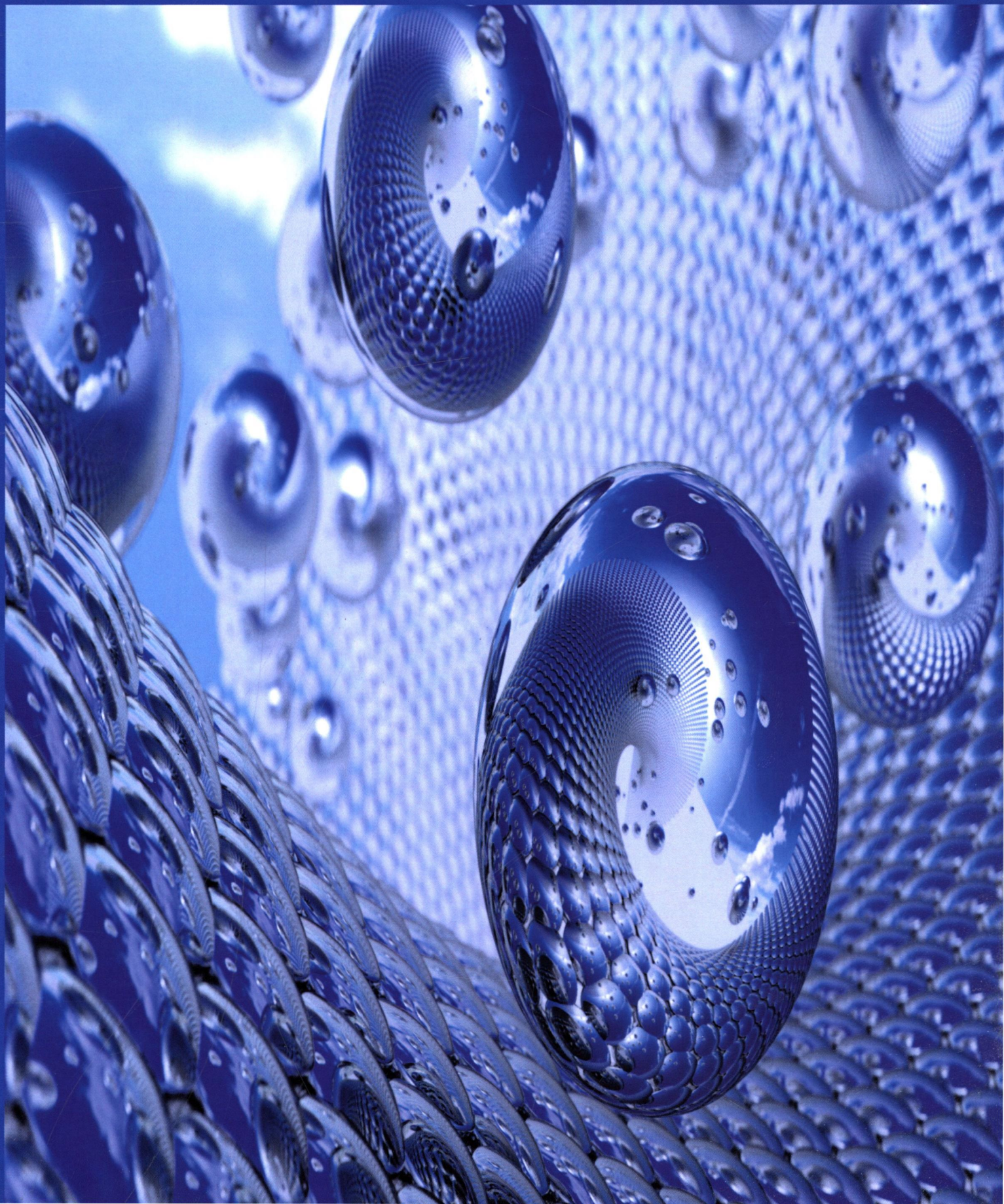


Advances in Nanoparticles: Issues and Developments

Contributors: Marina Usacheva, Buddhadev Layek, et al.



