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Gelatin coacervate microcapsules containing sulphamerazine: their preparation and the *in vitro* release of the drug

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An improved method is described for the preparation of gelatin coacervate microcapsules containing sulphamerazine as a fine deflocculated powder. The factors which control both the coacervation step and the recovery of the microcapsules are discussed. The *in vitro* release of sulphamerazine from microcapsules of different wall thickness which had been hardened by formaldehyde under different conditions has been studied. The method of preparation gave a high percentage of encapsulated material in comparison with other recovery techniques.

IN recent years investigations have been made into the ability of coacervate droplets to surround small particles dispersed in the coacervating system. Applications of this technique have been found in the preparation of carbonless copying paper (Green 1957; Green & Schleicher 1956a,b) and the microencapsulation of a number of pharmaceuticals (Phares & Sperandio, 1964; Luzzi & Gerraughty, 1964, 1967a, b). Although little difficulty appears to have been found in the coacervation step the recovery techniques reported indicate the difficulty encountered in producing a fine powder. In the patent literature spray-drying or freeze-drying of the microcapsule suspension is suggested or alternatively comminution of the hard cake produced after filtering the gelled microcapsules (Green & Schleicher, 1956a,b). None of these recovery techniques appears to have been critically evaluated. The methods of Phares & Sperandio (1964) and Luzzi & Gerraughty (1964) might be expected to produce a significant proportion of "free" material due to mechanical rupture during either comminution of the cake or passage of the gelled mass through a sieve.

We have examined the preparation of sulphamerazine microcapsules using the simple coacervating systems: gelatin–water–ethanol and gelatin–water–sodium sulphate. A fine powder essentially without "free" sulphamerazine was produced without the need of mechanical treatment. The *in vitro* release of the drug from these microcapsules was also examined.

Experimental

MATERIALS

Two samples of *gelatin* were used having the characteristics given in Table 1. The *gelatins* were dried in thin layers at 110° for 12 hr and stored in air-tight containers. *Absolute ethanol* and 20% w/w *sodium sulphate* (A.R. grade) solution were used as the coacervating agents. *Sulphamerazine* was of B.P. (1953) quality. Most of the particles were between 2 and 5 μ diameter under the microscope.

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* The work forms part of a thesis by S. A. H. Khalil submitted for the degree of Ph.D. in the University of London.

TABLE 1. CHARACTERISTICS OF GELATIN SAMPLES

Sample	Type	Source	Bloom No.	pH	Viscosity (Cps, 6·67%)	I.E.P.	% Ash
A	Lime-pretreated	Hide	240	6·6	8·1 (40°)	5·2	1·20
B	Acid-processed	Pigskin	252	4·7	5·5 (60°)	9·2	1·11

PREPARATION OF MICROCAPSULES

As a preliminary to microencapsulation, phase boundary triangular diagrams were prepared for each system (Nixon, Khalil & Carless, 1966), and suitable compositions within the coacervate region chosen. The general outline of the coating procedure followed that given in British Patent 751,600 (1956). The material to be encapsulated was dispersed in ethanol or 20% w/w sodium sulphate solution and added to the iso-electric gelatin solution. The mixture was continuously agitated by a variable speed paddle stirrer and maintained at 40°. Further coacervating agent was added over 30–40 min and the formation of coacervate coated particles was verified microscopically.

RECOVERY TECHNIQUES FOR MICROCAPSULES

(*a*) Depending upon which coacervating agent was used to prepare the microcapsules, the product was poured into five times its volume of either 30% w/w aqueous ethanol or 7% w/w sodium sulphate solution at 5°. This resulted in the gelling of the hitherto liquid shell of the microcapsules. The dispersion was continuously agitated for 30 min. After centrifugation at 500 rev/min for 5 min the mother liquor was decanted and the product washed with isopropanol at 5° for 30 min. It was then possible to filter the fine microcapsules and after washing with ethanol they were dried at room temperature. The coacervate shell of the recovered microcapsules was hardened by suspending 1·0 g of the microcapsules in 10 ml of formalin–isopropanol mixture (1:4 v/v). The formalization time ranged from 15 min to 3 days and the formalized microcapsules were filtered, washed with water and dried at 70° for 12 hr. This method was the most satisfactory, the product being recovered as a deflocculated fine powder. The porosity of the coacervate walls, particularly in sodium sulphate coacervated systems, depends upon the rate of gelling of the coacervate material. For this reason the experimental procedure was rigidly controlled.

