HYDROPHILE-LIPOPHILE BALANCE MEASUREMENTS OF CERTAIN EMULSIFIERS USING GAS-LIQUID CHROMATOGRAPHY

By

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CHAPTER I

INTRODUCTION

Emulsifiers are molecules that combine both hydrophilic and lipophilic groups. These compounds act in two ways. First, they improve the stability of an emulsion by producing finer particles of the discontinuous phase and "stabilizing" these particles in the continuous phase. Further, the relative solubility of an emulsifier controls the type of emulsion that is formed, i.e., oil-in-water or water-in-oil.

Emulsifiers have many uses in foods, some of which are: reduced sticking in caramels and nougats, retarded hardening in starch-based confections, resistance to dryness in ice creams, lengthened shelf-life of cake mixes, and improved palatability of cakes. The use of emulsifiers is not limited to food products. Some of these compounds also are essential ingredients of paints, lacquers, agricultural sprays, pharmaceuticals, cosmetics, asphalt emulsions, and many other products.

Although hundreds of emulsifiers are available, the prediction of their behavior is largely a matter of observation. Unfortunately no single emulsion theory exists to explain the classification of emulsifiers. Griffin's HLB concept, introduced in the late 1940's, has facilitated this formidable task. However, the use of this principle to select emulsifiers for foods has limited application because of the complex nature of food emulsions. Part of the confusion is a result of the fact that only crude techniques are currently available to measure

HLB. Therefore, it was the purpose of this work to develop an improved method of emulsifier measurement which could be used to classify emulsifiers and to aid in their selection.

CHAPTER II

REVIEW OF LITERATURE

Hydrophile-Lipophile Balance (HLB) is an abstract expression of the relative strength of polar and nonpolar groups in an emulsifier (10). This measurement ranges from 1-20 with lipophilic substances having numbers below 9.0. These values become lower as the molecule's lipophilic portion progressively overbalances the hydrophilic portion. HLB numbers of predominantly hydrophilic substances progress upward from 11.0. Numbers between 9.0 and 11.0 are neutral, i.e., the hydrophilic and lipophilic portions of the molecule are essentially of equal strength. Furthermore, HLB describes how emulsifiers differ in their functions in a food product. These functions are summarized in Table I.

TABLE I (1)

FUNCTION OF EMULSIFIERS OF VARIOUS HLB RANGES

HLB Range	Emulsifier Function
4-9	Water/oil emulsion
7-9	Wetting Agent
9-11	Neutral
11-18	Oil/water emulsion
13-15	Detergents
14-18	Solubilizers

Emulsifier systems may be blends of two or more emulsifiers. The ratio of the two emulsifiers (A and B) needed to obtain any desired HLB can be calculated algebraicly as follows (1):

$$\%A = \frac{100\ (X - HLB_b)}{HLB_a - HLB_b}$$

$$\%B = 100 - \%A$$

where A = emulsifier of known HLB "a"

B = emulsifier of known HLB "b"

X = HLB for mixture of A and B

If HLB numbers are not known, they can be calculated with the following formulas, given the appropriate measurements:

$$HLB = 20\ (1 - S/A)$$

where S = the saponification number for the ester portion of the emulsifier molecule

A = the acid number of the acid portion of the molecule

The HLB number of an emulsifier can be approximated by observing the emulsifier's water-solubility (1). A certain amount of emulsifier is vigorously agitated in a test tube containing a known quantity of water and the mixture allowed to settle for a certain time. The resultant characteristics are compared to the guide in Table II.

Griffin outlined a method for determining HLB numbers experimentally by blending an emulsifier of known HLB with an emulsifier of unknown

TABLE II

HLB RANGE OF EMULSIFIERS WITH CERTAIN DISPERSIBILITY CHARACTERISTICS IN WATER

Water Dispersibility Characteristic	HLB Range
No dispersibility in water	1-4
Poor dispersion	3-6
Milky dispersion	6-8
Stable milky dispersion	8-10
Translucent to clear dispersion	10-13
Clear solution	13+

HLB in varying ratios, then shaking these emulsifiers in a container with an oil of "known required HLB" (10). After standing overnight the samples are observed and the unknown HLB is calculated for the most stable system. This method is difficult to evaluate due to the large number of samples required (75 or more) and the subjective judgements involved. Preliminary work for this study showed that this technique was accurate only to ± 1.0 HLB unit. The modification of Griffin's method by Chun, et al. (5, 6) also was time consuming and its appraisal troublesome.

