Membrane Diffusion I: Design and Testing of a New Multifeatured Diffusion Cell

GORDON L. FLYNN and EDWIN W. SMITH

Abstract □ A new cell with a high diffusional area to diffusional volume ratio, precisely and positively controlled stirring planar with the plane of diffusion, variable speeds, facile assembly, provision for coupling compartments to flow cells or to an external reservoir, and maintenance of temperature by total immersion was designed, built, and checked for its functionality. Details of the shakedown experiments and design are presented.

Keyphrases □ Diffusion system—development of a vertical membrane equicompartment diffusion cell with high area to volume ratio □ Membrane transport, *in vitro*—development of a diffusion cell with high area to volume ratio

A perusal of the literature generates the observation that a formidable task facing the investigator concerned with *in vitro* membrane transport is the construction of a suitable diffusional system. Variables such as membrane integrity, area and thickness as well as temperature, compartmental concentrations, sampling, and stirring, must be provided for and/or controlled precisely. These must be accomplished without sacrifice of analytical sensitivity. Many cell prototypes can be found in the literature (1–12) and, considering that most were constructed for similar purposes, their diversity is remarkable. However, a rough classification based on gross similarity or dissimilarity of the donor and receptor compartment size and geometry is possible. Because of idiosyncrasies associated with membrane positioning, subclassification of the equicompartment systems according to membrane placement, *i.e.*, horizontal or vertical, is also convenient.

A relatively simple cell system to construct is a horizontal equicompartment unit. In such systems, the membrane is sandwiched between two cells stacked upon one another. The top compartment may be opened or sealed. Typical examples were described by Poulsen (1), Aguiar and Weiner (2), Scala *et al.* (3), Scheuplein (4), Tregear (5), and Haleblian *et al.* (6). The major problem with these systems is adequate and uniform control of stirring. Gravity allows for stirring of the lower compartment with a magnetic stirring device. Stirring of the upper compartment is solved either by neglecting agitation completely or by introducing a mechanical stirrer on a shaft. In any case, stirring in the respective compartments is dissimilar in kind and rate. Special concerns with these systems also include air bubble formation on the membrane undersurface, particularly at elevated temperatures, and the presence of a hydrostatic head. The latter can potentially distend the membrane. Other nonspecific problems common to all the diffusion cell types mentioned are awkward assembly and difficulty in obtaining a uniform seal on the membrane. Bolts are usually used to clamp the respective compartments together and these must be uniformly tightened.

A second type of system may be constructed by clamping a membrane over the end of a piece of tubing or bottle and immersing it in a large reservoir (beaker, tank, *etc.*). Examples were given by Garrett and Chemburkar (7), Kostenbauder *et al.* (8), and Most (9). Temperature is a bit easier to control relative to the first type of cell discussed because immersion is facilitated. Adequate stirring is more difficult to obtain. The assembly, air bubbles, and hydrostatic head may or may not be troublesome, depending on the overall design. With this type of system, sensitivity is generally compromised since the design precludes maximization of the diffusional area to compartmental volume ratio. In the case of Garrett and Chemburkar (7), this situation effectively masked the nonsteady-state portion of their diffusional curves and prevented assessment of lag times (10).

The third type of diffusional cell, including the present cell, is the vertical membrane equicompartment system. A complexity arising from turning the first type of cell on its side is that stirring in the plane of the membrane is most difficult to accomplish. *A priori*, it seems as if this would be required to minimize diffusional layer thicknesses and provide for diffusional layer uniformity. Thus, Shenouda and Mattocks (11) chose to use mechanical stirring perpendicular to the plane of diffusion. Stirring in the plane of diffusion has, on oc-

