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Exploiting guava leaf extract as an alternative natural reagent for flow injection determination of iron

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Abstract

Guava leaf extract is utilized as an alternative natural reagent for quantification of iron. The flow injection technique enables the use of the extract in acetate buffer solution without the need of further purification. Some properties of the extract such as its stability and ability to form a colored complex with iron were studied. The proposed system is an environmentally friendly method for determination of iron with less toxic chemical wastes.

Keywords: Guava leaf extract; Natural reagent; Flow injection; Iron

1. Introduction

Many scientific studies involve the use of chemicals that cause contamination in the environment. For example, water studies to determine the amount of different ions in natural water may need chemical reagents that are not normally present in the environment. If wastes from such studies are not properly stored or treated, these foreign substances could contaminate soil or water sources. Gaining popularity, the research area known as green chemistry aims to explore the use of alternative reagents or alternative synthetic methods that minimize the use of toxic chemicals.

In this study, a natural guava (*Psidium guajava* L., Myrtaceae family) leaf extract has been investigated as an alternative natural indicator for iron quantification by the flow injection technique. Reports have indicated that guava leaves contain chemicals that are useful for whitening [1], anti-pigmentation [2], anti-bacterial treatment [3], leather tanning [4], and diet food and beverage components [5], and in the prevention of diabetes. [6] In Thailand, there has been some local usage of guava leaves as an indicator of the presence of iron in ground water. A change in color of water to a darker color helps local villagers in making pre-treatment decisions such as adding alum to precipitate the iron. This use has never been published and no scientific explanation is available.

To determine the concentration of iron by a FI system, a water sample is introduced into a closed flow system and mixed with reagent that can form a colored complex, which can be detected by a colorimeter. Examples of some reagents [7] that have been used in determination of iron include 1,10-phenanthroline, 2,4,6-tri(2′-pyridyl)-1,3,5-triazine, azo-dye derivatives, mercaptoquinoline, thiocyanate and salicylate. [8] In this study, we investigate the use of guava leaf extract as an alternative reagent for quantification of iron using a FI system. Although the active chemical species in the extract is unknown and the extract may not be pure, the use of this guava leaf extract to determine the amount of iron in solution is made possible by the FI system. One of the features of FI systems is that they can be employed with reagents, which are not necessarily pure. This is because in the flow injection technique, an analyte to be determined goes through the

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system in exactly the same condition as that of the standard. [9]

The use of a natural and easily available material in place of a toxic and expensive reagent should be beneficial from both environmental and economic aspects. In this report, the authors conduct preliminary studies of the potential use of guava leaf extract as an alternative reagent to quantify iron using a simple FI system. Some properties of the extract, including its stability, ability to form a complex with iron (II) and (III), spectral properties, suitable extraction medium, and its potential to be used for quantification of iron in water samples have been investigated.

2. Experimental

2.1. Reagents

A 100 mL volume of 1000 ppm stock standard Fe (II) solution was prepared by dissolving 0.7022 g of $(NH_4)_2Fe(SO_4)_2{\cdot}6H_2O$ (Carlo Erba) in water containing 1% (v/v) concentrated H_2SO_4 (BDH). A 1000 ppm stock standard Fe (III) solution was prepared similarly but with 0.7240 g of $Fe(NO_3)_3{\cdot}9H_2O$ (Carlo Erba). A 250 mL volume of 0.2 M sodium acetate buffer pH 4.8 solution was prepared by dissolving 6.87 g $CH_3COONa{\cdot}3H_2O$ (Merck) in water containing 1.16% (v/v) acetic acid (Carlo Erba). HCl solution of the same pH 4.8 was prepared by diluting concentrated HCl (Merck) with deionized water to the desired pH. Ascorbic acid (Merck) was prepared at the concentration of 2% (w/v). Interference studies were done by spiking some cations, Ca^{2+} ($CaCl_2$, Merck), Mg^{2+} ($Mg(NO_3)$, Merck), Co^{2+} ($CoCl_2$, BDH), Ni^{2+} ($NiCl_2$, BDH) and Cr^{3+} ($CrCl_3$, BDH) into Fe (III) 10 ppm solutions at the final concentration of 1, 5 and 10 ppm (10:1, 2:1 and 1:1 concentration ratios of Fe (III):interference ion).