Robbers and Bhatia (14) modified Griffin's method of determining HLB values. Stock solutions of emulsifiers were used to shorten the procedure. Stable test emulsions were first prepared in a blender then centrifuged, accelerating phase separation. A comparison of relative emulsion stabilities was available in approximately one hour. This procedure is usually employed only as a screening method since the outcome can vary as a result of stock emulsion instability.

Recently attempts have been made to relate interfacial tension to HLB (2, 8, 9, 15). For example, Chun and Martin (7) measured interfacial tensions of aqueous solutions of surfactants (0.1%) overlayered with toluene as a means of estimating HLB numbers. A linear relationship resulted when the interfacial tensions were plotted against HLB values. However, the data from this interfacial tension method for HLB determination did not correspond with results obtained for the emulsion method of measuring HLB numbers of surfactant mixtures or single emulsifiers.

Progress in the area of analytical chemistry, especially gas-liquid chromatography (GLC), suggested to Becher and Birkmeier (4) that a more direct measurement of emulsifier polarity might be possible. Measurements of the retention time ratio were plotted as a function of known HLB for a number of polyoxyethylated fatty alcohols. It was found that even though the HLB number of a mixture of emulsifiers had long been calculated on the basis of algebraic additivity, this was not strictly accurate. Although the deviation from non-linearity was small, recent work indicated that curvilinear effects are observed under special conditions. Additionally, Becher (3) summarized the significant works on emulsions completed before 1965.

Huebner (11) was able to show in a limited way that his polarity index was linearly related to HLB number. The polarity index (P. I.) was defined in terms of the carbon number (C) where:

$$\text{P. I.} = 100 \log (C - 4.7) + 60$$

This method was based on a comparison of GLC retention times of ethanol and n-hydrocarbons when the emulsifier was used as the liquid phase in the GLC column. Usefulness of the method was limited due to the fact that few details of the scheme were outlined.

A procedure for the chromatographic analysis of the class of emulsifiers including the fatty acid esters of sorbitol and its anhydrides was explained by Sahasrabudhe and Chadha (16). After the total lipid material was fractionated by liquid partition column chromatography, each sample was analysed by GLC. The resultant peaks were identified by comparison with known standards and provided a quantitative estimation of the individual components of the emulsifiers. Another method for analysing surface-active agent composition was described by Suffis, _et al_. (17).

Although the works of Heubner (11) and Becher and Birkmeier (4) are more concise than previous methods, an improvement based on the needs of the Oklahoma State University Dairy Science Research Laboratory would be most useful. A combination of ideas expressed in these articles would provide a method that could reduce error, time, and equipment necessary to identify emulsifiers. The objective of this study was to seek such a method.

CHAPTER III

EXPERIMENTAL PROCEDURE

Apparatus: The GLC equipment was a Varian Aerograph Hy-Fi Model 600 in conjunction with a Sargent Recorder Model-SR and an Aerograph Hydrogen Generator Model 650. The column chamber was held at 80 ± 0.5 C and the injection port at 300 C. For each column the gas flow rates were adjusted using a soap bubble flow meter as follows: hydrogen, 18.75 ml/min.; nitrogen, 4.33 ml/min.; nitrogen and hydrogen, 23.08 ml/min.

The graph paper used in the recorder has ten major vertical divisions, each measuring 1.0 inch and ten 0.1 sub-divisions. Further, each principal horizontal division is one inch long with ten subdivisions. The recorder runs the graph paper at a speed of one inch per minute. The nitrogen flow rate was adjusted so the ether peak was eluted 1.4 minutes after sample injection. A 0.5 μl sample of solute was injected with a Hamilton microsyringe and the peak retention times determined with a minimum of three replicate values obtained. Iso-amyl alcohol in ethyl ether and methyl butyrate in ethyl ether were used to measure retention times.

Column Preparation: A 0.5 gram emulsifier sample was dissolved in 200 ml of a 25% solution of alcohol in ethyl ether in a 500 ml round bottomed flask. After 10.0 grams of Varian Aerograph Chromasorb-G (60-70 mesh) were added, the solvent was removed by vacuum using a rotary evaporator attached to a water aspirator. The dried and coated column packing was then transferred to an Erlenmeyer flask and placed in a vacuum oven at 60-70 C for 3 to 4 hours. A 5 ml sample of this material was packed into a 1/8 inch outside diameter (O. D.) copper column six feet in length. The column was preheated in the chromatograph oven for 24 hours at 125 C before being used.

The emulsifiers tested are listed in Table III.

