

**Collaborative Study of Molecular Weight Determination  
Applicable to Samples with Molecular Weights Less Than 500**

## **Collaborative Study of Molecular Weight Determination Applicable to Samples with Molecular Weights Less Than 500**

By C. L. OGG and L. H. SCROGGINS (Eastern Utilization Research and Development Division, U.S. Department of Agriculture, 600 E. Mermaid Lane, Philadelphia, Pa. 19118)

**A method for determining molecular weights employing the thermoelectric-vapor pressure technique was tested by 13 collaborators. The samples used in the study were benzoic and nicotinic acids, *n*-hexadecylamine, methylpentadecyl ketone, benzylisothiurea hydrochloride, and sulfanilamide. Data from the six samples were analyzed statistically by Youden's technique for collaborative tests. Most deviation between laboratories was due to random errors; systematic errors were low. Choice of solvent and standard were critical. Methanol was shown to be unsatisfactory as a solvent. It is recommended that the method be adopted as official, first action.**

The results of the study on molecular weight determination conducted in 1965 (1) indicated that the chief problem in the isothermal distillation method was selection of a suitable solvent and reference standard. In the method presented for study this year, preferred solvents and standards were recommended. Recommendations were based on the results of a study conducted by the authors (2) and on the data from the previous study.

The procedure, seven samples, and a report form were sent to all collaborators. Each was asked to make one analysis on each sample and to report the molecular weight found, along with other pertinent information on the procedure. Since no one solvent could be used for all samples, the method submitted listed different solvents in order of preference, along with a supplemental list of solvents to be used should the sample not be soluble in any of the recommended solvents. Standard reference materials were also specified. The samples

were: I, benzoic acid; II, nicotinic acid; III, *n*-hexadecylamine; IV, methylpentadecyl ketone; V, benzyl isothiouraea hydrochloride; VI, sulfanilamide; and VII, glycerol tristearate. The study was designed to follow Youden's proposed technique (3) for collaborative tests using paired samples; each collaborator performed single analyses on each sample.

## METHOD

### Apparatus and Reagents

(a) *Molecular weight apparatus*.—Vapor pressure osmometer, Hewlett-Packard; molecular wt app., Hitachi Perkin-Elmer; isothermal dist app., Arthur H. Thomas; or equiv. equipment using vapor pressure equilibrium technique. Instrument must use sensitive bridge system to measure temp. difference between solv. and test soln drops suspended on thermistors in constant temp. cell whose atmosphere is satd with solv. vapor.

(b) *Benzil std.*—Molecular wt 210.23, mp 94.5–95.5°, and C and H analyses within 0.2% of theoretical values (C, 79.99; H, 4.79).

Recrystallize from EtOAc, acetone, or CHCl<sub>3</sub>, if necessary. If sample is ionizable salt sol. in H<sub>2</sub>O, use reagent grade KCl as std. If sample is not salt and sol. only in H<sub>2</sub>O, use sucrose as std.

(c) *Solvents*.—Use reagent grade solv. from same lot and preferably from same bottle to sat. cell and to prep. sample and std solns. Solv. must completely dissolve sample, preferably without heating. (Proper choice of solv. and std is critical.) Preferred solvents are (number indicates order of choice):

Solvent	Nature of Sample				
	Un-known	Neu-tral	Acidic	Basic	Salt
Acetone	2	1	1	—	—
Ethyl acetate	1	2	2	1	—
Chloroform	3	3	—	2	—
Water	—	—	—	—	1

For samples not sol. in solvs listed, test solubility in H<sub>2</sub>O (if thermistor wiring is completely encased in glass or plastic), *n*-heptane, and benzene. Other solvs that may be used are: EtOH, CCl<sub>4</sub>, methylethyl ketone, dioxane, cyclohexane, CH<sub>2</sub>Cl<sub>2</sub>, dimethyl formamide, toluene, and acetonitrile. Use solvents such as esters, ketones, or alcohols for samples which tend to form dimers thru H bonding, e.g., org. acids.

