Bead Polymerization Technique for Sustained-Release Dosage Form

S. C. KHANNA*, T. JECKLIN†, and P. SPEISER

Abstract □ The bead polymerization technique has been studied for the preparation of a sustained-release dosage form. This technique has been employed in aqueous outer phase for many water-insoluble monomers with and without drug. Different concentrations of water-soluble acidic monomers (α- and β-methacrylic acids) have been incorporated with these water-insoluble monomers in an attempt to improve the solubility and swelling property of the beads and hence the release of drug embedded in them in artificial gastrointestinal buffer juices. To prove the applicability of this method for the preparation of a pharmaceutical dosage form, the beads of various monomers and their mixtures in the presence of different drugs have been prepared. The influence of drug on the property of the beads has also been studied.

Keyphrases □ Sustained-release dosage form—bead polymerization □ Polymerization process—sustained-release dosage form □ Diagram—bead polymerization apparatus □ Particle-size determination—bead polymers □ Drug effect—bead polymer formation

The sustained-release dosage form constitutes an important part of modern pharmaceutical technology. Such an oral dosage form is often desirable to impart sustained-release or long-acting therapeutic effects and is commercially useful. A variety of methods and techniques has been employed for preparing such a dosage form, and approximately 1000 pharmaceutical patents have been issued in the last 15 years. The natural and synthetic polymers, waxes, and many other materials, either alone or combined, have been frequently employed for coating powder particles or granules, using conventional (1), spray drying (2), and other methods of coating in an attempt to prepare sustained-release dosage forms. Beads of different waxes (3) and epoxy resins (4) have also been reported for obtaining such products. The methods so far have mostly employed polymers, but polymerization techniques in which the drug can be embedded during polymerization of a single monomer or the combination of a few monomers have received little or no attention.

The monomers and catalyst used for polymerization are relatively toxic substances. However, the pure polymers obtained from these monomers are nontoxic and harmless to human beings. Most monomers employed in this study are those whose polymers in one form or another have already been in use for pharmaceutical purposes, *e.g.*, acrylates (5) and vinyl acetate (6).

In the present study, the possibility of using one of the methods of polymerization, known as bead polymerization or pearl polymerization, for the preparation of a sustained-release dosage form has been explored (7). Various monomers alone or in combination with others have been prepared in bead form with and without drugs. The effect of drugs on the properties of the beads obtained has also been investigated.

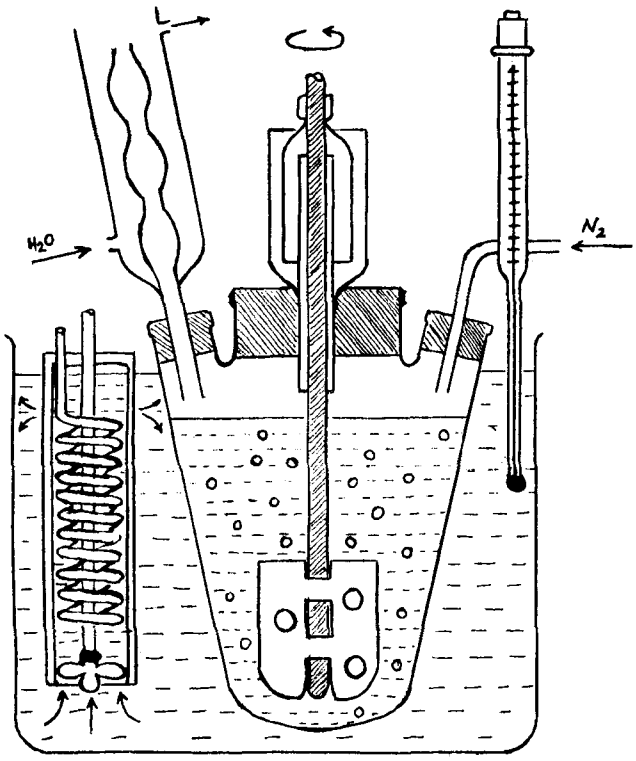

Figure 1—*Apparatus for bead polymerization.*

METHODS OF POLYMERIZATION

Polymerization can be carried out by the following four methods: (*a*) bulk or block polymerization; (*b*) bead or pearl polymerization; (*c*) emulsion polymerization; and (*d*) solution polymerization. The latter two polymerization methods can be employed for pharmaceutical purposes but are less common because of the high content of the monomers left in the polymers after the polymerization is over. The bulk polymerization method may become inconvenient for thermolabile drugs because heat of polymerization is evolved during the process. This can be quite high for many monomers and may cause decomposition of the drug.

