ACTA CHEMICA SCANDINAVICA 1 (1947) 328—334

The Diffusion Constant of Danish Penicillin and its Application to the Determination of the Molecular Weight

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Frieden [1] has determined the diffusion constant of American penicillin and found the value $D = 0.176$ at a temperature of 0.5° C. From this value the molecular weight was calculated, applying Einstein-Stokes' equation for the relation between D and the radius of the molecules. The value obtained was $M = 490$.

It has been the aim of the present work to determine the diffusion constant of Danish penicillin and its approximate molecular weight. These investigations can be carried out, although the substance is not so pure as it should be for the generally used methods of determination of the molecular weight.

EXPERIMENTAL

Fig. 1 shows the apparatus used. Concerning the principle of the apparatus and its application in practice the reader may be referred to Brodersen and Klenow [2].

In order to eliminate the disturbing influence of the electric charge of the penicillin ions, about 1 M KCl was used as a solvent. The lower liquid consisted of a Leo penicillin solution to which a corresponding quantity of KCl was added. The liquid was diluted to a concentration of 5 000—10 000 units per ml, and the molarity was 1 M with regard to KCl.

At the end of the diffusion period the diffusion liquid was displaced by mercury and collected from the outlet in fractions of about 1.5 g.

After several preliminary experiments the necessary period of diffusion was found to be about 72 hours, depending *i. a.* upon the strength of the penicillin solution and the dimensions of the apparatus.

The relative activity of the penicillin solutions was determined by testing their inhibition of growth of staphylococcus aureus seeded on agar bouillon plates. This method, the so-called cup method, has been fully described by Jensen, Møller, and Overgaard [3], and it has been further investigated by Vesterdal [4].

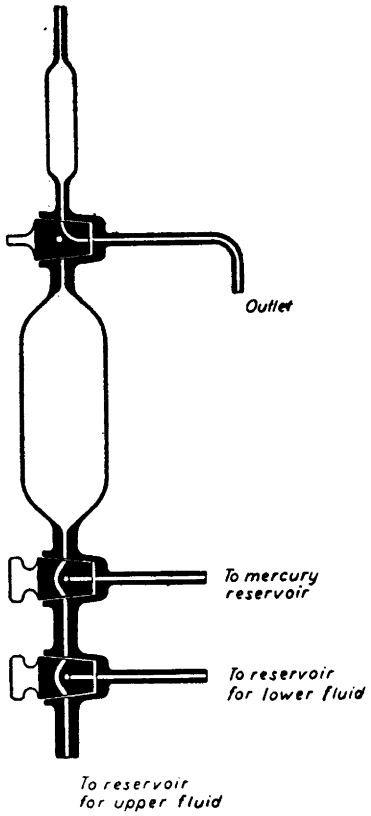

Fig. 1. The apparatus employed.

The activity of the penicillin samples was determined on the basis of a standard curve representing the inhibition produced by a series of dilutions of the last fraction of the diffusion liquid which had been removed from the apparatus. The diameters of the inhibition circles obtained from the diluted samples are plotted against the degree of dilution on logarithmic graph paper.

The curve obtained allows us to determine the relative concentrations of the individual fractions from the diameters of the inhibition circles. The mean of the two perpendicular inhibition diameters is used as a measure of the inhibitory strength.

In order to reduce the uncertainty of these determinations, the activity of each fraction is measured at several dilutions and, in the final calculation, the mean values are used.

Since both the diameter of the cylinder, the volumes of the individual fractions, and the volume of the displaced quantity of solvent are known, the mean distance x from the original boundary in the cylinder can be calculated for each fraction.

Table 1. First experiment. Temperature: 11.8—12.1° C. The penicillin solution displaced 6.50 g of the upper fluid. By testing the strength of fraction no. 15, it was found that the concentration in the region of the cylinder where the original boundary had been, actually was c_s.

