J. Pharm. Pharmac., 1966, **18**, 697–708 Received May 2, 1966

Accelerated crystal growth of sulphathiazole by temperature cycling

J. E. CARLESS AND A. A. FOSTER

A novel method is suggested for accelerating the physical changes in suspensions. The suspension is stirred in a jacketed vessel and is alternately heated and cooled. Results indicate a rectilinear relationship between the median particle size of sulphathiazole in suspension and the number of temperature cycles. Crystal growth is measured by the Coulter counter and growth rates are compared arithmetically to indicate which additives (surface-active agents) may be used to inhibit crystal growth.

THE crystal growth of particles suspended in fluids may be due to polymorphic change, Ostwald ripening or temperature fluctuations during storage. The last two factors will become more significant when the range of the particle size is wide, since the small particles will tend to dissolve and recrystallise on the large particles. This effect is described by the Ostwald-Freundlich equation (Ostwald, 1900; Freundlich, 1930) and is usually insignificant above 5 μ, because there is little change in solubility with change in size above this level (Jones & Partington, 1915; Fischer & Ferguson, 1950; Rumford & Bain, 1960). The relationship between solubility and particle size was correlated with the charge on the particles by the equations of Lewis (1909) and Knapp (1922). Polderman (1962) reviewed the relationship of surface-active agents, temperature changes, polymorphism and particle size to crystal growth in pharmaceutical suspensions and many references are available in the literature about crystal growth in essentially saturated suspensions and from supersaturated solutions.

Nogami & Nagai (1958) and Hasegawa & Nagai (1958) studied the effect of pH, surface-active agents and time on the crystal growth of aqueous suspensions of sulphadiazine stored at one of four constant elevated temperatures. They concluded that all of the factors interacted, but the most significant was temperature.

In general, the first polymorphic form to crystallise from a supersaturated solution will be the metastable form and this may quickly revert to the more stable form. This has been demonstrated clearly for cholesterol (Higuchi & Saad, 1965; Saad & Higuchi, 1965). Furthermore, milling may introduce polymorphic change (Polderman, Bloo & Fokkens, 1958; Lees, 1963) and the formation of the hydrates and solvates of organic materials may also be important in this context (Shefter & Higuchi, 1963). In some instances the metastable form of a medicament may have a greater pharmacological activity than the stable form and, where this is so, reversion to the less energetic, stable modification must be prevented (Higuchi & Lau, 1962).

Many workers have studied crystal growth from supersaturated solutions and have concluded that impurities, surface-active agents and other

From the Department of Pharmacy, Chelsea College of Science and Technology (University of London), Manresa Road, London, S.W.3.

additives, markedly retarded crystal growth rates (Fysh, 1951; Albon & Dunning, 1957, 1960; Okano, Kojima & Satake, 1957; Okano, Kojima & Akahosi, 1958; Michaels & Colville, 1960; Allen, Milosovich & Mattocks, 1965; Higuchi & Saad, 1965; Saad & Higuchi, 1965). However, our technique of producing crystal growth not only involves crystallisation rates, but also dissolution rates, because the large particles are made to grow at the expense of the small particles. The dissolution rates of several pharmaceutical materials have been studied with the Coulter counter (Edmundson & Lees, 1965; Higuchi & Saad, 1965; Saad & Higuchi, 1965).

It is desirable that an accelerated storage test should simulate, as closely as possible, the conditions of normal shelf storage. When a preparation is exposed to ambient conditions, the range of temperature experienced is variable within fairly closely defined limits, but it is always variable. The accelerated storage test imposed should supply exactly similar temperature fluctuations, but at greatly increased frequencies. However, accelerated tests have previously been designed to effect the acceleration of degradation by using elevated temperatures. The present test design demonstrates the effect of altering the frequency of temperature fluctuations at fairly low temperatures resulting in particle size changes, which are dependent upon (*a*) the slope of the solubility curve, (*b*) the rates of dissolution, crystallisation and diffusion, (*c*) the range and frequency of the temperature cycles.

Sulphathiazole was chosen since it showed a large increase in water solubility with temperature rise, i.e. over the temperature range 23–33° the solubility almost doubled in some vehicles. However, the solubility at constant temperature was insufficient to render particle size determinations difficult when using the Coulter counter or sedimentation techniques.

