Introduction

Determining the trace amounts of drugs at different stages of drug development and manufacturing, such as formulation, quality control, stability study, pharmacological properties, including pharmacokinetics and pharmacodynamics and drug performance evaluation are among the most important and vital issues for researchers. All of these studies require the development of sensitive, simple, inexpensive, effective, accurate, reproducible and reliable analytical methods, that measure drugs in various matrices, including biological fluids, such as urine, plasma and pharmaceutical formulations, without the need for hazardous solvents, separation steps and tedious pretreatments.

Pantoprazole (PAN) (Fig. 1), 5-difluoro methoxybenzimidazole-2-yl 3,4-dimethoxy-2-pyridylmethyl sulfoxide, as a benzimidazole derivative and proton-pump inhibitor (PPI), is widely used for the short-term treatment of gastro-esophageal reflux and any other acid-related disorders. The mechanism of action of PAN (like other PPIs) is to decrease gastric acid secretion by covalently binding and irreversibly inhibiting the H+/K+-ATPase proton pump in gastric parietal cells. At low-pH values, PAN turns into tetracyclic cationic sulfenamide (the active form of PAN), which can react with two cysteine residues (813 and 822) of a transmembran H+/K+ ATPase proton pump. The bioavailability of PAN is 77% and its metabolization is achieved by a hepatic cytochrome P450 system. It is also effective and helpful for treating Zollinger–Ellison syndrome and in preventing ulcer rebreeding. The drug was approved in 2001 and was officially listed in Martindale the extra pharmacopoeia in 2005. It has also been demonstrated that PPIs show antibacterial

A new sensitive, simple, rapid, reliable and selective fluorometric method for the determination of pantoprazole (PAN) in human plasma and a pharmaceutical formulation has been developed. This technique is based on a quenching effect of silver nanoparticles (AgNPs) on the emission intensity of a fluorescent probe, terbium(III)-1,10-phenanthroline (Tb(III)-phen) complex (due to a fluorescence resonance energy transfer (FRET) phenomenon between the Tb(III)-phen complex and AgNPs), and then restoring the fluorescence intensity of the Tb(III)-phen-AgNPs system upon the addition of PAN (turn off-on process). The effects of various factors on the proposed method including time, temperature, pH, order of the addition of various reagents and the concentration of AgNPs were investigated. Under the optimal conditions, a good linear relationship between the enhanced emission intensity of the Tb(III)-phen-AgNPs system and the PAN concentration was observed in the range of (10 – 1000) × 10⁻⁸ M. The limit of detection (LOD) and the limit of quantitation (LOQ) were 7.2 × 10⁻⁸ and 24.2 × 10⁻⁸ M, respectively. Also, the interferences of some common interfering species on the fluorescence intensity of the system were investigated. This simple and sensitive method was successfully applied for the determination of PAN in spiked human plasma samples and in its capsule formulation. The analytical recoveries were in the range of 88.54 – 101.33 and 90.07 – 98.85%, respectively.

Keywords Silver nanoparticles, pantoprazole, FRET phenomenon, turn off-on, nanosensor based method

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effects on *Helicobacter pylori*.3

Different analytical methods for the determination of PAN in biological fluids and pharmaceuticals were reported during the last years, which include high-performance liquid chromatography (HPLC),4–3 fluorescent chromatography-tandem mass spectrometry (LC-MS/MS),6,7 thin-layer chromatography (TLC),8,9 spectroscopic methods,10–13 electrochemical methods14–19 and iodometric assay.19 Nevertheless, most of these methods compared to spectrofluorimetric techniques have some disadvantages, which include a narrow range of determination, high cost, time consumed sample pretreatment and tedious preparation of the detection system. They are also complicated to operate. Often problems are the relatively high detection limit and sensitivity, the use of toxic solvents, the preparation long time for the reaction to be completed and the instability of the colored product formed. These limitations, have restricted their applications for an accurate and routine assay of PAN. Therefore, it is also important to develop convenient systems for determining simple, rapid and free from matrix effects of PAN in biological and pharmaceutical samples.

