Meditsiiniline keemia/Medical chemistryl LOKT.00.009

Photometric determination of albumin.

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1. Introduction

Albumin is a blood plasma protein that is produced in the liver and forms a large proportion of all plasma protein. It is also found in egg white. The normal range of albumin concentrations in human blood is 3.5 to 5.0 g/dL. A blue solution is formed when albumin reacts with bromcresol green (see Fig. 1) in buffer solution with pH = 4. Intensity of the blue color and absorbance of the solution at 670 nm are proportional to the albumin concentration in the solution (Beer's law). Concentration of albumin in the sample is calculated from the measured absorbances of the blank (or zero-solution), standard solution with known concentration of albumin and the sample.

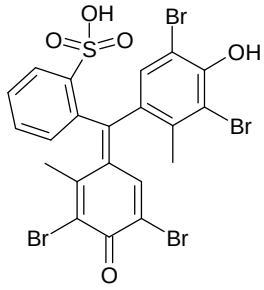

Fig. 1. Structure of bromcresol green

2. Apparatus, reagents and glassware

- Photometer (photoelectric colorimeter) and cuvettes (cells) with 1.0 cm optical path length are used for the analysis.
- The reagent for determination of albumin: solution of bromcresol green (0.01%) in buffer solution with pH = 4. (The buffer solution is 0.05 M solution of potassium hydrogen phthalate, pH at 25°C is 4.008.)
- The standard solution of albumin. (50.00 g of albumin dissolved in 1 liter of distilled water.)
- The physiological solution. (9.00 g of chemically pure NaCl dissolved in 1 liter of distilled water.)
- Use 10 cm^3 test-tubes, 5 cm^3 and 1.0 cm^3 graduated pipettes.

3. Analytical procedure

Solutions are prepared in test-tubes according to the following table.

	Blank	Albumin standard			Sample		
Reagents	**No. 1**	**No. 2**	**No. 3**	**No. 4**	**No. 5**	**No. 6**	**No. 7**
Sample, cm^3	-	-	-	-	0.2	0.2	0.2
Standard solution of albumin, cm^3	-	0.2	0.2	0.2	-	-	-
Physiological solution, cm^3	0.2	-	-	-	-	-	-
Reagent for determination of albumin, cm^3	5.00	5.00	5.00	5.00	5.00	5.00	5.00

The solutions should stand for 15 minutes after preparation. The absorbance is measured at 670 nm using distilled water as the reference. Concentration of albumin in the standard solution (c_{st}) is given by the supervisor.
The arithmetical mean is calculated from the measured absorbances. Concentration of albumin in the sample is calculated from the following equation:

$$c_x\left[mg/cm^3\right] = \frac{\overline{A}_x - \overline{A_0}}{\overline{A}_{st} - \overline{A_0}} \cdot c_{st}$$

where $\overline{A}$ – arithmetical mean of the corresponding absorbances,
c_{st} – concentration of albumin in the standard solution (mg/cm^3)

Photoelectric colorimeter КФК – 2Mn

1. Switch on the photoelectric colorimeter
   1.1. Plug in the photoelectric colorimeter (note the symbols appearing in the screen).
   1.2. Press the button ПУСК (note the blinking comma and light Р). If there is no change, press the button ПУСК again.
   1.3. Open the cuvette chamber lid and wait for 15 min.
   1.4. Close the lid and open it again, wait for 5 seconds and press button Ш(0). Note the value of n_0 on the screen, it should be in the range of 0.001-1.000.
   1.5. If the value of n_0 is not in the range, ask guidance from your supervisor.
2. Beginning of the measurements
   2.1. Place the cuvette with the reference solution (distilled water) on the distant cuvette holder. Place the solution to be measured on the nearer cuvette holder.
   2.2. Close the cuvette chamber lid.
   2.3. Turn the left button in the front of the photometer to select the suitable filter (see the suitable wavelength value from the practical work instructions) and turn the right button to change the correspondent photovoltaic cell.
   2.4. Place the handle for moving cuvette in the position “1” so the beam of light passes through the reference solution.
   2.5. Press the button К(I). Number “1” appears on the left side of the display.
3. Measurement of absorbance
   3.1. Place the handle for moving cuvette in the position “2” so the beam of light passes through the sample solution.
   3.2. Press the button D(5). Number “5” appears to the left side of the comma. This shows that the absorbance of the sample solution has been measured. The value of absorbance appears to the right from the blinking comma.
   3.3. Repeat the steps 2.4-3.2 at least 3-5 times. Find the arithmetical mean of the results and use this in your calculations.
   3.4. Open the cuvette chamber lid.
   3.5. Take out the cuvette and replace the sample solution with another sample solution, standard solution or blank sample.
   3.6. Close the cuvette chamber lid.
   3.7. Repeat the steps 2.4-3.6 with all solutions.
4. To finish the work - plug off the photometric colorimeter