

COLORIMETRIC DETERMINATION OF IRON IN BEER BY FLOW INJECTION ANALYSIS USING THE MERGING ZONES TECHNIQUE

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A flow injection analysis system with colorimetric detection for the iron determination in beer is described. The methodology is based on the formation of a complex produced by the reaction of iron (II) with 1,10-phenanthroline, after reduction of iron (III) to iron (II) by ascorbic acid. The asymmetric merging zones technique was used to allow the sequential blank and sample absorbance readings. The results obtained with the developed system were in good agreement with those provided by the reference method (relative deviations lower than 4%) and exhibited a precision (relative standard deviation) better than 7%. A sampling-rate of 60 determinations per hour can be achieved.

Key Words: *Flow injection analysis, colorimetric iron determination, beer, merging zones*

INTRODUCTION

The iron content in beer can affect its quality and taste. It has been reported⁴ that iron ions play an important role in catalysing reactions that lead to the oxidative degradation of beer, thus justifying the need to carry out the iron determination in breweries for routine quality control.

The European Brewery Convention (EBC) describes two methods² for the determination of iron in beer, using flame atomic absorption spectrophotometry or UV/VIS spectrophotometry as detection methods. The former involves addition of known amounts of iron to the samples (method of addition of standards) and their subsequent introduction in the atomic absorption spectrophotometer. The measurement is difficult to perform as proteins and dissolved solids in beer cause burner clogging. To minimise this problem, a flow injection analysis (FIA) manifold was reported³ to introduce the beer solutions into the atomic absorption spectrometer. However, this system does not avoid the need to resort to the method of standard additions, and so spiking of beer samples before injection is always necessary.

The other EBC method for the iron determination in beer is a colorimetric procedure based on the formation of an orange-red complex produced by the reaction of iron (II) with 1,10-phenanthroline, after reduction of iron (III) to iron (II) by ascorbic acid. The overall analytical process is rather time-consuming and labour intensive because reaction completeness is required (implying heating the solutions for 15 minutes, subsequent cooling and measurement), and also because blank readings must be performed for each sample.

In order to automatically carry out this colorimetric determination, a flow injection system based on the merging zones technique¹ is described in this paper. In the FIA merging zones mode, sample and reagent plugs are simultaneously injected into two different channels, which subsequently merge in a confluence. This technique has been mainly used to minimise reagent consumption, as only the required amount for the reaction is injected in the system, instead of its continuous aspiration into the manifold. In this work, advantage was taken from the merging zones principle to create two distinct

measuring zones, corresponding to the sample blank absorbance and to the sum of the blank and analyte signals. This was accomplished by the asymmetric merging of a large sample volume and a small reagent plug.

EXPERIMENTAL

Reagents and Solutions

All chemicals were of analytical-reagent grade and double de-ionised water was used throughout.

The acetate buffer solution was prepared by dissolving 46 ml of fuming acetic acid and 65.6 g of sodium acetate trihydrate in about 1 litre of water; subsequently the pH was adjusted to 4.0 by dropwise addition of concentrated sulphuric acid, and finally the solution was diluted to 4 litres with water.

The solution used as carrier in the FIA system was prepared by dissolving 10.0 g of L(+)-ascorbic acid in acetate buffer and the volume (500 ml) completed with the buffer solution. This solution was prepared daily.

The colour reagent solution injected in the manifold was prepared by dissolving 0.15 g of 1,10-phenanthroline in the carrier solution and made up to 100 ml with the same solution. The one used for the reference method presented a 1,10-phenanthroline concentration of 3% (wt/vol) and was prepared in water. Both solutions were also prepared daily.

The iron (II) stock solution (200 ppm) was obtained by dissolving 1.404 g of ammonium iron (II) sulphate hexahydrate in a mixture of 20 ml of concentrated sulphuric acid and 50 ml of water and subsequently diluted to 1.00 litre with water. A stock solution containing 200 ppm of iron (III) was prepared as previously described⁵: 1.404 g of ammonium iron (II) sulphate hexahydrate were dissolved in a mixture of 20 ml of concentrated sulphuric acid and 50 ml of water; after dropwise addition of 0.2 mol/l potassium permanganate solution until a persistent pink colour was obtained, the solution was diluted to 1 litre.

Working standard solutions containing iron (II) or iron (III) in the range 0.25–3.00 ppm were prepared by rigorous dilution of each stock solution in the acetate buffer.

All the solutions, including beer samples, were de-gassed in an ultrasonic bath for three minutes prior to their introduction in the FIA system.

