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METHOD OF REMOVING INORGANIC IODINE FROM BLOOD

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This invention relates to a method for separating inorganic iodine from organic iodine in a blood sample and, more specifically, to a method of removing inorganic iodine from a blood sample so that the organic iodine content of the sample may be determined.

The determination of iodine in organic and biological materials is of very great importance, particularly in the medical-clinical field where organic iodine or protein-bound iodine (PBI), in blood is used as an indication of the functional condition of the thyroid gland. Since a blood sample contains both organic iodine and inorganic iodine, it is obviously necessary in determining the organic iodine content of the sample to initially remove the inorganic iodine.

The conventional method used today for removing inorganic iodine from a blood sample requires several steps, expensive equipment and skilled technicians. In order to separate the inorganic iodine from a blood sample by using the conventional method, zinc sulfate and sodium hydroxide are initially added to the sample to coagulate the protein out of the sample. Then, the sample is centrifuged and the supernatant liquid, which contains inorganic iodine, is poured off from the precipitate. The precipitate contains the organic or protein-bound iodine, the amount of which is desired to be determined. The organic iodine is generally determined by using the standard alkaline ash technique described in detail in copending patent application of Matsuyama, entitled "Method and Apparatus for Determining Iodine in Organic and Biological Materials," Serial No. 289,179, filed concurrently herewith, now Patent No. 3,235,336, and assigned to the same assignee as the present invention.

Recently, another method has been described for removing inorganic iodine from a blood sample. This method involves filling a glass column with an anion exchange resin, Amberlite, and flowing a blood sample through the column whereby the inorganic iodine is removed from the sample by an anion exchange reaction. The present invention involves still another manner by which inorganic iodine may be removed from a blood sample which has the advantage over the presently known methods of being more rapid and efficient.

It is therefore the principal object of the present invention to provide a method for separating inorganic iodine from organic iodine in a blood sample.

Another object of the present invention is to provide a simple and rapid method for removing inorganic iodine from a blood sample whereby the organic iodine remaining in the supernatant sample may be readily determined.

According to the principal aspect of the present invention, it has been discovered that inorganic iodine may be separated from organic iodine by adding solid silver chloride to the blood sample, mixing and allowing a precipitate containing the inorganic iodine to settle out.

It is quite unexpected that the addition of silver chloride to a blood sample removes the inorganic iodine, that is iodide, from the sample. This can be best appreciated by considering the theoretical iodide content of blood, both before and after the addition of silver chloride. Normally there are about 1 to 2 micrograms of iodide in 100 ml. of blood. Thus, taking an average of about 1.5 micrograms of iodide in 100 ml. of blood, it is seen that the concentration of iodide would be approximately 0.1 micromolar (μM) which equals 10^{-7} molar.

The solubility product constant (K s.p.) of silver chloride is approximately 10^{-10}. The chloride ion concentration in blood is about 0.09 to 0.11 molar, which will be assumed as being 0.1 molar for the purposes of this analysis. Thus, if silver chloride salt is placed into a blood sample, the silver ion concentration will be approximately 10^{-9} M in accordance with the equations

$$[Ag^+]\ [Cl^-]=K\ s.p.=10^{-10}$$

$$[Ag^+]=\frac{10^{-10}}{[Cl^-]}=\frac{10^{-10}}{10^{-1}}=10^{-9}\ M$$

The solubility product constant of silver iodide which is formed by the addition of silver chloride to a blood sample is about 10^{-16}. Thus, knowing that the silver ion concentration in blood saturated with AgCl is 10^{-9} M, then the resulting iodide concentration in the blood sample after the reaction of the silver chloride with iodide forming silver iodide is approximately 10^{-7} M as is seen in the following equation

$$[I^-]=\frac{K\ s.p.\ of\ AgI}{[Ag^+]}=\frac{10^{-16}}{10^{-9}}=10^{-7}\ M$$

It is thus seen that the maximum concentration of inorganic iodine theoretically should be 10^{-7} M when a blood sample is saturated with silver chloride, which concentration is approximately the same as the iodide concentration in blood before the addition of silver chloride. Thus, one would reasonably expect that silver chloride added to blood would not remove the inorganic iodine therefrom. However, contradictory to theoretical considerations, it has been unexpectedly found that when silver chloride is added to blood sample, it does remove substantially all the inorganic iodine from the sample.

It has also been found that the amount of silver chloride added to a blood sample is not critical. All that is necessary is that enough silver chloride is added so that all the silver chloride does not dissolve in the sample, that is, the sample must be saturated with silver chloride to ensure that all the inorganic iodine in the blood reacts to form a precipitate containing the inorganic iodine. For example, it has been found that only 2 micrograms of silver chloride need be added to a sample of 10 cc. of blood to separate out all the inorganic iodine from the blood sample. However, as a practical matter, it would be difficult to measure out such small quantities of silver chloride. Therefore, 3 to 5 milligrams may be added to the blood sample to remove all the iodide from the sample. The additional amount of silver chloride does not adversely affect or contaminate the sample because of the limited solubility of AgCl.

