A Green chemosensor for Colorimetric Determination of Phosphate Ion in Soil, Bone and Water Samples using Curcumin Nanoparticles

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This article presents a sensitive and straightforward colorimetric chemosensor for determination of phosphate ion utilizing curcumin nanoparticles (CUNPs) as the sensing system. The color of as-prepared CUNPs can be changed from yellow to orange upon adding iron(III) ions due to the formation of a complex with CUNPs. However, in the presence of phosphate ions, iron(III) ions prefer to bind to phosphate ions and subsequently, the color of CUNPs is selectively recovered because of releasing the iron(III) ions from the CUNPs-iron(III) complex. Therefore, in this work the selective color changing of CUNPs-iron(III) system upon the addition of phosphate ions was used for quantitative sensing of phosphate ions. Various factors, such as pH, concentration of iron(III) and volume of CUNPs were examined and the optimum conditions were established. A linear calibration graph over the range of 10–400 ng mL\textsuperscript{-1} for phosphate (r=0.9995) was achieved using optimal conditions. Limit of detection (LOD) of the proposed method for phosphate was 7.1 ng mL\textsuperscript{-1} and the relative standard deviation (RSD) for measuring 50 ng mL\textsuperscript{-1} of phosphate was 3.7\% (n=8). The developed method was applied for the measurement of phosphate in water, soil and bone samples and satisfactory results were obtained.

Keywords: Curcumin nanoparticles; chemosensor; iron (III); phosphate; soil; bone.

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Introduction

Phosphate, as an inorganic polyatomic ion, is an important chemical in the metabolism of plants and animals. Phosphate in bones and proteins plays an important role in the energy production and leads to the stimulation of the plankton and aquatic plant growth which provides food for fish and hence it is very vital for the health of all living organisms. Besides phosphate fertilizers are used at very high concentrations and phosphate often enter natural waters and sediments. Thus, the presence of phosphate in drinking water is of great concern since its high concentration is considered as a pollutant in water. Consequently, determination of phosphate in natural waters is a priority for checking and controlling the quality of water.\(^1,2\)

Owing to the importance of phosphate determination, to date, several analytical methods have been employed for the assay of phosphate, including spectrophotometry,\(^1,3,4\) microcomplexation-spectrophotometry,\(^5\) solid phase extraction spectrophotometry,\(^6\) electrochemistry,\(^7,8\) micro nuclear magnetic resonance spectroscopy (NMR),\(^9\) flow injection analysis (FIA),\(^10\) near-infrared,\(^11\) spectrofluorimetry,\(^12,13\) paper-based analytical devices,\(^14\) ion chromatography,\(^15\) and high performance liquid chromatography mass spectrometric (HPLC-MS/MS).\(^16\) Compared to other techniques described above, UV–Vis spectrophotometric methods are still known as an attractive alternative because of rapidity, simple instrumental implementation, versatility and availability of the instrumentation.

Nanoparticles have attracted significant attention in analytical techniques due to their unique properties such as optical, electronic, chemical, magnetic, mechanical, catalytic and also high surface-to-volume ratio.\(^17\) Hence, to improve the sensitivity and selectivity of the analysis methods, several nanoparticles such as silver, gold, zinc oxide, palladium nanoparticles have been employed. For example Kaur et al. found that the silver nanoparticles coated with organic ligands can be used as a selective chemosensor for the detection of phosphate.\(^18\) Unfortunately, the use of some of these mentioned nanoparticles in the chemical
analysis has been limited because of toxicity, high costs and complexity of their synthesis methods.