Instrumentation

In the flow injection system, a Gilson Minipuls 3 peristaltic pump was used for propelling the solutions. The solutions were injected in the manifold with two Rheodyne type 5020 six-port rotary injection valves, connected by a metallic axle so that the switching of the valves positions would occur at the same time, thus allowing the simultaneous injection of samples or standards and colour reagent. Omnifit Teflon tubing (0.8 mm i.d.) with Gilson end-fittings and connectors, and a home-made perspex confluence point linked the components of the manifold. A Unicam 8625 UV/VIS spectrophotometer (wavelength set at 512 nm), equipped with an Hellma 178.713 flow cell (8 μ l of optical volume) and connected to a Kipp & Zonnen BD 111 chart recorder, was used as detection system.

A Bandelin-Sonorex RK100 ultrasonic bath was used for solutions degassing.

Reference Method

The colorimetric method suggested by the European Brewery Convention² was used as reference procedure. To each standard and sample, solid ascorbic acid and colour reagent solution (1,10-phenanthroline) were added. The blank sample solutions were prepared the same way as samples except for the addition of 1,10-phenanthroline solution. All the solutions were heated at 60°C for 15 minutes in a Unitronic S.320-100 thermostatic bath and absorbance measurements were performed at 512 nm. After subtracting the corresponding absorbance blank measurement to the sample absorbance, the concentration was calculated from the previously established calibration plot.

RESULTS AND DISCUSSION

FIA Manifold Configuration

The iron content in beer usually lies in the working concentration range of the colorimetric methodology in which the FIA system is based, often close to the detection limit. Therefore, no substantial dilution of the beer samples can be performed in order to eliminate their intrinsic absorption at the selected wavelength. In these conditions, a manifold configuration was sought to carry out inside the flow tubes the colour development reaction and allow the measurement of the signal corresponding to the sample blank and the one corresponding to the blank plus the coloured complex. In order to achieve these requirements, a manifold (Fig. 1) designed to implement the asymmetric merging zones technique was constructed.

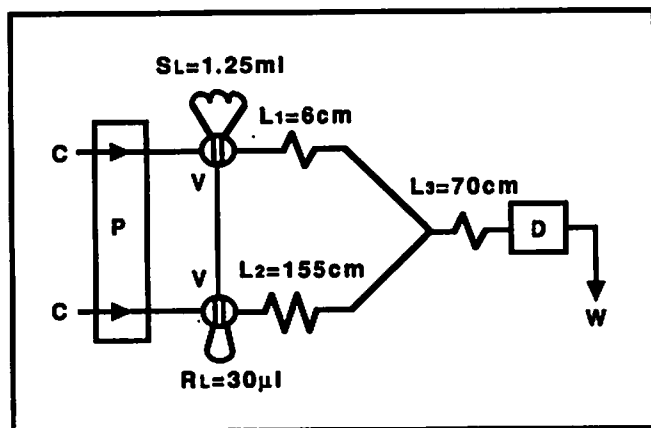


FIG. 1. FIA manifold used for the determination of iron in beer using the merging zones technique: C - carrier solution: 2% (w/vol) ascorbic acid in acetate buffer; D - detector (UV/VIS spectrophotometer); L_i - tubes length; P - peristaltic pump: flow rate of 2.3 ml/min; R_L - reagent loop: 0.15% (w/vol) 1,10-phenanthroline in the carrier solution; S_L - sample loop; V - injection valves; W - waste.

