Molecular Estimates for Molar Attraction Constants

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Abstract □ A semiempirical method is developed by which molar attraction constants and cohesive energy densities (the Hildebrand–Scott solubility parameter δ) can be calculated for relatively nonpolar substances. In the simplest possible modification of the method, only molecular polarizabilities and molar volumes must be known to effect the calculation. Good agreement between the experimental and theoretical estimates is obtained. To illustrate the method, the cohesive energy densities are calculated for some drug agents and formulation constituents.

Keyphrases □ Molar attraction constants—method of calculation for nonpolar substances □ Cohesive energy density—method of calculation for nonpolar substances, values for some drugs and excipients □ Molecular estimates—molar attraction constants, semiempirical method of calculation □ Polarizability—used in calculating molar attraction constants

Since the pioneering work of Chertkoff and Martin (1) and of Restaino and Martin (2), the application of the Hildebrand–Scott solubility theory (3) to pharmaceutical systems has been a goal striven toward by other investigators (4–7) in hope that, appropriately applied, this theory might alleviate some problems associated with formulation design. There are significant, but not insurmountable, difficulties intrinsic to the application of this theory (*cf.*, *Reference 8*), one of the more important being the determination of "cohesive energy densities" or "internal pressures," δ, for pharmaceutical substances. Thermodynamic measurements on formulation constituents that allow a determination of δ are few, especially for drug entities (see, however, *Reference 9*), and this condition will likely persist into at least the near future.

Small (10) described an additive–constitutive method for determining δ values using atomic and group contributions for the molar attraction constant F and the molar volume V. This approach is based on the relationships:

$$\delta = \left(\frac{E}{V}\right)^{1/2} = \frac{(EV)^{1/2}}{V} = \frac{F}{V} \qquad \text{(Eq. 1)}$$

in which E is the energy of vaporization for a pure substance. Atomic and group contributions to F are calculated using thermodynamic measurements made on simple model compounds, and these are subsequently used in an additive–constitutive manner to determine F values for other more complex compounds. In a similar manner, atomic and group contributions, such as were tabulated by Exner (11), can be used to calculate V. Ostrenga (12), following a statistical procedure due to Exner (13), showed that F values are only approximately additive–constitutive.

In this report, we present a theoretical analysis of the molar attraction constant which: (*a*) provides a basis for its approximate additive–constitutive nature, (*b*) indicates conditions under which this additivity should break down, and (*c*) leads to a framework for determining molar attraction constants in a semiempirical manner from polarization measures. An outline of the features essential to this analysis was reported previously (14) in connection with structure–activity relationships having a basis in regular solution theory. This report is much more detailed regarding the verification of the relationships developed.

THEORY

According to Hildebrand and Scott (3, pp. 94–96, 124–129), the molar attraction constant F corresponds to the parameter a, or something very similar, in the van der Waals equation of state. Therefore, it is given by the expression:

$$F^2 = -2\pi N^2 \int_d^\infty \epsilon_{mm}\rho(r)r^2\,dr \qquad \text{(Eq. 2)}$$

in which ϵ_{mm} is the interaction energy between two like molecules, $\rho(r)$ is a distribution function which can be interpreted as giving the probability of having a center-to-center distance r separating the two molecules, and N is Avogadro's number. In general, $\rho(r)$ is a complicated function of r, but the use of the simplifying assumption $\rho(r) = 1$ will be considered. This amounts to a presumption that loosely structured liquids are under consideration, none of which has minimum energy separation distances leading to an effective stabilization. The limit d in Eq. 2 corresponds to the least separation distance providing a stabilization, which is the choice simplification.

