Microencapsulation III: Preparation of Invertase Microcapsules

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Received July 15, 1981, from the *Laboratoire de Pharmacie Galénique, E.R.A. au C.N.R.S. n°319, Faculté de Pharmacie, 51, rue Cognacq-Jay, F51096 REIMS Cédex, France.* Accepted for publication October 5, 1981.

Abstract □ Invertase was incorporated into polyamide microcapsules. The following parameters were studied: pH of the aqueous phase during interfacial polymerization; duration of the polymerization; surfactant concentration; stirring rate; improvements in the isolation procedure; effect of lyophilization. The inactivation of the encapsulated enzyme by pepsin was shown to be related to the acidic incubation medium and prompted incorporation of protective proteins in the microcapsules. This process allowed relative protection of the enzyme. In a second set of experiments, an emulsification–reticulation method was developed, which encapsulated invertase in a cross-linked protein. Various proteins and bifunctional acylating agents were tested. Microcapsules of immobilized invertase were prepared through cross-linking of the enzyme protein itself.

Keyphrases □ Microencapsulation—preparation of invertase microcapsules □ Invertase—preparation of microcapsules □ Emulsification–reticulation—preparation of invertase microcapsules

Invertase (β-D-fructofuranoside-fructohydrolase[1]) is lacking in the intestinal mucosa of some patients with constitutional intolerance to sucrose (1–4).

Accumulation of sucrose in the lumen prompts intense diarrhea, amplified further by microbial fermentation. The palliative treatment consists of adding large doses (~1 g) of yeast invertase to the food. As the excessive amount of the supplemented enzyme is probably related to its partial hydrolysis in the digestive tract, it was decided to microencapsulate invertase. Invertase microencapsulation with a 40% retention of enzymatic activity was recently reported (5).

This report deals with the preparation of such microcapsules through two different procedures (6–8). While in the well-known interfacial polymerization procedure (9), the capsule wall is made of an interfacially prepared polyamide, in the emulsion–reticulation procedure, it is made up of an interfacially prepared cross-linked protein. This second technique was progressively devised, and is reported herein.

Although much research has been devoted to the cross-linking of proteins, only a few authors have used this reaction in the preparation of microcapsules: Chang (9, 10), and more recently Miyawaki (11), reported on the chemical interactions between a protein and the acylating agent used in interfacial polymerization. Apparently, only Chang (12) attempted preparation of microcapsules walled in cross-linked proteins. However, he encountered serious difficulties in obtaining stable preparations.

In the cross-linking experiments, the aim was no longer to prepare therapeutically useful invertase microcapsules, but rather to test the feasability and limits of the method, with invertase used as a tracer.

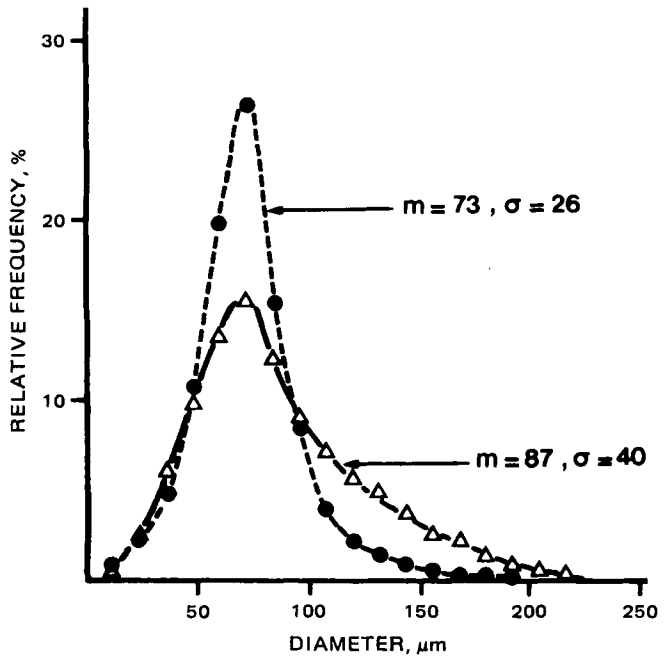

Figure 1—*Effect of the surfactant concentration on the size of microcapsules. Key: (●), sorbitan trioleate, 2%; (△), sorbitan trioleate, 1%.*

[1] E.C. No. 3.2.1.2.6.

EXPERIMENTAL

Reagents—Invertase from baker's yeast[2], exhibiting 34 U of activity/mg was used as a suspension (5%, w/v) in distilled water.

A buffer was adjusted to pH 9.8 by adding hydrochloric acid to a 0.45 *M* solution of Na_2CO_3. It was used to prepare a 6.67 g% (w/v) solution of alcalisoluble casein[3] and a 1.2 *M* solution of 1,6-hexamethylenediamine[4] (13.95%, w/v).

