SYNTHESIS OF SOLID-SUPPORTED SILVER NANOCOMPOSITES FOR ANTIMICROBIAL AND DYE ADSORPTION APPLICATIONS

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DEDICATION

This thesis is dedicated to my family for their endless support and encouragement that kept me going forward until the end. I also dedicate this thesis to my wonderful husband, Nik Ismail and my delightful children, Iqbal, Iqmal, Nur Syahirah and Muhammad Asyraf, for their love and inspiration

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ABSTRACT

Dyes and pathogenic bacteria from industrial effluents and pharmaceutical waste are a major concern when they are not efficiently removed by conventional methods. This is because many of these compounds can adversely affect human health. Nanotechnology-driven materials are being explored as efficient adsorbers and antimicrobial agents for removal of dye and microorganisms in water bodies. Silver nanoparticles (AgNPs) are known for their broad spectrum of antimicrobial and adsorption properties. However, AgNPs tend to agglomerate in aqueous media, thus reducing their stability and eventually deteriorate their capability to be used as adsorbent and antibacterial agents. In this study, to overcome aggregration problem, silver nanoparticles were loaded on kaolinite (Kln) and synthesized using chemical and physical methods producing silver kaolinite naocomposites (Ag/Kln NCs). To further enhance stability, biopolymer Chitosan (Cts) was added to the Ag/Kln NCs resulting in silver kaolinite bionanocomposites (Ag/Kln BNCs). Ag/Kln NCs and BNCs were successfully developed using two synthesis methods which using sodium borohydride and gamma-irradiation in an aqueous system at room temperature and under ambient pressure. The effect of different concentration of silver nitrate solution, irradiation dose and addition of biopolymer Cts was also investigated. The synthesized Ag/Kln NCs were characterized by UV-Vis spectrophotometer, X-Ray Diffraction, Transmission Electron Microscopy, Field Emmission Scanning Electron Microscopy and Fourier Transform Infra-Red Spectroscopy analysis. The successful formation of Ag/Kln NCs was confirmed by the colour change due to reduction and band appearance at the range of 386-425 nm due to surface plasmon resonance. The intensity of the surface plasmon resonance peak increased with the increase of either irradiation dose or $AgNO_3$ molar concentration. The XRD pattern indicated both Ag/Kln NCs and BNCs had a face-centred cubic crystalline structure. Shapes of AgNCs and BNCs produced were approximately spherical with the average diameter range of 2.92 – 5.81 nm depending on the irradiation dose and concentration. The characterization of the samples revealed the successful loading of Ag on the surface of the Kln instead of being intercalated between the layers of the Kln framework. The addition of Cts reduced the minimum size range of both chemical and gamma synthesized Ag BNCs and extended their storage stability. Generally, the structure of Ag/Kln NCs and BNCs remained same as the raw Kln in both synthesis method. The resultant Ag/Kln NCs and BNCs displayed excellent adsorption capacity evaluated through elimination of Methylene Blue dyes from the liquid phase. This observation was confirmed by a decrease in absorbance maximum values. The rate constant for reduction reaction for both dyes using the synthesized NCs and BNCs was found to increase with the increase of Ag molar concentration and irradiation dose. The synthesized NCs and BNCs showed efficient antimicrobial activity against Gram-negative and Gram-positive bacteria represented by *Escherichia coli, Enterococcus faecalis, Proteus vulgaris,* Methicilin resistant *Staphylococcus aureus* and *Candida albicans.* Overall, stable Ag/Kln NCs and BNCs with adsorbent and antibacterial agent were synthesized using a clean and safe process and material. This indicates that these NCs have the potential of removing dyes and pathogenic microorganisms in water.