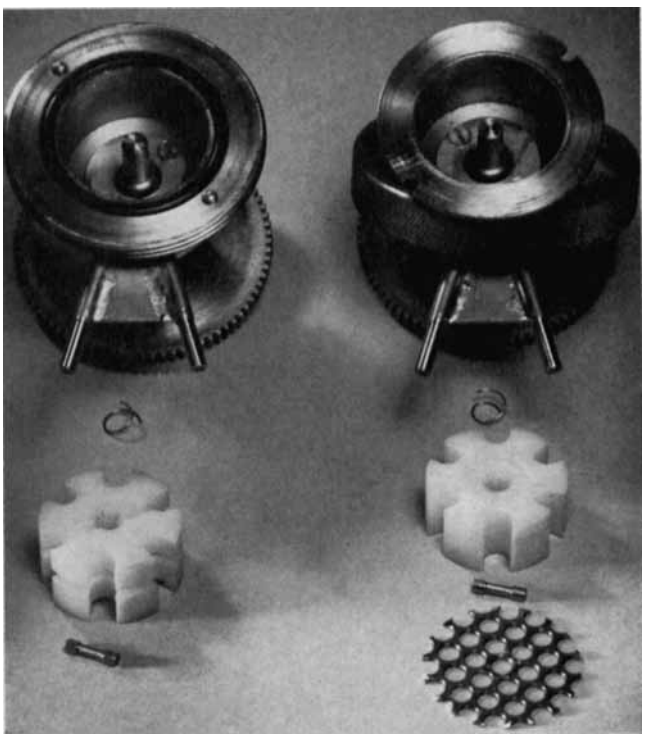

Figure 1—*Completely disassembled cell. (See text for description.)*

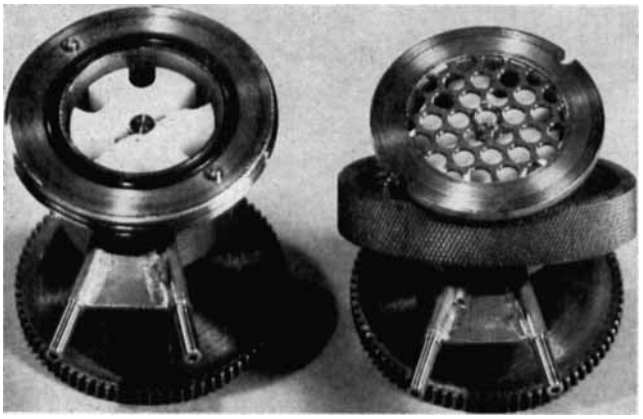

Figure 2—*Assembled cell halves.*

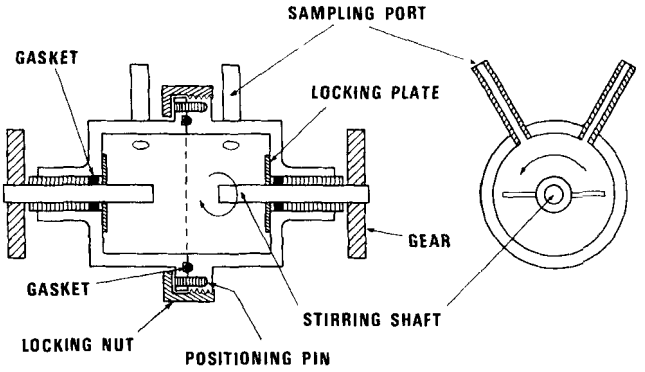

Figure 4—*Engineering drawing of diffusion cell.*

casion, been resolved. Misra *et al.* (12) and Olson *et al.* (13) published two ingenious designs in which magnetic stirrers are mounted on shafts protruding from the back walls of each cell half. Generally speaking, the advantages of this type of design are that cell immersion is feasible, air pocket formation is minimized, and the hydrostatic head is eliminated.

For reasons explicitly discussed in the paper by Olson *et al.* (13), the cell feature about which a diffusional experimenter should be most concerned is cell sensitivity. For any cell, sensitivity will be proportional to the diffusional area divided by the compartmental volume in which the appearance or disappearance of permeant is being followed. It has been shown (9, 10, 13) that nonsteady-state diffusion is fleeting for some practical systems and, without maximum sensitivity, may be indecipherable within the total diffusional curve. For these reasons, an attempt was made to construct a diffusional cell with a high area to volume ratio. At the same time, consideration was given to ease of assembly, temperature control by immersion, stirring planar with the diffusional plane, and other aforementioned factors. Since the care with which a cell is built and evaluated has much to do with the credibility of experimental conclusions, a rather complete analysis of cell performance is presented in this first membrane transport paper.

