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Ultra Stable Dual Functionalized Gold Nanoparticles for the Effective Colorimetric Detection of Clenbuterol

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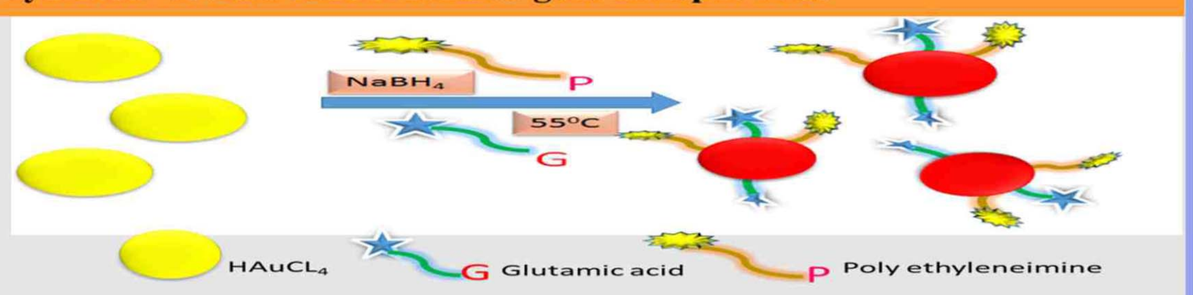
Abstract : A new ultra stable dual functionalized **PE-Glu-AuNPs** was developed and utilized towards naked eye colorimetric detection of clenbuterol (CLB). **PE-Glu-AuNPs** were synhesized by functionalization of AuNPs with Glutamic acid (Glu) and polyethyleneimine (PE) via $NaBH_4$ reduction method. We have elucidated the stability of **PE-Glu-AuNPs** over a period of six months. Further, the colorimetric assay of **PE-Glu-AuNPs** to CLB revealed that, its selective sensing ability starts from 300 nM visualized through naked eyes at pH 5. Additionally, the sub nanomolar detection of CLB has been estimated from linear fittings and standard deviation of UV-vis titration. It has also been well demonstrated that the probe does not evidence the selectivity towards any other interferences (A-Alamine; B-Phenylalanine; D-Nacl; E-$CaCl_2$; F-Threonine; G-Cysteine; H-Glycine; I-Glucose and J-Urea). Moreover, the aggregation induced detection of CLB was well confirmed by TEM studies. Moreover, the optimization process for the effective determination of clenbuterol in human urine sample were also investigated.
Keywords: Dual functionalized gold nanoparticles, Clenbuterol, Biosensor, Human urine sample

Synthesis of dual functionalized gold nanoparticles



Scheme 1.Schematic representation of synthesis of dual functionalized gold nanoparticles

Stability of ultra stable dual functionalized gold nanoparticles

Fig. 1. (a) Naked eye Photograph and (b) The SPR absorption of **PE-Glu-AuNPs** with different month interval.

Effect of pH on ultra stable dual functionalized gold nanoparticles

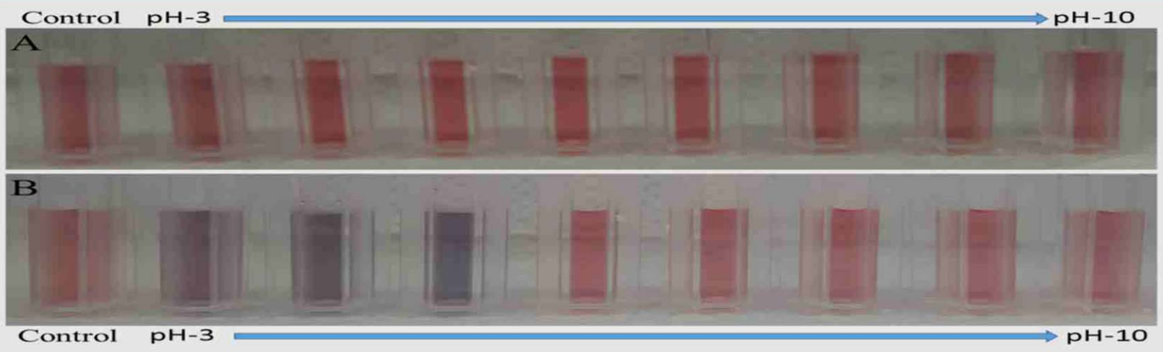
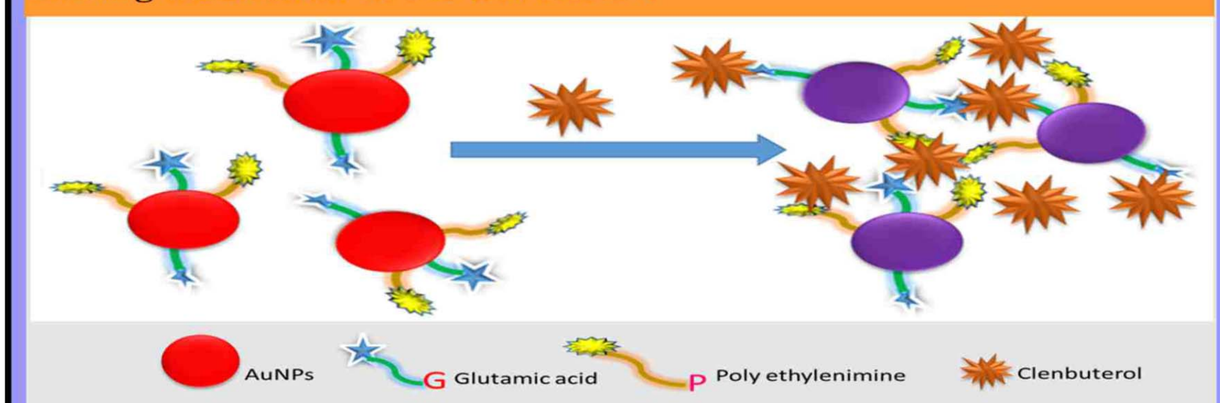


