SYNTHESIS OF SILICA NANOCOLLOIDS ENCAPSULATED DYES FOR BIO-IMAGING AND BIO-LABELING APPLICATIONS

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SYNTHESIS OF SILICA NANOCOLLOIDS ENCAPSULATED DYES FOR BIO-LABELING AND BIO-IMAGING APPLICATIONS

by

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

A549	Human epithelial cells
Ag	Silver
APTS	3-aminopropyltriethoxysilane
Au	Gold
CBMN	Cytokinesis Block Micronucleus Assay
CCC	Critical coagulation concentration
Cd^{2+}	Cadmium ion
CdSe	Cadmium selenide
cfu	Colony forming unit
DCFH	Dichloro-dihydro-fluorescein diacetate assay
DI	De-ionized water
DiI	1,1%-dioctadecyl 3,3,3%,3% tetramethylindocarbocyanine perchlorate dye
DLS	Dynamic light scattering
DLVO	Derjaguin, Landau, Verwey, and Overbeek theory
DNA	Deoxyribonucleic acid
EtOH	Ethanol
FITC	Fluorescein isothiocyanate dye
Fluo	Fluorescein dye
FMSNP	Fluorescent mesoporous silica nanoparticles
FRET	Förster resonance energy transfer
FSNPs	Fluorescence silica nanoparticles
GLP-1	Glucagon-like peptide-1
GSH	Reduced glutathione level assay
H^{+}	Hydrogen ion
HCl	Hydrochloric acid
HEL-30	Mouse keratinocytes
HEp-2	Human epithelial type 2 cells
HER2	Human epidermal growth factor receptor 2

HIPPNPs	Anti-HER2 ICG-encapsulated polyethylene glycol-coated poly(lactic-co-glycolic acid) nano-particles
HPRT	Hypoxanthine-guanine phosphoribosyltransferase
HUVEC	Human umbilical vein endothelial
ICG	Indocyanine green dye
LDH	Lactate dehydrogenase assays
M^-	Radicals of molecules
*M**	Photoexcitation of molecules
MCM-41	Silica mesophase structure in hexagonal phase
MCM-48	Silica mesophase structure in cubic phase
MCM-50	Silica mesophase structure in lamellar phase
MDA	Malondialdehyde assay
MeOH	Methanol
MET-5A	Human mesothelial cell
MonoMac 6	Macrophage
MSN	Mesoporous silica nanoparticles
MTT	Methyl tetrazolium
N2a	Mouse neural crest-derived **cell** line
Na^+	Sodium ion
NaCl	Sodium chloride
NaOH	Sodium hydroxide
NH_3	Ammonia
Ni	Nitrogen
NIR	Near infrared
NPs	Nanoparticles
O/W	Oil-in-water
O_2	Oxygen
ORMOSIL	Organically modified silica
O–Si–O	Silicon dioxide bonding
Pb^{2+}	Lead (II) ion
PbS	Lead sulfide
PBS	Phosphate buffer solution/saline
PDI	Polydispersity index

PEG	Polyethylene glycol
PLGA	Poly(lactic-co-glycolic acid)
PrOH	Isopropanol
QDs	Quantum dots
R6G	Rhodamine 6G
RBITC	Rhodamine B isothiocyanate dye
RBS	Rhodaminde B
RLE-6TN	Rat Lung Epithelial-6-T-antigen Negative
ROS	Reactive oxygen species
RPMI 2650	Human nasal septum quasidiploid tumour
$Ru(bpy)_3^{2+}$	Tris(bipyridine)ruthenium(II)
S*	Singlet excited state
S_0	Electrons from the ground state
S_1	Excited singlet state 1
S_2	Excited singlet state 2
Si	Silica
SiDiI	Silica nanoparticles encapsulated 1,1%-dioctadecyl 3,3,3%,3% tetramethylindocarbocyanine perchlorate dye
SiFluo	Silica nanoparticles encapsulated fluorescein dye
SiNPs	Silica nanoparticles
SiO_2	Silicon dioxide, silica
SPN-GLP-1	silica nanoparticles encapsulated glucagon-like peptide-1
SRB	Sulforhodamine B assay
T*	Triplet excited state
T_1	Lowest vibrational level
TEM	Transmission electron microscope
TEOS	Tetraethyl orthosilicate
THP-1	Macrophage
TMR	Tetramethyl rhodamine
TNF-a, IL-6, IL-8	Cytokine expression
TRITC	Tetramethylrhodamine isothiocyanate

TSTMP	Trisodium trimetaphosphate
Tween 80	Polysorbate 80
UV-Vis	UV visible spectrophotometer
UV-Vis	UV-Vis spectrophotometer
VCO1	Vibrio cholera O1
VTMS	Vinyltrimethoxysilane
W/O	Water-in-oil
WIL2-NS	Human B-cell Lymphoblastoid cell
W_o	Water-to-surfactant molar ratio
XRD	X-Ray diffraction
ZnSe	Zinc selenide

LIST OF SYMBOLS

%	Percentage
±	Plus minus
°	Degree
°C	Degree Celsius
µg	Microgram
µl	Microliter
µm	Micrometer
cfu	Colony-forming unit
d	Diameter
ε	Molar absorbance
g	Gram
h	Hour
kDa	Kilodaltons
l	Liter
M	Molarity
min	Minute
ml	Mililiter
mm	Milimeter
nm	Nanometer
ns	Nanosecond
$r°$	Fundamental emission anisotropy
rpm	Revolutions per minute
wt	Weight
α	Two-photonic cross-section
θ	Theta
λ	Wavelength
τ_F	Excited-state lifetime
φ	Fluorescence quantum yield