For lack of a better representation, the Lennard–Jones "6–12" potential is used to describe the interaction energy ϵ_{mm}. This potential energy function is written:

$$\epsilon_{mm} = -\frac{k'}{r^6} + \frac{j}{r^{12}} \qquad \text{(Eq. 3)}$$

where the first term ($\sim 1/r^6$) gives the attraction energy and the last term ($\sim 1/r^{12}$) gives the repulsion energy. At distances corresponding to the least separation for a minimum energy interaction (around 4–6 Å for noncoulombic interactions), the contribution

Table I—Molecular Dispersion Energies and Ionization Potentials

Simple Molecules[a]			Complex Molecules		
Compound	$h\nu_0$, ev.	I, ev.	Compound	$h\nu_0$, ev.[b]	I, ev.[c]
Helium	25.5	24.5	Diethyl ether	15.79	9.60
Neon	25.7	21.5	Ethane	15.44	11.5
Argon	17.5	15.4	Methanol	14.31	10.84
Krypton	14.7	13.3	Cyclohexane	14.31	9.80
Xenon	12.2	11.5	Cyclohexene	13.79	8.72
Nitrogen	17.2	17	Cyclohexanone	13.76	9.14
Oxygen	14.7	13	Acetone	13.52	9.69
			Methylene bromide	12.27	10.49
			Methylene chloride	12.10	11.35
			Methyl chloride	11.56	11.30
			Ethylene	11.51	10.5
			Acetylene	10.73	11.4
			Benzene	10.68	9.24

[a] From *Reference 15*. [b] Calculated from data presented in W. Kauzman, "Quantum Chemistry," Academic, New York, N. Y., 1957, p. 692. [c] From "Handbook of Chemistry and Physics," 50th ed., R. C. Weast, Ed., Chemical Rubber Co., Cleveland, Ohio, 1970, p. E-80.

Table II—Molecular Properties Characterizing Molar Attraction Constants

Compound	δ(cal./mole-cm.^{3})$^{1/2}$ Experimental[a]	δ(cal./mole-cm.^{3})$^{1/2}$ Estimated[b]	F (cal.-cm.^{3}/mole)$^{1/2}$	V^c, cm.^{3}/mole	I^d, ev.	α^e, cm.^{3}(×10^{24})	$-\chi^f$, cm.^{3}(×10^6)
Cyclopentane	8.10	8.08	762	94.07	10.53	9.21	59.2
Cyclohexane	8.20	7.51	892	108.82	9.80	11.05	68.1
Methylcyclohexane	7.85	6.00	1101	140.30	9.85	12.88	78.9
Benzene	9.15	8.76	819	89.55	9.24	9.91	54.8
Toluene	8.90	8.25	945	106.13	8.82	12.32	66.1
Ethylbenzene	8.80	7.60	1080	122.71	8.76	14.16	77.2
o-Xylene	9.00	7.52	1104	122.71	8.56	14.18	77.9
m-Xylene	8.80	7.67	1080	122.71	8.58	14.44	76.6
p-Xylene	8.75	7.52	1074	122.71	8.44	14.27	76.8
n-Propylbenzene	8.65	7.09	1205	139.29	8.72	16.02	89.2
Mesitylene	8.80	10.32	949	107.81	8.40	16.17	92.3
Styrene	9.30	7.51	1089	117.13	8.47	13.27	68.2
Naphthalene	9.90	9.03	1210	122.22	8.12	17.37	91.9
Pyridine	10.70	9.55	883	82.56	9.30	9.53	49.2
Chlorobenzene	9.50	9.50	927	97.61	9.07	12.34	70.0
Nitrobenzene	10.00	10.20	992	99.16	9.92	12.97	61.8
Carbon tetrachloride	8.60	12.36	676	78.62	11.47	10.32	66.6
Methane	6.80	7.34	315	46.38	12.60	2.65	12.2
Ethane	7.60	7.51	479	62.96	11.50	4.49	27.3
n-Propane	6.00	7.34	477	79.54	11.10	6.34	40.5
n-Butane	6.70	6.97	644	96.12	10.63	8.17	57.4
Isobutane	6.25	6.96	601	96.12	10.57	8.18	51.7
n-Pentane	7.05	6.69	795	112.10	10.35	10.02	63.1
Isopentane	6.75	6.62	761	112.70	10.32	10.00	64.4
Neopentane	6.25	6.70	704	112.70	10.35	10.11	63.1
n-Hexane	7.30	6.34	944	129.28	10.18	11.85	74.6
Chloroform	9.30	11.81	656	70.56	11.42	8.40	59.3
Bromoform	10.50	13.15	843	80.25	10.51	11.83	82.6
Methyl iodide	9.90	11.45	638	64.41	9.54	7.77	57.2
Nitromethane	12.60	9.61	705	55.99	11.10	4.90	21.1
Dioxane	10.00	7.75	891	89.14	9.13	8.75	52.2
Diethyl ether	7.40	6.49	761	102.86	9.60	8.86	55.1
n-Heptane	7.45	6.03	1090	145.86	9.90	13.70	85.2
Ethylene	7.90	7.90	453	57.38	10.50	4.30	12.0
Propene	7.60	7.45	562	73.96	9.73	6.16	31.5
cis-2-Butene	7.20	7.05	652	90.54	9.13	8.15	42.6
trans-2-Butene	7.00	7.05	634	90.54	9.13	8.15	43.3
2-Methylpropene	6.70	7.23	607	90.54	9.60	8.15	44.4
Methyl chloride	8.60	9.39	468	54.44	11.30	4.55	32.0
Methylene chloride	9.70	10.88	606	62.50	11.35	6.47	46.6
Ethyl bromide	8.90	9.32	661	74.25	10.29	7.54	54.7
Ethylene chloride	9.80	9.20	775	79.08	9.99	8.30	59.6
Ethylene bromide	10.40	10.34	890	85.54	9.80	10.60	78.8
cis-1,2-Dichloroethylene	9.10	9.71	669	73.50	9.65	7.99	51.0
trans-1,2-Dichloroethylene	9.00	9.86	662	73.50	9.64	8.12	48.9
Methyl ether	8.80	9.92	613	69.70	9.98	5.17	26.3
Dimethoxymethane	8.20	6.70	763	93.02	10.00	7.71	47.3

