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The *in vitro* evaluation of gelatin coacervate microcapsules

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Microcapsules of sulphadiazine have been prepared by the simple gelatin coacervation technique, using sodium sulphate as coacervating agent. The free flowing microcapsular material was hardened with formalin. There is no direct relation between particle size and sulphadiazine concentration nor between different starting gelatin percentages. The effects on size of hardening time, temperature and sampling time are small. *In vitro* dissolution studies show that first order release characteristics are exhibited by all the hardened materials. Temperature and pH effects indicate the dissolution of the sulphadiazine itself to be the controlling step rather than the rate of diffusion through the microcapsule wall.

Although coacervation has been applied to the encapsulation of a wide variety of products (Green & Schleicher, 1956a,b; Phares & Sperandio, 1964; Luzzi & Gerraughty, 1964; Nixon, Khalil & Carless, 1968), few published data are available about the size distribution of microcapsules or the *in vitro* release of inclusions, particularly for simple coacervate systems such as gelatin–water–sodium sulphate (Phares & Sperandio, 1964; Nixon & others, 1968). Release of material from the complex coacervate produced by gelatin and acacia has been extensively studied by Luzzi & Gerraughty (1967a,b) and Bell, Berdick & Holliday (1966), as has that from nylon (Luzzi, Zoglio & Maudling, 1970).

Whilst the sizing of microcapsules has been performed by a number of methods (*Editorials* 1963, 1968; Luzzi & others, 1970; Shigeri, Koishi & others, 1970), a detailed examination of temperature and hardening effects has not been reported.

In the present work we have examined the comparative effects of pH, temperature, % sulphadiazine content and hardening on the *in vitro* release of sulphadiazine from simple coacervate microcapsules and we have recorded the effect of temperature, % sulphadiazine content, and hardening on their size.

MATERIALS AND METHODS

Gelatins: acid pre-treated material, deionized by the method of Janus, Kenchington & Ward (1951) was used throughout. This material had the following characteristics. Bloom No. 274, pH 4·2, viscosity ($6\frac{2}{3}$%, 40°) 6·2 cP, I.E.P. 9·2.

Sulphadiazine: (May & Baker) recrystallized from ethanol; m.p. 254·5–255·5°. Differential scanning calorimetry and infrared analysis showed the presence of Form 1 only.

All *electrolytes* were of A.R. grade; *water* was triple distilled from an all glass still.

Sulphadiazine was assayed by the method of Bratton & Marshall (1939).

Preparation of microcapsules. The method of Nixon & others (1968) was used with the following variations: a 10% w/w starting gelatin concentration and a 4:1

ratio of 7% cold sodium sulphate solution (7–9°). Batches required 300 g of gelatin solution as the starting material.

Dissolution studies. The rate of dissolution from the various microcapsules was examined by stirring the sample and dissolution medium in a flask. The microcapsules (sufficient to give 20 mg of sulphadiazine litre^{-1}) were prepared as a slurry, to prevent agglomeration, poured into 1 litre of McIlvaine buffer solution and stirred at 100 rev/min. The temperature was controlled to $\pm 0{\cdot}5°$. Samples were removed at suitable intervals and filtered through a Swinnex Millipore adaptor fitted to a syringe, before assay for sulphadiazine.

Particle size analysis. A suitable count level dispersion of the microcapsules was prepared by pouring a concentrated slurry, deflocculated by exposure to ultrasonics for 15 s, into 250 ml of 0·9% saline contained in a double walled Coulter cell. The temperature was controlled to $\pm 0{\cdot}5°$. Counts were taken at zero time and 30 min intervals using a Coulter model B calibrated with pecan pollen (42·3 μm diam.). The results were processed using a computer program first developed by Marshall & Ord Smith and adapted by Raymond (1968).

RESULTS

Particle size analysis

The results in Table 1 show that increasing the temperature, particularly for hardened microcapsules, causes only a slight decrease in size compared with the effect of changes in sulphadiazine content. However, from a study of other sulphonamide contents no direct correlation was immediately apparent between microcapsule size and sulphonamide content.