The organic solvent was a chloroform–cyclohexane mixture (1:4, v/v), which was used to prepare solutions of sebacoylchloride[4] (1:250, v/v), terephthaloylchloride[4] (0.04 *M* and 0.12 *M*), succinyl chloride[5] (0.04 *M*), toluene diisocyanate[6] (0.14 *M*), and piperazine[6] (1.2 *M*).

The surfactants were sorbitan trioleate[7] (1% solution v/v in the organic solvent) and polysorbate[7] (50% solution v/v in distilled water).

The dye for the permeability studies was erioglaucine A[8].

The proteolytic enzymes used were pepsin[9], pancreatin[10], and pronase[11].

Materials—Stirring was performed with a motor[12] and a 3-bladed screw (each blade was 16 mm long). An homogenizer[13] was used in one experiment. Lyophilization was performed with a freeze dryer[14].

Evaluation of Enzymatic Activity—A qualitative test was made using reactive strips[15]. Quantitative determinations were performed through the automated method of Goldstein and Lampen (13). The ac-

[2] Grade V, Sigma Chemical Co.
[3] Merck Darmstadt.
[4] Aldrich Chemical Co.
[5] Fluka.
[6] Eastman Kodak.
[7] Seppic Montanoir.
[8] Bleu Patenté 5, Prolabo.
[9] From porcine stomach mucosa, Sigma Chemical Co.
[10] From porcine pancreas, Sigma Chemical Co.
[11] Calbiochem.
[12] Heidolph: stirring motor type RZRII—adaptation system type RK6.
[13] Potter's homogenizer, Braun.
[14] Leybold.
[15] Clinistix, Ames.

0022-3549/82/0700-0753$01.00/0

Table I—Effect of the Polymerization pH on the Enzymatic Activity of Invertase Microcapsules

pH		
Buffer	Aqueous Phase	Enzymatic Activity, U/mg
9.8	10.8	19.5
8.4	9.8	6.8
7.2	8.8	6.5

Table II—Effect of the Polymerization Duration on the Enzymatic Activity of Invertase Microcapsules

Duration of the Polymerization Step, min	Enzymatic Activity, U/mg
2	17.4
3	19.5
5	19.5
10	13
20	11.5

tivity of the microcapsules was calculated relative to 1 mg of invertase used in each experiment.

Microencapsulation Procedures—A standard emulsion–polymerization procedure is as follows: In a 100-ml beaker cooled in an ice bath, the casein solution (1.5 ml), the invertase suspension (1 ml, *i.e.*, 50 mg of invertase), the hexamethylene diamine solution (0.5 ml), and the 1% solution of sorbitan trioleate (15 ml) were mixed. The stirrer was set at 650 rpm, and the sebacoyl chloride solution (15 ml) was added.

After a 3-min agitation, the suspension was diluted with the organic solvent (30 ml) and centrifuged (350×*g*, 30 sec).

The sediment was dispersed in 40 ml of the polysorbate solution and then transferred to a 500-ml beaker. Dispersion of the microcapsules was achieved by stirring with a glass rod (30 sec) and then by mechanical stirring (650 rpm, 2 min). Water (120 ml) was added, the suspension was agitated (300 rpm, 3 min), and then diluted further with 300 ml of water. A prolonged sedimentation then allowed isolation of the microcapsules, which were again suspended in distilled water.

Variations were introduced in the pH of the aqueous phase, in the duration of the polymerization steps, surfactant concentration, stirring rate, method of isolation of microcapsules, nature of the polyamide, and nature of the protective protein.

In the emulsion–reticulation experiments, no diamine was used in the emulsification step, while a protein (100–800 mg) was reacted with a bifunctional reagent. Cross-linking of invertase itself was performed with terephthaloylchloride with no diamine and no protein added.

Membrane Permeability Test—The typical emulsion–polymerization procedure was performed with 3 mg of erioglaucine A (MW 1159) added during the emulsification step. Blue microcapsules were obtained and one could microscopically observe their progressive discoloration through washing with water.

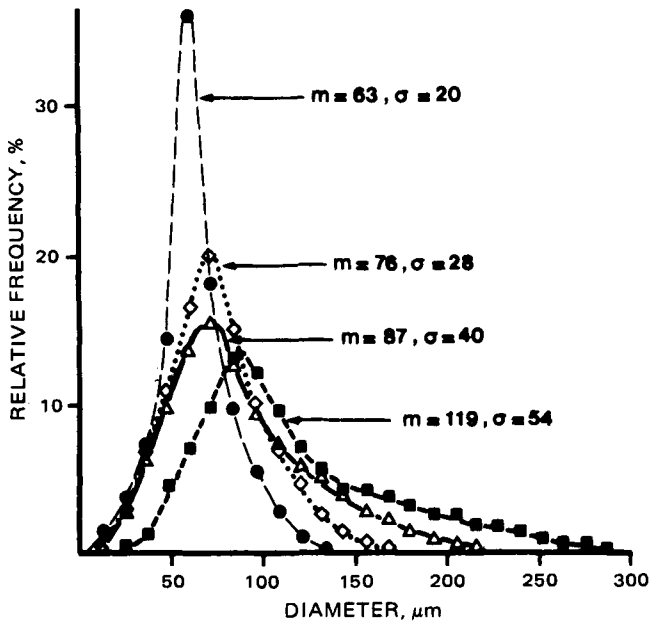

Figure 2—*Effect of the stirring rate on the size of microcapsules. Key: (■), 450 rpm; (△), 650 rpm; (◊), 900 rpm; (●), 1200 rpm.*

