**Lesson 5**

**Spectrophotometric determination by the method of**

**constructing a calibration graph.**

Calibration graphs are used only in physical and physico-chemical methods of analysis. Against the backdrop of victorious reports of theoretical physicists about the successful description of physical and physicochemical processes, the question arises: if everything is so well studied, then why do we need calibration graphs at all? They are needed in order to take into account all the factors that affect the analysis process, which are theoretically difficult to take into account. Factors of this kind include the complex kinetics of a chemical reaction, anomalous equilibrium constants, activity coefficients, etc. It follows from this that it is easier to build a calibration plot than to deal with tedious corrections to theoretical forecasts.

The principle of constructing a calibration graph is simple. Several standard solutions are prepared (5-6 solutions, rarely less than 4) with a known content of the analyte. In each standard solution, the analytical signal is measured by the instrument used in this type of analysis. According to the measurement results, a graph is plotted in the coordinates of the analytical signal - the content of the substance in the standard solution. The constructed graph is a calibration one. Further, everything becomes even simpler: measurements are taken in the analyzed solution, in which the concentration of the analyte should be determined. Having received the value of the analytical signal, using the calibration graph, the concentration that corresponds to this signal is found. This completes the analysis procedure.

 Simplicity is simplicity, but it is necessary to explain some technical details of constructing a calibration graph. When talking about a calibration graph, they always (with a few exceptions) mean a straight line. The straight line is either a natural function of the analytical signal versus concentration, or the experimental data is linearized to make the calibration straight. This raises the question: why should the gauge be represented as a straight line? In our time, when a computer is on every table, isn't it too primitive to build straight lines? Here the situation is not so much in the mathematical processing of the results, but in the need to once again make sure that the gauge line confirms the expected physicochemical law. It is easier to evaluate this by the parameters of a straight line than by the shape of a curved line. Thus, if, when constructing the calibration line, we are once again convinced that it is really a straight line, then with a clear conscience we can state the expected course of the reaction and the normal functioning of the device.

Let us give an example of constructing a calibration for the spectrophotometric method of analysis.

The optical density of colored solutions is linearly dependent on the concentration of the colored substance in the solution. This is evidenced by the Bouguer-Lambert-Beer law:

A = ε \* C \* l,

Where

A - optical density;

ε is the molar extinction coefficient;

C is the molar concentration of the solution, mol/l;

l is the cell thickness, cm.

Hence the conclusion about the form of the calibration graph is unambiguous: the graph must be a straight line and start from zero.

Reality brings surprises. First, calibrations often do not start from zero. Secondly, the calibration curve has concentration limits of linearity. It is possible to deal with the second feature for a long time and to no avail, so we will accept this fact and put up with it, using only the linear calibration region. With the first surprise, the situation is somewhat simpler. There are usually two reasons that prevent the gauge line from starting from zero. The first reason is not serious and can be easily eliminated. It consists in the fact that the cuvettes have slightly different sizes or glass defects. This difference is small and can reach 0.001-0.002 units of optical density. It is not difficult to measure this difference; it is enough to fill the cuvettes with a background solution that does not contain the analyte, and measure the optical density of the solutions relative to each other.



Options for constructing a calibration graph with a linear dependence of extinction on the concentration of the test substance:

A - the Bouguer-Lambert-Beer law is observed, the straight line leaves the zero point, the correct version of the calibration graph

B - there is an influence of a systematic factor, it is desirable to measure it in comparison with a blank sample, in which this factor would be taken into account.

B - the measurement is incorrect, since low concentrations of the substance are not measured. The graph must be redone with a different calibrator or on a different instrument.

If you get graph B, then you can use it, but there is a systematic error that must be taken into account when calculating the factor:



where a is a segment on the y-axis from 0 to the beginning of the calibration graph.

If graph B is obtained, then it cannot be used, since low concentrations of the calibrator are not determined from this graph.

If the calibration line does not start from zero by a greater value than the difference in cell sizes suggests, then we can talk about partial decomposition of the reagent, which causes the color of the solution with the analyte. In other words, as a result of the decomposition of the reagent, a small amount of a substance is formed, which reacts with the substance to be determined. Of course, you can turn a blind eye to this, but it is better not to do this and subject the reagent to purification. Why is this needed? And this must be done in order to exclude adverse reactions that significantly reduce the reliability of subsequent analyzes.

There are certain requirements when constructing a calibration graph.

1. It is necessary to establish linearity between the zero and the minimum calibration point: for this, low concentration calibrators must be used, that is, a calibration plot for very low concentrations must be drawn in order to be able to carry out calculations in the situation when a very low concentration of the analyte to be determined is encountered.

2. It is necessary to measure the concentration of the substance near the maximum calibration point to ensure that the law is maintained at high concentrations.

3. Approximately estimate the effect of the variation. To do this, determine the third (average) concentration 20 times, calculate the arithmetic mean and standard deviation (RMS). Draw a new graph through the arithmetic mean. All points on the main chart should not deviate from the newly drawn line by more than 1.2 SD.

There is one more rule that should be strictly observed: do not make analytical determinations outside the calibration curve. This does not lead to anything good, since there is a big risk of getting a big systematic error. But there is a delightful exception here. If the calibration line starts from zero (if it theoretically should start from zero), then it is possible to make determinations below the lower calibration limit. If the straight line should come from zero, but it does not come from it, then the risk of making a mistake when determining low concentrations of a substance inevitably increases many times over!