TABLE III

EMULSIFIERS USED AS COLUMN COATING MATERIALS AND THEIR HLB NUMBERS

Emulsifier or Emulsifier Combination[1]	HLB
Span 60	4.7[2]
Span 60 + Tween 60	9.5[3]
Tween 61	9.6[2]
Span 60 + Tween 60	9.75[3]
Tween 81 + Tween 61	9.8[3]
Span 60 + Tween 60	10.0[3]
Tween 81	10.0[2]
Span 60 + Tween 60	10.25[3]
Span 60 + Tween 60	11.0[3]
Tween 60	14.9[2]
Tween 80	15.0[2]
Tween 80	15.0[4]

[1] All were manufactured by Atlas Chemical Industries, Inc. Wilmington, Del.

[2] HLB according to manufacturer's label.

[3] HLB calculated using algebraic formula.

[4] Commercially coated column, obtained from Varian Aerograph, Walnut Creek, Calif.

CHAPTER IV

DISCUSSION OF RESULTS

The basic procedure used in this research was determined after several preliminary tests. During these periods, stainless steel and copper chromatograph tubing were compared and the copper was found to be easier to manipulate with no noticeable difference in test results. Several oven temperature settings of the chromatograph were evaluated and 80 C was chosen because the retention time range was wider than at higher temperatures. The fact that the temperature settings were critical under these conditions is in agreement with findings of Becher and Birkmeier (9). It was observed that a variation of 2.0 C in oven temperature resulted in peak retention time variations of approximately one minute. This sensitivity to column temperature appeared to be either an inherent trait of the GLC method or a change in HLB with that of temperature. An attempt was made to use a column three feet in length to conserve time and materials. A coated Chromasorb-G column packing was utilized with iso-amyl alcohol and methyl butyrate as test materials. However, due to the short length and packing characteristics of this particular column, the sample peaks came off under the ether peak and were not resolved.

Mixtures of heptyl alcohol, decyl alcohol, methyl caprylate, methyl caprate, iso-amyl alcohol, and methyl butyrate each dissolved in ethyl ether were used as test solutions and injected into the chromatograph column to determine the difference in retention times caused by various emulsifier coatings. The iso-amyl alcohol and methyl butyrate were used in further experimentation because they gave better resolution at this particular temperature.

The retention times for each column are measured from the origin of the ether peak. This position was designated as 0 time. As Figure 1 indicates, the retention time was measured from 0 time to the center of the iso-amyl alcohol or methyl butyrate peak.

The retention times of a commercial column packing coated with 5% Tween 80 were compared to those of a similar column coated by the previously described experimental method. The column with the commercial packing had shorter retention times for the iso-amyl alcohol (2.1 minutes) and methyl butyrate (0.4 minute), although both columns apparently were identical. This difference could be the result of experimental errors in the procedure and might be an indication of the size of other experimental errors in this work.

The data for methyl butyrate have been shown in Table IV and graphed in Figure 2 as retention time vs HLB number. These data had no apparent pattern. As the data in Table I indicate, the time differential with this standard was so small that the difference between emulsifiers was not readily apparent.

The data shown in Table V were graphed in Figure 3 as retention time of iso-amyl alcohol through the emulsifier columns plotted against HLB numbers. The pattern of these dots indicated that perhaps a straight or slightly curved line might fit the data even though the dots scattered over a large area. The dots for the single emulsifiers (Span 60, Tween 60, etc.) tended to fit a well defined straight or slightly curved line (Figure 4). Additional emulsifier samples were needed to provide intermediate HLB numbers with corresponding time values. However, the figures reported here seem to define a line from which HLB numbers might be measured with accuracy.

The emulsifiers in Figure 5 had a more limited range of HLB numbers than did the pure emulsifiers. These data formed a random pattern and did not fit the line formed by the single emulsifiers in Figure 4 . This discrepancy might have been caused by errors in the weighing or other parts of the procedure. In any case, these data were not reliable enough to make distinctions among HLB numbers nor for use in identifying HLB of emulsifiers. This random pattern could indicate that each blended sample acted as two entities rather than a single unified mixture.