### Determination

Follow manufacturer's instructions including recommended concn range for solns, instrument operation, and reading of  $\Delta R$  response.

Adjust cell temp. so vapor pressure of solv. is 150–350 mm, preferably 200–300 mm. If instrument is not equipped to cool cell, cell temp. must be enough above ambient (ca 5°) so thermostatic control maintains constant cell temp.

Construct calibration curve with std and solv. to be used in analysis. Det.  $\Delta R$  response at 4 std concns in recommended range and plot  $\Delta R$  against mole fraction (MF). Prep. sample soln in recommended range and obtain 3  $\Delta R$  readings. Use median  $\Delta R$  value to calc. molecular wt. If calibration curve is straight line, calc. molecular wt (MW) of sample by:

$$MW = (\text{g solute})(MW \text{ solv.})(K - \Delta R)/(\Delta R)(\text{g solv.})$$

where  $K = (\Delta R \text{ std})/(\text{MF std})$  and  $M \text{ std} = [(\text{g std})/(\text{MW std})]/[(\text{g std}/\text{MW std}) + (\text{g solv.}/\text{MW solv.})]$

If  $\Delta R$ -MF plot yields curved line, interpolate MF of sample from calibration curve and calc. molecular wt by:

$$MW = (\text{g solute})(MW \text{ solv.})(1 - \text{MF})/(\text{MF})(\text{g solv.})$$

### Results and Discussion

Thirteen collaborators reported molecular weight data. The data from samples I and II, the two acids with similar molecular weights, were paired and analyzed according to Youden. A plot of the data is shown in Fig. 1; the solid lines are the means for the two samples and the dotted lines are the theoretical values. The four pairs of values marked with an X are outliers at the 0.05 level according to Dixon's test (4). In addition to being an outlier the pair at X = 190 should be eliminated on the basis that the wrong standard was used in the analysis. The solvent used for all four pairs of outliers was methanol, which was included in the list of preferred solvents sent

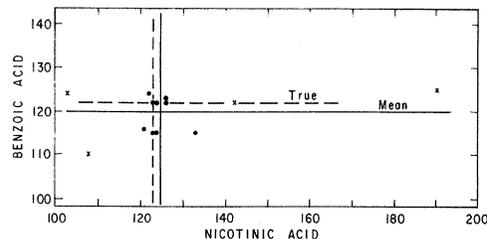


Fig. 1—Collaborative molecular weight data for sample pair I, plotted according to Youden (3).

to collaborators but has since been deleted because of the poor results.

Table 1 gives the molecular weight and statistical data for pair I, benzoic and nicotinic acids, with the four pairs of outliers eliminated. The deviation of the mean from theoretical molecular weight was 2.4 for benzoic acid and 1.6 for nicotinic acid. The low  $S_b$  and  $F$  values show that the systematic errors in the procedure are low. Although most of the distribution of the actual data is due to random errors ( $S_r = 3.16$ ), the interlaboratory precision ( $S_d = 3.68$ ; coefficient of variation = 3.0%) is reasonably good for this type of determination.

The second pair of samples, *n*-hexadecylamine and methylpentadecyl ketone, although of dissimilar composition, have similar molecular weights. One limitation on the amine was that acetone should not be used as the solvent because of the tendency toward high results with such a system. Two collaborators used acetone even though it was not recommended and both obtained results higher than the true

**Table 1. Molecular weight and statistical data for collaborative pair I: benzoic and nicotinic acids**

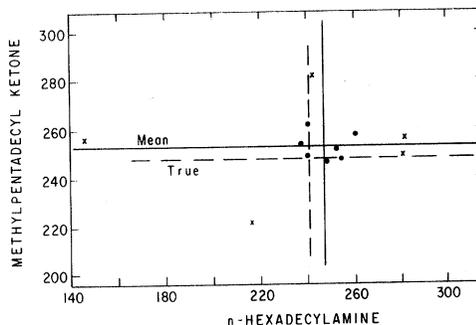
Coll.	Benzoic Acid	Nicotinic Acid	Differences (D)	Sums (T)
0	122	123	1	245
2	118	124	6	242
7	115	133	18	248
17	122	126	4	248
19	124	122	2	246
30	122	124	2	246
36	115	123	8	238
59	123	126	3	249
62	116	121	5	237
Total	1077	1122	49	2199
Mean	119.7	124.7	5.4	244.6
Theoretical	122.1	123.1	1.0	245.2