Bead polymerization was first carried out in 1931 (8). However, this method was successfully applied only after effective suspension stabilizers were found. The bead polymerization method is similar to the bulk polymerization with respect to reaction kinetics, but the monomer or the mixture of monomers is dispersed by strong mechanical agitation into droplets in a second liquid phase in which both the monomer and the polymer formed are essentially insoluble. The monomer droplets, which are larger than those of a true emulsion, are then polymerized by heating with the catalyst. The dispersion is maintained during this time by continuous agitation. The suspending liquid, mostly water, acts as a cooler when the high amount of the heat of polymerization is evolved during the process. Suitable agents are added to the suspended liquid to hinder the coalescence of the droplets during polymerization. The polymerization takes place in the individual monomer droplets; these polymerized droplets are called beads or pearls. These beads can easily be separated from the aqueous phase when stirring is discontinued. Thus the bead polymerization method overcomes the

Table I—Beads of Methyl Methacrylate and α-Methacrylic Acid with Chloramphenicol

Materials	Formula 1	1.1	2	2.1	3	3.1	4	4.1
Inner phase								
Methyl methacrylate, g.	100	80	66.6	53.0	33.3	27.0	—	—
α-Methacrylic acid, g.	—	—	33.3	27.0	66.6	53.0	100	80
Chloramphenicol, g.	—	20	—	20.0	—	20.0	—	20
Outer aqueous phase								
Carboxyvinyl polymer, g.	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9
Sodium sulfate, anhyd., g.	—	—	15.0	15.0	30.0	30.0	60.0	60.0
Sulfuric acid, concn., ml.	—	—	6.0	6.0	6.0	6.0	—	—
Water, ml.	300	300	300.0	300.0	300.0	300.0	300.0	300
Catalyst (benzoyl peroxide), g.	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Speed of stirrer, r.p.m.	120	120	120	120	120	120	120	120
Temp. of polymerizn.	70–75	70–75	74–80	74–80	74–80	74–80	80–95	80–95
Time of polymerizn., hr.	24	24	7	7	7	7	6	6
Drying temperature	60	60	60	60	60	60	60	60
Washing medium	Water	Water	Water	Water	Water	Water	HCl, 20% v/v	HCl, 20% v/v
Yield	94	80	93	87	95	75	96	80
Amount of drug in beads, %	—	7.0	—	19.5	—	15.0	—	9.5

Table II—Beads[a] of Vinyl Acetate and Crotonic Acid with Chlorothiazide

Materials	Formula 5	5.1	6	6.1	7	7.1	8	8.1	9	9.1
Inner phase										
Vinyl acetate, g.	98.0	78.4	95.0	76.0	92.0	73.6	89.0	71.2	86.0	68.8
Crotonic acid, g.	2.0	1.6	5.0	4.0	8.0	6.4	11.0	8.8	14.0	11.2
Chlorothiazide, g.	—	20.0	—	20.0	—	20.0	—	20.0	—	20.0
Outer aqueous phase[b]	300 ml.									
Yield, g.	85	71	95	78	88	80	95	82	86	76
Amount of drug in beads, %[c]										

[a] General conditions for polymerization: catalyst (α,α′-axo-isobutyronitrile), 1.0 g.; speed of stirrer, 100 r.p.m.; temperature of polymerization, 60–65°; time of polymerization, 36–38 hr.; washing medium, ice cold water; drying temperature, in vacuum at 30°. [b] Composition of the outer aqueous phase: polyvinylpyrrolidone 0.2 g., sodium hypophosphite 2.1 g.; water 300 ml. [c] In all cases, negligible (perhaps just on the surface of the beads).