[a] From *Reference 3*, pp. 435–439. [b] Based on Eq. 11*a*. [c] Calculated from values given in *Reference 10*. [d] From "Handbook of Chemistry and Physics," 50th ed., R. C. Weast, Ed., Chemical Rubber Co., Cleveland, Ohio, 1970, p. E-80. [e] Calculated from data given in A. I. Vogel, "Elementary Practical Organic Chemistry. Part 2: Qualitative Organic Analysis," 2nd ed., Wiley, New York, N. Y., 1966. [f] From "Handbook of Chemistry and Physics," 47th ed., R. C. Weast, Ed., Chemical Rubber Co., Cleveland, Ohio, 1967, pp. F9–F12.

of the repulsion term may be said to be small in comparison to the contribution of the attraction term. By taking this to be generally valid and by substituting only the attraction term of Eq. 3 and $\rho(r) \approx 1$ into Eq. 2, integration leads to the result:

$$F^2 = \frac{2\pi N^2}{3d^3}k' \qquad \text{(Eq. 4)}$$

where k' can be determined using quantum theory. For example, in the London theory (15) of intermolecular forces:

$$\epsilon_{mm} = -\frac{1}{r^6}\left(\frac{2\mu^4}{3kT} + 2\mu^2\alpha + \frac{3}{4}\alpha^2 h\nu_0\right) \qquad \text{(Eq. 5)}$$

where μ and α are the molecular dipole moment and polarizability, respectively; k is Boltzman's constant; h is Planck's constant; and ν_0 is the natural frequency that can be associated with oscillations of the valence-shell electrons of a molecule. The quantity k' in Eq. 4 thus corresponds to the terms given in parenthesis in Eq. 5.

From Eqs. 4 and 5, it is readily evident that the molar attraction constant becomes approximately additive only when the stabilization energy contributions due to dipole–dipole and dipole-induced dipole interactions are small in comparison with the stabilization energy contribution made by the induced dipole–induced dipole interaction. In other words, the molar attraction constants for highly polar substances should not fit within a simple additive–constitutive scheme. Even for compounds having little or no dipole moment, the molar attraction constant should be only approximately additive. Additivity in F is due to the additivity in α, and a departure from additivity in this ideal case is a consequence of variations in $h\nu_0$ and d in passing from one compound to another. For example, on the average, $h\nu_0$ is about 10 ev., but two substances whose F values are used to obtain an atomic or group contribution may have $h\nu_0$ as wide apart as 8 and 15 ev. Hence, by presuming that d varies over a very slight range so that it is effectively a true constant, a calculated constitutive contribution based on F values for two substances could differ substantially from the "best average" for the constitutive contribution determined from F values for a variety of similarly substituted compounds. Conversely, an F value calculated from a set of "best averages" for constitutive contributions could differ substantially from the thermodynamic F, since the average $h\nu_0$ (10 ev.) may differ considerably from the value of $h\nu_0$ specific to the compound. Each of these illustrative descriptions is, admittedly, the possible extreme, but they do serve to point out why, even under ideal circumstances, the molar attraction constant should be considered as only approximately additive–constitutive.

