CLXXIX. NOTE ON THE USE OF DIOXAN AS SOLVENT IN THE DETERMINATION OF MOLECULAR WEIGHTS BY THE CRYOSCOPIC METHOD.

By ALBERT EDWARD OXFORD.

From the Division of Biochemistry, London School of Hygiene and Tropical Medicine, University of London.

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IN the course of work carried out in this laboratory on the constitution of complex mould metabolic products—many of which are polybasic acids, or otherwise highly oxygenated compounds, very sensitive to the action of heat or mild reagents—the need for a neutral, chemically inert, non-hydroxylic liquid of extensive solvent powers and of convenient freezing-point just below room temperature, has often been felt. With such a solvent it would be possible to determine the molecular weight of almost any organic compound quickly and accurately. The need arises because many mould metabolic products are not sufficiently soluble in cold benzene, ethylene dibromide or nitrobenzene to allow of an accurate determination of the molecular weight by the depression of the freezing-points of these solvents, and other solvents such as water and glacial acetic acid are obviously unsuitable for this type of compound. Further, the Rast camphor method cannot be used with compounds which decompose below 170° , and has indeed often yielded abnormal results with polybasic acids apparently quite stable at this temperature. It has been found that the properties of dioxan (diethylene dioxide, $\text{O}:(\text{CH}_2\cdot\text{CH}_2)_2\text{O}$; B.P. 101.4° ; F.P. 11.67° ; density, 1.0) closely approach those of the ideal solvent mentioned above. This observation is not new, but appears not to be generally known since, as far as the author is aware, it has not previously been explicitly stated.

Dioxan is a colourless, stable liquid, chemically quite inert, the solvent powers of which at room temperature are wide and unique. Its freezing-point is very conveniently just below the ordinary room temperature and its molecular depression constant (K) is relatively large. Furthermore, no compound (neutral, acidic or basic) so far tested dissolves in dioxan to give a solution containing any considerable proportion of polymerised or dissociated molecules, provided that the solute contains no water or other solvent of crystallisation. Since dioxan is only slightly hygroscopic, it follows that reliable molecular weights can be determined in the usual Beckmann apparatus.

The use of dioxan for this purpose was first mentioned by Anschütz and Broeker [1926] and the method for its purification was improved by Eigenberger [1931], but these authors appear not to have made a detailed study of its solvent powers. Briefly it may be stated that hydrocarbons, sterols, alcohols and carbonyl compounds of the aliphatic and terpene series, aliphatic and aromatic mono- and poly-basic and hydroxy-acids (free or esterified), aromatic nitro-compounds, phenols (free, methylated or acetylated), amides (*e.g.* hippuric acid)

and many bases are in general so readily soluble in cold dioxan that depressions in F.P. of 0.4° or more can be obtained. Some of the results on which this statement is based are collected in Table I, from which it can be seen that the

Table I.

$$K = \frac{\Delta M}{C}, \text{ where } M = \text{molecular weight of solute.}$$

Δ = depression in F.P. in ° C.

C = g. solute dissolved in 100 g. dioxan.

Solute	M	C	Δ	K
Neutral substances:				
1. Choral hydrate. $\text{CCl}_3 \cdot \text{CH}(\text{OH})_2$	165.4	3.264	0.936	47.4
2. Triphenylmethane. $\text{C}_{19}\text{H}_{15}$. M.P. 92°	244.1	3.885	0.754	47.4
Phenols:				
3. 6:8-Dihydroxy-3-methylisocoumarin. $\text{C}_{10}\text{H}_8\text{O}_4$	192	0.998	0.235	45.2
4. Quinol. $\text{C}_6\text{H}_6\text{O}_2$	110	1.891	0.813	47.3
5. Kojic acid. $\text{C}_6\text{H}_6\text{O}_4$	142	2.079	0.663	45.3
Acids:				
6. Benzoic acid. $\text{C}_7\text{H}_6\text{O}_2$	122	1.780	0.678	46.5
7. Byssochlamic acid. $\text{C}_{18}\text{H}_{20}\text{O}_6$ [Raistrick and Smith, 1933]	332.2	3.493	0.472	44.9
8. Carlic acid. $\text{C}_{10}\text{H}_{10}\text{O}_6$ [Clutterbuck <i>et al.</i> , 1934]	226	1.814	0.352	43.6
9. Fumaric acid. $\text{C}_4\text{H}_4\text{O}_4$	116	1.398	0.578	48.0
10. 6-Methylsalicylic acid. $\text{C}_8\text{H}_8(\text{OH})(\text{CH}_3)(\text{COOH})$	152	1.956	0.595	46.2
11. Mycophenolic acid. $\text{C}_{17}\text{H}_{20}\text{O}_6$	320.2	3.641	0.521	45.8
12. Palmitic acid. $\text{C}_{15}\text{H}_{31} \cdot \text{COOH}$	256.2	3.840	0.722	48.1
13. Spiculisporic acid. $\text{C}_{17}\text{H}_{28}\text{O}_6$	328.2	5.799	0.857	48.5
14. Succinic acid. $\text{C}_4\text{H}_6\text{O}_4$	118	0.954	0.377	46.6
15. Terreic acid. $\text{C}_8\text{H}_{10}\text{O}_3$ [Raistrick and Smith, 1934]	154	2.702	0.815	46.5
16. Tartaric acid. $\text{C}_4\text{H}_6\text{O}_6$	150	4.344	1.337	46.2
Base:				
17. Caffeine (anhydrous). $\text{C}_8\text{H}_{10}\text{O}_2\text{N}_4$	194	1.184	0.297	48.7
			Mean	47

