Acta Pharm. **68** (2018) 243–250 Short communication
https://doi.org/10.2478/acph-2018-0020

Spectrofluorimetric method for atenolol determination based on gold nanoparticles

ESAM BAKIR[1,2]*
MOHAMED GOUDA[1,3]
AHMED ALNAJJAR[1]
WALEED E. BORAIE[1,2]

[1] *Department of Chemistry College of Science, King Faisal University, Hofuf, Saudi Arabia*

[2] *Department of Chemistry Faculty of Science, Ain Shams University, Cairo, Egypt*

[3] *Textile Research Division National Research Center, Dokki Cairo, Egypt*

Accepted January 21, 2018
Published online February 19, 2018

A simple and sensitive spectrofluorimetric method for determination of atenolol (ATE) using gold nanoparticles (AuNPs) was developed. The method is based on the quenching effect of atenolol on photoluminescence of AuNPs at λ_{em} = 705 nm. Variables affecting luminescence of gold nanoparticles such as the solvent, pH value and surfactant were studied and optimized. The method was preliminarily validated according to ICH guidelines. A linear correlation was recorded within the range of 1.0–10 μg mL^{-1} ATE with the coefficient of determination R^2 of 0.999. The limit of detection and limit of quantitation for atenolol were found to be 0.87 and 2.64 μg mL^{-1}, resp. Good recoveries in the range of 98.7–100.0 % were obtained for spiked samples. The proposed method was applied successfully to assaying atenolol in pharmaceuticals formulations.

Keywords: atenolol, spectrofluorimetry, gold nanoparticles

Atenolol, 4-(2-hydroxy-3-isopropylaminopropoxy)phenylacetamide (ATE), is a beta-blocker that affects the heart and circulation. Atenolol is used to treat angina and hypertension. Atenolol is also used to lower the risk of death after a heart attack (1).

Previous studies applied spectrofluorimetry to estimation of atenolol in aqueous solutions and samples of human urine because of its sensitivity, selectivity and low-cost instrumentation (2). Fluorescence was used to detect atenolol without and with solid-phase extraction (3). Photoluminescence of metal nanoparticles that offer emission of light without requiring conjugation with luminous dyes is the basis for the new method (4).

The photo-physical properties of colloidal gold nanoparticles are unique in showing stable and non-bleaching fluorescence, which was already widely used in a variety of molecularly targeted biological applications, including multiphoton and correlative microscopy (5). In this study, novel, simple, sensitive and selective gold nanoparticles (AuNPs) were developed for determination of ATE.

* Correspondence; ebakir@kfu.edu.sa; esambakir99@yahoo.com

EXPERIMENTAL

Materials

Atenolol, gold(III) chloride trihydrate ($HAuCl_4$), cetyl-trimethyl-ammonium bromide (CTAB), sodium dodecyl sulfate (SDS) surfactant and HPLC grade solvents such as methanol, acetonitrile (ACN) and dimethylsulphoxide (DMSO) were purchased from Sigma Aldrich (USA). A commercial pharmaceutical formulation (Normoten-100, Jazeera Pharmaceutical Industries, Saudi Arabia) was used.

Synthesis and physical characterization of gold nanoparticles

Gold nanoparticles were synthesized by the citrate reduction method according to the reported method (6).

Absorption and photo-luminescence spectra were measured on spectrophotometer and spectrofluorimeter models USB4000 and USB4000FL, resp. (Ocean Optics Devices, USA). Diameter and colloidal properties of gold nanoparticles were estimated using scanning electron microscopy (SEM) on a Phillips CM201C microscope at 80 kV (Phillips, The Netherlands).

ATE standard solutions and calibration

An accurately weighed amount of ATE was dissolved in methanol to get a stock solution of 50 μg mL^{-1}. Working ATE solutions in the concentration range of 1–10 μg mL^{-1} were transferred into a series of 10-mL calibrated flasks containing 300 μL of 0.277 mmol L^{-1} colloidal AuNPs.