2.2. Guava leaf extraction

Fresh guava leaves of 10.0 g were ground in 150 mL water (pH 7.0), diluted HCl (pH 4.8) or acetate buffer (pH 4.8) with a blending machine for 5 min. Then the suspension was filtered through filter paper no. 1 (Whatman) and the filtrate was kept at room temperature for further use. The extract was prepared daily.

2.3. Study of the conditions for guava leaf extract–iron complex formation

Both Fe (II) and Fe (III) solutions were prepared in three sets; in water, in HCl solution and in acetate buffer. Two iron concentrations of 10 and 100 ppm were used. Guava leaf extracts in the three media were mixed with iron solutions prepared in the matching medium at the ratio of 1:1 (v/v) (10 mL extract:10 mL Fe solution). Guava leaf extract without iron solution was used as a blank solution. Mixtures of guava leaf extract and iron solution were scanned for absorption spectra, measured against each medium, in the visible region 400–700 nm using a UV–vis spectrophotometer (Lambda 25, Perkin-Elmer).

2.4. Study of stability of guava leaf extract

After selection of the suitable medium for the extract from the previous experiment, guava leaf extract was prepared in that medium and allowed to stand in an uncovered beaker for 1, 2, 3, and 4 h. Mixtures of the extract and Fe (II and III) 10 and 100 ppm solutions were prepared as previously described. Mixtures were then scanned for absorption spectra to investigate absorption characteristics.

2.5. Apparatus

Peristaltic pumps (Ismatec) were used for reagent drawing. Pump tubing was Tygon and all the other tubing was PTFE (1/16 in. i.d.). Samples were introduced via a six-port injection valve (FIAlab). A Spectronic 21 spectrophotometer (Spectronic Instruments) with a flow through cell (80 μL, Hellma, Germany) was set at 570 nm and connected to a computer to record the FIA-grams using Stamp (Parallax, USA) and Microsoft Excel (Microsoft Corp., USA) software programs.

2.6. Manifold designs

2.6.1. Normal FI system

The simple one line normal FI manifold, where a sample is injected into the stream of reagent, is shown in Fig. 1(a).

2.6.2. Reverse FI system

In order to save reagent, the reverse FI manifold with two lines was tried where the reagent was injected into the stream of buffer that was later mixed with the stream of sample solution (Fig. 1(b)).

2.6.3. Column FI system

To shorten the extraction process, a column packed with ground guava leaves was connected in-line with the FI system, see Fig. 1(c). This column is made from a small Perspex glass tube with 3 mm i.d. and 2.5 cm length. Both ends were plugged with Teflon wool.

All three types of manifolds have an injection loop of 60 μL.

3. Results and discussion

3.1. Optimum condition of guava leaf extract-iron complex formation

The aim of this study was to find a suitable acidic solution for guava leaf extract–iron complex formation. Buffers