Curcumin (diferuloylmethane, or (E, E)-1, 7-bis (4-hydroxy-3-methoxy-phenyl)-1, 6-heptadiene-3, 5- dione) is a yellow polyphenol with low molecular weight that is extracted from the traditional spice turmeric (Curcuma longa). This main pigment of the curcuma longa possess several biological and pharmacological activities including antitumor, anti-inflammatory, antioxidation, anti-HIV, antivirus, immunomodulation anticancer, chemoprevention and anti-angiogenesis properties. Curcumin gains tremendous attention as a pharmacological drug and has preventive and therapeutic effects against different ailments. However it is a poorly water-soluble compound that leads to a decrease in its bioavailability in vivo. Hence, to solve the problem of poor solubility of curcumin, numerous approaches have been presented including nano-formulation and encapsulation in micelles, liposomes, cyclodextrin and hydrogels. We have utilized curcumin nanoparticle in several studies such as lab-on-paper device and a sensitive chemosensor for mercury detection and citrate using micelle mediated cloud point extraction.

Herein, CUNPs as natural, low-cost and non-toxic nanoparticles have been used as a colorimetric sensor for the analysis of phosphate ion in soil, water and bone samples. The method uses the effect of phosphate on CUNPs-iron(III) complex as the sensing system. The influence of pH, concentration of iron(III), reaction time and concentration of CUNPs were studied in order to optimize the conditions to achieve better sensitivity and selectivity.

Materials and Methods

Instruments
The absorption spectra and measurements were performed by GBC UV–visible spectrophotometer model Cintra 101 (Sidney, Australia) using 1cm path length glass cells. Transmission electron microscopy (TEM) images were obtained using a Zeiss- EM10C-80 KV TEM (Jena, Germany). A Metrohm pH meter model 632 (Herisau, Switzerland) and combined glass electrode was used for pH adjustments. A DSA 100-SK2 ultrasound bath (Fujian, China) 100 W power, 40 kHz frequency, was used during the synthesis of CUNPs. A Heidolph model Labrota 4000 rotary evaporator (Schwabach Germany) was utilized for removing solvent after the synthesis of CUNPs.

Chemicals

The chemicals and solvents were analytical grade and de-ionized water was used for preparing the solutions. 0.400 g of Na₃PO₄·12H₂O (Merck, Darmstadt, Germany) was dissolved in water and diluted to 100 mL to give a phosphate solution of 1000 µg mL⁻¹. Other concentrations were made daily from this solution by appropriate dilutions. An iron(III) solution of 1000 µg mL⁻¹ was prepared as follows: 0.4840 g of the FeCl₃·6H₂O (Merck) and 1 mL of hydrochloric acid (Merck) was added to 100 mL volumetric flask and diluted to the mark with water. For preparation of acetate buffer pH 4; 0.10 mol L⁻¹ of NaOH (Merck) was added to 0.01 mol L⁻¹ of acetic acid (Merck) and the pH was adjusted to 4 by a pH meter. Curcumin powder was purchased from Sigma-Aldrich (St. Louis, MO, USA).

CUNPs Preparation

Fabrication of CUNPs was carried out by formerly presented method introduced by Bhawana et al.²⁵ after slight modification of the procedure.²² For the fabrication of CUNPs two solutions were prepared. In the first solution, 125 mg of curcumin was dissolved in 25 mL of dichloromethane. In the second solution 10 mL of Triton X-100 5% (v/v) was slowly
added to 90 mL of boiling water. Then 2 mL of the first solution was added to the second solution dropwise at the rate of 10 drops/min under ultrasonic action (power of 100 W and a frequency of 40 kHz) for 20 min. The obtained solution was stirred by a magnetic stirrer at 1500 rpm and at room temperature for 20 min to obtain a yellow color solution. This solution was evaporated to remove dichloromethane using a rotary evaporator. The solution was transferred to a dark bottle for storing. The stability of the solution was checked by recording the spectra every week and it was found to be about 6 months (the variation of the absorption intensity at $\lambda_{\text{max}} = 436$ nm for CUNPs was less than 5%).