About the Book

Nowadays, research in nanoparticles is a very dynamic field that ranges across many areas of science, from physics to chemistry and medicine, but one of the clearest examples of the role of nanoscale in the properties of a material is the catalytic activity of nanoparticles. This is due mainly to their unique optical, electronic, and magnetic properties radically different from the properties observed in the bulk state. The use of particles with a well-defined dispersion makes it possible to relate various physicochemical properties (optical, magnetic, electronic, catalytic, and others) to the size and morphology of the particles in a more quantifiable way. Nanoparticles can be defined as objects ranging in size from 1-100 nm that due to their size may differ from the bulk material. Presently, different metallic nanomaterials are being produced using copper, zinc, titanium, magnesium, gold, alginate and silver.

Nanoparticles are being used for diverse purposes, from medical treatments, using in various branches of industry production such as solar and oxide fuel batteries for energy storage, to wide incorporation into diverse materials of everyday use such as cosmetics or clothes, optical devices, catalytic, bactericidal, electronic, sensor technology, biological labelling and treatment of some cancers. Due to their exceptional properties including antibacterial activity, high resistance to oxidation and high thermal conductivity, nanoparticles have attracted considerable attention in recent years. Nanoparticles can be synthesized chemically or biologically. Metallic nanoparticles that have immense applications in industries are of different types, namely, Gold, Silver, Alloy, magnetic etc. This study aims to present an overview of nanoparticles, with special reference to their mechanism of biosynthesis and types.

This book named with Advances in Nanoparticles Issues and Developments highlights classification, preparation techniques, characterization methods, application, health implications and clinical aspects of nanoparticles. The text is organized into eleven chapters. In this book potential use of Nano crystals and Nanoparticles in various important areas has been discussed. Special properties of these nanoparticles may offer new advancement in drug discovery. The book bridges the gap between nanomaterials synthesis and characterization, and catalysis. As such, this book will be a valuable resource for postgraduate students and researchers as well practitioners in these exciting fields.

Nanomedicine has tremendous prospects for the improvement of the diagnosis and treatment of human diseases. Use of microbes in biosynthesis of nanoparticles is an environmentally acceptable procedure. Nanotechnology has potential to revolutionize a wide array of tools in biotechnology so that they are more personalized, portable, cheaper, safer, and easier to administer. Due to their incredible properties, nanoparticles have become significant in many fields in recent years such as energy, health care, environment, agriculture etc. Nanoparticle technologies have great potentials, being able to convert poorly soluble, poorly absorbed and labile biologically active substance into promising deliverable substances.



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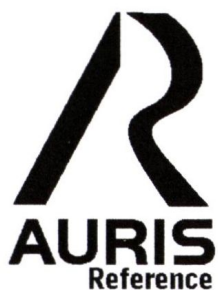
Advances in Mathematics and Developments



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Preface

Nowadays, research in nanoparticles is a very dynamic field that ranges across many areas of science, from physics to chemistry and medicine, but one of the clearest examples of the role of nanoscale in the properties of a material is the catalytic activity of nanoparticles. This is due mainly to their unique optical, electronic, and magnetic properties radically different from the properties observed in the bulk state. The use of particles with a well-defined dispersion makes it possible to relate various physicochemical properties (optical, magnetic, electronic, catalytic, and others) to the size and morphology of the particles in a more quantifiable way. Nanoparticles can be defined as objects ranging in size from 1-100 nm that due to their size may differ from the bulk material. Presently, different metallic nanomaterials are being produced using copper, zinc, titanium, magnesium, gold, alginate and silver.