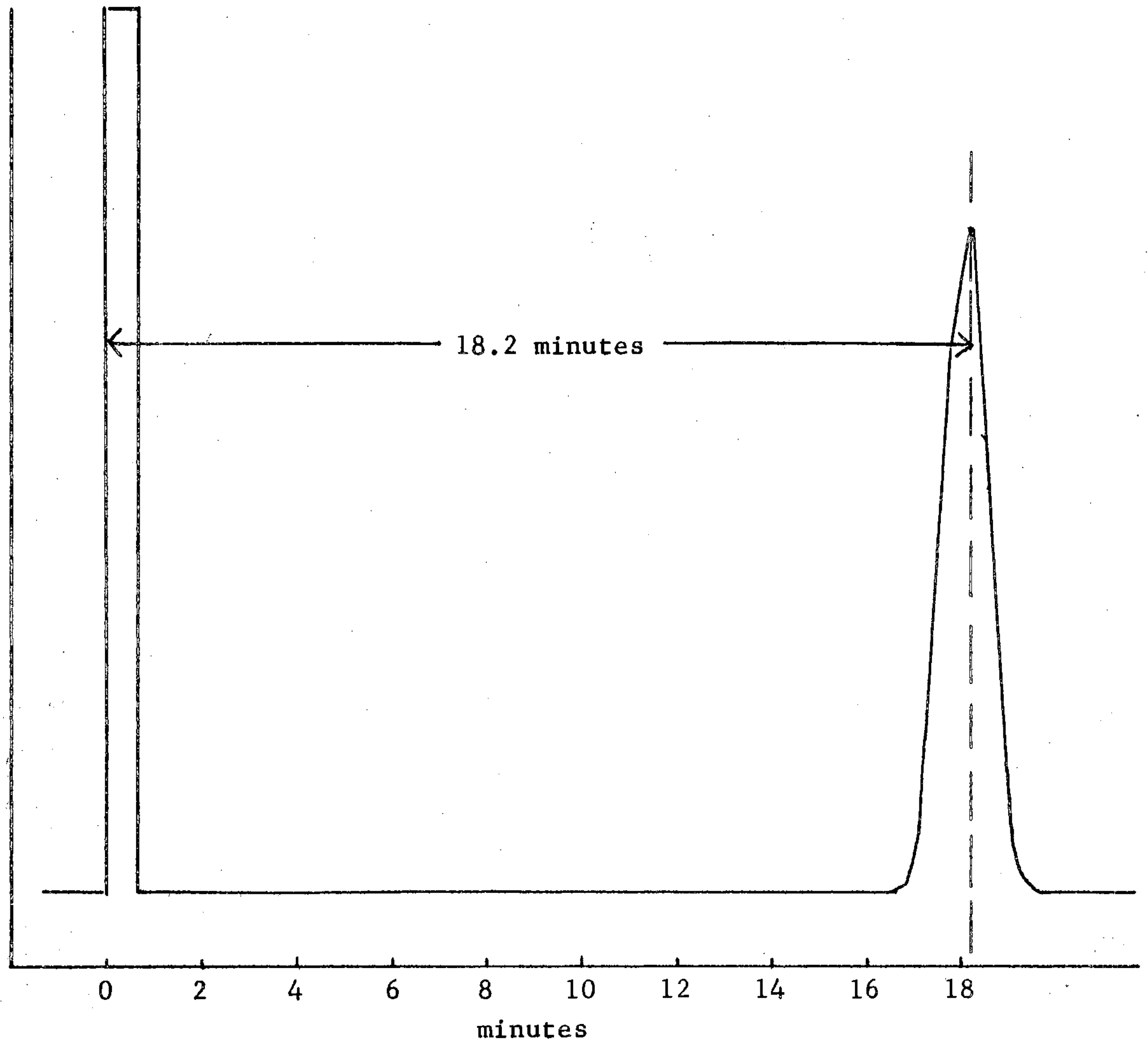

Figure 1. **Example of chromatograph chart and of data obtained from it. Span 60 + Tween 60 (HLB 9.5) in a 6 foot column at 80 ± 0.5 C.**

TABLE IV

RETENTION TIMES OF METHYL BUTYRATE ON CHROMASORB-G CHROMATOGRAPH COLUMNS COATED WITH VARIOUS EMULSIFIERS

Column Coating	HLB	Retention Time (minutes)[1] Replications 1	2	3	4	Average
Span 60	4.7[2]	4.4	4.4	4.4	4.3	4.4
Span 60 + Tween 60	9.5[3]	5.0	4.9	4.9	4.9	4.9
Span 60 + Tween 60	9.75[3]	4.6	4.6	4.6	4.5	4.6
Span 60 + Tween 60	10.0[3]	4.2	4.3	4.3	4.2	4.3
Span 60 + Tween 60	10.25[3]	4.3	4.3	4.3	4.3	4.3
Span 60 + Tween 60	11.0[3]	4.8	4.8	4.8	4.8	4.8
Tween 60	14.9[2]	4.6	4.6	4.6	4.6	4.6
Tween 61	9.6[2]	4.1	4.1	4.2	4.2	4.2
Tween 81 + Tween 61	9.8[3]	5.0	4.9	4.9	5.0	5.0
Tween 81	10.0[2]	4.4	4.4	4.4	4.3	4.4
Tween 80	15.0[2]	5.2	5.2	5.3	5.3	5.3
Tween 80[4]	15.0[2]	4.9	4.9	4.9	4.9	4.9

[1] Measured from leading edge of ether peak to center of alcohol peak.

