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Short communication

Application of Prussian blue-based optical sensor in pharmaceutical analysis

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Abstract

Optical flow-through cell-detector with incorporated transparent chemosensitive layer of Prussian blue has been applied in simple, single-channel flow-injection system for pharmaceutical analysis. The reductant analyte converts the Prussian blue based sensing layer to Prussian white form, and the attendant color change is used for sensing. Discoloration of the film is spectrophotometrically detected at 720 nm wavelength. The flow injection system has been successfully used for selective determination of ascorbic acid in simple and complex pharmaceuticals. The method is free from interferences caused by various ions and active ingredients commonly found in pharmaceuticals. The flow-through sensor is useful for spectrophotometric flow-injection analysis of intensively colored and turbid samples. The results of medicine analysis are comparable to those obtained using reference pharmacopeal method. The analytical system could be also used for determination of cysteine and hydrogen peroxide in medicines.

Keywords: Spectrophotometry; Prussian blue film; Flow-injection analysis; Ascorbic acid; Cysteine; Hydrogen peroxide; Pharmaceuticals

1. Introduction

For many pharmaceutical and biomedical analyses various dry colorimetric tests are developed. There are paper or plastic strips, plastic cuvettes and microtitrationplates coated with reagents layers that selectively react with an analyte forming color products detectable spectrophotometrically. For such tests no additional reagents are necessary. Recently, we have developed cuvette tests for selected redox species, having integrated sensing layer made of Prussian blue (PB). The tests are useful for selective determination of ascorbates [1] and some mercaptocompounds [2]. PB film plays double role in the developed determination system, first as an immobilized reagent (oxidant), and second as an optical sensing element. However, the tests are useful only as single-use, disposable devices.

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In this paper, application of PB film for construction of flow-through detector is presented. Utilization of the PB film-based reaction–detection system in flow-injection analysis (FIA) conditions enables both, spectrophotometric determination of analyte and regeneration of detector. In other words, the presented FIA system allows application of the non-indicating, stoichiometric scheme of analyte recognition [3,4] for reversible optical sensing. The presented optical PB-based-FIA system has been used for determination of vitamin C, cysteine and hydrogen peroxide in pharmaceutical products.

2. Experimental section

2.1. Reagents

4-(Pyrrol-1-yl)-benzoic acid was obtained from Aldrich (Germany). All other organic and inorganic reagents were of analytical grade and were used as received (POCh, Poland). All experiments were carried out with solutions prepared with doubly distilled and degassed water. All solutions of reagents and analytes were prepared immediately before use.

2.2. Flow-injection system and flow-through detector

For optical measurements Shimadzu 2401/PC Spectrophotometer connected with data collecting/processing personal computer has been used. Experiments were carried out using conventional system for FIA with a transmission flow-through cell having one of the windows coated with sensing film. Simple single line manifold (Fig. 1) consists of peristaltic pump Minipuls 3 (Gilson Medical Electronics), rotary injection valve and flow-through optical cell (both laboratory-made). The rectangular $1.2 \times 1.2 \times 4$ cm^3 cell made of transparent polystyrene was adjusted for use with polyester foils coated with sensing film (Fig. 1). The total effective volume of the flow-through cell was 0.15 ml.

The optical chemosensitive layer was prepared according to the procedure described previously [5]. The PB film was deposited from 0.1 M solution of potassium hexacyanoferrate(III) in 1.0 M hydrochloric acid saturated with 4(pyrrol-1-yl)-benzoic acid. A dust-free polyester foil (0.1 mm thickness) having one side protected with silicone rubber was used as the support. The foil was immersed in the reaction mixture exposed to ultraviolet radiation from conventional UV lamp (Philips, 15 W). The films were obtained after 1 day of the deposition process. The foil with the deposited film was intensively washed with 1.0 M hydrochloric acid and water. Then the silicon-protecting layer was removed and the sensor membrane was mounted so that it formed one wall of the flow-through cell (Fig. 1). Before first usage the cuvette with the sensing layer was conditioned in 0.2 M potassium phosphate buffer of pH 7.0.

2.3. Measurements

All spectrophotometric measurements were performed at the wavelength of 720 nm. No reference cell was used. The flow rate of carrier stream was 0.70 ml/min and the injection volume of standard or sample was 0.30 ml. As a carrier solution 0.2 M potassium phosphate buffer (pH 7.0) has been used. In some cases, the carrier buffer additionally contained a regeneration agent. In such experiments, the buffer was spiked with 0.005 M potassium hexacyanoferrate(III) or with 0.0005 M ascorbic acid depending on kind of the utilized scheme of sensing. If the regeneration agent was absent in the carrier buffer, the film was regenerated by injection of the reagent solution into carrier stream after standard or sample injection.

