SIZE SEPARATION OF UNCOATED AND POLYETHYLENE GLYCOL COATED SILVER NANOPARTICLES USING DENSITY GRADIENT CENTRIFUGATION

By

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree Master of Science

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Chair : Prof. Arbakariya bin Ariff, PhD
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Purification of silver nanoparticles (AgNPs) is essential for biomedical field, where high purity and application are difficulty to have compatible size ranges of AgNPs as drug delivery. AgNPs in different size ranges, such as 1-20 nm, 20-40 nm, 40-60 nm, and 60-80 nm, would size uniformity AgNPs are required for drug delivery. Currently, the limitations in biomedical improve drug delivery efficacy. However, AgNPs with a wide range of sizes have disadvantage such as being toxic to health cells. Therefore, surface-bound chemical modification using coating agents such as polyethylene glycol (PEG) and purification procedures are required to generate smaller size ranges of AgNPs, which will also give a better understanding on the role of AgNPs' physical properties in biomedical applications. In this study, uncoated AgNPs were produced via chemical and biological synthesis and then coated with PEG for its stability. UV-Visible spectrophotometer, high resolution-transmission electron microscope (HR-TEM), Fourier-transform infrared spectroscopy (FTIR), and dynamic light scattering (DLS) were used to characterise uncoated and PEG-coated AgNPs from chemical and biological synthesis for size, shape and morphology. The size distribution of PEG-coated AgNPs from both synthesis methods are slightly bigger around 1 – 3 nm compared to the uncoated AgNPs from both synthesis methods. Further, the shape of PEG-coated AgNPs from both synthesis methods revealed predominant spherical shape (86.86%). It showed that PEG-coated AgNPs from both synthesis methods was homogenous in term of size, shape and morphology compared to the uncoated AgNPs from both synthesis methods. In addition, a purification method which is density-gradient centrifugation (DGC) was carried out on the both uncoated and PEG-coated AgNPs from both synthesis methods by using sucrose at concentration ranging from 10% to 50% (w/v). Based on the results from UV-Visible spectrophotometer and HR-TEM, the size ranges of uncoated and PEG-coated AgNPs from both synthesis methods after purification was smaller than the crude one. In comparison to uncoated AgNPs from both synthesis methods, PEG-coated

AgNPs from both synthesis methods obtained desired separated size ranges. Hence, the PEG- coated AgNPs from both synthesis methods are ideal for drug delivery in biomedical applications.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Master Sains

PENGELASAN SAIZ NANOZARAH ARGENTUM YANG TIDAK DILAPIS DAN YANG DILAPIS DENGAN POLIETILENA GLIKOL MENGGUNAKAN SENTRIFUGASI KECERUNAN KETUMPATAN

Oleh

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Penulenan nanopartikel argentum (AgNPs) sangat penting bagi bidang bioperubatan, di mana ketulenan tinggi dan keseragaman saiz AgNPs diperlukan sebagai penghantaran ubat. Pada masa kini, pembatasan dalam aplikasi bioperubatan adalah kesukaran untuk mendapatkan julat saiz AgNPs yang sesuai sebagai penghantaran ubat. AgNPs dalam julat saiz yang berbeza, seperti 1-20 nm, 20-40 nm, 40-60 nm, dan 60-80 nm, akan meningkatkan keberkesanan penghantaran ubat. Walau bagaimanapun, AgNPs dengan pelbagai saiz mempunyai kelemahan seperti toksik pada sel yang sihat. Oleh itu, pengubahsuaian kimia untuk permukaan yang terikat menggunakan agen salutan seperti polietilena glikol (PEG) dan prosedur penulenan diperlukan untuk menghasilkan julat saiz AgNPs yang seragam, yang juga akan memberikan pemahaman yang lebih baik mengenai peranan sifat fizikal AgNPs dalam aplikasi bioperubatan. Dalam kajian ini, AgNPs yang tidak disaluti dihasilkan melalui sintesis kimia dan biologi dan kemudian disaluti dengan PEG untuk kestabilannya. Spektrofotometer, resolusi tinggi mikroskop elektron transmisi (HR-TEM), spektroskopi inframerah transformasi fourier, dan taburan cahaya dinamik (DLS) digunakan untuk menilai sifat AgNPs yang tidak disaluti dan disaluti PEG dari sintesis kimia dan biologi untuk saiz, bentuk, dan morfologi. Taburan saiz AgNPs bersalut PEG dari kedua-dua kaedah sintesis mempunyai saiz yang lebih besar sedikit, sekitar 1 - 3 nm berbanding dengan AgNPs yang tidak disaluti dari kedua-dua kaedah sintesis. Seterusnya, bentuk AgNPs yang disaluti PEG dari kedua-dua kaedah sintesis tersebut menunjukkan bentuk sfera yang dominan (86.86%). Ini menunjukkan bahawa AgNPs yang disaluti PEG dari kedua-dua kaedah sintesis adalah seragam dari segi saiz, bentuk, dan morfologi berbanding AgNPs yang tidak disaluti dari kedua-dua kaedah sintesis. Tambahan pula, kaedah penulenan yang merupakan sentrifugasi kecerunan