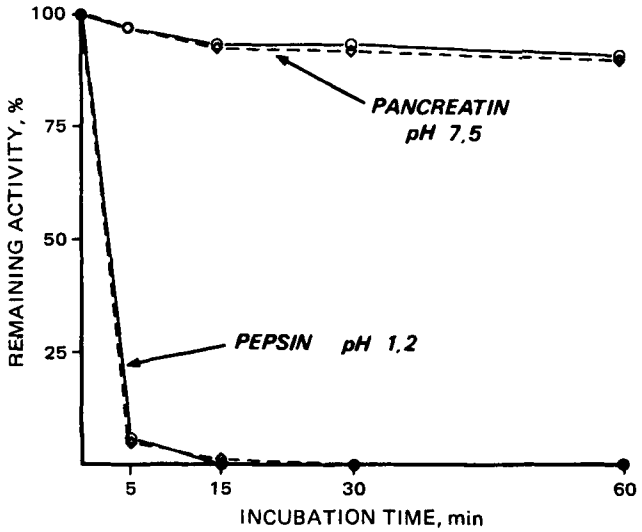

Figure 3—*Effect of proteases on the enzymatic activity of free and encapsulated invertase. Key: (○), free invertase; (◊), encapsulated invertase.*

RESULTS AND DISCUSSION

Interfacial Polymerization—When prepared through the standard procedure, the microcapsules were spherical, with their sizes ranging from 50 to 150 μm. A thin membrane, including insoluble particles of invertase, was to be seen. The permeability test was positive. When washed with water, the capsules became clear; the blue color passed into the surrounding liquid. Their enzymatic activity was 19.5 U/mg used in their preparation, *i.e.*, 57% of the activity (34 U) of free invertase.

Crushing of the microcapsules with an homogenizer[13] resulted only in a slight increase of activity: 22.2 U, *i.e.*, 61% of the activity (36.4 U) of crushed, free invertase. It was then concluded that invertase was lost or inactivated (to an extent of ~40%) during the microencapsulation process.

Lowering the pH of the aqueous phase during polymerization resulted in a loss of activity (Table I). Although the diamine solution increased the pH value, a rather basic medium was necessary for membrane formation.

The optimal duration of the polymerization step was shown (Table II) to be 3–5 min. Shorter time gave unstable capsules; longer time yielded thick membranes and eventually denatured the enzyme. With no surfactant used, no microcapsules were formed.

Increasing the concentration of the surfactant solution from 1 to 2%

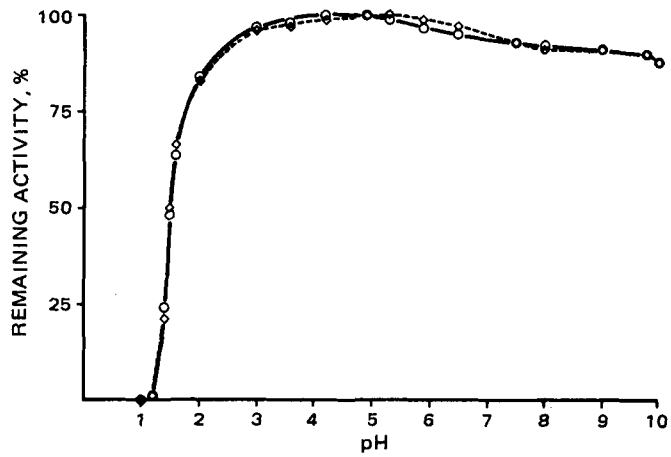

Figure 4—*Remaining activity of invertase (free and microencapsulated) after a 15-min incubation at different pH values. Key: (○), free invertase; (◊), microencapsulated invertase.*

Table III—Activity (U/mg, percent *versus* Free Invertase) of the Microcapsules Prepared from Various Diamines and Bifunctional Agents[a]

Bifunctional Agent	Diamine	Hexamethylene Diamine, 70 mg, 1.2 *M*	Piperazine, 52 mg, 1.2 *M*
Sebacoyl chloride	(67 mg): 0.018 *M*	26 U (76%)	—
Terephthaloyl chloride	(120 mg): 0.04 *M*	11.5 U (34%)	25.3 U (74%)
	(375 mg): 0.12 *M*	21 U (62%)	—
Toluene diisocyanate	(375 mg): 0.14 *M*	27 U (79%)	25.3 U (74%)

[a] Quantities in milligrams of the reagents used for 50 ml of invertase.

