Fig. 2.—Quantitative response of piperine in the HNO₃–KOH–thiourea method.

where X = micromoles of piperine per milliliter of reagent and Y = absorbance at 490 mμ.

Therefore

% piperine in sample =

$$\frac{X(0.28533)(\text{dilution factor})}{\text{sample wt. (mg.)}} \times 100$$

The red chromogen is stable for as long as 2 hours and has a molar extinction coefficient of 5.299×10^3. It is unstable in acids, strong alkalies, water, alcohols, chloroform, and many solvents tried.

All compounds with the benzene nucleus will interfere; the severity of interference depends on the ease of nitration.

Reproducibility of absorbance values from day to day was good. Over a period of 20 consecutive days, runs at three levels of 0.05 to 0.15 μmole of piperine and pepper extracts containing 5.0–7.2% piperine showed absorbance variations of only 0.01 to 0.02.

piperine in pepper samples. With four levels of piperine added to black pepper, good recoveries of 99.2% to 100.7% (Table III) were obtained.

The standard curve for piperine (Fig. 2) obeys Beer's law between the levels of 0.02 to 0.2 μmole of piperine per ml. of reagent. It can be described by:

$$X = 0.25785\ Y + 0.00123$$

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Effect of Ultrasound Energy on Hydrolysis of Aspirin

By ERNEST MARIO and ROBERT J. GERRAUGHTY

The effect of ultrasonic energy on the hydrolysis of aspirin solutions at various temperatures and pH values is described. The reaction kinetics followed a pseudo first-order rate, both with and without the influence of ultrasound. The rate of hydrolysis was increased in all cases by applying sound energy. A mathematical treatment of the data disclosed that the effect of ultrasonic energy on the hydrolysis rate is relatively constant and can be compared to the effect obtained by increasing the reaction temperature.

THE VERSATILITY of ultrasonic energy, particularly as it has been employed to increase the rate of a number of chemical reactions, has been demonstrated by several investigators in the field (1–5); however, no references to the use of ultrasound in accelerated drug stability studies were found in the literature. Since such an application might offer advantages over the use of traditional physical means for effecting acceleration of degradation, this study was undertaken. The extensive work reported on aspirin–water systems and their degradation without the influence of ultrasound (7) led to the selection of this system for such a study.

EXPERIMENTAL

Equipment.—The ultrasonic energy was supplied by a 100-kc. generator[1] operated at the maximum

plate voltage of 1000 v. The transducer consisted of a mounted barium titanate crystal. Fitted to the inner wall of the ultrasonic bath was a round copper coil connected through an inlet–outlet pump arrangement to a separate constant-temperature water bath,[2] and controlled so that the temperature of the two baths was both constant and identical, within the limits of ±0.2°, during the individual runs.

Procedure.—Two sets of duplicate samples of aspirin in appropriate buffer solutions were used for all degradations. Duplication was accomplished by preparing a stock solution containing 100 mg. of aspirin per 100 ml. of water. Dissolution of the aspirin was aided by the addition of 5.0 ml. of 95% ethanol. Aliquots were pipeted from the freshly prepared stock solutions and brought to volume with the appropriate buffer in 50-ml. volumetric flasks. When the reaction was followed under conditions of high aspirin stability, it could only be allowed to go to about 10% completion, since the instrument

efficiency can be affected when run for long periods of time (6). Of the duplicate samples involved in each degradation, one was subjected to ultrasound waves, and the other was used as a control by immersing it in the second bath. Temperatures of 21, 25, 35, and 45° were used.

After the samples were placed in their respective baths, several hours were allowed to pass before the zero time reading was taken to insure that equilibrium had been attained. Aliquots were withdrawn from the reaction vessels at accurately measured intervals of time and the absorption values recorded.