Today, photoluminescence methods, especially molecular fluorescence-based methods, have gained considerable attention for the study and measurement of various chemical and biological compounds, due to the particular advantages of fluorescence spectroscopy. One of these very useful and effective methods is the use of lanthanide ions, especially europium (Eu3+) and terbium (Tb3+), as chelates with special and exceptional luminescence properties (lanthanide-sensitized luminescence). These emission properties can be attributed to the large Stokes’ shift (the large difference between the maximum excitation and emission wavelengths), the narrow (sharp) emission bands (1–20 nm) and the long fluorescence lifetime (a few hundred microseconds), which enhances their ability in many analytical fields including, lasers, molecular sensing in biochemistry (as a luminescent probe), optical amplifiers and medically important compounds.20–24

On the other hand, the fluorescence quenching of chemical compounds (fluorophores) by multiple quenchers (such as nanomaterials) has also been of particular importance in recent decades, especially with nanosciences and nanosensors development. This method provides useful information on the nature of the interaction between fluorophore and quencher and is widely applied to the determination/detection of metal and inorganic ions (including Fe2+, Fe3+, Hg2+, Al3+, F–, citrate and chromate), biomolecules (such as amino acids, DNA, RNA, ATP, lysozyme, glucose, glutathione and cholesterol), pesticides, heparin, anti-cancer drugs and etc.25–34 Currently, most fluorescence quenching methods are based on the use of nanomaterials, which are performed in different strategies, including quenching (turn-off), quenching-recovery (turn-off-on), turn-on-off-on and turn off-on-off. Among these different modes, based on turn off-on fluorescence approaches have been widely used for the determination of different drugs and compounds in biological samples. These methods are very sensitive, rapid, simple, inexpensive and free from interferences.27,28,31,33 In a typical turn off-on based sensing system, the fluorescence intensity of a donor fluorophore is first quenched by nanomaterials, which are commonly used as energy acceptors, and then gradually and linearly restored by introducing competing analytes, and as a result, a turn off-on detection signal is generated. Indeed, due to the strong and specific bindings between analyte molecules and nanomaterials (quenching agents), the interaction between donor and acceptor molecules is weaken, consequently the quenched fluorescence of the system is gradually recovered. Various mechanisms can decrease the fluorescence intensity of the fluorophore in these system such as fluorescence resonance energy transfer (FRET), photo induced electron transfer (PET) and inner filter effect (IFE).26,31,36–38

Fluorescent biosensors based on nanomaterials, especially metal nanoparticles (MNPs; such as gold (Au) and silver (Ag) NPs) and Au and Ag nanoclusters (NCs) for the determination of biological samples, has recently gained much more attention. These biosensors compared to traditional fluorescent probes give some advantages involving high quenching coefficients, broad absorption bands, ease of synthesis and their surface chemical modification, high photostability, photobleaching, good solubility and because of their unique electronic and optical properties, act as an ideal of either the acceptors or donors for the FRET process.31,36,38–42

FRET is a powerful and sensitive spectroscopic method, that is widely used to study intermolecular reactions, to determine the distance between two molecules (chromophores) by a few nanometers, as well as to measure various drugs and biological compounds. In this method, the excitation energy of a donor fluorophore in the electronic excited state is non-radiatively transferred to a convenient acceptor chromophore near to itself. Förster showed that the FRET process efficiency is inversely proportional to the sixth power of the distance (r) between the donor and the acceptor chromophores.31,43

