General.—We may combine the equations for the viscosity dependence on aging at constant temperature and on temperature at equal ages into the form

$$2.303 \log \eta_D = 2.303\, a \log t - E/RT + g \quad \text{(Eq. 5)}$$

or

$$\eta_D = ht^a e^{-E/RT} \quad \text{(Eq. 6)}$$

where g and h are fit constants which are dependent upon units used and upon preparative history of the sample.

Equation 5 implies that, for any given viscosity level attained during aging, a plot of the reciprocal of temperature and the logarithm of the time required to attain that viscosity will be a straight line. In Fig. 7 are shown such plots for four viscosity levels of Veegum F. The lines are reasonably parallel as is required by the equation. Thus the times required to attain a given level of viscous build-up by varying temperature may be compared.

Equation 5 or 6 or their precursors imply an infinite viscosity with infinite time. This seems to have no practical likelihood of substantiation. Syneresis appears to be the phenomenon which dictates the boundaries within which Eqs. 5 and 6 are valid.

SUMMARY

The exponential aging relation has been found to hold for 2% Veegum HV and 2% Veegum F suspensions in water, so that the logarithm of the viscosity is a linear function of the logarithm of time over a time interval from a few hours to several months.

For samples of equivalent age, an Arrhenius-type relation holds to relate the viscosity with the temperature of preparation and storage. The apparent activation energy for both Veegum HV and Veegum F is about 8000 calories/mole.

The temperature and time relationship may be combined to one general equation which permits time to reach a given viscosity for a given temperature to be determined.

One constant of any of the above relationships is critically dependent on the method of mucilage preparation.

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Benzoic Acid as an Absorbance Standard in Infrared Spectrophotometry in Pharmaceutical Analyses

By J. P. COMER and A. M. RIBLEY

A solution of benzoic acid in chloroform measured at 5.91 μ was used to establish absorbance ratios for several drug substances. The ratios were used with daily measurements of benzoic acid as absorbance standards. The precision and accuracy of the technique was found to be equal to the customary method of using the drug substance as a reference standard.

THE PREDICTION of Carol (1) that the use of infrared spectrophotometry in pharmaceutical analyses would increase has certainly been correct. The maintenance of the multitude of chemical standards for infrared spectrophotometry has created many problems. The pharmaceutical laboratories must store and maintain in the original state these standard compounds and also supply governmental agencies with the proper standards. Many analysts (2–6) have reported on various methods for the standardization of instruments. Most of the work concerning the standardization of wavelength and possible use of solid standards does not consider some of the variables encountered in routine control of pharmaceuticals. Variable cell paths, temperature changes, and instrumental responses are corrected normally by performing standard readings of the known compound in solution. The purpose of this investigation was to attempt to find a suitable standard that could be used for quantitative analyses to replace the ever increasing number of individual standards that are encountered in pharmaceutical research programs.

Benzoic acid being readily available in high purity was selected as a trial substance since it has

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distinct absorption maxima scattered through the 3–10 μ range. The carboxylic acid absorption at 5.91 μ was the most desirable reference wavelength since it represents the carbonyl region characteristic of so many drug substances and because it exhibits maximum sensitivity for benzoic acid. The results of the initial work with benzoic acid was so encouraging that studies of other potential standards were not undertaken.

EXPERIMENTAL

The Beckman IR 2 instrument was used for all the measurements unless noted otherwise. The benzoic acid solutions were prepared fresh daily to avoid concentration by loss of chloroform. A solution of *d*-propoxyphene hydrogen chloride (130 mg.) was made alkaline and extracted with chloroform. The chloroform was evaporated and the residue dissolved in 4 ml. of chloroform. Another solution was prepared containing acetylsalicylic acid (908 mg.), caffeine (640 mg.), and acetophenetidin (130 mg.) in 50 ml. of chloroform. Benzoic acid solutions were prepared containing 100 mg. (BA) and 600 mg. (BAII) in 10 ml. chloroform. The absorbances of these solutions were measured and ratios calculated. Accurate location of the absorption maxima was required for each determination made through the study.

