

GREEN SYNTHESIS OF BIOPOLYMER MEDIATED ZINC OXIDE
NANOCOMPOSITES FOR ANTICANCER STUDY APPLICATION

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DEDICATION

This thesis is dedicated to my family, future wife and career

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ABSTRACT

The development of metal-oxide based composites can be drawn from advanced cancer treatment. For years, zinc oxide nanoparticles (ZnO-NPs) have gained great research attention for their potential applications in diverse areas including the medicine world. Chemicals are harmful to human health and the environment when used in the fabrication of various nanoparticles. This study aims to fabricate facile zinc oxide nanoparticles using carrageenan as a green stabilizer and simple sol-gel and combustion method at four different temperatures ranging from 300 to 700°C for 60 minutes. Data from X-ray and Fourier-transform infrared spectroscopy indicated that the ZnO nanoparticles fabricated at 600°C possessed better crystallinity, purity with least aggregation than other counterparts. This was followed by immersing the fabricated ZnO-nanoparticles (NPs) in the chitosan solution to synthesize facile Cs/ZnO nanocomposites (NCs) and develop cross-linked Chitosan/ZnO nanocomposites (NCs) using sodium tripolyphosphate (TPP). The successful chitosan coating on ZnO nanoparticles and synthesis of cross-linked Cs/ZnO-NCs were shown by ultraviolet-visible, X-ray, and Fourier-transform infrared spectroscopy. Cs/ZnO-NCs were produced for better stability in anticancer activities due to their biocompatibility and stability under harsh environments. Further, the potential anticancer activities of ZnO and Cs/ZnO-NCs were in vitro studied against various cancer cell lines of rhabdomyosarcoma (RD), pancreatic (AsPC-1), breast (MCF7), colon (Caco-2), lung (A-549), colon (HCT116) and embryonic normal kidney cells (293T). In in vitro anticancer assays, both ZnO and Cs/ZnO nanocomposites exhibited significant cytotoxicity on the tested cancer cells. The results indicated that Cs/ZnO-NCs coatings and cross-linked Cs/ZnO-NCs increased anticancer effect with the maximum killing effect of 85% and 100 % against Caco-2 and HCT116 cancer cells respectively. This discovery may provide a ray of hope for the easy and low-cost production of anticancer agents. These results also support the potential use of carrageenan-mediated ZnO-NPs coated with chitosan for prospective and low-cost anticancer efficacy. Therefore, the green synthesized CS/ZnO nanoparticles can be considered as a low-cost nanotherapeutic agent for cancer treatments.

ABSTRAK

Perkembangan komposit yang berasaskan logam-oksida boleh menjadi permulaan kepada rawatan kanser lanjutan. Selama bertahun-tahun, nanozarah zink oksida (ZnO-NPs) telah mendapat perhatian penyelidikan yang sewajarnya untuk potensi aplikasinya dalam pelbagai bidang termasuk dunia perubatan. Bahan kimia memudaratkan kesihatan manusia dan alam sekitar apabila digunakan dalam sintesis zarah nano yang berbeza. Kajian ini bertujuan untuk membuat facile zink oksida nanozarah menggunakan karagenan sebagai penstabil hijau dan kaedah sol-gel mudah dan pembakaran pada empat suhu berbeza antara 300 hingga 700°C selama 60 minit. Data daripada spektroskopi inframerah sinar-X dan *Fourier-transform* menunjukkan bahawa nanozarah ZnO yang difabrikasi pada suhu 600°C mempunyai kehabluran yang lebih baik, ketulenan dengan pengagregatan paling sedikit berbanding rakan sejawat lain. Ini diikuti dengan merendam ZnO-nanopartikel (NPs) yang direka dalam larutan kitosan untuk mensintesis nanokomposit (NCs) Cs/ZnO yang mudah dan membangunkan nanokomposit Chitosan/ZnO (NCs) berkait silang menggunakan natrium tripolifosfat (TPP). Salutan kitosan yang berjaya pada nanopartikel ZnO dan sintesis Cs/ZnO-NC berkait silang ditunjukkan oleh spektroskopi inframerah ultraungu-kelihatan, sinar-X dan *Fourier-transform*. Cs/ZnO-NCs dihasilkan untuk kestabilan yang lebih baik dalam aktiviti antikanser kerana biokompatibiliti dan kestabilannya di bawah persekitaran yang keras. Selanjutnya, potensi aktiviti antikanser ZnO dan Cs/ZnO-NCs telah dikaji secara *in vitro* terhadap pelbagai saluran sel kanser rhabdomyosarcoma (RD), pankreas (AsPC-1), payudara (MCF7), kolon (Caco-2), paru-paru (A-549), kolon (HCT116) dan sel buah pinggang normal embrio (293T). Ujian antikanser *in vitro*, kedua-dua nanokomposit ZnO dan Cs/ZnO menunjukkan sitotoksiti yang ketara pada sel-sel kanser yang diuji. Dapatan menunjukkan bahawa salutan Cs/ZnO-NCs dan Cs/ZnO-NCs berkait silang meningkatkan kesan antikanser dengan kesan membunuh maksimum masing-masing sebanyak 85% dan 100 % terhadap sel kanser Caco-2 dan HCT116. Penemuan ini mungkin memberikan sinar harapan untuk pengeluaran agen antikanser yang mudah dan kos rendah. Dapatan ini juga menyokong potensi penggunaan ZnO-NPs pengantara karagenan yang disalut dengan kitosan untuk keberkesanan antikanser yang mempunyai prospektif dan kos yang rendah. Oleh itu, nanozarah CS/ZnO yang disintesis hijau boleh dianggap sebagai agen nanoterapi kos rendah untuk rawatan kanser.

