

# **Anti-amyloidogenic property of gold nanoparticle decorated quercetin polymer nanorods in pH and temperature induced aggregation of lysozyme**

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## **1. Synthesis of citrate capped gold nanoparticle (Au nanoparticle)**

Citrate capped AuNPs synthesized by J. Turkevich method <sup>1</sup>. Chloroauric acid (HAuCl<sub>4</sub>) is a metallic nanoparticle precursor and tri-sodium citrate used as reducing agent. 100ml of 1mM HAuCl<sub>4</sub> was boiled under reflux condenser with continue stirring for 20 min. after boiling 10ml of 38.8mM tri sodium citrate was added dropwise into the boiling solution. Colour of the solution has been changed from pale yellow to colorless and finally dark reddish within few minutes.

## **2. Calibration curve**

### **2.1 Preparation of standard stock solution:**

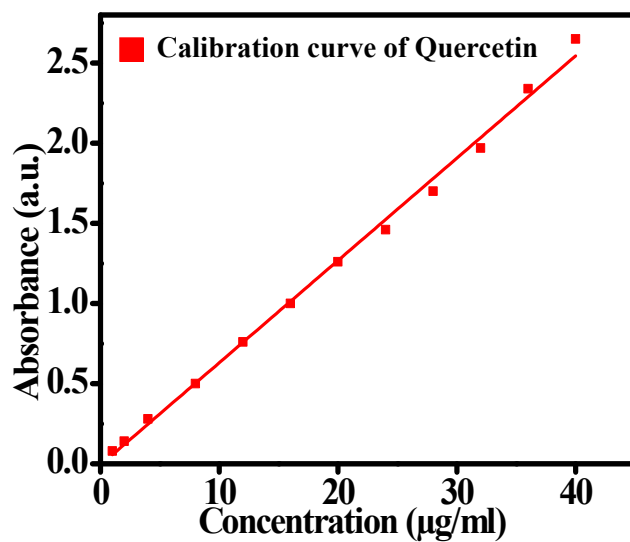
The standard stock solution of quercetin was prepared by dissolving 2 mg drug in 50 ml of methanol, in volumetric flask to get a solution containing 40µg/ml of drug. Series of solution (1µg/ml, 2µg/ml, 4µg/ml, 8µg/ml, 12µg/ml, 16µg/ml, 20µg/ml, 24µg/ml, 28µg/ml, 32µg/ml, 36µg/ml, 40µg/ml) for the calibration curve prepared in volumetric flask by taking appropriate aliquots of working standard solution of quercetin withdrawn and diluted up to 10 ml with methanol.

## 2.2 Construction of calibration curve:

Series of solution (1 µg/ml, 2 µg/ml, 4 µg/ml, 8 µg/ml, 12 µg/ml, 16 µg/ml, 20 µg/ml, 24 µg/ml, 28 µg/ml, 32 µg/ml, 36 µg/ml, 40 µg/ml) for the calibration curve prepared in volumetric flask by taking appropriate aliquots of working standard solution of quercetin withdrawn and diluted up to 10 ml with methanol. Quercetin shows absorption maxima at 372 nm. The concentration of drug in unknown samples from drug release studies were determined from the absorbance to concentration of the drug from Figure S1.

**Table S1:** Concentration of Quercetin use in calibration curve

Quercetin solution (µg/ml)	Absorbance at $\lambda_{\max}$ 372 nm
1 µg/ml	0.08
2 µg/ml	0.14
4 µg/ml	0.285
8 µg/ml	0.50
12 µg/ml	0.776
16 µg/ml	1.02
20 µg/ml	1.26
24 µg/ml	1.46
28 µg/ml	1.7
32 µg/ml	1.97
36 µg/ml	2.34
40 µg/ml	2.65



**Figure S1:** calibration curve for Quercetin at different concentration. Linear fitting gives  $R^2$  value of 0.9960

Parameters	Quercetin
$\lambda_{\max}$	372nm
Linearity Range	1 - 40µg/ml
$R^2$	0.9959
Slope	0.06379

### 3. FTIR stretching frequencies for Q, AuNP, Q-PVP and Q-PVP-Au

The new FTIR spectra for Q, AuNP, Q-PVP and Q-PVP-Au for the confirmation of additional group. We noted some additional groups are present. We observed the peak and intensity changes due to the conjugation of PVP and AuNP with quercetin.