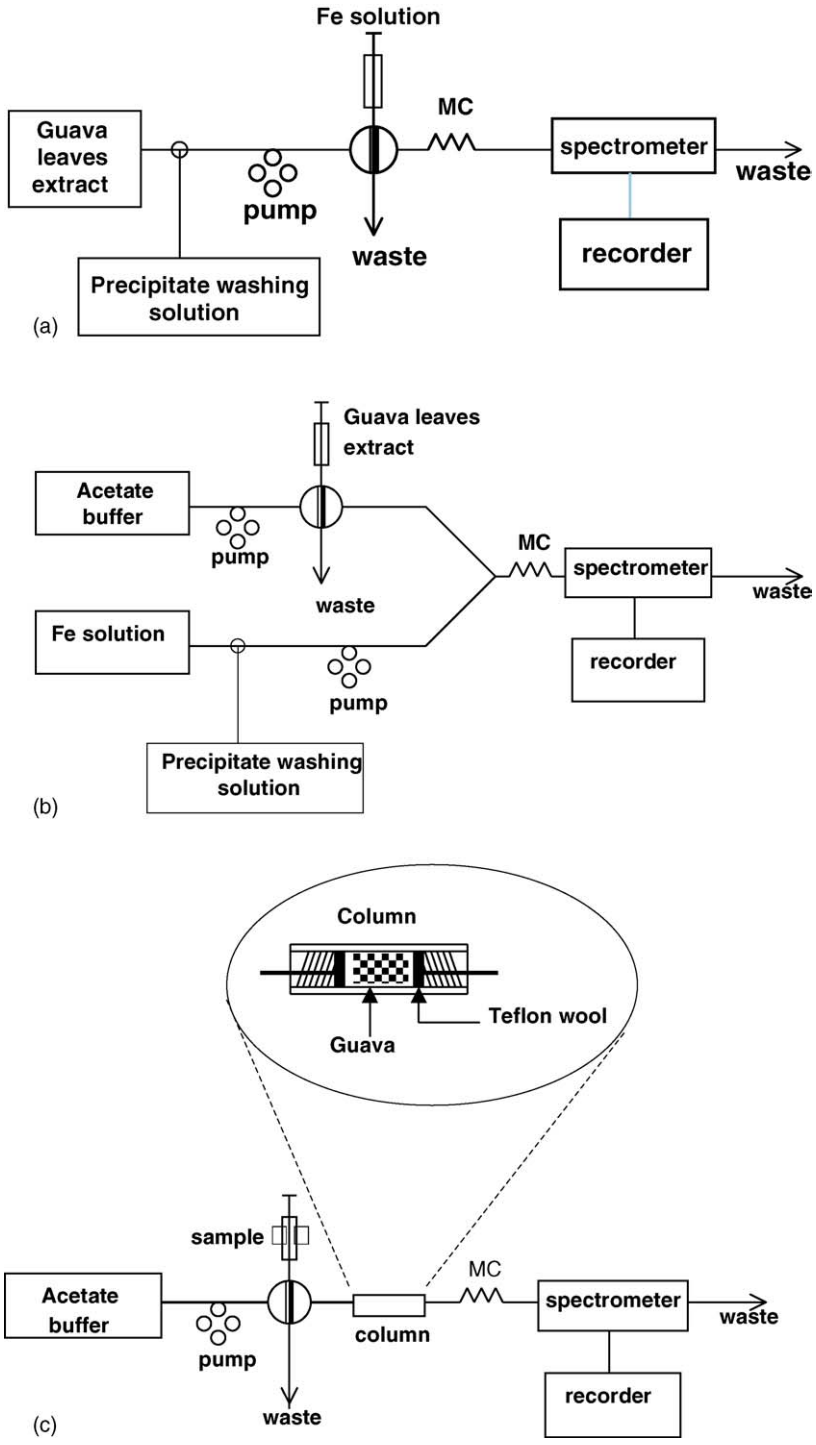

Fig. 1. (a) A normal FI manifold, (b) a reverse FI manifold and (c) a FI manifold with packed column.

of acidic pHs that can be prepared with easily available chemicals that would not interfere with the iron determination are rare. A suitable solution was sodium acetate buffer pH 4.8. Ammonium acetate was avoided because ammonium may form a complex with the metal ion of interest. To ensure the necessity of buffer solution in this study, water pH 7.0 and HCl diluted to pH 4.8 were also used as media for extraction to compare the results.

Fig. 2(a) and (b) represents the absorption spectra of guava leaf extract-iron complexes in water and buffer solution, respectively. In water (Fig. 2 (a)), no significant iron complex absorption is shown in the region of 450–700 nm, and except for high Fe (III) concentration, it is suppressed. This is likely because water cannot extract active species from the guava leaf. It could also be possible that there was no formation of iron complex with the active species or the