SINTESIS SILIKA NANOKOLOID TERKANDUNG PEWARNA UNTUK APLIKASI BIOPENGIMEJAN DAN BIOPELABELAN

ABSTRAK

Dua jenis pewarna pendarflor iaitu 1, 1%-dioctadecyl 3, 3, 3%, 3%-tetramethylindocarbocyanine perchlorate (DiI) dan fluorescein (Fluo) telah dikapsulkan ke dalam nanopartikel amorfus silika dengan mengunakan kaedah pembentukan misel. 2-butanol sebagai pelarut bersama digunakan bagi memudahkan reaksi hidrolisis semasa proses sintesis telah divariasikan untuk mengetahui kesan keatas saiz partikel. Sampel telah dianalisa untuk menentukan purata saiz partikel, morfologi partikel, ciri-ciri spektrum peresapan dan intensiti kependarfloran. Partikel bersaiz 26.2 hingga 53.4nm, berbentuk sfera dengan taburan partikel yang sekata telah dihasilkan dengan menggunakan 2, 4 dan 6 ml isipadu pelarut bersama. Kestabilan warna antara silika berkapsul pewarna Fluo, (SiFluo) dan silika berkapsul pewarna DiI (SiDiI) telah dijalankan. SiDiI mempunyai kestabilan warna yang tinggi berbanding SiFluo. Berikutan itu, SiDiI bersaiz 30.4, 40.0 and 53.4 nm telah diuji untuk menilai kestabilan partikel di dalam media biologi yang berbeza (larutan NaCl dan serum tikus), potensi sitotoksisiti dan paparan pengimejan pendarflor terhadap sel hidup manusia (human breast adenocarcinoma, MCF-7). SiDiI bersaiz 53.4 nm mempunyai kestabilan warna yang tinggi dengan hanya 11 % peratus pemudaran dan mempunyai kestabilan partikel yang bagus di dalam kedua-dua media biologi. Selain itu, SiDiI bersaiz 53.4 nm mempunyai kadar sitotoksisiti yang rendah terhadap sel MCF-7. Sel MCF-7 yang dirawat oleh SiDiI bersaiz 53.4 nm menunjukan kadar kecerahan dan intensiti kependarfloran yang tinggi diperolehi daripada paparan pengimejan pendarflor.

SYNTHESIS OF SILICA NANOCOLLOIDS ENCAPSULATED DYES FOR BIO-IMAGING AND BIO-LABELING APPLICATIONS

ABSTRACT

Two different types of fluorescent dyes which are 1,1%-dioctadecyl 3,3,3%,3%-tetramethylindocarbocyanine perchlorate (DiI) and fluorescein (Fluo) were encapsulated inside the amorphous silica nanoparticles (SiNPs) using micelle entrapment method. 2-butanol which acted as a co-solvent to facilitate hydrolysis reaction during the synthesis process was varied in order to investigate the effect on the particle size. The synthesised samples were analysed and characterised to determine the hydrodynamic size of particles, average particle size and particles morphology, absorbance properties, fluorescence properties and crystallinity of the samples. Spherical and monodispersed nanoparticles (NPs) with different sizes ranging from 26.2 to 53.4nm, were obtained when synthesised using 2, 4 and 6 ml volume of co-solvent, 2-butanol respectively. The photostability effect between SiNPs encapsulated with Fluo dye (SiFluo) and SiNPs encapsulated with DiI dye (SiDiI) were conducted. SiDiI showed good photostability effect compared to SiFluo. Therefore, selected particle sizes of SiDiI 30.4, 40.0 and 53.4 nm were further analysed to study the particles stability in different biological medium (NaCl and mouse serum), cytotoxicity and fluorescent cell imaging in living cells, human breast adenocarcinoma (MCF-7). SiDiI with size 53.4 nm showed good photostability with only 11 % of decolourisation and obtained good particles stability in both NaCl solution and mouse serum. The cytotoxicity study showed that cytotoxicity of SiDiI is size dependent whereby SiDiI with size 53.4 nm was less toxic. Furthermore, bright and high contrast fluorescent cell images of MCF-7 treated with SiDiI with size 53.4 nm was observed.

CHAPTER ONE

INTRODUCTION

1.1 Research background

Nanomaterials are defined as the set of particles which have a very small scale which is less than 100 nm in at least one dimension. Nanomaterials exhibit unique properties in optical, magnetic, electrical or other properties. In the past few decades, nanomaterials were introduced to industries and currently in continuous research due to the potential for great impact which can improve the quality of a product in electrical, medical and other field. Recently, numerous nanomaterials have been developed and employed as the most promising candidates for bio-analysis applications especially in optical bio-imaging and bio-labelling (Yan *et al.*, 2007).

Optical bio-imaging and bio-labelling are researches involving different experimental technique and materials that are being utilized to obtain optical contrast in biological specimens. Optical imaging method is expected to have a substantial impact on the prevention and treatment of diseases especially in cancer treatment. Fast and easy diagnosis process of optical imaging method enables researchers to visualize complete organ to complex biological process by multidimensional and multi parameter data (Deshmukh *et al.,* 2016). Optical bio-imaging and bio-labelling have attracted great attention in biomedical research due to their distinguished advantages in terms of the availability of biocompatible, high resolution and good sensitivity of imaging agents or biomarker (Arunkumar *et al.*, 2005).

In optical bio-imaging and bio-labelling, the imaging agents or biomarker should have high water-solubility, biocompatibility, good chemical stability and