Standard solutions were prepared in the carrier buffer without any regeneration agent. Sample pretreatment was not needed for analysis. An accurately weighed amount of a pharmaceutical sample (three subsamples of each medicine) was dissolved in carrier buffer. The obtained solutions were not filtered nor discolorated. Carbon dioxide was expelled from the sample solution by simple shaking, if necessary. Each subsample was injected three times.

Reference determinations of analyte content in the sample solutions were performed using iodometric titration with starch as an end-point indi-

cator (three titrations for each subsample), according to pharmacopeal recommendations [6].

3. Results and discussion

3.1. Sensing film

The mechanism of the non-electrochemical deposition of the sensing layer on non-conducting, plastic support has been investigated and discussed in detail in our previous paper [5]. In strongly acidic environment hexacyanoferrate complex anions are slowly decomposed forming film of PB ($Fe_4[Fe(CN)_6]_3$). The process of the film deposition is initialled and accelerated by UV irradiation.

The chemosensitive layer is composed of PB in a poly(pyrrolylbenzoic acid) network. The presence of organic polymer in this composite material is very low, but crucial for physical properties of the film. In contrast to pure PB films, the composite film is homogeneous, robust, crack-free and strongly adheres to the support. Hard scratching is necessary to mechanical removing of the material from the foil. The sensing layer exhibits very high stability. No changes of its physical or chemical properties were observed after 1 year of measurements as well as after 3 years of storage in ambient conditions.

The freshly prepared sensing layer is composed of 'insoluble' PB. Optical experiments with such form of the film were poorly reproducible. Before use the film should be converted into so-called

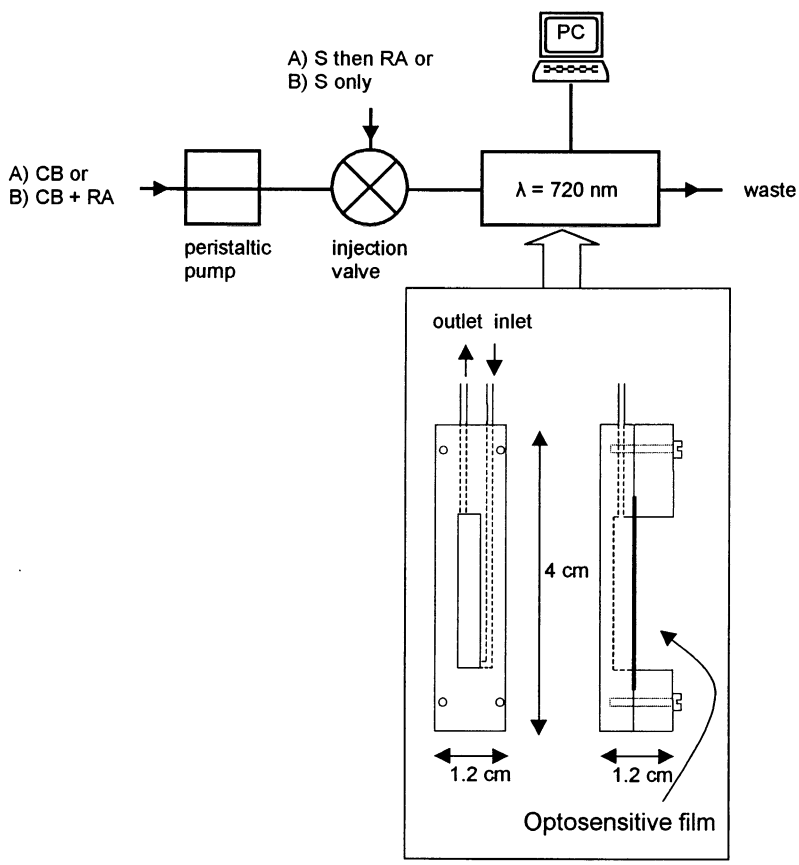

Fig. 1. Scheme of FIA system with flow-through optical detector (insert). S, analyte (sample or standard); RA, regeneration agent; CB, carrier buffer.