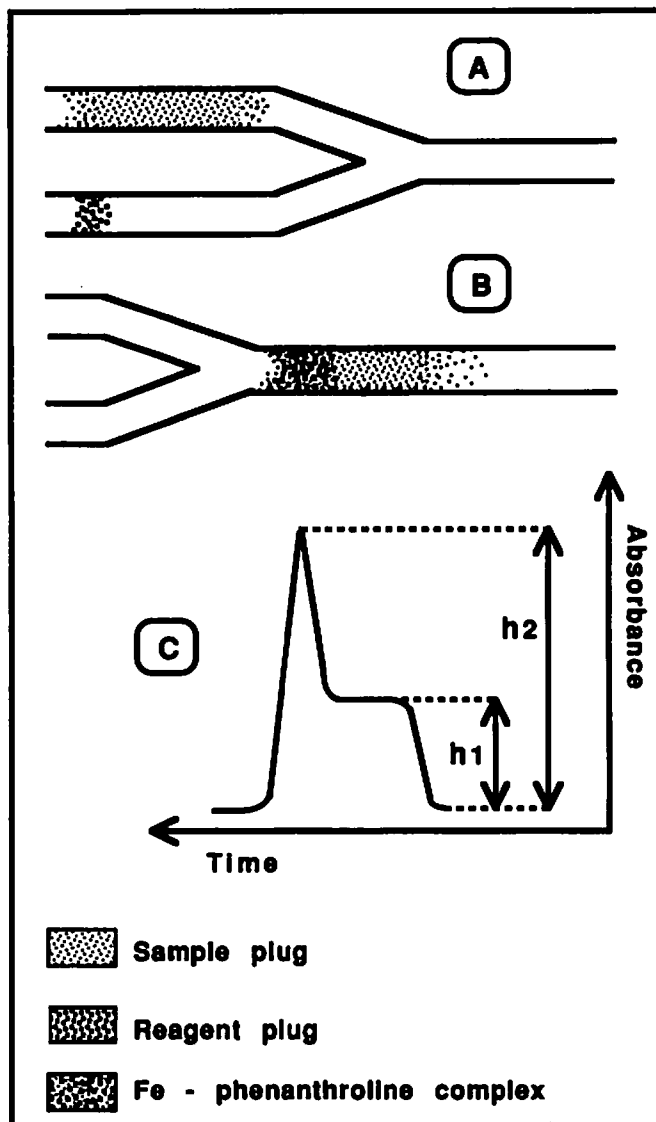


FIG. 2. Schematic representation of the asymmetric merging of the sample and reagent bolus inside the manifold, and the consequent analytical signal observed. (A) sample and reagent plug before the confluence point; (B) merging of the reagent plug in the tail of the sample bolus; (C) corresponding recorder output; h_1 - height corresponding to blank absorbance; h_2 - height corresponding to the sum of the absorbance of the blank and of the coloured complex.

This system is based on the simultaneous injection into two separate channels of a large volume of sample and a small volume of colour reagent, which subsequently merge at the confluence point in different times. For this purpose, equal flow-rates and different tube lengths (L_1 and L_2) were selected so that the sample plug front could arrive at the merging point before the colour reagent (Fig. 2A).

As the L_2 tube is longer than L_1 , the reagent bolus takes longer to get to the confluence, thus merging to the last portion of the sample plug (Fig. 2B). The reaction occurs in this section with formation of the iron (II)-1,10-phenanthroline coloured complex. Using this strategy, two measuring zones are created, yielding the analytical signal depicted in Fig. 2C. When the sample plug front, mixed with the acetate buffer, arrives at the flow cell, the absorbance signal starts to increase up to a point where a plateau is reached. The height of this plateau (h_1) corresponds to the blank signal as in this zone there are no concentration gradients, and no contact with the reagent bolus. Afterwards, the last portion of the sample plug, where the formation of the iron (II)-1,10-phenanthroline coloured complex occurs, flows through the

cell. It yields a peak whose height corresponds to the coloured complex plus matrix blank signal (h_2). Consequently, the difference ($h_2 - h_1$) is proportional to the iron concentration in the beer sample. Alternatively, a much larger sample volume could have been used, thus allowing to obtain two blank plateaux, before and after the coloured complex peak. However, this strategy would drastically decrease the sampling-rate and would not improve the quality of the results, as one plateau is sufficient to assess the blank absorbance signal.

Optimisation of the Manifold

After a previous assessment for selecting approximate values for each parameter, optimisation tests were carried out by varying each parameter independently and setting the remainder. This procedure aimed to adjust the best conditions in terms of signal resolution, sensitivity (taken as the slope of the calibration curve), detection limit, reproducibility, sampling rate and reagent consumption.

The combined selection of the injected volumes (sample and reagent) and the length of the L_2 tube is critical to provide two clearly defined measuring zones in the sample plug. The reagent volume was set to the minimum value (30 μ l) allowed by the injection valve. The sample volume was studied in the range 0.38–1.35 ml and the L_2 length from 30 to 175 cm. It was found that to obtain a distinct blank plateau, a sample volume of 1.25 ml and a L_2 tube of 155 cm should be used. Lower sample volumes increased the dispersion of the sample plug, and so it was not possible to achieve a zone in which there were no sample concentration gradients along the plug length (blank signal). Longer L_2 lengths were not used as the reagent plug would be more dispersed at the confluence point arrival, and so it would not be possible to make the reaction to occur in only one small part of the plug tail. Longer sample loops and L_2 lengths were not used as they would cause the signal broadening and consume much more sample, with no further improvement in the quality of the results.