ABSTRAK

Bahan pewarna dan bakteria patogenik yang terhasil dari efluen industri dan sisa farmaseutikal merupakan isu yang serius sekiranya tidak dilupuskan secara efektif menggunakan kaedah konvensional. Ini kerana kebanyakan kompaun ini boleh mengakibatkan kesan buruk kepada kesihatan manusia. Penggunaan bahan yang dihasilkan melalui teknologi nano sebagai penjerap yang berkesan dan juga ejen antimikrob yang boleh diaplikasikan dalam penyingkiran bahan pewarna dan mikroorganisma dari takungan air masih dalam penyelidikan. Nanopartikel perak (NPs) mempunyai spektrum yang luas untuk kebolehan antimikrob dan penjerapan yang efektif. Namun, NPs mudah beraglomerasi di dalam medium akueus mengakibatkan tahap kestabilannya menurun dan mengurangkan keupayaan bahan ini untuk digunakan sebagai bahan penjerap dan ejen antimikrob. Kajian ini dijalankan bertujuan untuk mengatasi masalah aglomerasi dengan memuatkan NPs dengan kaolinite (Kln) dan disintesis menggunakan kaedah kimia dan fizikal di mana nanokomposit perak/kaolinite (Ag/Kln NCs) terhasil. Biopolimer, kitosan (Cts) juga ditambah kepada nanokomposit Ag/Kln NPs untuk meningkatkan tahap kestabilan bahan ini menghasilkan bionanokomposit perak kaolinite (Ag/Kln BNCs). Bahan Ag/Kln NCs dan BNCs in berjaya dihasilkan melalui dua kaedah sintesis iaitu menggunakan natrium borohidrida dan pengunaan sinaran-γ pada suhu bilik serta suhu yang ambien. Kesan penggunaan kepekatan yang berbeza bagi larutan nitrat perak ($AgNO_3$), dos sinaran dan penambahan biopolimer Cts juga dikaji. Nanokomposit Ag/Kln tersintesis dianalisis menggunakan UV-Vis spektroskopi, kaedah pembelauan sinar-X (XRD), Mikroskopi Transmisi Elektron (TEM), Mikroskopi Imbasan Elektron (FESEM) dan Spektroskopi Inframerah (FTIR). Penghasilan Ag/Kln NCs disahkan melalui perubahan warna disebabkan tindakbalas reduksi dan kemunculan tanda gelombang dalam lingkungan 386-425 nm disebabkan resonan permukaan plasmon. Kekuatan puncak resonan permukaan plasmon meningkat seiring dengan peningkatan dos sinaran atau kepekatan larutan $AgNO_3$. Analisis XRD dan TEM menunjukkan kedua-dua Ag/Kln NCs dan Ag/Kln BNCs mempunyai struktur kristal kubik berpusat muka, berbentuk sfera dengan diameter berjulat 2.92 - 5.81 nm berdasarkan dos sinaran dan kepekatan larutan. Analisis XRD juga membuktikan nanokomposit Ag yang dimuatkan ke permukaan Kln dan bukan diantara lapisan struktur Kln. Penambahan Cts mengurangkan julat saiz minimum bagi kedua-dua Ag BNCs yang disintesis secara kimia dan sinaran gamma, juga melanjutkan tempoh kestabilan penyimpanannya. Secara umum, struktur Ag/Kln NCs dan BNCs kekal seperti Kln asli dalam kedua-dua kaedah sintesis yang menunjukkan kestabilannya yang tinggi. Penilaian Ag/Kln NCs dan BNCs yang terhasil memaparkan kapasiti penjerapan terbaik melalui penyingkiran bahan pewarna MB dari fasa cecair. Fungsi NCs dan BNCs sebagai ejen penjerap dan antimikrob dapat ditingkatkan dengan peningkatan kepekatan molar Ag dan dos sinaran. Hasil sintesis NCs dan BNCs juga menunjukkan keberkesanan terhadap aktiviti antimikrob bagi *Escherichia coli, Enterococcus faecalis, Proteus vulgaris,* Methicilin resistant *Staphylococcus aureus* and *Candida albicans*. Secara keseluruhannya, Ag/Kln NCs yang disintesis melalui sinaran gamma adalah agen penjerap dan antimikrob yang berkesan bagi menyingkirkan bahan pewarna dan mikroorganisma patogenik dalam aktiviti pembasmian air.

TABLE OF CONTENT

CHAPTER	TITLE	PAGE
	DECLARATION	**iii**
	DEDICATION	**iv**
	ACKNOWLEDGEMENT	**v**
	ABSTRACT	**vi**
	ABSTRAK	**vii**
	TABLE OF CONTENT	**vii**
	LIST OF TABLES	**xii**
	LIST OF FIGURES	**xiv**
	LIST OF ABBREVIATIONS	**xix**
	LIST OF SYMBOLS	**xxi**
CHAPTER 1	**INTRODUCTION**	**1**
1.1	Research Background	1
1.2	Problem Statement	3
1.3	Research Objectives	5
1.4	Research Scope	5
1.5	Significance of Study	6
1.6	Outline of the Thesis	7
CHAPTER 2	**LITERATURE REVIEW**	**9**
2.1	Introduction	9
2.2	Silver Nanoparticle	10
2.3	Kaolinite as Ceramic Supports	17
2.4	Chitosan as a stabilizing agent	21
2.5	Synthesis of Silver Nanocomposites	24
	2.5.1 Chemical Synthesis of Nanocomposites	26
	2.5.2 Physical Method of Nanoparticle Synthesis	27
	2.5.3 Synthesis using Gamma Irradiation	27
2.6	Antimicrobial Activity	29

2.7 Adsorbent properties 35
2.8 Adsorption isotherm 37
2.8.1 Langmuir isotherm 37
2.8.2 Freundlich Isotherm 38
2.9 Summary 39

CHAPTER 3 RESEARCH METHODOLOGY 41
3.1 Introduction 41
3.2 Materials 42
3.3 Sample Preparation for Nanocomposites and Bionanocomposites material 43
3.3.1 Synthesis of Ag/Kln NCs Using Chemical Reduction Method 43
3.3.2 Synthesis of Ag/Kln/Cts BNCs Using Chemical Reduction Method 44
3.3.3 Synthesis of Ag/Kln NCs Using Gamma Irradiation Method 44
3.3.4 Synthesis of Ag/Kln/Cts BNCs Using Gamma Irradiation Method 45
3.4 Characterization of Ag/Kln Nanocomposites 45
3.4.1 Absorption Spectra 46
3.4.2 Transmission Electron Microscope 46
3.4.3 X-ray Diffraction Spectroscopy 47
3.4.4 Fourier Transform Infrared Spectroscopy 47
3.4.5 Field Emission Scanning Electron Microscope and Energy-Dispersive X-ray spectroscopy 47
3.5 Antimicrobial Activities Test of the NCS and BNCs 48
3.5.1 Preparation of Inoculum 48
3.5.2 Determination of CFU 49
3.5.3 Determination of MIC 50
3.6 Statistical Analyses 51
3.6.1 Antimicrobial Assay by Well Diffusion Method 51
3.7 Dye Adsorption Capabilities of Ag/Kln NCs 52
3.7.1 Adsorption of Methylene Blue Dye 52
3.7.2 Adsorption Isotherms 54
3.7.3 Adsorption Kinetics 55
3.7.4 Reusability Studies 56