$S_d = 3.68 =$  s.d. due to distribution of actual data.  
 $S_r = 3.16 =$  s.d. due to random error.  
 $S_b = 1.88 =$  s.d. due to bias or systematic error.  
 $S_d^2 = 13.52$ .  
 $S_r^2 = 10.01$ .  
 $F = 1.35$ .  
 Coeff. of var. = 3.0%.

$$S_d = \sqrt{\frac{\sum(T_i - \bar{T})^2}{2(n-1)}}$$

$$S_r = \sqrt{\frac{\sum(D_i - \bar{D})^2}{2(n-1)}}$$

$$S_b = \sqrt{\frac{S_d^2 + S_r^2}{2}}$$



**Fig. 2.—Collaborative molecular weight data for sample pair II, plotted according to Youden (3).**

value though not among the outliers. Twelve pairs of values were reported for pair II. Of the six values eliminated, five were outliers by Dixon's test and the sixth result was excluded because it was obtained with methanol as solvent. Four of the five outliers can be explained on the basis of solvent used: methanol (or ethanol) for three and a solvent that was not recommended for the fourth, leaving only one high value unexplained.

Figure 2 is a plot of the values for pair II, and Table 2 gives the statistical data for the seven pairs remaining after the elimination of outlier values and those obtained with an unsatisfactory solvent. Unfortunately, the elimination of a value for one sample eliminates both paired values in the statistical analysis so that 6 bad values out of 24 reduced the number of pairs from 12 to 7. The mean

**Table 2. Molecular weight and statistical data for collaborative pair II: *n*-hexadecylamine and methylpentadecyl ketone**

Coll.	<i>n</i> -Hexadecylamine	Methyl-pentadecyl Ketone	Differences (D)	Sums (T)
0	240	249	9	489
2	260	258	2	518
7	252	252	0	504
17	237	254	17	491
31	240	252	22	502
41	254	248	6	502
62	248	247	1	495
Total	1731	1770	57	3501
Average	247.3	252.9	8.1	500
Theoretical	241	248	7	489

$S_d = 6.92$     $S_d^2 = 47.92$    Coeff. of var. = 2.8%  
 $S_r = 5.99$     $S_r^2 = 35.91$   
 $S_b = 2.45$     $F = 1.33$

values for both samples were high by 5 and 6 units and again the  $F$  and  $S_b$  values indicate a low systematic error. Most of the deviation is due to random error ( $S_d = 6.92$  and  $S_r = 5.99$ ) and the coefficient of variation of 2.8% agrees with that for pair I.

Samples V and VI, benzylisothiourea hydrochloride and sulfanilamide, were to constitute pair III in the study. However, too many hydrochloride results were outliers, as seen in Fig. 3. The recommended solvent and standard for a salt such as sample V were methanol and LiCl. Of the four collaborators who used this solvent and standard, two obtained good values and two low values. LiCl has since been eliminated as a possible choice for standard. Four collaborators who used water and potassium chloride obtained molecular weights near the true value. Three collaborators used a nonionizing standard and consequently the molecular weights found were very low. The six values not eliminated by Dixon's test for outliers had a standard deviation of 2.06 and a mean deviation from theoretical of 2.7.

Twelve values were reported for sulfanilamide, sample VI, and only one was markedly different from the theoretical; the collaborator had chosen the wrong standard and used methanol as solvent. The remaining 11 values had a standard deviation of 6.14 (coefficient of variation = 3.6%) and a mean deviation from theoretical of 3.8. When two outliers were eliminated, the statistical values became  $S = 2.35$ , coefficient of variation 1.4%, and mean deviation from theoretical 1.9. The data for samples V and VI are shown in Table 3.

