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Cost-effective flow injection spectrophotometric assay of iron content in pharmaceutical preparations using salicylate reagent☆

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Abstract

A new flow injection procedure for an assay of Fe(III) by using salicylate obtained from antipyretic powder, which is a cheap and easily available reagent, is proposed. A red complex was continuously monitored by a laboratory-made green LED colorimeter. A linear calibration was obtained in the range of 1–20 mg Fe l^{-1} with a detection limit of 0.5 mg Fe l^{-1} and R.S.D.s of 1.4–5.4% ($n = 3$, for 1–20 mg Fe l^{-1}). The new procedure was applied to assay iron contents in pharmaceutical preparations. The results were in good agreement with those of the USP standard method.

Keywords: Drugs; Flow injection; Iron; Salicylate; Aspirin

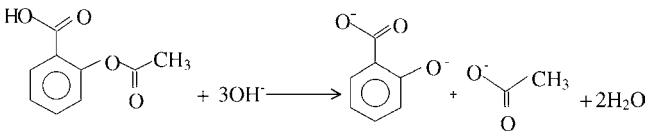
1. Introduction

Iron is a mineral essential to the human body. The average adult has 4–5 g of iron, of which 60–70% is present as heme in the circulating haemoglobin. The human body loses 0.5–1.5 mg iron per day, and sufficient amounts should be ingested (12 mg for adults, 15 mg during pregnancy and lactation and for adolescents, and 7.5–10.5 mg for children, rising to 13.5 mg in 11–14 years old group) [1]. Iron deficiencies are particularly common in premenopausal women, and even college-age women should pay particular attention to the amount of iron in their diets [2].

There are many methods for iron determination in pharmaceutical samples. Of these methods, USP and BP have served as the standard methods for a long time. However, both of the methods are based on a titration technique, which is tedious and involves many steps with consumption of large amounts of chemicals.

Spectrophotometric FI systems have been reported by using different color agents such as 1,10-phenanthroline [3,4], thiocyanate [5], Tiron [6] and 2,2-dipyridyl-2-pyridylhydrazone [7]. The last one was applied for the determination of iron in pharmaceutical samples.

Iron(III) was proposed to be a color agent for a batch spectrophotometric determination of acetylsalicylic acid in aspirin [8,9]. Acetylsalicylic acid in aspirin is hydrolyzed in an alkaline solution to yield salicylate dianion, as represented in Eq. (1).



(1)

Acidification, followed by addition of iron(III) ion, yields a soluble tetraaquasalicylatoiron(III) complex. The intensely purple solution exhibits a strong absorption at 520 nm [9]. In the presence of excess iron, the following complex is formed [9]:

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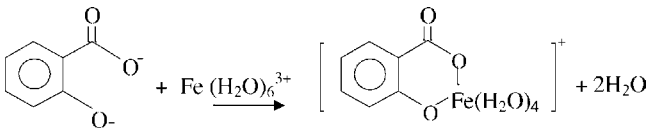

(2)

But in the presence of excess salicylic acid, as here, the iron can be present as the $Fe(III)(sal)_3$ chelate in solution. The absorption maximum was at 520 nm.

We have investigated the use of such reactions for a simple FI system to assay the iron content in pharmaceutical preparations using cheap salicylate reagent obtained from aspirin. This is a less expensive source of salicylic acid than ACS +99% grade salicylic acid.

2. Experimental

2.1. Chemicals and reagents

All reagents were of analytical grade, unless otherwise stated. Deionised water (Milli Q, Millipore) was used throughout. A stock solution of 1000 mg l^{-1} Fe(III) was prepared by dissolving 0.8774 g of ferric sulphate in a portion of water. Concentrated sulfuric acid (0.5 ml) was added before making up to a volume of 100.00 ml. Working Fe(III) standard solutions were obtained freshly by appropriate dilutions of the intermediate 100 mg l^{-1} Fe(III), obtaining from the stock solution. A salicylate reagent solution (0.01 M) was prepared by dissolving 1.20 g aspirin drug (Tumjai, Osodsapa, commercially available in a drug store in Thailand) in 20 ml of 2 M sodium hydroxide. The solution was placed in a boiling water bath for 15 min before making to a volume of 500 ml with water.

2.2. FI manifold

A laboratory-made flow injection analysis system was employed. The system consisted of a peristaltic pump (Ismatec, Switzerland), a six port injection valve (Upchurch, USA), a home-made flow through cell, a controller and a recorder as shown in Fig. 1. The green LED and the photodiode were used as light source and sensor, respectively. The controller was a laboratory designed and assembled device with microprocessor and was used to control the operation of the pump and injection valve, and to collect and to transfer the data to a recorder.

