propanolamine hydrochloride. After extraction of the phenylpropanolamine from chloroform into pH 5 citrate buffer and separation of the phases, the chloroform was discarded and a portion of the aqueous phase was mixed with ninhydrin reagent. This mixed stream was passed through a heating bath (95°) for color development, cooled to room temperature, and measured at 570 nm.

The developed simultaneous automated system significantly reduced the analysis time. Previously, about 8 and 12 hr. were required to perform the comparable manual procedures for two-component and three-component preparations, respectively. Based on a single-lot determination with the automated procedure, the time required for analysis was 3 hr. This represented a time savings of 5–9 hr., depending on the number of components present. Since the automated system is capable of analyzing 20 samples/day in duplicate, a reduction in analytical time to 0.4 hr./sample can be realized.

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Molecular-Scale Drug Entrapment as a Precise Method of Controlled Drug Release IV: Entrapment of Anionic Drugs by Polymeric Gelation

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Abstract □ A physicochemical approach to the preparation of drug-containing matrix systems is described in which a soluble anionic drug may be entrapped on a molecular scale in coagulated (gelled) polymer emulsion systems. The resultant dried product (or drug–xerogel system) was designed to provide controlled, prolonged release. The phenomenon of gelation of the polymer emulsions by the addition of a divalent cation (Mg^{+2}) was utilized for the entrapment of various drug materials. A solid, highly reproducible entrapment compound of sodium phenobarbital, magnesium sulfate, and a styrene-acrylic copolymer latex was prepared and subjected to *in vitro* and *in vivo* prolonged-release studies. The physical factors influencing both entrapment and drug release were investigated. A significantly increased duration of therapeutic effectiveness was established by the *in vivo* results. Rats, fed dry polymer powder in their diet, exhibited no toxic effects in a 27-day study. The validity and reproducibility of the entrapment procedure were demonstrated.

Keyphrases □ Polymer emulsion systems—entrapment of anionic drugs by gelation, prolonged-release rates, methods, prepared with sodium phenobarbital, tested in rats □ Timed-release formulations—molecular-scale drug entrapment as a precise method of controlled drug release, anionic drugs by polymeric gelation, prepared with sodium phenobarbital, release rates, rats □ Gelations, polymeric—entrapment of anionic drugs (sodium phenobarbital), methods of preparation, release rates, tested in rats □ Phenobarbital—prolonged-release formulation prepared by molecular-scale drug entrapment, release rates, rats □ Anionic drugs—prolonged-release sodium phenobarbital formulation prepared by molecular-scale drug entrapment, release rates, rats □ Drug release—sodium phenobarbital from a prolonged-release formulation prepared by molecular-scale drug entrapment (polymeric gelation), rats

The inclusion of soluble drugs in insoluble matrixes is well known as a means of controlling drug release rates from solid dosage forms. Diffusional models describing drug release from such systems were thoroughly described by T. Higuchi (1) and W. Higuchi (2). Systems have been designed in which "channeling agents" are added to the matrix to attract fluid into the system as well as to facilitate drug diffusion from the matrix (3). Recently, others described (4) the use of dry gels of cross-linked polymer which are charged by immersion in solutions of the drug. Charging of drugs into cross-linked polymers offers a unique method of releasing drug into the eye for very long periods from soft lens systems or other ocular inserts (5). Other drug release systems from matrixes have obvious application for intrauterine and assorted implantable devices, which might range from being totally insoluble to completely soluble or biodegradable.

In previous papers in this series (6–10), it was demonstrated that cationic drug materials could be entrapped in the solid matrix of a flocculated (linear acrylic acid–methacrylic acid copolymer) polymer emulsion system[1] in such a manner as to exhibit reproducible control of drug release and prolongation of drug action from the resultant dried material. The advantages of polymer emulsion systems for this purpose include a high solids

[1] Acrysol ASE 75, Rohm and Haas Co., Philadelphia, Pa.

Table I—Polymer Emulsions Studied and Their Designation

Polymer Emulsion Designation	Chemical Classification	Charge	Solids, %	pH
1	Styrene–acrylic latex[a]	Nonionic	46–48	8.0
2	Styrene–acrylic latex[b]	Anionic	46–48	9.0–9.5
3	Modified acrylic[c]	Nonionic	44–46	9.4–9.9
4	Modified acrylic[d]	Anionic	27.5–28.5	3.5
5	Modified acrylic[e]	Nonionic	19.5–20.5	3.0
6	Modified acrylic[f]	Nonionic	46	9.0–9.5
7	Modified acrylic[g]	Nonionic	45–47	9.0–10.0
8	Modified acrylic[h]	Anionic	38	9.5–10.0
9	Modified acrylic[i]	Nonionic	44.5–45.5	7.5–9.0
10	Ethyl acrylate–vinyl pyrrolidone[j]	Anionic	40	6–8
11	Styrene–vinyl pyrrolidone[k]	Anionic	40	2–4
12	Styrene–vinyl pyrrolidone[l]	Anionic	40	2–4
13	Vinyl acetate–vinyl pyrrolidone[m]	Emul. free	40	3–4
14	Polyethylene[n]	Nonionic	41	9.0
15	Acrylic copolymer[o]	Anionic	35	8.5–9.5
16	Styrene–acrylic[p]	Anionic	39–41	7–8
17	Vinyl–acrylic latex[q]	Anionic	45	4.3
18	Vinyl–acrylic copolymer[r]	Anionic	55	4.5
19	Vinyl–acetate copolymer[s]	Anionic	55	4.5
20	Acrylic copolymer[t]	—	49–51	5–6