'soluble' form ($KFeFe(CN)_6$) by conditioning in neutral buffer containing high level of potassium ions. The potassium-phosphate carrier buffer is very useful for such process. In the course of the conditioning potassium ions are incorporated into zeolitic structure of PB, according to the following reaction:

$$Fe_4[Fe(CN)_6]_3 + 3K^+ + 3H_2O \rightarrow 3KFeFe(CN)_6 + Fe(OH)_3 + 3H^+ \quad (1)$$

This protolytic, ion-exchange process is not associated with the destruction of the zeolitic lattice of PB. The process causes small decrease of absorbance of the film. The conditioned film reproducibly works in the optical sensing schemes described in the next paragraph.

3.2. Analytical system

Strong reductants cause discoloration of the sensing material. The changes of the film absorbance are connected with formation of Prussian white (PW):

$$KFe^{III}Fe^{II}(CN)_6 + e^-_{(\text{reducing analyte})} + K^+ \rightarrow K_2Fe^{II}Fe^{II}(CN)_6 \quad (2)$$

Oxidation/reduction processes of the film caused by redox species can be optically monitored at the wavelength of 720 nm i.e. at the maximum of absorbance of the PB film. PW exists in the form of transparent, colorless film, not absorbing the light in visual and near-infrared range of spectrum [1]. The reduced film is regenerated using hexacyanoferrate(III) — oxidant present or injected into flow stream:

$$K_2Fe^{II}Fe^{II}(CN)_6 + Fe^{III}(CN)_6^{3-} \rightarrow KFe^{III}Fe^{II}(CN)_6 + Fe^{II}(CN)_6^{4-} + K^+ \quad (3)$$

The presented optical flow-through detector is an example of sensor based on the integration of reaction and detection [4]. Determination of reductants according to the Eq. (2) is a typical 'stoichiometric', kinetic process [3]. The analyte reacts with the film and converts it into colorless form (PW film). The analytical signal (change of the film absorbance) is related to the total amount (not to concentration) of the analyte that has contacted/reacted with the film. This means, that the transport of analyte to the sensing layer and the time of the contact (reaction) should be strictly controlled. Such requirements are fulfilled by simple FIA system with flow-through cuvette, like this one here used (Fig. 1).

After contact of the sensing layer with analyte the partially reduced film should be renewed before next measurement. Potassium hexacyanoferrate(III) has been found as suitable, inexpensive and stable regeneration agent. In the presented FIA system (Fig. 1) two regeneration procedures are possible. The first one is based on injections of the regeneration agent into a carrier stream after each injection of sample (Fig. 2). In the second mode of the FIA measurements the regeneration agent is permanently present in the carrier stream, in which samples are successively injected (Fig. 3).

The first procedure of measurements is more time and work consuming, but also more sensitive. The second mode of measurements is simpler from instrumental point of view and faster, however, less sensitive, as the regeneration agent continuously reoxidized sensing film and partially consumed analyte. Obviously, the increase of the regeneration agent level in the carrier stream suppresses the sensitivity of and increases the detection limit of the FIA system, resulting in the shift of calibration graphs towards higher concentrations. As can be seen from the inserts in Figs. 2 and 3, the presented analytical system enables determination of ascorbic acid in the millimolar range of concentrations. Both presented modes of measurements are useful in pharmaceutical analysis.

3.3. Pharmaceutical analysis

The developed FIA system enables selective determination of vitamin C. The PB film detects ascorbate and selected thiols, but does not detects typical inorganic and organic reductants, like halides, oxalate, sulfite, arsenite, cyanide, tiocyanate, alcohols, aldehydes and reducing sugars. Commonly occurring anions and ligands (sulfate, carbonate, nitrate, phosphate, acetate, citrate, borate and ethylenediamine tetraacetic acid (EDTA)) did not interfere. The used buffer carrier suppresses effects from alkaline cations and pH of