Materials and apparatus

The crystal growth studies were made on sulphathiazole B.P. micromilled and of mean size 6 μ. Differential thermal calorimetry (Perkin Elmer D.S.C.I.*) indicated that two polymorphic forms were present and, in this respect, the sulphathiazole corresponded to the authentic sample of sulphathiazole supplied by the Department of Pharmaceutical Sciences, Pharmaceutical Society of Great Britain.

In some experiments the sulphathiazole, at a concentration of 0·3% w/v, was suspended in an aqueous vehicle containing 0·1% v/v Nonidet P42 (Shell Chemical Co. Ltd.) as wetting agent. This was used without further purification and contained 27% of a polyethylene oxide condensate. In other experiments, 0·3% w/v concentrations of sulphathiazole suspended in aqueous vehicles containing 0·006, 0·06 or 1·0% w/v of cetomacrogol 1000 B.P.C. were examined.

All concentrations of the wetting agents were in excess of their critical micelle concentrations.

* Perkin-Elmer Ltd., Beaconsfield, Bucks.

TEMPERATURE CYCLING APPARATUS

Suspensions were alternately heated and cooled in a jacketed, stainless-steel reaction vessel. The vessel was lagged and fitted with an inlet pipe to the base of the jacket and an outlet pipe at the top, diametrically opposite the inlet pipe. The reaction vessel had an essentially airtight lid, in the form of a cupola, so designed that condensation occurring on the lid could run back into the reaction suspension.

The suspension was stirred by a stainless-steel paddle-type stirrer at 680 rpm and the temperature of the suspension was recorded by a conventional thermometer passing through the lid.

"Hot" water was allowed to flow from a thermostatic bath, through the vessel jacket and back to the same bath during 8 min, then "cold" water from a second thermostatic bath was allowed to flow for 8 min. The 16 min cycle was repeated for the desired number of temperature fluctuations. This time cycle was selected to effect an accelerated temperature fluctuation simulating daily variations in temperature. A shorter cycle was less reproducible because the bath temperatures were necessarily more divergent.

The results quoted in this paper were obtained by the manual operation of the temperature cycling apparatus during continuous periods of up to 33 hr. This was avoided in later work by automating the process using more suitable water circuitry, and a "cyclothermostat" (Carless, Foster & Jolliffe, 1966). A comparison of the temperature hysteresis of manual and automatic cycling techniques is made in that paper.

COULTER COUNTER

A Coulter counter, model "A",* with a 100 μ orifice was used for the particle sizing. The electrolyte solution used for counting was prepared by saturating with sulphathiazole a solution containing 0·9% w/v sodium chloride and the preselected concentration of surface-active agent, shaking for three days at 23° and filtering through Whatman No. 1 filter paper and then through a No. 4 sintered glass filter. Corrections for background counts and coincidence were made in the usual way.

The sample of suspension at 23° was always removed from the reaction vessel $15\frac{3}{4}$ min after the beginning of a temperature cycle (see Carless & others, 1966, for details). A suitable aliquot was added to 150 ml of saturated electrolyte solution in the Coulter beaker and was stirred for 10 min before counting was commenced. Total counting time was approximately 9 min.

Methods and results

CRYSTAL GROWTH

Fig. 1 describes the change in particle size and particle size range when a suspension of sulphathiazole was submitted to temperature cycling

* Coulter Electronics Ltd., Dunstable, Beds., England.

in a vehicle containing 0·1% v/v Nonidet P42 in a saturated solution of sulphathiazole.

