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EXECUTIVE SUMMARY OF THE THESIS

Microwave Assisted *in-situ* Synthesis of Gold Nanoparticles in a Gel System

TESI MAGISTRALE IN INGEGNERIA CHIMICA - CHEMICAL ENGINEERING

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1. Introduction

Gold Nanoparticles (AuNPs) are nanoscale materials with unique size-dependent optical properties, most notably a phenomenon called Localized Surface Plasmon Resonance (LSPR). This collective oscillation of their conduction electrons results in strong absorption and scattering of visible light, which can be finely tuned by their size, shape, and environment. Coupled with their inherent biocompatibility and ease of functionalization, these properties make AuNPs powerful tools for biomedical applications such as biosensing, bioimaging, and drug delivery [1], [2], [3]. Hydrogels are three-dimensional networks of hydrophilic polymers and are equally vital in biomedicine. Their high-water content, soft mechanical properties, and ability to encapsulate and release therapeutic agents can mimic natural tissue, making them ideal scaffolds for drug delivery and tissue engineering [4].

This thesis aimed to merge these two technologies by creating a unified fabrication process. The central objective was to assess the feasibility of a one-pot, *in-situ* synthesis where AuNPs are formed directly during the gelation of a hydrogel. The goal was to identify a polymeric formulation whose components could simultaneously act as the reducing agent for the gold precursor ($HAuCl_4$) and the building blocks for the gel network. This approach seeks to streamline production, ensure intimate integration of the nanoparticles within the matrix, and create a composite material with inherent plasmonic functionality for advanced biomedical uses.

2. The AC-PEG synthesis platform

The experimental procedure began with identifying a suitable reaction medium. The classical Turkevich method, which uses citrate as reductant

and stabilising agent, fails to perform in physiologically relevant buffers like Phosphate-Buffered Saline (PBS) due to its high ionic strength. Therefore, a screening of alternative, biocompatible reducing agents was conducted. While several polymers and biomolecules were assessed, Polyethylene Glycol (PEG) emerged as the most promising candidate. At given conditions, its terminal hydroxyl groups demonstrated a reliable capacity to reduce Au^{3+} to Au^{0} in aqueous solution [5], [6], yielding AuNPs with the characteristic ruby-red colour and a distinct resonance wavelength peak near 527nm [1].

However, PEG alone could not stabilise the newly formed NPs in the harsh ionic environment of PBS. To address this, a composite solution was engineered based on previous studies [3]: the AC-PEG system. This formulation combines Agarose (a gelling agent), Carbomer (a polymer providing electrostatic and steric stabilisation via its carboxylic groups), and PEG (the primary reductant). This synergistic combination created a robust platform where reduction and stabilisation could occur efficiently, even in PBS, setting the stage for the *in-situ* synthesis.

3. One-Pot *in-situ* synthesis of AuNPs in AC-PEG solution

With the AC-PEG platform established, the first major phase of research focused on optimising the synthesis parameters within the liquid solution before gelation. A microwave-assisted heating method was employed for its rapid and uniform energy delivery. The effects of two critical variables (initial solution pH and $HAuCl_4$ concentration) were systematically investigated.

The results revealed a delicate balance. An initial pH of 6.5 was found to be a crucial starting point, providing an environment conducive to balanced nucleation and growth. Furthermore, the concentration of the gold precursor played a defining role in determining the final nanoparticle characteristics. The UV-Vis spectra of the analysed solutions are shown in Figure 1, exhibiting an increasing in the absorption intensity at higher concentrations of Au precursor; the summary of the relevant parameters is found on Figure 2, including the γ parameter, which represents the width of the absorption band and is a measure of polydispersity. Through qualitative and quantitative analysis using UV-Vis spectroscopy and Transmission Electron Microscopy (TEM), it was determined that $HAuCl_4$ concentrations in the range of 0.25 to 1.5mM yielded the most favourable outcomes. Under these preliminary optimised one-pot conditions, the synthesis successfully produced spherical AuNPs with a mean diameter of approximately 25nm, high circularity, and a low polydispersity index, a chosen sample description is shown in Figure 3. The results demonstrate that controlled AuNP formation within a complex polymeric medium was achievable.

