11th يوم البحث العلمي بكلية الصيدلة
College of Pharmacy Research Day

جامعة الملك سعود
King Saud University

Optimization of Apigenin stabilized gold nanoparticle synthesis as Targeted drug delivery for cancer therapy.

INTRODUCTION

The development of metallic nanoparticles delivery systems is a rapidly emerging area of nanotechnology applications where nanomaterials (NMs) are used to control the delivery of therapeutic agents to a specific site. Apigenin is a flavonoid, derived from edible fruits and vegetables, it is non-mutagenic and possesses antimicrobial, antiviral, and anticarcinogenic activities.

The development of nanotechnology has expedited our ability to design new nanoparticles for the diagnosis and treatment of cancer, which is one of the leading causes of death worldwide. Gold nanoparticles have been explored as drug carriers due to the several advantages. It provides large surface area with high loading capacity for drug loading and improves the hydrophilicity and stability of drugs. Also , it has The ability to modify surface with targeting ligands to enhance the tumor selective accumulation compared to free drugs. Besides, it provides enhanced permeability and retention(EPR) effect. It can be modified to control the release of loaded drugs in response to internal or external stimulus.

Moreover, the effect of Flavonoids is reported to be enhanced when they are in nano-formulations. Apigenin can be conjugated to gold nano-particles (ap-AuNPs) when apigenin reacts with Au3+ under appropriate conditions. This project demonstrates an optimized synthesis of a targeted delivery system of apigenin-gold nanoparticles (ap-AuNPs) to enhance a potential therapeutic solution for the treatment of various cancers.

OBJECTIVES

The main objective of this study is to optimize and characterize a targeted drug delivery nanocarrier for Apigenin, capable of improving the therapeutic effect of the chemotherapeutic agent itself and aimed at the treatment of several types of cancers.

1. To synthesis targeted delivery nanocarrier of Apigenin (gold nanoparticles).
2. To characterize and optimize the Ap-AuNPs (with and without target moiety).

METHODS

Synthesis of gold nanoparticles (coated and uncoated)

AuNP were synthesized using a modified Turkevich method by reducing gold chloride with sodium borohydride. Briefly, 1% w/v gold (III) chloride (HAuCl4) was dissolved in 100mL of ultrapure water. 1% w/v sodium borohydride was freshly prepared in cold ultrapure water. Then, HAuCl4 was reduced via adding 1 mL 1% sodium borohydride solution in a dropwise manner while stirring for 20 minutes. The color of the mixture changed from pale-yellow to black and then eventually to wine-red. For coating of AuNPs, Poly (ethylene glycol) bis(carboxymethyl)ether was used for coating. Free PEG was removed via centrifugation at 20,000 g for 60 minutes three times at room temperature. Supernatant then is discarded, and the residue was redispersed in phosphate-buffered saline (PBS) for future usage.

Synthesis of ap-AuNPs (coated and uncoated):

Ap-AuNPs are produced according to the instruction reported in literature and minor modified. Briefly, Apigenin was dissolved in DMSO at a concentration of 10 mM. the concentration and pH of apigenin solution were adjusted for different formulation condition as shown in the Table 2. HAuCl4 was added dropwise in to boiled apigenin solution. The solution was kept stirring at room temperature for 72 to 96 hours, with nanoparticles collected by centrifugation at 15000 g for 30 min. Supernatant then is discarded, and the residue was redispersed in phosphate-buffered saline (PBS) for future characterization. For coated ap-AuNPs, Poly (ethylene glycol) bis(carboxymethyl)ether was used for coating. Free PEG was removed via centrifugation at 20,000 g for 60 minutes three times at room temperature. Supernatant then is discarded, and the residue was redispersed in phosphate-buffered saline (PBS) for future characterization.

Synthesis of targeted Ap-AuNPs:

Folate was conjugated with synthesized AuNPs with and without apigenin using crosslinking with 1-ethyl-3-(3-dimethylaminopropyl-carbodiimide hydrochloride (EDC) in the presence of N-hydroxy-succinimide (NHS).

