acrylic copolymer entrapment product containing 25% drug, is quite different. The diffraction pattern indicates that crystalline drug is absent. The absence of crystalline drug in this sample demonstrates that the drug in the entrapment product is uniformly dispersed at a molecular level.

The mechanism by which the carboxylic acid anion facilitator increases the amount of drug bound by the polymer is of considerable theoretical interest and is the subject of a further study. Several modes of action appear possible. The formation of complex ions between the polymer carboxyl group, drug, and acid anions has been suggested, the resultant electrostatic forces binding the drug to the polymer. Presumably, inclusion-type complex formation may also be involved to some extent.

SUMMARY

A new method for the preparation of sustained-action pharmaceuticals by stoichiometric entrapment of drugs in polymer flocculates is described.

The advantages of the use of a suitable entrapment facilitator, an organic acid, to enhance the binding of drugs by polymers is demonstrated.

In vitro tests indicate that the products obtained by this technique could be used in either solid or liquid dosage forms.

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Molecular Scale Drug Entrapment as a Precise Method of Controlled Drug Release III: *In Vitro* and *In Vivo* Studies of Drug Release

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Abstract □ Controlled-release drug-entrapped systems of phenylephrine and phenylpropanolamine have been prepared by the facilitated molecular scale drug-entrapment method previously described. Variables influencing the entrapment process, such as flocculation pH and rate of agitation, have been investigated. These variables are readily controllable. The influence of various polymers and carboxylic acid anions on the entrapment and release of the drugs was examined. Tests applied to the products so obtained indicate that they are well suited for pharmaceutical use. An *in vivo* study verified the sustained-release properties of a molecular scale entrapped phenylpropanolamine.

Keyphrases □ Drug entrapment, molecular scale—release-rate control □ Flocculation pH effect—drug binding, release □ Stirring rate effect—drug binding □ Release rates, *in vivo*, *in vitro*—polymer-bound drug

Parts I and II of this series described the development of a method of molecular scale drug entrapment for the physicochemical preparation of controlled-release pharmaceuticals; the advantages offered by this new technique were also discussed (1, 2). Part I described the flocculation of polymeric colloidal dispersions as a mechanism of precise drug entrapment. It was demonstrated that the resultant drug–polymer flocculates, when administered as a fine powder, exhibited very satisfactory sustained-action characteristics. However, less success was obtained when weakly basic drugs such as phenylephrine were used as flocculants.

Table I—Efficiency of Various Carboxylic Acid Anions in Binding Phenylephrine to Acrylic Copolymer Emulsion 1

Facilitator	pKa	Drug Bound by Polymer, %[a]
(Hydrochloride)		25
Adipate, H	4.43	28
Citrate, H	4.74	32
Malonate, H	2.85	32
Citrate, HH	3.06	33
Glutarate, H	4.34	35
Glutarate	5.12	36
Tartrate, H	3.01	41
Tartrate	4.54	44
Fumarate	4.47	46
Succinate, H	4.19	49
Succinate	5.57	49
Maleate, H	2.20	49
Ascorbate, H	4.17	50
Adipate	5.27	53
Citrate	5.40	58
Ascorbate	11.57	60
Maleate	6.26	60

[a] Percentage of drug entrapped from solution.

In Part II the study of drug entrapment by polymer flocculation was considerably extended. It was shown that the presence of a carboxylic acid anion greatly facilitated drug entrapment, increasing the binding of the drugs by the polymer, which substantially increased the efficiency of the drug-entrapment process and provided added control over drug release.

Table II—Effect of pH upon Binding and Release of Phenylephrine Maleate by Acrylic Copolymer Emulsion 1

Emulsion pH	Concentration of Drug in Polymer Flocculate, % w/w	Drug Released after 30 min., %
1–1.5[a]	3.3	54
2.9	3.7	70
4.0	4.6	95
4.7	5.3	100
5.9	No flocculation	—

[a] Without pH adjustment.