Table IV—Enzymatic Activity of Invertase Microcapsules after Incubation in Protease-Containing Media

Polymer	Initial Activity, U/mg (Percent *versus* Free Invertase)	Remaining Activity After Incubation, U/mg (Percent Initial Activity)						
		Pepsin, pH 1.4			Pancreatin, pH 7.5		Pronase, pH 8	
		5 min	15 min	30 min	5 min	30 min	5 min	30 min
None: free invertase	34(100)	15.5(48)	8(25)	2(6)	33.3(98)	31.7(93)	33.4(98)	31.4(92)
Hexamethylene diamine–Sebacoyl chloride	26(76)	12.9(50)	6.8(26)	1.8(7)	24.9(96)	25(96)	26.5(102)	26(100)
Hexamethylene diamine–Terephthaloylchloride, 120 mg	11.5(34)	9.2(80)	6.3(55)	2.6(23)	13.3(121)	18.2(166)	15.1(137)	19.2(175)
Hexamethylene diamine–Terephthaloylchloride, 375 mg	21(62)	15.8(75)	8.2(39)	2.7(13)	23.6(112)	25(119)	24(114)	25.3(120)
Hexamethylene diamine–Toluene diisocyanate	27(79)	12.4(46)	6.2(23)	1.6(6)	26.5(98)	26.5(98)	26.8(99)	27(100)

(Fig. 1) had little effect on the average size (87–73 μm) but resulted in a more homogeneous repartition of the diameters. With a 5% solution, however, the membranes were altered and a large amount of invertase escaped microencapsulation.

When the stirring speed was raised from 450 to 1200 rpm, the size of the capsules decreased regularly, while the distribution of their diameter became more and more homogeneous (Fig. 2). Simultaneously, the enzymatic activity rose to a maximum of 21.3 U/mg (63% retention).

Stirring became more efficient when the 3-bladed screw was replaced by a 5-bladed screw (each blade was 20 mm long, 9 mm wide).

The separation and washing of the microcapsules from the reaction medium is the most time-consuming step in the process. Several attempts were unsuccessful (replacement of polysorbate by other surfactants and washing with organic solvents: ethanol, dioxane, and acetone inactivated the enzyme). Another technique afforded an improvement. The sediment from the first centrifugation was suspended in a mixture of glycerin–polysorbate (3:1, v/v, 5–10 ml) and 50 ml of distilled water was added. After agitation and centrifugation (350×*g*, 5 min) the sediment was washed several times with water.

After lyophilization, observation of the dried microcapsules (optical *versus* electron scattering microscopy) showed the integrity of the membranes; they rehydrated instantaneously with no modification of the shape. When held at +4° for 50 days, the aqueous suspension lost 15% of its initial activity, while the lyophilizate completely retained the enzymatic activity.

Evaluation of the Protection of the Enzyme—The protecting effect of the microcapsule wall was evaluated toward proteolytic enzymes. Figure 3 shows that no noticeable differences were observed regarding the protection of the free *versus* microencapsulated invertase toward pancreatin and pepsin. The same result was obtained with pronase since the activity curve was similar to the curve established with pancreatin.

This unsatisfying result was readily explained when the activity of invertase was tested after a short incubation time at different pH values, with no other enzyme added (Fig. 4).

In agreement with literature data (14), invertase (either free or microencapsulated) was irreversibly denatured by standing in acidic medium <pH 3.

Changes in the nature of the wall were studied according to Table III.

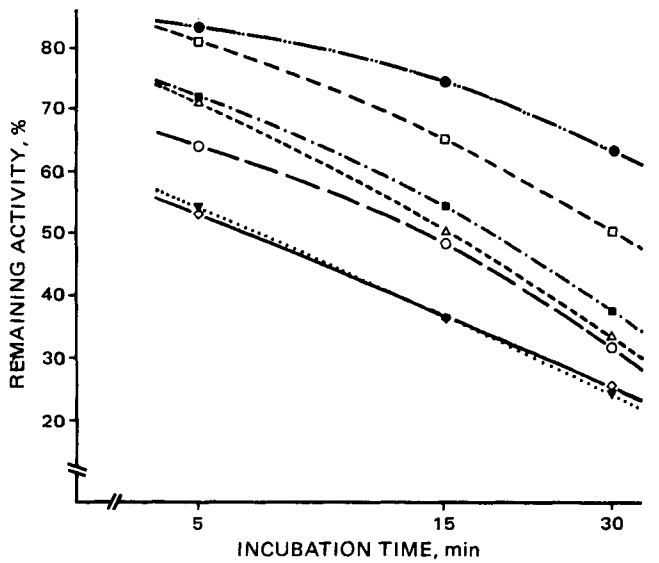

Figure 5—*Remaining activity of different invertase microcapsules after incubation at pH 1.5. Key: (●), hexamethylene diamine, terephthaloylchloride, 120 mg; (□), hexamethylene diamine, terephthaloylchloride, 375 mg; (■), hexamethylene diamine, toluene diisocyanate; (△), hexamethylene diamine, sebacoylchloride; (○), free invertase; (◊), piperazine, terephthaloylchloride; (▼), piperazine, toluene diisocyanate.*