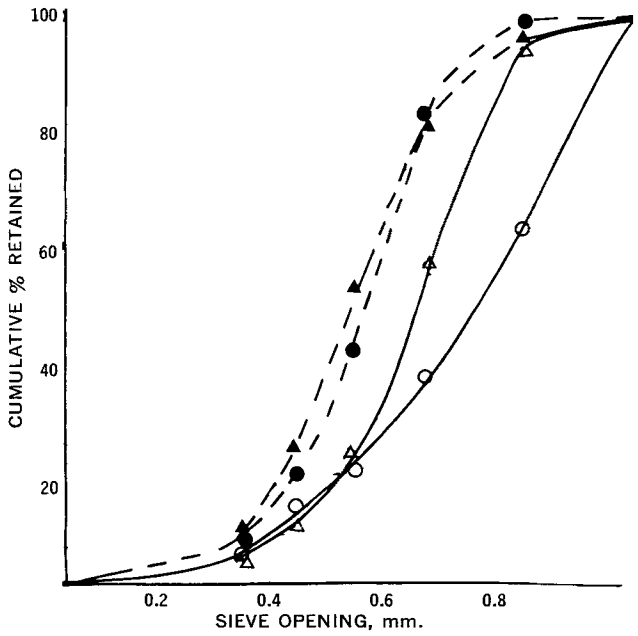

Figure 2—*Percent cumulative undersize of bead formulations 1, 1.1, 2, and 2.1. Key: ○, 1; ●, 1.1; △, 2; and ▲, 2.1.*

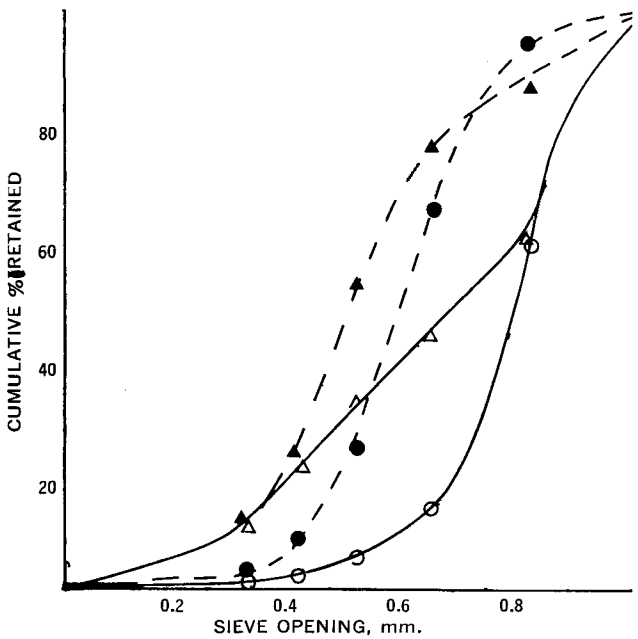

Figure 3—*Percent cumulative undersize of bead formulations 6, 6.1, 8, and 8.1. Key: ○, 6; ●, 6.1; △, 8; and ▲, 8.1.*

Table III—Beads of Composed Copolymers Containing N_1-6-Methoxy-2-(methoxymeth yl)-4-pyrimidinyl Sulfanilamide

Materials[a]	Formula 10	10.1	11.1	12.1	13.0	13.1	14	14.1	15	15.1	16	16.1	17	17.1
Inner phase														
Methyl methacrylate, g.	30.0	22.5	21.0	15.0	—	—	—	—	—	—	—	—	—	—
α-Methacrylic acid, g.	40.0	30.0	30.0	30.0	50	40	50	40	50	40	50	40	50	40
Polyvinylacetate-crotonic acid (92:8), g.	30	22.5	22.5	22.5	30	24	25	20	20	16	15	12	10	8
Divinylbenz. (50%)	—	—	1.5	2.5	20	16	25	20	30	24	35	28	40	32
Sulfa-drug	—	27.0	27.0	27.0	—	20	—	20	—	20	—	20	—	20
Outer aqueous phase														
Carboxyvinyl polymer, g.	0.80	0.96	0.96	0.96	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2
Sodium sulfate, anhydrous, g.	40.0	48.0	48.0	48.0	60.0	60	60	60	60	60	60	60	60	60
Sodium hydroxide, g.	0.5	0.8	0.8	0.8	—	—	—	—	—	—	—	—	—	—
Water, ml.	200	240	240	240	300	300	300	300	300	300	300	300	300	300
Catalyst (α,α′-axo-isobutyronitrile), g.	—	0.1	0.1	0.1	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Speed of stirrer, r.p.m.	300	300	300	300	300	300	300	300	300	300	300	300	300	300
Temp. of polymerizn.	60	60–70	60–70	60–70	70	70	70	70	70	70	70	70	70	70
Time of polymerizn., hr.	2	2	2	2	5–6	5–6	5–6	5–6	5–6	5–6	5–6	5–6	5–6	5–6
Drying temperature	40	40	40	40	40	40	40	40	40	40	40	40	40	40
Yield	—	88.0	81	86	96	92	95	92	95	89	98	92	95	85
Amount of drug in beads, %	—	26.5	25.8	26.0	—	18.6	—	18.8	—	15.8	—	18.8	—	15.9

[a] Washing medium—water in all cases.

disadvantages of the other polymerization methods and has the further advantage that the drug is homogeneously distributed in the uniform tiny spheres.