The aim of this study was to develop a very selective, sensitive, simple, rapid and applicable fluorimetric method based on a turn-off on fluorescent nanosensor for the determination of PAN in a biological fluid as well as pharmaceutical dosage form (capsule), using Tb(III)-phen-AgNPs, as a fluorescent nanoprobe. As expected, the Tb(III)-phen complex with specific emission properties (as a donor), as well as the high molar absorption coefficient of AgNPs (as an acceptor), increases the FRET phenomenon performance and the fluorescence intensity of the Tb(III)-phen complex, to be efficiently quenched by AgNPs (turn-off stage). Upon the addition of PAN, the quenched fluorescence of the Tb(III)-phen-AgNPs system is gradually recovered as a result of its strong adsorption on the surface of AgNPs and the removal of the Tb(III)-phen complex from the AgNPs surface and inhibition of the FRET process (turn-on stage). This method gives good precision, provides a low detection limit and is easily and successfully applied for the direct estimation of PAN and various medically important compounds in biological fluids without the need for preconcentration as well as separation methods and long pretreatments.

**Experimental**

**Reagents and chemicals**

To prepare a stock solution of terbium(III) (1.0 × 10–3 M), 0.373 g of terbium(III) chloride (TbCl3·6H2O; Acros Organics, USA) was dissolved in double-distilled water, and the volume was made up to 100 mL with double-distilled water. The prepared stock solution was stored in a polyethylene container.

A stock solution of 1.10-phenanthroline (1.0 × 10–2 M) (phen; Fluka, Switzerland) was prepared by dissolving 1.8021 g in 5.0 mL ethanol and then the volume was adjusted to 100 mL with double-distilled water.

A 26 × 10–5 M stock solution of pantoprazole (IRAN DAROU pharmaceutical company, Tehran, Iran) was prepared by dissolving 0.01 g of the compound in double-distilled water, and the volume was made up to 100 mL with double-distilled water.
To prepare the buffer solution of Tris-HCl (5.0 × 10^{-2} M), 0.788 g of Tris-HCl powder (Merck, Germany) was dissolved in double-distilled water, and then the volume was made up to 100 mL with distilled water and the pH was adjusted to 7.0 using HCl.

A 0.1 M, 2.0 × 10^{-3} M and 1.0% w/v stock solutions of silver nitrate (AgNO₃; Merck, Darmstadt, Germany), sodium borohydride (NaBH₄; Reagents Chemicals, Korea) and trisodium citrate (C₆H₅Na₃O₇·2H₂O; Merck, Darmstadt, Germany) were prepared by dissolving an appropriate amount of the compounds in double-distilled water.

To study the interferences of different substances, stock solutions of glucose (Glc), fructose (Frc), L-cysteine (Cys), L-tryptophan (Trp), glycine (Gly), NaCl, KCl, CuCl₂·2H₂O, FeCl₂·6H₂O, ZnCl₂, MgCl₂ and CaCl₂·2H₂O (all were obtained from Merck, Darmstadt, Germany) were prepared by dissolving a desired amount of these compounds in double-distilled water.

Healthy human plasma was taken from Iranian Blood Transfusion Organization (IBTO).

**Apparatus**

All fluorescence spectra were measured using a Jasco FP-750 spectrophotofluorometer (Kyoto, Japan) with a 1.0-cm quartz cell, equipped with a 150-W xenon lamp and a Peltier thermostated cell holder. The excitation wavelength was fixed at 300 nm and the emission wavelength was recorded in the range of 400 to 600 nm at room temperature (298 K). The emission peak position at 545 nm was used to measure the fluorescence intensity. All UV-visible absorption spectra were recorded using a T60 spectrophotometer (PG Instruments Limited, Leicestershire) at room temperature (298 K) with a 1.0-cm quartz cell.

SEM (scanning electron microscopy) MIRA3 FEG-SEM (TescanBrno, Czech Republic) was applied to a morphological study of synthesized AgNPs.