Procedure for commercial tablets

Fine powder of Normoten-100 tablets (equivalent to 100 mg of atenolol) was accurately weighed and placed in a 100-mL flask containing methanol. The solution was sonicated, filtered and then made up with methanol to 10 μg mL^{-1} of atenolol.

Quenching of photoluminescent AuNPs by ATE

The Stern-Volmer relationship was explored for the kinetics of photo-physical intermolecular deactivation of photoluminescent AuNPs by atenolol according to:

$$\frac{I_0}{I} - 1 = K_{SV}[Q]$$

where I_0 and I are luminescence intensity of AuNPs in the absence and in the presence of different atenolol concentrations at λ_{em} = 705 nm, [Q] is the concentration of the quencher and K_{SV} is the Stern-Volmer constant as a measure of sensor sensitivity (7).

Method validation

Validation of the method was done according to the ICH Q2 (R1) guideline (8).

Linearity and range. – Linearity was established from the calibration curve of photoluminescence of AuNPs *vs*. atenolol concentration estimating R^2. Limits of detection and

quantitation (*LOD* and *LOQ*, resp.) for the proposed method were evaluated as per ICH guidelines using the formulae:

$$LOD = 3.3\ \sigma / S \text{ and } LOQ = 10\ \sigma / S$$

where σ is standard deviation of the response (standard deviation of y-intercepts of the regression line), S is the slope of the calibration line.

Precision and accuracy. – Intra-day and inter-day precision were investigated by evaluating 3 sample replicates on the same day and on 3 different days of a week. Results are expressed as RSD.

Accuracy was established as the percent of recovery of ATE for three standard additions to the sample. Three different concentrations were added to a fixed amount of the prepared drug sample, standard Normoten, and the total was analyzed by the studied method. Each analysis was carried out three times.

Specificity. – Specificity of the method for atenolol was evaluated in the presence of interfering substances such as excipients, degradants and matrix components (starch, lactose, glucose and sodium chloride). Excipient concentrations were 5, 10 and 20 μg mL^{-1} mixed with a fixed concentration of atenolol (5 μg mL^{-1}).

Robustness. – For the determination of method robustness, three factors were examined: reaction time, change of temperature and change of the order of reagent addition.

RESULTS AND DISCUSSION

Diameter of AuNPs

AuNPs appeared as small dark spherical dots. Fig. 1 shows the SEM images of AuNPs, which reveal that the average size of AuNPs with the shape of nanoclusters was 22.8 ± 8.0 nm.

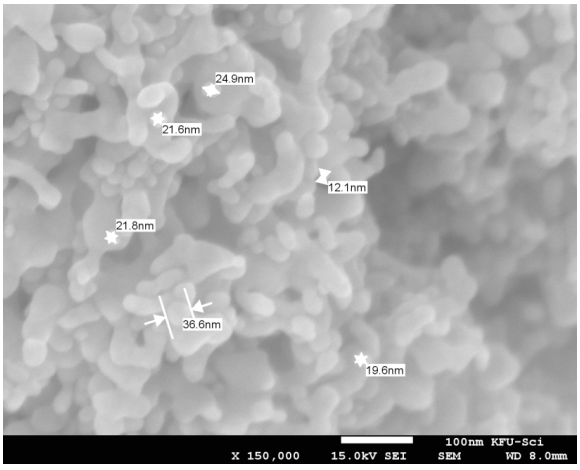

Fig. 1. SEM image of AuNPs prepared by the citrate reduction method in DMSO.

Factors affecting AuNP luminescence

Solvent effect. – The absorption and luminescence spectra are shown in Fig 2. Absorbance of Au nanoparticles at 525 nm decreased whenever the dipole moment of the solvent decreased. The gold nanoparticles aggregated and flocculated at room temperature in a nonpolar solvent such as cyclohexane while the strength of solvent polarity maintained the AuNP particles in suspension form.

In the case of protic solvents, the absorbance of AuNPs is slightly red-shifted as the polarity decreases. This might be attributed to the electrostatic interaction of the solvent OH group with AuNP citrate capping. In the case of acetonitrile, the absorbance is also red-shifted. It is worth noting that the peak in this case is shown to be broad. In the case of CAN, the AuNPs appear to be aggregated, since ACN has low affinity towards AuNPs (9). In the case of DMSO, the sulfuric group can envelope AuNPs as a further protection layer, resulting in the lowest quenching of AuNP luminescence (10).

