Investigating the interface in thiol-functionalized silver-gold nanoshells for lipase immobilization

Camila M. Kisukuri[†], Alexandra Macedo[§], Caio C. S. Oliveira[§], Pedro H. C. Camargo[§] and Leandro H. Andrade*[†]*

Universidade de São Paulo, Instituto de Química. Av. Prof. Lineu Prestes 748, SP 05508-900, São Paulo, Brazil.

[†]Laboratório de Química Fina e Biocatálise

[§]GrAND - Grupo para o Avanço no Design de Nanomateriais

E-mail = leandroh@iq.usp.br and camargo@iq.usp.br

Investigation of the AgAu NSs morpholgy

The characteristic hollow on silver/gold nanoshells can be evidenced through SEM and TEM images.[1] **Figure 1-3**.

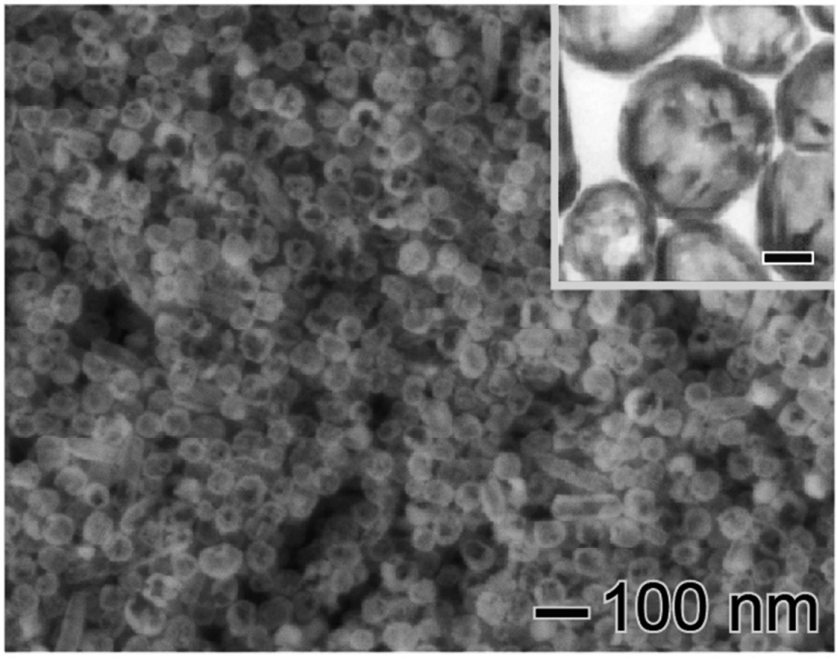


Figure 1: MEV and TEM (inset) images of the AgAu NSs. The scale bar in the inset corresponds to 15 nm.

In **Figure 1** (inset) we can see more clearly the difference in contrast presented by the TEM images. In **Figure 2-3** have the same SEM image, **Figure 3** is an enlarged and it can be seen more clearly the difference in contrast structure confirming the hollow nanoparticle.

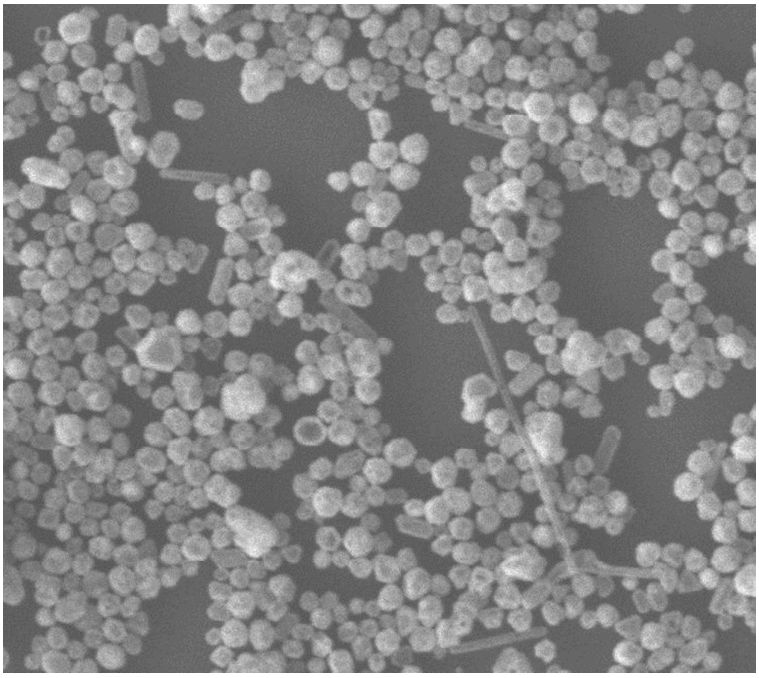


Figure 2: MEV image of the AgAu NSs.

