Simultaneous colorimetric determination of morphine and ibuprofen based on the aggregation of gold nanoparticles using partial least square

Morteza Bahram\textsuperscript{a,b}*, Tayyebeh Madrakian\textsuperscript{b}, Sakineh Alizadeh\textsuperscript{b}

\textsuperscript{a} Department of Chemistry, Faculty of Science, Urmia University, 571517976 Urmia, Iran
\textsuperscript{b} Department of Analytical Chemistry, Faculty of Chemistry, Bu-Ali Sina University, 65178638695 Hamadan, Iran

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Abstract
In this work a new method is presented for simultaneous colorimetric determination of morphine (MOR) and ibuprofen (IBU) based on the aggregation of citrate-capped gold nanoparticles (AuNPs). Citrate-capped AuNPs were aggregated in the presence of MOR and IBU. The difference in kinetics of AuNPs aggregation in the presence of MOR/IBU was used for simultaneous analysis of MOR and IBU. The formation and size of synthesized AuNPs and the aggregated forms were monitored by infra-red (IR) spectroscopy and transmission electron microscopy (TEM), respectively. By adding MOR or IBU the absorbance was decreased at 520 nm and increased at 620 nm. The difference in kinetic profiles of aggregation was applied for simultaneous analysis of MOR and IBU using partial least square (PLS) regression as an efficient multivariate calibration method. The number of PLS latent variables was optimized by leave-one-out cross-validation method using predicted residual error sum of square. The proposed model exhibited a high capability in simultaneous prediction of MOR and IBU concentrations in real samples. The results showed linear ranges of 1.33–33.29 μg/mL ($R^2=0.9904$) and 0.28–6.9 μg/mL ($R^2=0.9902$) for MOR and IBU respectively with low detection limits of 0.15 and 0.03 μg/mL(S/N=5).

1. Introduction

Analytical methods based on spectrophotometric measurements, such as UV–Vis, have attracted increasing interest due to their availability, simplicity and ease of operation [1]. Multivariate spectral calibration is becoming a general method that can be used in quantitative spectral analysis, allowing simultaneous determination of several analytes that coexist in samples with a minimum number of preparation steps [2]. Among multivariate calibration methods, partial least squares (PLS) regression as a powerful method can model the effect of the interfering components without primary separation [2].

Gold nanoparticles (AuNPs) are particularly attractive because they have surface modifying capability such as bio-labelling and are very useful for biological applications. Some unique characteristics of AuNPs are particularly large surface area, good bio-compatibility, high conductivity and electrocatalytic activity [3–5]. AuNPs are also useful colorimetric probes because of their distance-dependent optical properties and extremely high extinction coefficients in visible region that make them more sensitive than AgNPs [6,7]. Due to the localized surface plasmon resonance (LSPR), AuNPs exhibit color shift from red to blue corresponding to their dispersed and aggregated states respectively [8] and based on this, several colorimetric methods have been developed for the detection of biomolecules [9], 2,4,6-trinitrotoluene [10], drugs [9] and melamine [11,12].

Morphine (MOR, Scheme 1A), a non-steroidal anti-inflammatory drug, is used primarily to treat both acute and chronic severe pain. It is also used for pain due to myocardial infarction and for labor pains and its duration of analgesia is about three to seven hours [13].

Ibuprofen (IBU, Scheme 1B), is commonly used as an antipyretic drug especially for podiatry [14]. Many techniques have been applied to the quantitative determination of IBU, including non-aqueous titration [15], polarography [16], colorimetric titration [17], first and second derivative spectrophotometry [18], infra-red (IR) spectrophotometry [19] and H-nuclear magnetic resonance (H-NMR) spectroscopy [20]. In addition, several chromatographic methods have also been employed which although are the most common direct methods, they are time-consuming and in some cases need hazardous organic solvents [21–23].

Various analytical methods have been developed for the determination of MOR. The same as IBU determination, chromatographic methods [24,25] are the most common methods for MOR detection.

* Corresponding author.
E-mail addresses: m.bahram@urmia.ac.ir, morteza.bahram@gmail.com (M. Bahram).
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The AuNPs were synthesized according to Ferns method. Briefly, 100 mL of 1 mM aqueous solution of HAuCl₄ was heated to boil with stirring; then 10 mL of 1% (m/v) aqueous sodium citrate was added. The color of the mixed solution changed from yellow to wine red in a few minutes, indicating the formation of AuNPs. The boiling and stirring were continued for 15 min [3]. The solution was cooled to room temperature and was stored in a dark bottle at 4 ℃.