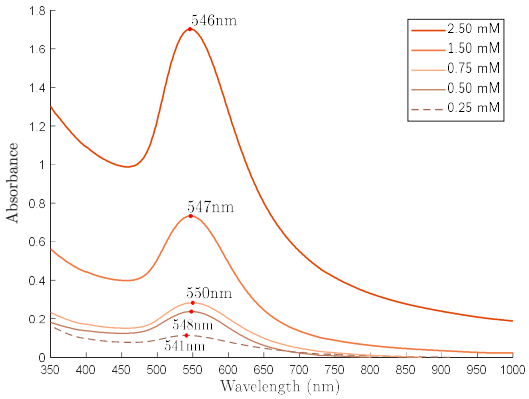

Figure 1. UV-Vis spectra of AuNPs colloidal solution at different concentration of Au precursor (HAuCl4). pH was fixed at 6.5.

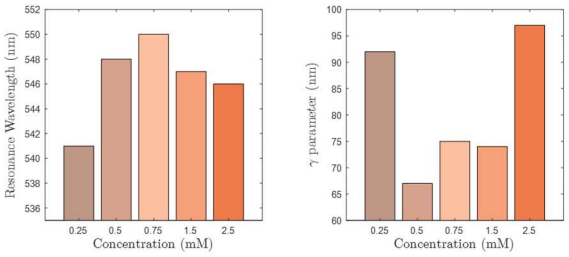

Figure 2. Bar chart summary with the principal UV-Vis data derived from Figure 1. Left: Resonance wavelength (nm); Right: γ parameter (nm).

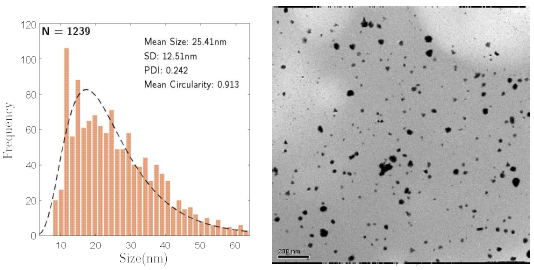

Figure 3. Statistical chart distribution with relevant TEM image (left), and selected TEM image (right). $[HAuCl_4] = 1.5$mM and initial pH = 6.5.

4. One-Pot *in-situ* synthesis + post-synthetic addition of NaOH in AC-PEG solution

A pivotal discovery was made when investigating the order of reagent addition. It was found that altering the quantity and the order of addition of NaOH in the synthesis protocol could profoundly influence the reduction kinetics. This led to the development of an enhanced protocol: the one-pot synthesis with post-synthetic NaOH addition.

In this method, a controlled amount of NaOH was introduced 60 seconds ***after*** the initial reaction between the AC-PEG solution and $HAuCl_4$ had commenced. This simple yet strategic modification acted as a "reaction booster", accelerating the reduction process and refining the nanoparticle morphology, the UV-Vis spectra of the analysed solutions are shown in Figure 4 analogous to the ones obtained in Figure 1, this time however with post-synthetic addition of NaOH; the summary of the relevant parameters is found on Figure 5. A comprehensive optimisation study allowed to determine an optimised molar ratio of NaOH to $HAuCl_4$ (Φ) to be between 30 and 35.

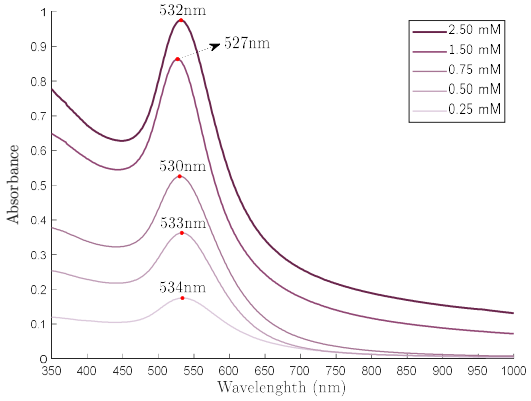

Figure 4. UV-Vis spectra of AuNPs colloidal solution at different concentration of HAuCl4 with delayed addition of NaOH. $\Phi \approx 30$ and pH was fixed at 6.5.