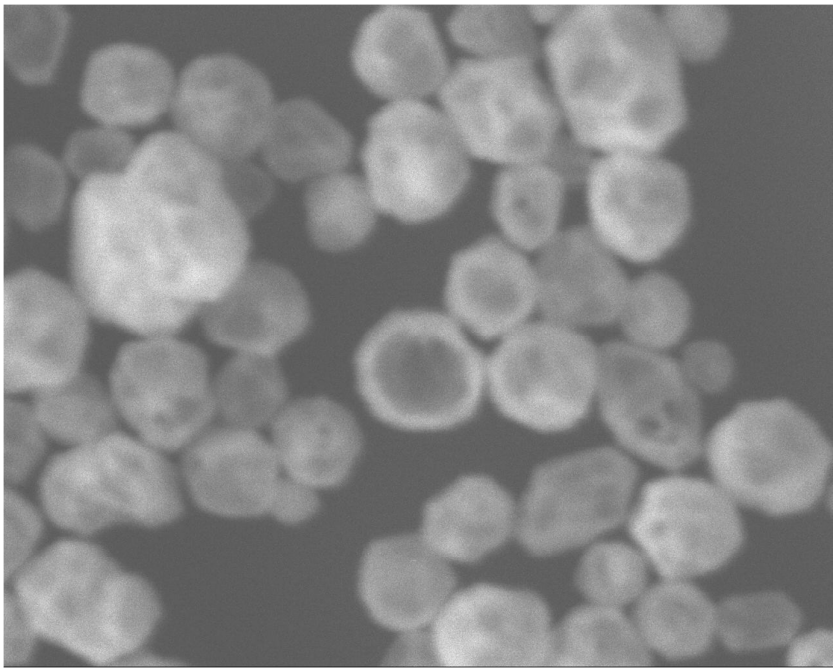
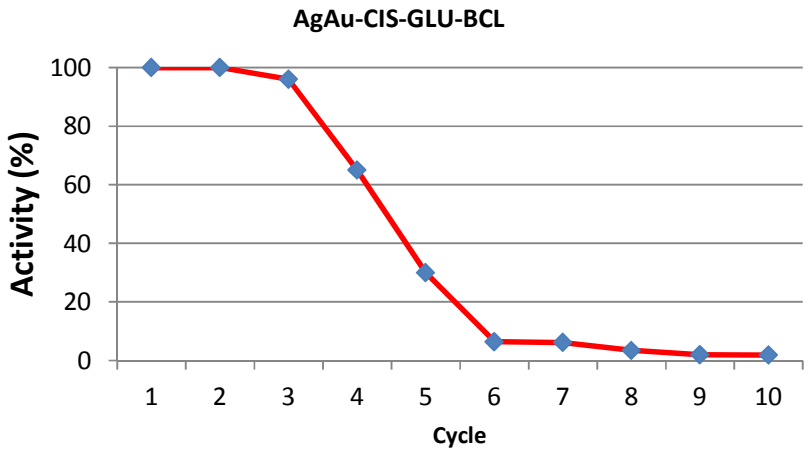


Figure 3: Zoom of MEV image from AgAu NSs, **Figure 2**.

Recycling study for the immobilized lipase

Study of recycling with AgAu-CIS-GLU-BCL has been done to complete loss of enzyme activity and is shown in the graph below, graph 1.



-

Graph 1: Recycling study of AgAu-CIS-GLU-BCL.

In the **Graph 1**, we can see that there is a large decrease in activity, about 35%, after four cycle and six cycle to almost total loss of activity.

Characterization of functionalized AgAu NSs and BCL immobilized in AgAu NSs via FT-IR.

FT-IR Analyses of AgAu-CIS, AgAu-MAC, AgAu-PROP, AgAu-UND, were performed to confirm the organic functionalization, **Figures 4-7**.