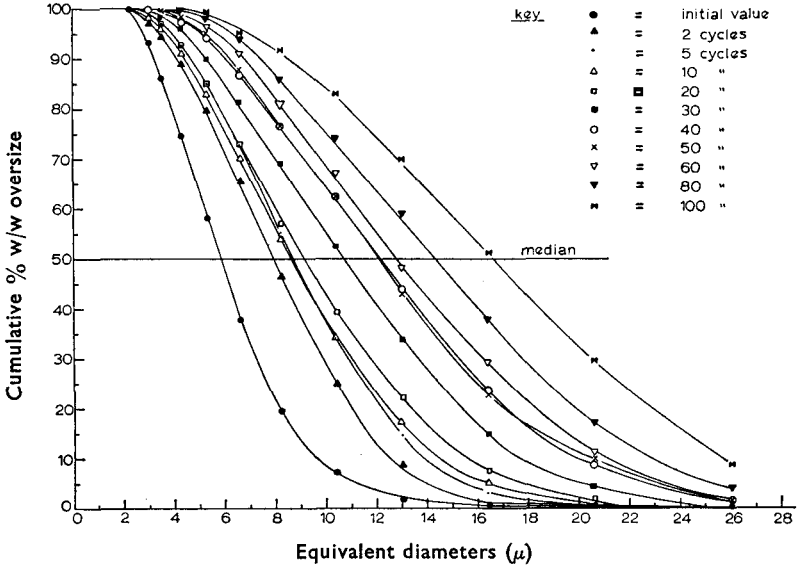
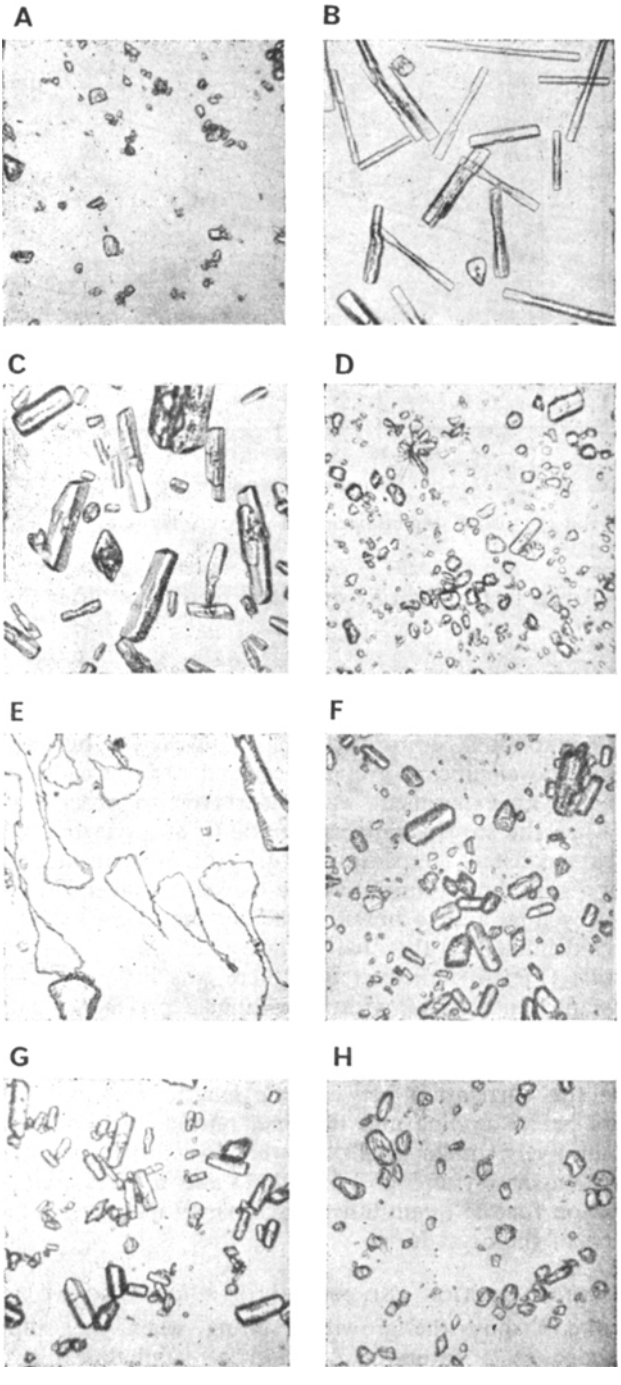


FIG. 1. Crystal growth of sulphathiazole in 0·1% Nonidet P42.