[2] HLB according to manufacturer's label.

[3] HLB determined using algebraic additivity.

[4] Commercially coated packing used.

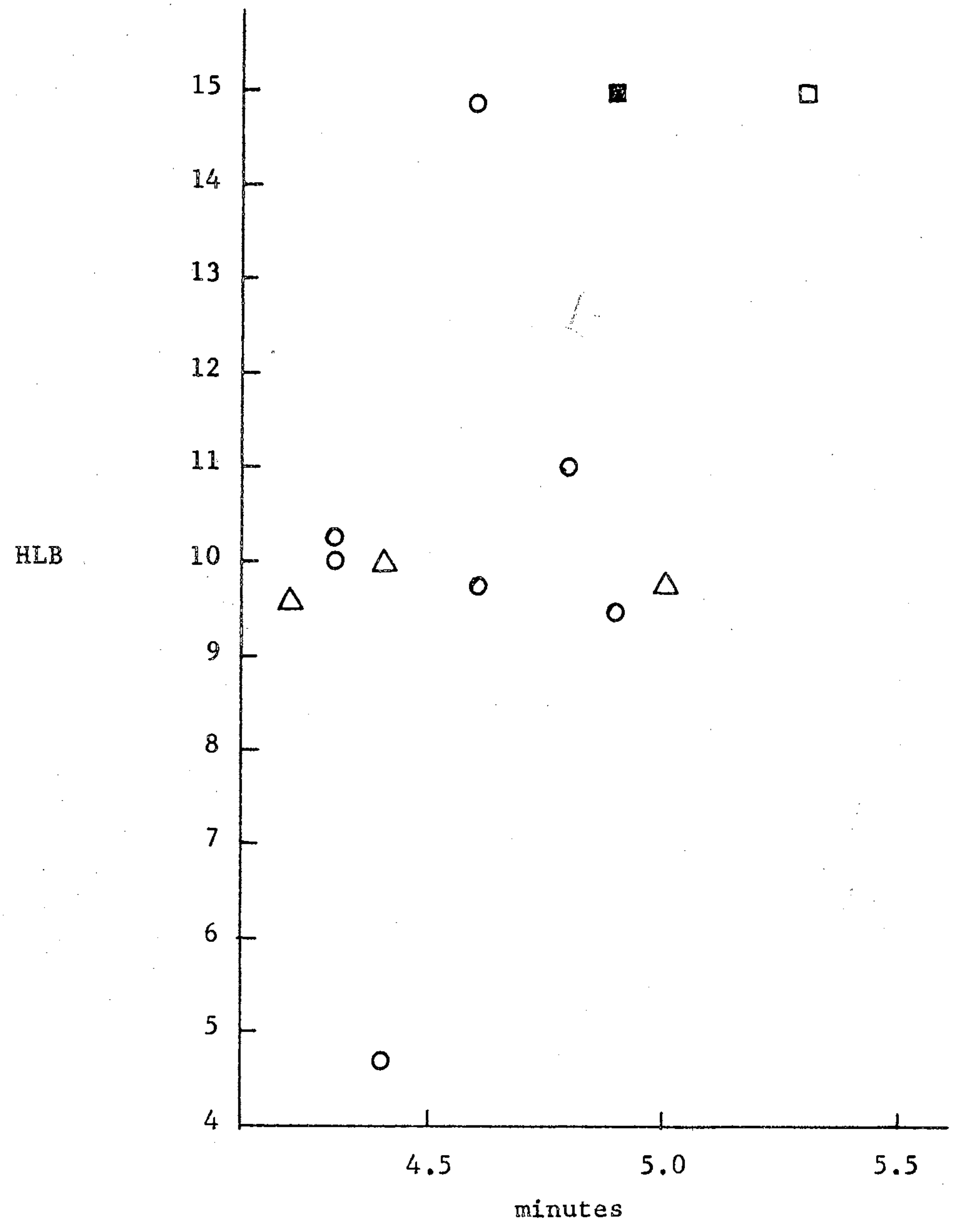

Figure 2. HLB vs retention times of methyl butyrate on Chromasorb-G chromatograph columns coated with various emulsifiers. ○ Span 60, Tween 60, and combinations; △ Tween 61, Tween 81, and combinations; □ Tween 80-experimentally coated packing; ■ Tween 80-commercially coated packing.

