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LIST OF ABBREVIATIONS

Ag	Silver
APTMS	Aminopropyl-trimethoxysilane
BCC	Base-centered cubic
Bi	Bismuth
$Bi(OH)_3$	Bismuth hydroxide
Bi_2O_3	Bismuth oxide
Bi_2Se_3	Bismuth selenide
BSA	Bovine Serum Albumin
CT	Computed tomography
DMEM	Dulbecco's Modified Eagle Medium
DNA	Deoxyribonucleic acid
DTPA	Diethylenetriaminepentaacetic
EDTA	Ethylenediaminetetraacetic acid
EDX	Energy Dispersive X-Ray Analysis
EGFR	Epidermal growth factor receptor
EPR	Enhanced permeability and retention
ESR	Erythrocyte sedimentation rate

FBS	Fetal bovine serum
FCC	Face-centered cubic
FTIR	Fourier-transform infrared spectroscopy
Gd	Gadolinium
H_2O	Water
HER-2	Human epidermal growth factor
HSA	Human serum albumin
IC_{50}	The half maximal inhibitory concentration
ICDD	International Centre for Diffraction Data
KOH	Potassium hydroxide
LSM	Strontium-doped lanthanum manganate
MBE	Molecular beam epitaxial
Mcf-7	Michigan Cancer Foundation 7: Type of breast cancer cells
MOCVD	Metalorganic Chemical Vapor Deposition
MRI	Magnetic resonance imaging
MTX	Methotrexate
Mw	Molecular weight

Na_2SO_4	Sodium sulfate
NaOH	Sodium hydroxide
NPs	Nanoparticles
O_2	Oxygen
OH^-	Hydroxide ions
PBS	Phosphate buffer saline
PDT	Photodynamic therapy
PEG	Polyethylene glycol
PFC	Perfluorocarban
Pt	Platinum
ROS	Reactive oxygen species
RT	Radiotherapy
SEM	Scanning electron microscopy
SER	Sensitisation enhancement ratio
Si	Silica
STEM	Scanning transmission electron microscope
TEM	Transmission electron microscopy
TiO_2	Titanium dioxide

TMBi	Trimethylbismuth
VS	Vapor-solid
XRD	X-Ray Diffraction
YSB	Yttria-stabilized bismuth oxide

LIST OF SYMBOLS

α	Alpha phase (monoclinic)
Z	Atomic number
β	Beta phase (tetragonal)
G	Degree of ellipticity
δ	Delta phase (face-centered cubic)
e_r	Diaelectric permittivity
e_r	Dielectric permittivity
γ	Gamma phase (body-centered cubic)
g	Gram
Gy	Gray (radiation dose)
h	Hour
KeV	Kilo-electronvolt
kPa	Kilopascal
kVp	Kilovolt photon
MeV	Mega-electronvolt
MV	Megavolt
$\mu g.mL^{-1}$	Microgram per milliliter

µL	Microlitre
µM	Micromolar
ml	Millilitre
mm	Millimeter
mM	Millimolar
nm	Nanometer
%	Percent
V	Potential
P	Probability
η	Refractive index
rpm	Revolutions per minute
θ˚	Theta

SINTESIS NANOPARTIKEL BISMUT OKSIDA UNTUK APLIKASI RADIOTERAPI

ABSTRAK

Nombor atom tinggi (Z) nanopartikel bismut oksida (Bi_2O_3 NPs) mempunyai penembusan sel yang lebih banyak dan kurang kesan buruk daripada pemeka sinaran konvensional. Pelbagai saiz Bi_2O_3 NPs telah berjaya dihasilkan dengan menggunakan kaedah hidroterma dan digunakan untuk aplikasi radioterapi. Beberapa sintesis parameter telah dikaji: kesan perbezaan suhu dan kesan perbezaan masa hidroterma, kesan perbezaan kepekatan bismuth nitrat, $Bi(NO_3)_3$, dan, kesan perbezaan kepekatan polietilena glikol (PEG). Sifat-sifat Bi_2O_3 NPs telah dikaji untuk menentukan kehadiran fasa, penghabluran, morfologi, kehadiran unsur dan saiz partikel. Analisa XRD membuktikan Bi_2O_3 tulen dengan fasa monoklinik telah dihasilkan (Kod Rujukan ICDD: 98-000-6260). Saiz Bi_2O_3 NPs didapati meningkat dengan peningkatan suhu dan masa tindakbalas hidrotherma. Walaubagaimanapun, apabila kepekatan $Bi(NO_3)_3$ meningkat, saiz partikel Bi_2O_3 NPs menurun disebabkan oleh kurangnya resapan ion ke dalam nucleus. Pemerhatian terhadap morphologi menunjukkan Bi_2O_3 NPs berbentuk rod. Nanopartikel yang disalut dengan PEG tidak menunjukkan sebarang peningkatan saiz. Berdasarkan analisa FTIR, keamatan Bi_2O_3 NPs berkurang apabila kepekatan PEG meningkat kerana molekul PEG dapat diserap ke permukaan kristal Bi melalui ikatan Bi–O. Bi_2O_3 NPs yang dihasilkan kemudiannya menjalani kajian ketoksikan dan radioterapi. Kajian ketoksikan terhadap Bi_2O_3 NPs tidak memberi kesan toksik kepada sel barah payudara (mcf-7) pada kepekatan 0.05 µM - 50 µM. Prestasi radioterapi oleh Bi_2O_3 NPs yang telah dihasilkan diperoleh dengan mengira nisbah peningkatan pemekaan (SER). Akhirnya, 60 nm Bi_2O_3 NPs diperolehi sebagai keputusan yang optimum dengan SER 1.26.