3.8 Concluding Remarks 57

CHAPTER 4 RESULTS AND DISCUSSION 59

4.1 Introduction 59

4.2 Synthesis of Ag/Kln NCs with Chemical Reduction Method 59

4.2.1 Ultraviolet-visible Spectroscopy 60

4.2.2 Structural Analysis 62

4.2.3 Morphology and Particle Size Analysis 65

4.2.4 Surface Morphology and Elemental Composition Analysis 68

4.2.5 Chemical Functional Group Analysis 71

4.2.6 Adsorption Kinetics 74

4.2.7 Antimicrobial Evaluation of the Chemically Synthesized Ag/Kln NCs 79

4.3 Synthesis of Ag/Kln/Cts BNCs with Chemical Reduction Method 84

4.3.1 Ultraviolet Visible Spectroscopy 85

4.3.2 Structural Analysis 87

4.3.3 Morphology and Particle Size Analysis 89

4.3.4 Surface Morphology and Elemental Composition Analysis 92

4.3.5 Chemical Functional Group Analysis 95

4.3.6 Adsorbent kinetics 98

4.3.7 Antimicrobial Evaluation of the Chemically Synthesized Ag/Kln/Cts BNCs 101

4.4 Synthesis of Ag/Kln NCs with Physical Reduction Method 105

4.4.1 Ultraviolet Visible Spectroscopy 106

4.4.2 Structural Analysis 107

4.4.3 Morphology and Particle Size Analysis 109

4.4.4 Surface Morphology and Elemental Composition Analysis 113

4.4.5 Gamma Functional Group Analysis 116

4.4.6 Adsorption Kinetics 118

4.4.7 Antimicrobial Evaluation of the Gamma Synthesized Ag/Kln NCs 121

4.5 Synthesis of Ag/Kln/Cts BNCs with Physical Reduction Method 124

4.5.1 Ultraviolet Visible Spectroscopy 125
4.5.2 Structural Analysis 127
4.5.3 Morphology and Particle Size Analysis 129
4.5.4 Surface Morphology and Elemental Composition Analysis 133
4.5.5 Gamma Functional Group Analysis 137
4.5.6 Adsorbtion Kinetics 140
4.5.7 Antimicrobial Activity of Ag/Kln/Cts Gamma Synthesized 143
4.5.8 Stability in Different Medium 146

CHAPTER 5 CONCLUSION AND RECOMMENDATIONS 149
5.1 Research Outcomes 149
5.2 Future Work 150

REFERENCES 152

LIST OF PUBLICATIONS 185

LIST OF TABLES

TABLE NO.	TITLE	PAGE
Table 2.1	Silver nanocomposite properties and their potential applications in various fields	13
Table 2.2	Kaolinite based nanocomposite products	20
Table 2.3	Antimicrobial of AgNCs synthesize with different matrix and their applications	33
Table 3.1	List of chemicals, their details and uses	42
Table 4.1	The mean particle size of Ag/Kln NCs of different $AgNO_3$ concentration	65
Table 4.2	Summary of infrared bands of Ag/Kln NCs	73
Table 4.3	Pseudo-first order and second order kinetic parameters attained via the kinetic study	75
Table 4.4	Langmuir and Freundlich adsorption parameters	77
Table 4.5	Summarizes the minimum inhibition concentrations of Ag/Kln NCs against Gram-positive and Gram-negative bacteria and yeast	80
Table 4.6	The average particle size of Ag/Kln NCs of different concentration.	91
Table 4.7	Summary of infrared bands of Ag/Kln/Cts BNCs of different concentration	96
Table 4.8	Pseudo-first order and second order kinetic parameters attained via the kinetic study	98
Table 4.9	Langmuir and Freundlich adsorption parameters	99
Table 4.10	Summarizes the minimum inhibition concentrations of Ag/Kln/Cts NCs against Gram-positive and Gram-negative bacteria and yeast	101
Table 4.11	The average particle size of Ag/Kln NCs for different dose of gamma irradiation	112
Table 4.12	Summary of infrared bands of Ag/Kln NCs synthesized using different irradiation dose	116

Table 4.13	Pseudo-first order and second order kinetic parameters attained via the kinetic study	118
Table 4.14	Langmuir and Freundlich adsorption parameters	119
Table 4.15	Summarizes the average inhibition zone of Ag/Kln NCs against Gram-positive and Gram-negative bacteria and yeast	121
Table 4.16	The average particle size of Ag/Kln/Cts BNCs for different concentration of $AgNO_3$	129
Table 4.17	Summary of infrared bands of Ag/Kln/Cts BNCs synthesized using different irradiation dose	138
Table 4.18	Pseudo-first order and second order kinetic parameters attained via the kinetic study	140
Table 4.19	Langmuir and Freundlich adsorption parameters	141
Table 4.20	Average diameters of inhibition zones of Ag/Kln/Cts BNCs synthesised using gamma irradiation	143

LIST OF FIGURES

FIGURE NO.	TITLE	PAGE
Figure 1.1	Various applications of silver nanoparticles [27]	2
Figure 2.1	Different structures of nanoscale and their approximate sizes [58]	9
Figure 2.2	Various applications of AgNPs [99]	11
Figure 2.3	Synthesis mechanism of AgNPs [131]	15
Figure 2.4	Schematic illustration of the main physicochemical properties of nanoparticles which effects their interaction in biological systems [165]	16
Figure 2.5	Structure of kaolinite [image reproduced from the virtual museum of minerals and molecules [18]	18
Figure 2.6	Industrial application of kaolinite	21
Figure 2.7	Chemical structure of chitin and chitosan [218]	22
Figure 2.8	Various methods and approaches for synthesizing nanocomposites [235]	25
Figure 2.9	The four most prominent routes of antimicrobial action of AgNPs [11]	31
Figure 3.1	Overall methodology with all the objectives	41
Figure 4.2	Visual observation of the formation AgNCs synthesis indicated by the gradual colour change from light to dark brown at different $AgNO_3$ concentration.	60
Figure 4.3	UV–vis absorption spectra and corresponding photo of the synthesized Ag/Kln NC suspension synthesized by $NaBH_4$ at different concentration of $AgNO_3$ solution (0.5, 1, 2, 5 and 10%)	61
Figure 4.4	Powder XRD pattern of kaolinite and Ag/Kln NCs, 2θ range between 10° and 80°, operated voltage of 40 kV and a current of 30 mA	63
Figure 4.5	Small angle X-ray diffraction patterns of Kln, 0.5, 1, 2, 5 and 10.0% of Ag/Kln NCs by chemical synthesis.	64

