Applications of Pulsed NMR to Fatty Emulsions[1]

J. TRUMBETAS, J.A. FIORITI, and **R.J. SIMS,** General Foods Corporation, Technical Center, White Plains, New York 10625

ABSTRACT

Pulsed NMR was used to measure solid fat content in oil-in-water type emulsions. The data obtained were useful in selection of optimum types and levels of emulsifiers for each system. By using a flow-through cell in the NMR magnet, the rate and extent of phase separation could be measured accurately. This approach provides a new, quantitative method to measure emulsion stability.

INTRODUCTION

Pulsed NMR has been applied to the determination of solid fat index (1). Recent reports from the Unilever Laboratories indicate that a rapid, accurate, and fully automatic method based on this technique has now been developed (2-4). In addition, other authors have applied pulsed NMR to the measurement of oil content in oilseeds (5).

To our knowledge, no one has reported on applications of pulsed NMR to emulsions. It was reasoned that dispersion of a fat into small droplets brings it into more intimate contact with the aqueous phase and should have an inhibiting effect on its rate of solidification during cooling. Since it is well known that the greater the degree of dispersion, the slower the rate of phase separation (6), pulsed NMR measurements might be used as an index of emulsion stability.

There was an incentive to develop improved techniques for selection of levels and types of emulsifiers, since this is still largely a trial and error procedure (7). Although the Hydrophilic and Lipophilic Balance (HLB) method for surfactant selection (8) has been extensively applied, a knowledge of both the required HLB for the system and the given HLB of the individual surfactants must be considered. In most cases, commercially available surfactants are mixtures, so that HLB cannot be determined easily from the chemical structure. In approaching the problem of selecting the proper combination of surfactants for use in food applications, one finds that the list of approved additives is quite large (9). Usually the choice for a particular application is based upon previous experience.

The methodology described here represents an attempt to provide a systematic approach to emulsifier selection. Also, a technique is suggested for measuring the stability of emulsions toward phase separation in quantitative terms. The lack of such a quantitative method, and excessive reliance on subjective evaluation, has hindered the development of a theory which would permit prediction of emulsion stability (10).

[1]Presented at the AOCS Fall Meeting in Cincinnati, September 1975.

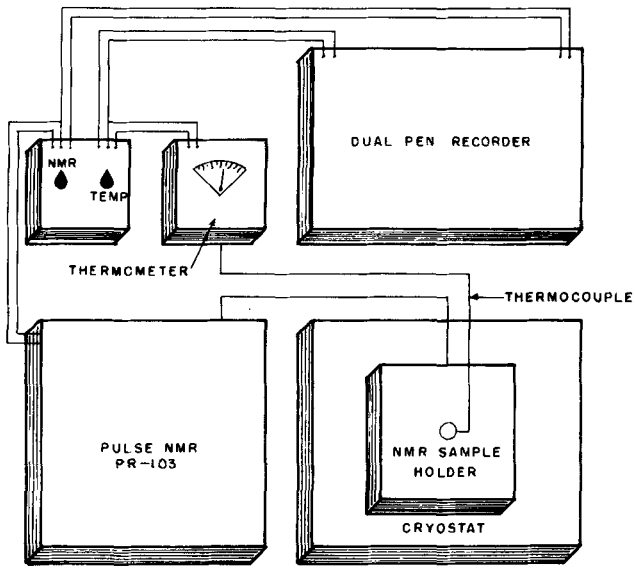

FIG. 1. Schematic diagram of cooling curve apparatus.

EXPERIMENTAL PROCEDURES

Materials

Refined soybean oil and refined palm oil were obtained from Best Foods Division, CPC International, Union, NJ. Coconut oil was from Welch, Holme and Clark Company, Inc., Harrison, NJ. Capital City Products Co., Columbus, OH, was the source of hydrogenated coconut oil. Samples of hydrogenated soybean oil and hydrogenated palm oil were prepared in a 1 gal pressure reactor (Paar Instrument Co., Inc., Moline, IL) at 200 C and 50 psi hydrogen pressure using 0.2% by wt of Rufert nickel catalyst (Harshaw Chemical Co., Cleveland, OH).

