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SHORT REPORT

FLAME AAS DETERMINATION OF Fe IN SERUM WITH DISCRETE SAMPLING

JUWADEE SHIOWATANA

Department of Chemistry, Faculty of Science Mahidol University Rama VI Road, Bangkok 10400, Thailand.

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ABSTRACT

A direct rapid method for flame AAS determination of iron in serum using discrete sample nebulization is developed. Effect of sample size and uptake rate of nebulizer on peak signals is studied. Either peak height or peak area can be employed in the measurement. Sample size can be adjusted accordingly with uptake rate depending on required sensitivity and sample limitation. The result obtained from the proposed method is superior to the method using pretreatment of sample prior to AAS measurement in its speed, sensitivity and precision.

INTRODUCTION

One of the shortcomings of the flame AAS with continuous pneumatic nebulization is the low efficiency and large sample requirement. This is particularly of importance when analyses of biological samples are to be performed. Although the use of graphite furnace has been found to overcome such shortcomings, the graphite furnace system may not always be available due to their higher cost, greater time per analysis and need more skill of operator. For this reason, in the analyses which the sensitivity of the flame system permits, it is often of the first choice.

The use of discrete sample nebulization has been introduced to overcome the problem of large sample requirement. Not only does it reduce the sample volume required, it can also solve the problem of clogging of nebulizer and burner due to high salt content of the samples. The use of discrete sample nebulization was thoroughly reviewed by Cresser.[1]

In this work, the method of discrete sample nebulization is utilized in the analysis of blood serum. A simple direct method for determination of iron in blood serum is presented. When the conventional continuous nebulization method is used, the sample volume required is usually not less than 1 mL. Although the graphite furnace method can be employed with a very small sample volume, the amount of iron present in normal blood serum is adequate for analysis to be performed by the flame system, it is therefore preferred. Several

reports[2-4] showed difficulties when using the flame system with samples containing high solid matter. Prior separation of protein was often suggested for flame AAS determination of metal ions in serum.

This work suggests a simple direct method without pretreatment for the analysis of iron in serum. Other elements can also be analysed using this method.

Instrumentation

An IL 457 AA/AE spectrophotometer equipped with a background corrector is used. A chart recorder is used to follow all signals except for the time-resolved signals in Fig 2.

Reagents

A commercial standard solution of 1000 ppm iron (Koch-Light Laboratory Ltd.) is used to prepare a diluted solution when needed. Other reagents are of analytical grade.

Procedure

Direct method

The sample volume (150 μL or specified otherwise) was pipetted to a small sample vial without any treatment. It was then totally aspirated for AA measurement.

Method with deproteinization

The sample volume of 500 μL was mixed with equal volume of 2M trichloroacetic acid to precipitate the protein. It was then centrifuged and the clear solution was analysed as in the direct method.

Effect of sample size and uptake rate on AA signal

When the discrete sample nebulization is employed with a constant uptake rate, it is important to select the optimum sample size to obtain a compromised performance between the precision of analytical result and the sample volume requirement. For example, the use of larger volume will increase the signal peak height to a steady state and precision is improved.

The effect of sample size on peak height and peak area was studied. The results were shown in Fig.1 with error bars included. It is evident that with peak height measurement, a minimum volume of 150 mL is required to obtain a steady state signal (which is the same peak height as in continuous nebulization). The relative standard deviation is improved at larger volumes. In the peak area mode, not only the precision is comparable with the peak height at higher volume, it is superior at small sample size. The plot between peak area and sample size is linear whereas that of the peak height curved. From the above advantages, the peak area seems to be more attractive than the height method although the latter has usually been performed in earlier reports.

The above evidence proved that peak height should be used only when a sample volume is large enough to obtain a steady state signal. When peak area is used, the sample volume can be selected independently according to the concentration of the analyte because the area of the signal obtained is related to the absolute amount of analyte, not the concentration.

Another advantage of the area measurement is that it is not necessary to dilute or to concentrate the sample to suit the calibration curve prepared. The linearity of the relationship shown in Fig.1 made it possible to vary the sample volume introduced as desired. This was confirmed by introducing same amount of sample with varying sample volumes and the area of the signal are compared.

Fig.2 showed AA traces recorded using a chart recorder as compared to the time-resolved traces from a storage oscilloscope. The volume required to obtain a steady state peak height was 100 μL when a storage oscilloscope was used to record the signal whereas the slow recorder system needed at least 150 μL. This is because of the smaller time constant of the former system.

