Discussion. The proposed ternary system is one of the most sensitive for U(VI) comparable to the reagent rhodamine B (*3*) which has a molar absorptivity of 1.02×10^5. The rhodamine B method, however, involves extraction of the uranium–rhodamine B complex into a benzene–ether–hexane solvent.

Other sensitive reagents for U(VI) include chlorophosphonazo III (*4*) with a molar absorptivity of 7.86×10^4.

The proposed reaction suffers from serious interferences from many ions, and preliminary separation of U(VI) by such techniques as ion exchange (*5*) is necessary before determination.

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Graphite Rod Atomizer in Atomic Absorption Spectrometry for Direct Determination of Iron in Serum

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DURING THE PAST FEW YEARS, several types of non-flame atomizers for atomic absorption spectrometry have been fully described (*1–3*). Because non-flame atomization has resulted in atomic absorption absolute detection limits in the picogram region, much attention has been given recently to the possible advantages for using non-flame atomic absorption spectrometry for the measurement of trace metals in real samples, *e.g.*, in jet engine oils (*4–7*) and also in biological materials (*8–10*). Most recently, Kubasik, Volosin, and Murray (*11*) have described a method for analysis of lead in whole blood where only a dilution of the whole blood sample was required. In the present study, a method for direct analysis of iron in 1-μl samples of serum is described, and a correlation study with an automated spectrophotometric procedure is presented.

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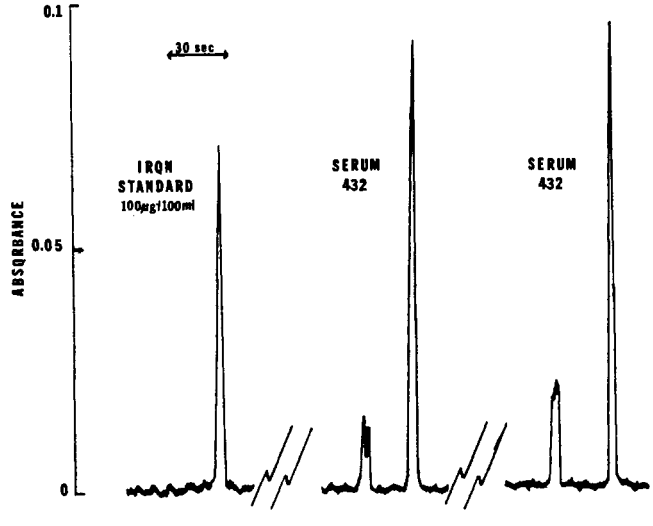

Figure 1. Typical recorder tracings

EXPERIMENTAL

Apparatus. A modified graphite rod atomizer (GRA) described by Molnar *et al.* (*12*) was mounted in place of the burner in a Perkin-Elmer 303 atomic absorption spectrophotometer (wavelength 284.3 nm, slit 3, scale expansion 1, noise 1) equipped with a recorder readout unit, deuterium background corrector, and a Brown strip chart recorder. The photomultiplier output was also connected to an Autolab 6230 digital integrator with print-out facility. A high intensity Perkin-Elmer iron hollow-cathode lamp was operated at manufacturer's recommended maximum current.

Graphite rods were machined from Poco FX91 graphite (Poco Graphite, Inc., Decatur, Texas 76234), and a cylindrical

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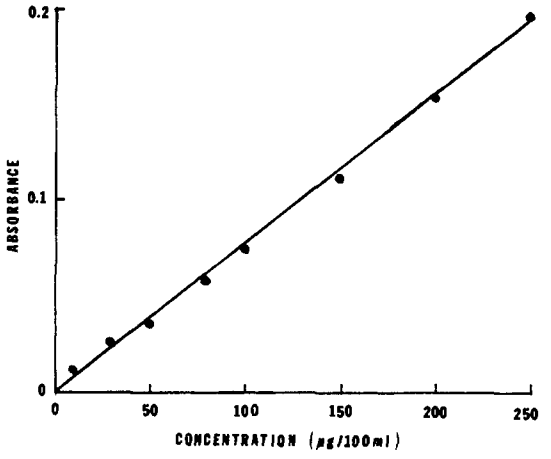