**Synthesis and characterization of AgNPs**

There are different methods for the synthesis of AgNPs. In this study, we used the chemical reduction method (one of the most common approaches), which is described in the literature. For this purpose, 2.0 mL of a stabilizer (1.0% w/v sodium citrate aqueous solution) was mixed with 50 mL of 2.0 × 10^{-3} M silver nitrate (AgNO₃) and stirred for 10 min under vigorous stirring. After 10 min, 10 μL of a reducing agent (ice-cold sodium borohydride) was added to the reaction mixture to reduce silver nitrate. The mixture was stirred for 30 min; during this time, color alteration of the reaction mixture to reduce silver nitrate. The mixture was transferred to the tube and diluted to 5.0 mL with double-distilled water, and then the volume was made up to 5.0 mL using double-distilled water. The final AgNPs concentration was in the range of 0.9 × 10^{-11} to 3.6 × 10^{-11} M. The emission intensity of the Tb(III)-phen-AgNPs system was recorded at 545 nm and the following equation (Eq. (1)) was used to calculate the quenched fluorescence intensity of Tb(III)-phen complex in the presence of AgNPs:

\[
\Delta I_f (%) = \left( \frac{I_0 - I_f}{I_0} \right) \times 100
\]

Here, \(I_f\) and \(I_0\) represent the fluorescence intensity of the Tb(III)-phen complex without and with AgNPs.

**Procedure for the determination of pantoprazole**

The following analytical approach was utilized to make the calibration curve and estimate the PAN, under the optimum condition: 100 μL of 1.0 × 10^{-3} M Tb³⁺, 700 μL of 1.0 × 10^{-2} M Tris-HCl buffer pH 7.0, 50 μL of 1.0 × 10⁻² M phen and 200 μL of synthesized AgNPs (1.8 × 10⁻¹¹ M) were mixed together. Subsequently, the desired amount of a standard solution of PAN was added to the prepared mixture and the final volume was adjusted to 5.0 mL using double-distilled water. The final concentration of PAN in the mixtures was in the range of (10 – 1000)×10⁻⁴ M. The fluorescence intensity of the mixtures was recorded and the enhanced emission intensity of the Tb(III)-phen-AgNPs system in the presence of PAN was represented as follows:

\[
\Delta I_f (%) = \left( \frac{I_f - I_0}{I_0} \right) \times 100
\]

where \(I_f\) and \(I_0\) are the fluorescence intensity of the Tb(III)-phen-AgNPs system with and without PAN.

**Sample preparation and constructing the calibration curve in a plasma sample and its capsule dosage form for the estimation of PAN**

The following procedure was used to make a calibration curve and to determine the PAN in human plasma samples under the optimum condition: drug-free plasma samples were spiked with an appropriate amount of standard solution of PAN before the experiment. To remove proteins, an aliquot of 0.5 mL spiked plasma samples (with a desired amount of PAN for total concentration in the range of (100 – 1100)×10⁻³ M and 1.5 mL acetonitrile were mixed and centrifuged at 13000 rpm for 5.0 min. Afterward, the supernatant was separated and transferred to the tube and diluted to 5.0 mL, with double-
distilled water. Then a 3.0-mL of prepared samples were transferred into a quartz cuvette (1.0 cm) and the fluorescence intensity was recorded against a blank sample. The same procedure was applied to prepare the blank solution by using drug-free plasma.

For the determination of PAN in its drug formulation, ten capsules of PAN were weighed in order to find the average mass of each capsule. Then, the contents were powdered and mixed. A portion of 10 mg of this powder was accurately weighed and dissolved in about 10 mL of a 0.1 M NaOH solution and filtered into a 100-mL volumetric flask. The residue was washed several times with distilled water, and the solution was diluted to the mark. A suitable aliquot of this solution was taken for a fluorimetric estimation of PAN.

Recovery studies were carried out by adding known concentrations of the PAN standard solution to healthy plasma and capsule samples, before pretreatment steps. We then determine the total PAN concentrations.

Method validation
To evaluate the assay method and to ensure the optimization of the method, the present method was validated through determining the limit of detection, linearity, the limit of quantification and the recovery.