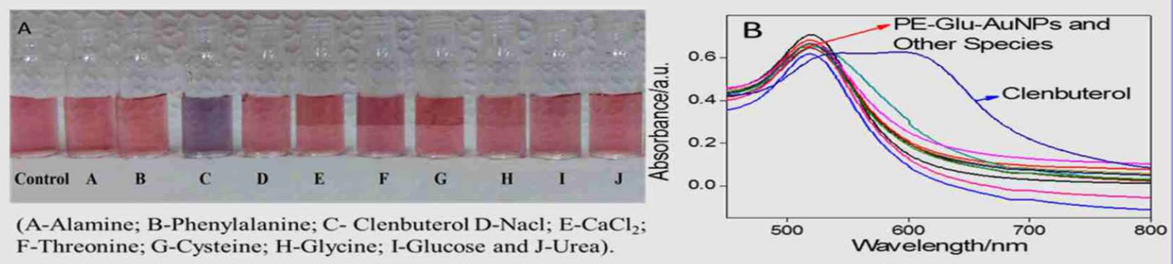
Fig. 2. pH effect on the **PE-Glu-AuNPs** (A) in the absence of CLB (B) presence of CLB 1000 nM

Sensing mechanism of PE-Glu-AuNPs



Scheme. 2. Schematic representation **PE-Glu-AuNPs** sensing mechanism with CLB

PE-Glu-AuNPs selectivity towards other analytes



(A-Alamine; B-Phenylalanine; C- Clenbuterol D-Nacl; E-$CaCl_2$; F-Threonine; G-Cysteine; H-Glycine; I-Glucose and J-Urea).

Fig. 3. (A) Naked eye Image and (B) The SPR absorption of the **PE-Glu-AuNPs** in the presence of various other interferences (1000 nM) at pH-5.

TEM Analysis

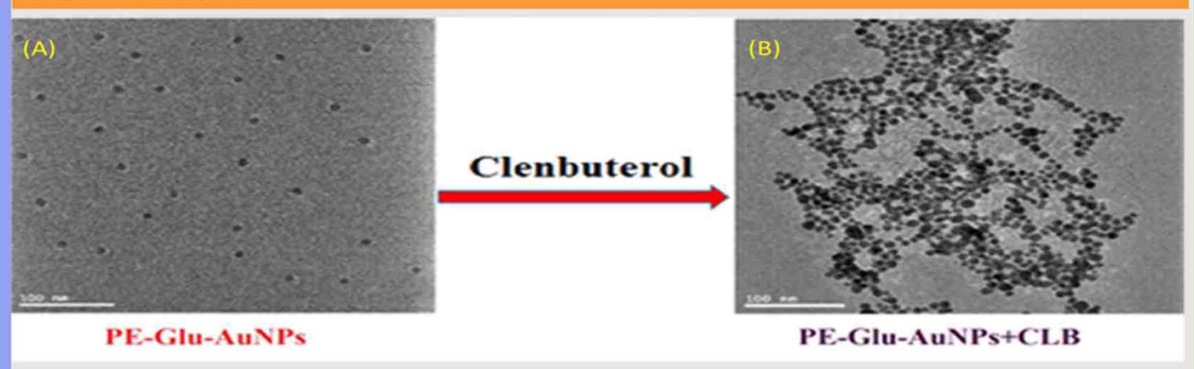


Fig. 4. TEM image of (A) **PE-Glu-AuNPs** at pH -5 (b) **PE-Glu-AuNPs** with CLB (1000 nM) at pH-5