EXPERIMENTAL

Materials Used—*Monomers*—α-Methacrylic acid,[1] methyl methacrylate,[1] vinyl acetate,[1] crotonic acid[1] (β-methacrylic acid), and divinylbenzene in ethylvinylbenzene as 45–55% solution.[1]

Polymers—Polyvinylacetate with 8% crotonic acid.[2]

Catalyst—α,α′-Axo-diisobutyronitrile[1] and benzoyl peroxide.[1]

Suspension Stabilizers—Carboxyvinyl polymers[3] and polyvinylpyrrolidone.[4]

Drugs—Chloramphenicol USP, N_1-6-methoxy-2-(methoxymethyl)-4-pyrimidinyl sulfanilamide,[5] chlorothiazide, pentobarbital and its sodium salt, papaverine base, hydrocortisone, and 1-ethyl-1-phenylglutarimide.[6]

Method of Preparation—*Apparatus*—A three-necked flask connected to a reflux condenser and an inert gas (nitrogen or carbon dioxide) supply was used. Through the remaining central wide opening, a stirrer was inserted. The flask was maintained at a constant temperature with a thermostatic water bath to control the reaction of the polymerization process (Fig. 1).

Procedure—The continuous outer phase consisting of water, protective colloid, and other additives (described under individual preparations) was heated in the three-necked flask to the desired temperature. The monomer, with or without drug, was added to the continuous phase and stirred at the optimum speed to form the desired size of monomer droplets. The catalyst was then added to effect the polymerization reaction under the inert gas flow. When the droplets were polymerized completely into solid beads, they were removed and washed with excess water or acidic aqueous solution to remove the impurities on the surface. These were then dried at an appropriate temperature with or without a vacuum. The different formulas prepared are given in Tables I–IV.

Determinations—*Yield of Beads*—The total amount of beads obtained after washing and drying was weighed, and this weight was taken as the yield for that preparation (Tables I–IV).

Amount of Drug in Beads—The polymer beads obtained were powdered and passed through a fine sieve. Approximately 0.5 g. accurately weighed powder was suspended in different 1-l. buffer solutions for 2 days. The amount of the drug dissolved in these buffer solutions from these powders was determined spectrophotometrically (Tables I–IV).

Particle-Size Distribution—A known amount of beads was used for sieve analyses to determine the particle-size distribution. The results for a few preparations of Tables I and II are presented as percent cumulative undersize of beads (Figs. 2 and 3).

RESULTS AND DISCUSSION

Choice of Monomers and Polymers for Polymerization—The choice of the monomers and the polymer to be added to them for polymerization in bead form depends on the following considerations:

1. The composition employed should be easily capable of polymerization at a relatively low temperature in the presence of the drug.
2. The polymer obtained should be mechanically hard, chemically stable, and free from monomers.
3. The beads should show specific properties of dissolution and/or diffusion of the embedded drug in gastrointestinal fluids. The presence of acidic groups in the beads increases the solubility in the neutral and alkaline buffer solutions (artificial duodenum and intestinal juices, *i.e.*, sustained dose) (9). The presence of network-

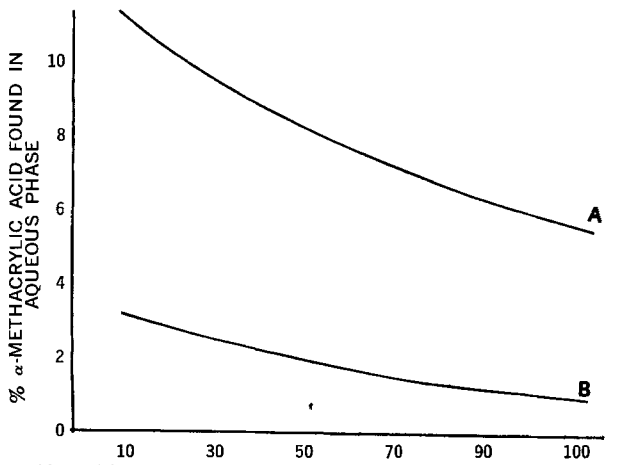

Figure 4—*Solubility of α-methacrylic acid in water and sodium sulfate solution. Key: A, in water; B, in 20% sodium sulfate solution.*