**Sensing procedure**

An aliquot of phosphate solution (to give a final concentration over the range of 10–400 ng mL$^{-1}$), 0.7 mL of as prepared CUNPs solution, 0.4 mL of 10 µg mL$^{-1}$ of iron(III) to give a final concentration of 400 ng mL$^{-1}$ of iron(III) and 2.5 mL of acetate buffer (pH 4) were placed into a 10 mL volumetric flask and diluted to the mark. After 20 min, the absorbance was measured at $\lambda_{\text{max}}=420$ nm for CUNPs-iron(III) using 1cm path length glass cell. The absorbance of the blank solution in the absence of phosphate was also measured following the same procedure. The difference of the absorbance of the blank solution and the sample solution ($\Delta A$) was calculated and used as an analytical signal for phosphate measurement.

**Sample pretreatment**

2.5 g of each soil sample (Mahshahr and Ahvaz soil, Iran) was placed into a beaker; 50 mL of water was added and shaken for 30 min. Then it was filtered using a Whatman filter paper No. 1 and diluted to 50 mL with water. An appropriate volume of this solution was used for phosphate analysis.
The bone sample (chicken and sheep bones) was washed and dried in an electric oven at 100 °C. After cooling, it was then cut into small pieces and powdered. One g of each bone sample was placed into a porcelain crucible and ashed in a furnace. The obtained ash was thoroughly mixed with water and then filtered using a Whatman filter paper No. 1 and diluted to 25 mL with water. An appropriate volume of this solution was analyzed using the procedure for phosphate determination.

Water samples were collected in glass bottle from different places (Karoon River, Ahvaz tap water and Mahshahr Petrochemical Refinery wastewater, Iran) boiled for few minutes and filtered using a Whatman filter paper No. 1. An aliquot of the filtrate was used to determine phosphate by the above procedure.

Results and Discussions

Sensing mechanism

Due to the presence of two phenolic groups and one active methylene group in curcumin structure, it shows strong chelating ability towards a number of metal ions. Among these, the complex formation of curcumin with iron(III) ions has been known and reported in previous studies. UV–Vis absorption spectra of the CUNPs (A) before adding phosphate and iron (III), (B) after adding iron (III) and (C) after adding both iron(III) and phosphate (the insets are photograph of color intensity change of the solutions) and the corresponding TEM images (D-F) are presented in Fig. 1. As shown in this figure, the presence of iron(III) in solutions containing CUNPs causes a decrease in the absorbance of CUNPs along with color changing from yellow ($\lambda_{\text{max}} = 436$ nm) to orange ($\lambda_{\text{max}} = 420$ nm) due to the formation of complex between CUNPs and iron(III), which subsequently leads to aggregation of CUNPs as the TEM image shows (Fig. 1E). After adding phosphate ions to the above solution, a number of iron ions are trapped by phosphate, because of the interaction of iron with phosphate.
Iron(III) forms different complexes such as FeH$_2$PO$_4^{2+}$ and FeHPO$_4^{+}$ in dilute acidic media (K$_f$ values of 1.7×10$^8$ and 3.1×10$^8$ for FeH$_2$PO$_4^{2+}$ and FeHPO$_4^{+}$, respectively) or FePO$_4$ at higher pH values (K$_{sp}$=1.58×10$^{-23}$). Thus at the pH 4 the dominant phosphate species is H$_2$PO$_4^{2-}$ (about 98%) and it is most probable that FeH$_2$PO$_4^{2+}$ complex is the final product. As the subsequence of this interaction, the color of CUNPs and the absorbance intensities are selectively recovered because of releasing the iron(III) ions from the CUNPs-iron(III) complex and de-aggregation occurs as the TEM image in Fig. 1F shows. The difference in the absorbance and consequently the selective color changing of CUNPs-iron(III) system from orange to yellow in the presence of phosphate was taken as the signal for determination of phosphate calorimetrically. The schematic illustration of the suggested sensing strategy is presented in Fig. 2.