value of the constant K is, with a few exceptions to be discussed later, always very close to 47, the mean of the most reliable determinations. This value also agrees well with that obtained by Eigenberger (*viz.* 48) who tested only four solutes. The following types of compounds are however not sufficiently soluble in cold dioxan (*i.e.* they give a saturated solution of concentration less than 0.04 M whose F.P. is less than 0.2° below that of pure dioxan) for an accurate determination of their molecular weights to be made: amino-acids (*e.g.* leucine, aspartic acid); alkali salts of organic acids; carbohydrates (*e.g.* glucose, arabinose); glucosides (*e.g.* salicin); sugar alcohols (*e.g.* mannitol, sorbitol); anthraquinone; certain mould pigments related to anthraquinone (*e.g.* helminthosporin) or xanthone (*e.g.* citromycetin); urea; certain complex bases (*e.g.* cinchonine); and salts of bases (*e.g.* aniline hydrochloride, quinine sulphate). All these compounds, with the exception of the amino-acids, alkali salts and carbohydrates, are however appreciably (some readily) soluble in boiling dioxan and crystallise well on cooling, so that their molecular weights could doubtless have been determined by the ebullioscopic method with dioxan as solvent (for which $K = 22$ according to Anschütz and Broeker), provided that the solute is stable at 101°.

It will be noticed that certain solutes in Table II, *viz.* citric and oxalic acids, the hydrated form of 3:5-dihydroxy-2-carboxybenzoylmethylketone [Oxford and Raistrick, 1933] and brucine give abnormally high values for K . These all contain water of crystallisation and the affinity of dioxan for water is such that water of crystallisation is split off from the solute molecules. Since the

constant is in no case as high as it ought to be if complete dissociation had taken place, it follows that the water molecules are shared between solvent and solute. It is worthy of note that with chloral hydrate as solute a perfectly normal value for K is obtained. The accepted idea that that compound contains water of constitution and not of crystallisation is thus confirmed. On the other hand, the molecular compound $\text{CH}(\text{C}_6\text{H}_5)_3$, C_6H_6 (triphenylmethane crystallised from benzene) is completely dissociated in dioxan solution, the value for K being almost exactly twice that for normal compounds.

The only solute so far tested which appears to polymerise appreciably in dioxan solution (or to form mixed crystals with the solvent) is carolic acid (see Table II). The abnormality in this case is however not very great, since, using the mean value of $K=47$, the molecular weight of carolic acid is found to be 209 instead of 182.

Table II. *Substances which give abnormal values for K when dissolved in dioxan.*

Associated:	Solute	M	C	Δ	K
	Carolic acid. $\text{C}_9\text{H}_{10}\text{O}_4$ [Clutterbuck <i>et al.</i> , 1934]	182	3.978	0.894	40.9
Dissociated:					
	1. Triphenylmethane (crystallised from C_6H_6). $\text{C}_{19}\text{H}_{16}$, C_6H_6 . M.P. 76°	322.2	3.459	0.993	92.5
	2. Citric acid. $\text{C}_6\text{H}_8\text{O}_7$, H_2O	210	2.293	0.911	83.4
	3. Hydrated form of 3:5-dihydroxy-2-carboxy-benzoyl methyl ketone. $\text{C}_{10}\text{H}_8\text{O}_8$, H_2O	242	4.107	1.363	80.3
	4. Minioluteic acid. $\text{C}_{16}\text{H}_{22}\text{O}_7$. Air-dried. (Contains some H_2O [Birkinshaw and Raistrick, 1934])	330.2	3.329	0.818	81.1*
	5. Oxalic acid. $\text{C}_2\text{H}_2\text{O}_4$, $2\text{H}_2\text{O}$	126	1.481	1.377	117.2
	6. Brucine. $\text{C}_{23}\text{H}_{26}\text{O}_4\text{N}_2$, $4\text{H}_2\text{O}$	466.3	1.657	0.372	104.7

* Minioluteic acid after drying at 110° for $3\frac{1}{2}$ hours gave an almost normal value for K , viz. 53.5.