Effect of pH. – The absorbance and photoluminescence of colloidal AuNP dispersions was measured at different pH values (4–10). At pH ≤ 4, the negative charge of citrate anions surrounding the gold nanoparticles became fully protonated and the Au nanoparticles were aggregated; hence the stability of the suspension form decreased. At pH ≥ 10.0, the gold nanoparticle surface was fully deprotonated and their stability was improved (11).

Effect of KCl solution. – The absorbance and photoluminescence properties of AuNPs were measured at 635 nm using different concentrations of KCl (0.1–1.00 mmol L^{-1}). At low ionic strength, small aggregates were formed of gold nanoparticles, which were stable over a long period of time. We found that the AuNPs showed faster aggregation and precipitation at 0.6 mmol L^{-1} KCl (12).

Effect of surfactant. – The absorption band of gold nanoparticles was affected by different concentrations of CTAB (10–50 mmol L^{-1}) and SDS (10–50 mmol L^{-1}). The AuNPs aggregated at 10 mmol L^{-1} of cationic CTAB micelles due to the strong interaction of the cationic head group

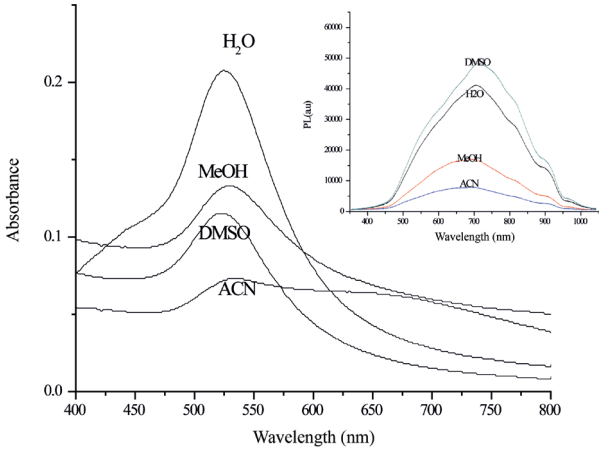

Fig. 2. Absorption and photoluminescence spectra of AuNPs using different solvents.

of CTAB micelles with citrate capping of AuNPs. At higher concentrations (> 10 mmol L^{-1}), the AuNPs became more stable because the CTAB micelles formed a bilayer over citrate capped AuNPs. In the case of 10 mmol L^{-1} of anionic SDS, the AuNPs did not aggregate due to the electrostatic repulsion between the negative head group ($-OSO_3^-$) and citrate capping on AuNPs. AuNPs were stabilized at > 10 mmol L^{-1} of SDS micelles since the SDS micelles adsorbed on the surface of citrate-capped AuNPs (13).

Analytical validation

Linearity. – The calibration line was established in the range of 0.1–10 μg mL^{-1} ATE, R^2 = 0.9998. Table I shows the *LOD* and *LOQ* values of 0.87 and 2.64 μg mL^{-1} ATE, resp.

Sensitivity and stability. – The high value of molar absorptivity (ε) and low values of *LOD*/*LOQ* indicate high sensitivity of the proposed method, as shown from Table I. The luminescence intensity of AuNPs was stable for 24 h. Using the spike standard, namely, after adding atenolol, recovery of luminescence of AuNPs-ATE was $99.5 \pm 0.0_1$ % (mean ± SD, n = 3) and the RSD value was less than 1 %. The luminescence of AuNPs-ATE was measured at time intervals of 1, 10 and 60 min and the temperature intervals were 20, 30 and 40 °C.

