concentrations and tested for its antifungal properties against *Candida albicans* (ATCC 140503), *Candida tropicalis* (ATCC 13803) and *Candida krusei* (ATCC 34135). The results showed that, compared to nanofiber structured conducting PANi, polyaniline doped with fluconazole have shown higher antifungal activity on all the species tested. It is very much evident that PANi doped fluconazole has considerable enhanced antifungal activity. *C. tropicalis* (ATCC 13803) is more susceptible than *C. albicans* (ATCC 140503) and *C. krusei* (ATCC 34135). Structural and morphological properties of PANi with fluconazole nanofibers were evaluated by SEM and FTIR.

1. Introduction

National Nanotechnology Initiative (NNI) define nanotechnology as the consumption of structures with at least one dimension of nanometer size for the production of materials, systems or devices with initially or extensively improved properties due to their nano size. Since nano-particles have high surface energy and a large surface area-to-volume ratio, it can provide high durability for fabrics, at the same time presenting good affinity for fabrics and enhance durability of the function. Nano-Tex known as a secondary of the US-based Burlington Industries have done the earliest work on nano-textiles.[1] To apply nano-particles onto textiles, the most frequently used technique is coating. Textiles are generally composed of nano-particles; a surfactant, ingredients and a carrier medium to entrap the nano-particles.[2]

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Spraying, transfer printing, washing, rinsing and padding are the several methods can apply coating onto fabrics. The method used for this is padding.[3] By using a padder, the nanoparticles are attached to the fabrics which is adjusted to suitable pressure and speed, followed by curing and drying. Textiles are omnipresent to us, covering our skin and environments by not only giving protective shield but they also serve artistic appeal and cultural value. Smart clothes were created from intelligence to textiles which are added from advances in material science. They have fascinated because of their potential applications such as in dust and germ free clothing,[4] cooling systems,[5] electrotherapy,[6] heat generation,[7] health monitoring shirts, drug delivery,[8] data transfer in clothing, electro chromic display, sensors and military applications like stealth technology. This smart textiles can be differentiated into three subtypes,[9] acting as sensors where as active smart textiles can sense and react to the stimuli from the environment, and have an actuator function and very smart textiles, having the reward to alter their behavior to the situations where else passive smart textiles can only sense the environment. Furthermore, for the development of smart nanotextiles there are some suitable materials such as inherently conducting polymers (ICPs), carbon nanotubes (CNT) and a number of materials in the form of nano-particles or nanofibers.[10] A type of ionic electro active polymer which changes the shape by mobility or diffusion of ions and conjugated substances defined as inherently conductive polymers.[11] Polyacetylene, polypyrrole, polyaniline and polythiophene are usually used ICPs[12] but Polyaniline (PANi) is one of the most commonly studied ICP. It has three possible oxidation states and is relatively steady in the environment.[10] In smart nanotextiles, especially polyaniline and polypyrrole may have a vital role in remote monitoring those undergoing rehabilitation or chronically ill patients. Besides that, to build up materials with motor functions a combination of ICP actuators in textiles can be used.[10] ICPs can also mimic and increases the sensory system of the skin by sensing external stimuliincluding proximity, touch, pressure, temperature, and chemical or biological substances.[3] Studies have been done by using anti-bacterial agents in textiles such as, nano-sized silver,[13] titanium dioxide[14] and zinc oxide.[15] The number of particles per unit area is increased with the use of nano-sized particles, so can maximize the anti-bacterial effects. A very big relative surface area can be caused by the nano-sliver particles. So, this will leads to rise in their contact with bacteria or fungi. Furthermore, greatly improving their antimicrobial efficiency which is usually applied to socks in order to prohibit the growth of bacteria. Synthetic compounds that have one or more azoles rings with three nitrogen atoms in the five membered rings known as antifungal triazoles. They primarily act by inhibiting CYP450-dependent conversion of lanosterol to ergosterol which leads to an accumulation of toxic 14-a-methylsterols, which alters the function and cell membrane properties leading to the inhibition of replication and cell growth.[16] The antifungal triazole which is used in this study is fluconazole. Treatment of candidemia over the past decade has been increased considerably by the introduction of fluconazole.[17] In order to widen its antifungal spectrum of activity and to enhance its *in vitro* potency, fluconazole's chemical structure has been modified.[18] It has unique pharmacokinetics with a long half-life, good water solubility, low molecular weight, weak protein binding, and a high level of cerebrospinal fluid penetration. It has been effective in treating both superficial[19] and systemic Candida infections.[20] The development of resistant strains of Candida after use of fluconazole as primary therapy or as a prophylactic agent for superficial candidosis that have been documented in several other reports. Basically, fluconazole thought to be fungistatic rather than fungicidal in standard *in vitro* susceptibility tests. In present study, we prepared nanofibers of PANi and PANi with fluconazole by simple and cost effective sol-gel process and investigate its enhanced antifungal activity on various candida species. Structural and morphological properties of PANi doped fluconazole will be evaluated by SEM and FTIR.

2. Materials and methods

2.1. *Chemicals*

Aniline, ammonium persulfate, camphor sulphonic acid and fluconazole obtained from Sigma Aldrich with 99.5% purity. Methanol, barium chloride, sulfuric acid, acetone and dimethlysulfoxide were reagent grade. Sabouraud agar and Nutrient broth were obtained from HiMedia.

2.2. *Fungal organisms*

Candida albicans (ATCC 140503), *Candida krusei* (ATCC 34135) and *Candida tropicalis* (ATCC 13803) used in this study were purchased from ATCC.

2.3. *Preparation of PANi with fluconazole*

Required quantity of fluconazole was dissolved in acetone and was mixed for 30 min. Aniline (An) monomer was distilled under reduced pressure. D-CSA as the dopant and ammonium persulfate ($(NH_4)_2S_2O_8$, APS) as the oxidant were used as received without further treatment. PANI–(D-CSA) nanofibres were prepared by oxidative polymerization of aniline at 0–5 °C (ice bath) using ammonium persulfate (APS) as the oxidant in the presence of D-CSA. A typical polymerization process of PANI–(D-CSA), briefly of aniline was been transferred to 100 ml beaker containing 10 ml of deionized water. The beaker was kept in ice bath (0–5 °C) and the contents were stirred for 5 min. The equivalent moles of ammonium persulfate were dissolved in 10 ml of deionized water. The beaker was kept in ice bath (0–5 °C) and the contents were stirred for 5 min. D-CSA and transferred into a 100 ml beaker containing 10 ml of deionized water and the contents were stirred for 5 min till a clear and homogeneous solution is obtained and added with fluconazole solution. After that the surfactant has been added to the monomer drop wise with constant stirring at 0–5 °C. After the addition of surfactant, oxidant has been added to the monomer contents drop by drop under constant stirring and temperature of 0–5 °C. After the addition of oxidant the contents color had slowly changed to dark green color indicating the polymerization of aniline to polyaniline. The final contents have been stirred for 10 min and kept in refrigerator at 0 °C for 24 h. After that the contents were filtered by washing with deionized water