[a] Acriflo-151, Chemical Division, The General Tire and Rubber Co., Akron, Ohio. [b] Lytron 680, Plastics Division, Monsanto Chemical Co., Springfield, Mass. [c] MC-4530, Rohm and Haas Co., Philadelphia, Pa. [d] Acrysol ASE 60, Rohm and Haas Co., Philadelphia, Pa. [e] Acrysol ASE 95, Rohm and Haas Co., Philadelphia, Pa. [f] Rhoplex AC 33, Rohm and Haas Co., Philadelphia, Pa. [g] Rhoplex AC 200, Rohm and Haas Co., Philadelphia, Pa. [h] Rhoplex B 85, Rohm and Haas Co., Philadelphia, Pa. [i] Rhoplex C 72, Rohm and Haas Co., Philadelphia, Pa. [j] Polectron 130, GAF Corp., New York, N. Y. [k] Polectron 430, GAF Corp., New York, N. Y. [l] Polectron 450, GAF Corp., New York, N. Y. [m] Polectron 845, GAF Corp., New York, N. Y. [n] Poly-Em 20017, Spencer Chemical Co., Kansas City, Mo. [o] NeoCryl W63M, Polyvinyl Chemicals Inc., Peabody, Mass. [p] NeoCryl A247H, Polyvinyl Chemicals Inc., Peabody, Mass. [q] Resyn 25-2833, National Starch and Chemical Corp., New York, N. Y. [r] Resyn 25-2243, National Starch and Chemical Corp., New York, N. Y. [s] Resyn 25-1255, National Starch and Chemical Corp., New York, N. Y. [t] Ubatol U-7001, UBS Chemical Co., Cambridge, Mass.

content (usually 40–50%) and a very low viscosity (normally 100–200 cps.).

In the previous studies (6–8), all polymer emulsion systems were observed to flocculate on being coagulated. The flocculated products were dense and nonporous and required polymer hydration and dissolution to achieve satisfactory drug release. Polymers containing carboxyl functionality had appropriate solubility and were available as anionic polymer emulsions. These emulsions were effective in the entrapment of cationic drugs but were ineffective in the entrapment of anionic drugs. Some other type of system was obviously required to achieve molecular-scale entrapment of anionic drugs. In addition, a polymer latex that would gel on coagulation rather than flocculate would provide a less dense, more porous entrapment matrix and might permit the use of less soluble or insoluble polymers as the matrix or carrier.

The purposes of this investigation were to: (*a*) study the phenomenon of gelation of polymer emulsions, as induced by anionic drugs with and without added electrolytes; (*b*) elucidate the factors affecting drug entrapment by polymeric gelation, including polymer concentration, method of preparation, and added electrolytes; (*c*) study the factors affecting drug release rate, including particle size, method of drying, and drug concentration; and (*d*) evaluate these drug–polymer systems using recognized *in vitro* and *in vivo* techniques.

EXPERIMENTAL

Materials and Equipment—The medicinal agents used were either USP or NF quality. The purity of each was checked utilizing melting-point determinations. Table I summarizes the main properties of the polymer emulsions screened and their designation in this paper.

Particle-size reduction was accomplished using a comminutor[1].

Polymer Emulsion Screening—Coagulating of the polymer emulsions of Table I in the presence of various salts was performed using a test tube method outlined by Jirgensons and Straumanis (11). Uni/univalent or bi/bivalent electrolytes (sodium chloride and magnesium sulfate) were used to coagulate the anionic and nonionic polymer emulsions studied. Sodium phenobarbital was used as an anionic model drug substance throughout the study.

Sodium Phenobarbital Assay Procedure—A method of determining the sodium phenobarbital content in the presence of polymer was developed using a differential UV spectrophotometric procedure[2] first suggested by Walker *et al.* (12) and later by others (13–16). The procedure used routinely was as follows. An accurate aliquot of supernatant solution was removed and buffered to pH 10.5 in a volumetric flask. A second identical aliquot was removed and brought to volume with 0.2 *M* NaOH solution.

By using the pH 10.5 solution in the reference side and the 0.2 *M* NaOH solution in the sample side, the differential absorbance was measured at 260 nm. A plot of sodium phenobarbital concentration *versus* absorption was found to be linear and passed through the origin, allowing the accurate determination of the drug in the presence of polymer and magnesium sulfate.

The Entrapment Procedure—The following procedure illustrates the entrapment method commonly employed. The formulation used (Formula I) was: sodium phenobarbital, 1.20 g.; magnesium sulfate, 0.80 g.; distilled water, 20.00 ml.; and Polymer Emulsion 2, 25.00 ml. (11.75 g. of solids). (The USP cathartic dose of magnesium sulfate is 15 g. Calculations show that a dose of this sustained product will give a patient a negligible 73 mg. of magnesium sulfate.)

Procedure A—Fifty milliliters of a solution of magnesium sulfate and sodium phenobarbital was prepared and transferred to a volumetric flask. A 20.0-ml. aliquot of this solution was then transferred to a 100-ml. beaker. The polymer emulsion was added to the drug solution from a freely draining 25-ml. pipet with constant mixing;

[1] Fitzpatrick model M.

[2] A double-beam, UV visible, automatic recording spectrophotometer (Bausch & Lomb Spectronic 505) was used to develop the assay procedure. A Beckman model DU spectrophotometer was used for routine UV spectral analysis. All IR spectra were obtained using a double-beam IR spectrophotometer (Perkin-Elmer model 21).

Table II—pH Gradient for the *In Vitro* Release Rate Test

Hours	Amount of Fluid Removed[a], ml.	pH of Test Fluid
0	—	1.5
0.5	50.0[b]	1.5
1.5	30.0	2.2
2.5	50.0	7.5
4.5	50.0	7.5
6.5	50.0	7.5
8	50.0	—

[a] With one exception (see *Footnote b*), the fluid removed was replaced by an equal amount of pH 7.5 buffer solution (prewarmed to 37°). [b] This sample bottle was removed after 0.5 hr. and assayed. All of the remaining samples were taken from a second identical sample bottle.

the mixture was stirred for an additional 30 sec. and was then allowed to stand for 2 min. During this interval, a solid white gel of firm semisolid consistency formed. This material was transferred to an uncovered Petri dish by means of a spatula. During the next hour, the small amount of water (usually about 3–5 ml.) that leached out of the gel due to syneresis was carefully siphoned off. The gel was then allowed to air dry for 12 hr. At the end of this time, the material was broken into small pieces by hand, oven dried at 45° for 24 hr., and air dried for an additional 24 hr. The dried material was ground in a comminuter (previously cooled by passing dry ice through the machine). This served to prevent sticking of the polymer due to the heat produced by processing. The comminuted material was then sized. Particles that passed through a 40-mesh screen were used for *in vitro* testing.