In this study, a new method is presented for simultaneous determination of MOR and IBU. The method is based on the difference in the aggregation rate of citrate-capped AuNPs in the presence of MOR and IBU. In the presence of MOR or IBU, the absorbance of the solution containing AuNPs decreased at 520 nm and increased at 620 nm, showing the aggregation of AuNPs. Difference in the aggregation kinetics (e.g. absorbance increase versus time at 620 nm) was applied for simultaneous analysis of MOR and IBU using PLS regression as an efficient multivariate calibration method. The proposed method is simple, selective, fast, low cost and in contrast to chromatographic methods, without the need to any sample preparation procedures. This method does not need any expensive apparatus and primary time-consuming separation process by applying the chemometrics methods.

2. Experimental

2.1. Chemicals and materials

All chemicals and reagents used were of analytical grade, solvents were of spectroscopic grade, and double distilled water (DDW) was used throughout the experiments. MOR and IBU pure drugs were obtained from the Department of Food and Drug Administration, Urmia, Iran. All chemicals in the experiments were used without further purification. Sodium citrate, HCl and NaOH were purchased from Merck (Darmstadt, Germany).

2.2. Apparatus

Absorption spectra were recorded with an Agilent 8453 UV–Visible spectrophotometer with 1 cm quartz cells. The size, morphology and structure of the synthesized AuNPs were characterized by transmission electron microscopy (TEM, Philips-CMC-300 kV). A Metrohm pH meter (713 pH-meter) was used for pH measurements. A 40 kHz ultrasonic cleaner water bath (Elmasonic E60H, Germany) was used. The calculations were performed in MATLAB (Hyper-cube Inc. Version10) software using PLS files.

2.3. Synthesis of AuNPs

The AuNPs were synthesized according to Ferns method. Briefly, 100 mL of 1 mM aqueous solution of HAuCl₄ was heated to boil with stirring; then 10 mL of 1% (m/v) aqueous sodium citrate was added. The color of the mixed solution changed from yellow to wine red in a few minutes, indicating the formation of AuNPs. The boiling and stirring were continued for 15 min [3]. The solution was cooled to room temperature and was stored in a dark bottle at 4 ℃. The solution of the prepared citrate-capped AuNPs was wine red, and had a characteristic localized surface plasmon resonance (LSPR) absorption band of AuNPs at 520 nm (λmax) with narrow peak. In the solution, monodisperse AuNPs were red and exhibited a relatively narrow surface plasmon absorption band centered at around 520 nm in the UV–Vis spectrum. In contrast, a solution containing aggregated AuNPs was blue corresponding to a characteristic red shift in the surface plasmon resonance to higher wavelength of 620 nm [3]. The results are presented in Figs. 1A and B. Figs. 1C and D show the IR spectra of the synthesized AuNPs, before and after aggregation. The concentration of the synthesized nanoparticles was estimated as 10⁻¹⁰ mol/L based on the extinction coefficient for 13 nm gold nanoparticles reported in the literatures as 2.8×10⁶. IR spectra demonstrated the functional groups of PhOH (3000–3600 cm⁻¹), RCOOH (2800–3200 cm⁻¹), OR₂ (1000–1300 cm⁻¹), and Allyl-OH (3000–3600 cm⁻¹). In the spectrum of aggregated AuNPs using the studied drugs, the N₉ bands (1000–1350 cm⁻¹) obviously exist, proving that drugs interacted with AuNPs. The intensity of IR spectrum of AuNPs decreased when drugs coated them or have interaction with them, while the intensity of the functional group of drug increased. The absorbance spectra of synthesized AuNPs before and after aggregation are shown in Fig. 1E. Fig. 1F represents the time-dependent evolution of UV-Vis spectra of AuNPs toward interaction with MOR.

3. Results and discussion

3.1. Optimization of AuNPs

Ionic strength has a crucial role in the aggregation of nanoparticles. It can be attributed to the ability of strong electrolytes to constrict the aroused electrical double-layer from the capping agent around the nanoparticles. Although the addition of an electrolyte is necessary for starting the aggregation, it was also found that by increasing the ion strength above a certain limit, the aggregation of nanoparticles occurred even in the absence of analytes [1]. Therefore, the effect of ionic strength (electrolyte concentration) should be studied. The results were presented in Fig. S1 and 1 mmol/L of NaCl was used in the study.

3.2. Optimization of pH

Because of the presence of hydroxyl, carboxyl and amine groups in drugs, pH is another critical parameter that should be taken into consideration. Although electrostatic interactions are dominantly responsible for aggregation of AuNPs [6], the presence of ionizable hydroxyl, carboxyl and amine groups in drugs makes pH another critical parameter which should be considered for optimization and the best results are achieved when the interactions between drugs and AuNPs are strong enough to cause aggregation [38]. Fig. S2 shows the effect of pH on the interaction of drugs with AuNPs. Results showed that the synthesized AuNPs were stable at pH 6 and the drugs had the most favorable ionic structure for interaction with AuNPs in the pH ranges of 6–7. Therefore, pH 6 was chosen for further studies.