The following emulsifiers were used: Myverol 1800 (glycerol monostearate), Myvacet 5-07 (acetylated monoglycerides), and SMG (succinoylated monoglycerides) from Distillation Products Industries, Rochester, NY; Tween 60 (polyoxyethylene 20 sorbitan monostearate) and Span 60 (sorbitan monostearate) from ICI America, Inc., Wilmington, DE; Acidan Z-1 (citric acid ester of monoglycerides) from Grinsted-Vaerket A/S, Denmark.

Equipment and Methods

The equipment used was a Praxis Pulsed NMR (PR-103) Spectrometer, Praxis Corporation, San Antonio, TX. The spectrometer settings were as follows: pulse interval, 0.1 sec; variable delay, 60 μ sec; display, free induction decay (FID). These were chosen so as to minimize the water signal response. This is possible because of the large difference in relaxation time between the water and the oil protons due to rf saturation of the former. A Bailey Laboratory Thermometer, Model BAT-4, fitted with a microprobe MT-4 (Bailey Instruments Co., Inc., Saddle Brook, NJ) was used to monitor the temperature of the sample. A dual pen recorder, Omniscribe, was from Houston Instrument, Austin, TX. The NMR Magnet was positioned in an International Cryostat CTR (Scientific Products, Evanston, IL) which was equilibrated at 4 C. A schematic diagram (Fig. 1) indicates how this equipment was assembled to obtain cooling curves. Each sample, initially at 80 C, was chilled in a test tube, 75 x 10 mm OD, and positioned in the magnet. A single tube was used throughout because the signal response is volume dependent, and each tube has a slightly different diameter. When the temperature of the sample dropped to 50 C, both NMR signal and temperature were plotted continuously over a 20 min period as the samples were chilled. The method for calculation of percent solids in the sample from the pulsed NMR signal has been described by Van Putte (2).

The Van Putte method of solid:liquid ratios calculations is performed by using a standard oil at various temperatures

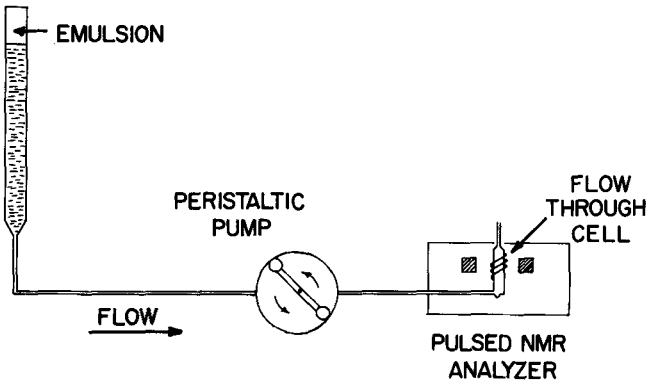

FIG. 2. Schematic diagram of stability determination apparatus.

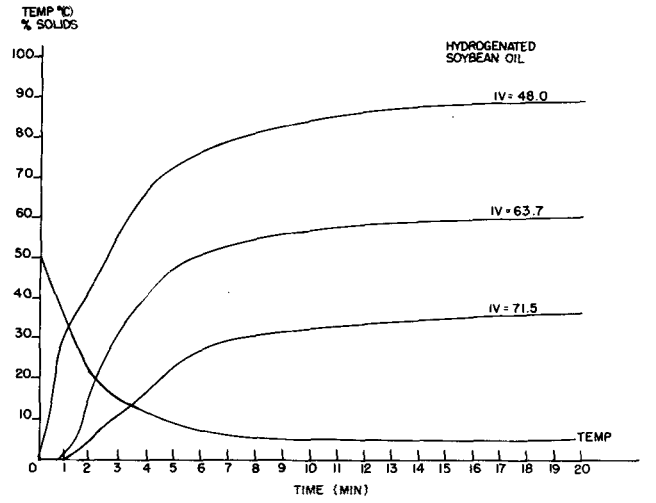

FIG. 3. Cooling curves on hydrogenated soybean oils.

as references. It was found that by heating the fat at least 20 degrees above its melting point and plotting NMR signal vs. temperature, a linear relationship exists until the sample reaches the temperature where the fat begins to solidify. By extrapolating this line to temperatures below the melting point of the fat and calling this line the 100% liquid curve at all temperatures, we can calculate the solid:liquid ratio without the use of a standard oil as described by Van Putte.