[1] Purchased from Fluka AG, Buchs, Switzerland. Pure monomers are stabilized with 0.01% hydroquinone. These were distilled under vacuum at appropriate temperature before use in presence of copper powder to remove hydroquinone.
[2] Supplied by Ciba AG, Basel, Switzerland.
[3] Carbopol 934 and 941, B. F. Goodrich Chemical Co., Ohio.
[4] Kollidone 25, BASF, Ludwigshafen, West Germany.
[5] Ba 35 092, Ciba AG, Basel, Switzerland.
[6] Doriden, Ciba AG, Basel, Switzerland.

Table IV—Beads[a] of Composed Copolymer in Presence of Different Drugs in Various Concentrations

Materials	Formula 18.1	18.2	18.3	19.1	20.1	20.2	20.3	20.4	21.1	22.1
	Pentobarbital			Sodium Pentobarbital	Papaverine				Hydrocortisone	1-Ethyl-1-phenyl Glutarimide
Inner phase										
Methyl methacrylate, g.	27	24	21	21	27	24	21	18	27	15
α-Methacrylic acid, g.	36	32	28	28	36	32	28	24	36	20
Polyvinylacetate-crotonic acid, g. (92:8)	27	24	21	21	27	24	21	18	27	15
Drug, g.	10	20	30	30	10	20	30	40	10	50
Outer aqueous phase										
Carboxyvinyl polymer, g.	1.0	1.0	1.0	0.8	1.2	1.2	1.6	1.6	0.8	0.8
Sodium sulfate, anhydrous, g.	48.0	48.0	48.0	40.0	60.0	60.0	75.0	75.0	40.0	40.0
Sodium hydroxide, g.	0.5	0.5	0.5	—	0.35	0.4	0.5	0.7	0.35	0.6
Water, ml.	240	240	240	200	300	300	375	375	200	200
Yield, g.	96	89	73	91	79	94	95	66	97	94
Amount of drug in beads, %	9.3	19.2	29	32.7	9.8	19.3	29.1	39.5	10.2	47

[a] General conditions for polymerization: catalyst (benzoyl peroxide), 0.6 g.; speed of stirrer, 250–300 r.p.m.; temperature of polymerization, 60°; time of polymerization, 2–2.5 hr.; drying temperature, 40°; and washing medium, water.

forming monomers in copolymers such as divinylbenzene decreases the swelling and solubility of copolymers in buffer solution.

Choice of Additives in the Outer Phase—The right type and amount of protective colloids in the aqueous outer phase prevent the monomer droplets from deforming and sticking to each other during polymerization. The choice in the outer phase depends mainly on the properties of the monomers used, the protective property at the selected pH, and the presence of electrolytes. The concentration of the protective colloid in the outer phase affects the bead size and may even lead to the formation of emulsions in the high concentrations. Hence, for each preparation, the optimum amount of protective colloid must be determined. The presence of the electrolyte, sodium sulfate, in the aqueous phase is necessary to decrease the solubility of the water-soluble monomer such as α-methacrylic acid in water (salting-out effect). Figure 4 shows the solubility of α-methacrylic acid from the mixtures of methyl methacrylate and α-methycrylic acid in water and 20% w/v sodium sulfate solution. However, the presence of such an electrolyte in water increases the interfacial tension between the monomeric phase and the outer aqueous phase. This latter effect may be strong enough to disturb the bead polymerization system, so it is always desirable to balance these two effects carefully. In principle, many protective colloids can be used, provided the electrolyte causes no salting-out effect on them in the aqueous phase. Carboxyvinyl polymers are stable in the presence of electrolytes between pH 4 and 11 and are employed in this study when electrolytes are present in the aqueous outer phase; otherwise, polyvinylpyrrolidone is the protective colloid used.