Signal peak height, uptake rate and sample volume

Most reports on discrete sample nebulization have employed peak height for the measurement of signals obtained. For an identical sample, peak height is dependent on sample volume and uptake rate used. Cresser (1) reported 50 μL as minimum sample to give equivalent signal as in continuous nebulization when a storage oscilloscope was used and more than 90 μL was required with a normal chart recorder. In fact, the minimum value is dependent on the sample uptake rate apart from the time constant of the detection and recording system. Fig.3 demonstrates the effect of uptake rate on the peak appearance.

For a small sample volume to be applicable with peak height reaching its steady state value, the uptake rate can be reduced with the sacrifice of decreased sensitivity. Fig.3 shows the effect of uptake rate on the volume required to reach the steady state value.

It can be concluded that both peak height and peak area are comparably applicable when discrete sample nebulization is employed. The reason for not using peak area in all the previous work was reported as the poor reproducibility of the latter. In this work, it shows the advantage of applicable to varying sample size by using the same calibration. In addition, it offers higher precision when signals are obtained below the steady state.

Standard Addition method for iron in serum

The standard addition method was applied to the determination of iron in serum with both peak area and peak height modes. The results comparing with aqueous calibration were presented in Fig.4.

It appears that the slope of standard addition calibration of serum is slightly smaller than the aqueous calibration for both methods. The origin of lower recovery of iron from serum is thought to be due to the lower nebulization efficiency according to the higher viscosity of the sample. To prove this, standard calibration of iron was performed in varying concentration of albumin. The slope of the standard calibration curve was found to be smaller at increasing albumin concentration as predicted (Fig.5). However, various serum samples exhibited identical slope of standard addition curve. Moreover, the concentration of iron in the same serum sample determined by height and area methods were no significant difference. Therefore, it could be recommended that determination be performed using standard calibration of iron in artificial serum.

