DETERMINATION OF SULFHYDRYL GROUPS WITH THE T-201 LABORATORY TITRATOR

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A method of determining SH groups with the T-201 laboratory titrator is suggested. The principle of the method is coulometric titration of SH groups with silver ions. The method is highly sensitive and has high reproducibility.

KEY WORDS: SH groups; determination; cysteine; proteins.

The T-201 laboratory titrator (Special Design Bureau of Analytical Instruments, Tbilisi), intended for quantitative determination of halides, is now in production. The principle of its operation is coulometric titration of silver ions. The same principle can also be used for titration of SH groups.

This paper describes an attempt to use the T-201 laboratory titrator for quantitative estimation of SH groups of proteins and low-molecular-weight compounds.*

EXPERIMENTAL METHOD

The principle of the method is that the solution is titrated with silver ions and the end point of the titration is determined amperometrically. After a current of under 4 μA is established in the circuit of the silver indicator electrodes, a direct current is passed through the pair of silver generator electrodes, leading to electrolytic formation of silver ions; the time counter is actuated at the same time. The silver ions are bound with titratable compounds.

After the appearance of free silver ions in the medium the power supply to the generator electrodes is automatically disconnected by means of relay systems. The titration and time counter are stopped. The quantity of titratable substance is calculated by the equation:

$$C = K \cdot I \cdot t,$$

where K is the electrochemical equivalent, I the magnitude of the generator current, and t the duration of titration measured with the counter.

It will be clear from the equation that after establishment of the electrochemical equivalent K all that is needed to estimate SH groups is the duration of titration.

EXPERIMENTAL RESULTS

To find a suitable medium for coulometric titration of SH groups on the T-201 instrument a series of media suggested by other authors [2, 5, 7] was investigated. Experiments showed that a medium consisting of a mixture of nitric and acetic acids (0.1N HNO_3 in 10% acetic acid solution) satisfies the requirements for coulometric titration of SH groups. After establishment of the generator current (1 mA), and the volume (15 ml) and optimal concentration of the medium, coefficients for the regression equation ($a = -0.05 \cdot 10^{-6}$ M, $b = 0.008 \cdot 10^{-6}$ M), the error of regression, and the level of significance of the results were calculated from the duration of titration (t) of empirically chosen concentrations of cysteine by regression analysis [3]. Hence the equation for calculation of SH groups assumed the following form:

$$C = (0.008 \cdot t - 0.05) \cdot 10^{-6} \text{ M}.$$

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TABLE 1. Experimental and Theoretical Values of Content of SH Groups (C) and Titration Time (t) for Their Determination with the T-201 Titrator

Protein content in sample, mg	0,05	0,01	0,15	0,20	0,25	0,30	Mean
Content of SH groups, 10^{-6} M in sample	0,41	0,83	1,24	1,65	2,06	2,48	1,44
Quantity of SH groups, 10^{-6} M, in sample based on value of t found experimentally	0,41	0,84	1,24	1,63	2,01	2,56	1,45
Deviation, %	—	+1,2	—	−1,2	+1,9	+3,2	+0,7
Experimental value of t	57	112	162	210	257	327	187
Theoretical value of t for SH groups in sample	56	110	162	215	267	322	188
Deviation, %	−1,8	−1,8	—	+2,4	+3,9	−1,5	+0,6

TABLE 2. Concentration of SH Groups in Samples Tested by Iodometric (I), Spectophotometric (S), and Coulometric (C) Methods

Cysteine content in sample, mg	Content of SH groups in 10^{-6} M			
	calculated theoretically	I	S	C
0,05	0,41	0,43 (+4,8)	0,42 (+2,4)	0,41
0,10	0,83	0,82 (−1,3)	—	0,84 (+1,2)
0,15	1,24	1,24	—	1,24
0,20	1,65	1,63 (−1,2)	—	1,63 (−1,2)
Mean 0,125	1,03	1,03	1,05	1,03

Legend. Deviation (in %) in parentheses.