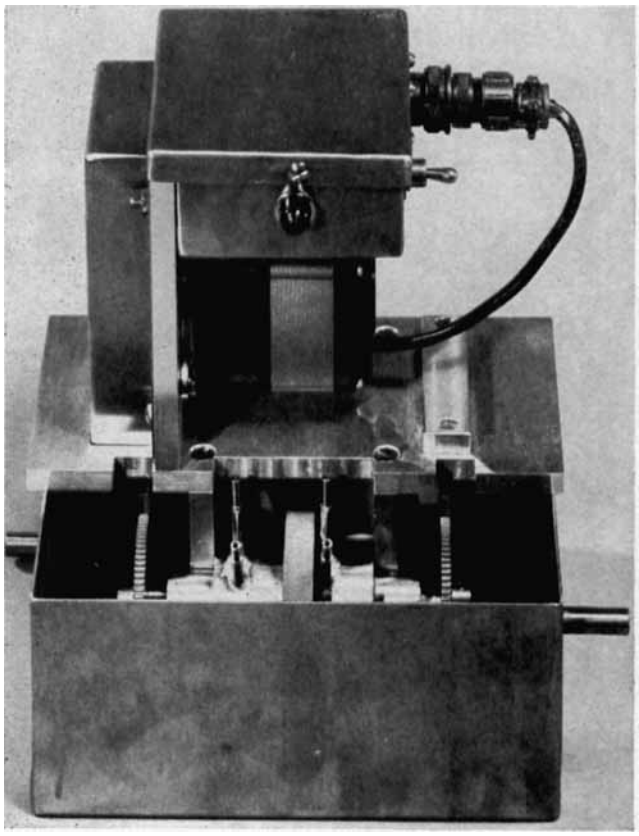

Figure 3—*Completely assembled cell.*

CELL DESCRIPTION

The features of the diffusion cell are best understood by examining Figs. 1–4. In Fig. 1, the cell is completely disassembled. The parts from the top of the picture to the bottom are the cell compartments, the stirring shaft O-ring seals, the springs for spring loading the stirrers, the Teflon stirrers, the stirrer locking pins, and the membrane supporting screen. The stirrer shafts inside the compartments are exposed. The O-ring seals fit tightly into a precisely machined die at the base of the stirring shaft, providing fluid-tight interfaces with the movable stirring shaft and the fixed compartment wall. These O-ring gaskets are securely positioned with a covering stainless steel template.

In Fig. 2, the respective cell halves are assembled. This picture shows the large brass nut (tilted with respect to the right compartment) and the threads at the rim of the left compartment. This provision for joining the cells assures that the fastened pressure will be uniform around the membrane circumference. The black ring in the flange of the left-hand compartment is a seated O-ring for sealing the membrane. Just outside this ring are two pins which are placed in the notches of the right-hand compartment during assembly to obtain reproducible positioning of the cell halves. At the base of each half cell is a gear attached externally to the stirring shaft. When the cells are fully assembled, these gears are connected through other gears to the stirring motor. The membrane support screen, which was alternately used or omitted, is in place in the right-hand compartment.

In Fig. 3, the completely assembled cell sits in the cell rack which also houses the motor. This whole assembly is in a small water bath. Constant temperature is maintained with an auxiliary Tamson force/suction circulating water bath. The cell is equipped with three interchangeable synchronous motors providing speeds of 30, 60, and 120 r.p.m.

Figure 4 provides details on relative dimensions and gives a contrasting perspective on cell design. The diffusional area is 10 $cm.^2$; with Teflon stirrers in place, the individual compartmental volumes are approximately 15 ml.

EXPERIMENTAL PROCEDURE

Sample Preparation—An excess of *p*-aminoacetophenone was placed in a 2-l. flask, and deionized water was added. This solution was stirred overnight and filtered. The concentration was determined to be 4.616 mg./ml. by UV spectrophotometry.

Table I—Compilation of Diffusional Steady-State Rates and Lag Times as a Function of Variables