TABLE V

RETENTION TIMES OF ISO-AMYL ALCOHOL ON CHROMASORB-G CHROMATOGRAPH COLUMNS COATED WITH VARIOUS EMULSIFIERS

Column Coating	HLB	Retention Time (minutes)[1] Replications 1	2	3	4	Average
Span 60	4.7[2]	13.4	13.4	13.6	13.6	13.5
Span 60 + Tween 60	9.5[3]	18.2	18.1	18.2	18.2	18.2
Span 60 + Tween 60	9.75[3]	17.1	17.0	17.0	17.0	17.0
Span 60 + Tween 60	10.0[3]	16.1	16.2	16.1	16.1	16.1
Span 60 + Tween 60	10.25[3]	16.8	16.7	16.7	16.7	16.7
Span 60 + Tween 60	11.0[3]	19.7	19.8	19.7	19.8	19.8
Tween 60	14.9[2]	19.0	19.0	19.1	19.1	19.1
Tween 61	9.6[2]	14.6	14.6	14.5		14.6
Tween 81 + Tween 61	9.8[3]	18.5	18.5	18.6		18.5
Tween 81	10.0[2]	16.1	16.1	16.2	16.1	16.1
Tween 80	15.0[2]	21.3	21.3	21.3	21.3	21.3
Tween 80[4]	15.0[2]	19.1	19.2	19.1	19.2	19.2

[1] Measured from leading edge of ether peak to the center of alcohol peak.

[2] HLB according to manufacturer's label.

[3] HLB determined using algebraic formula.

[4] Commercially coated packing.