Figure 4.6	TEM images of 0.5 and 1% Ag/Kln NCs and their particle size distribution prepared using $NaBH_4$ as a reducing agent	66
Figure 4.7	TEM images of 0.5, 1, 2, 5 and 10% Ag/Kln NCs and their particle size distribution prepared using $NaBH_4$ as a reducing agent	67
Figure 4.8	FESEM and EDX spectrum of Ag/Kln NCs with different concentration (0.5, 1, 2, 5 and 10%). synthesized using $NaBH_4$ as reducing agent and kaolinite as stabilizer	69
Figure 4.9	FTIR spectrum of Kaolin and Ag/Kln/NCs at different concentration of $AgNO_3$ are shown in figure above.	72
Figure 4.10	Pseudo-first order and second order kinetic model for the adsorption of MB dye on Ag/Kln NCs	76
Figure 4.11	Langmuir isotherm model for adsorption of methylene blue	77
Figure 4.12	The Freundlich isotherm model for adsorption of methylene blue	78
Figure 4.13	Electrostatic interaction proposed between silanol group on Kln surface and MB dye	79
Figure 4.13	Inhibition zone Ag/Kln NCs synthesised chemically at i) 0.5%; ii) 1.0%; iii) 2.0%; iv) 5.0%; and v) 10.0% towards *E. coli, E. faecalis, P. vulgaris,* MRSA and *C. albicans*. Gentamicin and Nystatin (Gm, 10 μg/ml) are used as positive control	81
Figure 4.14	Antibacterial activity of chemically synthesised Ag/Kln NCs samples and reference drug gentamycin against pathogen bacteria *E. coli, E. faecalis, P. vulgaris, MRSA* and *C. albicans*.	83
Figure 4.15	Photograph of Kln and Ag/Kln/Cts BNCs suspension at different $AgNO_3$ concentrations.	85
Figure 4.16	UV–vis absorption spectra of Ag/Kln/Cts BNCs synthesized by $NaBH_4$ at different concentration (0.5, 1, 2, 5 and 10% $AgNO_3$ solution)	86
Figure 4.17	Low angle X-ray diffraction patterns of Kln, 0.5, 1, 2, 5 and 10.0% of Kln/Cts BNCs by chemical synthesis	87
Figure 4.18	Wide angle XRD pattern of kaolinite and Ag/Kln/Cts BNCs, 2θ range between 10° and 80°, operated voltage of 40 kV and a current of 30 mA	88

Figure 4.19	TEM images of Ag/Kln/Cts of different concentration and particle size histogram	90
Figure 4.20	FESEM and EDX spectrum of kaolinite as carrier	92
Figure 4.20	FESEM and EDX spectrum of Ag/Kln/Cts BNCs with different concentration (0.5, 1, and 2%). synthesized using $NaBH_4$ as reducing agent and kaolinite and chitosan as stabilizer	93
Figure 4.21	FESEM and EDX spectrum of Ag/Kln/Cts BNCs with different concentration (0.5, 1, 2, 5 and 10%). synthesized using $NaBH_4$ as reducing agent and kaolinite and chitosan as stabilizer	94
Figure 4.22	FTIR spectrum of Kaolin, Chitosan and Ag/Kln/Cts BNCs at different concentration of $AgNO_3$ are shown in figure above	97
Figure 4.23	Pseudo-first order and second order of MB sorption onto Ag/Kln NCs	99
Figure 4.24	Langmuir isotherm model for adsorption of methylene blue	100
Figure 4.25	Freundlich isotherm model for adsorption of methylene blue	100
Figure 4.26	Inhibition zone Ag/Kln/Cts BNCs synthesised chemically at i) 0.5%; ii) 1.0%; iii) 2.0%; iv) 5.0%; and v) 10.0% towards *E. coli, E. faecalis, P. vulgaris*, MRSA and *C. albicans*. Gentamicin and Nystatin (Gm, 10 μg/ml) is used as positive control for bacteria and yeast respectively	103
Figure 4.27	Inhibition trend of microorganism s when subjected to treatments with chemically synthesised Ag/Kln/Cts BNCs.	104
Figure 4.28	Photograph of kaolinite and Ag/Kln suspension before irradiation and Ag/Kln NCs suspension after irradiation at different dose (7, 13. 20, 30, 40, 50 and 80 kGy).	105
Figure 4.29	UV–vis absorption spectra of aqueous Ag/Kln NCs prepared by γ-ray irradiation for different irradiation dose: 7, 13, 20, 30, 40, 50 and 80 kGy	106
Figure 4.30	Wide angle XRD pattern of kaolinite and Ag/Kln NCs, 2θ range between 10° and 80°, operated voltage of 40 kV and a current of 30 mA	108
Figure 4.31	Low angle X-ray diffraction patterns of Kln, 7, 13, 20, 30, 40, 65 and 80 kGy of Ag/Kln NCs by γ irradiation.	109