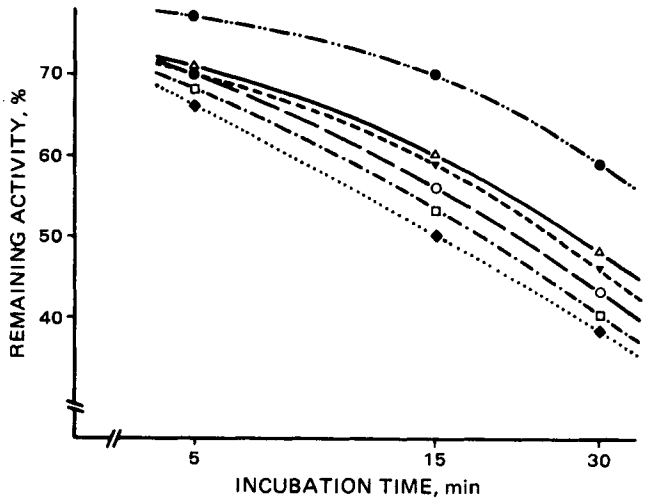

Figure 6—*Remaining activity of invertase microcapsules with nylon 6-10 membrane after incubation at pH 1.55. Protective effect of added proteins. Key: (●), gelatin; (△), casein, 100 mg; (▼), hemoglobin, 100 mg; (○), free invertase; (□), ovalbumin, 100 mg; (♦), albumin, 100 mg.*

Table V—Properties of Invertase Microcapsules with Nylon 6-10 Membranes Containing Different Proteins

Batch Number	Associated Protein (Weight)	Mean Diameter, μm	Invertase Activity, U/mg (% *versus* Free Invertase)	Effect of Incubation at pH 1.55 on the Invertase Activity, U/mg (% Initial Activity) 5 min	15 min	30 min
0	Free Invertase	—	34(100)	23.8(70)	19(56)	14.6(43)
1	Casein (100 mg)	50	26(76)	18.5(71)	15.6(60)	12.5(48)
2	Casein (50 mg)	45	25.7(75)	17.2(67)	13.6(53)	11.1(43)
3	Casein (200 mg)	60	23.2(68)	17.2(74)	14.2(61)	11.1(48)
4	Protamin (100 mg)	—	—[a]	—[a]	—[a]	—[a]
5	Albumin (100 mg)	50	25.3(74)	16.7(66)	12.7(50)	9.6(38)
6	Ovalbumin (100 mg)	50	26.3(77)	17.9(68)	13.9(53)	10.5(40)
7	Gelatin (100 mg)	40	20.4(60)	15.7(77)	14.3(70)	12(59)
8	Hemoglobin (50 mg)	50	21.1(62)	13.7(65)	10.3(49)	7.8(37)
9	Hemoglobin (100 mg)	45	20.8(61)	14.6(70)	12.3(59)	9.6(46)
10	Hemoglobin (200 mg)	50	21.6(64)	15.6(72)	13.2(61)	10.4(48)
11	Hemoglobin (400 mg)	65	19.9(59)	13.7(69)	11.1(56)	9.6(48)

[a] Isolation of microcapsules very difficult.

Table VI—Feasability of Microcapsules with Cross-Linked Protein Membrane

Protein–Acylating Agent		Feasability of Microcapsules
Casein–Sebacoyl chloride		Very unstable microcapsules
Casein–Terephthaloylchloride		Unstable but isolable
Albumin–Sebacoyl chloride		No microcapsules (after 3 min)
Albumin–Terephthaloylchloride	Albumin = 200 mg	Unstable but isolable
	Albumin = 400 mg	Stable microcapsules
Ovalbumin–Sebacoylchloride		No microcapsules (after 3 min)
Ovalbumin–Terephthaloylchloride	Ovalbumin = 200 mg	Unstable but isolable
	Ovalbumin = 400 mg	Stable microcapsules
Gelatin–Terephthaloylchloride		Unstable but isolable
Gelatin–Toluene diisocyanate		Stable microcapsules
Gelatin–Hexamethylene diisocyanate		Unstable but isolable
Gelatin–Succinyldichloride		Immediate coagulation after addition of succinyldichloride
Hemoglobin–Sebacoyl chloride		Stable microcapsules
Hemoglobin–Terephthaloylchloride	Hemoglobin = 400 mg	Very unstable microcapsules
	Hemoglobin = 600 mg	Stable microcapsules
Hemoglobin–Succinyldichloride		Stable microcapsules
Hemoglobin–Hexamethylene diisocyanate		Very unstable microcapsules

Stirring was effected by means of a 5-bladed screw at a speed of 1200 rpm.

The surfactant solution was 2% sorbitan trioleate. Isolation and washing of the microcapsules were performed through the glycerin–polysorbate method.

Formation of the microcapsules was satisfactory in every case except for the polymerization of piperazine with 0.04 *M* terephthaloylchloride. The mean size was 50 μm.