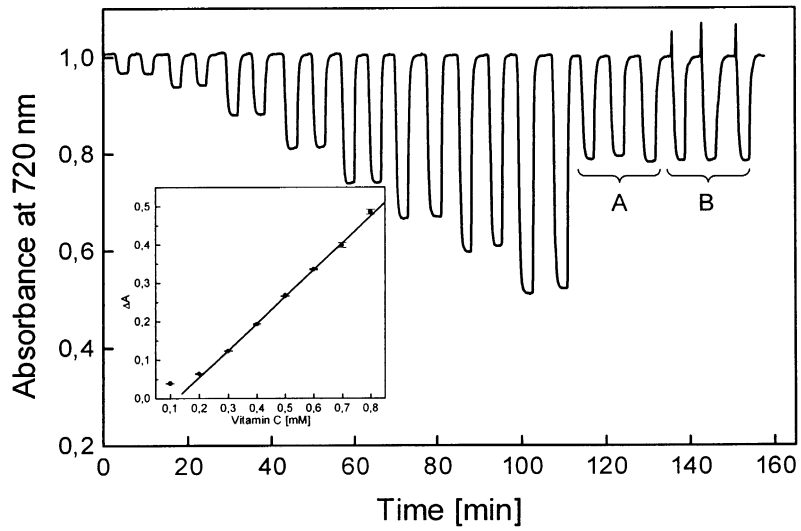

Fig. 2. Calibration of the FIA system on vitamin C in measurement mode with separated reaction and regeneration steps and analysis of filtered (A) and turbid (B) samples. Corresponding calibration graph is shown in the insert.

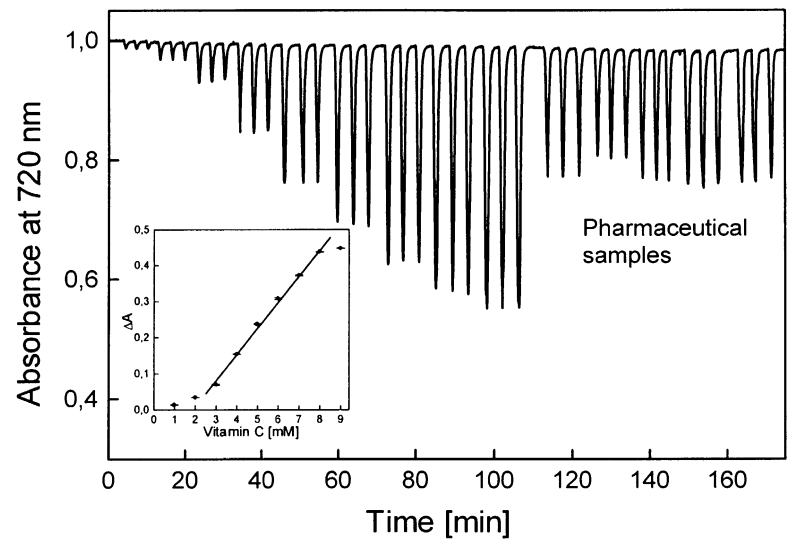

Fig. 3. Calibration of the FIA system on vitamin C in measurement mode with continuous regeneration of the sensing layer and analysis of real transparent samples. Corresponding calibration graph is shown in the insert.

real samples. Common components of pharmaceuticals, such as sugars, aspirin, paracetamol and other vitamins give no analytical signal.

It is worth to notice that the FIA system enables determination of vitamin C in strongly colored and turbid samples. It is possible in case of flow-injection measurements where reaction, detection and regeneration steps are separated (Fig. 2). The persistence of the color change after analyte exposure was utilized in detecting the analyte in turbid solutions. A clear analyte-free solution was passed through the cell after the turbid sample solution, enabling the transmission spectrum of the sensing layer to be recorded. The film was

Table 1
Results of analysis of medicines containing vitamin C

Product	Supplier	Main compound(s)	Content of ascorbic acid		
			Declared	PB-sensor in FIA-system	Iodometric titration
Vitamin C	Polfa	Ascorbic acid	100 mg per tablet	97 ± 2 mg per tablet	98 ± 2 mg per tablet
Aspirin C	Bayer	Acetylsalicylic acid	240 mg per tablet	246 ± 4 mg per tablet	245 ± 3 mg per tablet
Efferalgan C	Upsa	Paracetamol	200 mg per tablet	208 ± 4 mg per tablet	209 ± 5 mg per tablet
Magnesium	Polfa	Magnesium ascorbate	100 mg per amp	96 ± 2 mg per amp	98 ± 2 mg per amp
Calcium C	Polfa	Calcium gluconate	200 mg per tablet	213 ± 3 mg per tablet	214 ± 3 mg per tablet
Duovit	Krka	Vitamins	60 mg per tablet	55 ± 1 mg per tablet	56 ± 3 mg per tablet

reset to its initial redox state by running a regenerant (oxidant) through the cell. As can be seen from Fig. 2, changes of the PB film absorbance (analytical signal) for turbid and filtered samples are nearly the same and reproducible. It means, that the filtration of turbid samples (separation step) before injection into the FIA system is not necessary. Insoluble components of medicines (for example starch or chalk) or precipitates formed in the course of the analytical procedure (for example in reactions of calcium or magnesium ions from sample with phosphates present in the carrier buffer) did not disturb the spectrophotometric determinations of ascorbic acid. Similar procedure seems to be useful for analysis of samples showing significant absorption at the detection wavelength.