Characterization of synthesized formulation of AuNPs:

UV–vis absorption spectroscopy
Electron microscopic study (TEM)
Fourier transform infrared spectroscopy (FTIR)
Dynamic light scattering (DLS) and zeta potential
As for the statistical analysis, the data was presented as mean ± standard deviation (TEM) and the results were analyzed using GraphPad Prism 9, analysis with one-way analysis of variance (ANOVA).

RESULTS

Table 1 showed three trial formulations with and without apigenin at pH 10. Table 2 elicited coated gold NPs at two different pH (2.2 and 3.3) with and without apigenin. HAuCl4 served as reducing agent whereas apigenin is also reported to act as reducing agent. Table 3 is summary of evaluation parameters wherein size is slightly increased with coating and drug loading. And an average diameter of 20 nm to 35 nm and zeta potential of -32±8.1 mV. The stability of Ap-AuNPs in the biological environment was verified through UV-Vis spectroscopy. Furthermore, the FTIR spectroscopy analysis has illustrated that chemical binding of apigenin on the surface of Ap-AuNPs through hydroxyl and carbonyl functional groups was found to be the main reason for the stability of all Ap-AuNPs formulations.

Table 1: A summary of composition of trial uncoated gold nanoparticles

Components	GNF1	GNF2	GNF3
HAuCl4 (%w/v)	0.008	0.024	0.024
Apigenin (%w/v)	-	0.011	0.006
Sodium borohydride (%w/v)	0.001	-	-
DMSO (dimethyl sulfoxide) (%v/v)	-	4.76	3.7
Potassium carbonate (%w/v)	-	1.9	1.93
HCl (pH adjustment)	10	10	10

Table 2: A summary of composition of selected coated gold nanoparticles at two different pH

Components	CGN2.2	CGN3.3	ACGN2.2	ACGN3.3
HAuCl4 (%w/v)	0.008	0.008	0.008	0.008
Apigenin (%w/v)	-	-	0.2	0.2
Sodium borohydride (%w/v)	0.001	0.001	0.001	0.001
DMSO (dimethyl sulfoxide) (%v/v)	-	-	1.0	1.0
Sodium hydroxide (1 M) (%v/v)	-	1.25	-	1.25
*Diacid PEG (%v/v)	0.5	0.5	0.5	0.5
pH	2.2	3.3	2.2	3.3

Note: HAuCl4 = auric acid, *DPEG = Poly(ethylene glycol) bis(carboxymethyl)ether,

Table 3: Evaluation parameters of the selected coated gold nanoparticles with or without apigenin

Evaluation parameters	CGN2.2	CGN3.3	AGN3.3	ACGN3.3
Particle size (nm)	82	93	31	158
PDI	0.42	0.67	0.26	0.86
Zeta potential values	-18.3	-26.3	-7.45	-20.3
*FE (fold error)	4.09	4.5	1.5	1.4
Shape behavior	spherical	spherical	spherical	spherical
TDC (total drug content)	0%	0.2 %	0.2 %	0.2 %

Note: FE = Fold error estimated using particle size obtained from zetasizer and TEM (transmission electron microscopy), FE (fold error) = $10^{\log(\text{size, zetasizer/size, TEM})}$. Acceptable range of FE is < 2.0.