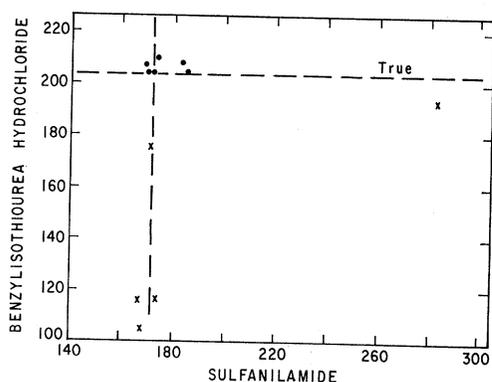


Fig. 3—Collaborative molecular weight data for sample pair III, plotted according to Youden (3).

Sample VII was included in the study to test the method on a higher molecular weight compound. The sample was supposed to have been 99% pure glycerol tristearate but, unfortunately, the authors did not analyze the sample before it was sent to the collaborators. Subsequent carbon, hydrogen, and infrared analysis showed the material to contain considerable free hydroxyl and to be nearer a distearate than the tristearate in elemental composition. The molecular weight data tended to confirm this, as most of the values were in the 660–700 range versus molecular weights of 625 for distearin and 891 for tristearin. Since the sample was not pure and the authors were not certain the sample was homogeneous, no analysis of the data has been made. The Associate Referee expresses his regrets to the collaborators for having asked them to analyze a worthless sample.

Table 4 indicates the outliers and summarizes some of the operational information gained from the data report form. The two commonly used instruments, Hewlett-Packard and Hitachi Perkin-Elmer, gave comparable data. Outliers usually occurred because the wrong solvent or standard was used. Eight of 14 values obtained with methanol as solvent and a suitable standard were outliers against only 3 of 32 values with acetone. None of the 14 values obtained with ethyl acetate, water, or chloroform as the solvent were outliers, whereas the use of solvents and standards other than those recommended accounted for the remaining outliers.

#### Conclusions and Recommendations

The study shows that choice of proper solvent and standard are critical factors in obtaining good results. There is a great temptation for the analyst to use the solvent already in the apparatus because changing solvent and re-equilibration takes time. This was true in the present study because at least three and preferably four solvents were required for good results, requiring a considerable amount of time and effort to complete the analyses.

The results of the study also show that methanol is not a suitable solvent. Whether the poor results with this solvent were due to a variable water content or to some other cause is not known, but they were not limited to one apparatus.

**Table 3. Molecular weight data, solvents, and standards used for samples V and VI**

Coll.	Benzylisothiurea Hydrochloride (V)			Sulfanilamide (VI)		
	MW	Solvent	Standard	MW	Solvent	Standard
0	204	H <sub>2</sub> O	KBr	172	acetone	benzil
2	209	H <sub>2</sub> O	KCl	173	acetone	benzil
7	207	H <sub>2</sub> O	KCl	183 <sup>a</sup>	acetone	benzil
17	175 <sup>a</sup>	95.6% C <sub>2</sub> H <sub>5</sub> OH	LiCl	171	acetone	benzil
19	105 <sup>a</sup>	95% C <sub>2</sub> H <sub>5</sub> OH	biphenyl	168	acetone	benzil
30	193 <sup>a</sup>	CH <sub>3</sub> OH	LiCl	282 <sup>a</sup>	CH <sub>3</sub> OH	LiCl
31	204	H <sub>2</sub> O	KCl	170	acetone	benzil
36	109 <sup>a</sup>	C <sub>2</sub> H <sub>5</sub> OH	benzil	—	—	—
41	206	CH <sub>3</sub> OH	LiCl	169	acetone	benzil
55	204	CH <sub>3</sub> OH	LiCl	185 <sup>a</sup>	acetone	benzil
59	—	—	—	172	acetone	benzil
62	116 <sup>a</sup>	CH <sub>3</sub> OH	LiCl	167	acetone	benzil
63	117 <sup>a</sup>	CH <sub>3</sub> OH	benzil	174	acetone	benzil

Theor. MW = 202.7  
s = 2.06  
Mean dev. from theor. = 2.7

Theor. MW = 172.2  
s = 2.35  
Mean dev. from theor. = 1.9

<sup>a</sup> Outliers eliminated before calculation of s.