The temperature was taken through a cycle from 23° to 43°. Fig. 2C shows the crystal shape and size after growth under these conditions, while Fig. 2 A represents the original suspension. When the median values from Fig. 1 were plotted against the number of temperature cycles, a rectilinear increase in the equivalent diameter occurred, after an initial period of 2–4 cycles (Fig. 3). However, many subsequent experiments indicated that the initial phase usually corresponded to 8–10 cycles. Fig. 3 shows that the data from Fig. 1 could be plotted as the median size derived from curves of percentage number oversize (N/N), or as percentage weight oversize (W/W) and that in both cases a rectilinear relationship existed.

Straight lines were obtained by the method of least squares, the significance limits of the Student *t*-distribution were calculated and P (the probability value) was found to be much less than 0·001, confirming that the experimental results fell on the straight line. Subsequently, more than 40 similar experiments have shown P to be less than 0·001. Therefore, it is safe to assume that, under these growth conditions, the response

FIG. 2. Key to photographs: A, Original suspension, no treatment. B, 0·4% v/v Nonidet P42 vehicle, 32 cycles. C, 0·1% v/v Nonidet P42 vehicle, 32 cycles. D, 0·1% v/v Nonidet P42 vehicle, stored at 43° during 63 hr. E, 0·1% v/v Nonidet P42 vehicle, stored at 43° during 63 hr, then 36 cycles, then stored at 43° during further 15 hr. F, 0·006% w/v cetomacrogol vehicle, 28 cycles. G, 0·06% w/v cetomacrogol vehicle, 28 cycles. H, 1·00% w/v cetomacrogol vehicle, 32 cycles.


A
B
C
D
E
F
G
H

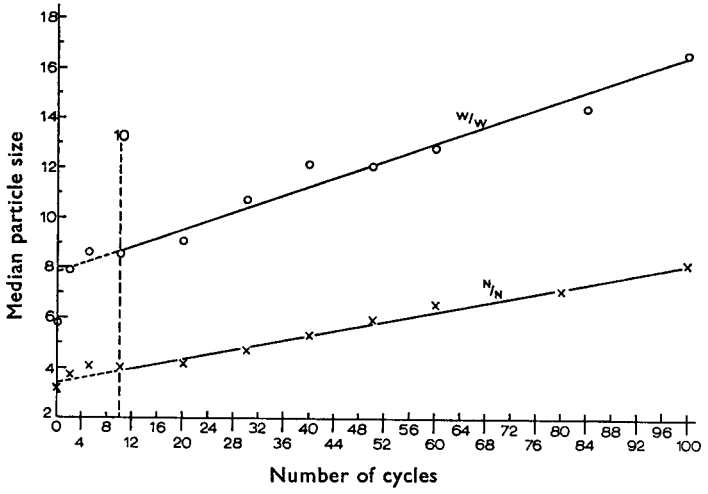


FIG. 3. Crystal growth of sulphathiazole in 0·1% v/v Nonidet P42. Lines of least squares, $P \ll 0{\cdot}001$.

of crystal growth to temperature cycling is rectilinear, after the first 8–10 cycles.

EFFECTS OF CONSTANT AND CYCLING TEMPERATURES

The results have clearly established the induction of rapid crystal growth by suitable temperature cycling. However, it became necessary to check that no significant growth occurred under steady temperature conditions and an experiment was undertaken to observe the growth produced when the suspension was stored: (i) at a constant temperature of 23°; (ii) at a constant temperature of 43°; (iii) at a constant temperature of 23° after a period of cycling; (iv) at a constant temperature of 43° after a period of cycling, and to observe whether the storage time before the cycling period influenced the final particle size.

The results (Fig. 4) indicated that there was little crystal growth at either constant temperature, with or without a previous history of temperature cycling. However, growth during the cycling period was considerable. Several replicates of this experiment were made and there was little correlation between the length of storage at constant temperature before cycling and the final particle size. Every replicate gave a qualitatively similar graph, but there was little quantitative correlation. A comparison of Fig. 2 C with D and E gives some indication that the reason for the quantitative discrepancy may be related to habit modification of the crystals.

EFFECT OF CONCENTRATION AND NATURE OF SURFACE-ACTIVE AGENT

Figs 1 and 3 show the growth resulting when the sulphathiazole was suspended in a saturated solution of sulphathiazole containing Nonidet P42 and exposed to temperature cycles between 23° and 43°.