(*b*) The coacervate microcapsules were gelled by reducing the temperature to 5°. They were not treated with isopropanol. Formaldehyde was added to the gelled microcapsules to produce a final concentration of 10% w/v formaldehyde. The mixture was stirred at 5–10° for 30 min, filtered, washed with water and dried at 70° for 12 hr. This method produced a cake which had to be further comminuted in a mortar or mill and the product fractionated.

(*c*) The prepared microcapsules were not gelled or treated with formaldehyde but spray-dried using a Portable Spray Drying Minor Unit (Niro Atomizer Ltd.). The feed rate of 5 ml/min was controlled by a pulsating pump. The operating conditions for spray drying are shown in Table 2.

TABLE 2. OPERATING CONDITIONS FOR SPRAY DRYING THE FORMALIZED SUSPENSION OF COACERVATE MICROCAPSULES CONTAINING SULPHAMERAZINE

Experiment*	Compressed air (kg/cm^2)	Heat control	Temp. ranges, °C		Cooling Ports
			Inlet	Outlet	
A	2	6	220–230	120–130	Fully open
B	3	4	130–140	75–80	Fully open
C	4	3	110–120	65–70	Partially open
D	5	2	100–110	55–60	Partially open
E	2	1	100	45–50	Partially open
F	4	2	100–110	50–55	Fully open

Feed rate in all experiments: 5 ml/min.
* Mean values from 5 replicates.

ASSAY OF SULPHAMERAZINE IN VARIOUS PRODUCTS

Total sulphamerazine. The formogelatin coat was decomposed with 50% v/v hydrochloric acid at 60° for 5–20 min (Bogue, 1922). A concentration of 0·1 g of the recovered powder per 40 ml of acid was used. Five ml of the clear solution produced was then assayed by the method of Bratton, Marshall & others (1939), using a photoelectric absorptiometer (Evans Electroselenium Ltd.) and a No. 605 filter. The mean of five determinations was calculated.

"*Free*" *sulphamerazine.* The product, 0·1 g, was shaken with 25 ml of acetone at room temperature to dissolve any unencapsulated sulphamerazine. After centrifuging, an aliquot was assayed for sulphamerazine content as previously.

MEASUREMENT OF *in vitro* RELEASE FROM MICROCAPSULES

A rotating bottle apparatus similar to that of Souder & Ellenbogen (1958) was used. About 0·9 g of the coacervate microcapsules, accurately weighed, was placed in a 90 ml screw-capped bottle containing 40 ml of the dissolution medium (acid pepsin solution B.P. or alkaline pancreatin solution B.P.). The bottles were rotated at 40 rev/min at 37° ± 0·05°. After various time intervals the dispersions were centrifuged for 1 min at 500 rev/min and an aliquot of the clear solution assayed for sulphamerazine released.

$$\% \text{ sulphamerazine released} = \frac{\text{mg sulphamerazine released/g microcapsule}}{\text{mg total sulphamerazine/g microcapsule}} \times 100\%$$

SIZE ANALYSIS OF MICROCAPSULES

The size of the microcapsules was determined by direct measurement of the diameter of the magnified microcapsules projected on the screen of a Projectina microscope. The results were expressed as number percentage cumulative curves.