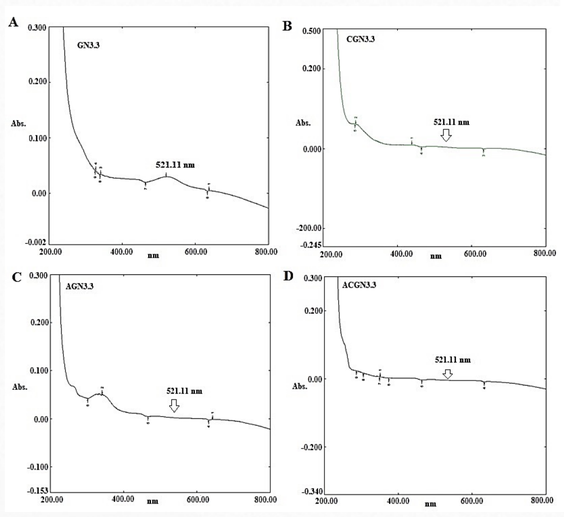

Figure 1: UV–Vis absorption spectra: (a) uncoated AuNPs at pH 3.3 (GN3.3); (b) coated AuNPs at pH 3.3 (CGN3.3), (c) apigenin loaded on uncoated AuNPs at pH 3.3, and (d) apigenin loaded on coated AuNPs at pH 3.3. Coating and the drug loading lost characteristic peak at 520 nm whereas the same peak was apparently observed in uncoated GN3.3.

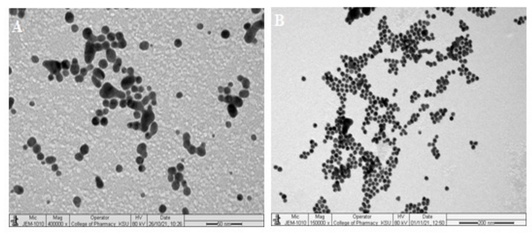

Figure 2: Representative images of transmission electron microscopy for (A) (AGN3.3), and (b) ACGN3.3

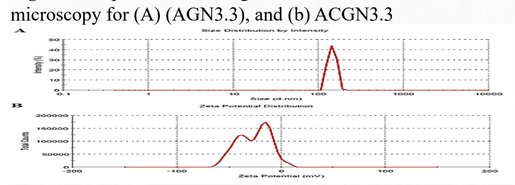

Figure 3: Particle size (a) and zeta potential (b) of ACGN3.3

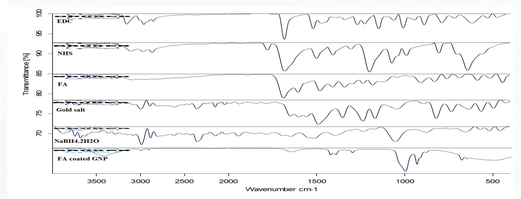

Figure 4: Compatibility study using FTIR

DISCUSSION/CONCLOSIONS

The promising results of optimized formula targeted delivery of flavonoids such as Apigenin using AuNP have great potential in improving the efficiency of cancer therapy.

REFERENCES

- Shouju Wang and Guangming Lu (December 20th 2017). Applications of Gold Nanoparticles in Cancer Imaging and Treatment, Noble and Precious Metals - Properties, Nanoscale Effects and Applications, Mohindar Singh Seehra and Alan D. Bristow, IntechOpen, DOI: 10.5772/intechopen.70901.
- Spadavecchia J, Movia D, Moore C, Maguire CM, Moustaoui H, Casale S, Volkov Y, Prina-Mello A. Targeted polyethylene glycol gold nanoparticles for the treatment of pancreatic cancer: from synthesis to proof-of-concept in vitro studies. Int J Nanomedicine. 2016 Feb 26;11:791-822.
- Manson, J., Kumar, D., Meenan, B.J. *et al.* Polyethylene glycol functionalized gold nanoparticles: the influence of capping density on stability in various media. *Gold Bull* **44,** 99–105 (2011
- Faiyaz Shakeel, Sultan Alshehri, Mohamed A. Ibrahim, Ehab M. Elzayat, Mohammad A. Altamimi, Kazi Mohsin, Fars K. Alanazi, Ibrahim A. Alsarra.Solubility and thermodynamic parameters of apigenin in different neat solvents at different temperatures,Journal of Molecular Liquids,Volume 234,2017,Pages 73-80.
- Dong YC, Hajfathalian M, Maidment PS, Hsu JC, Naha PC, Si-Mohamed S, Breuilly M, Kim J, Chhour P, Douek P, Litt HI. Effect of gold nanoparticle size on their properties as contrast agents for computed tomography. Scientific reports. 2019 Oct 17;9(1):1-3.