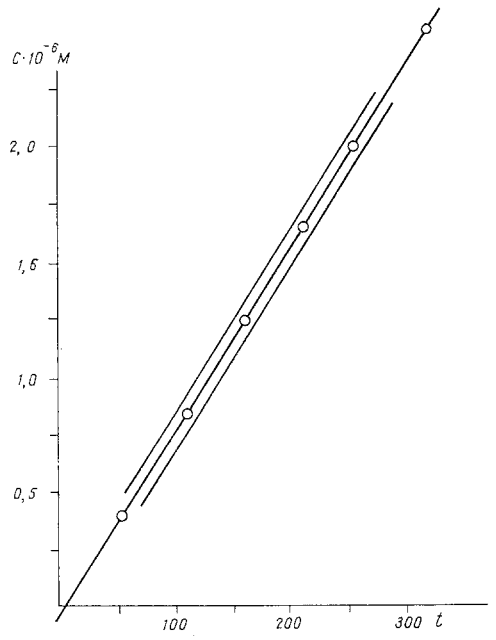

Fig. 1. Theoretical regression lines of SH groups (C) plotted against titration time (t). Outer lines show limits of deviation, circles represent experimental values of SH groups.

TABLE 3. Concentration of SH Groups in Homogenate of Cerebral Cortex of Animals (in 10^{-6} M/100 g wet weight of tissue)

Cerebral cortex of	Concentration of SH groups in 10^{-6} M
Cat	0.56-0.75 (Savich and Yakovlev [4])
Dog	0.78 (Gerasimovich [1])
Rat	0.75 (Our own data)

Complete agreement between the theoretical and experimental values for the content of cysteine SH groups in the sample is shown in Table 1. Deviations from theoretical values in individual experiments did not exceed on average 1.6-2.3%, and at a higher level (mean of all cysteine concentrations tested) 0.6-0.7%, within the limits of acceptable error for biological experiments.

A theoretical regression line was drawn on the graph by using coefficients of regression (a, b). As Fig. 1 shows, the experimental points for the concentration of SH groups do not deviate from the theoretical regression line; the error of regression in this case was 3.8%. According to calculations $t = 27.7$, which is higher than the value required at the $P < 0.001$ level of significance. Consequently, the sensitivity of the method is 0.15×10^{-6} M SH groups.

Special experiments were undertaken for the comparative analysis of determination of SH groups in the same samples by iodometric titration, by Ellman's spectrophotometric method [6], and by the method now suggested. It will be clear from Table 2 that the results of determination of SH groups in the test samples corresponded reliably to values for SH groups in cysteine calculated theoretically. Experiments to determine SH groups in homogenates of rat cerebral cortex confirmed the high reproducibility of the method. The results are in agreement with data in the literature [1, 4] relating to the study of the concentration of SH groups in the cerebral cortex of cats and dogs (Table 3).

COURSE OF DETERMINATION

The test solution is added to a solution of the titration medium and transferred to a glass titration cell. The electrodes are immersed in the solution of the mixture (15 ml). The counter is set at zero. The titration tumbler switch is moved to the on position. After the end of titration, the red lamp lights up. The time on the counter is read. The duration of the background titration (t) is determined in parallel tests and is subtracted from the values of t for titration of the background plus test substance.

EXAMPLE OF CALCULATION

Background titration time 20, titration time for background plus test substance 120, difference 100. Concentration of SH groups in test sample:

$$C = (0.008 \cdot 100 - 0.05) \cdot 10^{-6} \text{ M} = 0.75 \cdot 10^{-6} \text{ M SH groups per sample.}$$

(Details of the method of using the T-201 instrument and of testing will be found in the instructions supplied with the titrator.)

It can thus be concluded from these results that the simplified method of determining SH groups by means of the T-201 laboratory titrator now suggested is highly sensitive and gives good reproducibility. This method can be widely applied in all scientific and industrial biochemical laboratories where there is the need to determine SH groups in proteins or other compounds.

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