Since the breakdown or hydrolysis of aspirin yields salicylic acid in a mole-for-mole ratio, the

TABLE I.—k RATES AT VARYING pH'S AND TEMPERATURES

Temp., °C.	k without Ultrasound, Hr. $^{-1}$	k with Ultrasound, Hr. $^{-1}$
pH = 2.00		
21	0.00159	0.00209
25	0.00230	0.00305
35	0.00557	0.00676
45	0.01370	0.0160
pH = 4.00		
25	0.00799	0.0102
35	0.0218	0.0266
45	0.0593	0.0629
pH = 5.96		
25	0.0104	0.0137
35	0.0305	0.0368
45	0.0748	0.0902

concentrations of aspirin and a duplicate as a blank. The agreement of the duplicate runs was good and indicated that the experimental technique was reproducible.

RESULTS

Pseudo first-order rates were followed at all pH values and with varying temperatures, both with and without ultrasound. A typical plot is shown in Fig. 1.

The k rate at each pH and corresponding temperature was calculated, and these values appear in Table I. It was found that the Arrhenius relationship was followed in all cases and that the heat of activation of the hydrolytic degradation of aspirin was not changed by the introduction of ultrasound. The Arrhenius data appear in Fig. 2. The increase in rate must be effected therefore by a physical
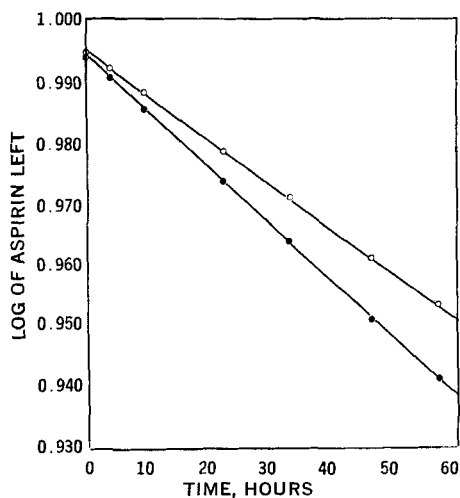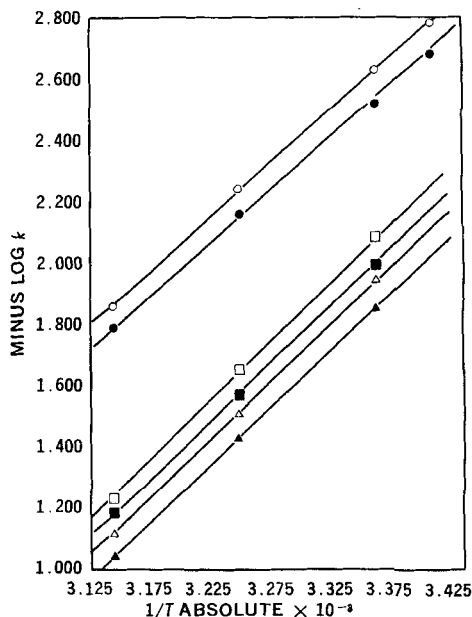

Fig. 1.—Pseudo first-order degradation of aspirin with and without ultrasound at pH 2.00 and 21°. Key: O, without ultrasound; ●, with ultrasound.

rate of degradation could be followed accurately by measuring the amount of salicylic acid formed. A Bausch & Lomb 500 spectrophotometer[3] was employed to determine the optimum wavelength of absorption for salicylic acid. A peak absorption for salicylic acid occurred at 302 mμ, and the absorption of aspirin at this wavelength was essentially zero. A Beer's law relationship at this wavelength was determined for salicylic acid at all pH values involved, and the molar absorptivity at pH 2.0 compared favorably with the results obtained by Edwards (7). A Beckman DU spectrophotometer[4] with automatic power supply was employed to analyze the reaction mixtures. The blank consisted of the buffer solution used for each trial. The following buffer systems were employed: (a) pH 2, hydrochloric acid in water; (b) pH 4, acetic acid and sodium acetate; (c) pH 5.96, disodium acid phosphate and sodium dihydrogen phosphate.