Table 1. *The effect of temperature and hardening time on the mean cumulative weight percentage oversize.*

Sampling time (min)	Temperature °C	% Sulphadiazine: 5 — Mean microcapsule size, μm, after hardening with formalin, at various times (min)					% Sulphadiazine: 30 — Mean microcapsule size, μm, after hardening with formalin, at various times (min)				
		0	30	60	120	180	0	60	120	180	240
0	10	84·5	85·5	84·5	86	86·7	—	43	37	38·5	35
30		84·4	85	—	87	88·7	—	43	37·5	38	34
0	20	80·5	86	85	83	83·5	28·5	41·5	33·5	35	36
30		78·5	85	86	84	84	28·5	37·5	32	33	35
0	30	—	83	82	83	83·5	18·5	28	28	27·5	26
30		—	82	82	83	82	12·0	26	27	27	26
0	40	—	82·2	80·9	81·3	80·5	—	—	26	—	26
30		—	81·3	82	80	80	—	—	26	—	—

Effects of hardening

Capsules containing 5% of the sulphonamide show little difference in size when hardened with formalin for up to 3 h. With 30% sulphadiazine the effect of hardening time is more marked; unhardened capsules were smaller than hardened ones. Hardening for 1 h with formaldehyde produces an increase in size which gradually decreases again as the hardening time is extended to 4 h. At temperatures below 30° there is no significant difference in capsule size up to 30 min after the

commencement of hardening. At 30° and above, the unhardened capsules were sensitive to sampling time, the hardened capsules were not.

The temperature at which the particle size analysis is made may cause size differences either by solution of the gelatin walls or the production of a diffuse hydrated layer. For both samples in Table 1 an increase in temperature up to 40° caused a fall in microcapsule size. With 5% sulphadiazine capsules (hardened) the effect is relatively small: for microcapsules containing 30% sulphonamide, particularly at 30–40°, the effect of temperature on the size was greater than that caused by the time of hardening.

The reaction between formaldehyde and the sulphonamide core material produces a soluble addition compound whose removal may cause a diminution in the microcapsule size. The other possible cause of particle size reduction could be dissolution from the microcapsules during the counting time.

Fig. 1, a typical series of dissolution data, shows that the initial release rate from the microcapsules is extremely rapid, but that after approximately 25% of the core material has been dissolved there is a gradual fall in the rate of release with the last 20% released very slowly. This pattern holds for all the dissolution rates investigated.

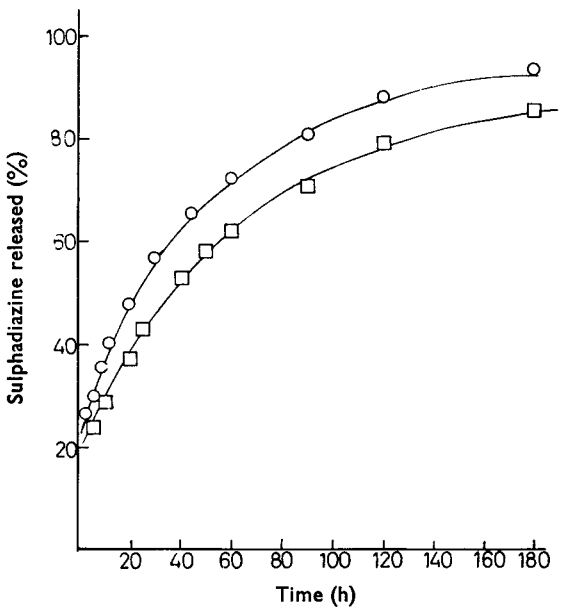

FIG. 1. Typical dissolution curves for sulphadiazine release from microcapsules. Temp. 20°, pH 6, Sulphadiazine ○, 5; □, 4%. 30 min hardening time.

The dissolution data have been examined by the methods of Gibaldi & Feldman (1967), Schwarz, Simonelli & Higuchi (1968) and Wagner (1969). Factors studied include the % sulphadiazine content of the microcapsules, the effect of hardening with formalin, the temperature of dissolution and the pH of the dissolution medium.

When the gelatin concentration was maintained constant, there was no apparent effect on the dissolution rate from an increasing sulphadiazine concentration in the microcapsules.