According to the improvements in the process, the activity of the microcapsules made from hexamethylene diamine and sebacoyl chloride reached 26 U/mg.

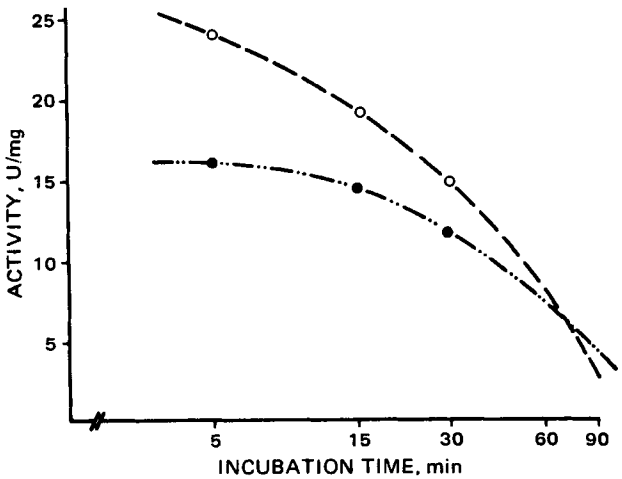

Figure 7—*Invertase microcapsules containing gelatin (nylon 6–10 membrane). Remaining activity after incubation at pH 1.55 as compared with free invertase. Key: (○), microcapsules with gelatin; (●), free invertase.*

These microcapsules were incubated at pH 1.5 for 5–30 min. The results of the activity measurements are plotted in Fig. 5: no radical improvement in the protection of invertase could be noticed. Moreover, the capsules made from hexamethylene diamine and terephthaloylchloride were destroyed by pancreatin, while an increase in the invertase activity was observed (Table IV).

Table VII—Microcapsules with Cross-Linked Protein Membrane: Mean Particle Size

Batch Number	Protein and Acylating Agent Nature and Weight, mg	Mean Diameter, μm
14	Casein, 200–Terephthaloylchloride, 375	50–100
15	Albumin, 400–Terephthaloylchloride, 750	40–80
16	Albumin, 600–Terephthaloylchloride, 750	40–70
17	Ovalbumin, 200–Terephthaloylchloride, 375	50–90
18	Ovalbumin, 400–Terephthaloylchloride, 375	20–100
19	Gelatin, 100–Terephthaloylchloride, 375	40–70
20	Gelatin, 100–Toluene diisocyanate, 750	20–40
21	Hemoglobin, 600–Terephthaloylchloride, 375	70–100
22	Hemoglobin, 800–Terephthaloylchloride, 375	120–150
23	Hemoglobin, 400–Sebacoyl chloride, 670	30–70
24	Hemoglobin, 600–Sebacoyl chloride, 670	40–80
25	Hemoglobin, 400–Succinyldichloride, 100	10–45

Table VIII—Microcapsules with Cross-Linked Protein Membrane: Effect of an Acidic Medium

Batch Number	Protein and Acylating Agent; Nature and Weight, mg	Activity, U/mg (Percent of Activity of Invertase Used)	Remaining Activity after Incubation at pH 1.5, U/mg (Percent of Initial Activity) 5 min	15 min	30 min
	Free Invertase	34(100)	21.6(64)	16.3(48)	10.5(31)
1	Nylon 6-10	26(76)	18.5(71)	13(50)	8.5(33)
14	Casein, 200–Terephthaloylchloride, 375	25(74)	14.5(58)	10(40)	6(24)
15	Albumin, 400–Terephthaloylchloride, 750	30(88)	21.6(72)	16.5(55)	11.4(38)
16	Albumin, 600–Terephthaloylchloride, 750	32(94)	24.3(76)	18.6(58)	12.8(40)
17	Ovalbumin, 200–Terephthaloylchloride, 375	26(76)	16.3(61)	11.5(44)	6.2(24)
18	Ovalbumin, 400–Terephthaloylchloride, 375	29.5(87)	15.1(51)	10(34)	5.3(18)
19	Gelatin, 100–Terephthaloylchloride, 375	10.4(31)	3.6(34)	2.7(26)	1.8(17)
20	Gelatin, 100–Toluene diisocyanate, 750	15(44)	6.6(44)	4.4(29)	3(20)
21	Hemoglobin, 600–Terephthaloylchloride, 375	32(94)	28.8(90)	23.4(73)	15.7(49)
22	Hemoglobin, 800–Terephthaloylchloride, 375	30(88)	27.6(92)	23.7(79)	18.9(63)
23	Hemoglobin, 400–Sebacoyl chloride, 670	32(94)	24(75)	18.6(58)	14.1(44)
24	Hemoglobin, 600–Sebacoyl chloride, 670	33(97)	27.7(84)	20.8(63)	15.5(47)
25	Hemoglobin, 400–Sucinyldichloride, 100	26(76)	21(81)	17.9(69)	15(58)

Table IX—Microcapsules of Cross-Linked Invertase (Terephthaloylchloride) Enzymatic Activity