This extrapolated 100% liquid signal curve below the fat's melting point has been verified by dissolving a fat in CCl_4 and obtaining NMR signals at temperatures below the melting point of the fat. The curve continued along the same slope as the fat alone at temperatures above its melting point.

We have modified Van Putte's method for calculating solid:liquid ratios. Instead of using a reference oil (external standard), the sample oil itself is used as an internal standard. Mansfield (3) shows clearly that a reference oil must be chosen very carefully in order to obtain acceptable results. He cites a few points to be remembered if a reference oil is to be used. First, the signals from one type of oil to another are different. Secondly, the hydrogen content from one oil to another varies because of different fatty acid compositions. By using the sample oil as its own reference (internal standard), both of the points that Mansfield brought up can be eliminated.

Emulsions were prepared by first dispersing the hydrophilic surfactant in water at 60 C (Tween 60, SMG, or Acidan Z-1). In the case of both SMG and Acidan, enough sodium hydroxide was then added to raise the pH to the desired level. Then the fat containing dispersed lipophilic

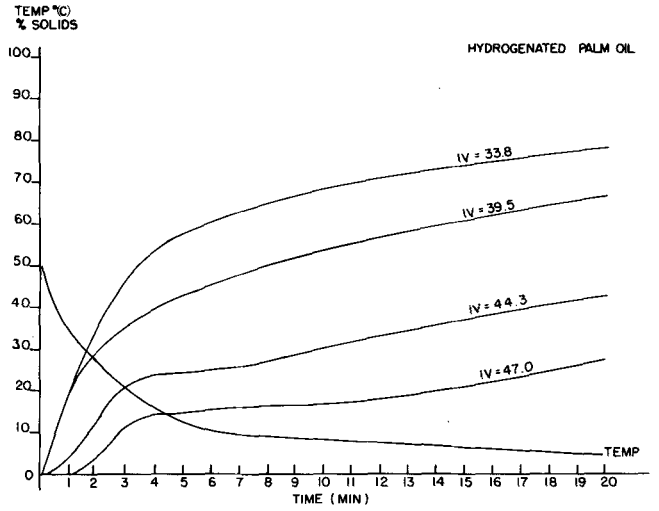

FIG. 4. Cooling curves on hydrogenated palm oils.

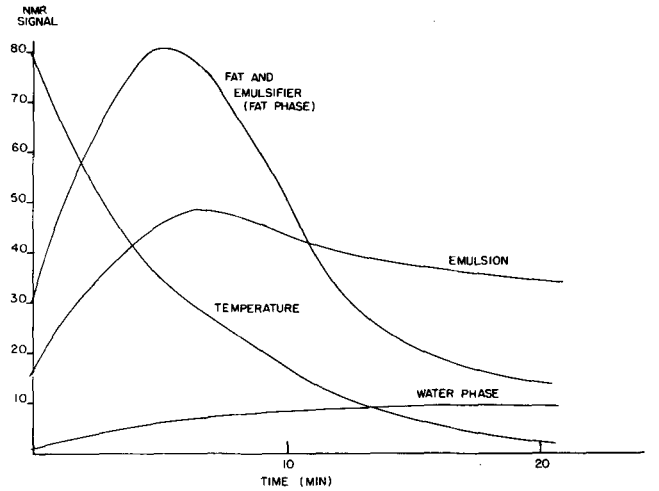

FIG. 5. Cooling curves on an emulsion and on its individual phases.

emulsifier (Myverol 1800 or Myvacet 5-07) at 60 C was mixed with the water phase at high speed in a Waring Blender for 2 min.