3.3. Optimization of incubation time

Different incubation times were examined, and the results showed that AuNPs started to aggregate right after mixing with MOR and IBU in...
optimum conditions and the aggregation nearly completed after 10 min of incubation which was considered to be the optimum incubation time. So we selected 10 min as the end time for simultaneous kinetic study (Fig. S3).

3.4. The effect of temperature

The results showed that by increasing the temperature the aggregation also increased (Fig. S4). Because the aim of our study was simultaneous kinetic analysis of MOR and IBU using PLS, a temperature should be selected so that a good difference between the kinetic profiles of the analytes is obtained. Based on the results, 25 °C was selected for further experiments.

3.5. PLS model development

PLS calibrations for both drugs were constructed by using nonlinear iterative partial least squares (NIPALS) algorithm. A training set of 27 standard samples (20 samples as calibration set and 7 samples as prediction set) were taken from different mixtures of MOR and IBU.
The correlation between different calibration samples has to be avoided because collinear component in the training set data tends to cause under-fitting in the PLS models [39].

### 3.6. Linear range of calibration curves

For the mentioned purposes, under optimum experimental conditions, a typical calibration curve was obtained for the determination of MOR and IBU separately. The calibration curves have a linear range of 1.33–33.29 and 0.28–6.9 µg/mL with $y=−0.0127x+1.2291$, $R=0.9904$ and $y=−0.0849x+1.1965$, $R=0.9902$ (Fig. 2) with the detection limit of 0.15 µg/mL (n=5) and 0.03 µg/mL (n=5) for MOR and IBU, respectively. Table 1 shows a comparison of the results obtained by the present method and those obtained by other methods reported for the determination of these drugs. As can be seen from Table 1, the present method had a good detection limit and linear range compared to those obtained from the reported methods [40–47].

### 3.7. Simultaneous determination of MOR and IBU

As mentioned above, in order to select the number of factors in PLS algorithm, a cross-validation method leaving out one sample at a time was employed. Increase in the intensity of the absorbance at 620 nm was directly related to the level of MOR and/or IBU in the sample (Fig. 3). Citrate-capped AuNPs were aggregated toward interaction with MOR and IBU with different rate constants. The calibration and prediction sets were collected by monitoring the absorbance increase at 620 nm and were used in PLS process. For the calibration set of 20 sample kinetic profiles, PLS1 and PLS2 algorithms were applied and the amount of the samples left out during the calibration process was calculated. Changes in predicted residual error sum of squares (PRESS) in PLS2 calibration as a function of the number of PLS latent variables are given in Fig. 4. As can be seen from Fig. 4, 3 components were enough to construct the PLS2 model. Nonlinearity in the absorbance–concentration relationship and interaction between the factors could be considered as the other sources of chemical factors.

The predicted values of MOR and IBU levels in the calibration and prediction samples and their corresponding relative prediction errors are listed in Tables 2–4. It was observed that the predicted values were very close to the actual amounts and the relative prediction errors were lower than 5.0%. This confirmed the success of PLS regression for accurate prediction amounts of MOR and IBU in samples. It would be beneficial to evaluate whether the results of PLS1 and PLS2 multivariate calibration resulted in a more appropriate model. For this comparison and the validation of the models, some statistical parameters including root mean square error of prediction (RMSEP), root mean square error of cross validation (RMSECV) and root mean square error of calibration (RMSEC) were calculated and are shown in Table 4. Tables 2 and 3 show that the calibration and prediction results of PLS are more consistent than those of univariate calibration.

The relative standard error and the total relative standard error were calculated using following equations and are shown in Tables 2 and 3.

### Table 1

Linear range and limit of detection of the proposed method in comparison with some previous methods for analysis of morphine and ibuprofen.

| Compound | Method | Linear range (µg/mL) | LOD (µg/mL) | Reference |
|----------|--------|----------------------|-------------|-----------|
| Morphine | RP–HPLC | 0.15–2 | 0.05 | [41] |
|          | LC–MS–MS | 0.002–2 | 0.001 | [42] |
|          | Direct aqueous derivatization | 0.008–5.0 | 0.002 | [43] |
|          | GC–MS | 0.02–20 | 0.003 | [44] |
|          | Spectrophotometry | 0.025–2 | 0.002 | [45] |
| Ibuprofen | Derivatives of the ratio spectra method | 2–32 | 0.53 | [46] |
|          | HPLC | 6.1–200 | 1.7 | [47] |
|          | Spectrophotometry | 0.28–6.90 (IBU) | 0.03 (IBU) | This work |
|          |          | 1.33–33.29 (MOR) | 0.15 (MOR) |          |