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LIST OF ABBREVIATIONS

ZnO-NPs	-	Zinc Oxide Nanoparticles
Cs/ZnO	-	Chitosan/Zinc Oxide
NCs	-	Nanocomposites
RD	-	Rhabdomyosarcoma Cancer Cells
ASPC1	-	Pancreatic Cancer Regar Cancer Cells
MCF7	-	Breast Adenocarcinoma Cancer Cell
CACO-2	-	Colon Adenocarcinoma Cancer Cells
A549	-	Lung Carcinoma Cancer Cells
T293	-	Embryonic Normal Human Kidney Cells
HCT116	-	Colorectal Carcinoma Cancer Cells
FWHM	-	Full Width at Half Maximum
OD	-	Optical Density
FTIR	-	Fourier Transform Infrared (FT-IR) Spectroscopy
XRD	-	X-ray Diffraction
FESEM	-	Field Emission Scanning Microscopy
HRTEM	-	High Resolution Transmission Electron Microscopy
UV-vis	-	Ultra Violet-Visible Spectroscopy
EDX	-	Energy Dispersive X-ray
TGA	-	Thermogravimetric Analysis
DTA	-	Differential Thermal Analysis
FWHM	-	Full Width at Half Maximum
OD	-	Optical Density
nm	-	Nanometers

LIST OF SYMBOLS

λ	-	X-ray Wavelength
β	-	Line Broadening at Half the Maximum Intensity (FWHM)
θ	-	Bragg angle

CHAPTER 1

INTRODUCTION

1.1 Research Background

Nanotechnology has attained great attention as an arising field of modern science and technology [1]. It is a branch of science concerned with the manipulation and production of nanoscale materials and electronics [2]. Nanomaterials are nanoparticles with nanoscale dimensions ranging from 1 to 100 nanometers [3, 4]. Because of their high surface area to volume ratio in comparison to bulk substances, these nanomaterials have unique physiochemical features such as increased thermal conductivity, catalytic reactivity, chemical stability, and non-linear optical performance [1, 5]. Nanoparticles aren't new to the environment; they're found in clays, minerals, and bacterial products. Engineered nanoparticles are made up of a range of nanoparticles in various shapes and sizes, which are separated from naturally occurring materials by a set of synthetic surface molecules [6].

Because of their exceptional optical, electrical, catalytic, and magnetic capabilities, metal and metal oxide nanoparticles are extremely important [7]. These nanoparticles are used in chemistry, physics, and materials research, among other applications. Their unique qualities enable them to be used in a wide range of medical, agricultural, environmental, and industrial domains. Traditional metal and metal oxide nanoparticle manufacturing processes use stabilising and reducing chemical agents that are detrimental to the environment, humans, and many wildlife. [7]. As a result, scientists are looking for alternate "green synthesis" approaches to eliminate or limit the use of chemicals in nanoparticle production.