Accuracy and precision. – Table II shows the accuracy and precision of the proposed method. RSD was ≤ 2.0 % (intra-day) and ≤ 0.4 % (inter-day). In addition, the accuracy of the proposed method was measured by calculating the percent relative error, which varied

Table I. Sensitivity and regression parameters for atenolol by AuNPs as a photo probe

Analyte	Atenolol
Wavelength (nm)	705 nm
Beer's law limits (μg mL^{-1})	1–10
Regression equation	$y = a + bx$ (a = 0.0346, b = 1.0012)
Coefficient of determination (R^2)	0.999
LOD (μg mL^{-1})	0.87
LOQ (μg mL^{-1})	2.64
Molar absorptivity (ε, L moL^{-1} cm^{-1})	3.7×10^2
RSD (%)	1.2

Table II. Intra-day and inter-day accuracy and precision

Atenolol taken (μg mL^{-1})	Intra-day accuracy and precision			Inter-day accuracy and precision		
	ATE found, average (μg mL^{-1})	RSD (%)	Accuracy*	ATE found, average (μg mL^{-1})	RSD (%)	Accuracy*
0.100	0.102	2.0	102.0 ± 1.0	0.103	0.3	101.0 ± 0.4
2.700	2.690	0.4	99.6 ± 1.0	2.720	0.4	101.1 ± 0.4
7.000	7.010	0.3	100.1 ± 1.0	7.020	0.1	100.1 ± 0.4

ATE – atenolol
* Recovery (%); mean ± SD, n = 3.

between 0.1 and 2.5 %, and the recovery was 99.6–102.0 % according to the standard addition method. Results presented in Table II reveal a slight effect on the accuracy of the method in various excipients.

Robustness. – The average value of ATE recovery was $99.5 \pm 0.0_1$ % using standard additions (mean ± SD, $n = 3$) and the RSD value was less than 1 %. This means that the proposed method is robust with small variations in temperature (20, 30 and 40 °C) and time intervals (1, 10 and 60 min).

Specificity. – Recovery of atenolol was found in the range 99.0–101.0 % with RSD < 2 % using the standard addition method.

Analysis of the commercial formulation

The proposed method was successfully applied to determination of ATE in the Normoten-100 formulation. The results are given in Table III.

Table IV shows the data obtained by the proposed method compared to reference methods (14, 15). Recovery of ATE in Normoten (100 mg ATE) by the proposed method was

Table III. Determination of atenolol in the pharmaceutical formulation using AuNPs as a photo probe

Formulation	Normoten (μg mL^{-1})	ATE added (μg mL^{-1})	Total ATE found (μg mL^{-1})	ATE recovered (%)[a]
Normoten-100	3.00	0.00	2.99	99.7 ± 0.1
	3.00	0.50	3.51	98.0 ± 2.0
	3.00	3.00	5.99	99.7 ± 0.3
	3.00	5.00	7.99	99.8 ± 0.2

[a] Mean ± SD, $n = 3$.

Table IV. Analysis of atenolol in tablets by the proposed and reference methods

Method	Label claim –100 mg ATE	Reference method (recovery, %) [a]	Linearity range/*LOD* (μg mL^{-1})	Remarks
Oxidation of drug using alkaline $KMnO_4$ (13)	Blokium	100.0 ± 0.5	2.0–14.0/ 0.081	Less sensitive under heating
Reaction with Phenol Red in acidic medium (14)	Normoten	98.9 ± 0.3	0.5–30/ 0.038	No interferences
Quenching of luminescence of AuNPs by atenolol (proposed method)	Normoten	100.2 ± 0.3	2.6–10/ 0.870	Simple, no heating, short response time, stable luminescent reagent

[a] Recovery ± RSD, $n = 3$.

100.2 ± 0.3 % (mean ± SD, n = 3). The advantages of this method compared to reference methods was that the proposed method had a short response time, ~5 s, and that the AuNP luminescence was stable, ~60 min.

CONCLUSIONS

A simple, sensitive, accurate and precise spectrofluorimetric method using AuNPs as a photo probe is suggested for determination of atenolol. The luminescence intensity of AuNPs in different solvents, at different pH values, at different KCl concentrations and in the presence of two types of surfactants was studied to obtain optimum conditions. The proposed method can be used for routine analysis of atenolol in pharmaceutical dosage forms.

Acknowledgments. – The authors are grateful to the Dean of Scientific Research at King Faisal University for financial support for project no. 140255.