RP–HPLC: Reverse phase–high performance liquid chromatography; LC–MS–MS: Liquid chromatography–tandem mass spectrometry; GC–MS: Gas chromatography–mass spectrometry; HPLC: High performance liquid chromatography
3.8. Interference effect

The influences of foreign coexisting substances such as naproxen, ascorbic acid, tramadol, codeine, acetaminophen, saccharides, amino acids and ions were tested. As listed in Table S1, some coexisting substances had remarkable interference on the assay. From the results, the interferences of naproxen, ascorbic acid, Na2NO2, tryptophan, tyrosine, glucose, sucrose, fructose and lactose were very weak. Among the tested substances K+, Na+, NO3−, I−, Cl−, Mg2+, Fe3+, Ca2+, codeine and cysteine could be present with relatively higher concentrations, but cefexime, ceftriaxone, NH2OH, Mn2+, Cd2+, SO42−, Ca2+, Zn2+, Co2+, Zn2+, Ni2+, Al3+, Fe2+ and Cu2+ could only be allowed with relatively low concentrations. The allowed concentrations of these interfering substances, however, were still rather higher than those of MOR and IBU, which indicated that this method had a good selectivity between drugs and other substances.

3.9. Real sample analysis

In order to test the applicability of the proposed method, it was applied to determine IBU and MOR in spiked blood serum and urine samples. The constructed PLS model was applied to estimate the concentration of IBU and MOR in these spiked samples. The obtained results showed good recoveries (99.4%–110.7%) (Table S2), and demonstrated the potential applicability of this method for simultaneous determination of IBU and MOR in real samples.

Table 3

| Reference value (µg/L) | Predicted value (µg/L) |
|-----------------------|------------------------|
|                       | IBU | MOR | PLS1 | PLS2 |
|                       | IBU | MOR | IBU  | MOR  |
| 1                     | 700 | 400 | 704.59 | 365.16 |
| 2                     | 520 | 260 | 524.96 | 262.55 |
| 3                     | 370 | 190 | 364.83 | 190.68 |
| 4                     | 290 | 160 | 293.77 | 165.03 |
| 5                     | 320 | 170 | 320.16 | 171.75 |
| 6                     | 320 | 170 | 315.20 | 173.44 |
| 7                     | 350 | 200 | 346.60 | 193.80 |
| 8                     | 460 | 270 | 464.14 | 232.29 |
| 9                     | 450 | 270 | 453.87 | 274.37 |
| 10                    | 740 | 290 | 737.16 | 299.62 |
| 11                    | 120 | 80  | 152.70 | 79.40  |
| 12                    | 170 | 100 | 152.46 | 103.04 |
| 13                    | 170 | 100 | 172.58 | 100.65 |
| 14                    | 330 | 220 | 331.50 | 218.00 |
| 15                    | 350 | 200 | 346.46 | 199.97 |
| 16                    | 160 | 90  | 162.17 | 91.43  |
| 17                    | 240 | 130 | 241.60 | 133.55 |
| 18                    | 650 | 370 | 655.49 | 376.18 |
| 19                    | 640 | 360 | 639.75 | 367.63 |
| 20                    | 200 | 100 | 194.34 | 110.29 |

| R.S.E. (%)           | 3.55 | 1.45 | 3.56 | 1.49 |
| R.S.E. t (%)         | 3.19 |

Table 4

| Statistical parameters       | Model of prediction |
|------------------------------|---------------------|
|                              | PLS1 (%) | PLS2 (%) |
| RMSEP                        | 4.7       | 4.69     |
| RMSECV                       | 4.1       | 4.2      |
| RMSEC                        | 5.1       | 5.2      |

3.9. Real sample analysis

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4. Conclusion

The LSPR of the AuNP synthesized by the reduction of gold ion with citrate was, used as a novel analytical tool for the determination of drugs and substances based on the aggregation of AuNPs. A direct relationship was found between the aggregation rates recorded in $\lambda_{\text{agg}}$ at about 520 and 620 nm and concentrations of MOR and IBU. Multivariate calibration modeling of the kinetic absorbance data by PLS regression produced accurate results and the relative prediction errors were almost lower than 5%. In comparison with available analytical methods for simultaneous determination of IBU and MOR, the proposed method had following advantages: (i) it needs smaller amounts of reagents; (ii) it is simple, fast, and low-cost; and (iii) in contrast to chromatographic methods, it does not need any expensive apparatus.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jpha.2017.03.001.

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