Table III—Effect of Stirring Rate on Percentage of Phenylephrine Maleate Bound by Acrylic Copolymer Emulsion 1

Stirring Rate (Variac Setting)	Concentration of Drug in Flocculate, % w/w
69	1.2
80	1.3
110	1.2
140	3.0

Phenylephrine and phenylpropanolamine, two weakly basic amine drugs which initially presented considerable difficulty as to efficient entrapment and adequately retarded release utilizing the entrapment procedure, were selected for detailed study to assess the utility and applicability of the facilitated entrapment technique in a rigorous manner. Results presented in this paper show that sustained-action products of both of these drugs may be readily prepared utilizing the facilitated entrapment method. Studies made to determine the optimum conditions for facilitated entrapment were also conducted.

EXPERIMENTAL

Phenylephrine Studies—*Efficiency of Various Dicarboxylic Acid Anions in Binding Drug to Polymer*—The binding of phenylephrine[1] by an acrylic copolymer latex,[2] hereafter referred to as acrylic copolymer emulsion 1 or acrylic copolymer 1, in the presence of a variety of anions was examined in the following manner. One-fortieth of a mole quantities of drugs, as the hydrochloride, together with an equivalent amount of carboxylic acid anion and sufficient sodium hydroxide to produce the facilitator moieties of Table I, was dissolved in 50 ml. of distilled water and added over a period of 5 min. to 50 ml. of the polymer emulsion, which was stirred continuously. The flocculate was separated from the supernatant liquid by filtration, and the drug content of both product and filtrate was determined by the peak height assay method (3). Results are shown in Table I.

Effect of Flocculation pH upon Binding and Release of Drug—A stock solution of phenylephrine maleate (0.25 *M*) was prepared, and 50-ml. quantities were added to 50-ml. volumes of polymer emulsion, the pH of which had been previously adjusted by addition of suitable amounts of sodium hydroxide solution. The flocculated products, which resulted from the addition of the drug electrolyte solutions to the polymeric colloidal dispersions, were collected by filtration, dried, and reduced to a 60-mesh powder. The concentration of drug in the dry flocculates and the amount of drug released after shaking for 30 min. in simulated gastric juice (without enzyme) at 37° are recorded in Table II.

Effect of Stirring Rate upon Binding of Phenylephrine—Solutions of phenylephrine maleate were used to flocculate acrylic copolymer emulsion 1, as described in the previous section but without pH adjustment. Flocculation was effected at different stirring rates using a standard three-bladed marine propeller, driven by a laboratory mixer employing Variac speed control. The effect of stirring rate upon percentage of drug bound is shown in Table III. Attempts to effect flocculation at higher stirring rates by the use of a high-shear mixer with a rotor/stator head were unsuccessful; the flocculate blocked the narrow aperture of the mixing head.

Influence of Polymer on Drug Entrapment—Goodman *et al.* (1) preliminarily investigated the usefulness of two related polymeric dispersions as entrapment matrices. In this phase of the research the influence of the polymer on drug entrapment was more closely studied. Twenty-five polymer dispersions were screened for pH solubility properties and apparent pKa values in order to select the potentially best materials for possible use as entrapment matrices. Those which demonstrated potentially useful solubility and coagulation properties were subjected to further drug flocculation studies. Polymeric systems with reasonable flocculation values (the volumes of a stock solution of phenylephrine maleate, 0.5 *M*, required to effect flocculation) are listed in Table IV. The physical properties of some of the flocculates, in particular the ease with which they could be dried and milled, are also noted in Table IV.

The two most satisfactory products, derived from a second acrylic acid copolymer emulsion[3] (hereafter referred to as acrylic copolymer emulsion 2 or acrylic copolymer 2) and a styrene–acrylic copolymer emulsion,[4] together with the previously described acrylic copolymer emulsion 1,[2] were further examined. The percentage of drug bound

Table IV—Results of Investigations of Use of a Number of Polymers as Matrices for Molecular Scale Entrapment and Controlled Release

Polymer Emulsion	Flocculation Value, ml.[a]	Physical Properties of Product
Crosslinked acrylic copolymer 2[b]	7.60	Good
Styrene–acrylic copolymer[c]	13.10	Good
Acrylic copolymer[d]	15.30	Product very soft and elastic, could not be milled
Polyvinyl acetate copolymer[e]	20	Product very soft and elastic, could not be milled
Acrylic copolymer[f]	14.50	Incomplete flocculation product not filterable
Acrylic copolymer[g]	8.70	Incomplete flocculation product not filterable

[a] Flocculation values are for a 0.5 *M* phenylephrine maleate solution. [b] Acrysol ASE 60, Rohm & Haas Co., Philadelphia, Pa. [c] Neocryl BT-4, Polyvinyl Chemicals, Inc., Peabody, Mass. [d] Rhoplex 4530, Rohm & Haas Co., Philadelphia, Pa. [e] Neo-Vac V-26-N, Polyvinyl Chemicals, Inc., Peabody, Mass. [f] Polyco 2715, Bordon Chemical Co., New York, N. Y. [g] Rhoplex B85, Rohm & Haas Co., Philadelphia, Pa.