Since dioxan may fairly be considered to supersede all the solvents usually employed for the determination of molecular weights by the cryoscopic method, it appeared feasible to develop a semi-micro method by replacing the customary Beckmann wide-bulbed thermometer by an ordinary thermometer with a narrow bulb (size of bulb 5×0.7 cm.), graduated in 0.02° over the range 10 – 12° only. Freezing-points were determined in a small test-tube ($15 \times 1\frac{1}{4}$ cm.) fitted with a cork carrying the thermometer, and for stirrer a length of nichrome wire (s.w.g. 26, such as is used for making platinum loops for bacteriological work) suitably bent at the lower end, was used. Its upper end was pushed into a cork in order that heat from the hand should not be conducted into the liquid, and a small groove was cut in the thermometer cork, through which the wire passed. The volume of solvent required was 5 ml. and reliable results were obtained with this simple apparatus by observing the following points:

(a) Stirring should be slow throughout, otherwise too much heat is developed by friction.

(b) No attempt should be made to cause the liquid to freeze until it is supercooled at least 0.4° .

The great advantage of this apparatus is that when freezing does take place the mercury thread rises very swiftly to a maximum at which it remains for a considerable time. The results obtained are given in Table III. No doubt a satisfactory micro-method could be devised by using a micro-Beckmann

Table III. *Molecular weights determined by the semi-micro method.*

Substance	Weight of substance in mg.	Weight of dioxan in g.	Δ	M (obs.)	M (theor.)
Benzoic acid	40.4	5.61	0.292	116	122
<i>p</i> -Nitraniline	43.8	5.25	0.287	137	138
Flavoglaucin. $C_{19}H_{28}O_3$ [Gould and Raistrick, 1934]	90.2	5.51	0.267	288	304
Auroglaucin. $C_{19}H_{22}O_3$ [Gould and Raistrick, 1934]	88.5	5.36	0.263	295	298
Citrinin. $C_{18}H_{14}O_5$	68.5	5.53	0.225	259	250

thermometer and an apparatus suitably designed for protection of the solvent from atmospheric moisture.

Since dioxan is miscible in all proportions with both water and light petroleum, most solutes may be recovered quantitatively by addition of one or other of these solvents.

EXPERIMENTAL.

Purification of dioxan.

Technical dioxan (B.D.H.), which contains considerable amounts of acetaldehyde and ethylene acetal, was purified by the method given by Eigenberger [1931]. 900 g. were boiled with *N* HCl (90 g.) during two working days, a slow stream of air being continuously drawn through the condenser to remove acetaldehyde formed by hydrolysis of the acetal. At the end of this period, a test portion of the liquid still gave a positive reaction with Brady's 2:4-dinitrophenylhydrazine reagent, although not nearly as strongly as at the beginning. Water and HCl were removed by treatment with solid KOH, the dioxan top layer being separated and further dried over solid KOH and finally over sodium for a day. The product was finally distilled over sodium, the distillate being collected in receivers protected from atmospheric moisture by $CaCl_2$ -tubes. The first fraction (B.P. 101–101.2°, 57 g.) gave a positive reaction with Brady's reagent and was not used for molecular weight determinations. The next fraction (B.P. 101.2–101.4°; 168 g.) had F.P. 11.5° and gave no reaction with Brady's reagent. The third and main fraction (525 g.) boiled constantly at 101.4° and had F.P. 11.67°* and moreover behaved like a pure liquid at its F.P. It gave no reaction with Brady's reagent. It was also noticed that just before the changing of the receivers previous to the collection of the main fraction, the sodium in the distillation flask had assumed the form of numerous silver-bright, smooth spheres. The end fraction (B.P. 101.4°) weighed only 31 g. The main fraction was regarded as pure dioxan and was used for the determination of most of the values of *K* recorded in Table I.

Method of determination of F.P.

Since dioxan is appreciably hygroscopic, the following modified method was used. A large quantity of the pure solvent was prepared and stored in a bottle with a well-fitting stopper. The freezing-point on the scale of a Beckmann thermometer appropriately set was determined once and for all and noted on the label, and was found not to vary more than a few thousandths of a degree over several months. The solute is finely powdered and weighed directly into a boiling-tube, which is afterwards corked and weighed. The requisite volume of

* According to Eigenberger, pure dioxan melts at 12.5–13°.

solvent is then added and the tube immediately corked and re-weighed; then gently shaken until all the solute has dissolved, with gentle warming if necessary. The cork is then replaced by another bearing the thermometer and stirrer (a nickel wire) and the F.P. determined immediately. The first reading is generally a few thousandths of a degree higher than the next and so on, since slow absorption of atmospheric moisture takes place, even if the glass tube through which the stirrer passes is fitted with narrow rubber tubing to encase the upper part of the stirrer. The highest reading (almost always the first) is recorded as the F.P. of the solution. The setting of the Beckmann thermometer must of course not be altered during the whole time this bottle of solvent is in use.

SUMMARY.

Many types of crystalline organic compounds, including most natural products other than sugars and amino-acids, are so readily soluble in cold dioxan that this solvent, being chemically quite inert, may not only supplement the use of, but also advantageously replace, all the others usually employed for the determination of molecular weight by the cryoscopic method. Pure dioxan has F.P. 11.67° and its molecular depression constant, K , is equal to 47.

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