The results were remarkably better. Compared to the standard one-pot method, this approach yielded AuNPs with a significantly smaller average size (a reduction of ~17.5 nm) and a narrow size distribution, evidenced by a polydispersity index of approximately 0.10, this contrast is clear when Figure 5 is compared with Figure 2. This level of monodispersity is exceptional for colloidal syntheses and underscores the superior control achieved. TEM images confirmed the presence of highly uniform, spherical nanoparticles, while UV-Vis spectra showed correspondingly sharper and more intense absorption intensity peaks. The TEM image and its derived statistical parameters for this trial are shown in Figure 6.

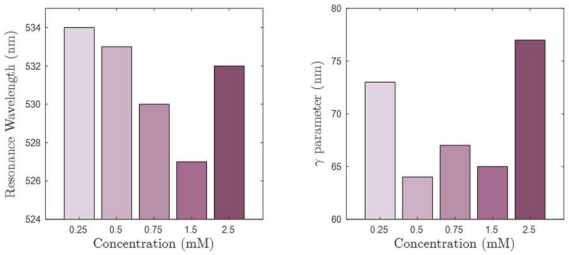

Figure 5. Bar chart summary with the principal UV-Vis data derived from Figure 4. Left: Resonance wavelength (nm); Right: γ parameter (nm).

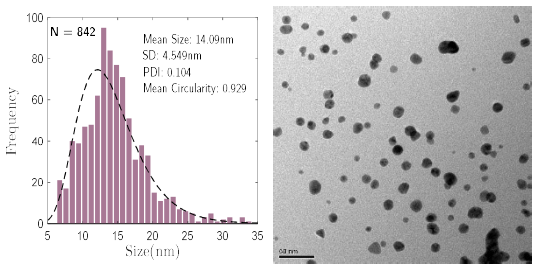

Figure 6. Statistical chart distribution with relevant parameters obtained from TEM image (left), and selected TEM image (right). Post-synthetic addition of NaOH. $[HAuCl_4] = 1.5$mM and $\Phi \approx 30$

5. Characterisation of the AC-PEG/AuNPs Nanocomposite

The optimised synthesis protocol (with post-addition of NaOH) was then successfully translated to form the final solid hydrogel nanocomposite. A critical question was whether the incorporated AuNPs would alter the fundamental properties of the hydrogel matrix. Characterization studies provided reassuring answers. FT-IR and DSC analyses confirmed that the chemical structure and thermal properties of the polymer network remained largely unchanged, proving that the *in-situ* synthesis was chemically non-disruptive.

The physical properties, however, presented some changes. Swelling studies showed a slight reduction in water uptake for AuNPs-loaded hydrogels, suggesting the nanoparticles act as additional

crosslinking points within the network and occupy free volume that otherwise would be occupied by water. Rheological measurements indicated that at lower concentrations, the AuNPs reinforced the gel, increasing its stiffness, while higher loadings had a slight softening effect due to the reduction of matrix-matrix interaction. Scanning Electron Microscopy (SEM) visually confirmed these findings, revealing a uniform, porous microstructure in optimally formulated composites (Figure 7), which became more heterogeneous at very high AuNPs concentrations (Figure 8).

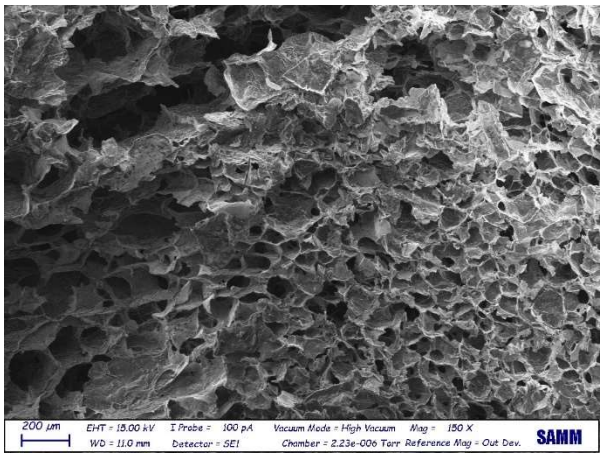

Figure 7. SEM selected image of *in-situ* formulated AC-PEG nanocomposite. [$HAuCl_4$]=0.50mM