Figure 4.32	TEM micrographs of Ag/Kln NCs (0.02 M) prepared by γ-ray irradiation of 80 kGy dose.	112
Figure 4.33	FESEM image of Ag/Kln NCs synthesized using different dose of gamma irradiation (7, 13, 20, 30, 40, 50, 65 and 80kGy) as reducing agent and kaolinite as stabilizer	114
Figure 4.34	FESEM image of Ag/Kln NC (a) and the corresponding energy-dispersive X-ray spectroscopy (EDS) elemental mapping for C, O, Al, Si and Ag. The scale bar is 10 μm.	115
Figure 4.35	FTIR spectrum of Kaolin and Ag/Kln/NCs at different concentration of $AgNO_3$ synthesized using γ-irradiation	117
Figure 4.36	Pseudo-first order and second order of MB sorption onto Ag/Kln NCs	119
Figure 4.37	Langmuir isotherm model for adsorption of methylene blue	120
Figure 4.38	Freundlich isotherm model for adsorption of methylene blue	120
Figure 4.39	Inhibition zone Ag/Kln synthesised physically at i) 7 ii) 13 iii) 20 iv) 40; and v) 50 kGy towards *E. coli, E. faecalis, P. Vulgaris,* MRSA and. *C. albicans.* Gentamicin (Gm, 10 μg/ml) and Nystatin (Nys) is used as positive control	122
Figure 4.40	Inhibition trend of microorganisms when subjected to treatments with gamma synthesised Ag/Kln NCs	123
Figure 4.41	Photograph of kaolinite and Ag/Kln suspension before irradiation and Ag/Kln/Cts BNCs suspension after irradiation at different dose (7, 13. 20, 30, 40 and 50 kGy)	124
Figure 4.42	UV–vis absorption spectra of aqueous Ag/Kln/Cts BNCs prepared by γ-ray irradiation for different irradiation dose: 7, 13, 20, 30, 40 and 50 kGy	126
Figure 4.43	Wide angle XRD patterns 2θ range between 10° and 80°, of kaolin and Ag/Kln/Cts BNCs for different dosage	128
Figure 4.44	Low angle X-ray diffraction patterns of Kln, 7, 13, 20, 30, 40 and 50 kGy of Ag/Kln/Cts BNCs by γ irradiation	128
Figure 4.45	TEM micrographs of Ag/Kln/Cts BNCs (1×10^{-2} M) prepared by γ-ray irradiation of different doses: 7 kGy, 13 kGy and 20 kGy	131
Figure 4.46	TEM micrographs of Ag/Kln/Cts BNCs (1×10^{-2} M) prepared by γ-ray irradiation of different doses: (d) 30 kGy, (e) 40 kGy and (f) 50 kGy	132

Figure 4.47	FESEM and EDX spectrum of Ag/Kln/Cts BNCs synthesized using different dose of gamma irradiation (7, 13, 20, 30, 40 and 50 kGy) as reducing agent and kaolinite and chitosan as stabilizer	134
Figure 4.48	FESEM and EDX spectrum of Ag/Kln/Cts BNCs synthesized using different dose of gamma irradiation (7, 13, 20, 30, 40 and 50 kGy) as reducing agent and kaolinite and chitosan as stabilizer	135
Figure 4.49	TEM image of Ag/Kln/Cts NC (a) and the corresponding energy-dispersive X-ray spectroscopy (EDS) elemental mapping for C (b), O (c), Ag (d), Fe (e), and Co (f). The scale bar is 10 μm	136
Figure 4.50	FTIR spectrum of Ag/Kln/Cts BNCs at different concentration of $AgNO_3$ synthesized using γ-irradiation	139
Figure 4.51	Pseudo-first order and second order of MB sorption onto Ag/Kln NCs	141
Figure 4.52	Langmuir isotherm model for adsorption of methylene blue	142
Figure 4.53	Freundlich isotherm model for adsorption of methylene blue	142
Figure 4.54	Inhibition zone Ag/Kln/Cts synthesised with gamma irradiation at i) 7 ii) 13 iii) 20 iv) 40; and v) 50 kGy towards *E. coli, E. faecalis, P. vulgaris*, MRSA and C. albicans	144
Figure 4.55	Inhibition zone of Ag/Kln/Cts synthesised using gamma irradiation at i) 7 ii) 13 iii) 20 iv) 40; and v) 50 kGy towards *E. coli, E. faecalis, P. vulgaris* and MRSA. Gentamicin (Gm, 10 μg/ml) and Nystatin (10 μg/ml) is used as positive control	145
Figure 4.56	Comparison of stability of suspension of Ag/Kln NCs without Cts and Ag/Kln BNCs with Cts	147

LIST OF ABBREVIATIONS

Ag/Kln	-	Silver Kaolinite
AgNCs	-	Silver nanocomposites
$AgNO_3$	-	Silver nitrate
AgNPs	-	Silver nanoparticles
ANOVA	-	Analysis of Variance
ATCC	-	American Type Culture Correction
BET	-	Brunauer-Emmett-Teller
BNCs	-	Bionanocomposites
C. albicans	-	*Candida albicans*
Cts	-	Chitosan
DIW	-	Deionized water
DMSO	-	Dimethyl sulfoxide
DW	-	Distilled water
E. coli	-	*Escherichia coli*
E. faecalis	-	*Enterococcus faecalis*
EDX	-	Energy Dispersive X-ray
FESEM	-	Field Emission Scanning-Electron Microscopy
FTIR	-	Fourier Transform Infrared Spectroscopy
g/l	-	Gram per liter
kGy	-	Kilo gray
Kln	-	Kaolinite
M	-	Molar
MB	-	Methylene blue
MIC	-	Minimum inhibitory concentration
ml	-	Mililitre (s)
mm	-	Millimetre (s)
mM	-	Millimolar
MRSA	-	Methicilin resistant *Staphylococcus aureus*
NaOH	-	Sodium hydroxide
NCs	-	Nanocomposites
nm	-	Nanometer
no.	-	Number