Absorption spectral changes of PE-Glu-AuNPs with respect to CLB concentration

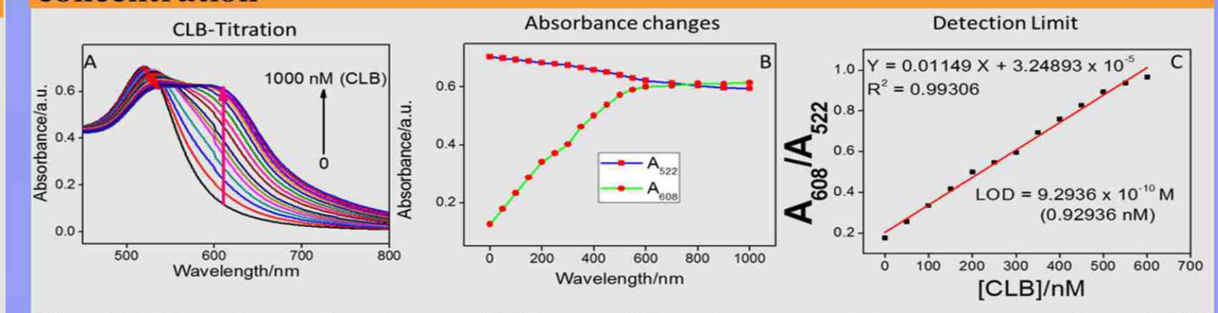


Fig.5. (A) Absorption spectral changes of **PE-Glu-AuNPs** in the presence of various concentrations of CLB (0−1000 nM) at pH-5, (B) absorbance ratio (A_{608}/A_{522}) changes of **PE-Glu-AuNPs** in the presence of various concentrations of CLB (0−1000 nM) and (C) detection limit calculated by standard deviation and linear fitting.

CLB detection in human urine sample

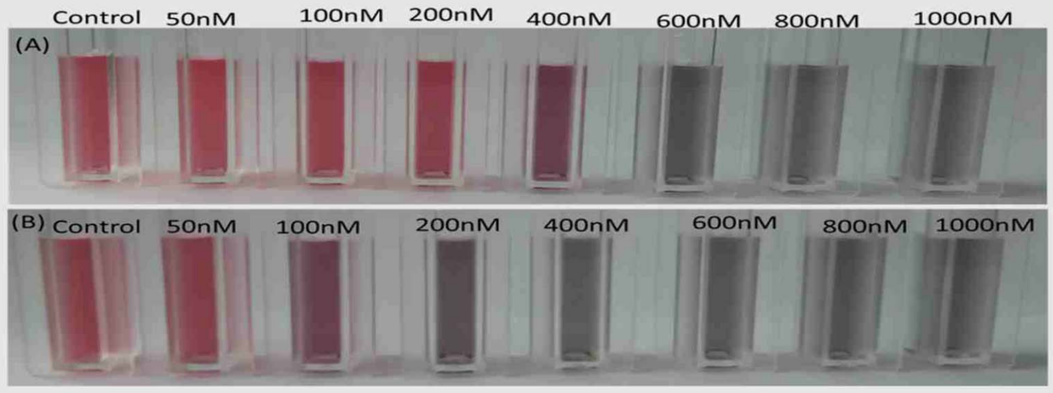


Fig. 6. (A) The image of the **PE-Glu-AuNPs** with CLB from 50 nM to 1000 nM: (B) **PE-Glu-AuNPs** with CLB form 50 nM to 1000 nM (0.7 µM CLB added in advance).

Conclusion

A novel dual functionalized gold nanoparticles (**PE-Glu-AuNPs**) with ultra-stability for more than 6 months has been reported first time as **CLB** sensor. It acts as a promising probe towards sensitive and selective colorimetric detection of **CLB**. Furthermore, the sub nanomolar detection of **CLB** has been estimated from linear fittings and standard deviation of UV-vis titration. This colorimetric **PE-Glu-AuNPs** based assay offers a fast method for **CLB** recognition at a low cost, simple synthesis step with 0.93 nM detection limit. The initial results on human urine sample analysis, illustrated that it can be further applied in real time biological studies.

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