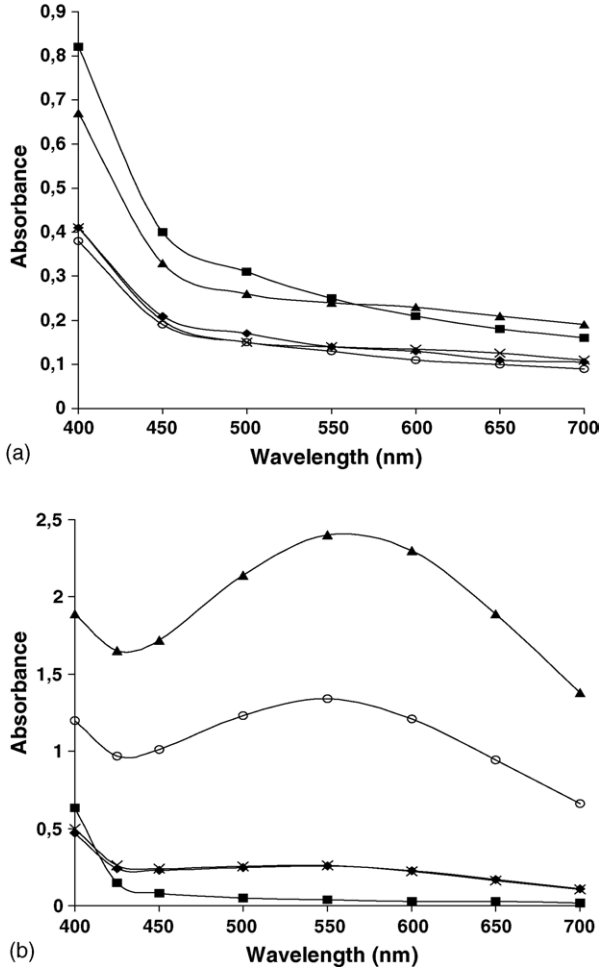

Fig. 2. (a) The absorbance spectra of guava leaf extract–iron complex in water, measured against water. (b) The absorbance spectra of guava leaf extract–iron complex in acetate buffer solution, measured against acetate buffer: (■) guava leaf extract; (▲) guava leaf extract with Fe (III) 100 ppm; (♦) guava leaf extract with Fe (III) 10 ppm; (◯) guava leaf extract with Fe (II) 100 ppm and (×) guava leaf extract with Fe (II) 10 ppm.

complex may not be stable in water. The exact cause of the results still needs further investigation, and the reason for the decreased absorbance is unknown. In acetate buffer solution pH 4.8 (Fig. 2 (b), both Fe (II) and (III) can form complexes with a chemical or chemicals in the guava leaf extract. Both Fe (II) and (III) complexes show maximum absorption at 570 ± 20 nm. The blank solution, guava leaf extract, did not show any significant absorption in the visible region. In a medium of diluted HCl pH 4.8, maximum absorption at 570 ± 20 nm was observed (not shown) only at high Fe (III) concentration but at much lower value as compared to the one in acetate buffer of the same pH. This indicates that guava leaf extract–iron complexes can only be formed at a suitable pH and that the use of buffer solution to control pH is necessary.

It has been observed that at low concentration of iron (10 ppm), the absorbances of Fe (II) and Fe (III) complexes were about the same while at high concentration of iron (100 ppm), the absorbance of the Fe (III) complex was higher

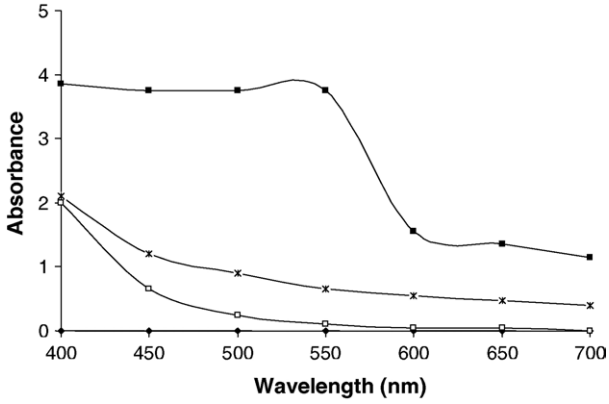

Fig. 3. The absorbance spectra studies for the reducing effect of ascorbic acid in. guava leaf extract: (♦) acetate buffer; (×) guava leaf extract and phenanthroline mixture; (□) Fe (III) 100 ppm with 0.3% (w/v) phenanthroline and (■) mixture of Fe (III) 100 ppm with 0.3% w/v phenanthroline and guava leaf extract.