Flow rates were studied within the range of 1.7–2.9 ml/min. To obtain a good signal resolution, a flow rate of at least 2.3 ml/min should be used. For smaller values, large fluctuations of the analytical signal were observed, probably due to an insufficient mixing of the merging solutions at the confluence point. Higher flow rates slightly improved the signal resolution but the sensitivity decreased about 3% for a 3 ppm concentration. So, a flow rate of 2.3 ml/min was selected as a compromise between signal resolution and sensitivity.

A length of 70 cm was set for the L_3 tube in order to obtain a distinct plateau and to allow the reaction to occur in a considerable extent so that a good sensitivity could be achieved. It also provides sufficient mixing of the solutions in order to get a stable baseline. The tube was helically coiled to improve radial mixing and minimise the dispersion of the sample plug. A further increase of the coil length slightly increased the sensitivity (2%), but it didn't allow the attainment of a distinct plateau due to increased plug dispersion. Additionally there was a broadening of the analytical signal decreasing significantly the sampling rate.

The ascorbic acid concentration was studied in order to find the minimum concentration capable of totally reducing iron (III) to iron (II). The ascorbic acid content in the carrier solution was varied between 0.28 and 2.5% (wt/vol). A minimum of 2% (wt/vol) ascorbic acid was necessary to obtain total reduction in the required concentration range (verified by obtaining equal absorbance values for injection of 3 ppm iron (II) and iron (III) solutions). An ascorbic acid concentration of 2.5% (wt/vol) caused baseline instability as well as a decrease in the reproducibility of the determinations, possibly due to a poorer mixing between this solution and the beer sample along the L_3 tube. Besides, a decrease in the sensitivity (about 4%) was observed.

The 1,10-phenanthroline content was changed within the range of 0.05–0.2% (wt/vol). The sensitivity increased up to 0.15% (wt/vol) and so this concentration was selected.

The pH of the acetate buffer was set to 4.0 units, as a compromise between reduction completeness and sensitivity. An increase of this value (pH = 4.2) caused incomplete reduction of iron (III) to iron (II). On the other hand, for a pH below 4 (pH = 3.7), a 6% decrease of the analytical signal was observed.

The sampling rate achieved with this set-up is of approximately 60 samples per hour.

The detection limit was 0.032 ppm, calculated as the concentration corresponding to three times the standard deviation of the plateau (blank signal) noise. This value is acceptable as iron can only be tasted³ at 0.050 ppm.

The developed flow injection methodology is very stable. Successive calibration curves carried out during each day, and also from day to day, presented similar values of slope and intercept. Therefore, no periodic calibrations between batches of sample determinations were necessary. Additionally, no baseline drift was observed, meaning that eventual problems of coloured complex retention on the flow cell walls did not occur.

Analysis of beer samples

In order to assess the quality of the results obtained with the developed methodology, determinations of total iron for a set of 22 beer samples (bottled and canned) were carried out by FIA and by the reference method. Seven samples presented concentrations below the detection limit. The results obtained for the other 15 samples are presented in Table I.

A relation of the type $FIA = C_0 + S \times (\text{Reference procedure})$ was established and the results were: $C_0 = 0.001$ ppm and $S = 0.984$, with a correlation coefficient of 0.9990. These results show a good agreement between the two methodologies, as can be perceived by a slope close to unit and an intercept near zero. The maximum relative deviation observed was 4.0%.

To assess the reproducibility of the FIA procedure, the relative standard deviation was calculated for 10 consecutive determinations of four samples, being the results (concentrations indicated between brackets): 6.6% (0.050 ppm), 5.4% (0.075 ppm), 1.9% (0.200 ppm) and 1.9% (0.335 ppm).

CONCLUSIONS

The developed FIA system for the determination of iron in beer is an advantageous alternative to the reference methods, as it provides good quality results with sampling rates of up to 60 determinations per hour.

The devised strategy for sequential measurement of blank and coloured complex plus blank signals in a single sample injection was well succeeded and presents great possibilities of

TABLE I. Comparison of the results obtained in the determination of iron in beer using the developed FIA methodology and the reference method

Sample	FIA (ppm)	Reference method (ppm)	Relative deviation (%)
1	0.495	0.512	-3.3
2	0.074	0.077	-3.9
3	0.242	0.252	-4.0
4	0.101	0.103	-1.9
5	0.051	0.053	-3.8
6	0.103	0.100	+3.0
7	0.102	0.103	-0.97
8	0.039	0.038	+2.6
9	0.128	0.126	+1.6
10	0.086	0.084	+2.4
11	0.106	0.108	-1.9
12	0.120	0.122	-1.6
13	0.115	0.116	-0.86
14	0.160	0.164	-2.4
15	0.389	0.376	+3.5

application to other analytical situations (other analytes and in different matrices) in which the same problem occurs.

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