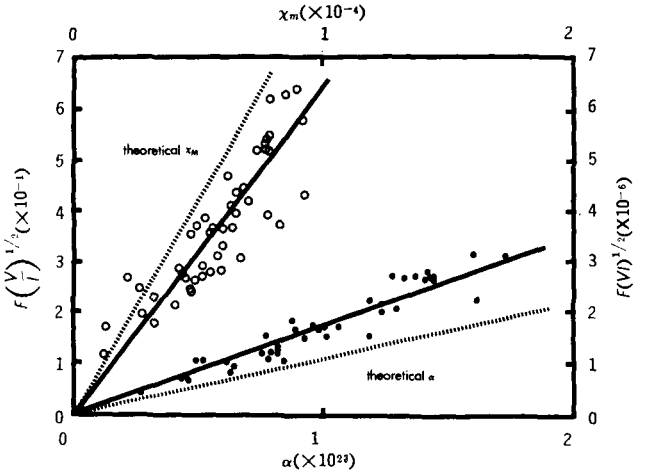

Figure 1—*Relationship of experimentally derived molar attraction constants to molecular polarizability* (●) *and its magnetic equivalent, diamagnetic susceptibility* (○). *The dotted line is the theoretical slope when the London approximation* (z = *1 in Eqs. 11*a *and 11*b) *is used.*

When the dispersion energy makes a dominant contribution to the intermolecular interaction energy ϵ_{mn}, a variety of equivalent theoretical and experimental estimates of ϵ_{mn} are possible. Linder (16) surveyed the more usual methods of estimation. In this report, only the two simple approaches originally considered by London (15) are discussed. They make use of the experimentally accessible quantities of electronic polarizability α and its magnetic equivalent, diamagnetic susceptibility χ.

Following London, the dispersion energy for the interaction between two unlike molecules m and n may be expressed:

$$\epsilon_{mn} = -\frac{2}{3r^6}\frac{(M_0^2)_m(M_0^2)_n}{h\nu_{0,m} + h\nu_{0,n}} \qquad \text{(Eq. 6)}$$

in which $(M_0^2)_m$ and $(M_0^2)_n$ are the electronic ground-state transition probabilities for each molecule. For two like molecules m and m, Eq. 6 becomes:

$$\epsilon_{mm} = -\frac{1}{3r^6}\frac{(M_0^2)^2}{h\nu_0} \qquad \text{(Eq. 7)}$$

so that, depending on the manner by which the transition moment (M_0^2) is to be estimated, a number of differing, but equivalent, expressions for the dispersion energy can be presented. Thus, evaluating (M_0^2) in terms of electronic polarizability leads (15, 17) to the relationship:

$$(M_0^2) = \frac{3}{2}\alpha h\nu_0 \qquad \text{(Eq. 8}a\text{)}$$

whereas evaluating (M_0^2) in terms of diamagnetic susceptibility

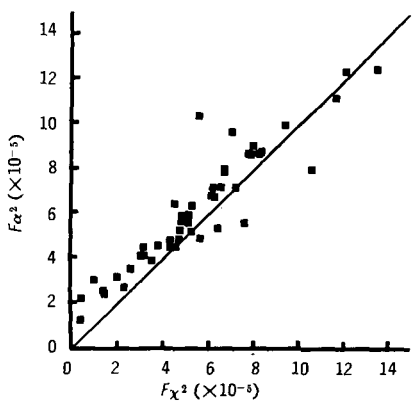

Figure 2—*Comparison of molar attraction constants calculated from molecular polarizabilities* (F_α) *and from diamagnetic susceptibilities* (F_χ).