Invertase, mg	Terephthaloylchloride, mg	Activity U/mg	% Activity of the Invertase Used	Total Activity, U Calculated	Measured	Difference
100	375	7.5	22	3400	750	−2650
200	375	16.3	48	6,800	3250	−3550
400	375	25.2	74	13,600	10,050	−3550

Changes in the Nature of the Protective Protein—To protect the microencapsulated invertase from the effect of lower pH values, changes were introduced in the nature and concentration of the accompanying protein (*i.e.*, alcalisoluble casein in the standard procedure). The following proteins were checked: protamine[16], human serum albumin, egg albumin, gelatin, human hemoglobin. These proteins were incorporated as 4% (w/v) solutions in the buffer, pH 9.8. The membrane was made from hexamethylene diamine and sebacoyl chloride (nylon 6-10) as before.

Protamine failed to give well-constituted microcapsules, as a reaction between protamine and the diacylchloride occurred. This observation prompted the development of the reticulation method. The results of successive assays are summarized in Table V.

The initial activity of all microcapsules prepared was in the 20–26 U/mg range. The slight decrease of activity observed with the higher concentrations in proteins may be due to a slowing down of the diffusion rate inside the capsules.

Figure 6 plots for six assays the effect of incubation at pH 1.55 on the enzymatic activity as a percentage of the initial activity of each batch. The good performance of gelatin is better illustrated in Fig. 7, where the invertase activity is now plotted as a percentage of the activity of the invertase used in the preparation. The slopes of the curves indicate that after ~1 hr, the microcapsules retain more enzymatic activity than does an equal amount of free invertase. However, only 6 U/mg (18% of activity) is still available.

Emulsification–Reticulation Procedure—While protamine actually afforded well-defined capsules, this protein rapidly was shown not to be an ideal material. With sebacoyl chloride (whatever the protamine concentration), the reticulation period necessary for gaining solid capsules had to be raised to 30 min. However, the membranes were very thin and vanished in water after 24 hr at +4°. With terephthaloylchloride, the results were somewhat better but the initial activity was low: with 200 mg of protamine and 750 mg of terephthaloylchloride (batch 12), it was 8.5 U (25%); with 400 mg of protamine (batch 13) it was 11 U (32%).

Using other cross-linked proteins gave better results. Table VI reports

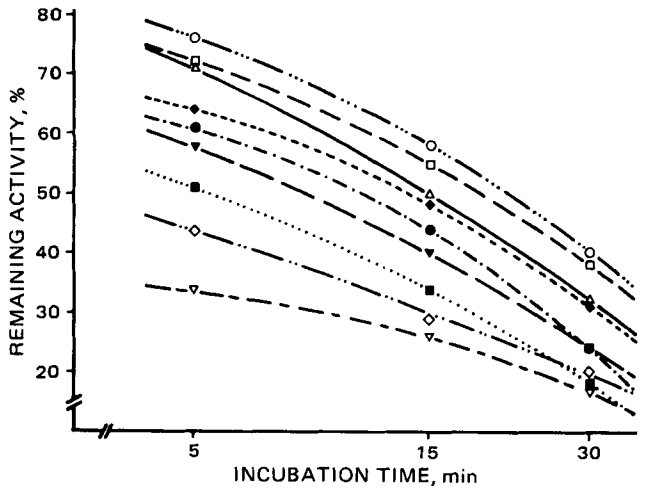

Figure 8—*Invertase microcapsules with cross-linked protein membrane: remaining activity after incubation at pH 1.5. Key: (○), batch 16 (Albumin, 600 mg–Terephthaloylchloride, 750 mg); (□), batch 15 (Albumin, 400 mg–Terephthaloylchloride, 750 mg); (△), batch 1 (Nylon 6-10); (♦), free invertase; (●), batch 17 (ovalbumin, 200 mg–terephthaloylchloride, 375 mg); (▼), batch 14 (casein, 200 mg–terephthaloylchloride, 375 mg); (■), batch 18 (ovalbumin, 400 mg–terephthaloylchloride, 375 mg); (◇), batch 20 (gelatin, 100 mg–toluene diisocyanate, 750 mg); (▽), batch 19 (gelatin, 100 mg–terephthaloylchloride, 375 mg).*