ketumpatan (DGC) dilakukan pada kedua-dua AgNPs yang tidak disaluti dan disaluti PEG dari kedua-dua kaedah sintesis dengan menggunakan sukrosa pada kepekatan antara 10% hingga 50% (w / v). Berdasarkan keputusan daripada spektrofotometer dan HR-TEM, julat saiz AgNPs yang tidak disaluti dan disaluti PEG dari kedua-dua kaedah sintesis setelah penulenan, lebih kecil daripada yang mentah. Sebagai perbandingan dengan AgNPs yang tidak disaluti dari kedua-dua kaedah sintesis, AgNPs yang disaluti PEG dari kedua-dua kaedah sintesis mempunyai julat saiz yang diinginkan. Maka, AgNPs yang disaluti PEG dari kedua-dua kaedah sintesis sangat sesuai digunakan sebagai penghantaran ubat dalam aplikasi bioperubatan.

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LIST OF ABBREVIATIONS

ρ	Density
γ	Gamma
°C	Degree celsius
%	Percentage
Ag^+	Silver ion
AFM	Atomic force microscope
AGE	Agarose gel electrophoresis
$AgNO_3$	Silver nitrate
AgNPs	Silver nanopartilces
ANVA	Analysis of variance
BET	Brunauer-emmet-teller
CFF	Cross flow-filtration
D_{BET}	Average diameter of spherical nanoparticles
DGC	Density gradient centrifugation
DLS	Dynamic light scattering
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
FTIR	Fourier transform infrared spectroscopy
g	Gravitaional force
HR-TEM	High resolution transmission electron microscope
kV	Kilovolt
M	Molar
mg	Milligram

mL	Millilter
µL	Microliter
µm	Micrometer
mm	Millimeter
mV	Millivolt
M	Molar
$NaBH_4$	Sodium borohydride
nm	Nanometer
NPs	Nanoparticles
PdI	Polydispersity index
PEG	Polyethylene glycol
ROS	Reactive oxygen species
RZC	Rate zonal centrifugation
S	Specific surface area
S.D	Standard deviation
SEC	Size exclusion chromatography
SPR	Surface plasmon resonance
TBE	Tris-borate-EDTA
TEM	Transmission electron microscope
ZP	Zeta potential
XRD	X-ray diffraction

CHAPTER 1

INTRODUCTION

1.1 Background of study

Nanoparticles (NPs) can be defined differently, depending on the types of materials, fields, and applications (Ng et al., 2013). However, the particles in the size range from 1 nm to 100 nm are generally considered to be NPs (Hosokawa et al., 2012). Additionally, NPs have different physical and chemical properties from their bulk materials; these properties are affording the exploitation of NPs for various applications (Ardani et al., 2017). The NPs with smaller size have larger specific surface area; thus, the total surface area of a particle is inversely proportional to its diameter (Cataxo, 2011; Hasan, 2015). Silver nanoparticles (AgNPs) are one of the important metallic nanoparticles that have received great attention because of their usage in biomedical application (Ahmed et al., 2016). AgNPs have been intensely studied due to their intrinsic unique properties (i.e., optical behaviour, conductivity, chemical stability, and catalytic activity) (Singh et al., 2015).