Results

PREPARATION AND RECOVERY OF THE MICROCAPSULES

The effect of gelatin type and the coated material on the coacervation step was studied. The results are shown in Tables 3 and 4. Factors

TABLE 3. EFFECT OF GELATIN TYPE ON MICROENCAPSULATION OF SULPHAMERAZINE

Gelatin type	pH of the gelatin solution	% w/w coacervating agent added to produce phase change		Microencapsulation	
		Ethanol	Sodium sulphate	Ethanol system	Sodium sulphate system
Lime-pretreated	6·6*	45·9	8·7	+	+
Acid-processed	4·7*	62·4	8·0	– (Floccules)	+
	6·1	60·1	8·6	– (Floccules)	+
	7·0	57·4	8·9	+	+
	8·4	55·2	9·2	+	+

* pH of the gelatin solution without adjustment.
Initial gelatin concentration: 8% w/w.

TABLE 4. EFFECT OF THE MATERIAL TO BE COATED ON THE OCCURRENCE OF COACERVATION. Minimum ethanol or sodium sulphate concentrations (% w/w in the total system) required to initiate coacervation

Material*	Gelatin–water–ethanol system			Gelatin–water–sodium sulphate system		
	Initial gelatin concentration (% w/w)					
	4	8	16	4	8	16
Blank (no material)	43·1	41·4	37·7	8·4	8·2	8·0
Sulphamerazine	43·6	41·8	36·1	8·4	8·1	7·9
Aspirin	54·2	49·5	44·2	8·0	7·7	7·3
Glass beads (53μ)	46·3	44·7	40·4	8·9	8·6	8·7
Phenolphthalein	68·7	63·4	58·5	9·3	9·2	9·0

* 10 g of the material dispersed in 100 g of the isoelectric gelatin solution maintained at 40° ± 0·1°.
Gelatin used: 240 Bloom, lime-pretreated sample.

which produced changes in pH or interaction with the gelatin molecule influenced the onset of coacervation and therefore microencapsulation. In this category was the alkalinity of the glass beads and the acidity due to aspirin, whilst with phenolphthalein the lyotropic effect of the phenolic group on the gelatin molecule (Gustavson, 1956) suppressed coacervation particularly in the ethanol system.

Changes due to gelatin type followed a similar pattern to that reported in our previous paper (Khalil, Nixon & Carless, 1968). With alkali-processed gelatin without pH adjustment it was possible to encapsulate sulphamerazine using either ethanol or sodium sulphate as the coacervating agent. The acid processed gelatin had to be adjusted to pH values in the vicinity of its isoelectric point before encapsulation was achieved using ethanol, but with sodium sulphate no adjustment was required within the pH range studied. This was in accordance with previous results (Khalil & others, 1968) where it was found that with ethanol systems, using alkali commercial gelatins, the effective range for coacervation was between pH values 4·6 and 9·2 and for acid processed gelatins 6·9–10·8. With sodium sulphate coacervates the effective pH range was found to be 2·1–10·5 irrespective of the gelatin type. The encapsulated material appeared to be evenly distributed throughout the coacervate droplets. These droplets tend to aggregate if the amount of coacervating

agent used to produce them was close to either of the boundaries of the coacervate region (Fig. 1). At the onset of coacervation only the higher molecular weight fractions of the gelatin coacervated and formed the mantle of the microcapsule. The lower molecular weight fractions remained in the equilibrium liquid and on gelling were deposited on the outside of the microcapsules which then tended to agglomerate on contact. This occurred with both coacervating systems. Near the three phase zone of the triangular diagram, the coacervate phase on analysis was found to be richer in gelatin and poorer in water whilst the equilibrium liquid contained negligible amounts of gelatin (Nixon & others, 1966). The highly viscous nature of the coacervate under these conditions enhanced the agglomeration of the coacervate droplets.