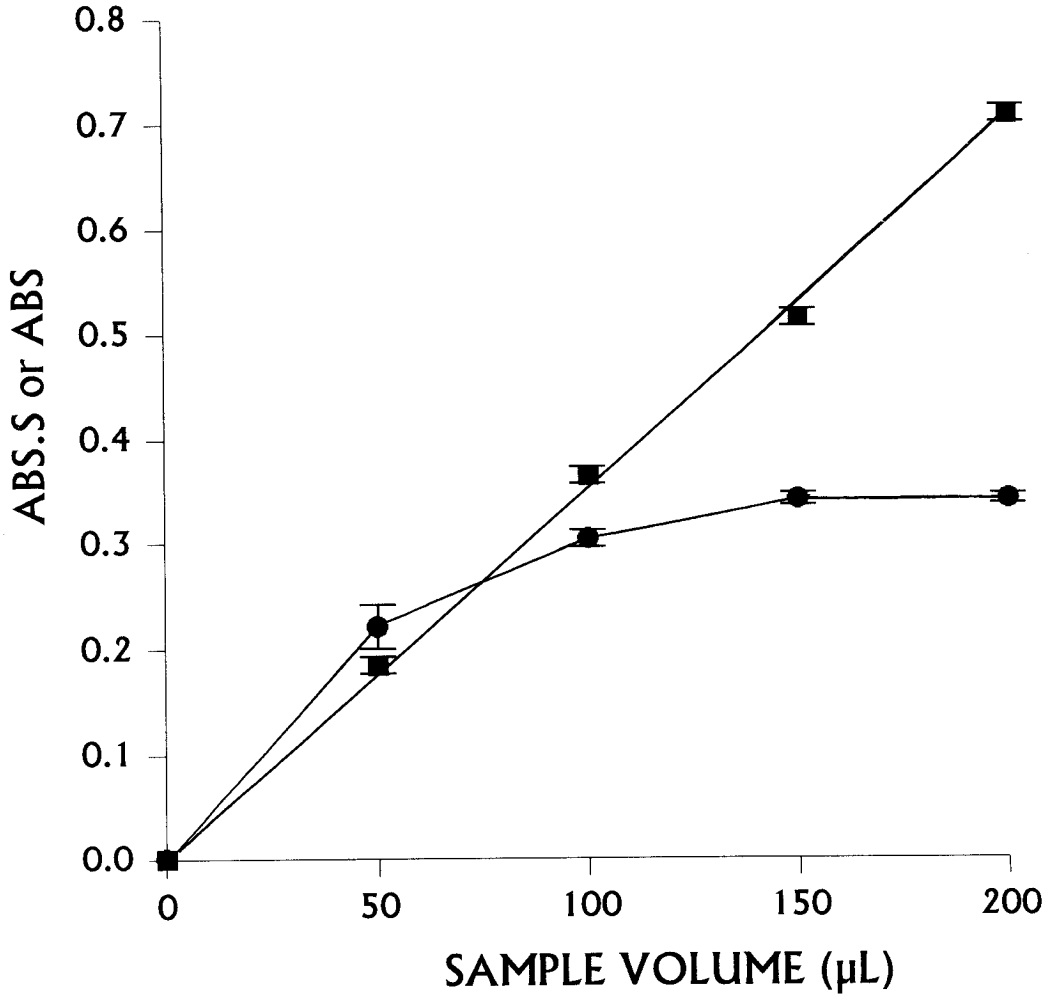

Fig.1. Effect of sample volume on peak height (●) and peak-area (■) for 5.0 ppm Fe. Standard deviations calculated from 10 replicate determinations were also presented using error bars. Uptake rate was 5 mL/min.

