

A Micro Method for Determination of Dextran in Blood

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G rönwall and Ingelman ^{1, 2, 3, 4}, suggested that dextran, when hydrolyzed to a suitable molecular size, could be used as a plasma substitute. Dextran, thus treated, has been tried out clinically by Bohmansson *et al.*⁵ and has been found to possess valuable properties for the prevention and treatment of shock and hypoproteinaemic conditions.

Earlier methods for the determination of dextran were based on its optic activity or on the determination of glucose after complete hydrolyzation of dextran ⁶. The clinical applicability of these methods is, however, limited owing to the relatively large amounts of blood required and to the complexity of the procedure.

These disadvantages have been overcome in the method described below which is therefore better suited for clinical purposes.

PRINCIPLE

Dextran is precipitated by copper sulphate in the presence of sodium hydroxide, copper being bound up quantitatively but not stoichiometrically. After the precipitate has been centrifuged off, the remaining concentration of copper is determined photometrically.

REAGENTS

1. 0.6 *N* trichloroacetic acid.
2. 2.5 *N* sodium hydroxide.
3. Copper reagent. A fresh reagent is prepared for each determination from the following stock solution:

CuSO ₄ · 5H ₂ O	3.000 g
Sodium citrate	30.000 g
Dist. water to	1000.0 ml

Copper sulphate and sodium citrate are dissolved separately in a relatively small amount of distilled water, then mixed and filled up to 1000.0 ml with distilled water.

The stock solution is stable for several months if kept in a dark room. In order to prepare the reagent, 20 ml of the stock solution are filled to 100.0 ml with distilled water.

4. 0.2 % sodium diethyl-dithiocarbamate in distilled water.

PROCEDURE

Precipitation of proteins

The blood proteins are precipitated with trichloroacetic acid. This is carried out by diluting 0.2 ml of heparinized plasma or whole blood in 4.8 ml of distilled water after which 5 ml of 0.6 *N* trichloroacetic acid are added. The mixture is then shaken vigorously and placed in a water bath at 70—80° C for about 5 minutes. The protein sediment is then filtrated off. It is preferable to use a fine filter, *e. g.*, Berzelius 00.

Precipitation of dextran

2.0 ml of 2.5 *N* sodium hydroxide are pipetted in an ordinary 10 ml centrifuge tube (or special glass-stoppered tube). Then 5.0 ml of the filtrate and 2.0 ml of the copper reagent are added. The tube is closed with a stopper and the contents are well mixed. When ordinary centrifuge tubes are used, the stopper is coated with plastic. Without protective coating, the stopper may liberate substances which influence the reaction. The tubes are then placed in an agitating apparatus for 4 hours or more, to allow ample time for the formation of the dextran-copper compound. The resulting sediment is then centrifuged at 2000-3000 r.p.m. for at least 10 minutes. The remaining solution should be clear and the sediment of a homogeneous bright blue colour. A discoloured sediment is probably due to incomplete removal of the proteins.

Colour reaction

6.0 ml of the clear solution are pipetted in a 100 ml volumetric flask which has previously been filled up to 70—80 ml with distilled water. Care should be taken not to pipette away any traces of the precipitate. It is therefore advisable to remove the stopper before centrifuging, as small particles of the precipitate tend to gather around the stopper. The flask is then shaken vigorously and 3.0 ml of the sodium diethyl-dithiocarbamate reagent are added and mixed well immediately. After this the flask is filled up to 100.0 ml with distilled water and then shaken once again.

It is of the utmost importance that the above description is followed meticulously. If the volumetric flask is filled with less water or insufficiently shaken before adding the reagent, the ensuing formation of copper carbamate may become massive enough to cause opalescence which is not revertible and greatly affects the results.

The solution will be clear, of a yellow straw colour and slightly fluorescent. The colour is fairly stable and the reading need not be performed immediately.

D e t e r m i n a t i o n

The readings are done in the Zeiss Pulfrich photometer against the blank value of the following mixture: 3.0 ml of the sodium diethyl-dithiocarbamate reagent; 1 ml of 2.5 *N* sodium hydroxide; distilled water to 100.0 ml. This is to compensate for the possible presence of copper in distilled water. 5 or 3 cm cuvettes are used for the readings. The filter is S 47 (470 $m\mu$). When other types of photometers are used, the method should be adapted accordingly.