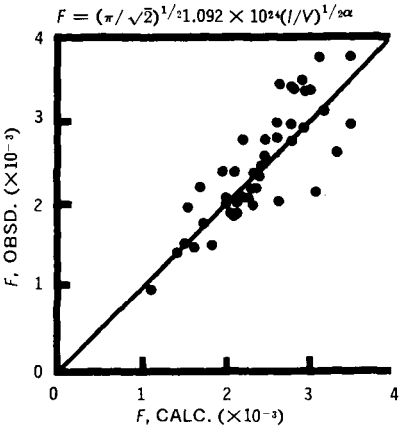

Figure 3—*Agreement between experimental molar attraction constants and the corresponding semiempirical estimates using molecular polarizabilities.*

leads (15, 18) to the relationship:

$$(M_0^2) = -\frac{6m_ec^2}{N}\chi \qquad \text{(Eq. 8}b\text{)}$$

in which m_e is the rest-mass of an electron and c is the velocity of light.

Substitution of Eqs. 8*a* and 8*b*, in turn, into Eq. 7 and each result subsequently into Eq. 4 thus provides two equivalent expressions for F, each of which can be written:

$$F^2 = \frac{\pi N^2}{2d^3}\alpha^2 h\nu_0 = z1.193 \times 10^{48}\left(\frac{I}{V}\right)\alpha^2 \qquad \text{(Eq. 9}a\text{)}$$

$$F^2 = \frac{8\pi m_e^2c^4}{d^3}\frac{\chi^2}{h\nu_0} = \frac{1}{z}\,7.303 \times 10^{21}\left(\frac{1}{IV}\right)\chi^2 \qquad \text{(Eq. 9}b\text{)}$$

where, in making the numerical evaluation, d has been estimated (crudely) from the molar volume V by means of the equation:

$$V = \frac{2\pi N}{3}d^3 \qquad \text{(Eq. 10)}$$

and I is taken to have units of calories per mole.

It is usual to follow London in identifying $h\nu_0$ with the molecular ionization potential I. As London showed (Table I), $h\nu_0$ is very nearly equal to the ionization potential I, at least for small molecules. However, in dealing with larger molecules, it seems more appropriate to make the assumption $h\nu_0 = zI$, where z is an empirically determined parameter. This identification is reasonable since, with large molecules, $h\nu_0$ is often found to be considerably larger than I (Table I). It is also found (*vide infra*) that this assumption is

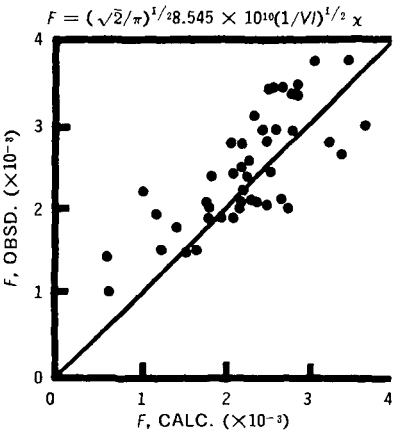

Figure 4—*Agreement between experimental molar attraction constants and the corresponding semiempirical estimates using diamagnetic susceptibilities.*

Table III—Calculated Dispersion Energy Contributions to the Cohesive Energy Densities of Some Pharmaceutical Substances