Sufficient materials were obtained so that the same lot numbers could be used for the preparation of the solutions for the complete wavelengths studies. The solutions were prepared immediately before use.

TABLE I.—ABSORBANCES OF BENZOIC ACID IN CHLOROFORM WITH CELL PATH 0.1 MM.

Concn. mg./ml.	Microns	Slit, mm.	Absorbance
10	5.91	0.3	0.393
10	5.91	0.35	0.366
10	5.91	0.4	0.347
10	5.91	0.45	0.327
10	5.91	0.50	0.311
25	6.91	0.45	0.153
25	7.08	0.45	0.311
25	7.59	0.45	0.306
60	3.92	0.15	0.405
60	6.25	0.3	0.293
60	8.50	0.7	0.312
60	8.87	0.6	0.242
60	9.34	0.8	0.180
60	9.75	0.8	0.171

RESULTS AND DISCUSSION

The use of a single standard would not be practical unless the absorption ratios of compound to standard would be constant in respect to time and changes of absorption cells thickness. Wichers (7) reported the use of benzoic acid as a thermometric standard but indicated possible instability problems. It was found that chloroform solutions of benzoic acid were stable. (Zero time A = 0.394, 1 hr. A = 0.392, 5 hr. A = 0.393, and 48 hr. A = 0.396.) The maxima of benzoic acid in chloroform solutions observed on a Beckman IR 2 instrument are shown in Table I.

The *d*-propoxyphene hydrogen chloride and the acetylsalicylic acid, caffeine, and acetophenetidin solutions were prepared at an interval of 30 months and the ratios of absorbances were calculated. Table II lists the comparative results. The changes in the ratios were not considered significant for the number of observations so a study was started for the selection of the optimum standard wavelengths to be used for a series of compounds. Table III shows the concentration, wavelength, cell path, etc. The calculated ratios from the absorb-

TABLE III.—CHLOROFORM SOLUTIONS USED IN ABSORBANCE STUDIES

Compound	Concn./ml.	Microns	Slit
Acetylsalicylic acid (ASA)	20 mg.	5.70	0.3
d-Propoxyphene HCl	32.5 mg.	5.80	0.3
Benzoic acid (BAI)	10 mg.	5.91	0.3
Salicylic acid	8 mg.	6.02	0.3
Caffeine	4 mg.	6.04	0.4
Benzoic acid (BAII)	60 mg.	6.34	0.3
Acetophenetidin (API)	10 mg.	6.61	0.6
Acetophenetidin (APII)	12.8 mg.	6.61	0.6
Benzoic acid (BAIII)	60 mg.	8.51	0.6
Aniline (AN)	0.08 ml.	8.52	0.6
Benzoic acid (BAIV)	60 mg.	9.75	0.8
Phenaglycodol (PHEI)	25 mg.	9.88	0.9
Isopropanol (ISO)	0.02 ml.	10.60	0.9
Phenaglycodol (PHEII)	100 mg.	10.93	0.9
Ethanol (ETH)	0.04 ml.	11.40	1.5

TABLE II.—EFFECT OF TIME ON RATIOS OF ABSORBANCES OF BENZOIC ACID TO OTHER COMPOUNDS

Compound[a]	Months Elapsed	Microns	Slit, mm.	Denominator	Ratio of Absorb.
d-Propoxyphene	0	5.80	0.3	BA	0.814
d-Propoxyphene	30	5.81	0.3	BA	0.813
Acetylsalicylic acid	0	5.69	0.3	BA	1.06
Acetylsalicylic acid	30	5.71	0.3	BA	1.07
Caffeine	0	6.04	0.4	BA	1.10
Caffeine	30	6.05	0.4	BA	1.14
Acetophenetidin	0	6.61	0.6	BAII	1.19
Acetophenetidin	30	6.61	0.6	BAII	1.18
Benzoic acid (BA)		5.91	0.4		
Benzoic acid (BAII)		6.34	0.35		

[a] See text for preparation of solutions.