REFERENCES

1. J. G. Hardman, L. E. Limbird and A. G. Gilman, *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, 10th ed., McGraw-Hill, New York, 2001, 1011–1021.
2. F. Belal, S. Al-Shaboury and A. Al-Tamrah, Spectrofluorometric determination of labetolol in pharmaceutical preparations and spiked human urine through the formation of coumarin derivative, *J. Pharm. Biomed. Anal.* **30** (2002) 1191–1196; https://doi.org/10.1016/s0731-7085(02)00471-5
3. H. Basan and S. Yarımkaya, A novel solid-phase extraction-spectrofluorimetric method for the direct determination of atenolol in human urine, *Luminescence* **29** (2013) 225–229; https://doi.org/10.1002/bio.2532
4. A. Bavili-Tabrizi, F. Bahrami and H. Badrouj, A very simple and sensitive spectrofluorimetric method based on the oxidation with cerium(IV) for the determination of four different drugs in their pharmaceutical formulations, *Pharm. Sci.* **23** (2017) 50–58; https://doi.org/10.15171/ps.2017.08
5. Y. Jiang, N. N. Horimoto, K. Imura, H. Okamoto, K. Matsui and R. Shigemoto, Bioimaging with two-photon-induced luminescence from triangular nanoplates and nanoparticle aggregates of gold, *Adv. Mat.* **21** (2009) 2309–2313; https://doi.org/10.1002/adma.200802312
6. A. D. McFarland, C. L. Haynes, C. A. Mirkin, R. P. V. Duyne and H. A. Godwin, Color my nanoworld, *J. Chem. Ed*. **81** (2004) 544A–544B; https://doi.org/10.1021/ed081p544a
7. O. Stern and M. Volmer, Decay of fluorescence, *J. Röntgen Soc.* **15** (1919) 133–133; https://doi.org/10.1259/lrs.1919.0087
8. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, *ICH Harmonised Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology Q2(R1)*, Current Step 4 version, November 2005; https://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4 /Q2_R1_Guideline.pdf; last access date Dec 11, 2017.
9. K. G. Thomas, B. I. Ipe and P. K. Sudeep, Photochemistry of chromophore-functionalized gold nanoparticles, *Pure Appl. Chem.* **74** (2002) 1731–1738; https:// doi.org/10.1351/pac200274091731
10. V. Amendola, G. A. Rizzi, S. Polizzi and M. Meneghetti, Synthesis of gold nanoparticles by laser ablation in toluene: quenching and recovery of the surface plasmon absorption, *J. Phys. Chem. B* **109** (2005) 23125–23128; https://doi.org/10.1021/jp055783v

11. K. Wagers, T. Chui and S. Adem, Effect of pH on the stability of gold nanoparticles and their application for melamine detection in infant formula, *IOSR J. Appl. Chem.* **7** (2014) 15–20; https://doi.org/10.9790/5736-07821520
12. R. Pamies, J. G. H. Cifre, V. F. Espín, M. Collado-González, F. G. D. Baños and J. G. de la Torre, Aggregation behaviour of gold nanoparticles in saline aqueous media, *J. Nanopart. Res.* **16** (2014) Article ID 2376 (11 pages); https://doi.org/10.1007/s11051-014-2376-4
13. J. Lim, N.-E. Lee, E. Lee and S. Yoon, Surface modification of citrate-capped gold nanoparticles using CTAB micelles, *Bull. Kor. Chem. Soc.* **35** (2014) 2567–2569; https://doi.org 10.5012/bkcs.2014.35.8.25674
14. A. M. El-Didamony and M. A. Moustafa, Direct spectrophotometric determination of atenolol and timolol anti-hypertensive drugs, *Int. J. Pharm. Pharm. Sci.* **9** (2017) 47–53; https://doi.org/10.22159/ijpps.2017v9i3.16198
15. I. E. H. Elgailani and T. H. Alghamdi, Development of spectrophotometric method for the determination of atenolol in Normoten drug, *Int. J. Chem.* **9** (2017) 58–64; https://doi.org/0.5539/ijc.v9n1p58