NPs	-	Nanoparticles
P. vulgaris	-	*Proteus vulgaris*
pH	-	Potential of hydrogen
SAED	-	Surface Area Electron Diffraction
SEM	-	Scanning Electron Microscopy
SPR	-	Surface Plasmon Resonance
TEM	-	Transmission Electron Microscopy
Temp.	-	Temperature
UV-Vis	-	Ultraviolet / Visible spectroscopy
WHO	-	World Health Organization
XRD	-	X Ray Diffraction spectroscopy

LIST OF SYMBOLS

Ag	-	Silver
Ag^{+}	-	Silver ions
δ	-	Minimal error
%	-	Percentage
°C	-	Degree celsius
g	-	Gram
L	-	Liter
~	-	Approxiamately
±	-	Error
&	-	And
µL	-	Micro Liter
µM	-	Micro mole
À	-	Angstrom
γ	-	Gamma
λ	-	X-ray wavelength
θ	-	Bragg angle

CHAPTER 1

INTRODUCTION

1.1 Research Background

Water is a vital component of life that plays an essential role in human and animal lives and access to quality water should be a priority [1]. However, pollution compromises water quality and the quantity discharged into wastewater treatment plants from different sources. Dyes and phatogenic bacteria from industrial effluents and pharmaceutical waste are a major concern when they are not efficiently removed because many of these compounds can adversely affect human health [2, 3]. This untreated or partially treated industrial effluent containing synthetic dye compounds is eventually discharged into water systems, where it causes damage to the aquatic ecosystem [4]. Several treatment strategies have been developed based on hybrid systems, filtrations, bioremediation, UV irradiation, photocatalysis, and chlorination [1, 4]. However, they are not very efficient when the effluent has a low content of suspended colloidal particle and a high load of organic matter. A promising alternative to the treatments mentioned is adsorption, due to its simplicity of operation and effectiveness [4]. Therefore, the search for new adsorbent materials with antimicrobial properties that can be used to remedy aquatic contamination has been stimulated.

NPs are diversely ultrastructured particles within the range from 1 to 100 nm [5, 6] with unique properties such as size-dependent qualities, high surface-to-volume ratio and unique optical properties at a critical length scale [7, 8]. Metals NPs exhibit remarkable physical, chemical, and biological properties and are being explored as a promising approach to combating resistance to antibiotic. Since they effectively bridge bulk materials and atomic or molecular structures, metal NPs are now an important area of scientific research.

Silver nanoparticles (AgNPs) are among the most favoured metal NPs due to the multiple functionalities making them significant in the field of medicine and health care. Their broad-spectrum antimicrobial activity [10], antifungal [11, 12], anti-cancer [13], anti-inflammatory [14], antiviral [15], anti-angiogenesis [16] and antiplatelet [17] activities, has made them the most used NPs to treat various diseases and infections [18]. Silver has also been most studied NPs for use in the disinfection of various waterborne disease-causing by microorganisms [19, 20]. AgNPs also demonstrated good adsorption properties in the field of dye detoxification, heavy metal removal and remediation [21–25] and incorporated into a variety of household and consumer products [26] (Figure 1.1) including textiles, cosmetics, paints, coatings, sensor, agricultural and food packaging, with a relatively high usage compared to other metals and alkaline earth metals in NPs applications [5]. Thus, AgNPs are promising tools for applicability in various wastewater ecosystems.

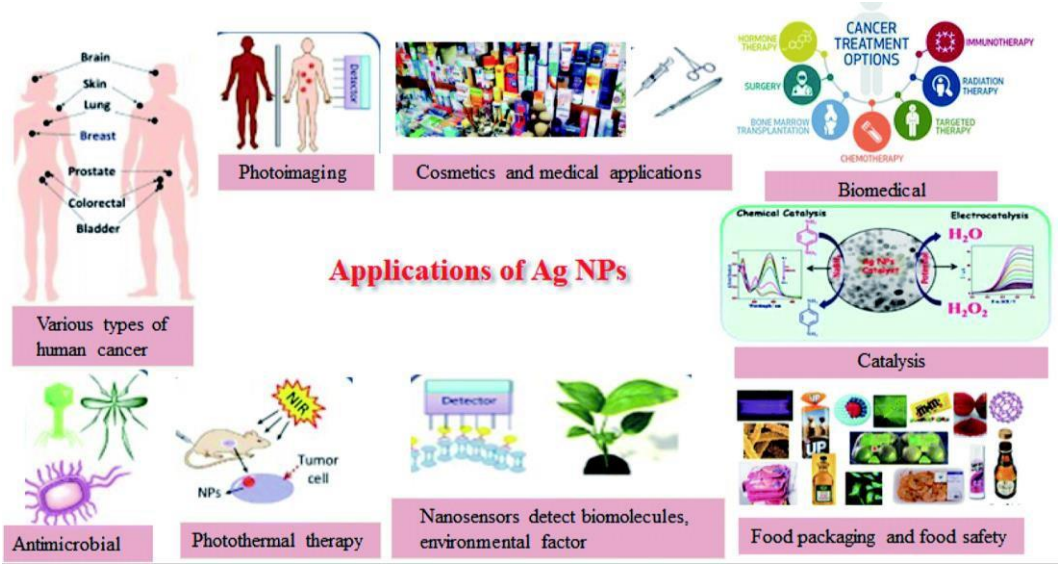

Figure 1.1 Various applications of silver nanoparticles [27]

Ag NPs tend to agglomerate in solution which will reduce its functionality as an adsorber and antimicrobial agent. To stabilize, AgNPs can be impregnated on a supporting material such as clay which was proved to remove metal cations [28]. AgNPs can be engineered to combine two or more materials, to develop nanocomposites (NC) with desirable properties with multi-functionality and greater performance [29, 30]. To further enhance stability of the NCs, biopolymer such as chitosan (Cts) were added to produce silver bionanocomposits (BNCs).

