doi:10.1017/S1431927618014885
Microsc. Microanal. 24 (Suppl 2), 2018

Correlation between ICP-OES and Synchrotron-XRF in Detecting the Penetration of Gold Nanorods into Excised Human Skin Layers

Nouf N. Mahmoud [a, *], Messaoud Harfouche [b], Alaaldin M. Alkilany [c], Amal G. Al-Bakri [c], Enam A. Khalil [c]

a Department of Pharmacy, Faculty of Pharmacy, Al-Zaytoonah University of Jordan, Amman 11733, Jordan
b Synchrotron Light for Experimental Science and Applications in the Middle East (SESAME), Allan 19252, Jordan
c Department of Pharmaceutics and Pharmaceutical Technology, School of Pharmacy, The University of Jordan, Amman 11942, Jordan

* Corresponding author: nouf.mahmoud@zuj.edu.jo

Gold nanoparticles have attracted interest in the biomedical and nanomedicine fields due to their unique optical properties, photothermal properties and ease of synthesis and localization within the biological systems [1, 2].
In this work, gold nanorods (GNR) of length ~ 40 nm, width ~10 nm and aspect ratio of 4, were synthesized using cetyltrimethylammonium bromide (CTAB) as a shape directing agent [3]. The synthesized GNR were functionalized with poly ethylene glycol thiol (PEG-SH) and polystyrene thiol (PS-SH), and resulted in hydrophilic PEG-GNR and hydrophobic PS-GNR, respectively. The synthesized GNR demonstrated typical peaks of GNR without significant broadening or tailing which indicate excellent stability. Furthermore, the surface effective charge was neutral for PEG-GNR and their transmission electron microscope (TEM) images confirmed their shape and size.
The synthesized GNR of different surface chemistry were applied to excised human full thickness skin using Franz diffusion cells and incubated for 24 hr. Skin layers (stratum corneum (SC) and dermis) were isolated, digested with aqua regia and the amount of GNR in each separated skin layer was estimated using inductively coupled plasma-optical emission spectroscopy (ICP-OES). The results were presented as percentage of the initial amount of gold applied onto the skin.
Hydrophilic PEG-GNR demonstrated about ~11% and ~17% accumulation into SC and the dermis respectively **(Figure 1)**. However, the hydrophilic PS-GNR exhibited low uptake into either the SC or the dermis (7% and 5% respectively, **Figure 1**). The hydrophobic-hydrophilic properties of PEG contributed to the high accumulation percentage of PEG-GNR, which facilitate their penetration through the lipid and aqueous pores of SC [4]. These results are supported by our recent work where we found that the hydrophobic PS-GNR were preferentially accumulated into the sebum-rich hair follicles, while PEG-GNR were accumulated into hair follicles as well as dermis because of its hydrophilic properties [5].

The current ICP-OES results are well correlated with our previous work, where the accumulation of hydrophilic PEG-GNR and hydrophobic PS-GNR into skin layers was investigated using Synchrotron X-ray fluorescence microscopy (XRF), in addition to other methods such as TEM and confocal microscopy [6].
Fluorescence maps were collected at different regions of skin to examine the gold localization. The previous results of GNR mapping using synchrotron-XRF were consistent with the current ICP-OES findings, where low and random distribution of GNR were observed in the maps of the upper SC and dermis of skin samples exposed to hydrophobic PS-GNR (**Figure 2 A,** [7]). On the contrary, gold

distribution maps of skin samples exposed to PEG-GNR revealed the presence of localized regions corresponding to GNR in the upper and lower regions of skin, where relatively higher concentration of gold was observed compared to samples treated with PS-GNR (**Figure 2 B,** [7]).
The current study demonstrated that the quantitative analysis of the penetrated GNR into skin by ICP-OES was supported and well correlated with the previous synchrotron-XRF mapping. In addition, synchrotron-XRF provided useful information regarding the correlation between the spatial distribution of gold and other tissue elements such as phosphorous and sulfur in order to understand the mechanism of GNR penetration into skin [6].

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[8] The authors acknowledge the Deanship of Academic Research at Al-Zaytoonah University of Jordan and the Deanship of Academic Research (DAR) at the University of Jordan for financial supports.

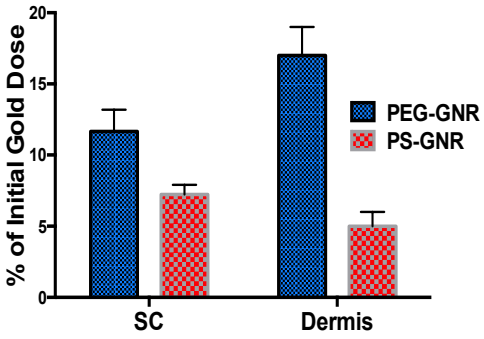

Figure 1: Percentage of PEG-GNR and PS-GNR penetrated into stratum corneum and dermis by ICP-OES. Data are given as mean ± SD; n = 4.

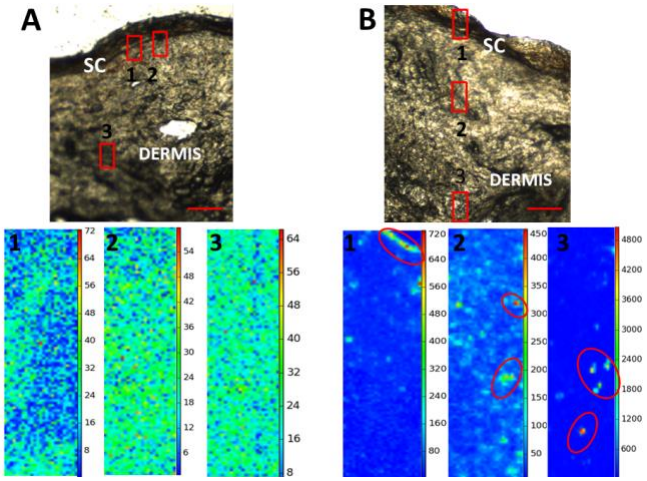

Figure 2: Synchrotron X-ray fluorescence elemental maps of gold (presented as number of counts) of skin sample incubated with **(A)** PS-GNR (dermis region) or **(B)** PEG-GNR (dermis region). Scale bar = 10 μm. The dimensions of the scanned map are (H × V) = 41 × 10 μm^2 [7].

https://doi.org/10.1017/S1431927618014885 Published online by Cambridge University Press