TABLE IV.—RATIOS OF ABSORBANCES USING BECKMAN IR 2 INSTRUMENT

Time Elapsed, Weeks	API[a] / BAII	API / BAI	AN / BAIII	AN / BAI	PHEI / BAIV
0	0.8540	0.7015	...	...	1.431
1	0.8602	0.7013	...	...	...
2	0.8825	0.7020	0.7034	0.6288	1.415
3	0.8870	0.7069	0.7168	0.6375	1.415
4	0.8782	0.6990	0.7135	0.6352	1.412
5	0.8836	0.7096	0.7252	0.6464	1.424
Av.	0.874	0.703	0.714	0.637	1.419
S.D./Av. × 100	1.57	0.569	1.26	1.14	0.5562

Time Elapsed, Weeks	PHEI / BAI	ETH / BAIV	ETH / BAI	ISO / BAIV	ISO / BAI
0	0.6352	...	...	1.810	0.8036
1	...	...	...	1.751	0.8025
2	0.6288	1.631	0.7247	1.807	0.8030
3	0.6401	1.631	0.7378	1.801	0.8342
4	0.6378	1.638	0.7398	1.819	0.8214
5	0.6364	1.638	0.7323	1.780	0.7954
Av.	0.634	1.63	0.734	1.79	0.810
S.D./Av. × 100	0.662	0.248	0.919	1.39	1.81

[a] See Table III for identification of solutions.

TABLE V.—PRECISION OF RATIO METHOD

Time Elapsed, Weeks	ASA Soln. 1 / BAI	ASA Soln. 2 / BAI	ASA Soln. 1 / ASA Soln. 2
0	1.016	1.009	1.007
2	1.005	1.016	0.989
4	1.016	1.021	0.995
6	1.010	1.019	0.991
8	1.014	1.016	0.998
12	1.007	1.007	0.999
Av.	1.011	1.014	0.9966
S.D.	0.0047	0.0055	0.0064

ance data are shown in Table IV. Precision is expressed as coefficient of variation (100 × S.D./Av.) since the ratios differ in magnitude.

General observations indicated that a single benzoic acid solution (BAI) at 0.3 slit and 5.91 μ could be used as a suitable standard. Precision and accuracy studies were started based on these observations.

The precisions of ratios derived from absorbance of acetylsalicylic acid and benzoic acid (BAI) were obtained over a three-month span. The ratios in Table V were calculated from the average absorbance of three solutions. There is no statistically significant difference (F-test) in the precision of the ratios calculated by using benzoic acid as the reference or by using acetylsalicylic acid as reference.

Another cell having 80% of the cell path of the one used for data in Table V was used for obtaining absorbances and ratios for four averages of three solutions each. The ratios found for ASA/BA were 0.995, 1.007, 1.006, 1.012, illustrating that cell path changes occurring in practical use will not significantly change the ratios.

After the ratios were established by numerous replicate determinations between BAI and each of the several compounds previously mentioned, a measure of the accuracy of this system for its proposed use was made. Observed absorbance values at weekly intervals for the individual standard materials were compared to absorbance values calculated by multiplying the current BAI absorbance by the previously established ratios.

Observation of the data from this experiment shown in Table VI indicates an accuracy and precision equivalent to that expected in customary standardization techniques.

Since the manually operated Beckman IR 2 was used for the preceding experiments, the precision of the technique was evaluated on the Perkin-Elmer model 21 recording instrument. Ratios of the five drugs *vs.* BAI were determined on three different days using an average of three replicates for benzoic acid and a single observation of the samples. An extensive realignment of the instrument was made between the first and second series. The data in Table VII show that satisfactory precision may be obtained using benzoic acid as a standard on a recording instrument if careful control of instrument parameters (recording speed, pen sensitivity, slit width, etc.) is maintained.

SUMMARY

Chloroform solutions of benzoic acid (BAI) were shown to be stable over two-day periods, but were prepared fresh daily to avoid concentration by evaporation. Ratios of *d*-propoxyphene, caffeine, and acetophenetidin solutions were found to be constant over a 30-month period (Table II). The precision of ratios calculated from maxima of benzoic acid nearest the maxima of various drug substances were compared to those ratios calculated from benzoic acid at 5.91 μ and the maxima of the drug substances (Table IV). Statistical analysis showed a significant difference for acetophenetidin and ethanol but not for aniline, phenaglycodol, or isopropanol. For acetophenetidin the best precision was by the ratio calculated from the 5.91 μ BA solution.