The supernatant sample containing the protein-bound iodine may then be readily removed from the precipitate by merely pouring the supernatant from the precipitate. The supernatant sample containing the PBI may then be treated to determine the protein-bound iodine content of the sample in accordance with the method described in said patent application of Matsuyama entitled "Method and Apparatus for Determining Iodine in Organic and Biological Materials."

To increase the speed of removal of iodide, it is preferable to shake the sample containing the silver chloride for a short period of time which permits the inorganic iodine in the sample to more quickly react. The method of the present invention is obviously much more rapid than the conventional method requiring centrifuging utilized in laboratories today. Also, it is more rapid to precipitate the inorganic iodine from a blood sample than to pass the sample through a column containing an anion exchange resin as has been recently described.

In order to prove the accuracy of the present invention, many tests have been performed on blood samples to determine the protein-bound iodine content of the samples after inorganic iodine has been removed therefrom, some of the results being indicated in Table I.

TABLE I

Sample No.	μg. Percent PBI Determined by Conventional Method	μg. Percent PBI Determined by New Method
1	5.6	5.5
2	5.3	5.1
3	4.7	4.8
4	5.6	5.5
5	7.2	7.2
6	6.2	6.0
7	5.4	5.5
8	5.6	5.6
9	2.7	2.6
10	2.8	2.6
11	5.4	5.5
12	5.6	5.6
13	5.4	5.3
14	6.3	6.4
15	8.4	8.8

The first column of Table I indicates the sample number whereas the second column indcates the protein-bound iodine content of the sample as determined by the conventional alkaline ash method utilized in the medical laboratories and clinics today and described in detail in said application of Matsuyama. The third column of Table I shows the protein-bound iodine content of the blood samples as determined by adding silver chloride to the sample to remove the inorganic iodine from the sample in accordance with the novel method of the present invention and by determining the PBI content of the supernatant sample in accordance with the method described in said application of Matsuyama. As can be seen, the results of the tests performed in accordance with the method of the present invention compares almost exactly to the results obtained by utilizing the conventional alkaline ash technique. As a pratcical matter, the very small differences which appear between these results are of virtually no consequence in the field of medical-clinical analysis.

Efforts have been made to see whether other salts having similar characteristics to silver chloride would be capable of removing the inorganic iodine from a blood sample. Silver bromide and mercurous chloride have been tried but without favorable results. Thus, even though silver chloride theoretically should not remove the inorganic iodine from a blood sample, it has been discovered that it does so with a high degree of efficiency and accuracy and, thus, there is provided by the present invention a very simple, efficient and rapid means by which inorganic iodine may be removed from a blood sample whereby the supernatant sample may be tested for PBI content.

It will be understood that the details of the above description are intended as exemplary rather than limiting, and that various modifications of the method may be employed without departing from the spirit and scope of the invention as defined by the appended claims.

What is claimed is:

1. In a method for separating inorganic iodine from organic iodine in a blood sample, the steps of:
providing a sample of blood;
placing solid AgCl into said sample to form a precipitate containing inorganic iodine; and
allowing the precipitate to settle out.

2. In a method for separating inorganic iodine from organic iodine in a blood sample, the steps of:
providing a sample of blood;
placing sufficient solid AgCl into said sample to react with substantially all iodide in said sample to form a precipitate containing inorganic iodine; and
allowing the precipitate to settle out.

3. In a method for separating inorganic iodine from organic iodine in a blood sample, the steps of:
providing a sample of blood;
placing sufficient solid AgCl into said sample so that an excess of AgCl is present over the amount of which dissolves in the sample; and
allowing a precipitate containing inorganic iodine to settle out.

4. In a method for separating inorganic iodine from organic iodine in a blood sample, the steps of:
providing a sample of blood;
placing solid AgCl into said sample;
shaking the sample containing AgCl to form a precipitate containing inorganic iodine; and
allowing the precipitate to settle out.

5. In a method for removing inorganic iodine from a blood sample, the steps of:
providing a sample of blood;
placing solid AgCl into said sample to form a precipitate containing inorganic iodine;
allowing the precipitate to settle out; and
removing the supernatant sample from said precipitate.

6. In a method for removing inorganic iodine from a blood sample, the steps of:
providing a sample of blood;
placing sufficient AgCl into said sample to react with substantially all iodide in said sample to form a precipitate containing inorganic iodine;
shaking the sample containing AgCl to hasten the formation of said precipitate; and
removing the supernatant sample from said precipitate.

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