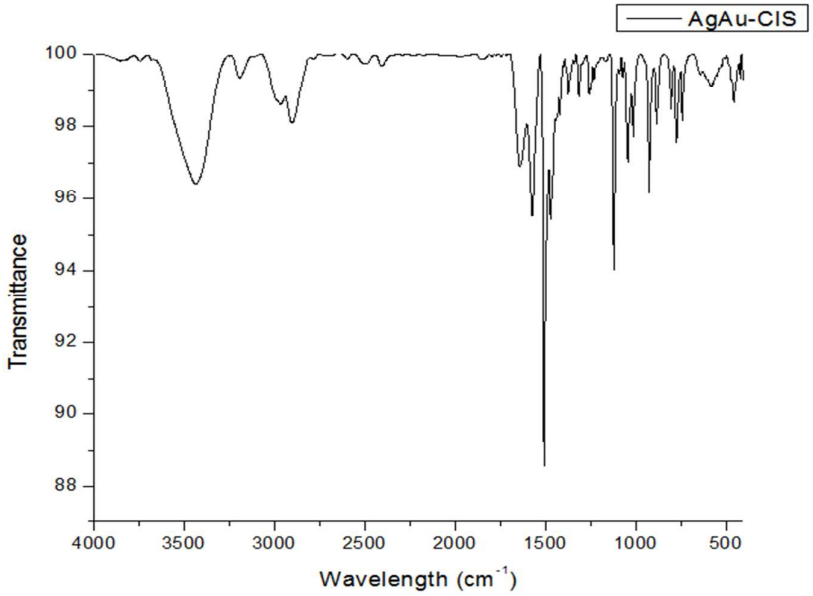


Figure 4: FT-IR Spectra of AgAu-CIS

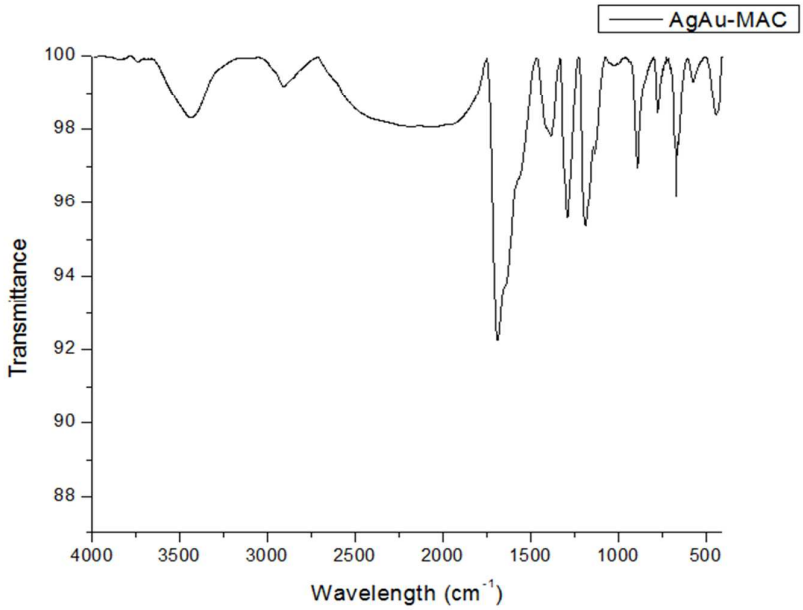


Figure 5: FT-IR Spectra of AgAu-MAC

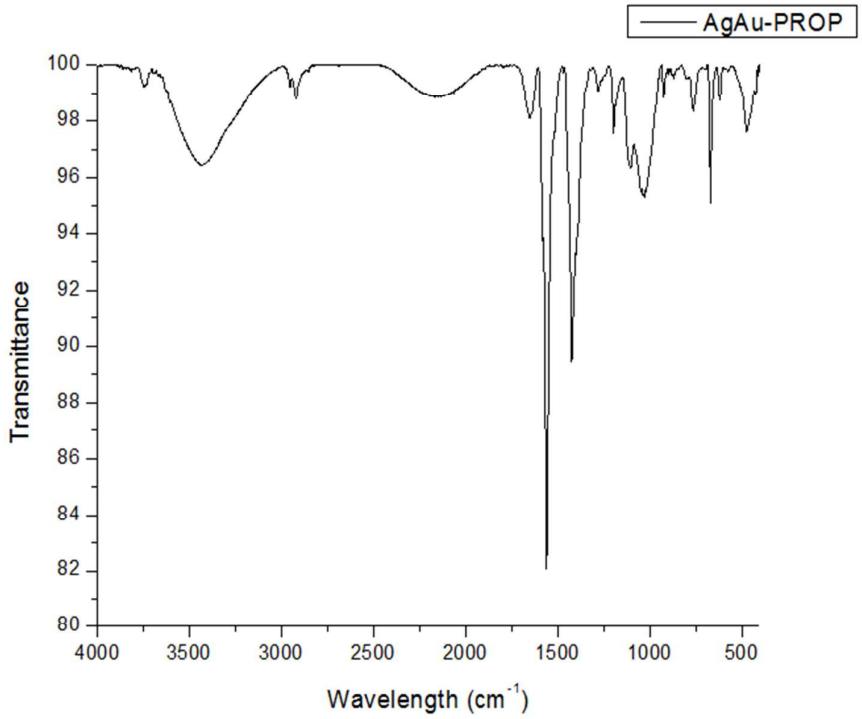


Figure 6: FT-IR Spectra of AgAu-PROP

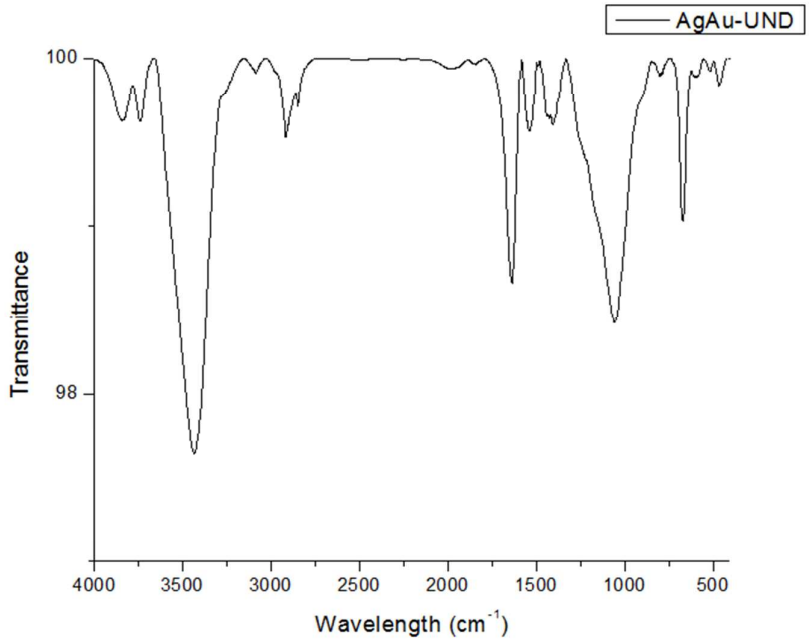