Generally, synthesis of AgNPs falls into three broad categories namely, chemical, physical and green [31, 32]. Chemical methods have been used more often [33] due to its ease of production, low cost, high yield and able to synthesize AgNPs on a large scale [34]. However, the use of chemical reducing agents is harmful to living organisms and poses hazardous risk to the environment [20]. This leads to the advancement of green syntheses, where the use of biological entities like microorganisms, plant extract or plant biomass to produce NPs [35]. Green synthesis is a good alternative to chemical and physical methods as it does not employ toxic chemicals and hence proving to become an eco-friendly process. The disadvantage of green synthesis is, there is a significant variation in chemical compositions of plant extract of same species when it collected from different parts of world and may lead to different results in different laboratories [36]. Furthermore, the combination of the already existing biomolecules such as proteins, polysaccharides, terpenoids in the plant extracts can be complicated affect the structure of NPs [37].

In comparison, synthesis using γ-irradiation has been shown to have a variety of highly advantageous properties [38]. Synthesis using γ-irradiation is simple, can be performed using aqueous system, at ambient pressure and room temperature. While the AgNPs produced are fully reduced without trace of chemical and the reductant is generated uniformly in the solution and suitable for large-scale production [39]. Beside biological resources, green chemistry is also defined as approaches of clean chemistry, and environmentally benign chemistry. Therefore, irradiation technique is considered a "green technique" as it does not require chemical initiator for reduction unlike the conventional methods [40].

1.2 Problem Statement

Two major limitations to apply AgNPs in large scale in water or wastewater treatment, are aggregation and instability. Aggregation and instability will restrict the abilities to adsorb contaminants and disinfect pathogenic microorganisms progressively [41]. In this regard, the incorporation of AgNPs on insoluble supports

has been verified as an efficient approach to enhance the dispersity and stability of AgNCs, thereby improving their adsorption efficiency [31, 42–44].

Kaolinite (Kln) a type of clay found abundant in nature, has been used as a carrier of antibacterial inorganic material with favorable results. This is due to its high ion-exchange capacity, high surface area, excellent sorption capacity, a negative surface charge, chemical inertness and low or null toxicity [45, 46]. Therefore, exploring the possibility of embedding AgNPs on Kln in developing silver kaolinite nanocomposites (Ag/Kln NCs) is one of the main objectives of further efforts to overcome this limitation and provides a promising alternative. To further enhance the stability of the produced AgNCs, the application of chitosan (Cts), a polymer binder was studied. Ag based composites with Cts demonstrated effective bionanocomposites (BNCs) with enhanced antibacterial performance and an effective stabilizer for silver with great biocompatibility, biodegradability and hydrophilic properties, which endow them opportunities for various applications [47, 48]. The incorporation of Cts, results a hybrid polymer, silver kaolinite chitosan bionanocomposites (Ag/Kln/Cts BNCs) which enhanced the properties of both polymer and NCs.

Though, there are several chemical and physical approaches available for the synthesis process, chemical reduction leaves residual reducing agents and poses hazardous risk to the environment and are relatively more expensive [34]. Thus, a better alternative which is ecofriendly, cost effective and provides single step is required for the synthesis of NPs [49–53]. γ-irradiation provides a more powerful and eco-friendly technique to synthesize NCs of controlled size and shape, better distribution and prevention of NCs aggregation [54–56]. High energy radiation, reduce metal ions homogeneously, without the use of reducing agents and is considered a "green technique" as it does not require chemical initiator for reduction unlike the conventional methods, producing highly pure, narrow particle size distributions and stable at room temperature [57].

1.3 Research Objectives

Based on the background of the study and the problem statements addressed, the main objective of this study is to develop stable NCs with good adsorption and antimicrobial properties through a clean technique and environmentally safe material. Thus, Ag was embedded in Kln as a support to avoid aggregation and to enable the NCs to be retrieved and reuse and is a crucial part in saving the environment, due to the release of AgNCs in soil or water.

This study investigates the adsorption capability antimicrobial activity and of Ag/Kln NCs and BNCs obtained by chemical and gamma irradiation synthesis method including:

(a) To characterize and optimize Ag/Kln NCs and Ag/Kln/Cts BNCs synthesized using sodium borohydride and gamma irradiation methods.

(b) To investigate the antimicrobial activity of all the synthesized Ag/Kln NCs and Ag/Kln/Cts BNCs against gram positive and negative bacteria and yeast.

(c) To evaluate the adsorbent properties of all the synthesized Ag/Kln NCs and Ag/Kln/Cts BNCs against methylene blue.

1.4 Research Scope

In order to achieve the above-mentioned objectives, several scopes are outlined as follows:

(a) In this work, silver a precursor was loaded on a solid kaolinite for stabilization and to prevent aggregation. One portion of the produced Ag/Kln NCs is further enhanced by adding chitosan biopolymer resulting silver kaolinite chitosan to produce Ag/Kln/Cts BNCs. Both Ag/Kln NCs and Ag/Kln/Cts BNCs were synthesized according to two different synthesis techniques, the chemical

technique using $NaBH_4$ and clean reduction technique using gamma irradiation as the reducing agent. Effect of different silver concentration and dose of gamma irradiation to the size and shape of NCs were investigated.

(b) The effect of physicochemical parameters that influences the size, shape and distribution of AgNCs, such as effect of different synthesis techniques, silver salt concentrations, radiation dose, effect of polymer, were studied. The NCs and BNCs were characterized by UV-Vis, XRD, TEM, FT-IR and FESEM-EDX analysis was used to determine the properties of AgNCs.

(c) At the next stage, the antibacterial activity of studied samples against *Escherichia coli, Enterococcus faecalis, Proteus vulgaris, Methicilin resistant Staphylococcus aureus* and *Candida albicans* was determined by Minimum Inhibition Concentration assay.