Results and Discussion

Investigating the fluorescence quenching of the Tb(III)-phen complex by AgNPs (turn-off process)
It is well known that the complex formation between phen and Tb³⁺ ions increases in fluorescence intensity, and stronger emission bands appear in the fluorescence spectrum of the Tb(III)-phen complex, the main characteristic of Tb³⁺ ion fluorescence, in the range of 450 to 600 nm, which is due to an intramolecular energy transfer process. The emission spectra of the Tb(III)-phen complex and Tb³⁺ under a 300-nm excitation wavelength, and also their absorption spectra are shown in Fig. S2 (Figs. S2a and S2b, respectively). The emission peaks, appearing at 490 and 545 nm, correspond to the 5D₄ → 7F₅ and 5D₄ → 7F₆ electronic transition of the Tb³⁺ ions, respectively.

Figure S3 shows a great overlap between the emission spectrum of the donor (Tb(III)-phen complex) and absorption spectrum of the acceptor (AgNPs), which is an important factor for FRET to occur. It is well known that AgNPs are negatively charged (because they are capped with citrate ions), while the Tb(III)-phen complex is a cationic complex. Thus, by adding AgNPs into the Tb(III)-phen complex solution, due to an electrostatic interaction between the Tb(III)-phen complex and AgNPs and the noncovalent binding of the Tb(III)-phen complex on AgNPs surfaces and, then charge neutralization, the Tb(III)-phen-AgNPs system was formed and the FRET process (energy transfer) occurred, which caused to a decrease in the fluorescence intensity of the Tb(III)-phen complex.

The emission spectra of the Tb(III)-phen complex without and with additional concentrations of AgNPs (in the range of 0.9 × 10⁻¹¹ – 3.6 × 10⁻¹¹ M) are shown in Fig. 3. Figure 3 reveals that the emission intensity of the Tb(III)-phen complex decreased regularly in the presence of an increasing concentration of AgNPs. These results confirmed the Tb(III)-phen-AgNPs system formation and energy transfer from Tb(III)-phen to AgNPs by the FRET process. The Stern-Volmer equation was applied to a fluorescence quenching data analysis.

\[
F/F = 1 + K_{sv}[Q]
\]  

(3)

here, F₀ and F represent the fluorescence intensity of the Tb(III)-phen complex without and with a quencher [AgNPs], respectively. Ksv and [Q] denote the Stern-Volmer quenching constant and the total concentration of the quencher, respectively. Figure 4 shows a Stern-Volmer plot of the emission quenching of the Tb(III)-phen complex in the presence of AgNPs. It is obvious that there is a good linear relationship. The linear part of this plot (0.9 × 10⁻¹¹ – 3.6 × 10⁻¹¹ M) was applied to obtain the Ksv value. The calculated Ksv was 2.08 × 10⁹ M⁻¹. Such a large value indicates that there is a strong interaction between the Tb(III)-phen complex and AgNPs, and that the energy transfer efficiency is high. Such behavior can probably be attributed to the following factors: a) the close proximity of AgNPs to the Tb(III)-phen complex and the great overlap between the emission spectrum of the Tb(III)-phen complex and the absorption spectrum of AgNPs; b) the dependence of the FRET phenomenon efficiency to the sixth-power inverse of the distance (r) between the donor (Tb(III)-phen complex) and the acceptor (AgNPs); c) the higher optical absorption and the extinction coefficient; and d) the larger surface area and increase in the quenching yield.
Study of the effect of various factors on the fluorescence quenching of system

In the present study, the effect of different factors including, the concentration of phen, the concentration of Tb³⁺, pH value, temperature, time and the order of the addition of various reagents on the fluorescence intensity of the Tb(III)-phen-AgNPs system, was investigated.

A series of tubes, containing different pH values of the Tris-HCl buffer (in the range of 2.0 – 12) but constant concentrations of other reagents, was prepared and the fluorescence intensities of samples were recorded at 545 nm after excitation at 300 nm. The quenching efficiency (ΔIᵢ (%) of the Tb(III)-phen-AgNPs system reached the maximum amount at pH 7.0 (Fig. S4). At higher and lower pH values the quenching intensity decreased due to the sedimentation of AgNPs and terbium hydroxide.³¹ So, pH 7.0 was considered to be as an optimum pH for following investigations. To investigate the effect of temperature on the ΔIᵢ (%) of the Tb(III)-phen-AgNPs system, the decreased fluorescence intensity of the system was recorded at different temperatures in the range of 0.0 – 40°C. Based on the obtained data (Fig. S5), ΔIᵢ (%) of the system reached a maximum amount when the temperature was 25°C, and remained stable at over 25°C. Thus, room temperature (25°C) was selected as the optimum temperature for all emission intensity measurements.