The flow-through sensor described here has been successfully applied for determination of vitamin C in pharmaceutical preparations. The results of ascorbic acid determinations in simple and complex pharmaceutical products are collected in Table 1. The results are comparable with those obtained using iodometric titration recommended by pharmacopoeia [6] for such analyses.

The presented flow-through sensor is optically sensitive to some organic thiols such as cysteine. The system could be used for determination of the amino acid in some medicines. No effects from other natural amino acids were observed. Only homocysteine and penicylamine slightly interfere. Cysteine was determined in Lobamine (medicine supplied by Pierre Fabre). The results of the determinations (142 ± 1 mg per tablet) were comparable with those obtained by iodometric titration (143 ± 2 mg per tablet) and close to declared content (150 mg per tablet). Methionine, the main compound of this amino acid antidote (350 mg per tablet) did not interfere.

Finally, it should be noted that it is possible to reverse the roles of analyte and regeneration agent in the analytical system [5]. The flow-through sensor based on reduced PB layer can be used for detection of various oxidants. Although, the PW film-based sensor is not selective it can be useful for pharmaceutical analysis. In the Fig. 4 utilization of the sensing scheme for hydrogen peroxide determination is shown. In these measurements the regeneration agent (0.0005 M ascorbic acid) has been continuously transported with carrier buffer through the flow cell coated with PW film. The FIA system was used for determination of H_2O_2 in Pertlenon (supplied by Filopharm). Main components of the pharmaceutical product are percarbamide (urea hydrogen peroxide), citrate and EDTA. The content of the peroxide in samples of the medicine solutions determined by iodometric titration and with use of the PW based sensor/FIA system was 341 ± 3 and 339 ± 5 mg per tablet, respectively (declared content, 362 mg per tablet). Similarly comparable results were obtained for samples of 3% solutions of H_2O_2, commercially available in pharmacies.

4. Conclusions

New inorganic material predominantly composed of PB is useful for optical chemosensing purposes. Incorporation of the PB film into flow-injection system enables multiple detection and

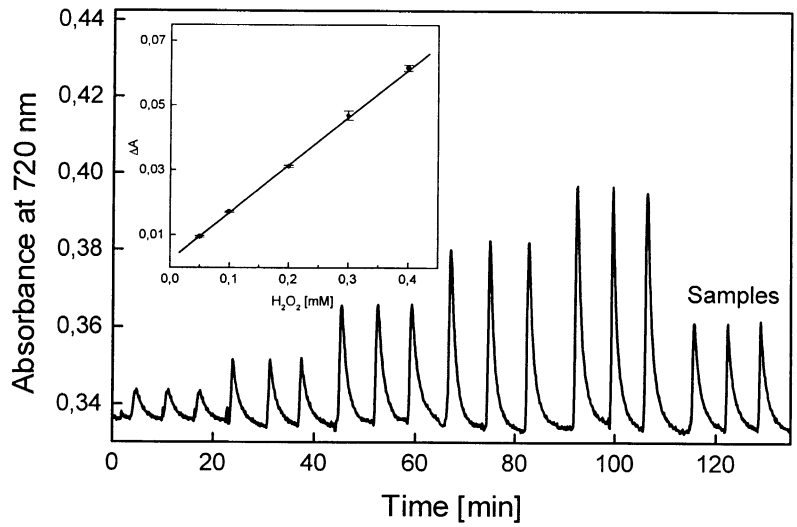

Fig. 4. Calibration of the FIA system on hydrogen peroxide in reverse mode of measurement with continuous regeneration of the sensing layer and analysis of real samples. Corresponding calibration graph is shown in the insert.

regeneration of the sensing layer. The spectrophotometric FIA system based on the flow-through sensor is useful for pharmaceutical analysis.

In conclusions, we would like to stress main advantages of the developed sensor. The shape of the flow-through cell detector is fully compatible with standard spectrophotometers. The reference cell is not necessary because as analytical signal changes of the film absorbance (not absolute values) are measured. The sensing film is optically compatible with economic near-infrared light emitting diodes. Only small amounts of cheap reagents are consumed in the course of analysis. Due to high selectivity and possibility of separation of reaction and detection steps the sensor can be used for analysis of complex and turbid samples working in very simple flow-injection system without any additional elements like separators, reactors, etc. No special procedures for sample pretreatment are necessary. Finally, the sensor is cheap, very stable and robust.

Acknowledgements

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