(d) To evaluate the sorption properties of all the NCs and BNCs as adsorbents for the removal of dye from water. The effect of initial methylene blue concentration, adsorbent dosage, and kinetic parameters influencing the sorption processes were also studied.

1.5 Significance of Study

AgNCs and BNCs constitute a very promising approach for the development of new antimicrobial systems due to their remarkable adsorption and antimicrobial, properties. To avoid aggregation and ensure sufficient stability, Kln was used as a support and carrier and Cts was added as a stabilizing agent. Kln has excellent adsorption ability, which are especially interesting for the impregnation of nanosized metals. However, Kln has strong hydrogen bond that does not allow intercalation and can only be broken by using organic solvents. Kln based NCs were not much explored for biomedical field in the past for this reason. Previous discoveries have proven that synthesis method influence the characteristic of the NCs produced. Chemical approach is the most popular method due to its convenience and inexpensive equipment, but it leaves hazardous chemical residues and produces chemical waste. An alternative

technique which does not involve chemical residues are much sought after especially for biological applications.

Therefore, in this work, two synthesis methods were applied, the common chemical synthesis and a clean technique using gamma irradiation. The produced NCs are compared to study and understand the influence of diverse synthesis methods, reducing agents and stabilizers to the size, distribution, morphological shape, and surface properties of the AgNCs. As the attention has been also focused on the synthesis method with minimal or no environmental impact, water was selected as the medium in this study and no other solvents was used. The novelty of this work is the development of NCs with high antimicrobial and adsorption capabilities through a clean technique using gamma irradiation method and environmentally safe material. This environmentally friendly and simple synthesis can be performed at room temperature without using harsh organic solvents. This work aims to produce Ag/Kln NCs with distinguished antimicrobial and adsorption activities using simple yet controllable environmentally friendly synthesis method.

1.6 Outline of the Thesis

This thesis consists of five chapters. Chapter 1 introduces the research background and highlights the objectives, the research scopes, and the significance of study.

Chapter 2 is a literature review of past and current issues of NPs research related to the types of synthesis methods, carriers, stabilizers used related to silver nanoparticle. This chapter also discusses various combination of metal, stabilizers and polymers resulting in silver nanocomposites with various properties. The properties and applications were briefly described. An overview of adsorption and antimicrobial properties of various methods were used as comparison. Research gap related to this project based on the previous studies were identified.

Chapter 3 shows a detailed explanation of methodology carried out to achieve all the objectives of the study. This includes the materials used and characterization methods including UV-Vis, XRD, TEM, FT-IR and FESEM-EDX analysis to determine the properties of AgNCs. A flowchart of research methodology is presented at the beginning of the chapter to provide an overview of how the research was conducted.

Chapter 4 presents the results of physico-chemical characterization for synthesized Ag/Kln NCs and BNCs using silver nitrate solution of different concentration and dose of irradiation. The evaluation of adsorption and antimicrobial properties for synthesized Ag/Kln NCs and BNCs were discussed.

Chapter 5 concludes the research work and all the findings from the study were summarised. Lastly, several suggestions were also proposed to improve the research study.

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LIST OF PUBLICATIONS

Journal with Impact Factor

1. Salmah Moosa, Anis Nadia Mohd Faisol Mahadeven , Kamyar Shameli, 2019, Synthesis of Silver Nanoparticles in Kaolinite and Their Antibacterial Behaviour, International Journal of Engineering Research & Technology (IJERT) Volume 08, Issue 08 (August 2019)

Indexed Journal

1. Moosa, S., Mahadeven, A. N. M. F., & Shameli, K. (2021). Physiochemical synthesis of Silver/Kaolinite nanocomposites and study their antibacterial properties. Journal of Research in Nanoscience and Nanotechnology, 1(1), 1-11.

2. Mossa, S., & Shameli, K. (2021). Gamma Irradiation-Assisted Synthesis of Silver Nanoparticle and Their Antimicrobial Applications: A Review. *Journal of Research in Nanoscience and Nanotechnology*, *3*(1), 53-75.

Non-Indexed Journal

Indexed Conference Proceedings

1. Anis Nadia Mohd Faisol Mahadeven, & Salmah Moosa (2019). Antimicrobial Susceptibility Testing of Silver Nanoparticles: A Comparison Between Chemical Synthesis and Physical Synthesis Using γ Irradiation. In Fairuz Faisal (Ed.). Proceedings of the Research and Development Seminar Nuklear Malaysia 2018 Nuclear Technology Towards Sustainable Development, (p. 275). Malaysia: Malaysian Nuclear Agency.

2. Salmah Moosa, Anis Nadia Mohd Faisol Mahadeven and Kamyar Shameli, 2018, A Comparative Study Of Chemical And Gamma-Irradiation Method For Synthesis of Silver Nanoparticles on Kaolinite, Research & Development Seminar Nuclear Malaysia.

3. Salmah Moosa, Anis Nadia Mohd Faisol Mahadeven and Kamyar Shameli, 2018, Irradiaation Using γ-Ray Route to Synthesis Silver Nanoparticles: Characterization And Antibacterial Evaluation, WIN Conference 2018, Nuclear Malaysia.

4. Salmah Moosa & Anis Nadia Mohd Faisol Mahadeven 2018, Bio-Nanotechnology and It's Role in Agriculture and Food, Nucl ear Technical Conference 2019, Nuclear Malaysia.

5. Salmah Moosa, Anis Nadia Mohd Faisol Mahadeven and Maizatul Akmam Md. Nasir, 2020, Synthesis by Gamma Irradiation Enhance Antimicrobial Properties of Silver Nanoparticles, Research & Development Seminar 2020, Nuclear Malaysia.

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