Run Number	Membrane Thickness, μ	Stirring Speed, r.p.m.	Steady-State Rate, A/min.	Uncorrected Lag Time, min.	Screen[a]	General Comment
1	126.0	30	0.1177	N.R.[b]	−	—
2	126.0	30	0.1242	N.R.	−	Reversed direction through flow cell
3	126.0	30	0.1137	N.R.	−	Repeat of 2
4	126.0	30	0.1149	N.R.	−	Repeat of 2
5	126.0	30	0.1199	N.R.	−	Repeat of 2
6	126.0	30	0.1183	N.R.	−	Repeat of 2
7	126.0	30	0.1130	N.R.	−	Repeat of 2
8	126.0	30	0.1199	N.R.	−	Flow rate changed to 30 ml./min.[c]
9	126.0	30	0.1013	N.R.	+	Repeat of 8—with screen
10	126.0	30	0.1157	N.R.	+	Repeat of 8—with screen
11	126.0	30	0.1212	N.R.	+	Donor solution preheated to 37° -w/screen
12	126.0	30	0.1355	N.R.	+	Repeat of 11
13	126.0	30	0.1214	N.R.	+	Repeat of 11
14	126.0	60	0.1130	N.R.	−	Changed stirrer to 60 r.p.m.
15	126.0	60	0.1290	N.R.	−	Repeat of 14
16	126.0	60	0.1400	N.R.	−	Repeat of 14
17	481.6	60	0.0249	19.5	−	Changed to 0.051-cm. (0.019-in.) membrane
18	481.6	60	0.0238	19.3	−	Repeat of 17
19	481.6	60	0.0253	19.3	−	Repeat of 17
20	481.6	60	0.0244	N.R.	−	Repeat of 17
21	481.6	60	0.0244	N.R.	−	Repeat of 17
22	281.7	60	0.0440	9.75	−	Changed to 0.025-cm. (0.010-in.) membrane
23	126.0	60	0.1605	N.R.	−	Changed to used 0.013-cm. (0.00495-in.) membrane
24	126.0	60	0.1282	2.75	−	Changed to fresh 0.013-cm. (0.00495-in.) membrane
25	281.7	60	0.0426	10.00	−	Changed to 0.025-cm. (0.011-in.) membrane
26	281.7	60	0.0421	10.75	−	Repeat of 25
27	281.7	60	0.0483	9.25	−	Repeat of 25
28	281.7	60	0.0430	9.50	−	Repeat of 25
29	281.7	60	0.0449	9.25	−	Repeat of 25
30	281.7	60	0.0430	9.75	−	Repeat of 25
31	126.0	60	0.1515	N.R.	−	Changed to used 0.013-cm. (0.00495-in.) membrane
32	126.0	60	0.1554	2.00	−	Repeat of 31
33	126.0	60	0.1193	2.25	−	Changed to fresh 0.013-cm. (0.00495-in.) membrane
34	126.0	60	0.1253	2.10	−	Repeat of 33
35	126.0	60	0.1186	2.40	−	Repeat of 33
36	126.0	60	0.1173	2.30	−	Repeat of 33
37	481.6	60	0.0245	18.5	+	0.051-cm. (0.019-in.) membrane with screen
38	481.6	60	0.0246	15.8	+	Repeat of 37
39	481.6	60	0.0246	19.25	+	Repeat of 37
40	126.0	60	0.1211	1.73	+	0.025-cm. (0.011-in.) membrane with screen
41	126.0	60	0.1211	2.00	+	Repeat of 40
42	281.7	60	0.0440	8.75	+	0.013-cm. (0.00495-in.) membrane with screen
43	281.7	60	0.0440	9.50	+	Repeat of 42

[a] −, no screen; +, screen in place. [b] N.R. = not recorded. [c] Normal flow rate was 2.5 ml./min.

Preparation of Membranes—The membranes were cut from sheets of dimethylpolysiloxane, reportedly 0.013, 0.025, and 0.051 cm. (0.005, 0.010, and 0.020 in.) thick, respectively, with a die specially made for the diffusional cell. They were thoroughly rinsed and then allowed to equilibrate with water for 48 hr. or longer. No absorbance was observed from membranes treated in this manner.

Diffusion Studies—In each run the diffusion cell was assembled with a dimethylpolysiloxane membrane of the indicated thickness sandwiched between the compartments. The cell was then placed in the bath maintained at exactly 37°.

The receptor phase was connected by chemically resistant black tubing to a Technicon proportionating pump and through the pump to a 1-cm. flow cell mounted in a spectrophotometer[1]. Deionized water was drawn from a reservoir into and through the flow cell and to the empty receptor compartment by the action of the pump. Eventually the compartment filled, and the water was drawn through the remaining lines to and through the pump. When the cell and lines were thoroughly flushed, the loose ends were connected under the surface of the deionized water reservoir to prevent air from getting into the system as it was closed. After a moment to allow for temperature equilibration, the donor compartment was filled by pumping approximately 25 ml. of solution into it with a 30-ml. air-free syringe. The overflow was piped to a beaker and discarded. Then, by joining the two donor ports with a short piece of tubing, the donor compartment was sealed. All runs were at 37 ± 0.01°.

[1] Beckman DB.

EXPERIMENTAL RESULTS

Table I lists the information gathered from 43 runs. The variables manipulated from run to run are either cited in the comments column or tabulated. These variables include the stirring rate, membrane thickness, presence or absence of the membrane supporting screen, flow rate, direction of flow, and preheating of the donor phase. Also evaluated was the reuse of membranes. The steady-

Table II—Thickness of Dimethylpolysiloxane Nonreinforced Membranes

Measurement Number	Thickness, in. H-0574[a]	H-0640[a]	H-0596[a]
1	0.0049	0.0110	0.0187
2	0.0049	0.0110	0.0187
3	0.0047	0.0112	0.0188
4	0.0053	0.0110	0.0189
5	0.0043	0.0111	0.0192
6	0.0053	0.0113	0.0189
7	0.0055	0.0110	0.0192
8	0.0048	0.0109	0.0191
Average values			
Inches	0.00496	0.01109	0.01896
Centimeters	0.0126	0.0282	0.0482
Microns	126.0	282.0	482.0

[a] Dow Chemical Co.

state rate is reported as it was taken off the chart paper in units of absorbance per minute. Conversion to more conventional units will be discussed in subsequent papers (10, 16).