One of the most important metal oxide nanoparticles is zinc oxide nanoparticles (ZnO-NPs), due to its unique physical and chemical properties, such as physical stability, chemical stability, high photosensitivity, non-toxicity, high thermal conductivity, wide band gap (3.37 eV)

together with large excitation binding energy (60 meV) [4, 8]. It is a non-toxic and biocompatible material with a UV-filtering properties utilized as a UV blocker in sunscreen products [4]. Because of its UV filtering properties, it has been widely utilised in cosmetics such as sunscreen creams [5]. Bulk ZnO is also regarded to be a generally recognised as safe (GRAS) material by the US Food and Drug Administration (FDA), and ZnO-NPs larger than 100 nm are considered to be reasonably biocompatible, allowing for its usage in biomedical applications [9]. The ZnO-NPs are regularly used as delivery vehicles of various biomolecules such as drugs, proteins, and genes [10]. It's because ZnO-NPs are a pH-sensitive nanocarrier that's ideal for intracellular drug release and tumor-targeted drug delivery because they dissolve quickly at low pH [10]. Beside that it has a wide scope of biomedical applications where they could be employed for the therapy of various diseases such as cancer, diabetes, cardiovascular disease, microbial and fungal infection and ischemic [1]. Furthermore, an average individual need 2-3 g of zinc and 10-15 mg of zinc every day for optimal growth and physiology [11]. Apart from these characteristics, ZnO nanostructures have high catalytic efficiency and solid adsorption capacity, and are rapidly being used in the manufacture of sunscreens, fungicide, rubber processing, ceramics and wastewater treatment. Due to its low toxicity in vivo and vitro without stimulation, synthetic techniques that is both simple and environmentally friendly, with high catalytic efficiency, strong adsorption ability, and stable petrochemical properties, it has a wide range of applications in other fields such as agrochemicals, pharmaceuticals, perfumes, dyes, biology, and petroleum [12]. Interestingly, because of its durability in hostile environments, quick electron transfer kinetics, high biocompatibility, and extended life than organic-based disinfectants, the use of ZnO-NPs as an antibacterial agent has recently piqued interest in the development of microbiology and biomaterials [12]. Antimicrobial agents, medication delivery, bio imaging probes, and cancer treatment are among the next-generation biological uses that nano-crystalline ZnO is being studied for [13]. When ZnO-NPs combine with cell membrane lipids, they produce reactive oxygen species (ROS) and have increased toxicity against cancer cells in vitro [14]. Many studies have discovered that cancer cells are extremely vulnerable to ZnO-NPs toxicity, which occurs in a dose and time dependent way.

ZnO-NPs can be fabricated using chemical, physical and biological methodical approaches. Various methods for preparing ZnO-NPs have been reported, including solvothermal and hydrothermal synthesis [15], precipitation [16], polymerization method [17], laser ablation

[18], microwave irradiation [19], wet chemical method [20], photochemical method [21], sonochemical [22], and sol gel methods [23]. However, chemical and physical techniques have a number of drawbacks, including the use of high temperature and pressure, long reaction times, hazardous reagents, and the need for external additives such as a specific stabiliser, promoter, or base during the reaction, all of which limit the purity of the final product. Generally, nanoparticle synthesis is a complicated process with a wide range of variables that might influence the qualities of the final product when using various techniques of preparation. The ability to manipulate the ZnO-NPs' morphology and produce a restricted size distribution of the end product is critical. Chemical approaches result in the presence of harmful compounds adsorbed on the surface, which could have negative consequences in medical applications [24]. It is critical to synthesis ZnO-NPs using a simple, environmentally acceptable, low-cost approach that can manufacture nanomaterials with stable particle sizes. Because chemical and physical approaches are harmful and expensive, biological processes for green nanoparticle manufacturing involving microbes, enzymes, plants, and algae have been proposed as viable eco-friendly alternatives [25]. Natural polymers like starch, gelatin, and different proteins are all promising materials for nanomaterial production since they are bioabsorbable and biodegradable, with non-toxic degradation products [26].

For the manufacture of different metal oxide nanoparticles, natural biopolymers such as starch [27], carrageenan [28], cellulose [29], pullulan [30], chitosan [31] and gelatin [32] are employed. It is the recently discovered and promising candidates that have the potential to be manufactured as medication delivery vehicles [33]. Tissue adhesive and drug-carrying capabilities of natural polymers are unique [34]. As a green stabiliser, nanoparticles are produced from a variety of natural polymers.