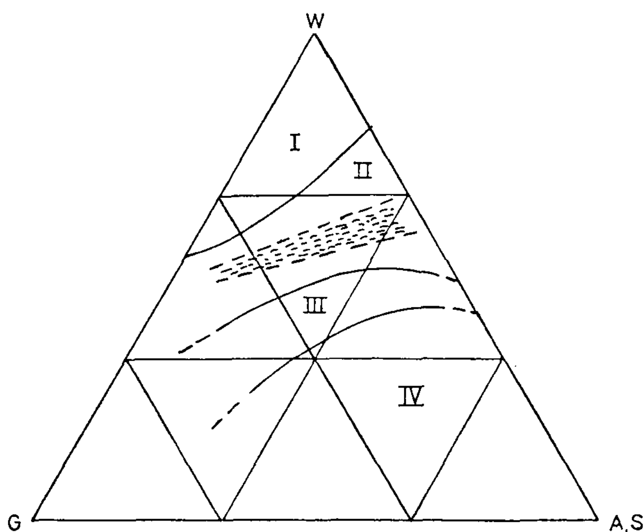

FIG. 1. Schematic representation of the optimum region for microencapsulation. I, clear solution. II, coacervate region (etched portion produces optimum conditions for microencapsulation). III, three phase region. IV, flocculate region.

The optimum conditions for the production of microcapsules is shown schematically in Fig. 1 and occurs in a region almost midway in the coacervation zone. In this region all the gelatin fractions had coacervated yet the viscosity had not appreciably increased.

Whilst the formation of a coacervate coat around a particle was easily accomplished from a study of the physical characteristics of the system, the recovery of the microcapsules in an acceptable form as a fine powder presented difficulties.

Filtration under varying conditions quickly led to blockage of the filter pores and production of a rubbery cake. Prior treatment with formalin did not produce any marked improvement. The cake produced after drying was comminuted and sieved to give three fractions. These were then assayed for "free" sulphamerazine (Table 5). The two coarser

fractions contained up to 25% w/w unencapsulated material whilst the fine fraction was almost completely in the "free" form. This adequately indicates that comminution would be accompanied by rupture of the coacervate shell depending on the extent of comminution. This result was also anticipated from the use of coacervate microcapsules in the preparation of carbonless copying paper.

TABLE 5. "FREE" SULPHAMERAZINE IN PRODUCTS RECOVERED BY VARIOUS METHODS*

Method of recovery	Average % of free sulphamarazine	
	Ethanol system	Sodium sulphate system
A Isopropanol method		
(a) Before acetone washing	7·1	4·3
(b) After acetone washing at 20° for 15 min	3·6	1·8
B Comminution of the filter cake		
(a) Fraction > 44 mesh	11·4	9·8
(b) Fraction >170 mesh	25·3	24·1
(c) Fraction <170 mesh	97·0	98·2
C Spray drying (all products obtained) ..	98·3	98·6

* All products obtained were prepared under the same conditions and the ratio of gelatin to sulphamerazine was 8·4. The products were formalized for 30 min.

Spray dried products needed no further comminution and consisted of fine free-flowing powder which passed through a 300 mesh sieve. The best conditions for operating the spray drier were D and F (Table 2). However, the spray dried products were entirely unacceptable as almost all the sulphamerazine was unencapsulated. Microscopic examination showed empty spherical particles 2–5 μ in diameter. This was much smaller than the size of the microcapsules in the feed suspension. The gelled microcapsules could probably not withstand the shearing force of the atomizer which ruptured the microcapsules. Spray drying, under far gentler conditions than here available, may be possible.

The only successful method of producing microcapsules as a dry deflocculated powder was by treating the centrifuged slurry with water-miscible aliphatic alcohols which possess the property of precipitating fibres from soluble collagens. Isopropanol was found to be superior to n-propanol or ethanol in that it gave a product with a negligible amount of agglomerates. The sedimentation time and the type of product

TABLE 6. COMPARATIVE EFFECTS OF ETHANOL, PROPANOL AND ISOPROPANOL ON SULPHAMERAZINE COACERVATE MICROCAPSULES*

The alcohol used	Time of complete sedimentation (min)	Sedimentation volume (ml)	Quality of the sediment
Ethanol	6	42	lumpy agglomerates
n-Propanol	10	31	coarse aggregates
Isopropanol	24	18	very fine particles

* 25 g of the centrifuged microcapsule slurry were separately treated with equal volume of the alcohol in a 100 ml measuring cylinder.

obtained after treatment are shown in Table 6. In this experiment 25 g of the centrifuged microcapsules slurry were treated with equal volumes of the alcohol in a 100 ml cylinder.

