SYNTHESIS AND CHARACTERIZATION OF CHITOSAN/ZINC OXIDE NANOPARTICLES FOR ANTIBACTERIAL ACTIVITY

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SUPERVISOR'S DECLARATION

I hereby declare that I have checked this thesis, and in my opinion, this thesis is adequate in terms of scope and quality for the award of the degree of Master of Science

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I hereby declare that the work in this thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at Universiti Malaysia Pahang or any other institutions.

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ABSTRAK

Nanopartikel logam oksida mempunyai ciri-ciri fizikal dan kimia yang unik yang berkaitan dengan saiz nanopartikel. Selain itu, nanopartikel logam oksida juga mempunyai aktiviti antibakteria yang sangat baik sebagai agen antibakteria yang berkesan. Tujuan kajian ini adalah untuk mengenal pasti dan menentukan ciri antibakteria nanopartikel kitosan/ZnO terhadap bakteria Gram-positif dan Gram-negatif, dan kesan nanopartikel kitosan/ZnO terhadap ciri antibakteria filem hidrogel. Oleh itu, nanopartikel zink oksida (ZnO) telah disintesiskan menggunakan garam zink nitrat melalui kaedah pemanasan gelombang mikro. Parameter operasi seperti penstabil (kitosan), kuasa pemanasan (400, 600 dan 800 Watt) dan masa pemanasan (4, 6 dan 8 minit) memainkan peranan penting dalam sintesis nanopartikel kitosan/ZnO. Kewujudan kitosan menghalang nanopartikel dari bergumpal, dengan menghasilkan larutan putih tanpa sebarang mendapan. *Stafilokokus aures* (*S. aures*) dan *Eskericia koli* (*E. koli*) digunakan sebagai mikroorganisma yang diuji. UV- vis spektrofotometer mengesahkan kehadiran nanopartikel kitosan/ZnO dengan puncak jalur pada 360 nm. Kehadiran satu puncak jalur baru sekitar 427 cm^{-1} pada spektrum FTIR membuktikan kewujudan fasa ZnO. Keputusan XRD menunjukkan bahawa bahan nanopartikel kitosan/ZnO yang disintesis adalah tulen dan bersesuaian dengan struktur heksagon-wurzite. FESEM juga mendedahkan taburan nanopartikel kitosan/ZnO adalah seragam dengan purata saiz adalah 70 nm dan berbentuk sfera. Kesan kuasa dan masa pemanasan terhadap saiz nanopartikel kitosan/ZnO ditunjukkan melalui taburan saiz nanopartikel dengan purata 30 hingga 90 nm. Peningkatan kuasa dan masa pemanasan menyebabkan peningkatan saiz disebabkan oleh kerana nukleasi. Permukaan potensi zeta adalah negatif dan berubah dari -29.6 ke -20.9 mV. Kitosan/ZnO nanopartikel menghasilkan penyahaktifan yang lebih tinggi terhadap *S. aures* berbanding *E. koli*. Keputusan menunjukkan nanopartikel kitosan/ZnO telah memaparkan zon perencatan antibakteria terhadap *S. aures* dan *E. koli* sebanyak 16.0 dan 13.4 mm. Kehadiran nanopartikel kitosan/ZnO dalam hidrogel juga memaparkan permukaan yang kasar pada hidrogel. Filem hidrogel yang digabungkan dengan nanopartikel kitosan/ZnO menunjukkan kesan penyahaktifan yang lebih kuat ke arah *S. aures* berbanding *E. koli*., dengan zon perencatan yang lebih menonjol dan baik bersaiz 25.0 mm yang dilihat pada *S. aures*. Keseluruhannya, kajian ini telah berjaya menentukan parameter operasi optimum untuk sintesis nanopartikel kitosan/ZnO.

ABSTRACT

Metal oxide nanoparticles possess unique physical and chemical characteristics linked to their nanoscale size. Moreover, the metal oxide nanoparticles have an excellent antibacterial activity which could be used as an effective antibacterial agent. The aim of this study is to characterize and determine the antibacterial properties of the chitosan/ZnO nanoparticles against Gram-positive and Gram-negative bacteria, and the effect of chitosan/ZnO nanoparticles incorporated with hydrogel film on antibacterial properties. Considering that, nanoparticles of zinc oxide (ZnO) has been synthesized using zinc nitrate salt, sodium hydroxide by a microwave-assisted method. The operating parameter such as a stabilizer (chitosan), power heating (400 W, 600 W and 800 W) and time heating (4 min, 6 min and 8 min) play an important role in the synthesised of chitosan/ZnO nanoparticles. The presence of chitosan prevented the nanoparticles from agglomeration by producing a milky solution of chitosan/ZnO nanoparticles without any suspensions. *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) were used as a test microorganism. Uv-vis spectrophotometer indicated the presence of the chitosan/ZnO by a single peak at 360 nm. The presence of a new peak at around 427 cm^{-1} in the FTIR spectrum confirmed the existence of the ZnO phase. XRD patterns show that the chitosan/ZnO nanoparticles materials are good crystallinity and completely matched the hexagonal-wurtzite structure. FESEM revealed that chitosan/ZnO nanoparticles were uniformly distributed with the mean value of size is 70 nm and spherical shape. The effect of power and time heating on the size of the chitosan/ZnO nanoparticles can be shown by a nanoparticles size distribution with the average of 30 to 90 nm. The increasing power and heating time resulted in the increasing of the size due to the nucleation of nanoparticles. Surface zeta potential was negative for all the nanoparticles and varied from -29.6 to -20.9 mV. Chitosan/ZnO nanoparticles resulted in higher inactivation of *S. aureus* compared to *E.coli.* The results showed that chitosan/ZnO nanoparticles have displayed an antibacterial inhibition zone against *S. aureus* and *E.coli* which 16.0 and 13.4 mm, respectively. The chitosan/ZnO nanoparticles displayed a same antibacterial effect of *S. aureus* compared to *E. coli* when tested using growth curve analysis. The occurrence of chitosan/ZnO nanoparticles in hydrogel film detected at 522 cm^{-1} of absorption spectra. The presence of chitosan/ZnO nanoparticles in hydrogel film displayed a rough surface of hydrogel film. Hydrogel film incorporated with chitosan/ZnO nanoparticles showed effective inactivation effect towards *S. aureus* compared to *E.coli*. The more prominent and good zone of inhibition with size 25.0 mm was seen on the *S. aureus* bacteria. Overall, this study has successfully determined the optimum operating parameter for the synthesized of the chitosan/ZnO nanoparticles.

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Å	Lattice parameter
β	Full width at half maximum
θ	The Bragg angle
λ	The X-ray wavelength of Cu-Kα radiation source
°	Degree
v/v	Volume per volume
ζ	Zeta potential

LIST OF ABBREVIATIONS

ABS	Absorbance
FDA	Food and drug administration
FTIR	Fourier transform infrared spectroscopy
FESEM	Field emission scanning electron microscope
GRAS	Generally recognized as safe
KCl	Potassium chloride
MIC	Minimum inhibitory concentration
MBC	Minimum bactericidal concentration
OD	Optical density
TSA	Tryptone soya agar
TSB	Tryptone soya broth
UVA	Ultraviolet A
UVB	Ultraviolet B
UV-vis	Ultraviolet visible
XRD	X-ray powder diffraction
ZnO	Zinc oxide

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