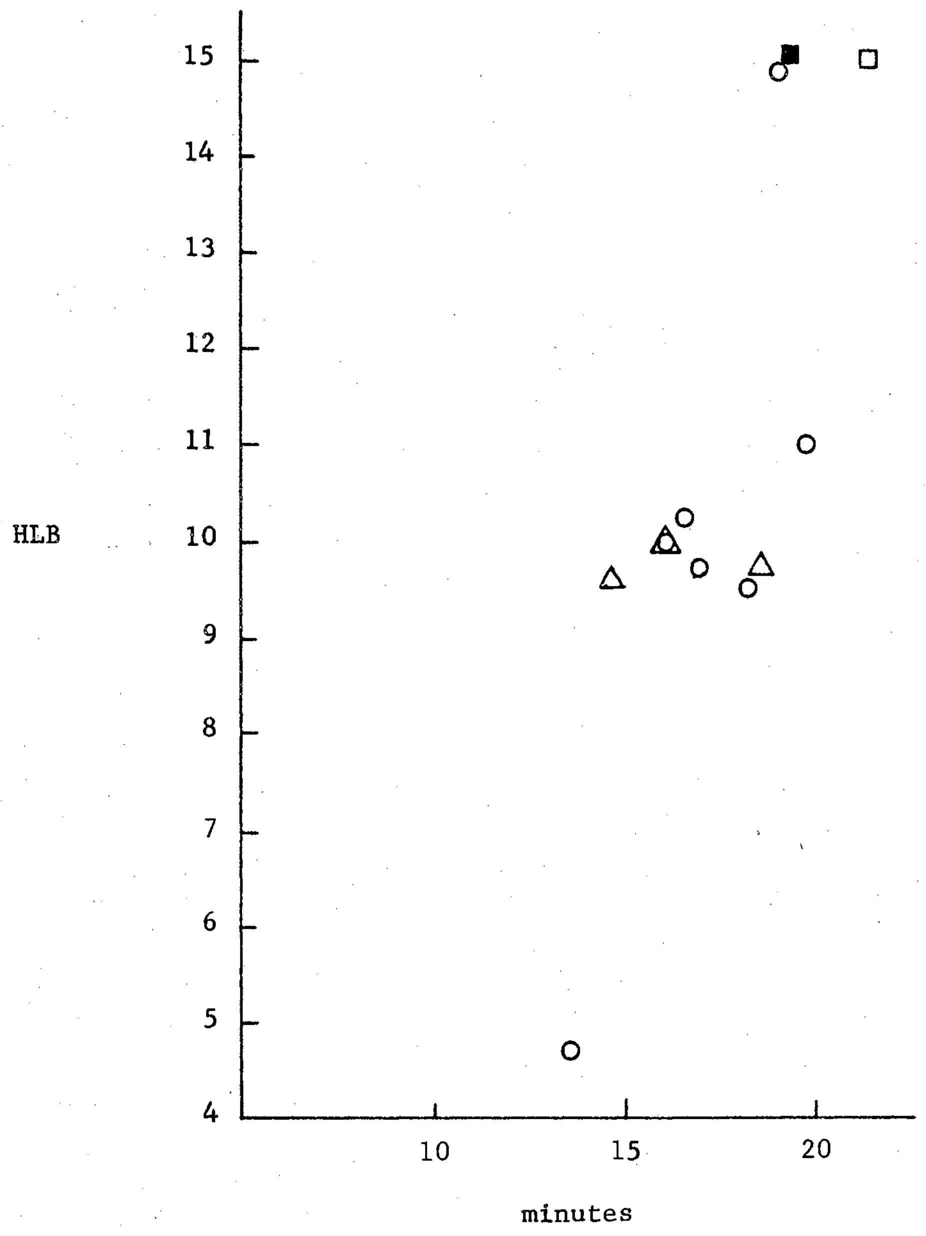

Figure 3. HLB vs retention times for iso-amyl alcohol on Chromasorb-G chromatograph columns coated with various emulsifiers. ○ Span 60, Tween 60, and combinations; △ Tween 61, Tween 81, and combinations; ☐ Tween 80-experimentally coated packing; ■ Tween 80-commercially coated packing.

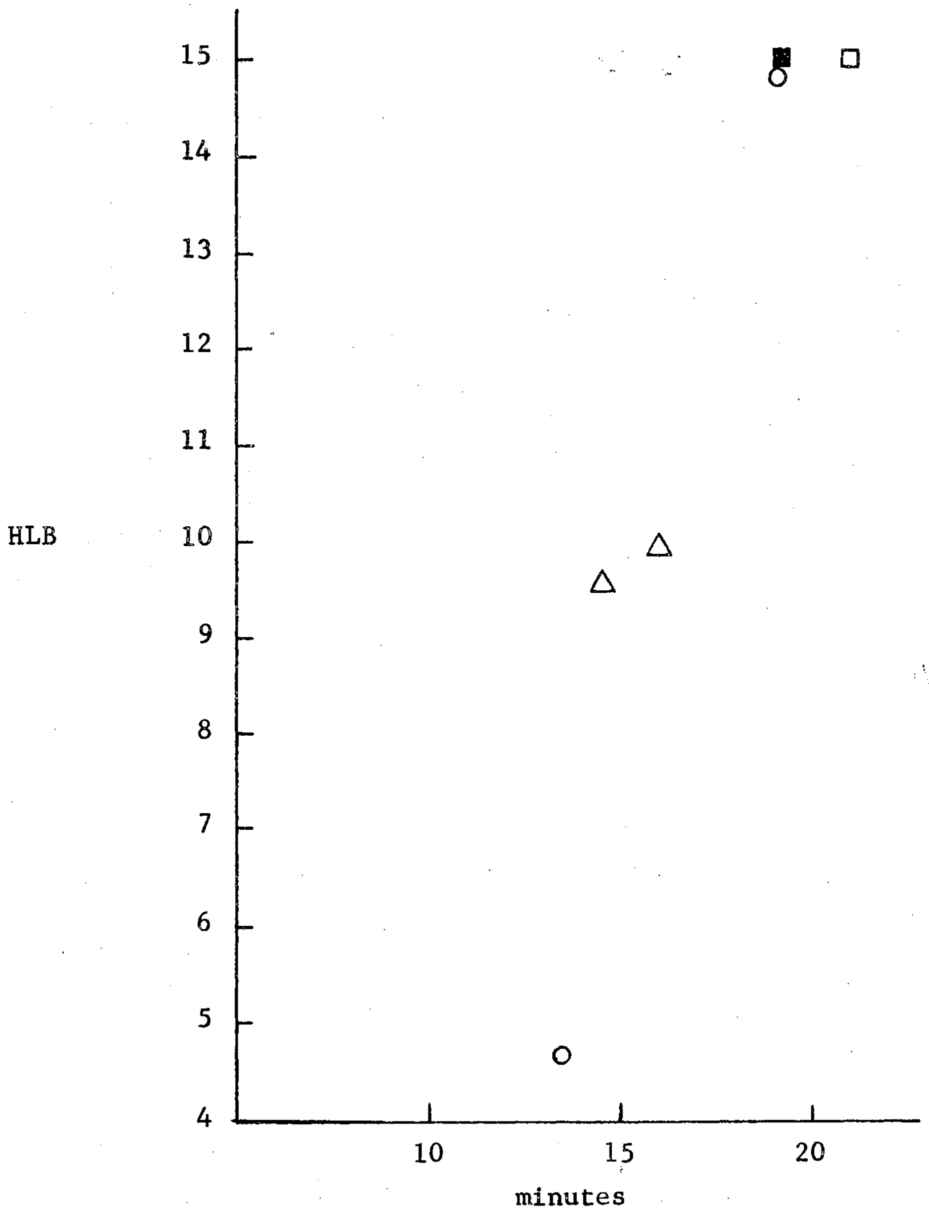

Figure 4. HLB vs retention times for iso-amyl alcohol on Chromasorb-G chromatograph columns coated with various single emulsifiers. ○ Span 60, Tween 60; △ Tween 61, Tween 81; □ Tween 80-experimentally coated packing; ■ Tween 80-commercially coated packing.