Time of hardening

The effect of hardening on the capsule size and the leaching effect of the formalin on the sulphonamide complicate the study of hardening effects on dissolution rates. Wagner plots (Fig. 2Ai) derived from hardened microcapsules no longer give straight lines over the whole range. As in all the dissolution experiments on this system, the initial release of drug was rapid and the amount released was slightly increased for longer hardening periods. Because of the cross linking effect of formaldehyde with the gelatin, the 50% release time was increased from that of unhardened capsules; hardening time to 50% release time (min) are: 0/13, 30/37, 120/37, 180/26. Higuchi plots (Fig. 2Aiii) show that hardening the microcapsules resulted in the dissolution obeying the Schwarz & others (1968) model.

The first order release plots (Fig. 2Aii) give straight lines for the hardened materials. As the hardening time was increased the initial rapid release of sulphadiazine was greater. Once the straight line portion of the curve is attained, the slope of the line is marginally dependent on the hardening time [Hardening time (min)/slope $\times 10^3$ are 30/–4·9, 120/–3·7, 180/–4·6]. Shorter hardening times still produce a faster dissolution rate; the unhardened sample gives a shallow curve with a faster release time than the formalin-treated material.

Unhardened gelatin microcapsules exhibit a tendency to disperse or dissolve above 30°. Hardening, as shown by Table 1, is effective in preventing solution of the capsule wall particularly with the large capsules of low sulphonamide content. Dissolution from unhardened microcapsules gave the usual straight line Wagner plots; above 30° solution occurred. An example of the effect of temperature on dissolution from microcapsules hardened for 30 min is shown in Fig. 2B.

At temperatures where the unhardened gelatin would not normally soften and dissolve, the plots are curvilinear, but at higher temperatures, where unhardened gelatin would dissolve, a straight line relation exists. At temperatures up to 60° the hardened microcapsules showed no tendency to disperse. A plot of the log mean release time against reciprocal temperature for this hardened sample gave a straight line.

Higuchi plots of the above data for hardened microcapsules produce no straightforward relation between temperature and dissolution rate.

First order release plots give a straight line relation, but when relatively small amounts of the sulphonamide core material remained to be dissolved the slope of the line changed to give a much slower release. An Arrhenius plot derived from the first order dissolution graphs gives a good temperature-rate relation (Fig. 3).

For the effect of the pH of the dissolution medium, Wagner plots gave straight lines at all pH values studied, although with low pH values there was a tendency at initial sampling times to depart slightly from the straight line. Higuchi plots were curvilinear and could not be readily interpreted; a plot of log % drug remaining against time showed a straight line down to 10% remaining, after which a slower dissolution rate occurred at low pH values. A plot of the slopes is shown in Fig. 4. At pH values between 2 and 6 there is little effect on the rate of dissolution, but once the pK_a of the NH group is passed and higher pH values are reached, the dissolution is extremely rapid. The McIlvaine buffer systems used do not go below pH 2·2 and the use of 0·1N HCl to give a pH of 1·5 produced a dissolution rate which did not fit the remainder of the data.