The effect of time on ΔIᵢ (%) of the system (under the optimum condition) was studied. After the addition of all reagents, the fluorescence intensity of the prepared solution was recorded every 5.0 min. In the first minutes, the ΔIᵢ (%) of the system reached a the maximum amount, and then remained stable for over 2.0 h (Fig. S6). So, in the present study 5.0 min was chosen for further research.

The effect of different concentrations of phen and Tb³⁺ ions on ΔIᵢ (%) of the system was also investigated. The obtained results indicated that ΔIᵢ (%) of the Tb(III)-phen-AgNPs system reached a maximum amount at 1.0 × 10⁻⁴ and 2.0 × 10⁻⁴ M of phen and Tb³⁺ ions, respectively (Figs. S7 and S8). So, these concentrations of phen and Tb³⁺ ions were chosen for further studies. Also, the obtained results indicated that the fluorescence intensity of Tb(III)-phen complex, at optimum concentrations of phen and Tb³⁺ ions, is entirely quenched in the presence of AgNPs. This shows that all of the Tb(III)-phen complex molecules at this concentration are adsorbed on the AgNPs surface. From the obtained experimental results, a concentration of 1.8 × 10⁻¹¹ M AgNPs was selected for subsequent analyzes.

To study the effect of the order of adding various reagents on ΔIᵢ (%) of the system, a series of different orders of adding various reagents was prepared under the optimum condition, and their fluorescence intensity was recorded at 545 nm. Based on the results, the best order of adding was chosen to be Tb³⁺ ions, Tris-HCl buffer, phen and AgNPs.

Study the interaction of PAN with Tb(III)-phen-AgNPs system (turn on process)

To investigate the analytical applicability of the proposed nanosensor system, the effect of PAN on the emission intensity of the Tb(III)-phen-AgNPs system was studied. Figure 5 shows the emission intensity of Tb(III)-phen (a), Tb(III)-phen-AgNPs system (b), Tb(III)-phen-AgNPs-PAN assembly (c) and PAN (d) under the same and optimal conditions. It can be seen from Fig. 5a that Tb(III)-phen shows two main and strong emission peaks (at 490 and 545 nm) due to energy transfer between them (Fig. 5b). However, when a desired amount of PAN (27.5 × 10⁻⁸ M was added to the Tb(III)-phen-AgNPs solution, the emission intensity of Tb(III)-phen complex was restored (Fig. 5c). This result indicated that Tb(III)-phen molecules were released from the surfaces of AgNPs due to the displacement of Tb(III)-phen molecules with PAN and the binding of PAN molecules onto the surface of the AgNPs instead of Tb(III)-phen.²⁶,³¹,33,36,39,54–57

It has been reported that the MNPs (which are 12 – 100 nm in diameter) can bind to fluorophore molecules and quench or enhance their fluorescence intensity without changing their structure. The intensity of the quenching or enhancement is strongly related to the size and shape of the MNPs. It is well known that electron transfer and energy transfer, such as the FRET process, are two main mechanisms for the emission quenching of fluorophores on the MNPs surfaces. Kavitha et al.⁵⁶ and Ghosh and co-workers⁵⁷ reported that the electron transfer and energy transfer processes occur when the particle sizes are smaller than 5.0 nm (<5.0 nm) and larger than 5.0 nm (>5.0 nm), respectively. As mentioned above, the FRET process takes place when the donor and acceptor molecules are positioned in their vicinity (within Förster distance) and a great overlap between the emission spectrum of the donor and the absorption spectrum of the acceptor is observed. If there is no overlap between the emission spectrum of the fluorophore and the absorption spectrum of MNPs, the dominant mechanism will be electron transfer.¹⁴,⁵⁷ In the present study, the fluorescence emission intensity of the Tb(III)-phen complex was strongly quenched in the presence of AgNPs (Kₑ = 2.08 × 10¹¹ M⁻¹). Since there is a good overlap between the emission and absorption spectra of the donor and acceptor and the interaction between them is strong, and the fluorescent probe is placed in the vicinity of a AgNPs, as well as the size of AgNPs used in the present study was larger than 5.0 nm, therefore, the observed fluorescence quenching of the Tb(III)-phen complex in the presence of AgNPs is probably due to energy transfer between the Tb(III)-phen complex and AgNPs.