Two methods, biological and chemical, could be used to synthesise AgNPs. Chemical synthesis of AgNPs are typically generated by chemical reduction and obtained an effective yield; however, they can lead to the limitations (i.e., usage of hazardous chemicals, high cost, and increased energy consumption) (Srikar et al., 2016). AgNPs can also be prepared using biological sources (e.g., plant extract, bacteria, and fungi); this approach is therefore more environmentally-friendly and cost-effective (Ajitha et al., 2014). However, plant extract-based synthesis appears to be the most appealing approach due to the easier subsequent extraction process compared to microbial routes, which require aseptic conditions for cultivation and laborious work in maintaining the cells (Awwad et al., 2013). In addition, the usage of plant extract may also offer many benefits, including high source availability, eco-friendliness, safety to handle, and containing a wide range of plant metabolites (Kulkarni et al., 2011). In addition, the synthesised AgNPs are preferable compared to the commercial AgNPs due to the long-term effects of commercial AgNPs exposure on human physiology and has given high negative impact as their release into the environment (Stensberg et al., 2011).

The biggest challenge associated with AgNPs synthesis is their instability and susceptibility to agglomeration intrinsic characteristics, which are resulting in the formation of larger-size AgNPs (Ardani et al., 2017). Additionally, Sarkar et al (2005) highlighted that polymers (e.g., polysaccharides, polyacrylamide, and PEG) and ligands (e.g., citrates, amines, peptides, and lipids) are widely used as capping agents for the surface modification because these substances can control the rate of reduction of metal ions and the aggregation process of the metal clusters. However, eventhough the synthesised AgNPs from chemical and biological methods has been coated with polymer, these will still lead to the wide size range and shapes of NPs (Suresh et al., 2015).

Based on the disadvantages of unseparated synthesised AgNPs, it is important to obtain well-separated size and shape of NPs for characterisation and also for biomedical application (Suresh et al., 2015). In previous study, spherical shaped AgNPs with size range 50 – 100 nm were tested towards pathogen bacteria (e.g., *Pseudomonas aeruginosa, Klebsiella pneumoniae,* and *Enterococcus faecalis*) by agar diffusion method and showed as good potential antibacterial agents (Ivanova et al., 2018). Other researcher has been tested spherical shape AgNPs with size range below 20 nm towards *Lactobacillus salivarius* as potential antibacterial agents, to be further used in dental implantology (Ghiuță and Cristea, 2020).

Purification of NPs into smaller sizes and similar shapes may not only enhance the NPs condition, but also provide ways to recognise which physical properties of NPs can be useful for a drug delivery vector (Robertson et al., 2016). The synthesis of NPs produced polydisperse mixtures, however the potential used of these NPs in biomedical application required monodispersed populations (Akbulut et al., 2012). In order to obtain homogeneous NPs populations, post-synthetic separation methods (i.e., density gradient centrifugation, gel electrophoresis and size exclusion chromatography) is required (Shin et al. 2013).

In this study, the uncoated AgNPs were synthesised by chemical and biological methods and then coated with PEG for its stability. Then, the uncoated and PEG-coated AgNPs from chemical and biological synthesis were characterised in terms of their size, shape, surface charge, and morphology using UV-Visible spectrophotometer, high resolution-transmission electron microscope (HR-TEM), Fourier-transform infrared spectroscopy (FTIR), and dynamic light scattering (DLS). Furthermore, a purification method which is density gradient centrifugation (DGC) was conducted for both uncoated and PEG-coated AgNPs from chemical and biological synthesis to distinguish the size range of AgNPs. Multiple layers of a common solvent which was sucrose in water with different densities in ascending order of its concentration was applied in DGC (Mace et al., 2012). Shin et al. (2013) claimed that DGC was one of the effective purification methods used to separate gold nanoparticles, resulting in small distributions of its diameters, shapes and aggregation state.

Generally, this research aimed at characterising the uncoated and PEG-coated AgNPs from chemical and biological synthesis and to evaluate the feasibility of DGC for the size separation of uncoated and PEG-coated AgNPs from chemical and biological synthesis. Development of size classification technique to enhance the properties of sub-micrometer particles has also been reported (Mori, 2015). Nevertheless, reports on the purification of uncoated and PEG-coated AgNPs from chemical and biological synthesis and the characterisation of homogenous nanoparticles are scarce. It is hypothesised that, DGC has the ability to separate size of synthesised AgNPs according to the different concentrations of solvent used. The NPs separation was proven by optical confirmation, spectrophotometric and transmission electron microscopy measurements (Suresh et al., 2015).

1.2 Specific objectives

Hence, the specific objectives of this research were:

1. To characterise the uncoated and PEG-coated AgNPS, synthesised by chemical and biological methods.
2. To evaluate the effect of size ranges within the uncoated and PEG-coated AgNPS from different synthesis methods using density gradient centrifugation (DGC).

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