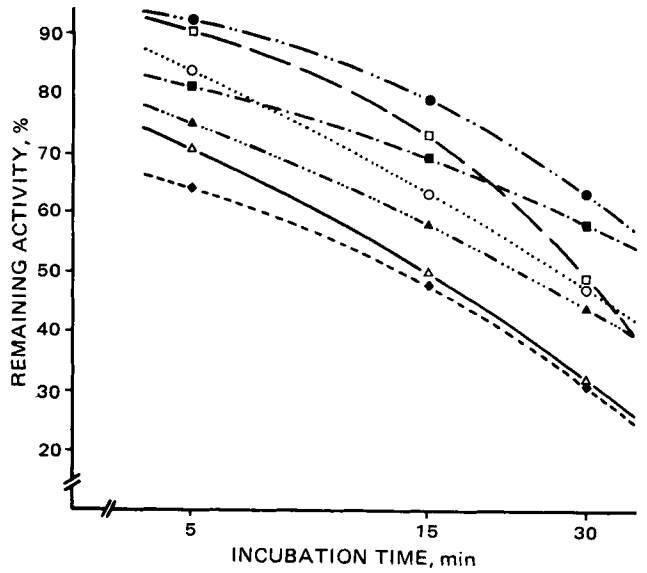

Figure 9—*Invertase microcapsules with cross-linked hemoglobin membrane: remaining activity after incubation at pH 1.5. Key: (●), batch 22 (hemoglobin, 800 mg–terephthaloylchloride, 375 mg); (□), batch 21 (hemoglobin, 600 mg–terephthaloylchloride, 375 mg); (○), batch 24 (hemoglobin, 600 mg–sebacoylchloride, 670 mg); (■), batch 25 (hemoglobin, 400 mg–succinyldichloride, 100 mg); (▲), batch 23 (hemoglobin, 400 mg–sebacoylchloride, 670 mg); (△), batch 1 (Nylon 6-10); (♦), free invertase.*

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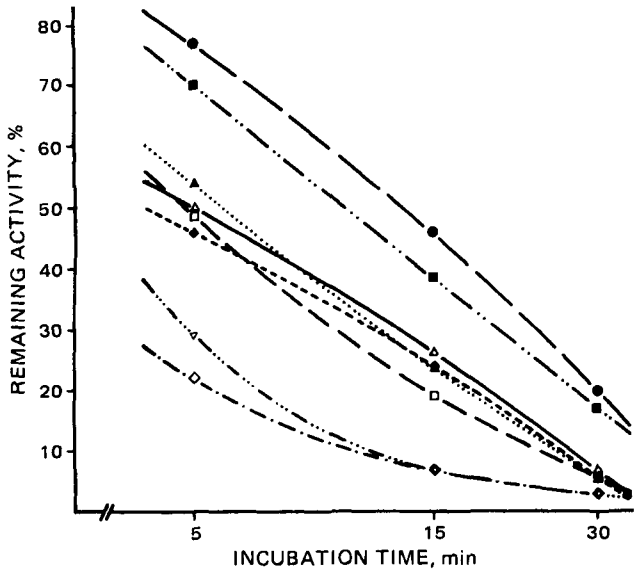

Figure 10—*Invertase microcapsules with acylated protein membrane: remaining activity after incubation with pepsin at pH 1.4. Key: (●), batch 25 (hemoglobin, 400 mg–succinyldichloride, 100 mg); (■), batch 22 (hemoglobin, 800 mg–terephthaloylchloride, 375 mg); (▲), batch 16 (albumin, 600 mg—terephthaloylchloride, 750 mg); (□), batch 17 (ovalbumin, 200 mg—terephthaloylchloride, 375 mg); (△), batch 1 (nylon 6-10); (♦), free invertase; (▽), batch 14 (casein, 200 mg–terephthaloylchloride, 375 mg); (◇), batch 20 (gelatin, 100 mg–toluene diisocyanate, 750 mg).*

on the feasability of various microcapsules while Tables VII and VIII summarize the results on batches 14–25.

The high retention of activity obtained with hemoglobin (94–97%), serum albumin (88–94%), and egg albumin (87%) is of interest.

Batches 15 and 16 (Fig. 8), and especially the microcapsules prepared from hemoglobin (batches 21–25, Fig. 9), were more resistant than the free enzyme toward inactivation caused by standing at pH 1.5.

The membrane was digested by the proteases. The microcapsules were destroyed within 5 (hemoglobin)–15 min (ovalbumin) in pepsin, pancreatin, or pronase. However, in some cases, and especially with hemoglobin (Fig. 10), a significant retention of activity was observed, even after the membrane was no longer visible.

Immobilized Invertase—An attempt was made to acylate invertase with no protein added. The reticulation agent was terephthaloylchloride. The amount of invertase admixed to 375 mg of terephthaloylchloride (2.5% solution (w/v) in the organic solvent) varied from 50 to 400 mg.

Though somewhat unstable, spherical microcapsules were obtained. Washing and transfer to water were only possible with the 100–400-mg capsules. The membranes did not survive a 3–4 day stay in water at +4°.

When evaluated immediately after the preparation, the enzymatic activity was as reported in Table IX. The loss of activity due to acylation tends to be a constant value. Terephthaloylchloride (375 mg) stoichiometrically combines with invertase so as to inhibit 3550 U of activity.

While invertase encapsulated in nylon membranes was irreversibly denatured by incubation in acidic medium, incorporation of various proteins in the capsules allowed relative protection. However, an interaction between the proteins and the bifunctional acylating agent was suspected and prompted the preparation of invertase encapsulated in cross-linked proteins. Invertase itself could be used both as the contents and as the cross-linked membrane. Application of this microencapsulation process to various proteins and polyhydroxylated substrates is under current investigation.

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