Fraction no.	Weight of fraction in g	Distance from boundary in cm	Dilution	Inhibition in mm	Relative concentration $\frac{c}{c_s}$	$\log\frac{c}{c_s}$		Dt
1	2.66							
2	1.50							
3	1.47							
4	1.22	5.17	1 : 1	29.3	$2 \times 0.30 \times 10^{-5}$	—4.22		0.80
5	1.68	4.73	1 : 1	34.0	$2 \times 0.95 \times 10^{-4}$	—3.72	—3.72	0.79
			1 : 2	30.5	$4 \times 0.44 \times 10^{-4}$	—3.74		
			1 : 4	28.3	$8 \times 0.25 \times 10^{-4}$	—3.70		
6	1.40	4.27	1 : 1	41.0	$2 \times 0.46 \times 10^{-3}$	—3.04	—3.12	0.80
			1 : 2	37.5	$4 \times 0.22 \times 10^{-3}$	—3.06		
			1 : 4	33.8	$8 \times 0.97 \times 10^{-4}$	—3.11		
			1 : 8	29.6	$16 \times 0.34 \times 10^{-4}$	—3.26		
7	1.69	3.81	1 : 1	43.5	$2 \times 0.77 \times 10^{-3}$	—2.81	—2.77	0.74
			1 : 2	40.5	$4 \times 0.40 \times 10^{-3}$	—2.80		
			1 : 4	37.8	$8 \times 0.23 \times 10^{-3}$	—2.74		
			1 : 8	34.2	$16 \times 0.10 \times 10^{-3}$	—2.80		
			1 : 16	33.0	$32 \times 0.63 \times 10^{-4}$	—2.70		
8	1.42	3.34	1 : 4	44.0	$8 \times 0.84 \times 10^{-3}$	—2.18	—2.21	0.74
			1 : 8	40.0	$16 \times 0.37 \times 10^{-3}$	—2.23		
			1 : 16	36.9	$32 \times 0.19 \times 10^{-3}$	—2.23		
			1 : 32	34.0	$64 \times 0.95 \times 10^{-4}$	—2.20		
9	1.95	2.84	1 : 10	46.0	$20 \times 0.13 \times 10^{-2}$	—1.60	—1.46	0.90
			1 : 20	43.0	$40 \times 0.72 \times 10^{-3}$	—1.54		
			1 : 40	41.0	$80 \times 0.42 \times 10^{-3}$	—1.48		
			1 : 80	39.0	$160 \times 0.29 \times 10^{-2}$	—1.33		
			1 : 160	35.7	$320 \times 0.14 \times 10^{-3}$	—1.34		
10	1.73	2.28						
11	1.60	1.78						

Fraction no.	Weight of fraction in g	Distance from boundary in cm	Dilution	Inhibition in mm	Relative concentration $\frac{c}{c_s}$	$\log\frac{c}{c_s}$		Dt
12	1.78	1.28						
13	1.43	0.80						
14	1.21	0.40						
15	1.77	—0.05	1 : 1000	41.5	$2000 \times 0.50 \times 10^{-3}$	0.00	—0.11	
			1 : 2000	37.7	$4000 \times 0.83 \times 10^{-3}$	—0.08		
			1 : 4000	33.0	$8000 \times 0.67 \times 10^{-3}$	—0.21		
			1 : 8000	30.6	$16000 \times 0.70 \times 10^{-3}$	—0.16		
16	1.73							
17	1.48							
18	1.64							
19	0.61		1 : 1000	45.0	Series of standard dilutions			
			1 : 2000	40.8				
			1 : 4000	38.5				
			1 : 6000	36.3				
			1 : 8000	35.3				
			1 : 12000	34.0				
			1 : 16000	32.0				
			1 : 32000	29.2				
							Mean:	0.79_5

$D_{12}t = 0.79_5$, time $t = 2.90$ days, $D_{12} = 0.27_4$, $D_{10} = 0.26_1 \pm 0.00_6$ cm²/day.

Table 2. Second experiment. Temperature: 10.7—11.3° C.

Fraction no.	Distance from boundary in cm	$\log\frac{c}{c_s}$	Dt
5	4.92	—4.00	0.78
6	4.44	—3.39	0.78
7	4.05	—3.00	0.73
8	3.62	—2.47	0.77
9	3.15	—1.76	0.89
10	2.72	—1.68	0.70
		Mean:	0.775

$D_{11}t = 0.77_5$, time $t = 2.75$ days, $D_{11} = 0.28_2$, $D_{10} = 0.27_5 \pm 0.00_9$ cm²/day.