Analytical figures of merit

As can be seen from Fig. 6A, the fluorescence intensity of the system increased with raising the concentration of PAN. Under the optimum condition, the calibration curve was plotted for PAN, which was linear in the range of (10 – 1000) × 10⁻⁸ M,

\[ [\text{PAN}] = 27.5 \times 10^{-6} \text{ M}. \]

The effect of time on \( \Delta I_i \) (%) of the system (under the optimum condition) was studied. After the addition of all reagents, the fluorescence intensity of the prepared solution was recorded every 5.0 min. In the first minutes, the \( \Delta I_i \) (%) of the system reached a the maximum amount, and then remained stable for over 2.0 h (Fig. S6). So, in the present study 5.0 min was chosen for further research.

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To study the effect of the order of adding various reagents on \( \Delta I_i \) (%) of the system, a series of different orders of adding various reagents was prepared under the optimum condition, and their fluorescence intensity was recorded at 545 nm. Based on the results, the best order of adding was chosen to be Tb³⁺ ions, Tris-HCl buffer, phen and AgNPs.

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Analytical figures of merit

As can be seen from Fig. 6A, the fluorescence intensity of the system increased with raising the concentration of PAN. Under the optimum condition, the calibration curve was plotted for PAN, which was linear in the range of (10 – 1000) × 10⁻⁸ M,
and its equation was $\Delta I_f (%) = 0.0161C + 4.6471$ ($R^2 = 0.9896$) (Fig. 6B). In this equation $\Delta I_f (%)$ and $C$ represent the enhanced fluorescence intensity of the system in the presence of PAN and the concentration of PAN (in M), respectively. The limit of detection (LOD) was obtained to be $7.2 \times 10^{-8}$ M, according to the $C_{LOD} = 3S_b/m$ equation, where $S_b$ and $m$ are the standard deviation of the blank and the slope of the calibration curve, respectively. Moreover, the limit of quantitation (LOQ) was calculated to be $24.2 \times 10^{-8}$ M according to $C_{LOQ} = 10S_b/m$, where $S_b$ and $m$ are the same as in the above equation. The relative standard deviation (RSD%) was 0.001 – 0.02% for five replicate determinations of three different concentrations of PAN ($200, 400$ and $800) \times 10^{-8}$ M under the optimal condition.

Some previously proposed methods for the determination of PAN are summarized in Table 1. As is shown in Table 1 and compared with previous methods, the present method has a better LOD than some other methods and can be used as an alternative analytical approach for fast and routine assay of PAN.

**Interference studies**

To investigate the selectivity and practical applicability of the proposed turn-off-on method, the possible interferences of coexisting species including amino-acids, metal ions and carbohydrates (under optimal condition) on the $\Delta I_f (%)$ was studied by adding desired amounts of these species to $7.0 \times 10^{-6}$ M PAN until a variation greater than 5.0% in the fluorescence emission intensity of the solutions (without interfering species).