Table V—Data on Binding and Release of Phenylephrine by Different Polymers

Polymer	Drug Bound, %	Bound Drug Released after 30 min. in Gastric Juice, %
Acrylic copolymer emulsion 1	60	54
Acrylic copolymer emulsion 2	58	39
Styrene–acrylic copolymer emulsion	60	48

[1] Delchem, Chicago, Ill.

[2] Acrysol ASE-75; a 100% linear, anionically charged acrylic acid copolymer with a molecular weight in excess of 300,000, supplied in emulsion form, containing 40% solids; Rohm & Haas Co., Philadelphia, Pa.

[3] Acrysol ASE-60; a crosslined acrylic acid copolymer containing approximately 17% carboxyl functionality (by weight), supplied in emulsion form, containing 27.7% solids; Rohm & Haas Co., Philadelphia, Pa.

[4] Neocryl BT-4; a styrene–acrylic copolymer, existing in emulsion form; Polyvinyl Chemicals, Peabody, Mass.

Table VI—*In Vitro* Release Data for Phenylephrine Maleate–Acrylic Copolymer 1 Entrapment Products

Time, hr.	pH of Media	Cumulative Drug Release, % (1)[a]	(2)[b]	(3)[c]	(4)[d]
0.5	1.3	52	54	70	34
1.5	1.3	63	65	77	54
2	1.3	64	—	—	—
2.5	2.3	—	70	—	—
4.5	7.3	—	90	95	—
6.5	7.3	—	91	—	—
8	7.3	90	94	—	—

[a] (1) 11.3% drug, 20 mesh. [b] (2) 3.3% drug, 50 mesh. [c] (3) Phenylephrine hydrogen maleate, 1.7% drug, 50 mesh. [d] (4) Product 2 pretreated for 10 min. by washing with 0.1 *N* HCl.

by the various polymers was determined, and the amount of drug released after shaking a 60-mesh powder at 37° in simulated gastric juice (without enzyme) for 30 min. was measured (Table V).

In Vitro *Release Study*—The *in vitro* release rates of phenylephrine from phenylephrine maleate–acrylic copolymer entrapment products were examined by the method described in Part I of this series (Table VI).

Phenylpropanolamine Studies—*Efficiency of Various Carboxylic Acid Anions in Drug Binding*—Experiments similar to those performed with phenylephrine were conducted. The results are shown in Table VII.

In Vitro *Release Study*—An *in vitro* study of the release of drug from a phenylpropanolamine acetate–acrylic copolymer 1 entrapment product, as a 60-mesh powder, was performed (Table VIII) in the same manner as was employed for the phenylephrine entrapment products.

In Vivo *Release Study*—Five adult healthy male subjects were used in this test. They were given a capsule dose of 50 mg. of phenylpropanolamine: (*a*) in the form of the USP product and (*b*) as a 60-mesh powder of the phenylpropanolamine acetate–acrylic copolymer 1 entrapment product. The amount of drug appearing in the urine was determined over a 24-hr. period by the method described by Heimlich *et al.* (4). From the data so obtained, the time for half the drug to be excreted, the biological half-life, was determined (Table IX).

DISCUSSION

The results in Tables I and VII show considerable variation in the efficiency with which the various facilitators assist in the binding of these weak basic amine drugs to the acrylic polymer. For both drugs (Tables I and VII) the order of facilitator entrapment efficiency is very similar. Further, replacement of all acidic hydrogen atoms on the polybasic facilitators appears to enhance entrapment efficiency. No direct relationship between facilitator efficiency and pKa is immediately discernible. It is thought that both electronic and steric factors control the activity of the acid anions in the entrapment process.