Nanoparticles are being used for diverse purposes, from medical treatments, using in various branches of industry production such as solar and oxide fuel batteries for energy storage, to wide incorporation into diverse materials of everyday use such as cosmetics or clothes, optical devices, catalytic, bactericidal, electronic, sensor technology, biological labelling and treatment of some cancers. Due to their exceptional properties including antibacterial activity, high resistance to oxidation and high thermal conductivity, nanoparticles have attracted considerable attention in recent years. Nanoparticles can be synthesized chemically or biologically. Metallic nanoparticles that have immense applications in industries are of different types, namely, Gold, Silver, Alloy, magnetic etc. This study aims to present an overview of nanoparticles, with special reference to their mechanism of biosynthesis and types. This book named with *Advances in Nanoparticles: Issues and Developments* highlights classification, preparation techniques, characterization methods, application, health implications and clinical aspects of nanoparticles. The text is organized into eleven chapters. In this book potential use of Nano crystals and Nanoparticles in various important areas has been discussed. Special properties of these nanoparticles may offer new advancement in drug discovery. The book bridges the gap between nanomaterials synthesis and characterization, and catalysis. As such, this book will be a valuable resource for postgraduate students and researchers as well practitioners in these exciting fields. Nanomedicine has tremendous prospects for the improvement of the diagnosis and treatment of human diseases. Use of microbes in biosynthesis of nanoparticles is an environmentally acceptable procedure. Nanotechnology has potential to revolutionize a wide array of tools in biotechnology so that they are more personalized, portable, cheaper, safer, and easier to administer. Due to their incredible properties, nanoparticles have become significant in many fields in recent years such as energy, health care, environment, agriculture etc. Nanoparticle technologies have great potentials, being able to convert poorly soluble, poorly absorbed and labile biologically active substance into promising deliverable substances.

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NANOPARTICLE-MEDIATED PHOTODYNAMIC THERAPY FOR MIXED BIOFILMS

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ABSTRACT

Wounds associated with diabetes are difficult to heal and often stay unhealed, leading to higher morbidity and mortality in the diabetic population. A major barrier to the successful healing of chronic diabetic wounds is the presence of biofilm-mediated infections. In recent years, photodynamic therapy (PDT) has emerged as a promising treatment modality for the management of chronic wounds. The objective of the present study was to evaluate the effectiveness of toluidine blue- (TB-) loaded nanoparticle-mediated PDT in eradicating antibiotic-resistant polymicrobial biofilms of *Pseudomonas aeruginosa* and methicillin-resistant *Staphylococcus aureus* in an in vitro model. Dioctyl sodium sulfosuccinate (aerosol OT, AOT)-alginate nanoparticles with high TB loading (%) were formulated using a double emulsification cross-linking method. TB nanoparticles induced effective killing of planktonic *P. aeruginosa* ($3.5 \log_{10}$ CFU) and *S. aureus* ($>5 \log_{10}$ CFU) and their combined biofilms ($2.8 \log_{10}$ CFU for *P. aeruginosa* versus $3.4 \log_{10}$ CFU for *S. aureus*). While *P. aeruginosa* biofilm was more resistant when compared to that of *S. aureus*, our results demonstrated effective eradication of complex biofilms of dual bacterial strains in vitro.

INTRODUCTION

Chronic wounds are a major health problem throughout the world, with limited effective methods of treatment. Wounds associated with diabetes are difficult to heal and often remain unhealed leading to increased morbidity and mortality in diabetic patients [1, 2]. A key barrier to the successful healing of chronic diabetic wounds is the presence of biofilm-mediated infections [3–5]. Biofilms are structured communities of microorganisms growing on a living or nonliving surface and enclosed in a self-produced matrix of extracellular polymeric substances (EPS) [6, 7]. The matrix provides enormous protection to the biofilm against hostile environmental conditions, such as host immunological system and antimicrobial agents [8–11]. The polymicrobial nature of diabetic wounds appears to promote the production of several virulence factors that contribute to the chronicity of the infection [12, 13]. In addition, presence of antibiotic-resistant bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa* (*P. aeruginosa*) is more common in diabetic wounds [14].

In recent years, photodynamic therapy (PDT) has emerged as a promising alternative treatment for the management of chronic infections. PDT is based on the concept that certain compounds, known as photosensitizers, when exposed to light of a specific wavelength, are capable of generating reactive oxygen species (ROS), such as singlet oxygen and free radicals [15–18]. Subsequently, ROS mediate cellular toxicity via oxidative stress to cell membranes and other cellular components [19, 20]. One of the key advantages of PDT as a therapeutic tool is the concept of dual selectivity. Collateral damage to normal tissues can be reduced by the preferential accumulation of photosensitizers in the target tissue and localized light irradiation in a spatially focused manner [21].