Carrageenan, for example, is a biodegradable linear polysaccharide polymer derived from marine red seaweed. Carrageenan is widely utilised in the food business for its physical qualities such as thickening, gelling, and stabilising [35]. Chitosan (Cs) is a chitin-derived linear polysaccharide polymer. It is also well studied for possessing good mechanical properties, non-toxicity, abundant availability, biodegradability, antimicrobial activity and low cost [36, 37]. Moreover, Cs together with amine and hydroxyl functional groups makes it notable choice to

utilize as a stabilizer and a cross-linker to connect diverse drugs to a metal oxide core [38]. In the intracellular environment, the chitosan amine group will promote solubility and provide hemocompatibility [39]. Furthermore, the surface of nanoparticles interacts with numerous cellular components which are exceptionally anionic in nature. The mucoadhesive property of Cs will allow the encapsulated component to interact with the cells for longer. The presence of Cs on the particle surface may also aid in the opening of tight intracellular junctions, thereby boosting cellular absorption and thus therapeutic efficiency [39]. Several biopolymers have been employed in medication delivery studies to increase therapeutic efficacy while reducing negative effects [34]. Many research also demonstrated a simple, economical, and environmentally friendly fabrication of ZnO-NPs employing the biopolymer *kappa carrageenan* as the stabilising, capping, and reducing agent, as well as Cs as the stabilising, capping, and reducing agent. [40].

In just one year, 18.1 million new cancer cases were displayed over the world [29]. Cancer is a terrible disease that arises as a result of a succession of molecular-level genetic events that disrupt the control over cell division, resulting in unregulated division and, finally, cancer [41]. Radiotherapies and chemotherapies are ineffective and aggressive, therefore cancer therapy remains a major issue for medicine [42]. These traditional anticancer medicines have side drawbacks like nausea, fatigue, sleeplessness, vomiting and delirium, which are all typical complaints among cancer patients [42]. Several investigations have been conducted on the anti-cancer properties of ZnO-NPs. ZnO-NPs cross-linked with biopolymer have shown to have a strong anticancer impact against human breast cancer cell (MCF-7 cell line) [14], AGS gastric cancer cells [43], Human Cervical cancer (HeLa) [44], human colon cancer HCT-116 [45], colorectal cancer (HCT116) [29], human colorectal adenocarcinoma cells (Caco-2 cell line) [46]. Up to date, there is no report the preparation of cross-linked biopolymer mixed with ZnO-NPs for cancer treatment activities.

Based on these considerations, the current research concentrated on the integration of nanoparticles with biopolymers. The goal of this study was to use a green synthesis approach to make biopolymer carrageenan-based ZnO-NPs. Thermal decomposition was utilised to successfully fabricate ZnO-NPs at varied temperatures. The syntheses of ZnO-NPs via green method at various calcination temperatures, as well as the fabrication of Chitosan/ZnO

nanocomposite (Cs/ZnO-NCs) coatings and cross-linked Chitosan/ZnO nanocomposite (Cs/ZnO-NCs), are the main emphasis of this research.

Fourier transform infrared (FTIR) spectroscopy, X-ray diffraction pattern (XRD), UV-vis spectroscopy, High resolution transmission electron microscopy (HR-TEM), energy dispersive X-ray analysis (EDX), field emission-scanning electron microscopy (FESEM) and thermogravimetric analysis (TGA) were used to characterise the physiochemical properties of ZnO-NPs and nanocomposites. At five different concentrations, MTS in vitro cytotoxicity assays assessed the cytotoxicity of NPs and NCs against pancreatic cancer, human rhabdomyosarcoma RD, breast adenocarcinoma, colon adenocarcinoma, lung carcinoma, colorectal carcinoma and embryonic kidney (normal human cell) cells after 24 hours.

1.2 Problem Statement

Physical, chemical, and green synthesis approaches have all been applied in the production of nanoparticles. NPs have been synthesised using a variety of ways, the chemical and physical methods were found has a serious hazardous and high toxicity for living organisms then is the biggest problems that limit the involvement of nanoparticles in the biomedical industry. Indeed, the synthesis of this material plays a huge role for the development and improvement of our technology today.

Cancer is one of the lethal diseases which has low survival rate. Cancer treatment approaches like chemotherapy and radiotherapy are aggressive and ineffective, therefore, cancer treatment approaches remains a major issue in medicine world. For decades, anticancer medications such as 5-FU have been the preferred chemotherapy treatment. On the other hand, the negative side effects of administering 5-FU-based chemotherapy can result in stomatitis, diarrhoea, and gastrointestinal mucosal injury, among other things [47]. The extended chemotherapy time and nonspecific defensive mechanisms of 5-FU may cause damage in normal healthy cells. [48]. Chemotherapy along with the severe side effects of commonly used drugs has raised the urgency in the search for alternative cancer treatments that are generally safe. Furthermore, as compared to

other nanoparticles, ZnO-NPs are well-known for their cytotoxicity against cancerous cells in vitro [49].

1.3 Research Objectives

The objective is to develop Chitosan/ZnO nanocomposite through green synthesized zinc oxide nanoparticles and to study in vitro cytotoxicity and anticancer effects for further applications.