Several alternative methods of preparing entrapment products were studied to evaluate their effects on the entrapment process and release of entrapped drug. These alternative procedures were as follows.

Procedure B—The solution containing the sodium phenobarbital and the magnesium sulfate was added to Polymer Emulsion 2 (the reverse was done in *Procedure A*).

Procedure C—An aqueous solution of magnesium sulfate was added to an aqueous mixture of the sodium phenobarbital and polymer emulsion.

Procedure D—The product was vacuum dried.

Procedure E—Alcohol USP was used as the drug solvent.

Solubility being a factor, magnesium sulfate was not used and phenobarbital replaced sodium phenobarbital.

Dialysis Procedure—Dialysis studies were used as an *in vitro* technique to ascertain whether or not the sodium phenobarbital and polymer were physically or chemically bound since permanent drug binding would be expected to reduce the therapeutic effectiveness of the sodium phenobarbital. The dialysis sacs were prepared from a semipermeable cellulosic membrane[4]. The closed sac, containing 20 ml. of sample solution, was placed in a 90-ml. (3-oz.) amber glass jar containing simulated gastric fluid USP (without pepsin). The jar was capped, sealed with a water-resistant tape, and

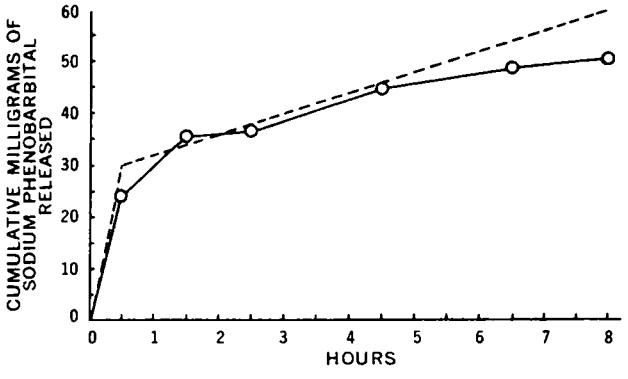


Figure 1—In vitro *release pattern—idealized* versus *experimental—for the four gels of Table VI. Key: - - -, idealized curve; and ——, experimental curve.*

[4] NoJax Casing, size 30, Visking Co., Chicago, Ill.

Table III—Oral Chronic Toxicity of Polymer Emulsion 2 Using Urine and Feces Analyses

Test[a]	Sensitivity
Proteinuria[b]	Detects less than 30 mg./100 ml. urine
Glucose[c]	Detects less than 0.5% in urine
Ketouria[d]	Detects 10 mg. of acetoacetic acid/100 ml. urine; reacts readily with acetoacetic acid but not with acetone
Bilirubin[e]	Detects 0.05 mg./100 urine
Occult blood[f]	Detects 1 part in 20,000

[a] All of the tests used are products of the Ames Division of Miles Laboratories, Elkhart, Ind. [b] Albustix test (urine). [c] Clinistix test (urine). [d] Ketostix (urine). [e] Ictotest (urine). [f] Hematest (feces).

allowed to tumble in the rotating-bottle apparatus for the specified time at 37°. Aliquot samples were taken both inside and outside the dialysis sac and assayed.

***In Vitro* Release Rate Procedure**—A rotating-bottle method (17, 18) was selected to evaluate the granular xerogel products. The comminuted samples were weighed in two individual 1.250-g. dosage units, and each sample was placed in a 90-ml. (3-oz.) amber glass jar. Fifty milliliters of simulated gastric fluid USP (without pepsin) was added, and the bottles were sealed as described earlier and were rotated at 41 r.p.m. at 37°. The first bottle was removed after 0.5 hr., the liquid was quantitatively filtered into a volumetric flask, and the solution (after proper dilutions) was assayed spectrophotometrically. The pH of the fluid in the second bottle was gradually changed from 1.5 to 7.5 over 2.5 hr. (Table II). Samples were assayed at each interval

The pH 7.5 intestinal buffer was not simulated intestinal fluid USP. The 0.2 *M* NaOH solution required in the sodium phenobarbital assay procedure precipitated the monobasic potassium phosphate of the USP fluid, but a pH 7.5 borate buffer was substituted with good results.

***In Vivo* Procedure**—*Activity Cages*—Williamson activity cages (19) located in an isolated, dark room were used to evaluate the degree and length of sedation of sodium phenobarbital administered in solution (as evidenced by a decrease in animal activity) *versus* a suspension of the sodium phenobarbital–magnesium sulfate–Polymer Emulsion 2 xerogel. The studies were performed on previously nondrugged, male, Holtzman strain, albino rats, weighing from 280 to 340 g. Male animals were selected because they demonstrate a shorter induction time as well as shorter duration of phenobarbital narcosis as compared to female rats (20). Four animals were randomly selected from the colony. Following 24 hr. of food deprivation (water was given *ad libitum*), the animals were weighed and placed in individual activity cages for a 0.5-hr. period of acclimatization. The animals were then briefly removed to a separate room for

Table IV—Coagulation of Polymer Emulsions

Polymer Emulsion Number[b]	Millimolar Coagulation Values[a]: Sodium Phenobarbital (Maximum Concentration 400 mmoles/l.)	Sodium Chloride (Maximum Concentration 1375 mmoles/l.)	Magnesium Sulfate (Maximum Concentration 325 mmoles/l.)
1	—	—	50–60[c]
2	—	—	50–60
4	180–200	350–400	65–80
5	180–200	1500	—
8	—	500	30–40
16	—	600–700	40–50
20	—	1000–1200	200–250

[a] Coagulation value is the concentration of the salt in millimoles per liter in the final system required to cause complete coagulation (gelation or flocculation) within 0.5 hr. A blank space indicates no coagulation occurred at the upper concentration stated for each salt. Coagulation, if it occurred, was usually immediate. If this rapid coagulation did not occur, the tubes were left undisturbed for 0.5 hr. and then reexamined for coagulation. [b] Polymer emulsion designations are given in Table I. [c] The concentration of the drug or electrolyte in millimoles per liter in the final coagulated system.