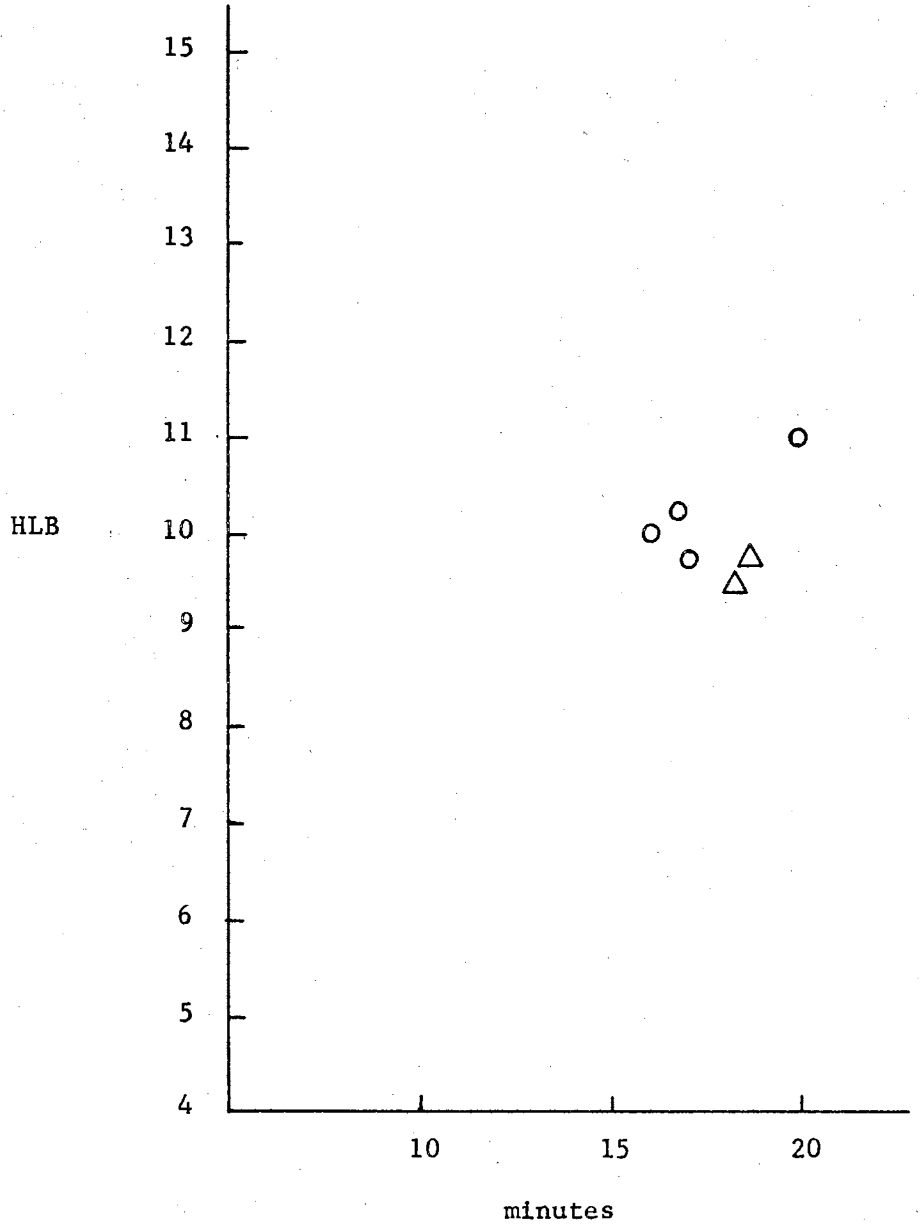

Figure 5. HLB vs retention time for iso-amyl alcohol on Chromasorb-G chromatograph columns coated with various emulsifier mixtures. ○ Span 60, Tween 60 mixtures; △ Tween 61, Tween 81 mixtures.

CHAPTER V

SUMMARY AND CONCLUSIONS

The purpose of this work was to seek an improved method of emulsifier measurement which could be used to classify emulsifiers and to aid in their selection. A 5% level of pure and mixed emulsifiers were coated on Chromasorb-G, packed in six-foot copper columns, and analysed by means of a Varian Aerograph gas-liquid chromatograph using iso-amyl alcohol in ethyl ether as a test solute. This procedure provided a means of predicting HLB numbers for certain pure emulsifiers based on retention times. However, the HLB of emulsifier mixtures could not be determined by this method.

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