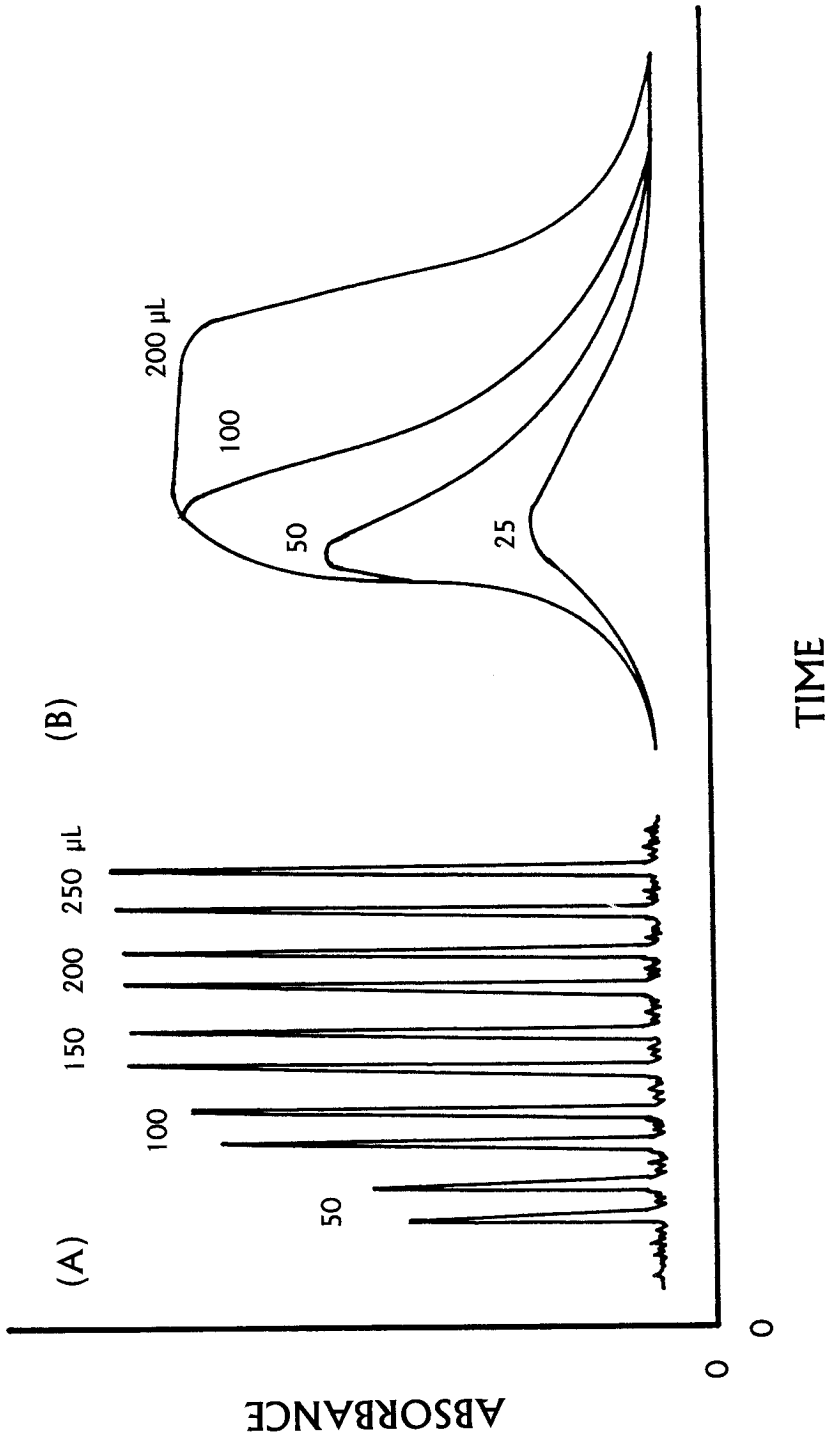

Fig.2. Recorder tracings of 5.0 ppm Fe obtained using a chart recorder (A) and a storage oscilloscope (B).

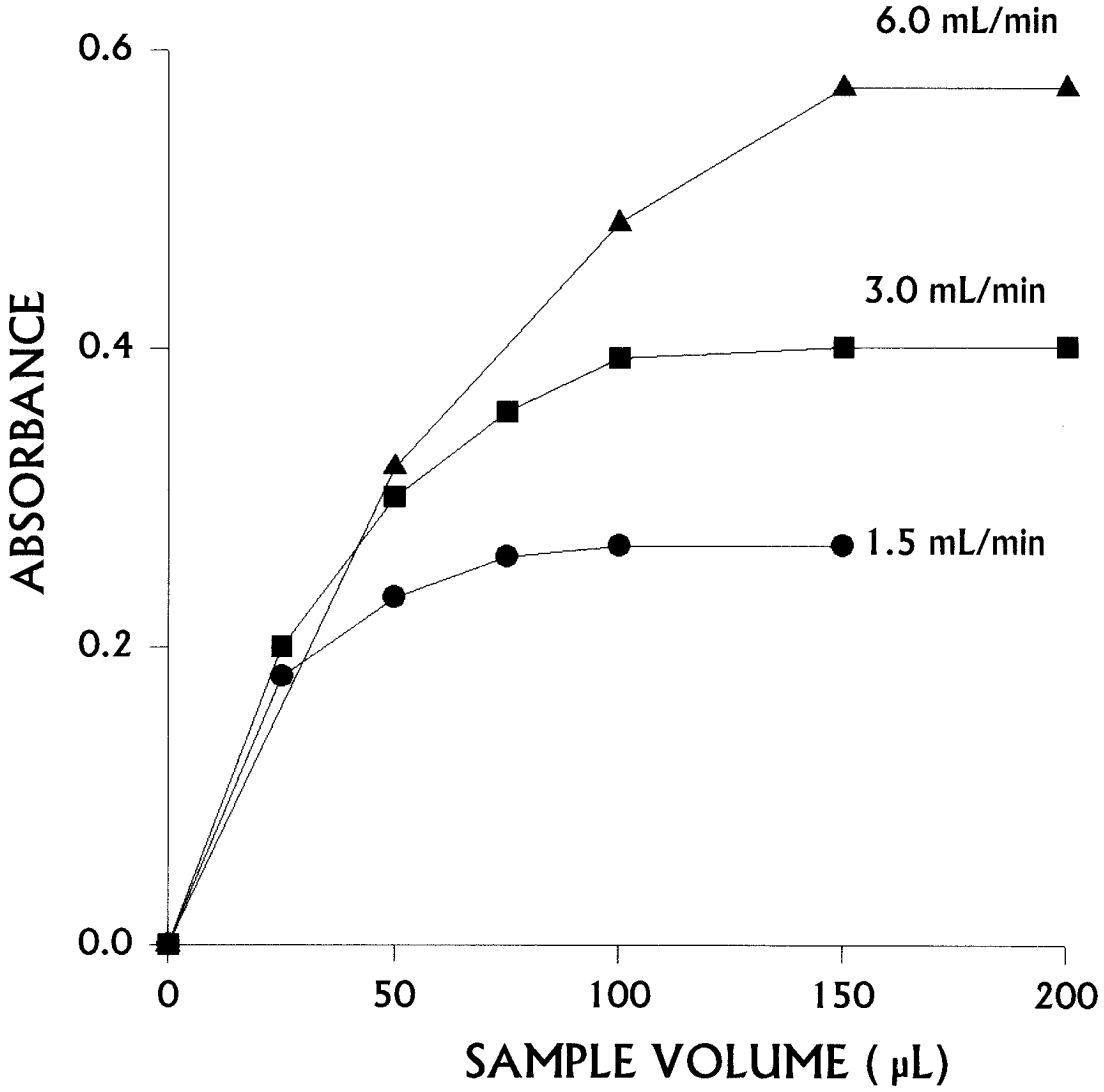

Fig.3. Effect of uptake rate and sample volume on absorbance peak height.