**Table 4. Summary of outliers and operational details in collaborative study of isothermal distillation method of molecular weight determination**

Coll.	Outliers <sup>a</sup>						Equi- librium Time (min) <sup>c</sup>	No. of Drops	$\Delta R$ Reading Time (min) <sup>d</sup>	Apparatus
	I	II	III	IV	V	VI				
0	—	—	—	—	—	—	15	6	8-15	Noncommercial
2	—	—	—	—	—	—	5	8	2	Hewlett-Packard
7	—	—	—	—	—	H	30	6	2-3	Hewlett-Packard
17	—	—	—	—	L	—	30-60	6-8	2-10	Hewlett-Packard
19	—	—	L	—	L	—	5	10	3-6	Hitachi Perkin-Elmer
30	—	—	H	—	L	H	6	8	1.5-6	Hitachi Perkin-Elmer
31	—	L	—	—	—	—	5-10	10	1.5-4	Hitachi Perkin-Elmer
36	—	—	L	L	L	0	20	6	2-18	Hewlett-Packard
41	L	L	—	—	—	—	—	8	2-4	Hitachi Perkin-Elmer
55	—	H	—	H	—	H	20	8-10	2-3	Hewlett-Packard
59	—	—	0	—	0	—	10-35	10	2	Hewlett-Packard
62	—	—	—	—	L	—	5-6	6	3.5-7	A. H. Thomas
63	—	—	—	—	L	—	15	5	2	Hewlett-Packard

<sup>a</sup> H = high outlier, L = low outlier, 0 = no data.

<sup>b</sup> I, benzoic acid; II, nicotinic acid; III, *n*-hexadecylamine; IV, methylpentadecyl ketone; V, benzyl isothiurea hydrochloride; VI, sulfanilamide.

<sup>c</sup> Time between inserting syringe and placing sample on thermistor.

<sup>d</sup> Time between placing sample on thermistor and reading  $\Delta R$  value.

It is recommended—

(1) That the isothermal distillation method be adopted as official, first action for materials with molecular weights below 500.

(2) That the study of the method be continued to test its applicability to higher molecular weight materials.

#### Acknowledgments

The cooperation of the following collaborators is appreciated:

L. H. Bowen, North Carolina State University, Raleigh, N.C.

L. M. Brancone, Lederle Laboratories, American Cyanamid Co., Pearl River, N.Y.

C. J. Dalton, Hercules Powder Co., Wilmington, Del.

H. J. Francis, Jr., Pennsalt Chemical Corp., King of Prussia, Pa.

H. R. Friedberg, Crobaugh Laboratories, Cleveland, Ohio

D. F. Ketchum, Eastman Kodak Co., Rochester, N.Y.

J. Kobliska, American Cyanamid Co., Bound Brook, N.J.

J. H. McClure, E. I. du Pont de Nemours and Co., Wilmington, Del.

C. McGrew, U.S. Department of Agriculture, Peoria, Ill.

J. Mitchell, Jr., E. I. du Pont de Nemours and Co., Wilmington, Del.

R. Sreaton, Minneosta Mining and Manufacturing Co., St. Paul, Minn.

C. S. Yeh, Purdue University, Lafayette, Ind.

#### REFERENCES

- (1) Ogg, C. L., *This Journal* **49**, 744-749 (1966).
- (2) Scroggins, L. H., and Ogg, C. L., *ibid.* **51**, 992-999 (1968).
- (3) Youden, W. J., *Statistical Techniques for Collaborative Tests*, Association of Official Analytical Chemists, Washington, D.C., 1967.
- (4) Dixon, W. J., and Massey, F. J., Jr., *Introduction to Statistical Analysis*, 2nd Ed., McGraw-Hill Book Co., New York, 1957.