Followings are the objectives proposed for this study:

- (a) To synthesize biopolymer mediated ZnO-NPs and further fabrication of Cs/ZnO-NCs coatings and cross-linked Cs/ZnO-NCs via green method.
- (b) To evaluate the physicochemical properties of ZnO-NPs, Cs/ZnO-NCs coatings and cross-linked Cs/ZnO-NCs.
- (c) To determine the efficiency of cytotoxicity assay for ZnO-NPs, Cs/ZnO-NCs coatings and cross-linked Cs/ZnO-NCs.

1.4 Scope of the Study

To complete all the objectives in this project, the scopes must comply with all the listed objectives. Hence, below are some brief descriptions of the research scope for this study.

- (1) Fabrications of ZnO-NPs were carried out using a green method using carrageenan as a stabilizing agent by sol-gel technique following thermal calcination in a furnace. Later, Cs/ZnO-NCs coatings and cross-linked Cs/ZnO-NCs were synthesized for further applications.
- (2) Fabricated ZnO-NPs were characterized structurally, morphologically, and electrically using various techniques such as XRD, FTIR, UV-vis, TGA, FESEM, EDX, and TEM for

the stable formulations of nanoparticles. Successful green synthesis of Cs/ZnO-NCs was also proved using XRD, FTIR, TEM and EDX analyzing tools.

- (3) Anticancer effects of ZnO-NPs, Cs/ZnO-NCs coating and cross-linked Cs/ZnO-NCs were evaluated using MTS against normal human cell and various cancer cells.

1.5 Significance of the Research

The unique features of ZnO-NPs made with biopolymer make them appealing for usage in medicinal and industrial applications, hence environmentally benign methods for their synthesis are required. This can be achieved by using Cs instead of typical chemical compounds to reduce zinc ions to ZnO-NPs during production. The production of cross-linked Cs/ZnO-NCs with reinforced features, with the goal of controlling the enteric release of ZnO-NPs, is the most important aspect of the research. The composite architectures were developed to keep acid-soluble chitosan in the internal phase of the complex to shield ZnO from the gastric acid environment [50]. Cross-linked chitosan's cross-linking density, crystallinity, and hydrophilicity can be used to modulate ZnO-NPs and expand their spectrum of possible uses in drug delivery systems [51].

Cancer is one of the world's major causes of death. It cannot be denied that cancer diagnoses are increasing in younger generations nowadays which may be due to the modern lifestyle. Many researchers are interested and working hard in developing a cure which can eliminate cancer cells utterly. Since ZnO-NPs are designed to be applied in human body, the biocompatibility and toxicity are also the major concerns of researchers. Thus, the outcome of this research will become the main interest to many anticancer researchers who are looking towards designing and fabricating new anticancer drug delivery agents. This research would also provide an understanding of how the ZnO-NPs can be applied in cytotoxicity with enhanced properties with selective cancer killing ability.

1.6 Outline of Thesis

Introduction, literature review, methods, results and discussion, and conclusion are the five key chapters of this thesis. As for the introduction, which is the first chapter, it consists of the background of this study which elaborates on the overall idea. Then, current problems related to this study are addressed in the problem statement followed by the objectives which are the main part of the introduction. The research scope and the significance of the study are stated in brief about the overall flow project in this study.

Next, the second chapter is the literature review. This part is about finding the research gap related to this project based on the previous studies. Apart from that, it also discussed in deep on the previous works of literature that are similar to the project to get the ideas and come up with a new solution that can be done in this project. For example, the metal and metal oxide nanoparticles, ZnO-NPs, synthesis methods of ZnO-NPs, green synthesis method, biomedical applications including cytotoxicity and anticancer treatment effects. The methodology is the third chapter in this study. This part is describing on the research flow and the methods used on how to achieve the objectives. Flow charts and the list of the chemical are present in this chapter.

Chapter four discusses both the synthesis of ZnO-NPs, Cs/ZnO-NPs coating and cross-linked Cs/ZnO-NCs with their characterizations followed by the efficiency of cytotoxicity of those NPs and NCs against various cancer cells. Lastly, chapter five presents the research conclusion of green synthesized ZnO-NPs, Cs/ZnO-NPs coating and cross-linked Cs/ZnO-NCs followed by the recommendation for future works in the synthesis.

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LIST OF PUBLICATIONS

Indexed Conference Proceedings

1. **H. Hamrayev**, and K. Shameli. *Biopolymer-Based Green Synthesis of Zinc Oxide (Zno) Nanoparticles*. in *IOP Conference Series: Materials Science and Engineering*. 2021. IOP Publishing. **(IOP Publishing)**

Non Indexed Journal

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