Figure 2. Analytical curves for iron

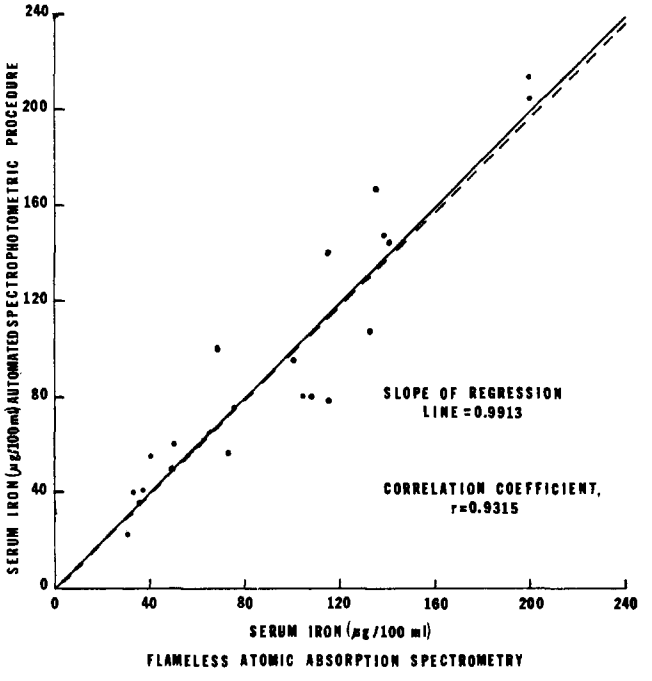

Figure 3. Comparison of measurements of serum iron by the automated spectrophotometric and flameless atomic absorption spectrometric methods

cavity 1.4 mm in diameter and 1.0 mm deep was drilled in the center of the top of each rod (*12*). The cavity volume of about 2 μl was suitable for containing and ashing the 1-μl serum samples.

Samples were dispensed onto the graphite rod with a Hamilton 10-μl syringe with a Kel-F guide. A 10-μl syringe was used instead of a 5- or 1-μl to minimize clogging of the syringe by serum. To further minimize clogging, the syringe was rinsed several times with deionized water between samples.

Power was supplied to the graphite rod by a 250-A, 10-V dc supply (SCR Power Supply, Electronic Measurements, Inc., Oceanport, N. J. 07757) controlled by a preset program in an adjustable timing circuit (*12*). Argon (14.0 l. min^{-1}) and hydrogen (2.6 l. min^{-1}) were premixed. The argon–hydrogen–entrained air flame greatly enhanced preservation of the atom population so that the light beam requirements were not as critical as in other types of systems.

Reagents. A stock solution of 1000 mg/l. iron was prepared by dissolving the appropriate amount of iron in hydrochloric acid. Aqueous standards (10, 30, 50, 80, 100, 150, 200, 250 μg/100 ml) were made by diluting appropriate amounts of the stock iron solution with deionized water and then storing in acid washed polyethylene bottles to prevent iron contamination.

Table I. Recovery of Iron When Added to Serum

Sample	Total content	Amount of iron recovered	Recovery, %
Serum: iron content 183 μg/100 ml			
+ 480 μg/100 ml	665	482	100.4
+ 320 μg/100 ml	459	276	86.3
+ 170 μg/100 ml	373	190	111.8
+ 90 μg/100 ml	275	92	102.2
Serum: iron content 50 μg/100 ml			
+ 100 μg/100 ml	154	104	104.0
+ 200 μg/100 ml	256	206	103.0
+ 300 μg/100 ml	344	294	98.0

Procedure. Serum iron was measured both by the graphite rod atomizer (GRA) and by Young and Hicks' (*13*) continuous flow automated spectrophotometric procedure utilizing 2,4,6-tripyridyl-1,3,5-triazine. The GRA procedure involved placing 1 μl of sample (aqueous standard or serum) in the cavity of the graphite rod with a 10-μl Hamilton syringe. The sample was dried for 20 seconds at 200 °C (20 A), ashed 30 seconds at 400 °C (40 A), and then atomized for 3 seconds at 1920 °C (125 A). The rod was allowed to cool for 30 seconds before application of another sample.

RESULTS AND DISCUSSION

In Figure 1, typical recorder tracings of the ashing and atomization signals for serum and aqueous standards are shown. Atomization conditions were such to ensure that the "absorption signal" during ashing (due to scatter and molecular absorption) was complete prior to the atomization step. Comparison of recorder tracings for serum samples with and without using the deuterium background corrector further demonstrated that ashing was complete. Thus, the deuterium background corrector was not necessary for most of the studies. No memory effect occurred in blank runs immediately after each sample. Duplicate serum samples were measured in most cases. A third serum sample was measured only if the iron results differed by more than 5%. For each series of serum samples, a complete analytical curve was also determined. In addition, if the graphite rod had to be replaced during a series of serums, a new analytical curve was again determined.