Compound	I, ev.	P_E[a], cm.^{3}/mole	V[b], cm.^{3}/mole	δ(cal./mole-cm.^{3})$^{1/2}$ Estimated	δ(cal./mole-cm.^{3})$^{1/2}$ Experimental[c]
Methyl alcohol	10.85	8.2	41.73	9.73	—
Ethyl alcohol	10.48	12.9	58.31	9.11	—
Acetone	9.69	16.2	73.50	7.77	9.9
Isopropanol	10.16	17.6	74.89	8.40	—
Propanol	10.20	17.5	74.89	8.37	—
Urethan	9.0	23.2	85.23	8.59	11.9
Ethyl ether	9.53	22.5	102.86	6.46	7.4
Butanol	10.04	22.1	91.47	7.77	—
Antipyrine	7.7	29.8	158.16	4.03	—
Pyridine	9.32	24.1	82.56	9.52	10.7
Chloroform	11.42	21.4	70.56	11.85	9.3
Hydroquinone	8.1	29.4	80.25	11.30	—
Aniline	7.70	31.6	92.32	9.60	10.7
Benzyl alcohol	8.8	32.5	101.48	9.16	—
Acetanilide	8.4	30.5	123.64	6.24	—
Pentanol	9.85	26.8	108.05	7.27	10.9
Phenol	8.50	27.8	84.90	10.06	—
Toluene	8.82	31.1	106.13	8.20	8.9
Hexanol	9.75	31.4	124.63	6.84	10.7
Nitrobenzene	9.92	32.5	99.16	10.06	10.0
Quinoline	8.3	42.1	112.51	9.87	—
8-Hydroxyquinoline	8.1	44.7	122.76	9.08	—
Heptanol	9.7	36.0	141.21	6.49	10.6
2-Naphthol	8.1	45.4	119.77	9.57	—
Methyl anthranilate	8.1	48.9	143.30	7.88	—
Octanol	9.65	40.6	157.79	6.18	10.3
Thymol	8.7	47.3	151.22	7.28	—
o-Phenanthroline	8.0	57.8	135.47	10.07	—
Ephedrine	9.1	50.2	158.52	7.37	—
Procaine	8.1	67.0	221.11	5.63	—
Xylocaine	8.0	72.5	232.93	5.60	—
Diphenhydramine	8.5	79.5	248.75	5.73	—
Tetracaine	7.76	79.7	258.80	5.19	—
Phenyltoloxamine	8.8	79.9	248.75	5.86	—
Eserine	8.5	82.4	212.24	7.54	—
Caramiphen	8.8	87.0	299.19	4.84	—
Dibucaine	8.25	103.6	312.37	5.23	—

[a] Molecular polarizability α can be obtained from the molar polarizability P_E using the relationship $P_E = {}^4/_3\pi N\alpha$. [b] Calculated from values given in *Reference 11*. [c] Experimental values as found in *Reference 3*.

necessary to obtain agreement between experimental and theoretical values of F.

RESULTS AND DISCUSSION

Cohesive energy densities, δ, given by Hildebrand and Scott (3) were used to calculate F according to Eq. 1. These subsequently were plotted against α and χ based on the relationships (from Eqs. 9*a* and 9*b*):

$$F\left(\frac{V}{I}\right)^{1/2} = z^{1/2}\,1.09 \times 10^{24}\alpha \qquad \text{(Eq. 11}a\text{)}$$

$$F(IV)^{1/2} = \left(\frac{1}{z^{1/2}}\right)8.55 \times 10^{10}\chi \qquad \text{(Eq. 11}b\text{)}$$

Table II presents the values for the quantities involved. As Fig. 1 shows, reasonable agreement with Eqs. 11*a* and 11*b* is observed. The dotted line in the figure is the slope of Eqs. 11*a* and 11*b* taking $z = 1$ (the London approximation). A more definite linear relationship is obtained when α is used as a measure of dispersion energy than when χ is used as a measure. This, no doubt, reflects the greater experimental accuracy with which α can be determined as opposed to χ. In the former case, an index of refraction is the experimental measure; in the latter case the experimental measure is the deflection of a sample suspended in a rapidly imposed homogeneous magnetic field. Considering the crude nature of the theory on which Fig. 1 is based, the agreement between theory and experiment can be considered adequate.

A value for z that is near 2 brings the experimental and theoretical lines shown in Fig. 1 into coincidence. We take $z = \pi/2^{1/2}$ since this assignment leads to a convenient representation of the theoretical form of the slope in Eqs. 11*a* and 11*b*. A value slightly different from the one used is necessary, however, to bring F values calculated on the basis of Eqs. 9*a* and 9*b* into agreement. Figure 2 gives an indication of the magnitude of the discrepancy between the theoretical estimates of F with our value for z. For most practical purposes, though, the choice of z does not lead to a theoretical value for F that is appreciably different from the experimental F, as shown by Figs. 3 and 4.

Some of the compounds in Table II have a large dipole moment. The fact that Eqs. 11*a* and 11*b* apply to these compounds tends to indicate that the dispersion energy dominates over dipole interaction energies with these substances.