Emulsion stability measurements were made in a constant temperature room at 25 C. If such a room is not available, it is then necessary to measure the NMR signal from the fat phase over the range of temperatures encountered during the storage period. The signal observed from the emulsion is then corrected to compensate for room temperature variations. Emulsions were stored in a series of glass columns (12 x 1¼ in. ID). At suitable intervals, a sample (200 ml) was pumped from the bottom of the column through the magnet at 24 ml/min. NMR signal response was plotted vs. volume through the magnet. For this purpose, a glass cell was designed to permit a constant flow through the magnet (Fig. 2). A Masterflex Peristaltic Pump (Cole Parmer Instrument Co., Chicago, IL) fitted with a 0.0655 in. ID tubing to prevent mixing was used to pump the emulsions. Only one such measurement was made on each column so that each sample was previously undisturbed prior to pumping it through the magnet.

RESULTS AND DISCUSSION

The cooling curve method was applied initially to a series of partially hydrogenated vegetable oils. Curves for three partially hydrogenated soybean oils are shown in

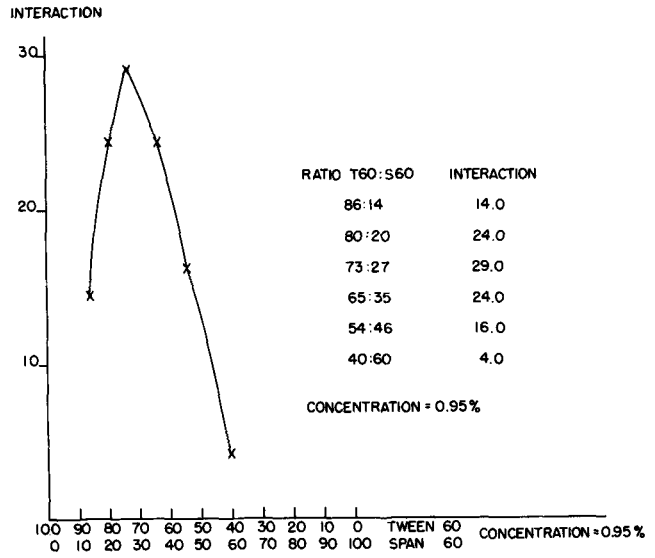

FIG. 6. Percent interaction at 25 C vs. Tween:Span ratio in an emulsion.

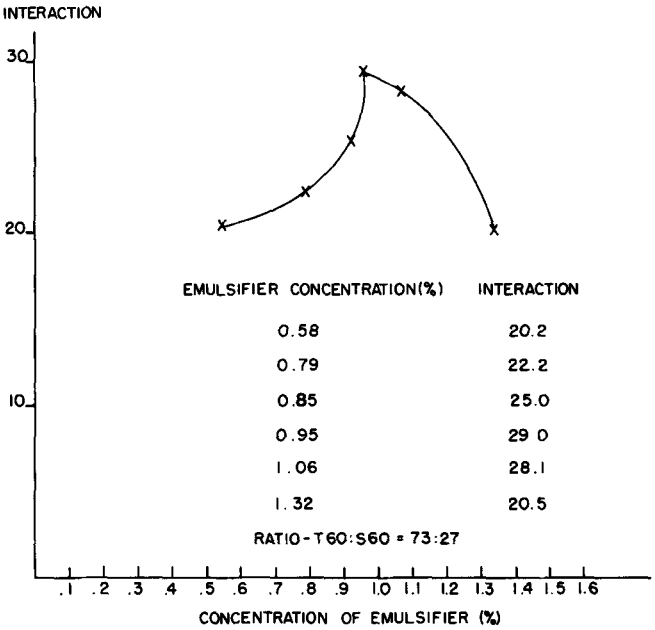

FIG. 7. Percent interaction at 25 C vs. emulsifier concentration in an emulsion.

Figure 3. The temperature of each sample reached 4 C within about the first 8 min of cooling, so that a single temperature curve applied to all three samples. The percent solids curves were drawn from a minimum of 20 calculated values at equally spaced intervals on the NMR signal curve using the procedure given previously. Within the 20 min cooling period, the samples all appear to have reached their equilibrium solids levels.

Similar data on a series of partially hydrogenated palm oils were also plotted (Fig. 4). These fats do not equilibrate as rapidly as the soybean oils since, even after 20 min of cooling, the solids levels are still increasing at a detectable rate.