The size distribution of the microcapsules and the effect of the isopropanol treatment is shown in Fig. 2. This recovery technique produced the smallest quantity of "free" sulphamerazine. The small amount of "free" material is ascribed to the solubility of the sulphamerazine used in the system. This produces external contamination of the surface layers of the microcapsules. Washing with acetone reduced this surface contamination to less than 2%.

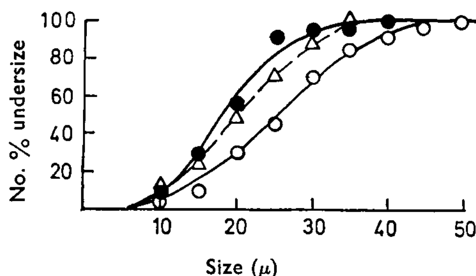

FIG. 2. Number size distribution of empty coacervate droplets, ●; freshly prepared microcapsules of sulphamerazine, ○; and microcapsules after hardening by isopropanol, △. System: gelatin–water–ethanol. % w/w gelatin/sulphamerazine ratio 8:4.

In vitro RELEASE OF SULPHAMERAZINE FROM COACERVATE MICROCAPSULES

This was examined under a number of conditions. A maximum of 6 hr of formalization was used, as longer periods allowed interaction between sulphamerazine and the diffused formaldehyde solution which resulted in the formation of a soluble complex which was leached out into the formalin–isopropanol mixture. At formalization times less than 6 hr no loss of sulphamerazine from the microcapsule could be detected.

The results of release experiments, Figs 3–6, showed that hardened microcapsules retarded the release of sulphamerazine into both acid pepsin and alkaline pancreatin solutions. The effect was more pronounced in the acid pepsin solution and depended on both the formalization time and the gelatin/sulphamerazine ratio. After 8 hr in this dissolution medium the formalized microcapsules were still intact, but in alkaline pancreatin solution they ruptured after various time intervals depending on their length of pretreatment with formalin-isopropanol mixture (Fig. 4). When compared with the effect in acid pepsin the thickness of the gelatin coat, as measured by the gelatin/sulphamerazine ratio, produced an insignificant effect on the release pattern in alkaline pancreatin solution (Fig. 5). It should also be noted that the release of the drug was faster from sodium sulphate coacervated microcapsules than from similar formulations using ethanol as the coacervating agent.

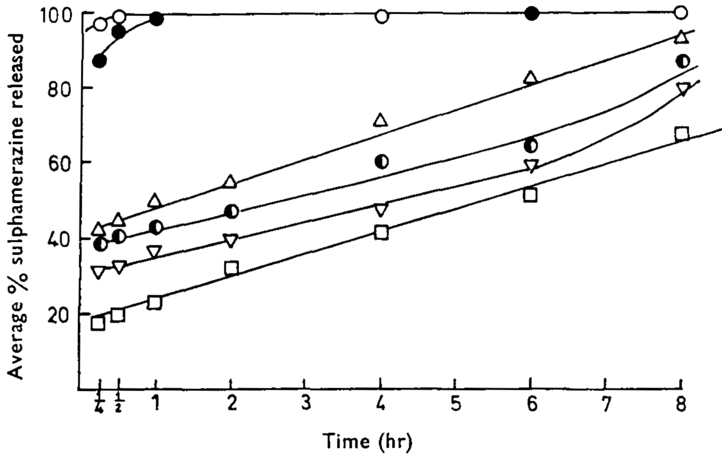

FIG. 3. *In vitro* release of sulphamerazine from ethanol coacervated microcapsules. % w/w gelatin/sulphamerazine ratio 8:4. Dissolution medium: acid pepsin solution B.P. Temperature 37° ± 0·05°. ○, Sulphamerazine crystals; ●, unformalized microcapsules; △, microcapsules formalized for 15 min; ◐, microcapsules formalized for 1 hr; ▽, microcapsules formalized for 3 hr; □, microcapsules formalized for 6 hr.