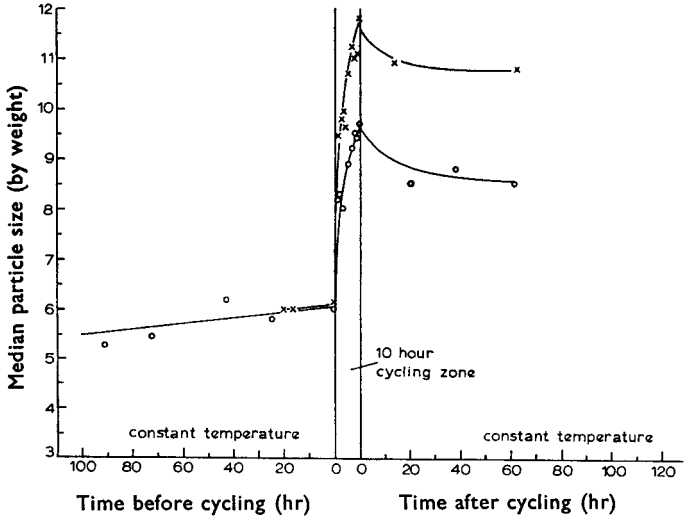


FIG. 4. Crystal growth at constant and cycling temperatures. X, under constant temperature of 23°. O, under constant temperature of 43°.

Fig. 5 represents the crystal growth of sulphathiazole, suspended in triple distilled water containing cetomacrogol, when exposed to temperature fluctuations between 23° and 33°. This Figure includes averaged data from experiments replicated up to five times.

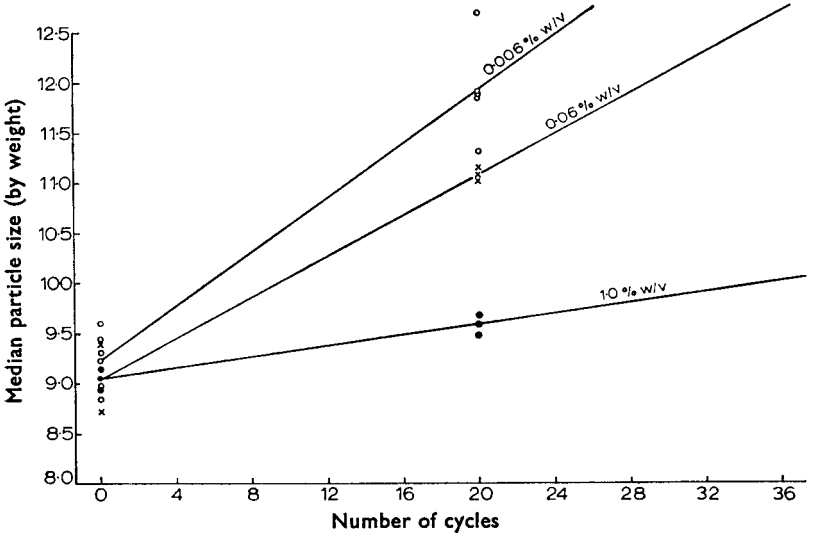


FIG. 5. Effect of concentration of cetomacrogol as % w/v on crystal growth of sulphathiazole.

Comparison of Figs 3 and 5 indicates that a change in the conditions of growth produced essentially linear growth rates, even though the shape and form of the final crystals were markedly different (Fig. 2 F, G and H). The same observation holds for other concentrations of Nonidet P42 (Fig. 2 B and C).

Fig. 5 also indicates that, under similar conditions, crystal growth rates were inhibited by increasing concentrations of cetomacrogol. It was considered that the observed change in growth rate may have been due to a change in the solubility of sulphathiazole resulting from the introduction of cetomacrogol.

Fig. 6 describes the solubility of sulphathiazole in several concentrations of cetomacrogol over the range of temperature studied.