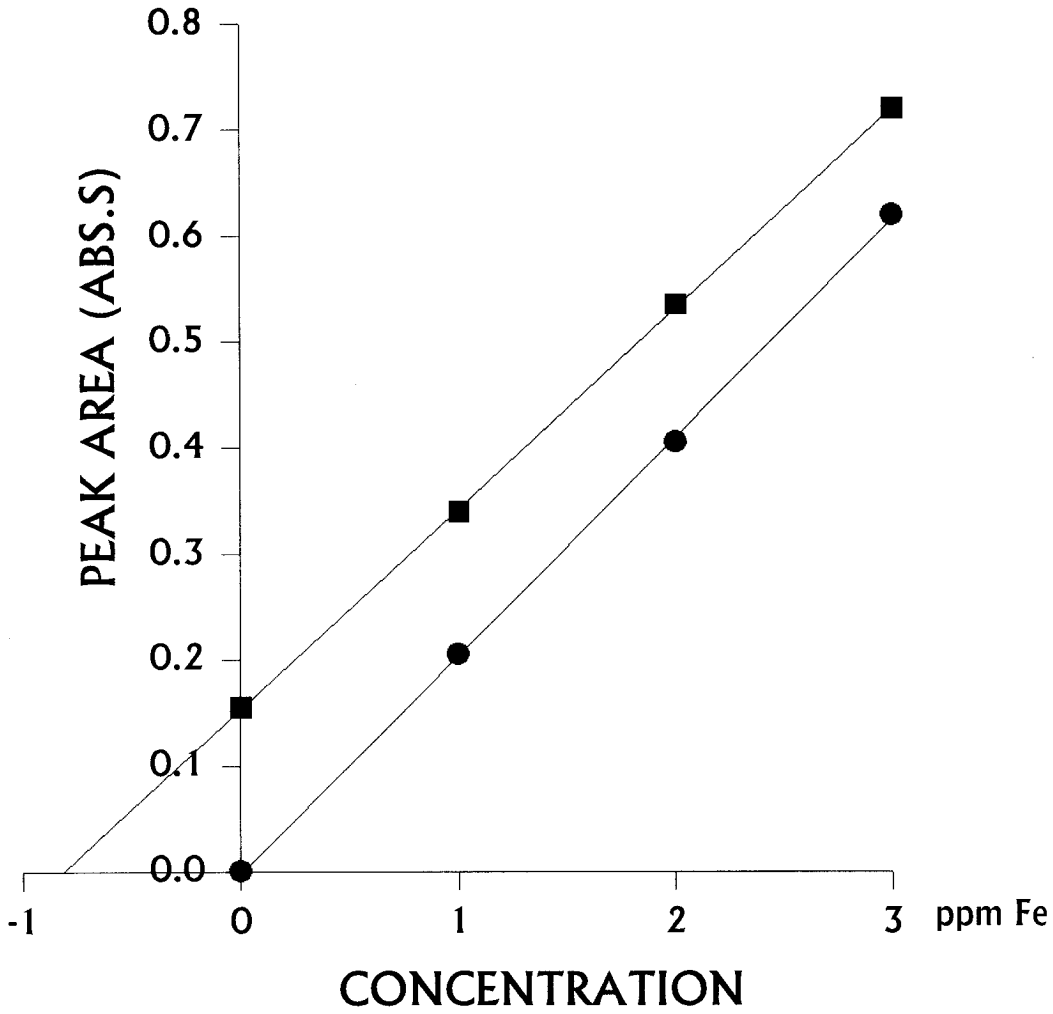

Fig.4. Standard addition analysis of serum sample (■) and aqueous calibration (●) measured at Fe 248.3 nm. Sample volume 150 μL. Uptake rate 5 mL/min..