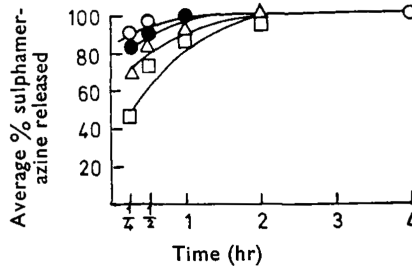

FIG. 4. *In vitro* release of sulphamerazine from ethanol coacervated microcapsules. % w/w gelatin/sulphamerazine ratio 8:4. Dissolution medium: alkaline pancreatin solution B.P. Temperature 37° ± 0·05°. ○, Sulphamerazine crystals; ●, microcapsules formalized for 15 min; △, microcapsules formalized for 1 hr; □, microcapsules formalized for 6 hr.

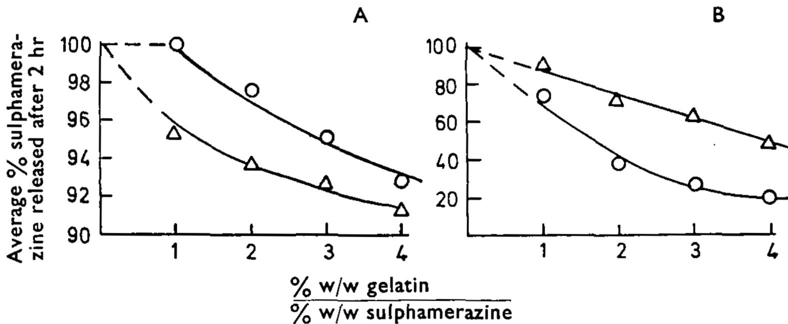

FIG. 5. Effect of thickness of microcapsule coat on the release of sulphamerazine. Dissolution medium A: alkaline pancreatin solution. B: acid pepsin solution B.P. B.P. Formalization time: 3 hr. Coacervating agent: △, sodium sulphate; ○, ethanol. Temp. 37° ± 0·05°.

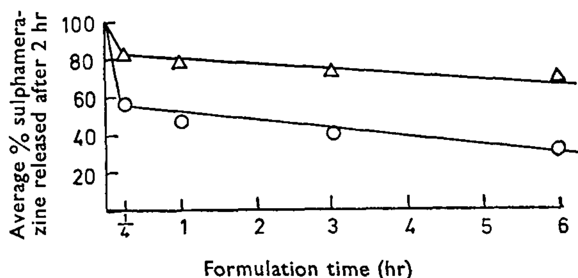

FIG. 6. Effect of formalization time on the release of sulphamerazine. % w/w gelatin/sulphamerazine ratio: 8:4. Dissolution medium: acid pepsin solution B.P. Coacervating agent: △, sodium sulphate, ○, ethanol. Temp. 37° ± 0·05°.

Discussion

Since no detailed studies have been reported on microencapsulation of pharmaceuticals by gelatin coacervation, it was necessary to investigate the various conditions under which a solid could be coated and recovered as a dry powder. Because only the coacervate phase shows the unique property of encapsulating solid materials it is important to study those factors which influence the location of the coacervate phase within the triangular diagram. Materials which produce changes in pH or interact with the gelatin could result in the displacement of the coacervate region inside the triangular diagram (Nixon & others, 1966; Khalil & others, 1968). Even within the coacervate region there was an optimum zone in which the coacervate droplets possessed a minimum aggregation potential and it was only within the zone that a deflocculated product was successfully prepared.