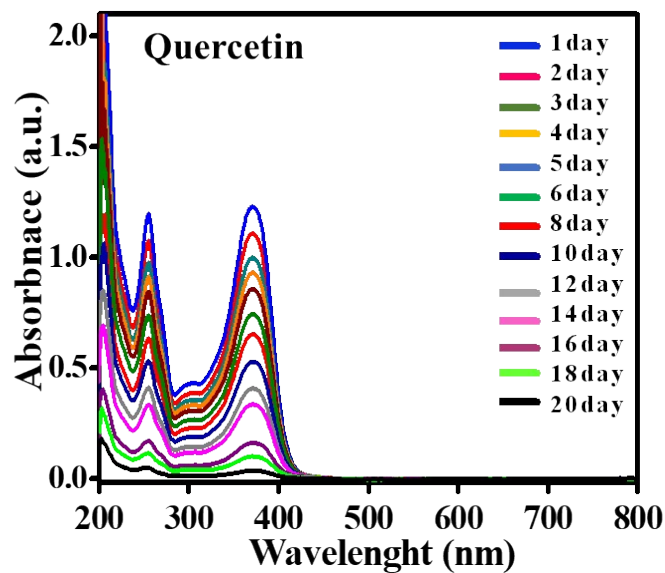
**Table S2: FTIR stretching frequencies for Q, AuNP, Q-PVP and Q-PVP-Au**

Name	Reference peak $\text{cm}^{-1}$	Obtained peak $\text{cm}^{-1}$	Functional group
Quercetin	3300-3500 $\text{cm}^{-1}$	3326 $\text{cm}^{-1}$	OH from the enol and phenolic -OH group
Q-PVP	3300-3500 $\text{cm}^{-1}$	3313 $\text{cm}^{-1}$	OH from the enol and phenolic -OH group
Q-PVP-Au	3300-3500 $\text{cm}^{-1}$	3363 $\text{cm}^{-1}$	OH from the enol

			and phenolic -OH group
Quercetin	2840-2950 $\text{cm}^{-1}$	2927 $\text{cm}^{-1}$	C-H Alkane group
Q-PVP	2840-2950 $\text{cm}^{-1}$	2927 $\text{cm}^{-1}$	C-H Alkane group
Q-PVP-Au	2840-2950 $\text{cm}^{-1}$	2927 $\text{cm}^{-1}$	C-H Alkane group
Quercetin	1660 $\text{cm}^{-1}$	1659 $\text{cm}^{-1}$	C=O Aryl ketone group
Q-PVP	1660 $\text{cm}^{-1}$	1659 $\text{cm}^{-1}$	C=O Aryl ketone group
Quercetin	1610-1630 $\text{cm}^{-1}$	1610 $\text{cm}^{-1}$	C=C Aromatic ring
Q-PVP	1610-1630 $\text{cm}^{-1}$	1619 $\text{cm}^{-1}$	C=C Aromatic ring
Q-PVP-Au	1610-1630 $\text{cm}^{-1}$	1610 $\text{cm}^{-1}$	C=C Aromatic ring
Q-PVP	1385 $\text{cm}^{-1}$	1383 $\text{cm}^{-1}$	Bending -OH of quercetin
Q-PVP-Au	1385 $\text{cm}^{-1}$	1383 $\text{cm}^{-1}$	Bending -OH of quercetin
PVP	3400-3700 $\text{cm}^{-1}$	3413 $\text{cm}^{-1}$	OH group from PVP
PVP	1600-1800 $\text{cm}^{-1}$	1756 $\text{cm}^{-1}$	C=O
Quercetin	1000 $\text{cm}^{-1}$	1000 $\text{cm}^{-1}$	C-H Aromatic hydrocarbon
Q-PVP	1000 $\text{cm}^{-1}$	1021 $\text{cm}^{-1}$	C-H Aromatic hydrocarbon
Q-PVP-Au	1000 $\text{cm}^{-1}$	1021 $\text{cm}^{-1}$	C-H Aromatic hydrocarbon

#### 4. Stability profile of Quercetin.

Stability profile of Q was studied by the UV-visible spectroscopy for the several days. Stability of Q examined with a specific time interval by dissolving the free Q in Milli Q water at room temperature.