It was not necessary to recrystallize the aspirin extensively since any salicylic acid present initially would be accounted for in the time zero reading. All the trials were made at least twice with varying

Fig. 2.—Composite of Arrhenius data. At pH 2.00, O, without ultrasound and ●, with ultrasound (H_{act} = 16,560 cal.). At pH 4.00, □, without ultrasound and ■, with ultrasound (H_{act} = 17,540 cal.). At pH 5.96, △, without ultrasound and ▲, with ultrasound (H_{act} = 17,660 cal.).

mechanism. (For example, an increase in the frequency of molecular bombardment within the reaction mixture.) Ultrasound produced an increase in the degradation rate that was equivalent to increasing the reaction mixture temperature within the range of 1.8–2.9°. This temperature effect was calculated by employing the following combined form of the Arrhenius equation.

$$\frac{\log k}{\log k_1} = \frac{H_{act}}{2.3R} \left(\frac{1}{T'} - \frac{1}{T} \right)$$

The various values calculated appear in Table II.

DISCUSSION

A review of the tabulated data showing k rates of the reaction appears to indicate that the influence of ultrasound had a diminishing effect as the tem-

TABLE II.—EFFECT OF ULTRASOUND IN TERMS OF ABSOLUTE TEMPERATURE

Absolute Temp.	Equiv. Temp. with Ultrasound
pH = 2.00	
294	296.9
298	300.6
308	310.3
318	319.8
pH = 4.00	
298	300.0
308	310.0
318	320.1
pH = 5.96	
298	300.2
308	310.0
318	320.0

perature of the reaction mixture increased at a given pH. However, a more careful examination of the data shows that ultrasonic energy did in fact exert a nearly constant effect on the reaction mixture at varying temperatures. The use of a combination form of the Arrhenius equation has disclosed the fact that ultrasound has an effect equal in acceleration to that brought about by increasing the temperature from approximately 1.8–2.9°. This range was consistent, regardless of the pH or temperature used.

It is not surprising that changes in pH did not affect the per cent increase noted through the use of ultrasound. At pH 2, for example, the apparent or observed k was essentially the result of the expression $k = k^\circ$ (Asp)(H$^+$).

The concentrations of molecular aspirin and (H$_3$O$^+$) were so much greater than the ionic species and (OH$^-$) at this pH that any contributions of

these latter species toward the observed k values could be considered negligible. Thus, in the expression

$$\frac{k}{k_1} = \frac{k^\circ \text{ (Asp) (H}^+)}{k_1^\circ \text{ (Asp) (H}^+)}$$

the (H$^+$) cancels in both numerator and denominator since the system is buffered. Similar conditions held for pH's 4 and 6. A more detailed description of the various k's involved in reactions of aspirin in water can be found in the work by Edwards (7) referred to earlier in this paper.

It had originally been postulated that a different order might result when the reaction was accelerated with sound energy, but this proposal proved to be erroneous. If the effect of ultrasound had varied with the changing concentrations of reaction products and starting material, a new order would probably have resulted. That the kinetic order remained the same suggests that ultrasound has a constant effect on the reaction mixture, regardless of any change in the composition of the reactants.

The slight variations observed in the Arrhenius plot might have been due to experimental error, or they might have been caused by slight variations in the sound power delivered to the bath. The error which might have been incurred in plotting the slope is magnified by the 2.3 factor involved in reactions of this kinetic type.

CONCLUSIONS

Therefore, it can be concluded that ultrasonic energy might be a useful tool in the acceleration of certain reactions. Also, in hydrolytic reactions containing very heat labile ingredients, which degrade by means other than those involving frequency of collision, the use of ultrasound may be indicated in accelerating such reactions since there was no heat factor involved in the use of sound energy. The use of low-frequency sound is suggested since little or no cavitation ensues, and the possibility of secondary products is minimized or eliminated.

The interesting effect of sound energy in terms of equivalent temperature increase is noteworthy. It would be superfluous to draw any concrete conclusions from this single study, but a further extension of this type of investigation is indicated to determine whether the effect noted in this work is reproducible in other systems of hydrolytic degradation.

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