Figure 7: FT-IR Spectra of AgAu-UND

Aiming comparison with the functionalized nanoparticles containing the immobilized lipase they were also analyzed pr FT-IR. These spectra are showed bellow, **Figures 8-11**.

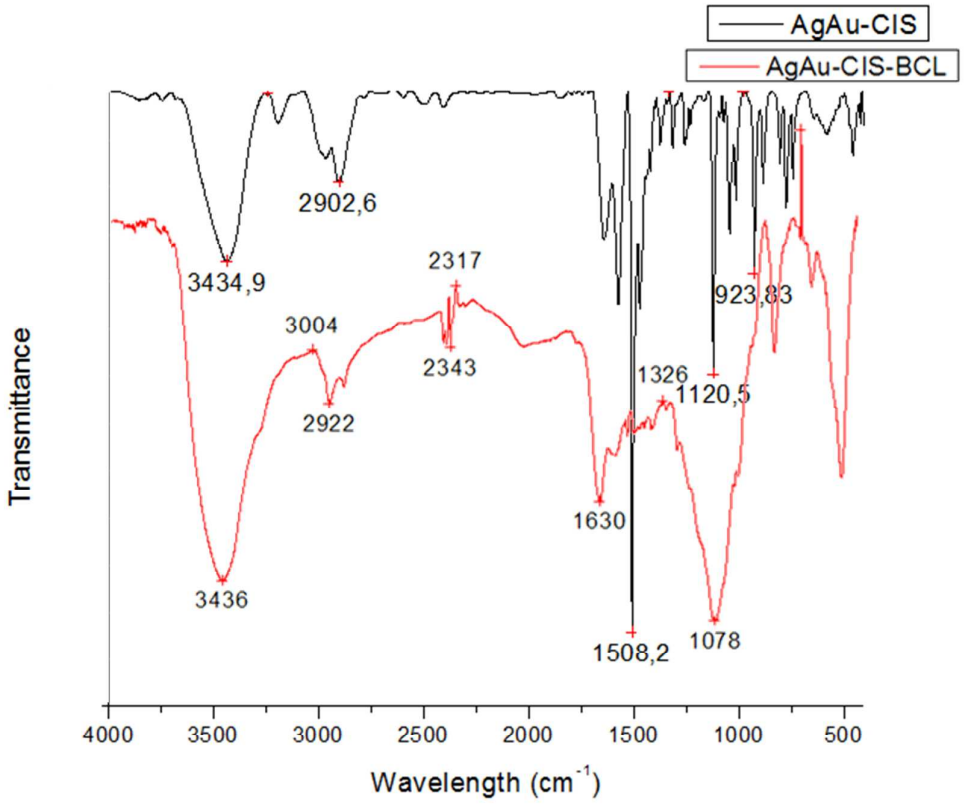


Figure 8: FT-IR Spectra of AgAu-CIS-GLU-BCL.