than that of the Fe (II) complex. This might be explained as follows; at low concentration of iron, some chemicals (e.g., tannin) naturally present in guava leaves may reduce Fe (III) to Fe (II) and form guava leaf extract–Fe (II) complex. At high concentration of iron, there may be insufficient amount of reducing agents naturally present in the guava leaf extract to reduce all Fe (III). Therefore complexes formed were both Fe (II) and Fe (III) complexes. To test this hypothesis that guava leaf has a natural reducing effect, an additional experiment was carried out. Phenanthroline does not form a reddish complex with Fe (III) but does so with Fe (II). [10] Therefore, no significant absorbance should be observed at about 500 ± 20 nm when Fe (III) solution was mixed with phenanthroline. However, if some natural occurring substances in the guava leaf can reduce Fe (III) to Fe (II), the Fe (II)–phenanthroline complex should be formed when adding guava leaf extract into the mixture of Fe (III)–phenanthroline and the reddish colored complex with the absorption maxima at 500 ± 20 nm should appear. The absorbance at 570 ± 20 nm from the guava leaf extract–Fe (III) complex from excess Fe (III) that has not been reduced should also be observed.

In this experiment 3% (w/v) 1,10-phenanthroline, 100 ppm Fe (III) solution and guava leaf extract were mixed 1:1:1 by volume. The results shown in Fig. 3, comparing absorption spectra of 100 ppm Fe (III)–phenanthroline solution and 100 ppm Fe (III)–phenanthroline–guava leaf extract solution, confirm that guava leaf has a natural reducing effect. The mixture of Fe (III)–phenanthroline shows only slight absorbance at 500 nm and lower wavelengths. Guava leaf extract–phenanthroline mixture in the absence of Fe (III) was slightly turbid with white precipitate and might block the light, showing the result as if the solution had absorbed the light at 500 nm and lower wavelengths. The increase of absorbance at that region, when adding guava leaf extract into the Fe (III)–phenanthroline solution, was due to the reducing effect of guava leaf extract that changed Fe (III) to Fe

(II) which formed a reddish complex with phenanthroline that was observed with bare eyes. The comparable graph for 100 ppm Fe (III) with guava extract in acetate buffer is shown in Fig. 2(b) with the absorbance being about one half that when phenanthroline is added.

The results from both experiments in this study also indicate that the guava leaf extract–Fe (III) complex has higher absorbance (higher molar absorptivity) than that of the Fe (II) complex at 570 nm.

3.2. Stability of the guava leaf extract indicator

Guava leaf extract in acetate buffer was set-aside for 1, 2, 3, and 4 h before mixing with Fe (II) and Fe (III) solutions. Absorbances at 570 nm were recorded every hour. It was found that there were no significant changes in absorbance with time. This indicates that guava leaf extract is stable for at least 4 h, which is sufficient for a continuous experiment using FI.

3.3. Comparison of different manifold designs

In order to reduce the consumption of guava leaf extract, a reverse two line FI manifold was developed. Guava leaf extract was injected into the acetate buffer stream before merging with Fe solution.

In both the normal and reverse FI systems, purple precipitate adhering to the tubing and the detection cell was observed. Such a precipitate can cause unreliable and faulty results. Therefore, the procedure was modified by having a precipitate washing step with ascorbic acid. Fig. 4 shows the characteristics of the signals obtained from the reverse FI system which are similar to those obtained from the normal FI system. Signals from the Spectronic 21 are recorded as transmittance. Peaks between each run, which are constant throughout the experiment, are due to the Schlieren effect that occurs from injection of ascorbic acid for line washing.

Apart from minimizing the consumption of guava leaf extract, the reverse FI also showed better sensitivity than the normal FI system. This is possibly due to higher amount and less dilution of the Fe solution introduced by pumping as compared to injection. In terms of precision, the results obtained from the reverse FI system showed a slightly higher relative standard deviation (3–10%) as compared to the normal FI system (1–7%), for the Fe (III) concentration range of 1–10 ppm, which could be due to the high diffusion of reagent into the Fe solution stream thereby causing inconsistency of the complex formation.

Another attempt was to reduce the process of guava leaf extraction by packing the ground guava leaves in a column and placing it in the FI manifold. It was found that the guava leaves packed column-FI system could not be used effectively. The chemical from the leaves only came out at the edge of each piece, yielding a very small amount of color reagent in the flow stream. In addition, the active chemical was used up quickly, resulting in the need to change the column for every analysis, which is not practical.