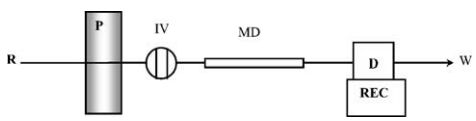

Fig. 1. FI manifold for determination of iron using salicylate: R: 0.01 M salicylate solution, P: peristaltic pump, IV: six-port injection valve, MD: mixing device, D: detector, REC: recorder, W: waste.

A series of Fe(III) standard solutions was injected into the salicylate reagent stream via an injection valve. The complex formed was continuously monitored. Calibration was made by plotting peak height versus Fe(III) concentration.

2.3. Assay of iron in pharmaceutical preparation samples

Pharmaceutical samples were taken from local drug stores. Sample preparation was adapted from AOAC 977.30 [10] as follows. Twenty tablets of a drug sample were weighed and powdered. A portion of the powder, equal in weight to the average of one tablet, was accurately weighed and transferred to a 250 ml flask. A 50 ml portion of water and 2 ml conc. hydrochloric acid were added. The mixture was boiled on a steam bath for 30 min before cooling to room temperature and was diluted with water to a volume of 100.0 ml. The mixture was filtered through Whatman No. 1 paper. A 1 ml aliquot of the filtrate was transferred to a 50 ml volumetric flask, and 0.5 ml of hydrogen peroxide was added before making to a volume of 50 ml with water. The samples were then analyzed by the proposed method.

3. Results and discussion

3.1. FI set optimization

A simple one-line manifold FIA system for iron determination using salicylate is depicted in Fig. 1. The parameters, namely, concentration, flow rate and pH of the salicylate reagent solution, mixing coil type and length were investigated. The injection volume (40 μl) and Fe(III) standard (0.5 mg Fe l^{-1}) were fixed for all studies.

Various concentrations (0.01, 0.05, 0.1 and 0.5 M) of salicylate were tried by using a flow rate of 4.0 ml min^{-1} and a mixing coil length of 100 cm. The higher the concentration of salicylate, the higher was the peak obtained. The Schlieren effect was pronounced when salicylate concentration was 0.05 M or more. Using a 200 cm mixing coil resulted in a similar observation. The mixing coil was replaced by a glass bead column (0.3 mm glass beads packed in 0.3 mm i.d. × 5 cm Tygon tube, in which both ends were plugged with 0.3 mm o.d. × 2.0 cm Tygon tubing for the connection to the system tubings) as a mixing device to promote better mixing [11]. A flow rate of 8.8 ml min^{-1} instead of 4.0 ml min^{-1} was tried, but a lower peak was obtained (0.68 cm instead of 0.75 cm).

A set of conditions was then selected: 0.01 M salicylate reagent, 40 μl injection volume, a glass bead column with the dimension described above and a flow rate of 4 ml min^{-1}.

3.2. Calibration

Using the selected conditions, a linear calibration was obtained in the range of 1.0–20.0 mg l^{-1}: peak height (cm) = 0.4618[Fe(III)(mg l^{-1})] + 0.1627, r^2 = 0.9997. Relative

Table 1
Assay of iron contents in pharmaceutical preparation samples by the proposed FI and USP method [13] (average of triplicate results)

Sample no.	Form of iron	Label (mg per tablet)	USP method		The proposed FI method			
					Modified AOAC 977.30 (HCl alone)[a]		Strong acid digestion (HNO_3 + $HClO_4$) with HCl[a]	
			mg g^{-1}	mg per tablet	mg g^{-1}	mg per tablet	mg g^{-1}	mg per tablet
1	Fumarate	200	459	221	455	219	443	213
2	Fumarate	90	67.7	90	69.8	93	72.0	96
3	Fumarate	200	446	214	436	209	481	231
4	Fumarate	200	390	197	376	190	395	200
5	Fumarate	400	424	415	405	396	400	391
6	Fumarate	200	352	194	355	196	375	208
7	Gluconate	200	509	330	296	192	330	213
8	Gluconate	150	231	161	205	157	179	138
9	Sulphate	200	322	149	438	202	444	205
10	Citrate	470.9	1094	604	688	380	909	502

[a] Sample preparation procedure

standard deviations ($n = 3$) over this range (1–20 mg l^{-1}) varied from 1.4–5.4%. A detection limit (3σ of the blank signal [12]) of 0.5 mg l^{-1} was estimated.

3.3. Application to pharmaceutical preparations

Pharmaceutical samples available in local drug stores were taken. They were in forms of fumarate, gluconate, sulphate and citrate. The sample preparation (Modified AOAC 977.30 procedure) described in the experimental section was employed. The obtained solution was analyzed following the proposed FI procedure. The results are summarized in Table 1.