The percentage of dextran is read off from a curve which has been plotted previously in accordance with known dextran values. For the construction of this curve, multiple determinations are made of the following 6 concentrations: 0.25; 0.5; 1.0; 1.5; 2.0; 2.5 per cent dextran in heparinized plasma. 0.2 ml from each is taken for the determination.

Lower dextran concentrations than 0.25 % can be determined when bigger amounts of blood are used. Up to 2.0 ml of blood can be used, diluted with water to 5.0 ml. Thus, the lowest determinable concentration of dextran will be 0.025 %.

When whole blood is used, the concentration of dextran in plasma can be computed by means of the hematocrit correction since the amount of dextran taken up by the red blood cells is negligible.

ERRORS

The values of dextran to be determined depend on the concentration of copper sulphate as well as sodium hydroxide, trichloroacetic acid and sodium citrate. Consequently, citrate or oxalate blood should not be used.

Cellulose combines with copper as does dextran. Alkaline solutions should therefore not come into contact with filter paper, cork or similar substances.

The blood samples should be freshly taken, since the use of old plasma may yield a dirty grey sediment which sticks to the walls of the tube, rendering the values obtained too high.

The applicability of this method for the determination of dextran in other body fluids has as yet not been fully established. It can, however, be adopted with fluids such as exsudates, transsudates, cerebrospinal fluid and different organ extracts, but not with urine.

Glycogen does not give sediment, nor does its presence affect the results.

As mentioned above copper carbamate may give disturbing opalescence. This compound is only slightly soluble in water and the concentration of copper in the reagent is chosen accordingly in order to keep the compound in solution.

It is evident from what has been said above that a strict copper »sterility» should be maintained. While working with the Pulfrich photometer, caution should be taken not to close the covers of the cuvette containers if they are made of brass.

EXAMPLE

Average error

For the construction of the curve as a rule 2—3 determinations of each concentration will suffice. In the following, however, 15 determinations were performed for calculation of the average error. The average extinction coefficients were plotted on the millimeter paper, the curve was constructed and the deviation of each extinction coefficient was expressed in the corresponding deviation of the dextran concentration. From the dispersion the average error was then calculated and expressed in per cents of dextran concentration. See Table and Figure.

The average (with standard error) and the standard deviation of the extinction coefficients have been calculated according to current statistical methods.

The standard deviations of the concentration percentage are obtained by multiplying the resp. standard deviations of the extinction coefficients by the slope of the curve. The slope of the curve has been determined graphically.

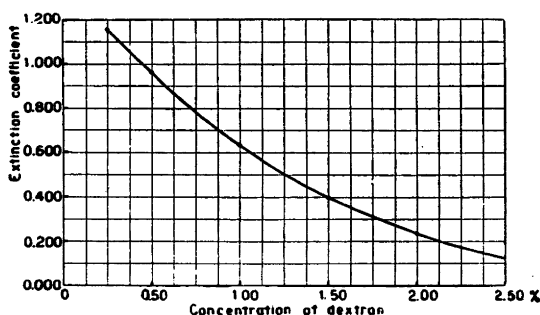


Fig. 1. Interpolation curve.

Table 1. Calculation of average error from 15 determinations.

Concentration of dextran	0.25 %	0.50 %	1.00 %	1.50 %	2.00 %	2.50 %
Average of obtained extinction coefficients in g cm cuvettes	1.155 ± 0.0053	0.963 ± 0.0036	0.634 ± 0.0026	0.395 ± 0.0016	0.234 ± 0.0015	0.123 ± 0.0011
Standard deviation of ext. coefficient	0.0206	0.0140	0.0102	0.0061	0.0057	0.0041
Slope of the (concentration) curve	1.29	1.39	1.76	2.55	3.55	4.80
Standard deviation of concentrations	0.027	0.019	0.018	0.016	0.020	0.020
Standard deviation in per cent of concentration	10.6	3.9	1.8	1.0	1.0	0.8

SUMMARY

A micromethod for determination of dextran — a plasmasubstitute — is described. Using 0.2—2.0 ml of fluid to be analyzed, concentrations from 0.025—2.5 % of dextran in body fluids, urine excluded, can be determined with sufficient accuracy for clinical purposes.

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