When this cooling technique is applied to oil-in-water emulsions, contributions to the total NMR signal are made by both the oil and aqueous phases (11). The fat phase is composed of a mixture of the oil and lipophilic emulsifiers, and the aqueous phase is composed of a mixture of water and hydrophilic emulsifiers. The signal from the aqueous

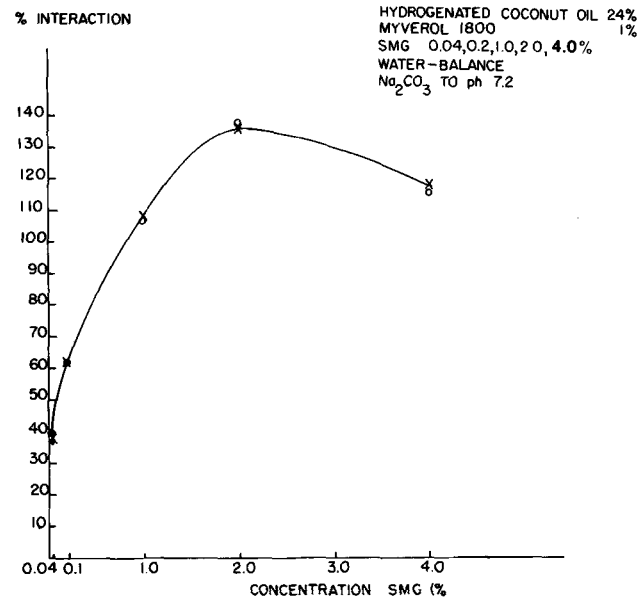

FIG. 8. Percent interaction at 25 C vs. succinoylated monoglyceride (SMG) concentration in an emulsion.

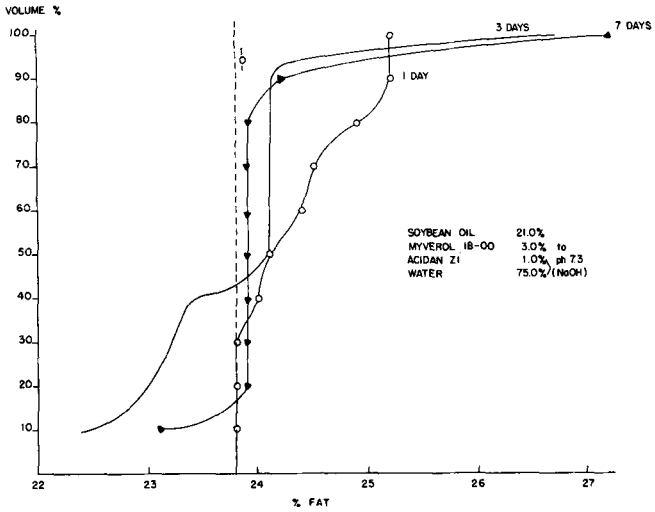

FIG. 9. Rate of phase separation in an emulsion at pH 7.3.

phase is very small relative to that from the fat phase because of rf saturation. The signals from the fat phase and from the aqueous phase are measured separately before the phases are mixed. In this work, we have measured the signal from the fat plus dispersed emulsifiers, from the aqueous phase, and from the emulsion itself. If emulsification had no effect on the rate and extent of fat solidification, then the signal obtained from the emulsion should be predictable; but, actually emulsification does have an inhibiting effect on fat solidification so that the NMR signal from the emulsion is always larger (the sample has a higher liquid content) than expected based on the data from the fat phase and the aqueous phase alone.

To illustrate this effect, data from one experiment are plotted in Figure 5. The emulsion used here contained 48% hydrogenated coconut oil, 1% acetylated monoglycerides (49% total, fat phase), 1% Tween 20, and the balance was water (51% aqueous phase). Measurements on the aqueous phase show that it gives a very small signal during the entire 20 min cooling period. As the fat phase is cooled, the signal response increases until solidification begins and then decreases as the sample solidifies (12). At 25 C, the expected emulsion signal is then calculated as follows:

Fat phase signal = 71.5 x 0.49	=	35.0
Aqueous phase signal = 7.5 x 0.51	=	3.8
Expected emulsion signal	=	38.8
Observed emulsion signal	=	41.5
Percent interaction = $\frac{45.5 \times 100}{38.8} - 100$	=	7.0 (at 25 C)

Actually, the emulsion described in Figure 5 is relatively unstable. When the above type of data was obtained for other, more stable emulsions, the "percent interaction" was found to be much higher. From these observations, it was reasoned that there might be a correlation between the amount of interaction and the actual resistance to phase separation.