Table V—Dialysis Data for Sodium Phenobarbital–Polymer Emulsion 2 Xerogels in Gastric Fluid

Gel (and Replicates)	Preparation Procedure	Milligrams of Drug Entrapped/ 250 mg.[a]	Original Molarity, $M \times 10^{-3}$	Molarity Inside after 72 hr., $M \times 10^{-4}$	Molarity Outside after 72 hr., $M \times 10^{-4}$	Average Concentration Inside and Outside, $M \times 10^{-4}$	Percent Free Drug	Average Percent Free Drug
1	A	22	4.3	5.3	5.3	5.3	55	
1_1	A	22	4.3	5.4	5.6	5.5	58	
1_2	A	22	4.3	5.4	5.3	5.35	55	56.5
1_3	A	22	4.3	5.4	5.4	5.4	58	
2	C	22	4.3	3.0	4.8	3.9	46	
2_1	C	22	4.3	4.6	4.5	4.55	48	48.0
2_2	C	22	4.3	4.7	4.9	4.8	51	
3	B	22	4.3	7.1	7.1	7.1	74	
3_1	B	22	4.3	8.9	6.5	7.7	73	73.5
4	B	28	5.3	8.9	9.9	9.4	82	
4_1	B	28	5.3	9.1	9.2	9.15	77	79.5
5	C	28	5.3	6.3	7.6	6.95	62	
5_1	C	28	5.3	7.0	7.4	7.2	62	62.0

[a] Gels containing 22 mg./250 mg. were prepared by Formula I. [b] These values were obtained by dividing the total milligrams of drug found in solution after 72 hr. (inside and outside the sac) by the milligrams of xerogel entrapped drug and multiplying by 100.

oral dosing. All animals were dosed between 3:10 and 4:00 p.m. to eliminate diurnal activity variation (rats being more active at night).

The treatments were: (*a*) sodium phenobarbital, 50 mg./kg.; (*b*) sufficient suspended sodium phenobarbital–gel to release 50 mg./kg. of drug initially and a second 50 mg./kg. dose in sustained form (previously determined by the *in vitro* method already described); and (*c*) an equivalent volume of suspending agent alone (one part syrup USP and two parts water was found to be a good suspending agent for the drug-gel particles). The 50-mg./kg. dose of sodium phenobarbital was chosen from preliminary studies because of its ability to sedate the animals without inducing sleep. Malone *et al.* (20) reported that the volume of the vehicle influences the onset of sodium phenobarbital action. Consequently, fluid volume was maintained at 8 ml. After dosing, the rats were returned to the activity cages for a predetermined period.

Acute Toxicity—The purpose of this study was to determine any gross difference in acute toxicity between sodium phenobarbital and the drug-xerogel product. Since the action of sodium phenobarbital lasts much longer in the female rats as compared to the male (20), females were used. The oral LD_{50} of sodium phenobarbital in female rats reported from different laboratories was found to be quite variable (150–350 mg./kg.).

All animals used in the acute toxicity evaluation were fasted for 24 hr. prior to dosing. Starting with 150 mg./kg., rats were orally dosed with sodium phenobarbital in 25-mg. increments. The vehicle used was the same syrup–water combination previously mentioned. An approximate LD_{50} of 200 mg./kg. was established for the experimental conditions.

Twenty previously nondrugged, female, Holtzman strain, albino rats, weighing from 130 to 275 g., were used in the final acute toxicity evaluation. Ten rats received 200 mg./kg. of unmodified drug, and 10 were given an amount of drug–xerogel powder sufficient to release 200 mg./kg. of drug initially and a second 200-mg./kg. dose in sustained form (previously determined by the *in vitro* method).

Chronic Toxicity–Twenty 50-g. female rats were involved in this phase of the research. Ten animals were kept in a common cage and served as a control group, and the other 10 were kept in a separate, common cage. The control group was fed a daily measured quantity of powdered chow. The experimental group was fed in an identical manner except a quantity of solid, gelled, dried polymer (see Formula II) equivalent to 7.50 g./kg. of drug per rat was mixed daily with the rat chow powder. The rats were kept on their diets for 27 days.

The second formulation tested (Formula II) consisted of: Polymer Emulsion 2, 25.0 g.; and magnesium sulfate, 2.5 g. (The solid polymer was very tacky and difficult to work with. The presence of this small amount of magnesium sulfate made the solid polymer of Emulsion 2 much easier to handle.)

The animals were examined daily for gross physical changes. Twice weekly the animals were weighed. On these same days, they were placed in metabolism cages and their urine and feces were collected. Table III summarizes the tests run on the collected material.

At the conclusion of the 27 days, the animals were sacrificed. The kidneys, liver, and spleen were grossly examined and removed, and the net weight of each was determined. The organs were then placed in an oven (overnight) and weighed again to obtain a measure of the dry weight. In this manner, any differences in tissue weights between the control and experimental groups could be attributed to a change in either water content or tissue content.

Table VI—Reproducibility of *In Vitro* Release Rates of Sodium Phenobarbital–Magnesium Sulfate–Polymer Emulsion 2 Xerogels

Gel Replicate Number	Milligrams of Drug[a] 0.5 hr.	1 hr.	2 hr.	4 hr.	6 hr.	8 hr.
1	24.0	38.7	38.6	45.8	47.8	49.0
1_1	24.8	36.0	38.8	48.2	42.0	53.0
1_2	24.0	32.7	32.8	41.0	46.0	48.9
1_3	24.0	34.3	36.3	44.3	48.9	51.5
Mean	24.2	35.4	36.6	44.8	48.7	50.6
SD	0.40	2.56	2.79	3.02	2.52	2.00
Coefficient of variation	1.7%	7.2%	7.6%	6.7%	5.2%	4.0%

[a] Cumulative release from 1.250-g. gel sample.