Phenothiazine dyes such as methylene blue (MB) [22] and toluidine blue (TB) [23] have been extensively used for PDT-mediated eradication of a wide range of Gram-positive and Gram-negative bacteria as well as fungi. The high quantum yield of singlet oxygen production [24, 25] coupled with relatively low dark toxicity [26, 27] makes them attractive candidates for PDT. However, several studies have demonstrated incomplete destruction of biofilms using MB [28, 29] and TB-mediated PDT [30]. The diminished susceptibility of biofilms to PDT was primarily attributed to poor penetration of the photosensitizer inside the cells [28, 29]. Furthermore, both MB and TB are substrates of bacterial multidrug resistance efflux pumps, thus reducing the efficiency of these photosensitizers [31].

Therefore, a potential strategy to improve the efficacy of photosensitizers is to encapsulate them in a suitable drug carrier such as nanoparticles. Photosensitizers encapsulated in nanoparticles offer several distinct advantages over free photosensitizing molecules, which include increased accumulation of the photosensitizer in the target cells, and inhibition of target cell's ability to pump out the photosensitizers [32]. Furthermore, incorporation of the photosensitizer in nanoparticles could prevent the inactivation of photosensitizer by plasma reductases and thus protects its photodynamic activity [33]. In this study, the nanoparticles were formulated using anionic surfactant dioctyl sodium sulfosuccinate (aerosol OT, AOT) and a naturally occurring polysaccharide sodium alginate. Besides being nontoxic, the AOT-alginate nanoparticles have also been shown to improve ROS yield of photosensitizers [34]. The objective of the present study was to investigate the *in vitro* photodynamic efficacy of TB-loaded nanoparticles against both planktonic and biofilm phases of *P. aeruginosa*, MRSA, and their mixed culture. In addition, the *in vitro* photobactericidal efficacy of TB nanoparticles was compared with that of free TB.

MATERIALS AND METHODS

Materials

TB, polyvinyl alcohol (PVA), sodium alginate, and bovine serum albumin (BSA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). AOT and chloroform were procured from Fisher Scientific (Chicago, IL, USA). Luria Broth (LB) medium and LB agar medium were purchased from MP Biomedicals, LLC (Solon, Ohio, USA). *P. aeruginosa* (ATCC 27853) was received from Dr. Karunya Kandimalla (Department of Pharmaceutics, University of Minnesota, Minneapolis, MN) and *S. aureus* (ATCC 33591) from Dr. Christine Salomon (Center for Drug Design, University of Minnesota, Minneapolis, MN). All other reagents are chemical grade and used without further modification.

Nanoparticle Formulation

AOT-alginate nanoparticles encapsulating TB were prepared using a double emulsification cross-linking method [35, 36]. Briefly, 1 mL of 1% w/v aqueous sodium alginate solution containing 5 mg of TB was emulsified into 2 mL of 2% w/v AOT solution in chloroform by sonication at 20 W for 5 min over an ice bath (Model W-375, Heat Systems Ultrasonics Inc., CT). The water-in-oil (w/o) primary emulsion was re-emulsified into 15 mL of 2% w/v aqueous PVA solution to form a water-in-oil-in-water (w/o/w) multiple emulsion. To the final emulsion, 5 mL of 60% w/v aqueous calcium chloride solution was added dropwise with constant stirring at 1200 rpm using a magnetic stirrer. Subsequently, chloroform was evaporated by continuously stirring the emulsion at ambient conditions for 18 h followed by 1.5 h stirring under vacuum. The nanoparticles were harvested by ultracentrifugation at 35000 rpm for 30 min (Beckman Optima™ LE-80K, Palo Alto, CA), washed three times with deionized water to eliminate excess PVA and untrapped TB. After the final wash, the nanoparticle pellet was redispersed in 1 mL of deionized water, and nanoparticles were separated from large aggregates by centrifugation at 1000 rpm for 5 min (Eppendorf® 5810 R, Eppendorf, Westbury, NY). The supernatant nanoparticle suspension was then freeze dried (FreeZone 4.5®, Labconco, Kansas City, MO) to obtain dry nanoparticles. Blank nanoparticles were synthesized in the absence of TB.

Nanoparticle Characterization

The average hydrodynamic diameter and zeta potential of the nanoparticles were measured using a Delsa™ Nano C particle size analyzer (Beckman Coulter Inc., Fullerton, CA) equipped with dual 30 mW laser diodes operating at