The size of the material to be coated did not limit the ability of the coacervate droplets to coat them and it was also found that encapsulation occurred whether the particles were dispersed in the gelatin solution before the coacervation step or added to an already coacervated system. This suggests that microencapsulation can occur by two mechanisms: (*a*) by the dispersed particles acting as seeding nuclei around which the coacervate droplets form; or (*b*) the droplets in the presence of dispersed particles may absorb them by an invagination mechanism.

The recovery of encapsulated material has been reported by a number of workers without any detailed evaluation of the proposed methods being attempted. Filtration appears to produce a rubbery mass which on drying yields a hard cake. The comminution of the cake (Phares & Sperandio 1964, Brit. Pat. 1956) or the passage of the rubbery mass through a sieve (Luzzi & Gerraughty, 1964) would affect the integrity of the coacervate coat. As seen from Table 5 a large proportion of the sulphamerazine was unencapsulated or in cracked microcapsules. This we anticipated, since the basic principle of the use of coacervate microcapsules in carbonless copying paper is that they will rupture under the mechanical pressure of writing or typing. The present method resulted in the complete encapsulation of the sulphamerazine provided adequate

coacervate material was present. As shown in Table 5, 7% by ethanol coacervation or 4% by sodium sulphate coacervation was either unencapsulated or present in broken capsules.

The simple procedure suggested in the present paper was based on hardening the coacervate shell by dehydration. Formalin was unsatisfactory due to its water content. The work of Bensusan (1960) showed that high concentrations of water-miscible aliphatic alcohols favoured precipitation of soluble collagens in the order: methanol > ethanol > n-propanol > isopropanol. Ethanol possessed too strong a dehydrating effect for the present purpose and resulted in shrinkage and agglomeration of the microcapsules. However, isopropanol, with its milder dehydrating effect, resulted in the production of a very fine free-flowing powder and in contrast to other methods of recovery, no rupturing of the coacervate coat occurred.

The *in vitro* release studies were only possible on the powders recovered by the isopropanol method. The coacervate coat retarded the release of the encapsulated sulphamerazine, the effect depending on the dissolution medium, time of formalization and thickness of the gelatin coat. It was found by Tanaka, Takino & Utsumi (1963) that formalized gelatin was less sensitive to hydrolysis by protease enzyme. This meant that by varying formalization time the rate of release could be controlled. Due to the reaction between formaldehyde and the primary amino-group of the sulphamerazine the formalization time was limited to 6 hr. Nevertheless the effect of formalization time is well shown in Fig. 3. After only 15 min in the acid pepsin, approximately 20% of the sulphonamide was released even when long periods of formalization were used. The degree of protection afforded against acid pepsin is much less than when a hard gelatin capsule is treated for enteric coating as the walls of the microcapsule are much thinner. Similarly the thickness of the coacervate coat effectively delayed the release, most probably by inhibiting the rate of diffusion of the dissolution medium into the microcapsules. The release of encapsulated material into alkaline pancreatin solution exhibited a totally different pattern from acid pepsin solution as the gelatin coat ruptured. The release from sodium sulphate-coacervated microcapsules was relatively higher than with similar ethanol coacervated products. The sodium sulphate not only hinders the hardening effect of the isopropanol (Bensusan & Hoyt, 1958) during the recovery technique, but also yields a highly porous coat under the conditions of the *in vitro* release experiments as the sodium sulphate dissolves from the microcapsule shell.

Whether the structure of the coacervate coat differs from that of a gel is still not certain in spite of the marked birefringence of the former (Bungenberg de Jong, 1938). Maier & Scheuerman (1960), from electron microscopy and diffusion studies, have found that coacervate films of cellulose nitrate of a graded porosity could be prepared by the correct choice of the solvent-non solvent system and drying conditions.

Diffusion through the coacervate coat may well occur through the intermolecular spaces of the coiled structure as well as through the tiny coacervate vacuoles which can be seen in the coacervate droplets.

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