RESULTS AND DISCUSSION

Polymer Screening—The results of the test tube emulsion coagulation procedure are shown in Table IV. Only those polymer emulsions that were coagulated by the drug system are shown. Four of the seven polymer emulsions were eliminated[b] because they appeared to flocculate rather than gel on coagulation and were listed only as "modified acrylics" in company literature. It was felt that the structures were too indefinite and probably very similar to the structure of the acrylic copolymer used by Goodman and Banker (6) to warrant extensive study in this project. Of the polymer emulsions left, all of which were styrene–acrylic latexes, Polymer Emulsion 2 was chosen for further investigation. The solid polymeric material precipitated from Polymer Emulsion 2 using 0.1 *N* HCl was insoluble in aqueous media over a wide pH range (from pH 1.0 to 0.2 *M* NaOH).

Dialysis Procedure—It was clearly established by spectrophotometric and gravimetric analyses that the drug could indeed pass freely through the porous membrane whereas the polymer could not cross this barrier.

[b] Polymer Emulsions 4, 5, 8, and 20 (Table I).

Table VII—Effect of Polymer–Drug Ratio on Release Rate Pattern of Sodium Phenobarbital (Cumulative Milligrams Released)[a]

Hours	Percent Drug Entrapped			
	3.1	5.9	8.7	11.3
0.5	7.0	17.5	24.2	60.0
1.5	10.2	32.7	35.4	75.0
2.5	13.7	31.6	36.6	76.6
4.5	16.8	37.0	44.8	84.1
6.5	19.2	39.5	48.7	86.1
8	20.3	40.7	50.6	87.0

[a] In all cases, a 1.250-g. sample of the drug–polymer gel was evaluated.

Drug Release from Polymer Gels—Table V summarizes the dialysis data obtained for a series of five drug–polymer xerogels. The concentration of materials in these gels and the method by which each was made are also given. Gels were prepared in at least two replicates, permitting comparison of the reproducibility of drug entrapment for a given method of preparation and concentration of drug. The effect of drug concentration on gels made by an identical method and the effect of the method of preparation on the reproducibility of gel entrapment and equilibrium dialysis for gels prepared utilizing the same concentration of drug are also shown in Table V.

Gel Series 1, 2, and 3 (Table V) contained the same initial concentration of drug. Three different methods were used to prepare the various gel products. The sodium phenobarbital available after 72 hr. ranged from 46 to 74% of the amount of drug originally entrapped (22 mg.). Gel Series 4 and 5, containing a greater amount of sodium phenobarbital but prepared by different methods, exhibited drug release varying from 62 to 82% of the original amount enclosed in the gel matrix (28 mg.). Despite this wide variance among the series (the five series in Table V), there is good agreement within each series.

***In Vitro* Release Rate Procedure**—*Rate of Release*—The *in vitro* release rate pattern was obtained initially with gels prepared using Formula I and Procedure A. Figure 1 represents a composite release curve drawn from four identically prepared gels (Gel Series 1) as shown in Tables V and VI. Except for a tailing-off between the 5th and 8th hr., the experimental data follow the "idealized" curve very closely. The idealized curve was constructed on the premise that in an oral sustained-action product involving phenobarbital, the patient should receive a dose of the drug material immediately (*i.e.*, within 0.5 hr.) and a second dose uniformly over the next 7.5-hr. period (for a total of 8 hr.). Ultimately, of course, the therapeutic response of the drug in man is the final criterion of the preparation (21).

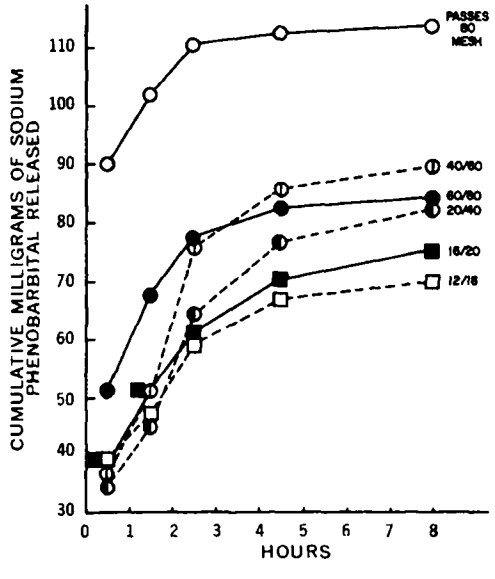


Figure 2—*Effect of particle size on release rate (Formula I and Procedure A).*

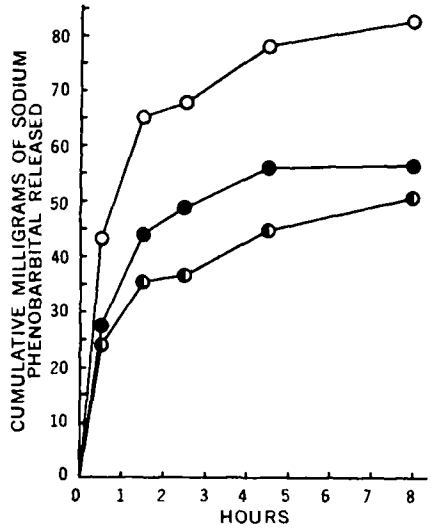


Figure 3—*Effect of method of preparation on the* in vitro *release rate of sodium phenobarbital. Key:* ◐, *Procedure A;* ●, *Procedure B; and* ○, *Procedure C. All samples contained 109 mg. of drug.*

Reproducibility—In this portion of the study, four gels[6] were prepared using Formula I and Procedure A. These gels were then subjected to *in vitro* analysis (Table VI), and excellent reproducibility of *in vitro* drug release was achieved.

Effect of Drug Concentration—This phase of the study was designed to establish limits of satisfactory drug concentrations in the Polymer Emulsion 2–magnesium sulfate gel system prepared using Procedure A. The results illustrating the effect of drug entrapment concentration on release rate are shown in Table VII.