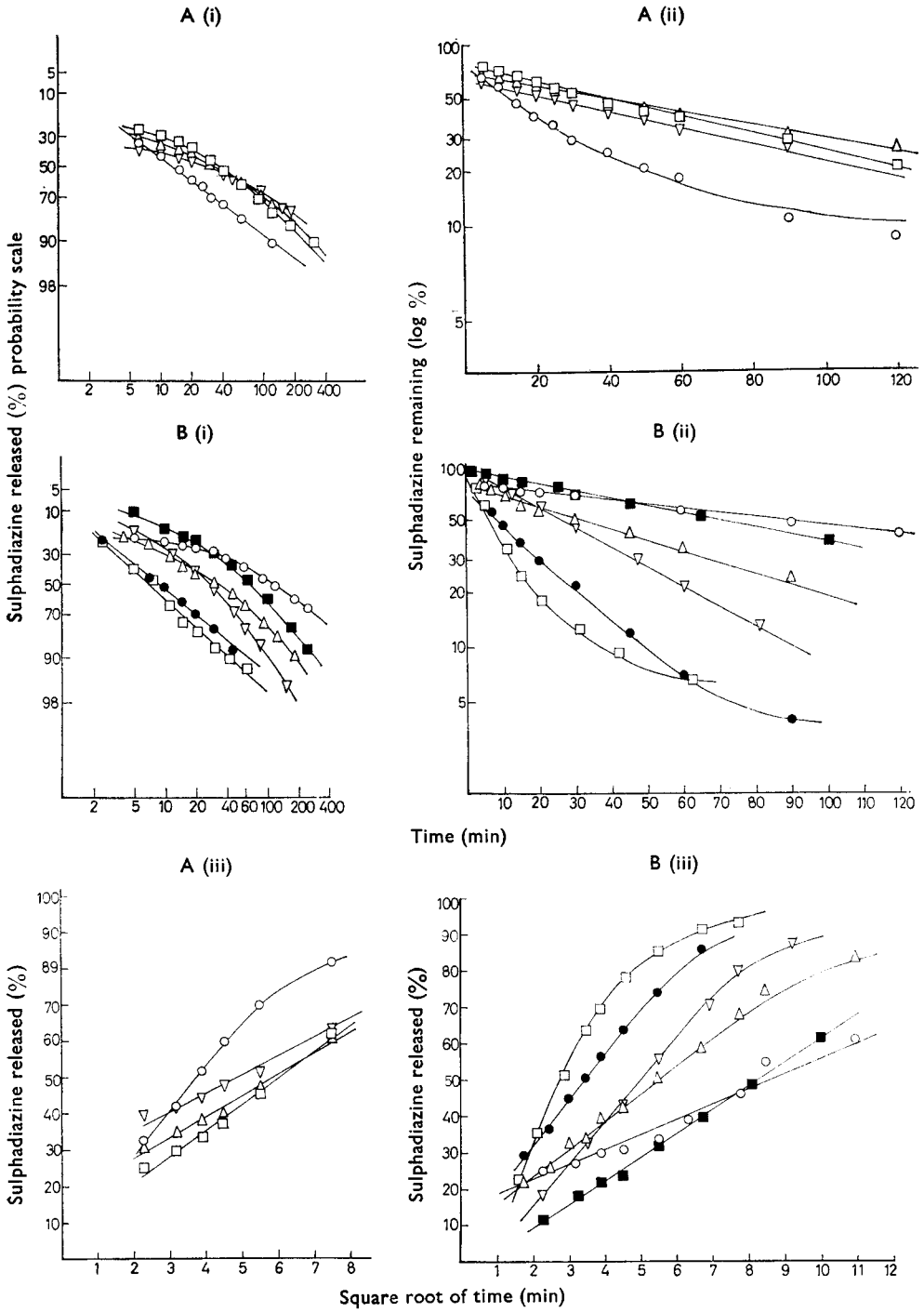

FIG. 2. A. Dissolution plots illustrating the effect of hardening. Temp. 20°, pH 6. Sulphadiazine 5%; (i) Wagner plots; (ii) First Order plots; (iii) Higuchi plots. Time of hardening: ○, unhardened; □, 30; △, 120; ▽, 180 min.

B. Dissolution plots illustrating the effect of temperature. Sulphadiazine 5%, 30 min hardening time, pH 6. (i) Wagner plots; (ii) First Order plots; (iii) Higuchi plots. Temperature: ○, 10; ■, 15; △, 20; ▽, 25; ●, 30; □, 40°.

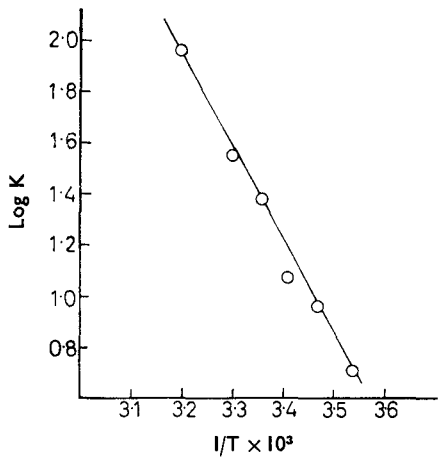

FIG. 3. Arrhenius plot of first order release rates from Fig. 2Bii.