This relationship was observed in a semiquantitative manner by making measurements on a series of emulsions (Fig. 6). As the ratio of Tween:Span was increased, the percent interaction increased, reaching a maximum at the 73:27 ratio. When the six emulsions described in Figure 6 were stored, this particular sample (73:27 ratio) showed somewhat better resistance to phase separation than did the two samples nearest in Tween:Span ratio, and considerably better stability than the remaining three emulsions which diverged widely from the 73:27 ratio.

A second series of emulsions was then prepared at the 73:27, Tween:Span ratio, but over a range of total emulsifier concentration (Fig. 7). Here a maximum "percent interaction" was observed at the 0.95% concentration of total emulsifiers. When these six emulsions were stored at room temperature, this particular sample appeared to have somewhat better resistance to phase separation than did any of the others at either higher or lower total emulsifier concentrations.

Another series of emulsions was then prepared using the sodium salt of SMG and glycerol monostearate as the emulsifiers. Since it is well known that oil-in-water emulsions prepared using anionic surfactants are relatively stable to phase separation, we anticipated that the interactions would be quite large. Actually, this proved to be the case (Fig. 8). When the level of SMG was raised from 0.4% to 2.0%, the interactions increased from 38 to 135%. Here a maximum interaction was observed at about the 2% SMG level, which also results in a maximum stability to phase separation. The data in Figure 8 were obtained by two individuals working independently on duplicate sets of emulsions (points marked X and O).

It appears that the "percent interaction" can be determined in a precise and reproducible manner for each emulsion, but a real problem arises in the selection of a method to measure emulsion stability. In preliminary tests, samples were stored at room temperature in graduated cylinders. After suitable intervals, the volumes of top fat phase, middle cream phase, and bottom water phase were estimated. In many cases, the phase boundaries were indistinct, and, as a result, an accurate estimate of rate of separation could be not obtained.

By modifying the pulsed NMR equipment to accommodate a flow-through cell (Fig. 2), the rate of phase separation could be monitored in a quantitative manner. Samples of emulsions, stored in glass columns, were pumped through the magnet to obtain profiles of fat distribution from bottom to top of the column.

Figure 9 illustrates the progressive separation with storage time at 25 C. At pH 7.3, this emulsion is relatively stable so that the initial reading, taken immediately after the emulsion had been prepared and cooled, shows a uniform fat distribution throughout the column. From the curves after 1, 3, and 7 days, it is evident that the fat has begun to rise to the top of the column and that this change progresses with time of storage. At any level in the column, the percent fat can be determined so that a profile of rate

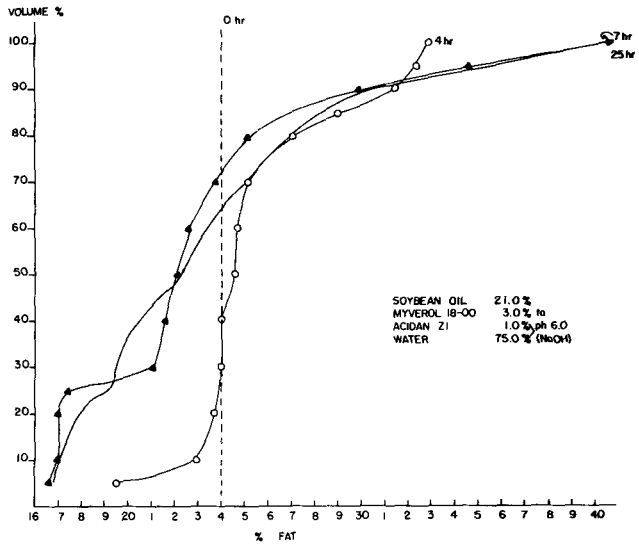

FIG. 10. Rate of phase separation in an emulsion at pH 6.0.