The xerogels containing 5.9 and 8.7% sodium phenobarbital exhibited a desirable 2:1 release ratio (twice as much drug released after 8 hr. as after 0.5 hr.). The xerogel containing 3.1% drug had a 3:1 release ratio. Hence, at this concentration, the initial dose of drug might not be sufficient. At the highest drug concentration studied (11.3% entrapped), most of the drug was released initially (a 1:1.5 ratio), producing a poor release pattern. The limits of sodium phenobarbital concentration for good sustained action appear to be between 5.9 and 8.7% for this polymer system.

Effect of Particle Size—It would be reasonable to assume that the size of the final gel particles would influence the rate of drug release from the insoluble matrix. A smaller granule should release more drug and probably release it more rapidly due to greater surface exposure and a higher relative drug concentration at the xerogel surface.

The release rates of selected particle-size fractions were determined using the rotating-bottle technique. The results are summarized in Fig. 2. With minor exceptions, it can readily be seen that the smaller the particles, the more rapidly and completely the drug is released. Figure 2 also suggests the possibility of using mixed particle-size fractions to achieve a desired release rate.

Effect of Method of Preparation—In the section concerning dialysis, it was shown that, all other factors remaining constant, the method of preparing the sodium phenobarbital–magnesium sulfate–Polymer Emulsion 2 xerogel significantly altered the percent drug entrapment. As a result, an *in vitro* analysis was conducted using gels containing identical amounts of the three ingredients and varying only in the method of preparation (Fig. 3).

In Fig. 3, the shapes of curves A and C are nearly identical. However, Procedure C permits the release of approximately 30% more drug. This could result in greater economy in the manufacturing process through a savings in the amount of drug required and would produce a preparation having greater pharmacological reliability by virtue of more drug being accounted for as available drug. A possible explanation of this difference is that in Procedure A the polymer emulsion is added to a concentrated solution of the salts. As each emulsion drop enters the system, it comes into contact with a large excess of electrolyte solution, thereby gelling immediately. In Pro-

[6] These xerogels are listed as Gels 1, 1_1, 1_2, and 1_3 in Table V.

Table VIII—Effect of Drying on *In Vitro* Release Rate of Sodium Phenobarbital from Polymer Emulsion 2 Xerogels

Method of Drying	Milligrams Released after— 0.5 hr.	8 hr.	Percent Released after— 0.5 hr.	8 hr.
Air 12 hr., oven (45°) 24 hr.	24.2	50.6	22.2	46.5
Vacuum oven 24 hr., no heat	43.5	62.5	39.9	57.3
Air 1 hr., vacuum oven (60°) 12 hr.	60.0	62.5	55.0	57.3

Table IX—Effect of Polymer Concentration on Release of Sodium Phenobarbital from Polymer Emulsion 2 Xerogels

Percent Solids In Emulsion	Drug in System, mg.	Drug Released after 72 hr., mg.	Percent Drug Released
56–48	109	62.4	57
35–36	109	72.0	66
23–24	109	102.0	93

cedure C, however, the magnesium sulfate solution is slowly added to an aqueous mixture of the sodium phenobarbital and the polymer emulsion. Gelation does not occur until a sufficient concentration of electrolyte is present in the system. When this concentration is reached, the entire emulsion tends to gel simultaneously and more slowly. Consequently, the two processes result in a completely different rate of matrix formation. Of the three methods, Procedure C would be expected to produce a more porous xerogel and, in fact, resulted in the most rapid and complete dissolution release.

Another change in the method of preparing the gel involved the use of a vacuum oven to dry the product rather than conventional air and oven drying (Table VIII). Conventional drying produced a gel with the the best release rate of drug by far. One vacuum-dried product released nearly all of its unbound sodium phenobarbital within the first 0.5 hr. This was probably due to the fact that most of the water normally entrapped in the gel was rapidly drawn out of the matrix by the combination of vacuum and heat, thereby promoting drug migration to the gel surface. Sodium phenobarbital, being very water soluble, would have a greater tendency to migrate under these drying conditions. After comminution, probably very little of the unbound drug remained in the interstices of the xerogel.

When alcohol was used as the solvent in place of water, the polymer was precipitated as a solid mass. The alcohol simply "broke" the emulsion, causing the polymer to coagulate. *In vitro* analysis showed that this drug-polymer system released nearly all of its drug in the first 0.5 hr. Therefore, this product was unsuitable for sustaining the release of sodium phenobarbital.

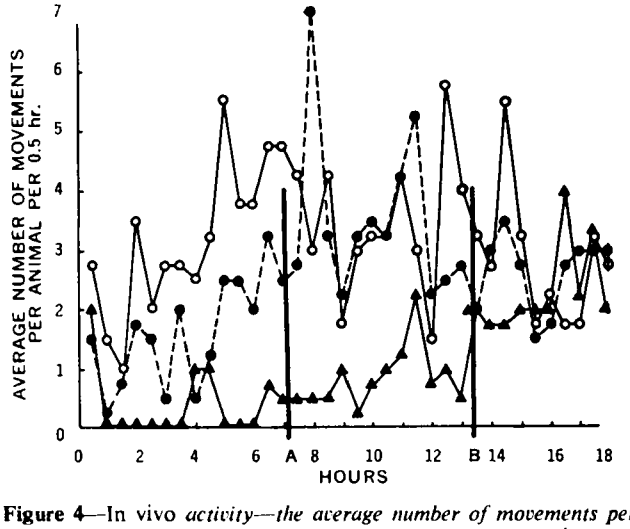


Figure 4—In vivo *activity—the average number of movements per animal per 0.5-hr. interval* versus *time. Key:* O—, *control group;* ●- - -, *sodium phenobarbital group, 50 mg./kg.; and* ▲—, *sodium phenobarbital-polymer group, 50 mg./kg.*