Table 2 Tolerance limits of some interfering substances in estimating $7.0 \times 10^{-8}$ M PAN under the optimum condition

| Coexisting substance | Ratio of coexisting substance to PAN | $\Delta I_f, \%$ |
|----------------------|-------------------------------------|-----------------|
| Na⁺                  | 98:1                                | 3.5             |
| K⁺                   | 28:1                                | -4.7            |
| Cu²⁺                 | 0.25:1                              | 4.5             |
| Fe²⁺                 | 0.3:1                               | 5.0             |
| Zn²⁺                 | 0.5:1                               | 2.0             |
| Mg²⁺                 | 7:1                                 | 4.5             |
| Ca²⁺                 | 62:1                                | 2.0             |
| Fr⁻                  | 36:1                                | 1.4             |
| Gla⁻                 | 18:1                                | -2.0            |
| Glycine              | 35:1                                | -1.5            |
| Tryptophan           | 24:1                                | 1.3             |
| Cysteine             | 13:1                                | 3.3             |

a. Fructose. b. Glucose. c. Glycine. d. Tryptophan. e. Cysteine.

Table 1 Comparison of proposed method with other methods used for the determination of PAN

| Method                        | Real samples          | Linear range/M | LOD/M  | Reference |
|-------------------------------|-----------------------|----------------|--------|-----------|
| LC-MS/MS⁺                     | Human plasma          | (0.013 - 13)×10⁻⁶ | —      | 7         |
| RP-HPLC⁺                      | Human plasma          | (0.026 - 1.3)×10⁻⁴ | —      | 5         |
| (Electrochemical method, voltammetric) | Human plasma          | (6.6 - 360)×10⁻⁴ | 2.2 × 10⁻⁸ | 16        |
| (Electrochemical method, square wave voltammetry) | Tablet and human plasma | (0.039 - 6.5)×10⁻⁴ | 1.25 × 10⁻⁷ | 17        |
| (Electrochemical method, differential pulse and square wave techniques) | Tablet and human urine | (0.5 - 7.5)×10⁻⁴ and (0.675 - 4.375)×10⁻⁶ | 0.0318 × 10⁻⁴ and 0.106 × 10⁻⁶ | 18        |
| Spectrophotometric            | Tablet                | 1.0 × 10⁻⁸ - 1.0 × 10⁻⁶ | 6.31 × 10⁻⁸ | 15        |
| Spectrofluorimetric           | Tablet and human plasma | (0.065 - 1.04)×10⁻⁵ and (0.65 - 13)×10⁻⁷ | 1.04 × 10⁻⁷ and 0.83 × 10⁻⁷ | 13        |
| SPE coupled with spectrophotometry | Human plasma          | (0.26 - 3.9)×10⁻⁶ | 0.36 × 10⁻⁷ | 11        |
| Spectrofluorimetric (Tb-phen-AgNPs; as a fluorescent nanosensor) | Pharmaceutical dosage form (capsule) and human plasma | (10 - 1000)×10⁻⁴ | 7.2 × 10⁻⁴ | This work |

a. Liquid chromatography tandem mass spectrometry. b. Reversed-phase high performance liquid chromatography. c. Solid phase extraction.
was achieved. The obtained results are summarized in Table 2. These results indicated that most interfering species have no significant effect on the proposed system. Therefore, the proposed method has good selectivity, and can be used as a simple, selective and sensitive nanosensor to determine PAN in plasma and capsule samples.

**Application**

To evaluate the practical applicability and sensitivity of the presented method, the proposed nanosensor was used for the analysis of PAN in plasma samples according to the analytical procedure described previously. For this purpose, after the addition of an appropriate amount of PAN into the plasma sample, the general procedure was used for the analysis of spiked samples. The obtained results are summarized in Table 3. A good linear relationship between the emission intensity of the AgNPs. Compared with some other reported methods, our proposed method for estimating PAN is a simple, rapid, sensitive and inexpensive. A good linearity for estimation of PAN was observed in the range of (10 – 1000) × 10⁻⁸ M and the limit of detection was calculated to be 7.2 × 10⁻⁹ M. Therefore, the present analytical procedure can be used for estimating PAN in real samples with great sensitivity and selectivity.

**Supporting Information**

This material is available free of charge on the Web at http://www.jsac.or.jp/analsci/

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