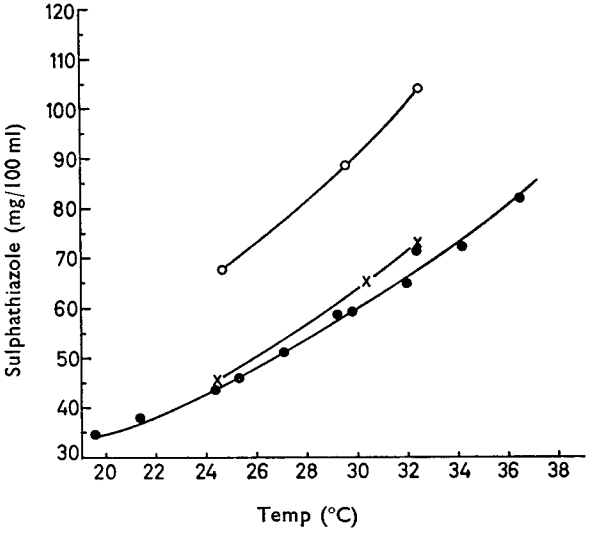


FIG. 6. Solubility of sulphathiazole in solutions of cetomacrogol. ○ = 1·0% w/v, × = 0·06% w/v, ● = 0·006% w/v.

The solubility values were obtained by shaking excess sulphathiazole with the solution of cetomacrogol for 3 days and filtering through a Millipore membrane filter* of nominal pore size, 0·45 μ, and assaying by the method of Bratton & Marshall (1939).

Solubility determinations were made on similar systems containing 0·9% w/v sodium chloride in addition and the results were identical to those in Fig. 6.

Thus, there was no likelihood of a change in particle size on addition of the suspension to the electrolyte vehicle in the Coulter counter. The inhibition of crystal growth by increasing concentrations of cetomacrogol occurred in spite of the enhanced solubility caused by the cetomacrogol.

* Millipore Filter Corporation, Bedford, Massachusetts, U.S.A.

Discussion

There are many combinations of large and small particles in a bulk suspension and the change in the mean diameter may be accepted as the integrated function of such combinations (Hasegawa & Nagai, 1958). However, the method employed for measuring particle size changes in suspensions must be highly sensitive, reproducible and statistically significant (Higuchi & Lau, 1962). A mean diameter may be obtained with this degree of accuracy from Coulter counter analysis and, when the particle size distribution is logarithmic normal, the statistical significance is increased further (Thornton, 1963; Lewis & Goldman, 1965).

We measured particles of 2·5 μ and over and assumed that these represented all the particles present. We noted that the particle size was log normal initially when calculated on a weight basis but that during the accelerated storage there was an increasing deviation from log normality. This agrees with the dissolution results of Higuchi & Hiestand (1963), but Hasegawa & Nagai (1958) found that the particle size distribution remained log normal even after much crystal growth in suspensions of sulphadiazine which resulted from storage at constant temperature for four months. Because of the difficulty of interpretation of distributions which were not log normal, we concluded that median values of the distributions would be a suitable criterion for crystal growth.

Under conditions of temperature cycling, up to a total of 100 cycles, the median particle size and the particle size range of the sulphathiazole suspension continued to increase. This differs from the results of Hasegawa & Nagai (1958) and Nogami & Nagai (1958), who noted that the size distribution became narrower as the median particle size increased and postulated that the effective growth rate and particle size would reach limiting values.

Allen & others (1965) recorded growth rates proportional to time, but Okano & others (1958), studying crystallisation by cooling supersaturated solutions, obtained growth curves very similar to those we report, i.e. curving steeply initially and then becoming rectilinear. Okano & others (1958) studied crystal growth in suspensions containing surface-active agents in excess of their critical micelle concentrations and this factor may account for the agreement with our results.

It is our opinion that the initial steep slope of the growth curves may be due to one or more of the following factors:

1. Milled material will have a large number of active growth sites because of particle fracture and these will gradually heal over as growth proceeds (Kolthoff & Rosenblum, 1935).

2. The inner perfection of the crystals by recrystallisation may result in the cementing together of small particles so causing a rapid loss of small particles in the initial stages (Kolthoff & Rosenblum, 1935; Kolthoff & Eggertsen, 1941).

3. The possible presence of an "easily soluble layer" (Clelland,

Cumming & Ritchie, 1952; Gibb, Ritchie & Sharpe, 1953; Bergman & Paterson, 1961; Bergman, 1962, 1963).