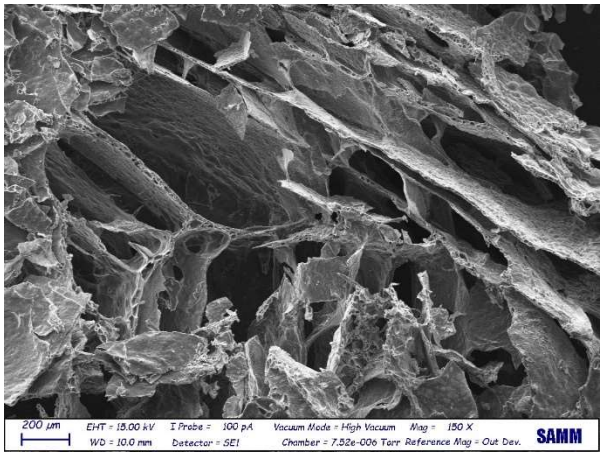

Figure 8. SEM selected image of *in-situ* formulated AC-PEG nanocomposite. [$HAuCl_4$]=2.50mM

6. Drug Release tests and Laser assisted Tests

The ultimate validation of this nanocomposite lies in its functionality as a drug delivery platform. To assess this, passive release studies were conducted using three drug-mimetic molecules with different physicochemical properties: fluorescein (a small, hydrophilic molecule), FITC (a more hydrophobic analogue), and BSA-FITC (a large functionalised protein). This selection was crucial to evaluate the hydrogel's release behaviour across a range of potential therapeutic agents. The results were clear and significant: for all three mimetics, the cumulative release profiles from the AuNP-loaded hydrogels were statistically not significant from those of the blank hydrogel. This demonstrates that the presence of the *in-situ* synthesized AuNPs does not occlude the polymer mesh or hinder the hydrogel's innate diffusion properties, a critical finding that confirms the matrix's integrity and its suitability for passive delivery applications.

Nonetheless, the defining experiment that unlocks the nanocomposite's advanced functionality was the laser-assisted drug release test. This test allowed to exploit the core property of the embedded AuNPs: their photothermal effect [1], [3]. When irradiated with a laser with a specific wavelength, the AuNPs efficiently converted light energy into heat. It is hypothesised that this localised heating induces dynamic, cyclical stretching and relaxation of the surrounding polymer matrix, actively enhancing the diffusion of the drug mimetics. The final result was a controllable release mechanism, achieving more than 20% of absolute increase in cumulative release compared to the non-irradiated control, the profiles are shown in Figure 9. This finding allows to transform the nanocomposite from a passive carrier into an active, externally triggered drug delivery system, opening doors for applications in precision medicine.

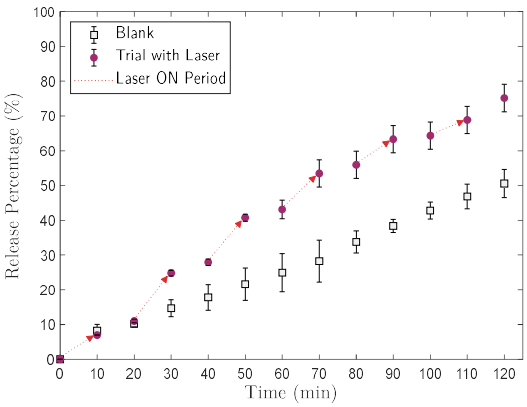

Figure 9. Release profile of the irradiated sample at room temperature in PBS 1X solution. Arrows indicate the windows of time in which sample was irradiated.

7. Conclusions

This research successfully demonstrates an streamline pathway for *in-situ* synthesis of AuNPs nanocomposites. By developing and optimising a one-pot *in-situ* protocol with a critical post-synthetic NaOH modification, it was possible to achieve a high level of control over nanoparticle size and distribution directly within a biocompatible matrix. The resulting material retains the desirable properties of the hydrogel while gaining the unique plasmonic capabilities of AuNPs, culminating in a proven capability for spatially and temporally controlled drug release via photothermal actuation.

This platform holds significant promise for future biomedical applications. The rational next steps involve rigorous biological validation, including in-vitro cytocompatibility studies and in-vivo models to assess therapeutic efficacy, biodistribution, and long-term stability, paving the way for its translation into a new class of smart, responsive biomaterials.

8. Bibliography

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