SYNTHESIS OF BISMUTH OXIDE NANOPARTICLES FOR RADIOTHERAPY APPLICATION

ABSTRACT

High atomic number (Z) of bismuth oxide nanoparticles (Bi_2O_3 NPs) has more cell penetration and less adverse effects than conventional radiosensitisers. In this work, various sizes of Bi_2O_3 NPs were successfully synthesised using hydrothermal method. Several synthesis parameters were studied: effect of hydrothermal reaction temperature, effect of hydrothermal reaction time, effect of bismuth nitrate, $Bi(NO_3)_3$ concentration and effect of polyethylene glycol (PEG) concentration. The properties of Bi_2O_3 NPs were then characterised to determine the phase presence, crystallinity, morphology, elemental presence and size of nanoparticles. The as-synthesised Bi_2O_3 NPs was in monoclinic Bi_2O_3 phase (ICDD 98-008-5622). Increasing reaction temperature and time increased the size of Bi_2O_3 NPs. However, as the $Bi(NO_3)_3$ concentration increased, the particle size of Bi_2O_3 NPs decreased due to less ions diffusion per nuclei. The morphology observation showed that Bi_2O_3 NPs were in rods form. Coating with PEG did not show any increase in nanoparticles size. Based on Fourier-transform infrared spectroscopy (FTIR) analysis, by increasing the PEG concentration, the intensity of Bi_2O_3 NPs band diminished because PEG molecules could adsorb onto the surface of Bi crystals through Bi–O bonding. After that, the produced Bi_2O_3 NPs were subjected to cytotoxicity analysis and radiotherapy. Bi_2O_3 NPs did not induce cytotoxicity in breast cancer (mcf-7) cell lines at concentration from 0.05 µM to 50 µM. The radiotherapy performance of the as-prepared Bi_2O_3 NPs was obtained by calculating the sensitisation enhancement ratio (SER). The optimum result was obtained for 60 nm Bi_2O_3 NPs with SER of 1.26.

CHAPTER ONE

INTRODUCTION

1.1 Research background

Nanomaterials with diameters ranging between 1 and 100 nanometers, are natural bridges between molecules and extended solids. Nanomaterials are complex of many-electron systems, where reduced sizes and quantum confinement of electrons and phonons give birth to fascinating new effects, potentially tunable with particle size (Van Dijk et al., 2005). The use of nanomaterials in medical application has been developed into a promising research area known as nanobiotechnology (Ahmed et al., 2012). The application of nanomaterials in medical field is very much welcomed nowadays especially in biological labelling and sensing, and cancer therapy. The main advantage of using nanomaterials is that its properties can be tuned or manipulated in order to meet the specific requirements of particular applications (Sumer and Gao, 2008).

Recently, application of nanomaterials in cancer therapy has gained a great interest. One of the main modalities for the treatment of cancer is radiotherapy. Radiotherapy is a critical component of the modern approach for curative and adjuvant treatment of cancers. Radiotherapy controls the growth of cancerous cells by bombardment with ionizing radiation, causing deoxyribonucleic acid (DNA) damage by direct ionization or through generation of free radicals by ionization of water or oxygen molecules. Sufficient damage to DNA can halt cell growth and prevent metastasis. The primary drawback is collateral damage: there is little distinction in

absorption between healthy and malignant tissues, and thus doses must be limited in order to mitigate unwanted damage to the tumour surroundings (Cooper et al., 2014).

Nanomaterials with high atomic number (Z), have recently received wide interests for their excellent radiosensitisation effect in order to overcome the weaknesses, as well as to selectively increase the radioactivity deposition in the cancer region (Kaur et al., 2013, Xiao et al., 2011). The strong photoelectric absorbance capacities and the numerous short-range secondary electrons generated on the particle surface can accelerate the DNA break and thus kill more tumour cells when undergo the radiotherapy. Meanwhile, nanoparticles down to 100 nm in diameter could increase tumour accumulation in the intravenous administration by virtue of the enhanced permeability and retention (EPR) effect of leaky tumour vasculature. Thus, it is believed that the nanometer size radiosensitiser could efficiently integrate the radiosensitisation effect as well as showing a great potential in delivering sufficient radiation dosage to the targeted tumour.