The effect of an increase in the flocculation pH value was twofold. It increased both the amount of drug bound and the rate at which drug was released. These effects are probably due to one or

Table VII—Efficiency of Various Carboxylic Acid Anions in Binding Phenylpropanolamine to Acrylic Copolymer 1 during Flocculation Entrapment

Facilitator	Drug Bound, %	Bound Drug Released after 30 min. in Gastric Juice, %
Citrate, HH	29	85
Citrate H	30	57
Tartrate	38	88
Fumarate	43	69
Citrate	49	61
Acetate	50	49
Maleate	51	55

Table VIII—*In Vitro* Release Data for a Phenylpropanolamine Acetate–Acrylic Copolymer 1 Entrapment Product[a]

Time, hr.	pH of Media	Cumulative Drug Release, %
0.5	1.3	48
1.5	1.3	67
2.5	1.3	69
4.5	2.3	74
6	7.3	94
8	7.3	97

[a] Product contained 9.76% drug.

both of two factors. First, an increase in pH will increase the amount of phenylephrine present in the unionized form and reduce the proportion present as the ionized species. The increase in the amount of drug bound may reflect the ability of the unionized species to interact with the polymer, possibly by a different mechanism to that operating in the binding of the ionized drug. Second, an increase in flocculation pH will alter the solubility of the polymer, since it will produce an increase in the proportion of ionized polymer carboxyl groups. The results shown in Table II do not suggest that an increase in flocculation pH is advantageous in the preparation of sustained-action pharmaceuticals by the carboxylic acid facilitated entrapment method.

The increase in the amount of drug bound with an increase in stirring rate (Table III) may possibly be attributed to the effect of high shear increasing the area of polymer surface available to drug in the aqueous phase by dispersing particle flocs, thus facilitating interaction.

Examination of a number of polymer emulsions for use as molecular scale entrapment matrices for potential controlled drug-release applications resulted in the discovery of two additional polymers, acrylic copolymer emulsion 2 and the styrene–acrylic copolymer emulsion, which appeared to be well suited for this purpose (Tables IV and V). These two polymer emulsions flocculated completely; the products obtained could be easily filtered, dried, and milled. Results of preliminary *in vitro* release studies compare favorably with those obtained earlier with acrylic copolymer 1.

The results in Table VI indicate that the phenylephrine maleate–acrylic copolymer 1 product possesses very satisfactory *in vitro* release characteristics, with approximately one-half of the total drug being released in the first 30 min. for two of the products and the remainder becoming available at a steady rate during the next 6.5 hr. Similar results are shown in Table VIII for the phenylpropanolamine acetate–acrylic copolymer 1 product.

Preliminary *in vivo* tests of the phenylpropanolamine entrapment product also indicate the sustained-action properties of the phenylpropanolamine product. For all subjects the rate of excretion of drug from the polymer flocculate was lower than from the untreated drug. The mean time for half the total dose to be excreted was 4.6 hr. for the USP product, as compared with 8.0 hr. for polymer–drug entrapment product (Table IX).

SUMMARY

Further studies of the facilitated molecular scale entrapment technique for the preparation of controlled-release or sustained-action pharmaceuticals are reported. The efficiency of a number of carboxylic acid anions in facilitating drug entrapment was studied, as were pH and mechanical effects on the facilitated entrapment.

Table IX—*In Vivo* Release Data for Phenylpropanolamine–Acrylic Copolymer 1 Entrapment Sustained-Release Product

Subject Weight, lb.	Biological Half-Life of Phenylpropanolamine, hr.	Biological Half-Life of Phenylpropanolamine Polymer Product, hr.
205	5.0	7.8
175	6.0	9.8
139	5.3	7.0
139	3.0	8.5
130	3.8	6.8
Average	4.6	8.0

In vitro and *in vivo* tests of phenylephrine and phenylpropanolamine products, obtained by the facilitated molecular entrapment method, confirmed the pharmaceutical utility of this technique.

These results indicate that the drug–polymer entrapment products prepared by the facilitation method possess considerable potential for exploitation as sustained-action or controlled-release pharmaceuticals.