**Figure S2:** Stability profile of Quercetin.

### 5. Drug loading at different morphology of Q-PVP and Q-PVP-Au

We have calculated the loading amount of Q under different morphology of Q-PVP, Q-PVP-Au. The Q drug loading for the different morphology in Q-PVP have been included for the table S3 and the Q drug loading for the different morphology for Q-PVP-Au have been included in the table S4 along with specific time.

**Table S3:** Q drug loading at different time in Q-PVP

Time	% of drug loading
1h	75
1h:30min	78
2h	81
2h:30min	86
3h	88

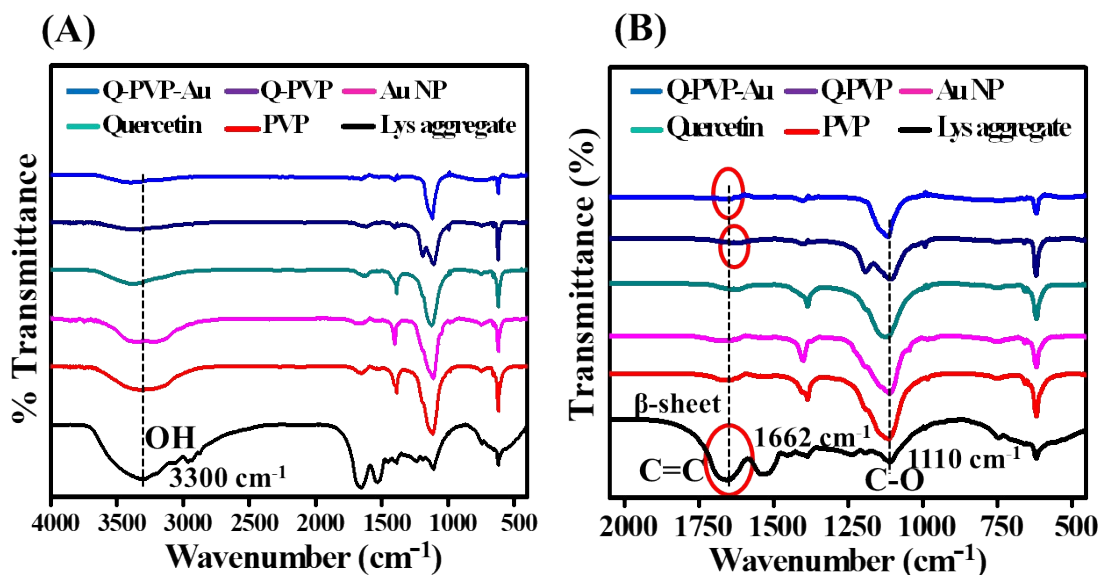
**Table S4:** Q drug loading at different time in Q-PVP-Au

Time	% of drug loading
15 min	79
30min	83
1h	86

1h:30min	90
2h	94

## 5. Fourier transform infrared (FT-IR) measurement of lysozyme aggregates

FTIR spectra for validation of functional groups of lysozyme aggregates treated by PVP, Quercetin, Au NP, Q-PVP and Q-PVP-Au.  $\beta$ -sheet structure is likely to form mature fibrils in HEWL, hence forth the secondary structure of HEWL was verified by FT-IR spectroscopy. FTIR analysis was used to characterize the secondary structure of HEWL aggregates. HEWL, 0.2 mg/mL was dissolved in 2mL of 0.1% HCl (pH 1.6) and heated up to 65°C for 24h and stirred at 550 rpm. Then, samples were mixed with the KBr to make a pallet, followed by air drying before FTIR measurements. HEWL samples with and without nanoconjugates were taken and analyzed for FT-IR measurement.



**Figure S3:** **A)** FTIR spectra for validation of functional groups of lysozyme aggregates treated by PVP (red line), Quercetin (green line), Au NP (pink line), Q-PVP (purple line), Q-PVP-Au (blue line); **B)** Shows the same spectra but zoomed into area of interest from 2000  $\text{cm}^{-1}$  to 500  $\text{cm}^{-1}$  to assign specific transmission bands to some functional groups.

### Reference

1. J. Kimling, M. Maier, B. Okenve, V. Kotaidis, H. Ballot and A. Plech, *The Journal of Physical Chemistry B*, 2006, **110**, 15700-15707.