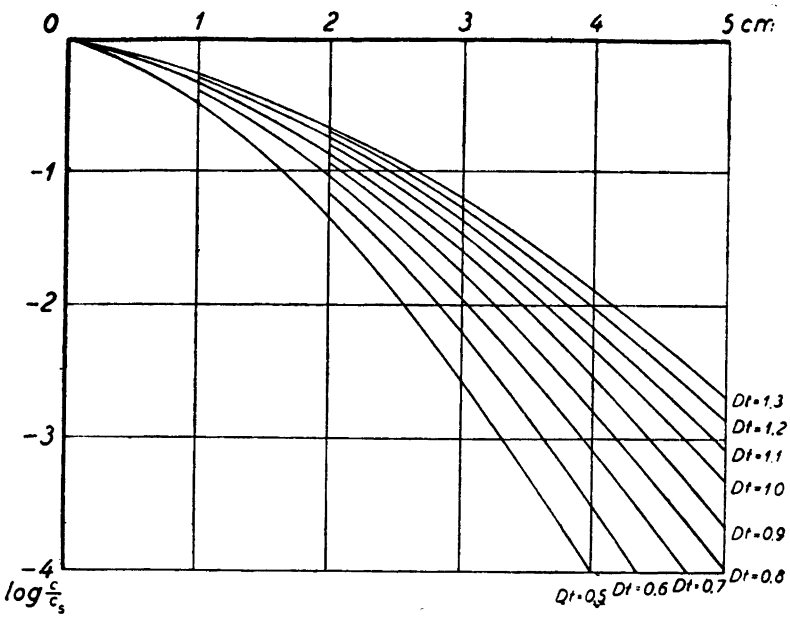

Fig. 2. The interdependence between the distance from boundary and $\log \frac{c}{c_s}$, *for different values of Dt.*

METHOD OF CALCULATION

By means of tables for the error integral which resembles the equation describing the dependence of the concentration on the path of diffusion after the lapse of time t, curves are drawn for different values of Dt; the path of diffusion is plotted as abscissa and the logarithm of the relative penicillin concentration as ordinate; here, half of the concentration of the initial penicillin solution (c_s) is taken as a unit. In this way, we arrive at the curves of fig. 2 (Brodersen and Klenow [2]).

When the points representing the experimental results are plotted, the corresponding values of Dt may be found by interpolation. The shape of the curves indicates that the most accurate determinations are obtained when the fractions of weakest concentration are used, because, in this case, a lower accuracy of the method of testing is required. Therefore, only the first ten fractions were applied; the first three or four figures were, however, discarded in view of the fact that these samples originated from the upper conical part of the cylinder, where diffusion did not take place according to Fick's law.

From the mean values of Dt for different fractions, D is calculated, the time of diffusion being known. For the sake of comparison, the experimental

results must be converted to the same temperature, *viz.* 10° C. The temperature coefficient was assumed to be 2.6 % per degree, as found by Jander and Winkel [5].

As the two experiments were performed analogously, the measurements from the first experiment only are given (table 1), while the results of the second experiment can be found from table 2. Mean value of the two experiments: $D_{10} = 0.26_8 \pm 0.00_9$ cm²/day.

ESTIMATION OF THE MOLECULAR WEIGHT

No unambiguous relation between the diffusion constant of a substance and its molecular weight M (ionic weight) is known. Several methods of calculation have been used in order to find M, when D is known. The empiric formula given by Riecke [6], *i. e.* $D\sqrt{M} = k$, where k is constant, leads to the best results for molecular weights below 500 (Stumpf [7]; Brodersen and Klenow [2]). The value for k was found by Øholm [8] to be 7.0—7.8 at 20° C, corresponding to 5.2—5.8 at 10° C. More recently, other investigators arrived at similar results. Brodersen and Klenow [2], in experiments with various substances, performed with the method and the apparatus described, found $D_{10}\sqrt{M}$ to be about 5.0.

Using this value for $D_{10}\sqrt{M}$, the ionic weight of Danish penicillin will turn out to be 346. The correctness of this figure depends upon whether 5.0 is the correct value for $D_{10}\sqrt{M}$ in the case of penicillin. In the experimental series just mentioned, this value varied for the different substances tested so that the value obtained for M (in our case the ionic weight) is encumbered with a greater uncertainty than that of the determination of D.

On the basis of the constitutional formulas stated by British and American authors (Committee on Medical Research, and the Medical Research Council') the ionic weights of various penicillins are from 312 to 350.

By means of decomposition experiments, Brodersen [10] showed that Danish penicillin consists primarily of one component, the other components being present in so minute quantities that they can be regarded as insignificant in the present experiments. It was also shown that decomposition takes place according to a reaction of the first order. Therefore, a decomposition of penicillin, if at all occurring, need not be taken into consideration.

SUMMARY

In diffusion experiments with a solution of Danish penicillin (Leo), using the cup method for the concentration determinations, the diffusion constant

at 10° C was found to be $D_{10} = 0.26_8 \pm 0.00_9$ cm²/day. Applying the formula $D_{10} \sqrt{M} = 5.0$, the ionic weight was found to be 346.

From the Anglo-American formulas for the different penicillins the values 312—350 were calculated.

The present work was performed with samples of Leo penicillin most kindly furnished by Professor K. A. Jensen, M. D.

The work was made possible by a University scholarship placed at the disposal of Rolf Brodersen, A. M., to whom my thanks are furthermore due for valuable advice and suggestions during the performance of the experiments.

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Received March 16, 1947.