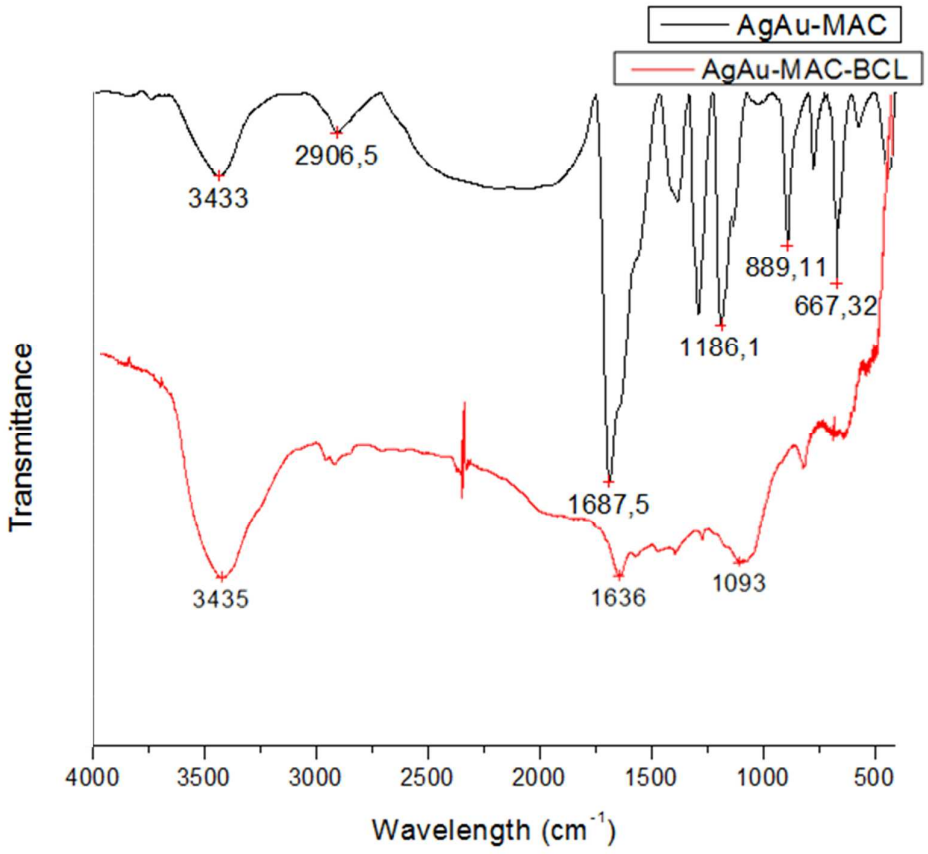


Figure 9: FT-IR Spectra of AgAu-MAC-BCL

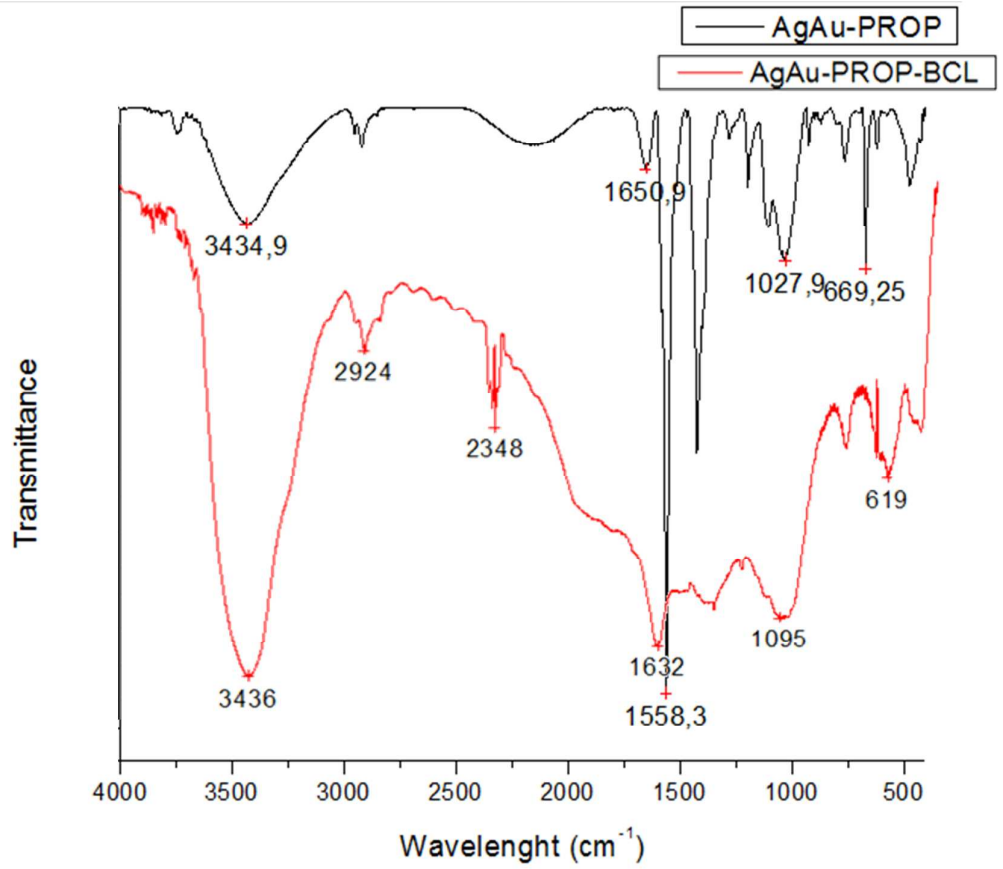


Figure 10: FT-IR Spectra of AgAu-PROP-BCL

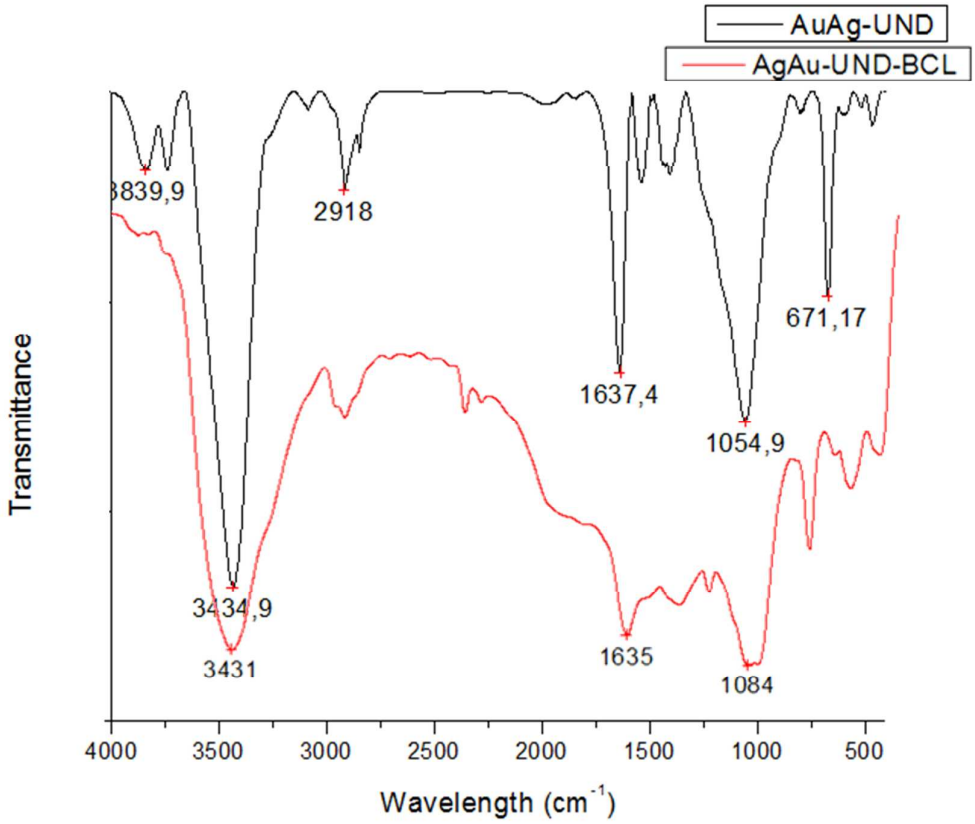


Figure 11: FT-IR Spectra of AgAu-UND-BCL

Morphology investigation of AgAu NSs containing immobilized BCL.

To illustrate the BCL and PPL immobilization onto AgAu NSs, TEM images were performed, **Figures 12-15**.