4. A large proportion of ultrafine material may be present initially, which would result in a very high solubility rate and hence a relatively rapid decrease in small particles.

5. Slow adsorption of the surface-active agent on to the growth sites of the crystal faces.

6. The existence of some material of a metastable modification, which rapidly reverts to the stable form.

The median particle size of the sulphathiazole studied was 6 μ and it is not to be expected that Ostwald ripening would be important. However, the process of temperature cycling involves two factors which will affect the net crystal growth, i.e. dissolution and crystallisation, and it would be impossible to separate the effects of each in the present experimental design. We have already discussed the crystal growth observed during storage at constant temperature and Edmundson & Lees (1965) have studied the dissolution rates of hydrocortisone acetate in very dilute solutions. They observed that the decrease in the diameter of the particles was proportional to time. Similar effects were noted at several agitation rates in excess of 400 rpm (Niebergall & Goyan, 1963; Wurster & Taylor, 1965). The stirring rate, used in the present work, was 680 rpm and, although it is appreciated that the rate limiting process is dependent upon the stirring rate, it has not yet been possible to carry out work at different agitation rates.

Higuchi & Saad (1965) and Saad & Higuchi (1965) noted that growth and dissolution of cholesterol were both retarded by the presence of impurity. They postulated an interfacial interaction producing a barrier to interfacial transport between solution and solid and vice versa. Similar growth retardation was observed by Michaels & Colville (1960) who stated that small particles were more sensitive to surface-active agents than were large particles. They deduced that the method of growth of small particles (i.e. two dimensional) was more sensitive to adsorbed contaminants than that for large particles (viz. growth from dislocations). Undoubtedly, this effect is significant in the crystal growth results quoted in this paper, but the nature and concentration of the surface-active agent produce two additional effects: (*a*) a net decrease in the crystal growth rate of all particles; (*b*) habit modification.

The net crystal growth retardation, observed with increasing concentrations of surface-active agents, must be a result of interfacial interaction between the cetomacrogol and sulphathiazole and occurred despite the increase in solubility of sulphathiazole in the presence of the cetomacrogol.

Reference to Fig. 2 B, C, E and H indicates the extreme habit modification and consequent crystal breakage, which Michaels & Colville (1960) and Allen & others (1965) stated was a result of selective adsorption onto specific faces of the crystals.

However, such crystal growth and modification will also be influenced by the initial polymorphic form of the crystals under study. With sulphathiazole, there are at least three polymorphs (Grove & Keenan, 1941; Miyazaki, 1947; Frederick, 1961) and Miyazaki stated that commercial samples consisted of a mixture of two polymorphs. We have shown, by differential thermal calorimetry, that the sample of sulphathiazole used was a mixture of two or more polymorphs and, although this must affect the crystal growth, we have been concerned primarily with the reproducibility of the growth without defining the actual mechanism of the physical changes.

Higuchi & Lau (1962) indicated the use of inhibiting agents in "stabilising" suspensions of the metastable modification of methyl prednisolone and the inhibition of growth of "stable" suspensions is now receiving an increasing amount of study.

To accelerate the change in particle size we subjected the suspension to temperature fluctuations producing a cycle of events resulting in saturation-undersaturation-saturation-supersaturation and then again saturation. Thus it was possible to effect accelerated storage under conditions closely simulating shelf storage, but differing in that the rate of temperature fluctuations was greatly increased and controlled.

To study the system over more protracted periods and to encourage better reproducibility and reliability, it was found desirable to automate the temperature cycling process (Carless & others, 1966).