When the right type and amount of the protective colloid are used in the outer aqueous phase, the polymers prepared show mostly spherical form. However, the formation of a certain percentage of small agglomerates, twins, and deformed beads cannot be completely avoided. The appearance of the beads, whether transparent or opaque, depends on the solubility of the drugs in the inner monomeric phase. The preparation takes mostly the color of the drug used.

The yield of the beads ranges from 75 to 95% for different preparations. This loss may be due either to the formation of micelles or tiny spherical particles which are lost during filtration and washing of the beads or to the evaporation of a small amount of monomer at the polymerization temperature. Hence the polymerization should be carried out below the boiling temperature of the monomer to avoid the loss due to the latter reason. Further, it may be observed from Tables I–IV that the yield in the presence of drug is always less than when the drug is absent. This loss can be due to the preferential solubility of the drug in, or the affinity of the drug for, the aqueous outer phase rather than those in or for the monomeric phase. This type of loss is further proved by determining the amount of drug present in the beads after polymerization is over (Tables I–IV). Drugs having no affinity for the monomeric phase could not even be embedded in the beads during polymerization as in the examples of chlorothiazide with vinyl acetate and crotonic acid (Table II).

The beads show a large range of particle size distribution varying between 0.3- and 1.0-mm. diameter. In the presence of drugs—either soluble in both the phases as chloramphenicol or insoluble in both the phases as chlorothiazide—the beads obtained are smaller in size than in the absence of drugs (Figs. 2 and 3). These two examples are chosen to investigate the influence of incorporation of soluble and insoluble drugs in the monomeric phase on the size distribution of beads. Chloramphenicol is soluble in the monomeric phase as well as in the outer aqueous phase while chlorothiazide is practically insoluble in both phases. The drug soluble in the outer phase may be affecting the interfacial tension between the two phases while the insoluble drug may be acting as a fine-mesh barrier between droplets and hence decreasing the particle size of the beads (10).

SUMMARY

The method of bead polymerization in aqueous phase has been studied for embedding drugs in polymers. Various polymers and copolymers, either in the presence or in the absence of the drugs, have been studied to prove the widespread applicability of the bead polymerization method. The presence of the drug, either soluble or insoluble, in the outer aqueous phase decreased the bead size and yield of the products. The amount of the drug which could be embedded in the polymer beads depended on the affinity and solubility of the drug for the monomers.

REFERENCES

(1) F. Rosenthal, U. S. pat. 2,895,880 (1959).
(2) A. F. Asker and C. H. Becker, *J. Pharm. Sci.*, **55**, 90(1966).
(3) E. B. Draper and C. H. Becker, *ibid.*, **55**, 376(1966).
(4) S. C. Khanna and P. Speiser, *ibid.*, **58**, 1114(1969).
(5) K. Lehmann, *Pharm. Ind.*, **29**, 396(1967).
(6) W. A. Ritschel, "Die Tablette," Editio Cantor KG, Aulendorg i. Württ, p. 104.
(7) U. S. pat. 3,390,050 (1968).
(8) W. Bauer and H. Lauth, DRP 656 134, 1931.

(9) H. Logemann, "Methoden der Organischen Chemie (Houben-Weyl)," vol. 14/1, Makromolekulare Chemie, Themie/ Stuttgart, p. 414.

(10) E. Trommsdroff and C. Schildknecht, "High Polymers," vol. X, Interscience, New York, N. Y., 1956, pp. 69–109.

ACKNOWLEDGMENTS AND ADDRESSES

Received October 6, 1969, from the *School of Pharmacy, Federal Institute of Technology, Zurich, Switzerland.*

Accepted for publication December 2, 1969.

Abstracted from the dissertations submitted by S. C. Khanna and T. Jecklin to the Swiss Federal Institute of Technology in fulfillment of Doctor of Natural Sciences degree requirements.

* Present address: J. R. Geigy S. A., Galenic Research Section, Basel, Switzerland.

† Present address: Sihlfeld Apotheke, Zurich, Switzerland.