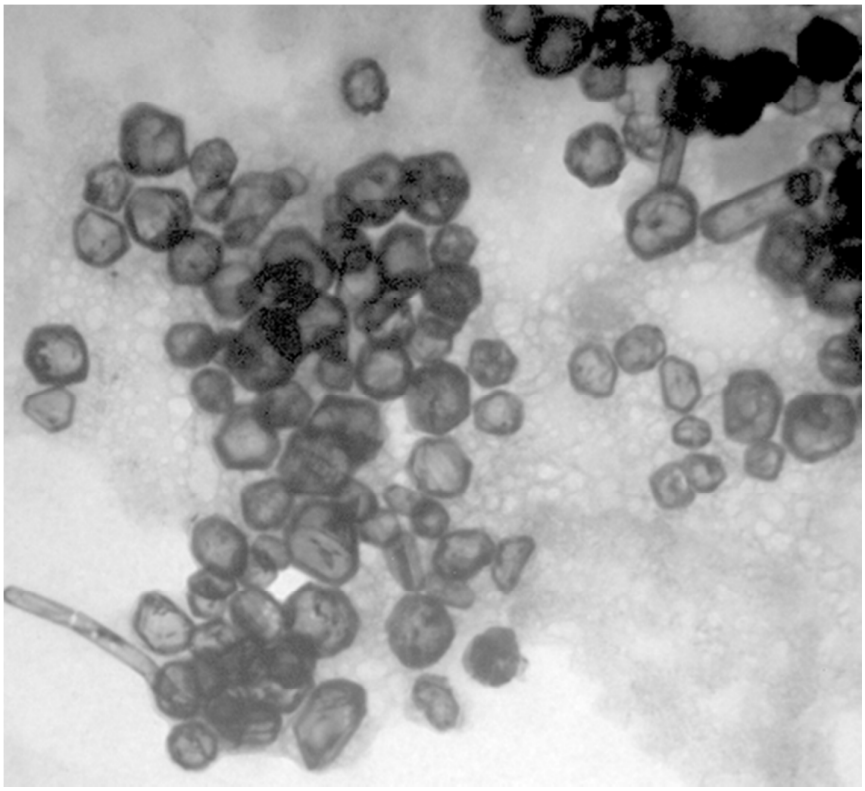


Figure 12: TEM Image of AgAu-CIS-GLU-BCL

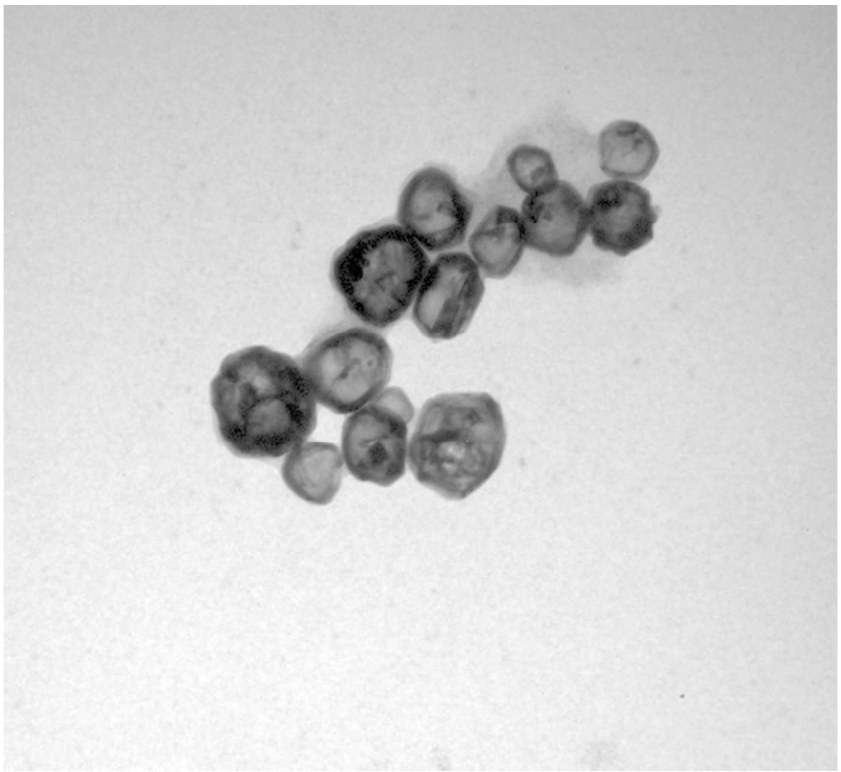


Figure 13: TEM Image of AgAu-MAC-BCL

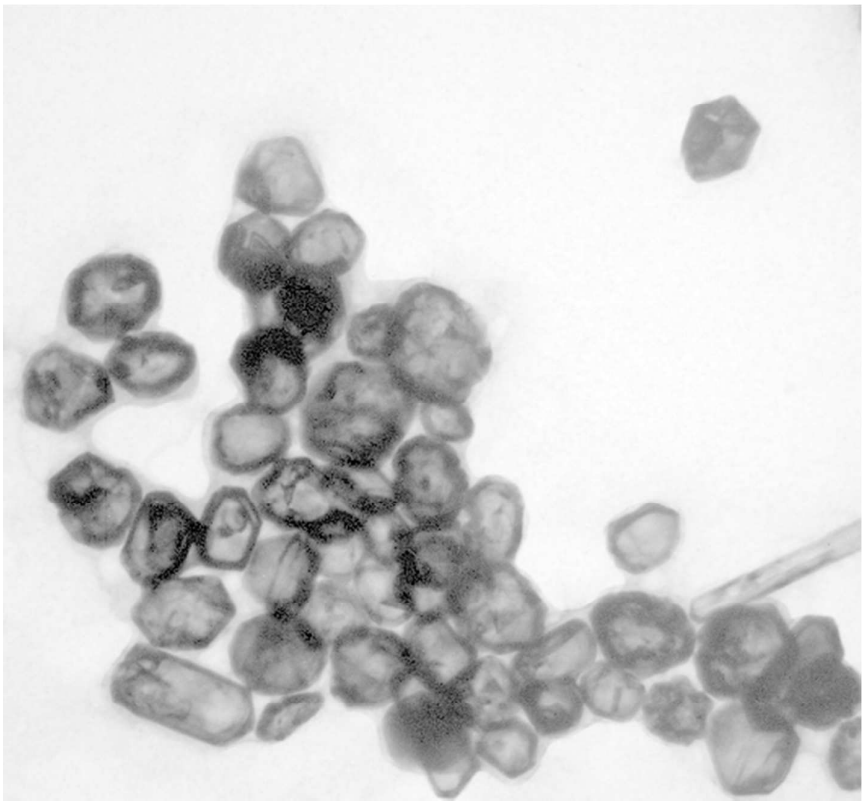


Figure 14: TEM Image of AgAu-PROP-BCL

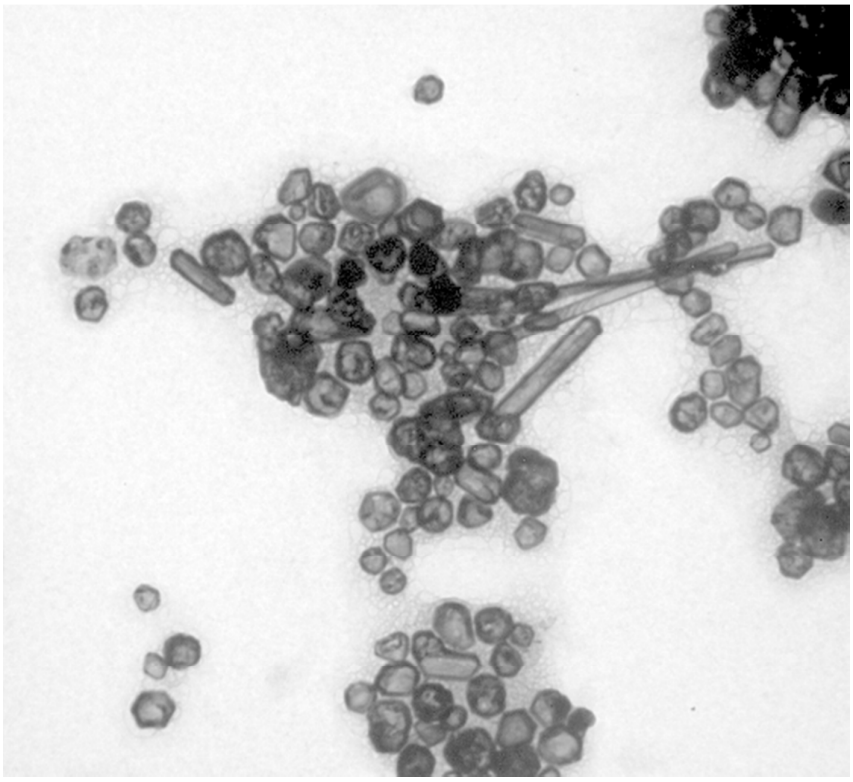


Figure 15: TEM Image of AgAu-UND-BCL

Assay of lipase activity for the hydrolysis of *p*-nitrophenyl palmitate in n-heptane.

The assay for lipase activity was based in the method developed by Pencreac'h and Baratti.[2][3]. In this method some amounts of free and immobilized enzyme were mixed with 1 mL of heptane solution containing 10 mM of *p*-NPP in a vial (2 mL, *eppendorf*®), **Table 1**. The mixture was incubated at 32°C in *Thermomixer*®, 800 rpm for 10 minutes. After that, an aliquot of 50 μL was withdrawn and immediately mixed with 1 mL of 0.1 M NaOH, contained in a vial (2 mL, *eppendorf*®). The absorbance was read at 410 nm against a blank without enzyme. The *p*NP (*para*-nitrophenol) liberated in this reaction can be transformed into the sodium *para*-nitrophenolate by aqueous alkaline phase which has a yellow color.

Table 1. Amounts of free and immobilized lipase used for the hydrolysis of *p*-nitrophenyl palmitate.

Systems	Amount of lipase (mg)
BCL free	0.278
AgAu-CIS-BCL	0.275
AgAu-MAC-BCL	0.278
AgAu-PROP-BCL	0.152
AgAu-UND-BCL	0.190