Agin *et al.* (19) reported a study where the ionization potentials of drugs were estimated. Their estimation procedure is based on the use of the simple Hückel molecular orbital theory to calculate the energy of the highest occupied molecular orbital (HOMO) for a compound. The ionization potential is obtained from this calculated energy value by substituting it into the least-squares equation relating I with HOMO for a standard set of compounds. The data of Agin *et al.* were used to calculate the cohesive energy densities δ for select drugs or formulation constituents (Table III). However, the δ values in Table III were calculated assuming dispersion forces were dominant. They, therefore, represent, at best, only a good estimate for the lower limit to the "true" δ values. Better estimates would require dipolar and/or hydrogen-bonding energies to be taken into account.

CONCLUSIONS

The simplified theory of intermolecular forces described by London seems adequate for use in gaining estimates of molar attraction constants. At present, however, it is not certain whether this simple theory will apply in gaining estimates for the molar attraction constants of compounds where dispersion as well as dipolar or hydrogen-bonding effects is important. By accepting this shortcoming,

for certain substances, at least, it is now possible to obtain at least a lower limit to the cohesive energy density of pharmaceutically important compounds by very simple calculations. These calculations can be simplified further by noting that the ionization potentials in Tables II and III are not far from 10 ev. generally, so this value can be used in place of specific ionization potentials.

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Comparative Stabilities of Ampicillin and Hetacillin in Aqueous Solution

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Abstract □ Ampicillin and hetacillin in aqueous solution showed distinctive chemical alterations within 1 day. Ampicillin formed polymers which were separated according to molecular size by gel filtration on columns of an acrylamide gel. Hetacillin exhibited a rapid generation of a substance with a characteristic absorbance at 317 nm. This material was probably a penicillenic acid. A mechanism for these reactions is suggested.

Keyphrases □ Ampicillin—stability in aqueous solution, compared to hetacillin, mechanisms proposed for chemical alterations □ Hetacillin—stability in aqueous solution, compared to ampicillin, mechanisms proposed for chemical alterations □ Antibiotics, stability in aqueous solution—comparison of ampicillin and hetacillin, mechanisms proposed for chemical alterations

In investigations of the biochemical properties of some semisynthetic penicillins, it was noted that stock solutions of the antibiotics showed colorations, became viscous, and developed precipitates when left to stand at room temperature for longer than a few days. Since the allergic reactions to penicillins have been variously attributed to reactions of penicillins with tissue proteins, endogenous polymer formation, and contaminations with protein impurities among other possibilities, it was felt necessary to investigate more definitively the stability of several semisynthetic penicillins in aqueous solution. This report is specifically concerned with ampicillin [D(−)-α-aminobenzylpenicillin] and hetacillin [6-(2,2-dimethyl-5-oxo-4-phenyl-1-imidazolidinyl)-penicillanic acid] (1). Preliminary studies were reported earlier (2).

EXPERIMENTAL[1]

Ampicillin trihydrate, potassium ampicillin, hetacillin, and potassium hetacillin were obtained from a commercial source[2]. Solutions of the penicillins were prepared in water at 10% w/v concentration and stored in ground-glass-stoppered flasks in darkness at room temperature. At intervals, the pH's of the stock solutions were measured, and 250-μl. aliquots were subjected to gel filtration on columns (43 × 1.8 cm.) of an acrylamide gel[3] with a stated exclusion limit of approximately 1800 daltons. The columns were equilibrated and eluted with 0.05 *M* K^+–PO_4^{-3} buffer, pH 7.4, or 0.05 *M* KBr. Elution patterns with either solvent were identical as detected by the absorbance at 270 nm. of eluant fractions. The latter solvent was necessary for those gel filtration runs whose fractions were examined by IR.

An estimation of polymer size was derived from a correlation of the ratio of absorbances at 1600 and 1765 $cm.^{-1}$ and the position in the elution pattern. These wavelengths (1, 3) correspond to the IR absorbances of the ionized carboxyl group and β-lactam structure, respectively. This estimation is based upon the assumption of a linear structure for the polymer with a terminal unit containing an intact β-lactam.

Assays for antimicrobial activity against *Escherichia coli* K12, a Gram-negative organism, were performed in broth cultures employ-

[1] IR spectral analyses were performed on KBr pellets of lyophilized material using the Perkin-Elmer 21. UV spectra were obtained with the Cary 14.

[2] Bristol-Myers Co., International Division.

[3] Bio-Gel P-2.