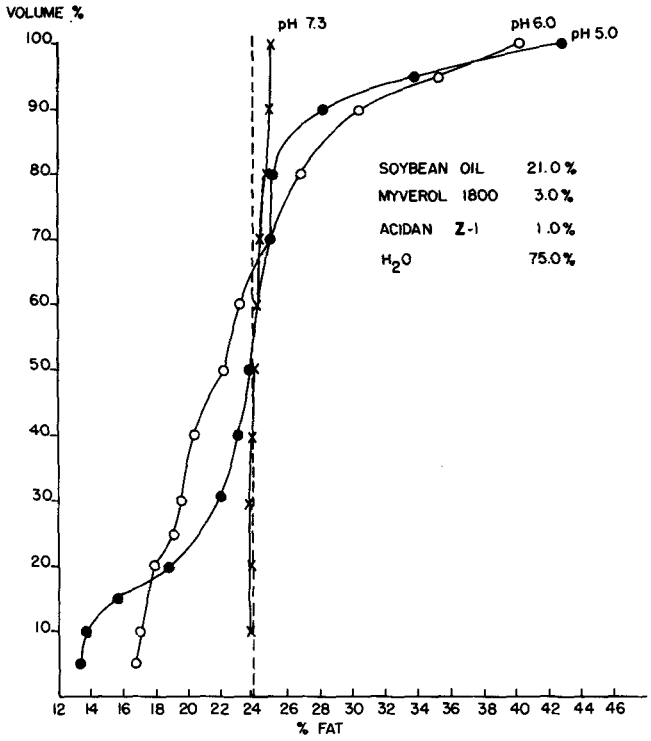

FIG. 11. Effect of pH on rate of phase separation in an emulsion.

and extent of phase separation is observed.

In Figure 10, the same emulsion formula is described, but here the pH was adjusted to 6.0. Since much less of the Acidan Z-1 is now present in the anionic form, its efficiency as an emulsifier is considerably reduced. Separation begins within a matter of hours. Within 4 hr, this emulsion is almost as badly separated as is the one at pH 7.3 after 1 day.

Finally, Figure 11 shows a comparison, after 24 hr of storage, between these two emulsions and a third at pH 5.0. At pH 7.3, separation is slight; but separations at pH 6.0 and 5.0 are substantial.

At pH 5.0, separation began almost immediately after the emulsion had been prepared, so that even the initial measurement at zero time showed an uneven distribution of fat.

REFERENCES

1. Preliminary Measurement of "Percent Solids" in Fats Using the Praxis PR-102 Transient NMR Analyzer, Applications Laboratory, The Praxis Corporation, San Antonio, TX.
2. Van Putte, K., and J. Van Den Enden, JAOCS 51:316 (1974).
3. Mansfield, P., Ibid. 48:4 (1971).
4. Van Putte, K., L. Vermaas, J. Van Den Enden, and C. Den Hollander, Ibid. 52:179 (1975).
5. Tiwari, P.N., P.N. Gambhir, and T.S. Rajan, Ibid. 51:104 (1974).
6. Levins, H.P., and F.G. Drommond, J. Pharm. Pharmacol, 5:743 (1953).
7. Griffin, W.C., "Kirk-Othmer Encyclopedia of Chemical Technology," Vol. 8, 2nd edition, John Wiley & Sons, Inc., New York, NY, 1965, p. 135.
8. Griffin, W.C., Proc. Sci. Sect. Toilet Goods Assoc. 6:43 (1946).
9. Petrowski, G.E., Food Technol. 29:52 (1975).
10. Becher, P., "Emulsions, Theory, and Practice," 2nd edition, Reinhold Publishing Corp., New York, NY, 1965, p. 424.
11. Shanbhag, S., M.P. Steinberg, and A.I. Nelson, JAOCS 48:11 (1971).
12. Haighton, A.J., L.F. Vermaas, and C. Den Hollander, Ibid. 48:7 (1971).

[Received December 19, 1975]