**Influence of pH**

pH has an impact in both formation of CUNPs-iron(III) complex and its subsequent reaction with phosphate. For achieving the highest sensitivity, pH was the first parameter which was tested over the range of 2-5. The effect of pH on the reaction illustrated in Fig. 3A revealed that the maximum absorbance was found at pH 4 which was chosen for further experiments. The intensity of absorbance was decreased at pH values higher than 4 due to the formation of Fe(OH)$_3$ precipitate. To stabilize the pH of the solution at pH 4 some buffer systems such as formate and acetate with pH 4 (1–3 mL) were added to the solutions. The results showed that acetate buffer gave better performance. Therefore acetate buffer pH 4 was selected and 2.5 mL of the buffer was needed for maintaining the pH at optimum value.

**Effect of iron(III) concentration**

In this assay, phosphate ion was measured using CUNPs in the presence of iron (III) as the sensing probe. As expected the concentrations iron(III) could affect the performance of
the sensing system due to the interaction of iron with phosphate. Hence, the effect of various concentrations of iron(III) was examined using concentration range from 200 to 800 ng mL\(^{-1}\) and as the results illustrated in Fig. 3B show ΔA value was increased up to 400 ng mL\(^{-1}\) of iron(III) followed by a decrease at higher concentrations. Therefore, the concentration of 400 ng mL\(^{-1}\) of iron (III) was selected and used in next experiments.

**Effect of CUNPs volume**

Since CUNPs is the base of this sensing probe, its amount is important. Thus the effect of CUNPs volume as the sensing probe was studied by applying different volumes of the synthesized CUNPs. From the results shown in Fig. 3C, it can be seen that the absorbance remains constant for CUNPs volumes of 0.6 mL and higher. Thus, 0.7 mL of the synthesized CUNPs was selected as the optimal concentration.

**Reaction time**

The preliminary investigations revealed that the reaction is time dependent. Reaction time may affect the extent of the complex formation and therefore affect the absorbance of the system. Thus, the effect of the reaction time was studied from 5 to 30 min. The absorption spectra of CUNPs-iron(III) and CUNPs-iron(III)-phosphate at 5, 10 and 15 min were recorded and are presented in Fig.4A to show the time dependent of the reaction. Above 15 min the spectra overlap could not be shown. The experimental results shown in Fig. 4B also reveals that the value of ΔA is increased up to 15 min and above that the change in ΔA is negligible. It seems that at least 15 min is required for the reaction to attain equilibrium. Hence, all the measurements were performed 20 min after mixing the solutions.
Analytical figures of merit

The UV-Vis absorption spectra shown in Fig. 5A illustrate that the absorbance of CUNPs-iron(III) is increased by adding different concentration of phosphate ion. In this analytical method, the calibration graph is described by the equation $\Delta A = 0.0009C + 0.0213$ over the concentrations of 10–400 ng mL$^{-1}$ of phosphate ($r=0.9997$) under the selected experimental conditions ($\Delta A$ is the analytical signal as previously described and $C$ is the concentration of phosphate in ng mL$^{-1}$) (Fig.5B). The limit of detection (LOD) calculated from the formula $3S_b/m$ (where $S_b$ is the standard deviation of the blank and $m$ is the slope of the calibration graph) was 7.1 ng mL$^{-1}$ of phosphate. The precision of the method was determined by calculating the relative standard deviations (RSD) for measuring 50 ng mL$^{-1}$ of phosphate ($n=8$) and was found to be 3.7%. This method was compared with some of the previous methods reported for phosphate detection (Table 1). As it is observed the analytical parameters of the method for colorimetric measurement of phosphate are better than or comparable with some of these methods. Although some of the methods give better limit of detection, they use preconcentration procedures.$^5$

Effect of interferences

The selectivity of the method was assessed by studying the interference effects of several coexistent ions on the determination of phosphate. Hence, samples containing 300 ng mL$^{-1}$ concentration of phosphate and different concentrations of the potential interfering species were studied and the results are presented in Table 2. A substance was considered as interference if the variation in the absorbance value of the sample was larger than ±5.0%. As can be seen from Table 2 most of these species showed minimal interference with the determination. The effect of carbonate can easily be removed by boiling the solution in slightly acidic media for few minutes.
Applications of the method