The use of Poco graphite resulted in several advantages compared to other forms of graphite (*12*). The useful lifetime of these rods was 150–200 determinations, and the soaking of the serum and aqueous standards in the graphite rod was so similar that impregnating the rod with an organic nonpolar solvent as proposed by other workers (*8*, *9*, *11*), was not necessary. The per cent relative standard deviations were determined for ten replicate measurements of an aqueous iron standard and a pooled serum sample. These precision values were 1.9% for a 100 μg/100 ml aqueous solution and 4.1% for the pooled serum sample (50 μg/100 ml).

The linear analytical curve for aqueous standards of iron (useful range for iron in serum is 10 to 400 μg/ml) is given in Figure 2. A standard addition analytical curve (aqueous iron added to a pooled serum) also was linear with the same

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slope. Therefore, an aqueous standard analytical curve was used for all correlation studies.

Known amounts of iron were added to two different pooled serum samples, and the results demonstrating quantitative recoveries over a wide range of iron concentration are given in Table I.

A study was carried out comparing serum iron levels from 22 patients at the Shands Teaching Hospital (Gainesville, Fla. 32601) by the GRA and the automated spectrophotometric procedure. The results of the study are given in Figure 3. The equation of the regression line was $y = mx$ (y = spectrophotometric method, x = GRA atomic absorption spectrometry). For the GRA method, a serum iron mean of 93.3 μg/100 ml was found and a serum iron range from 32 to 198 μg/100 ml. For the automated spectrophotometric method, a serum iron mean of 91.8 μg/100 ml and a serum iron range of 22 to 215 μg/100 ml were obtained. The standard error of estimate for the correlation was 19.6 μg/100 ml. These studies demonstrate a close correlation between the GRA method and the reference spectrophotometric procedure.

The only interferences with the GRA method resulted when the iron concentration was less than 50 μg/100 ml and/or the serum sample was more viscous than normal; in such cases, the problem was solved by diluting the serum sample 1:1 with deionized water.

From the results of the study, the GRA system in atomic absorption spectrometry is an extremely promising system providing rapid, precise, and accurate means for atomizing and measuring iron in serum with no prior sample treatment.

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Trichloride Ion Formation Constant in Acetonitrile Solutions

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THE ELECTROCHEMICAL BEHAVIOR of X_2/X^- (X = I, Br, Cl) systems at platinum electrodes in acetonitrile solutions has been the subject of previous inevstigations (*1–3*). The experimental results were explained by the existence of the equilibrium $X_2 + X^- \rightleftharpoons X_3^-$, where the relative magnitude of the equilibrium constant would determine the differences among them.

The stability of the trihalide complexes in various solvents has been estimated. Voltammetry had shown that the species X_3^- are produced in acetonitrile (*4*); subsequently, the following formation constants were calculated from a set of half-wave potentials (*5*): triiodide, $10^{6.6}$; tribromide, 10^7; and trichloride, 10^{10}. Recently, these values were reviewed (*6*) and it was concluded that the stability constant sequence would be analogous to that found in water:

$$K_{I_3^-} > K_{Br_3^-} \gg K_{Cl_3^-}$$

Similar results were found in sulfolane (*7*) and nitromethane (*6*), which closely resemble acetonitrile physicochemical properties.

The aim of the work described in this paper was to obtain the value of the stability constant of the Cl_3^- formation in acetonitrile under the same conditions used in the electrochemical study of the chlorine–chloride electrode (*3*). The experimental method is based on the determination of the distribution ratio of a volatile solute between two miscible solvents (*8*).

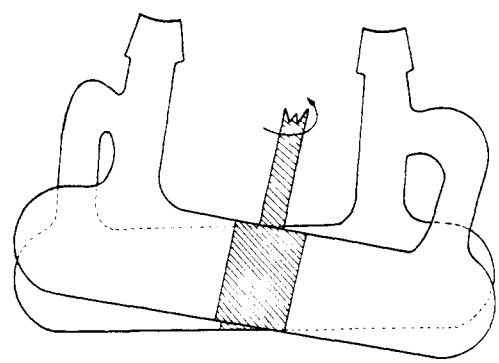

Figure 1. Apparatus side view

EXPERIMENTAL

Apparatus. The device is a closed borosilicate glass apparatus containing the two separated solutions under investigation which permits by mere rotation a continuous circulation of vapor through the two solutions. The essential features (Figure 1) are two cylindrical containers of about 200-ml volume each, provided with ground stoppers, and connected at the top by glass tubes.

The stoppers are replaced at the end of the run by delivery tubes which are ground to fit the same openings.

Reagents. Chlorine gas, LiCl, and $LiClO_4$ in acetonitrile solutions were used. Chemical and solvent purification, as well as solution preparation, have been described in a previous paper (*3*).

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