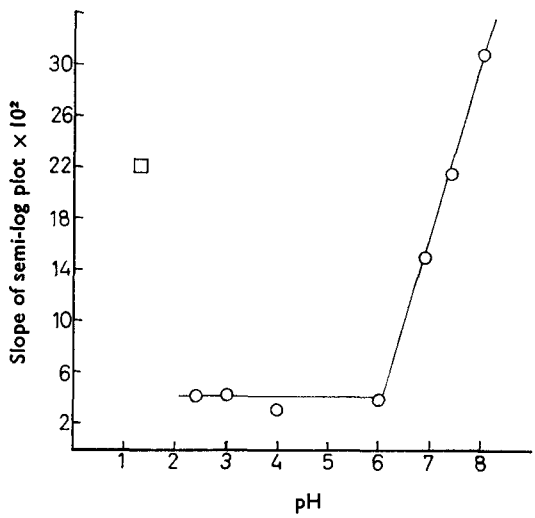

FIG. 4. Effect of pH on the slope of the first order dissolution plots. Sulphadiazine 5%, 30 min hardening time. Temp. 40°; ○, McIlvaine buffer; □, 0·1N HCl.

DISCUSSION

During the preparation of the microcapsules, before extraction and hardening and whilst the temperature is still high enough to keep the gelatin coacervate liquid, they are spherical. On hardening, the gelatin wall shrinks and the capsules tend to take on the shape of the crystals of sulphadiazine dispersed in them. Unlike microcapsules made from liquids or emulsified inclusions, which usually contain only one droplet, the individual capsules will be composed of a number of crystals which tend to be arranged towards the centre of the microcapsule.

The effect on unhardened microcapsules of temperatures over 30° may be interpreted by assuming solution of the gelatin wall. With both high and low sulphonamide:gelatin ratios the size of the capsules was relatively unaffected at lower

temperatures. Even at 10° or 20° a certain amount of solution appears to take place because there is an increase in size with capsules that have been hardened for up to 1 h. This effect is very much more pronounced at the higher temperatures where hardening with formalin renders the gelatin wall almost insoluble.

All capsules show an almost immediate hydration and swelling effect in water. Hardening reduces the degree of hydration and this is reflected by the slight fall in capsule size when longer periods of hardening are used. This decrease in size is greatest at 10° and 20° with the microcapsules containing 30% sulphadiazine.

One complicating factor in these particle size studies was the removal of sulphadiazine from the microcapsules in the form of a soluble compound with the formalin. Other hardening agents such as gluteraldehyde and acrolein reduced the sulphonamide content more rapidly and to a greater extent. The large microcapsules, with a small sulphonamide content, were far less susceptible to the dissolution of their contents than the smaller thin-walled capsules containing a relatively high proportion of inclusion (Table 2). With these thin-walled specimens, hardening times in excess of 1 h could not be used. Comparison of particle size data in Table 1 for 0 and 30 min sampling times does not suggest collapse of the capsules on the removal of the inclusion and the space occupied by the sulphadiazine appears to remain as a vacuole within the microcapsule.

Table 2. *The effect of hardening time on the sulphadiazine content of microcapsules at* 25°.

Starting % sulphadiazine	Sulphadiazine % after hardening with formalin for: 0 min	30 min	60 min	120 min	180 min	240 min
5	100	94	81·5	70	58	—
30	100	—	70	19	15·5	10

Dissolution of an encapsulated material will be controlled by a number of factors. The rate at which the solvent penetrates the wall material, p, the rate at which the drug dissolves in the solvent, s, and the rate at which the dissolved drug can penetrate the wall and disperse from the surface, p_1. The total rate of release, R, can thus be described as $R = f(p + s + p_1)$. It is not necessary that all these factors will have an equal importance and any may exert an overriding influence on the others.

Dissolution from the microcapsules is further complicated because they always contain a small proportion of sulphadiazine either unencapsulated or contained in cracked capsules (Nixon & others, 1968). This is termed "free sulphonamide" and accounted for approximately 2–10% of the total sulphonamide present: it is the material released rapidly during the dissolution studies. In the remainder of the inclusion contained in complete microcapsules, a certain amount will be associated with the wall material, but the bulk will exist as individual crystals in the centre of the microcapsules.

The dissolution and *in vitro* release of the drug from its formulation is open to treatment in different ways that are not always easy to correlate (Schwarz & others, 1968; Wagner, 1969; Langenbucher, 1969; Luzzi, Zoglio & Maudling, 1970). Further, the application of any one of these treatments to drug release from microcapsules presents a number of exceptions, no one model being able to adequately describe all release situations.