The application of the method was evaluated by the analysis of some real samples. The method was utilized for the phosphate measurement in soil, bone and industrial and environmental water samples. The samples were prepared as described above (sample pretreatment section). The results of the present work for phosphate determination in soil and bone samples are given in Table 3. The water samples were analyzed according to the sensing procedure for phosphate in these samples. The samples were spiked with two different concentration of phosphate and the analysis was performed. The obtained results are presented in Table 4. The results of the sample analysis demonstrate that acceptable recoveries (95.6-105.0%) are obtained for the phosphate determination by this procedure and soil, bone and water samples matrices did not show considerable effect on the analysis results. The amounts of phosphate found in Mahshahr soil, Ahvaz soil, chicken bone and sheep bone were 2.25, 4.50, 39.39 and 22.47 µg g\(^{-1}\), respectively.

Conclusions

In the present study, we have successfully demonstrated a green, sensitive and selective method using CUNPs for colorimetric measurement of phosphate. The method is eco-friendly and requires simple and available instrument such as spectrophotometry. The selective color changing of CUNPs-iron(III) system from orange to yellow upon the addition of phosphate ions, was utilized for quantitative sensing of phosphate. Important factors affecting the performance of developed analytical method were investigated and it was exploited for measuring phosphate in various water, soil and bone samples. The results revealed the great capability of the present method for measuring phosphate ions in real samples without significant interference from other species present in sample matrix. The linear range of the method was 10–400 ng mL\(^{-1}\) of phosphate (\(r=0.9997\)) with a detection limit of 7.1 ng mL\(^{-1}\) of
phosphate. Therefore the significant advantage of the developed method for phosphate sensing is the analytical characteristics of the method which are better or comparable with some of the previous methods for the determination of phosphate.

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Figure captions

Fig. 1. UV–vis spectra and color changing of the CUNPs s: (A) without adding phosphate and iron (III), (B) after adding iron (III) and (C) after adding both phosphate and iron (III) and the corresponding TEM images of CUNPs in solution, (D) without adding phosphate and iron (III), (E) after adding iron (III), and (F) after adding both phosphate and iron (III).

Fig. 2. Schematic illustration of phosphate ion sensing.

Fig. 3. (A) The effect of pH on the absorption intensity of CUNPs-iron(III), (B) The effect of iron (III) concentration on the absorbance CUNPs-iron(III), and (C) The effect of CUNPs volume on the absorbance of CUNPs-iron(III). In all cases phosphate concentration was 300 ng mL$^{-1}$.

Fig. 4. (A) The effect of time on the absorption spectra of CUNPs-iron(III) (a) and CUNPs-iron(III)-phosphate at times 5, 10, 15 min (b,c,d), respectively and (B) on the ΔA of CUNPs-iron(III)-phosphate.

Fig. 5. (A) The UV–Vis absorption spectra of CUNPs-iron(III) in the presence of different concentration of phosphate ion and (B) The calibration curve under optimum conditions: 400 ng mL$^{-1}$ of iron (III); 2.5 mL of acetate buffer (pH 4); 0.7 mL of the synthesized CUNPs; time of 20 min.
Fig. 1
Fig. 2
Fig. 3

(A) Effect of pH on ∆A

(B) Effect of concentration of Fe(III) on ∆A

(C) Effect of volume of CUNPs on ∆A

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Fig. 4
Fig. 5

(A) Absorbance vs. Wavelength (nm) for different concentrations of phosphate.

(B) Calibration curve for phosphate concentration.