TABLE VI.—ACCURACY STUDY

Solution[a]	Absorbance Observed	Absorbance Calcd.	Absorbance Diff.	% Error
d-Propoxyphene HCl	0.285	0.280	−0.005	−1.8
d-Propoxyphene HCl	0.280	0.279	−0.001	−0.4
d-Propoxyphene HCl	0.277	0.279	+0.002	+0.7
d-Propoxyphene HCl	0.283	0.280	−0.003	−1.1
Acetylsalicylic acid	0.378	0.381	+0.003	+0.8
Acetylsalicylic acid	0.376	0.380	+0.004	+1.1
Acetylsalicylic acid	0.375	0.380	+0.005	+1.3
Acetylsalicylic acid	0.389	0.388	−0.001	−0.3
Salicylic acid	0.251	0.252	+0.001	+0.4
Salicylic acid	0.250	0.251	+0.001	+0.4
Salicylic acid	0.248	0.251	+0.003	+1.2
Phenaglycodol (PHEII)	0.488	0.480	−0.008	−1.6
Phenaglycodol (PHEII)	0.478	0.480	+0.002	+0.4
Phenaglycodol (PHEII)	0.474	0.479	+0.005	+1.1
Caffeine	0.277	0.277	0.000	0.0
Caffeine	0.267	0.267	−0.000	0.0
Caffeine	0.277	0.276	−0.001	−0.4
Caffeine	0.275	0.276	+0.001	+0.4
Acetophenetidin (APII)	0.319	0.323	+0.004	+1.3
Acetophenetidin (APII)	0.320	0.321	+0.001	+0.3
Acetophenetidin (APII)	0.323	0.322	−0.001	−0.3

[a] See Table III for identification of solutions.

TABLE VII.—PRECISION OF RATIOS WITH A RECORDING INSTRUMENT

Time Elapsed, Months	Salicylic Acid[a] BAI	Caffeine[a] BAI	Acetophenetidin[a] II BAI	Acetylsalicylic[a] Acid BAI	Phenaglycodol[a] BAI
0	0.609	0.849	1.21	0.895	1.16
1	0.621	0.962	1.19	0.920	1.16
2	0.622	0.848	1.21	0.904	1.18
$\frac{\text{S.D.}}{\text{Av.}} \times 100$	1.17	0.916	0.962	1.40	0.987

[a] Ratio of absorbance.

The difference for ethanol is not considered practically significant for the coefficient of variation for the ethanol/BAI is only 0.92%. It was concluded from this study that the absorbance of BAI at 5.91 μ could be used for a reference standard for all drugs listed in this experiment.

The ratios for acetylsalicylic acid/BAI and acetylsalicylic acid/acetylsalicylic acid were measured during a three-month period (Table V). No statistically significant difference (F-test) was observed, and the coefficient of variation was less than 1%. Cell path changes occurring in practical use did not change the ratios.

Accuracy studies (Table VI) showed an average difference between customary standardization techniques and the ratio method of +0.16% with a pooled standard deviation of ±0.9%.

Ratios must be established for each instrument, for changes in slit width, optics, or measuring systems of various instruments will change the ratios. For example the ratio found for acetylsalicylic acid/BAI for single beam Beckman IR 2 was 1.01 and the ratio found for double beam Perkin-Elmer No. 21 was 0.907.

Noticeable changes in ratios on a single instrument can be used as an indication of needed maintenance or instrumental calibration by techniques described in references (2–6).

CONCLUSION

The accuracy and precision of the use of benzoic acid solutions in chloroform as secondary reference standards for quantitative infrared spectrophotometry was found to be for practical purposes equivalent to the use of individual standards of the drugs being measured. The successful use of the technique of using the secondary reference depends upon the establishment of accurate ratios on a single instrument by suitable replication and careful control of slit width and instrument calibration.

The technique would be useful to laboratories doing large numbers of measurements on a variety of drug substances that require the preparation of individual standards.

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