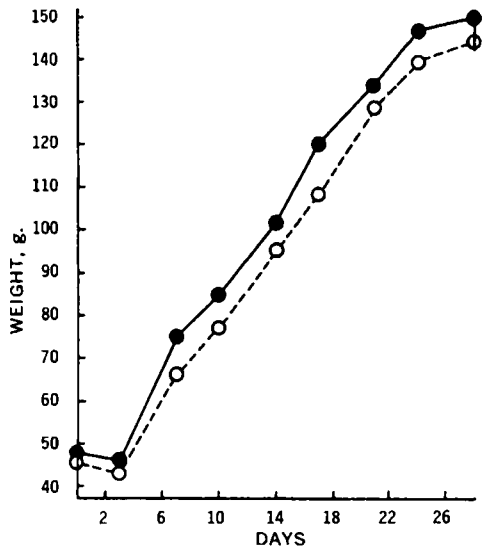


Figure 5—*Growth rate of rats on polymer feed diet* versus *control group. Key:* O- - -, *control group; and* ●—— *polymer group.*

Effect of Polymer Concentration—One potentially undesirable feature of the gel system was the "permanent" entrapment of 25–50% of the drug (depending on the procedure used). It was theorized that if the polymer emulsion were diluted and the drug-xerogel system prepared as before, the resulting matrix would be a looser and more open network. If this were the case, more drug should then be released from the interstices of the matrix.

To test this theory, the aqueous emulsion was diluted with varying amounts of distilled water. Gels were then prepared using Procedure A. The most dilute system that produced a satisfactory gel was prepared from an emulsion containing 23–24% solids (reduced from the original 46–48% solids). Table IX shows that dilution of the emulsion did, indeed, permit a substantially greater amount of the entrapped drug material to be released. This gives the formulator still another method of controlling the release of drugs from the polymer matrix.

***In Vivo* Procedures**—*Activity Cages*—The Williamson activity cages, utilized as previously described, yielded the data shown in Fig. 4. Each point represents an average value for four animals. The results clearly show a lengthening of the therapeutic effectiveness of sodium phenobarbital in the drug-polymer gel group. Furthermore, the *in vivo* drug effect observed permitted analysis of the general intensity (degree of central motor depression) of drug response throughout the test period. Over the entire period of prolonged action of the drug entrapped system, a consistent and continuous drug effect was seen. In Fig. 4, line A represents the sample

Table X—Wet Weights and Dry Weights of Various Organs of the Female Rat

Organ, g.	Polymer Group[a]	Control Group
Spleen		
Wet weight	0.502	0.569
Dry weight	0.113	0.138
Difference	0.389	0.431
Kidney		
Wet weight	1.59	1.56
Dry weight	0.42	0.41
Difference	1.17	1.15
Liver		
Wet weight	6.93	6.59
Dry weight	1.99	2.10
Difference	4.94	4.49

[a] The figures represent an average value for 10 animals (hence, a total of 20 animals was involved in this study).

Table XI—Coagulation of Polymer Emulsion 2 by Various Medicinal Agents[a]

Compound	Chemical	pH of Solution	Coagulation Type
Sodium phenobarbital	Cyclic	9.8	Gelation
Sodium citrate	Salt of an aliphatic acid	5.8	None[b]
Sodium salicylate	Salt of an aromatic acid	5.35	Gelation
Chloral hydrate	Aldehyde	4.5	Gelation
Caffeine alkaloid	Xanthine derivative	6.25	Gelation
Ephedrine sulfate	Secondary amine	5.6	Gelation
Ephedrine hydrochloride	Secondary amine	5.45	Gelation
Methapyrilene hydrochloride	Tertiary amine	6.2	Flocculation
Dextroamphetamine sulfate	Primary amine	5.9	Flocculation
Dihydrocodeinone bitartrate	Tertiary amine	1.75	Flocculation
Pyrilamine maleate	Tertiary amine	4.2	Flocculation

[a] All solutions (20 ml.) contained 1.20 g. of drug and 0.80 g. of magnesium sulfate, except the caffeine solution. This solution contained 0.80 g. of magnesium sulfate, but solubility limited the amount of caffeine to 0.40 g. [b] Gelation did occur when the concentration of magnesium sulfate was increased to 1.60 g.

mean of duration of effect for the group receiving drug alone and line B indicates the sample mean for the rats dosed with the drug–xerogel. For free drug:

$$\bar{x}_f = 7.1 \text{ hr.}$$
$$s^2 = 1.17$$
$$s = 1.08 \text{ hr.}$$
$$90\% \text{ confidence interval} = \pm 1.3 \text{ hr.}$$

where $\bar{x}_f$ is the sample mean, s^2 is the sample variance, and s is the standard deviation.

For drug xerogel:

$$\bar{x}_g = 13.4 \text{ hr.}$$
$$s^2 = 4.94$$
$$s = 2.2 \text{ hr.}$$
$$90\% \text{ confidence interval} = \pm 2.6 \text{ hr. (22)}$$

A sustained therapeutic effect was clearly achieved in that the free drug exhibited a duration of action of 7.1 ± 1.3 hr. whereas the drug–xerogel showed an effectiveness of 13.4 ± 2.6 hr. This portion of the *in vivo* work also served to substantiate the *in vitro* rotating-bottle procedure as a reliable method of predicting drug release for the systems of this study under the test conditions.

Acute Toxicity—Preliminary work suggested that, under the conditions previously described, an oral dose of 200 mg./kg. of sodium phenobarbital represented an approximate LD_{50} for female rats. Accordingly, 10 female rats were given this dose of free drug. Five of the animals died and an equal number lived. A second group of 10 animals were given the drug–polymer gel in an amount sufficient to release 200 mg./kg. of drug initially and an additional 200 mg./kg. of drug gradually. In this group, four animals died and six animals lived.

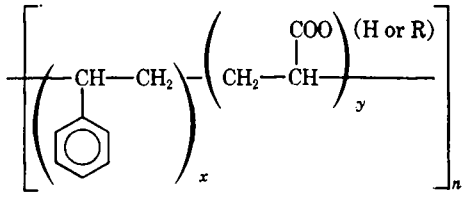
Many sustained-action preparations lower the acute toxicity of the modified drug product (23, 24). This also appeared to be true for sodium phenobarbital in the system used. The xerogel group received twice as much drug in the 8-hr. period as did the group that received unaltered sodium phenobarbital, yet one less animal receiving the sustained-release form died.