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Selected Pharmacological Studies of a Series of Substituted Imidazo(4,5-*d*)pyridazines

G. G. FERGUSON, H. C. HEIM, and G. D. APPELT

Abstract □ A general pharmacological screening program was applied to a series of 10 substituted imidazo(4,5-*d*)pyridazine compounds. All of the compounds produced depression of spontaneous activity in rats, and six of the compounds produced significant lengthening of hexobarbital "sleeping times." In addition, two of the compounds produced a partial reversal of reserpine hypothermia, measured by a rectal temperature monitoring apparatus, and seven of the compounds produced significant inhibition of monoamine oxidase using a Warburg apparatus. One compound produced monoamine oxidase inhibition when a spectrophotometric assay was employed.

Keyphrases □ Imidazo(4,5-*d*)pyridazines—pharmacological screening □ CNS activity—imidazo(4,5-*d*)pyridazines □ Monoamine oxidase inhibition—imidazo(4,5-*d*)pyridazines □ Reserpine-induced hypothermia—imidazo(4,5-*d*)pyridazine blocking effect

In recent years, considerable interest has arisen in the development of compounds having potential antineoplastic effects. Robins (1), for example, reported the synthesis of a series of over 1300 purine derivatives and included antineoplastic screening data on active compounds. The compounds studied in this report, the imidazo(4,5-*d*)pyridazines, were synthesized by Gerhardt *et al.* (2) as possible purine antimetabolites and are representative of 75 compounds in that series (Table I).

Little has been reported concerning the pharmacology of imidazopyridazines or similar compounds, other than antineoplastic screening data. Dimmling and Hein (3) found that certain types of imidazole derivatives decreased contractility of the frog heart and produced damage to leukocytes and macrophages. Certain pyrimidazole derivatives have shown local anesthetic effects (4), and other pyridine and pyridine–pyrrolidine imidazole compounds have been shown to have CNS depressant effects (5). Rinaldi *et al.* (6) showed some protection of mice to the effects of X-ray exposure with imidazole and benzimidazole derivatives. Certain derivatives of phenylimidazopyridine carboxylic acid have been shown to have diuretic effects (7). It was felt that a survey of the general pharmacological properties of this series of compounds might be useful to further consideration of them as possible antineoplastic agents. In this paper, studies of certain effects of these compounds on the CNS are reported.

Table I—Chemical Names of the Compounds

Compound	Chemical Name
1	1*H*-Imidazo[4,5-*d*]pyridazine-2,4,7-triol
2	1-Benzyl-2-mercapto-1*H*-imidazo[4,5-*d*]pyridazine-4,7-diol
3	1-Benzyl-2-(isopentylthio)-1*H*-imidazo[4,5-*d*]pyridazine-4,7-diol
4	1-Benzyl-2-(cyclopentylthio)-1*H*-imidazo[4,5-*d*]pyridazine-4,7-diol
5	1-Benzyl-2-[(*p*-fluorobenzyl)thio]-1*H*-imidazo[4,5-*d*]-pyridazine-4,7-diol
6	1-Benzyl-2-[(*m*-fluorobenzyl)thio]-1*H*-imidazo[4,5-*d*]-pyridazine-4,7-diol
7	1-Benzyl-2-[(2,4-dichlorobenzyl)thio]-1*H*-imidazo[4,5-*d*]-pyridazine-4,7-diol
8	1-Benzyl-2-[(2,6-dichlorobenzyl)thio]-1*H*-imidazo-[4,5-*d*]pyridazine-4,7-diol
9	1-Benzyl-2-[(*p*-bromobenzyl)thio]-1*H*-imidazo[4,5-*d*]-pyridazine-4,7-diol
10	1-Benzyl-2-[(*o*-iodobenzyl)thio]-1*H*-imidazo[4,5-*d*]-pyridazine-4,7-diol

EXPERIMENTAL

Evaluation of Gross CNS Effects—Adult Houston-Cheek albino rats, weighing between 200 and 350 g., were used for measurements of spontaneous activity and for determinations of hexobarbital "sleeping time." Spontaneous activity measurements were done using a "light box" similar to that proposed by Dews (8). Each animal was injected with a dose intraperitoneally of drug suspended in alkalinized distilled water (pH 9), placed in a stimulus-free chamber for 15 min. to allow for absorption of the drug, then placed in the activity box, and monitored for spontaneous activity for 1 hr.