Wagner (1969) plots, because they tend to produce straight lines, allow easy comparison of the T50 release time and it has been suggested that this parameter, coupled with σ of the curve would serve to define the release pattern. His system, a tablet, had initially a low surface area which subsequently increased on disintegration. The method of calculation took this factor into account. The data here presented are not always amenable to this type of plot. In no case is a straight line obtained from the commencement of dissolution: there are other differences depending on hardening and temperature.

The Higuchi equation (Schwarz & others, 1968) was developed to define the release from wax matrices. These were tabletted and presented a rather low constant surface area throughout the course of dissolution, but because of removal of drug via the tortuous channels, which could present a constantly changing area for drug dissolution, porosity and "tortuosity" factors had to be introduced. In Higuchi's systems it was necessary to study slow release rates and the experiments did not, in general, proceed past 30% release in approximately 100 h.

Hardened microcapsules present an extreme case of the Higuchi model. The surface area is very large, but remains approximately constant throughout the experiment. Because of the coacervate nature of the original wall, it is possible that porosity and "tortuosity" will play an important part in the release of the inclusion. Even so, straight line relations were not obtained in all cases, though where these do occur, they remain linear to greater than 60% release. The fit of the data to this equation may be entirely fortuitous and with unhardened microcapsules a linear relation was never obtained, although the size remained little changed throughout the course of the dissolution.

As our system retained its gross particle size throughout the course of the experiment, the use of the Langenbucher (1969) cube root treatment for non-disintegrating granules might be expected to apply. Whilst this treatment adequately described his systems down to about 85% dissolution, application to microcapsules at no time produced a single straight line.

The best fit for the bulk of the data was a first order kinetics plot. The equation used, which applies in conditions of exponential change of surface area and under sink conditions was $\log (W^{\infty} - W) = \log M - \frac{k_s}{2{\cdot}303} (t - t_0)$ for $t \geqslant t_0$ where $W^{\infty} - W$ is the amount remaining undissolved, $M = (K/k_s)C_sS^{\circ}$ = intercept, t_0 is zero time, t is sampling time, S° is surface area available for dissolution at the commencement of the experiment, C_s is the equilibrium solubility and K and k_s are constants.

This gave straight lines down to approximately 10% sulphadiazine remaining, with hardened material for all parameters studied. An Arrhenius plot from hardened material was also a straight line. Even with this treatment, however, the unhardened microcapsules did not produce a straight line relation.

The effect of hardening is complicated by the relation between formalin and sulphonamides. Due to the leaching effect it is suggested that a gradient of sulphadiazine is set up in the microcapsule wall. The longer times of hardening produce more of this "wall material", which will be released faster than the crystalline inclusion at the capsule centre.

The nature of the cross linking process for hardening the capsules appears unable to significantly reduce further the rate of dissolution of the sulphadiazine for hardening

times greater than 30 min. The coacervating agent, sodium sulphate, will be rapidly dissolved from the microcapsule wall by the aqueous dissolution medium to leave pores through which the dissolution process can take place. The cross linking process cannot prevent this and the number of dissolution channels produced by removal of the sodium sulphate is probably the same irrespective of hardening time. The unhardened material does not exhibit first order release characteristics, probably due to changes in the coacervate wall brought about by the gelatin forming a diffuse hydrated layer. Although no straight line relation exists, tangents to the curve give a greater slope, faster release rate, than any of the hardened materials.

The gelatin wall of unhardened microcapsules dissolved at temperatures above 30°, but with hardened samples there appears to be little effect on the size. All the plots show that at the higher temperatures the dissolution pattern changes slightly. Even so, down to about 85% dissolution there is a straightforward Arrhenius relation (Fig. 3): only at 40° is there a secondary slower release rate from the sample. Thus the effect of temperature appears to be only on the solubility of the sulphonamide and not on the permeability of the gelatin wall.

The effect of pH is straightforward. Until sulphadiazine becomes ionized there is no effect of pH on the rate of dissolution in McIlvaine buffer. Because the sodium salt is far more soluble the rate of dissolution at pH values in excess of 6 rises very rapidly. This appears to be simply a solubility effect.

It would appear that the *in vitro* dissolution of sulphadiazine from gelatin microcapsules is not amenable to any one type of treatment. Whilst first order rate kinetics allow the interpretation of most data it is necessary that other treatments of the results be applied to obtain the full picture.

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