Chronic Toxicity—It was believed that the solid polymer material used in this study would not be toxic because of its apparent complete insolubility in aqueous media. It was feared, however, that contaminants (*e.g.*, catalysts, monomers, and surfactants) could be present and might themselves be toxic. Based on preliminary testing conducted in one animal species, the rat, no toxicity was noted. The growth curves of the two groups parallel each other closely (Fig. 5). Frequent urinalysis revealed no sign of any internal damage or structural malfunction (see Table III for the test procedures used). The only material detected in measurable quantity in the urine was a constant 30 mg./100 ml. of albumin in both the control and experimental groups.

The terminal study on the sacrificed animals revealed no visual abnormalities in any rats. Selected portions of the GI tract of several animals from each group revealed no visible irritation. The wet weight *versus* dry weight data of the various test organs are summarized in Table X. Considering the relatively small number of animals used, the organ weights show good experimental agreement.

Gelation Phenomenon—Physical examination and centrifugal testing established that the sodium phenobarbital–magnesium sulfate–Polymer Emulsion 2 product was a gel. It was clearly established that the magnesium ion (present as magnesium sulfate) was the coagulating ion in the system.

The best estimated structure of the copolymer latex as obtained from information from the manufacturer and an IR spectrum of a film of the emulsion is shown below. The ratio of styrene monomer



to acrylic monomer is probably not 1 to 1 (as shown), but rather 1 part styrene to between 0.5 to 0.7 part of acrylic monomer (25). Latex gels are normally highly cross-linked (11), which would contribute to their insolubility.

The gelation of selected polymer emulsions (colloidal suspensions prepared by emulsion polymerization) provides a unique physicochemical method of preparing drug–matrix and drug–xerogel systems. The xerogel entrapment technique utilizing polymer emulsions, as well as flocculation entrapment in such systems (6–8), offers physicochemical approaches to drug dispersion and entrapment with numerous applications in controlled release and drug delivery.

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NOTES

Stability of Aspirin in Liquid and Semisolid Bases I: Substituted and Nonsubstituted Polyethylene Glycols

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Abstract □ The stability of aspirin in methoxypolyethylene glycol, polyethylene glycol acetate, or a mixture of polyethylene glycols was studied at three temperatures. Degradation proceeded to the highest extent in a mixture of polyethylene glycols and to a lesser extent in methoxypolyethylene glycol. Degradation in polyethylene glycol acetate for 30 days at 45° was insignificant. The data confirm that decomposition of aspirin in polyethylene glycol is due primarily to a transesterification reaction.

Keyphrases □ Aspirin—stability in liquid and semisolid bases, polyethylene glycols □ Polyethylene glycols—stability of aspirin, liquid and semisolid bases □ Stability, aspirin—in polyethylene glycols, liquid and semisolid bases

In a previous paper (1) on the decomposition of aspirin in polyethylene glycols, these laboratories provided data showing that degradation was due at least in part to a transesterification reaction between aspirin and the polyethylene glycols. This conclusion was reached when it was found that acetic acid was not among the degradation products when a mixture of aspirin and polyethylene glycols was stored at different temperatures. Further evidence of a transesterification reaction was the degradation of aspirin in various polyethylene glycols in the apparent absence of moisture and the resultant appearance of acetylated polyethylene glycol. Both the absence of acetic acid and the presence of acetylated polyethylene glycol were established using NMR techniques (1).

The implications of these findings are far reaching and, more specifically, may be applied to cases where polyethylene glycols may be desirable as a vehicle (either liquid, semisolid, or solid) for aspirin preparation. This being the case then, the next logical step was to establish the stability of aspirin when the transesterification pathway is inhibited. It was thought that inhibition of the transesterification mechanism could be brought about by reducing the number of sites available for this reaction, namely, by blocking free hydroxyl groups on the polyethylene glycols.

Methoxypolyethylene glycol has the formula CH_3—O—CH_2—$(CH_2$—O—$CH_2)_nCH_2OH$, which has a methoxy radical at one terminal and only one remaining hydroxyl group that may enter into the reaction. Acetylation of polyethylene glycol also reduces the number of hydroxyl groups and likewise was expected to retard the decomposition of aspirin.

EXPERIMENTAL

Materials—Aspirin USP[1] and polyethylene glycols[2] 400, 1540, and 6000 were used as received. Chloroform[3] was spectroscopic grade. Monosubstituted methoxypolyethylene glycol 550[4] was purchased, and polyethylene glycol 400 acetate was prepared in these laboratories.

Analytical Method—UV spectrophotometric techniques[5] were employed to measure aspirin and salicylic acid (2). Samples were taken at specific time intervals, dissolved in 1% acetic acid–chloroform, and read at 278 nm. for aspirin and 308 nm. for salicylic acid after appropriate dilutions. Standard curves were prepared for aspirin and salicylic acid. A correction factor was obtained to account for the overlapping of absorbance at absorption maxima of each drug. The quantification was based on the standard method of simultaneous spectrophotometric determinations (3).

Procedure—The samples were prepared by the incorporation of 12% of aspirin in methoxypolyethylene glycol 550, polyethylene glycol 400 acetate, and a mixture of polyethylene glycols [polyethylene glycol 400–1540–6000 (27:31:42)] at the melting points of the individual bases. Preparations were kept in airtight amber containers and stored in a desiccator at 4, 26, and 45°. At various time intervals a weighed amount (2.5 g.) of samples was dissolved in 1% acetic acid–chloroform. Spectrophotometric readings were taken immediately after dilutions for aspirin and salicylic acid.

[1] Merck & Co., Inc., Rahway, N. J.
[2] Matheson, Coleman and Bell, Norwood, Ohio.
[3] J. T. Baker Chemical Co., Phillipsburg, N. J.
[4] Union Carbide, New York, N. Y.
[5] Beckman DU spectrophotometer.