Absorbance = 0.0009x + 0.0213

$R^2 = 0.9990$
Table 1

Comparison of the developed method with some of the previously reported methods

| Method                                | Linear range (ng mL\(^{-1}\)) | Detection limit (ng mL\(^{-1}\)) | Ref. |
|---------------------------------------|-------------------------------|---------------------------------|------|
| Spectrophotometry                     | 500-10000                     | -                               | 1    |
| Flow injection                        | 200-1500                      | 54.0                            | 3    |
| Sequential injection                  | 200-700                       | 100.0                           | 4    |
| Microcomplexation Spectrophotometry   | 5-200                         | 5.0                             | 5    |
| Phosphate ion selective electrode     | 95-95000                      | 95.0                            | 7    |
| near-infrared                         | 388-7760                      | 37.3                            | 10   |
| Paper-based device                    | 200-10000                     | 50.0                            | 13   |
| Colorimetric with Curcumin nanoparticles | 10-400                        | 7.1                             | This work |

This work
Table 2

Effect of interfering species on the determination of 300 ng mL\(^{-1}\) of phosphate

| Interfering ion                  | Tolerance ratio [interfering]/[Phosphate] |
|----------------------------------|-------------------------------------------|
| K\(^+\), NO\(_3^-\), Cl\(^-\), Ca\(^{2+}\), NH\(_4^+\), Co\(^{2+}\) | 1000                                      |
| Cu\(^{2+}\), Pb\(^{2+}\), Bi\(^{3+}\), Ni\(^{2+}\), Mg\(^{2+}\) |                                           |
| Cd\(^{2+}\), SO\(_4^{2-}\)                       |                                           |
| Glucose                          | 800                                       |
| F\(^-\), I\(^-\)                  | 500                                       |
| Sucrose                          | 250                                       |
| HCO\(_3^-\)                      | 50                                        |
| Citrate                          | 10                                        |
| C\(_2\)O\(_4^{2-}\), Tartarate    | 5                                         |
| CO\(_3^{2-}\)                     | 1                                         |
### Table 3

**Determination of phosphate in soil and bone samples**

| Sample         | Added (ng mL\(^{-1}\)) | Found (±s)\(^a\) (ng mL\(^{-1}\)) | Recovery (%) |
|----------------|-------------------------|------------------------------------|--------------|
|                |                         |                                    |              |
| Mahshahr soil  | –                       | 56.1±2.1                           | –            |
|                | 50                      | 107.4±3.9                          | 102.6        |
|                | 100                     | 154.9±5.7                          | 98.8         |
|                | –                       | 112.4±4.1                          | –            |
| Ahvaz soil     | 50                      | 164.9±3.1                          | 105.0        |
|                | 100                     | 209.9±2.1                          | 97.5         |
|                | –                       | 157.4±5.8                          | –            |
| Chicken bone   | 50                      | 208.6±1.5                          | 102.4        |
|                | 100                     | 258.6±1.8                          | 101.2        |
|                | –                       | 89.9±3.3                           | –            |
| Sheep bone     | 50                      | 138.6±5.1                          | 97.4         |
|                | 100                     | 189.9±5.9                          | 100.0        |

\(^a\) Standard deviation (n=5)
Table 4

Determination of phosphate in water samples.

| Sample                  | Added (ng mL\(^{-1}\)) | Found (±s)\(^a\) (ng mL\(^{-1}\)) | Recovery (%) |
|-------------------------|------------------------|------------------------------------|--------------|
| Karun River water       | –                      | 71.9±2.7                           | –            |
|                         | 50                     | 121.9±4.5                          | 100.0        |
|                         | 100                    | 171.9±3.2                          | 100.0        |
| Mahshahr Refinery Wastewater | –                      | 104.1±3.8                           | –            |
|                         | 50                     | 151.9±5.6                          | 95.6         |
|                         | 100                    | 199.7±5.9                          | 95.6         |
| Tap water               | –                      | N.D\(^b\)                           | –            |
|                         | 50                     | 51.9±1.9                           | 103.8        |
|                         | 100                    | 99.7±3.7                           | 99.7         |

\(^a\) Standard deviation (n=5)

\(^b\) Not Detected