3.4. Standard calibration graph and figures of merit

Calibration graphs for Fe (III) were prepared using both normal and reverse FI systems. Only calibration graphs of Fe (III) were constructed because species found in real samples such as water samples should be in the form of Fe (III).

Calibration graphs were obtained from standard Fe (III) solutions of 1, 2, 4, 6, 8 and 10 ppm. Both the normal FI and the reverse FI systems offer linear calibration graphs, represented as $Y = 11.09X + 4.06$, $R^2 = 0.9982$ and $Y = 24.93X - 20.05$, $R^2 = 0.9997$, respectively (where Y is peak height in mV and X is concentration of iron in ppm). The normal FI system showed higher blank signal due to the color of the guava leaf extract in the carrier line. In the reverse FI system, guava leaf extract was injected into the stream of colorless Fe solution and, therefore, the blank signal was low.

Even though the relative standard deviation is higher (3–10% R.S.D. in the Fe (III) working range of 1–10 ppm), the reverse FI system offers much better sensitivity than the normal FI system does. The practical lowest detectable concentration of Fe (III) obtained from the reverse FI system (estimated based on 3σ of blank signals [11]) was 1 ppm. Sample throughput was 20 injections/h. A sample with simple matrices, tap water, was spiked with Fe (III) standard solution at 2, 5, 7 and 30 ppm. Percent recoveries were found to be 95–97%.

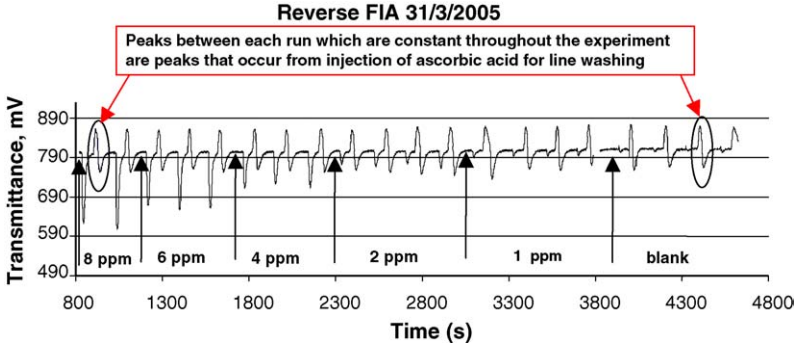

Fig. 4. Characteristics of the signals obtained from the reverse FIA system.

3.5. Interferences

It was found that the proposed system for iron (III) determination could tolerate Ca^{2+}, Mg^{2+}, Co^{2+}, Ni^{2+} and Cr^{3+} at least up to 1:1 concentration ratio (10 ppm Fe:10 ppm interference ion). Additional studies are required for general applicability.

4. Conclusion and future works

The results obtained from these preliminary studies indicate the potential use of guava leaf extract as an alternative reagent for quantification of iron in samples with simple matrices. The detection limit may possibly be improved by increasing the concentration of the guava leaf extract. No sample treatment is needed for iron concentrations at or below 30 ppm since Fe (III) and Fe (II) absorbances are similar, probably due to complete reduction of the Fe (III) by some chemicals naturally present in the guava leaves. Higher concentrations might be measured by adding a reducing agent such as hydroxylamine to convert the Fe (III) to Fe (II). Further studies are needed. Prior to applying the guava leaf extract in studies of samples containing complicated matrices, further studies should be made. Extensive interference studies, especially from other cations, should be evaluated. Even if the extract proves non-selective, it would serve for post-column detection of iron in separation or speciation studies. Although impure reagents can be used in a FI system, it would be interesting to find out what the exact chemical in guava leaf is that forms complexes with iron so that leaves of other plant species with similar chemical contents can be explored. Comparison of extracts from young and old leaves together with an additional separation technique such as HPLC or GC and NMR may help to identify the active chemical species. The use of dry leaves rather than fresh ones and the method of stabilizing the extract to be kept for future use should also be investigated. This will extend the use of the extract when guava leaf is not in season.

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