Gold nanoparticles (AuNPs) (Z = 79) is the most well developed nanoparticle platform. Hainfeld et al. (2013) reported that gold nanoparticles (AuNPs) (Z = 79) showed high-resolution CT-scan when AuNPs was injected into mice and evidently increased approximately three times local radiation dose in radiotherapy. The AuNPs was found to be effective as radiosensitiser to kill tumour cell by surrounded or loaded to specific cancer cells. However, the high cost of gold could limit the widespread use of AuNPs. The cost of 1kg bismuth is around USD29, while 1kg gold is around USD39 000 (Argus 2018, Goldprice 2018). Bismuth nanoparticles (BiNPs) (Z = 83), is a cost effective alternative and a better candidate for high Z radiosensitiser research. Furthermore, bismuth compounds are biodegradable and biocompatible with a long history in biomedicine. To date, most investigations centering on the use of bismuth

for nanoparticulate radiosensitiser. However, comprehensive cytotoxicity information of BiNPs is not available. BiNPs was detected in the nervous system and other organs of the mice (Stoltenberg et al., 2003). Therefore, the approval of Bi as a non-toxic alternative to Pb has been questioned by scientists who raised concerns about the lack of knowledge about Bi toxicity.

Bismuth-based nanoparticles have been studied as radiosensitiser. For example, bismuth sulphide nanoparticles (Bi_2S_3 NPs) could be successfully realized on the tumour-bearing mice model in the X-ray radiotherapy research, resulting in significantly higher inhibition effect for the tumour growth (Yao et al., 2014). Bismuth selenide nanoparticles (Bi_2Se_3) had exhibited remarkably enhanced DNA damage suggesting the strong radiotherapy enhancement effect of Bi-based nanoparticles (Song et al., 2015). Most recently, bismuth oxide nanoparticles (Bi_2O_3 NPs) also has be used to kill tumour in radiotherapy. Bi_2O_3 NPs also has been used in many medical and cosmetic applications for many years (Kim et al., 2008). Stewart et al. (2014) showed that Bi_2O_3 NPs could be tailored with different oxygen contents to induce cell proliferation or toxicity. Another research highlighted that a theranostic system based on Bi_2O_3 NPs would be highly effective in the treatment of cancer (Bogusz et al., 2014). Thus, it is suggested that Bi_2O_3 NPs is an ideal alternative to evaluate the therapeutic effect of nanomedicine based radiosensitiser in the radiotherapy research.

Therefore, several approaches have been employed to synthesis Bi_2O_3 NPs in materials science and engineering. Bi_2O_3 NPs has been synthesised via sol gel method (Armelao et al., 1998, Xiaohong et al., 2007), citrate gel process (Anilkumar et al., 2005), oxidation of bismuth metal at 800 °C or thermal decomposition of bismuth salt solution (Krüger et al., 2000, Davidge, 1986), flame spray pyrolysis (Mädler and Pratsinis, 2002), polyol method (Jungk and Feldmann, 2001) and atomic-pressure

chemical vapor deposition (CVD) (Shen et al., 2007). Although these methods have been proven to be successful in the synthesis of Bi_2O_3 NPs, they normally require high temperature heat treatment, long synthesis period, and post treatment, which is far from low cost and apparently require sophisticated equipments. Hence, a more simple synthesis method, which is precipitant-free, additive-free, and low cost, is sought after for the synthesis of Bi_2O_3 NPs. A hydrothermal method is a powerful method in synthesis of Bi_2O_3 NPs at a considerably low temperature, energy saving and cost effective benefits (Wu et al., 2011). The hydrothermal method also has the advantages such as controllable particle size, morphology and the degree of crystallinity by simply changing the experimental parameters (Xu et al., 2005). Table 1.1 shows in detail the comparison of several approaches to produce Bi_2O_3 NPs.

Table 1.1: The comparison of several approaches to produce Bi_2O_3 NPs

Method	Advantages	Disadvantages	Reference
Sol-gel method	Successful in producing Bi_2O_3 NPs	• Substrate dependent • Non-uniform thermal expansion	(Armelao et al., 1998, Xiaohong et al., 2007)
Citrate gel method			(Anilkumar et al., 2005)
Thermal decomposition method		High temperature	(Krüger et al., 2000)
Flame spray pyrolysis method		• Requires line of sight to the surface being coated • Difficult to handle	(Mädler and Pratsinis, 2002)
Polyol method		Required alkali as precipitation reagent	(Jungk and Feldmann, 2001)