References

Albon, N. & Dunning, W. J. (1957). *Nature, Lond.*, **180**, 1348–1349.
Albon, N. & Dunning, W. J. (1960). *Acta crystallogr.*, **13**, 495–498.
Allen, D. J., Milosovich, G. & Mattocks, A. M. (1965). *J. pharm. Sci.*, **54**, 383–386.
Bergman, I. & Paterson, M. S. (1961). *J. appl. Chem.*, **11**, 369–376.
Bergman, I. (1962). *Ibid.*, **12**, 336–341.
Bergman, I. (1963). *Ibid.*, **13**, 319–323, 356–361.
Bratton, A. C. & Marshall, E. K. (1939). *J. biol. Chem.*, **128**, 537–550.
Carless, J. E., Foster, A. A. & Joliffe, G. O. (1966). *J. Pharm. Pharmac.*, **18**, in the press.
Clelland, D. W., Cumming, W. M. & Ritchie, P. D. (1952). *J. appl. Chem.*, **2**, 31–41.
Edmundson, I. C. & Lees, K. A. (1965). *J. Pharm. Pharmac.*, **17**, 193–201.
Fischer, R. B. & Ferguson, B. L. (1950). *Proc. Indiana Acad. Sci.*, **60**, 145–152.
Frederick, K. J. (1961). *J. pharm. Sci.*, **50**, 531–535.
Freundlich, H. M. F. (1930). *Kapillar chemie*, **1**, Leipzig.
Fysh, D. (1951). Private communication cited by Moilliet, J. L. & Collie, B. (1951). *Surface Activity*, p. 144. New York: Van Nostrand Co. Inc.
Gibb, J. G., Ritchie, P. D. & Sharpe, J. W. (1953). *J. appl. Chem.*, **3**, 213–218.
Grove, D. C. & Keenan, G. L. (1941). *J. Am. chem. Soc.*, **63**, 97–99.
Hasegawa, J. & Nagai, T. (1958). *Chem. pharm. Bull., Tokyo*, **6**, 129–137.
Higuchi, W. I. & Hiestand, E. N. (1963). *J. pharm. Sci.*, **52**, 67–71.
Higuchi, W. I. & Lau, P. K. (1962). *Ibid.*, **51**, 1081–1084.
Higuchi, W. I. & Saad, H. Y. (1965). *Ibid.*, **54**, 74–78.
Jones, M. & Partington, J. R. (1915). *J. chem. Soc.*, **107**, 1019–1025.
Knapp, L. F. (1922). *Trans. Faraday Soc.*, **17**, 457–465.
Kolthoff, I. M. & Eggertsen, F. T. (1941). *J. Am. chem. Soc.*, **63**, 1412–1418.
Kolthoff, I. M. & Rosenblum, C. (1935). *Ibid.*, **57**, 597–600.
Lees, K. A. (1963). *J. Pharm. Pharmac.*, **15**, *Suppl.* 43*T*–55*T*.
Lewis, H. D. & Goldman, A. (1965). *Rev. scient. Instrum.*, **36**, 868–869.
Lewis, W. C. Mc C. (1909). *Kolloidzeitschrift*, **5**, 91–93.
Michaels, A. S. & Colville, A. R. (1960). *J. phys. Chem., Ithaca*, **64**, 13–19.

Miyazaki, H. (1947). *Japan J. pharm. Chem.*, **19**, 133–134.
Niebergall, P. J. & Goyan, J. E. (1963). *J. pharm. Sci.*, **52**, 29–33.
Nogami, H. & Nagai, T. (1958). *Yakkyoku*, **9**, 29–30.
Okano, S., Kojima, S. & Akahosi, T. (1958). *J. pharm. Soc., Japan*, **78**, 36–40.
Okano, S., Kojima, S. & Satake (1957). *Yaku-zaigaku*, **17**, 170–175, through *Chem. Abstr.* (1958), **52**, 11358b.
Ostwald, W. (1900). *Z. phys. Chem.*, **34**, 495–503.
Polderman, J. (1962). *Boll. chim.-farm.*, **101**, 105–122.
Polderman, J., Bloo, J. H. & Fokkens, J. (1958). *Pharm. Weekbl. Ned.*, **93**, 45–50.
Rumford, F. & Bain, J. (1960). *Trans. Instn chem. Engrs*, **38**, 10–29.
Saad, H. Y. & Higuchi, W. I. (1965). *J. pharm. Sci.*, **54**, 1205–1206, 1303–1307.
Shefter, E. & Higuchi, T. (1963). *Ibid.*, **52**, 781–791.
Thornton, M. J. (1963). *J. Pharm. Pharmac.*, **15**, 742–752.
Wurster, D. E. & Taylor, P. W. (1965). *J. pharm. Sci.*, **54**, 670–676.