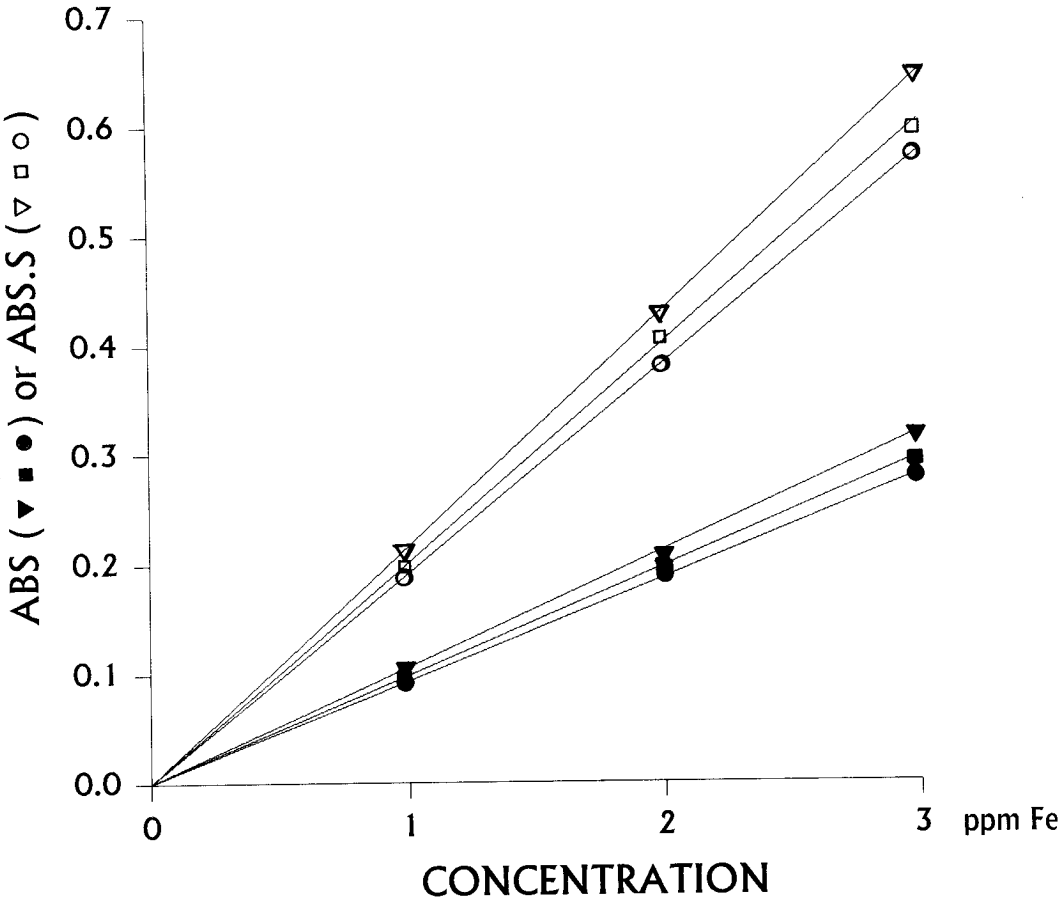

Fig.5. Effect of albumin concentration on slope of standard calibration

▽ ▼ 0% Albumin

❑ ■ 2.0% Albumin

○ ● 3.0% Albumin

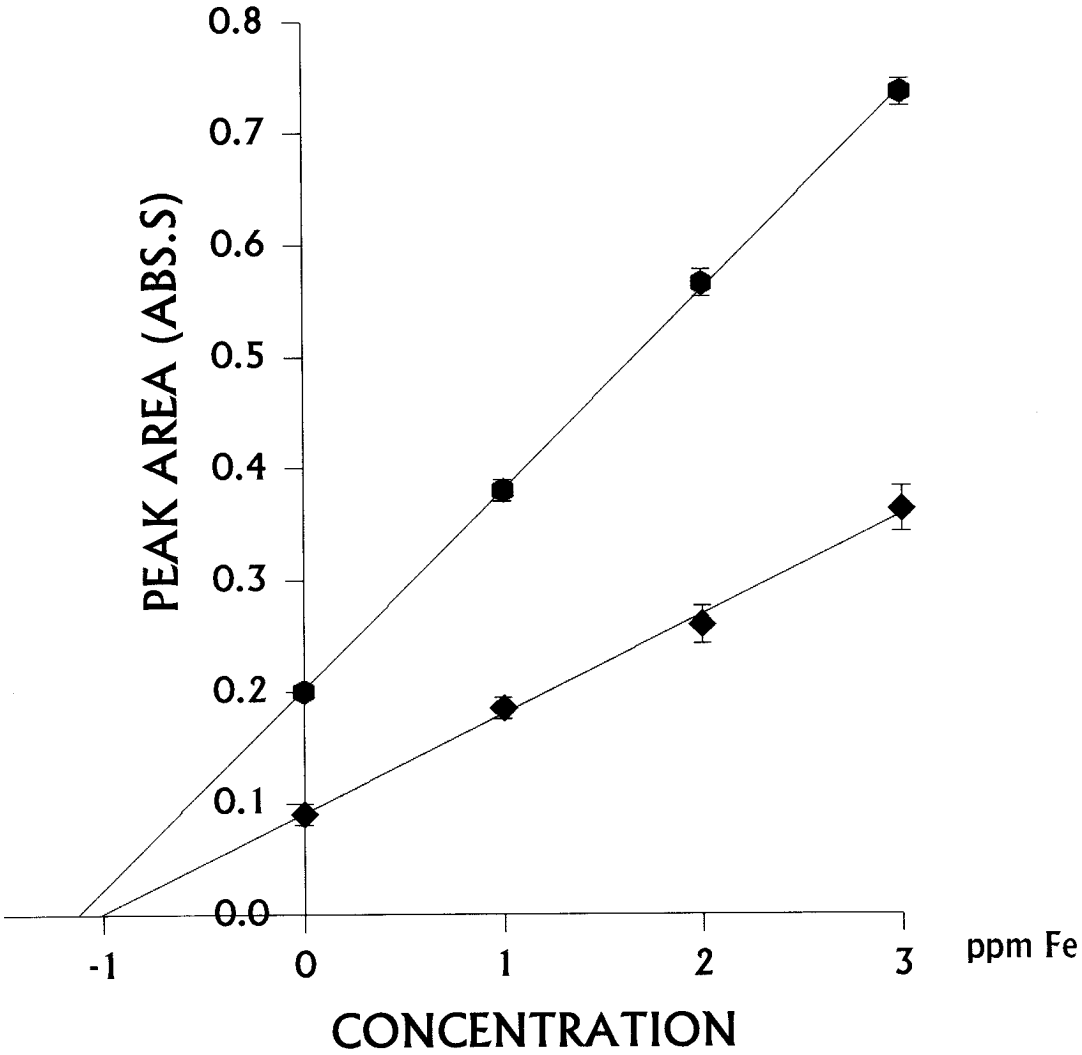

Fig.6. Comparison of standard addition method with direct method (●) and with deproteinization method (◆)

Comparison with other method

The direct determination of iron without deproteinization is compared with the result when protein is removed from the serum prior to atomic absorption determination (Fig.6). Both methods showed no significant difference in the analytical results. The slope of calibration of the deproteinization method was found to be about half that of the non-deproteinization method due to dilution effect following the addition of trichloroacetic acid for deproteinization. The deproteinization method not only worsens the detection limit through dilution effect, but also causes poorer reproducibility because of more steps of analysis