Another sample preparation procedure with very strong acid digestion was employed for comparison and to confirm that iron was converted into iron(III) and dissolved in the solution. The procedure is as follows. Twenty tablets of a drug sample were weighed and powdered. A portion equal to the weight of one tablet was transferred to a 250 ml flask. A 25 ml volume of water, 15 ml conc. nitric acid and 5 ml conc. perchloric acid were added into it. The mixture was boiled on a hot plate until nearly dry, then cooled to room temperature before adding 4 ml conc. hydrochloric acid and 10 ml water and making to a volume of 100 ml with water. The mixture was filtered through Whatman No. 1 paper. A 1 ml aliquot of the filtrate was transferred to a 50 ml volumetric flask and diluted to the mark with water. It was found that the results from the both sample preparation procedures for FI were in good correlation (Table 1), except for the citrate form (sample #10). This indicates that iron in the sample can be quantitatively converted into free Fe(III) by the sample preparation procedure using HCl and H_2O_2 in place of HNO_3 and $HClO_4$ with HCl.

The USP method was employed as a reference for validating the proposed FI method. The procedures followed the USP 24 [13] for the drug samples in the forms of fumarate, gluconate and sulphate. The sample in citrate form was also analyzed by following the USP procedure for sulphate since there is no USP method for the citrate form. It was found that the results from the proposed FI methods using both sample preparation procedures agree with that of USP method as seen from the t test ($t = 1.37$ for that of the sample preparation procedure with HCl and H_2O_2 and $t = 1.44$ for the procedure using HNO_3 and $HClO_4$ with HCl at the 95% confidence level). And there was no significant difference between the two procedures for sample preparation ($t = 0.08$ at 95% confidence level). However, although the results from the t test indicated that there were no significant differences between the methods, it can be seen from Table 1 that the milligrams per tablet obtained by the USP method for sample numbers 7–10, of which iron contents are in the forms of gluconate, sulfate and citrate, differed from that of the labels, whereas that of the proposed FI method were practically the same, except for sample number 10 (citrate form). The errors from the USP method for gluconate forms may be from zinc dust (getting through the filter paper in a step to convert Fe(III) to Fe(II) [13]), which may be contaminated in the solution and which made the values higher than labeled. For the sulphate form, the results are lower than the labeled, and this may come from the long time of the filtration step, during which some of Fe(II) may be oxidized to Fe(III). For the citrate form, using FI, both the procedures for sample preparation are not suitable and the USP procedure for sulphate is not suitable for citrate form. However, the proposed method can be well applied to the samples in which the iron contents are in the forms of fumarate, gluconate and sulphate. Further study in more detail in such cases should be made.

This study demonstrates that the proposed FI procedure is simple. Other advantages gained, in comparing to the USP method, include economics (simple FI system, cheap

and readily available reagent, simpler sample preparation), less risk from toxic and hazardous chemical wastes. The procedure offers feasibility in automation.

4. Conclusions

A new cost-effective FI spectrophotometric assay of iron in pharmaceutical preparations (in a form of fumarate, gluconate or sulphate) is proposed. Salicylate is obtained from alkaline hydrolysis of the antipyretic drug, aspirin, which is cheap and easily available as reagent. Various advantages can be benefit from the proposed FI procedure, especially in some remote places.

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References

[1] A.E. Bender, Dictionary of Nutrition and Food Technology, 6th ed., Butterworths, London, 1990.
[2] J.S. Levine, K.R. Miller, Biology, 2nd ed., D.C. Heath and Company, Massachusetts, 1994.
[3] P. Ampan, S. Lapanantnoppakhun, P. Sooksamiti, J. Jakmunee, S. Kradtap Hartwell, S. Jayasvati, G.D. Christian, K. Grudpan, Talanta 58 (2002) 1327.
[4] H.A. Bruno, F.J. Andrade, P.C. Luna, M.B. Tudino, Analyst 127 (2002) 990.
[5] Y. Kanai, Analyst 115 (1990) 809.
[6] M.J. Pullin, S.E. Cabaniss, Water Res. 35 (2001) 363.
[7] A.C.L. da Conceicao, M.T. Tena, M.M.C. dos Santos, M.L.S. Goncalves, M.D.L. de Castro, Anal. Chim. Acta 343 (1997) 191.
[8] http://www.usi.edu/science/chemistry/mkrahlin/courses/CHEM_321/Spectrophotometric%20Aspirin.pdf.
[9] http://www.ic.sunysb.edu/class/che134/susb/susb013.pdf.
[10] P. Cunniff, Official Methods of Anaysis of AOAC International, 16th ed., AOAC International, Gaitherburg, 1995.
[11] K. Grudpan, S. Lairaungrath, P. Sooksamiti, Analyst 120 (1995) 2110.
[12] J.C. Miller, J.N. Miller, Statistics for Analytical Chemistry, 2nd ed., Ellis Horwood, Chichester, 1988.
[13] The United States Pharmarcopeia, Official Monographs, Asian ed., United States Pharmacopeial Convention, Rockville, 2000.