The activity of lipase was calculated from the slope of the absorbance versus the concentration of *para*-nitrophenol curve by using an apparent molar extinction coefficient of 1.08E4 L/mol.cm for *p*NP. This value was determined from the absorbance of standard solutions of *p*NP in the reaction mixture. One unit of lipase activity was defined as the amount of enzyme that liberated 1 μmol of *para*-nitrophenol from pNPP per min.

The results by the assay of *p*-NPP in heptane using BCL free and immobilized were: BCL free 0.25 U/mg, AgAu-CIS-BCL 0.36 U/mg, AgAu-MAC-BCL 0.37 U/mg, AgAu-PROP-BCL 0.50 U/mg and AgAu-UND-BCL 0.51 U/mg. All tests were done in duplicate.

This lipase activity test for the hydrolysis of *p*-NPP confirmed what we had seen in our studies of transesterification reaction, the immobilized lipase had better activity than the free lipase.

References:

1. Yang, L.: Yan, B.; Reinhard, B. M. Correlated Optical Spectroscopy and Transmission Electron Microscopy of Individual Hollow Nanoparticles and their Dimers. *J. Phys. Chem. C. Nanomater Interfaces*, **2008**, *112*, 15989–15996.
2. Pencreac`h, G.; Baratti, J. C. A Hydrolysis of p-nitrophenyl palmitate in n-heptane by the Pseudomonas cepacia lipase: A simple test for the determination of lipase activity in organic media *Emzyme Microb. Technol.* **1996**, *18*, 417-422.
3. Pencreac'h, G.; Baratti, J. C. A model of the pressure dependence of the enantioselectivity of Candida rugosa lipase towards (+/-)-menthol. *Enzyme Microb.Technol.* **2001**, *28*, 473-479.