involved. Therefore, the proposed direct method is considered to be feasible with the advantage of simple procedure, shorter analysis time and better reproducibility.

CONCLUSION

The method described is considered to be suitable for a rapid determination of iron content in serum at both normal and abnormal levels. The workable range is between 5 μg/100 mL to 300 μg/100 mL.

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บทคัดย่อ

การวิเคราะห์ปริมาณของเหล็กในซีรัม สามารถทำได้รวดเร็วขึ้น และขจัดปัญหาการอุดตันของหัวตะเกียงของเครื่องอะตอมมิกแอบซอร์พชันสเปกโทรมิเตอร์ โดยวิธี discrete sampling ศึกษาอิทธิพลของขนาดตัวอย่าง และอัตราเร็วของการดูดของ nebulizer ที่มีต่อสัญญาณที่ได้ การวิเคราะห์ผลของสัญญาณทั้งที่เป็น peak height และ peak area ช่วยให้สามารถเลือกใช้ปริมาณของสารตัวอย่าง และอัตราเร็วของการดูดของ nebulizer ให้เหมาะสมกับความไวที่ต้องการ และความจำกัดของปริมาณตัวอย่าง วิธีนี้มีข้อได้เปรียบกว่าวิธีที่ต้องมีการ pretreat ตัวอย่าง หลายประการ คือ ความรวดเร็ว ความไว และความแม่นยำ