Mechanism of Dimethyl Sulfoxide-Induced Hemolysis

WILLIAM P. NORRED, HOWARD C. ANSEL, IVAN L. ROTH*, and JAMES J. PEIFER†

Abstract □ A study has been made of the possible mechanism by which dimethyl sulfoxide induces hemolysis of rabbit erythrocytes *in vitro*. Erythrocytes were shown by spectral analysis to remove dimethyl sulfoxide from aqueous solution and to resist the agent's release upon washing. Electron microscopy revealed the increased formation of lesions in the erythrocyte membrane with increasing concentrations of dimethyl sulfoxide. Dimethyl sulfoxide was also shown to be increasingly capable of removing fatty acids from the erythrocyte membrane with an increase in concentration. Results of the study indicate that dimethyl sulfoxide is capable of causing the hemolysis of erythrocytes by virtue of its affinity for the erythrocyte membrane and the disruption of its integrity, in part due to its lipid solvency action.

Keyphrases □ Erythrocyte hemolysis—dimethyl sulfoxide induced □ Mechanism—dimethyl sulfoxide-induced hemolysis □ Dimethyl sulfoxide retention—erythrocytes □ Fatty acid removal, erythrocytes—dimethyl sulfoxide □ Electron microscopy—erythrocyte lesion determination □ UV spectrophotometry—analysis

Although dimethyl sulfoxide (DMSO) was first synthesized in 1867 (1), it has only recently become the object of intensive scientific investigation. Used for years by the chemical industry as a solvent, the compound became of special interest to biologists after it was found to have a wide range of solvent action for chemicals employed in various laboratory procedures (2–7). DMSO was subsequently utilized investigationally as the solvent for poorly soluble drugs to be employed parenterally in the clinical treatment of cancer and leprosy (8, 9).

Much of the recent flourish of investigation activity centered about DMSO has been prompted by reports of the agent's apparent great ability to traverse biologic membranes and exert its pharmacologic activity (10–14), as well as to increase the degree and rate of penetration of other drugs across biologic membranes (15–17).

DMSO has also received considerable attention concerning its ability to serve effectively as a cryophylactic agent in the preservation of various body organs, tissues, and cells (7, 17–35), including red blood cells (31–35).

One difficulty concerning the use of DMSO as a cryophylactic agent in the preservation of blood for transfusion has been its nature to permeate the erythrocyte along with the subsequent difficulty experienced in its removal from the blood preparative to transfusion (32, 34, 35). Cellular destruction, resulting in hemolysis, commonly accompanies attempts to rid the cells of DMSO by washing (25, 36).

The hemolytic activity of DMSO, *in vitro* and *in vivo*, has been noted in reports from this (36) and from other laboratories (25, 37, 38). It was the purpose of the present work to investigate the mechanism by which DMSO exerts its hemolytic effects against the erythrocyte.

EXPERIMENTAL

Materials—The DMSO employed in this investigation was reagent grade and was obtained commercially.

Blood Samples—In previously reported studies the hemolytic activity of DMSO against human and rabbit erythrocytes was quite comparable (36, 37). This was verified in preliminary experiments to the present work using blood obtained from the forearm veins of several Caucasian donors, 20–25 years of age, and from rabbits by cardiac puncture. For convenience, rabbit blood was employed throughout this study. Fresh blood was obtained immediately prior to each experiment and defibrinated by gentle swirling with glass beads for approximately 5 min. The defibrinated blood was then transferred by decantation to a clean container and employed in the following procedures.

Absorption of DMSO by Red Blood Cells—To assess the capabilities of DMSO to enter and/or bind with the erythrocyte, red blood cells were incubated with solutions of DMSO, and any alteration in the absorbance of the original DMSO test solution was determined spectrophotometrically.

Erythrocytes were separated from the defibrinated blood sample by centrifugation and decantation. Then the cells were washed five times with normal saline solution and reconstituted to the original blood volume. This cell suspension was then employed to prepare test samples containing volumes of erythrocytes ranging in concentration from 0.06 to 0.48%, with 0.004% DMSO in normal saline. The suspensions were allowed to incubate for 30 min. at 37°, after which time the cells were settled by centrifugation at 13,500×g for 15 min. The supernatant was then examined spectrophotometrically with a Beckman DU for its DMSO content, using a wavelength of 208 mμ. The amounts found were compared to the amounts originally present in the test solutions, and the amounts absorbed determined by difference.

Preparation of Erythrocyte Ghosts for Electron Microscopy—One percent suspensions of defibrinated blood were prepared in 0.9% sodium chloride and varying proportions of DMSO, and incubated at 37° for 45 min. Control suspensions of blood in 0.9% sodium chloride were prepared and concurrently processed with the DMSO-containing suspensions.