The reported thicknesses of the purchased dimethylpolysiloxane membranes[2] were, in ascending order, 0.013, 0.025, and 0.051 cm. (0.005, 0.010, and 0.020 in.). These were checked using a micrometer by the method of Garrett and Chemburkar (7). These data are reported in Table II. A check on these values was made by simultaneously measuring eight membrane thicknesses. The average thicknesses by this method were 0.012, 0.027, and 0.048 cm. (0.00479, 0.01091, and 0.01890 in.).

DISCUSSION

In general, the evaluation of the cell went smoothly. Reliable estimates of both the steady-state velocity and the lag time were possible. The lag time gives an assessment of the apparent diffusivity, D', from the equation of Daynes (14) and Barrer (15):

$$t_{lag} = \frac{h^2}{6D'} \qquad \text{(Eq. 1)}$$

where t_{lag} represents the lag time, and h is the membrane thickness. Most (9) showed that the diffusivity so obtained is not equivalent to the specific diffusion constant through pure polymer for dimethylpolysiloxane membranes due to physical adsorption on silica filler in the membranes. The full significance of this fact will be treated in a subsequent paper (16). It is worthy of note here that the high membrane area-to-receptor compartment volume made estimation of the lag time possible.

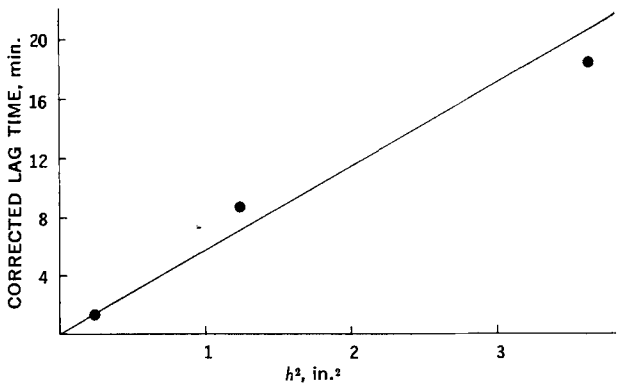

Figure 5—*Plot of average corrected lag time at a given membrane thickness* versus *the square of membrane thickness*, h^2. *Corrected lag times equal observed lag times minus 1.25 min. or the time required to pump diffusion cell contents into the spectrophotometer lightpath. The data are reasonably consistent with expected linear dependency.*

[2] Dow Chemical Co.

Table III—Use of the Membrane Support Screen

Thickness, μ	Steady-State Velocity, A/min. With	Without
126	0.1181	0.1211
282	0.0433	0.0440
482	0.0240	0.0246

These experiments were carried out specifically to determine the experimental sensitivity to stirring rate, preheating of the donor phase by varying the membrane thickness, reusing membranes, and placing the membrane support screen in the system. Reproducibility and the associated statistical parameters were also sought.

Varying Stirring Rate—A comparison of Runs 2–8 with Runs 14–16, 24, and 33–36 indicates that stirring rate is not critical above 30 r.p.m. The average for the former, which were run at 30 r.p.m., is 0.1173, while the latter 60-r.p.m. set is 0.1238. Both are within the 95% confidence limits for runs at 37° with a 126-μ membrane (see *Reproducibility*). Obviously, no benefit would be derived by going to 120 r.p.m.

Preheating Donor Phase—The average value for Runs 11–13, which were preheated, is 0.1260. These values can be compared directly with Runs 9, 10, 40, and 41 (average value of 0.1148). The former is just above the upper 95% confidence limit, 0.1256, while the latter is just below the lower limit, 0.1167. This finding suggests a significant increase in rate when preheating is performed. The actual increase, if real, is at best marginal. This result is not surprising, because the cell is made of a good heat-conducting material, stainless steel, and is itself preequilibrated to 37°.

Varying Membrane Thickness—As expected, the steady-state velocity decreased and the lag time increased as the membrane thickness increased. For a given membrane thickness, both the steady-state rate and lag time were reasonably reproducible. Based upon Eq. 1, a plot of lag time *versus* the membrane thickness squared should yield a straight line, and this was the observed case (Fig. 5). The lag times used in this plot were corrected for the time required for solution in the cell to be pumped into the spectrophotometer flow cell, 1.25 min., called the mechanical lag time. Therefore, despite physical adsorption on filler, lag times apparently show the same dependency on thickness (h^2) as is found in simpler membrane cases.

Reusing Membranes—Runs 23, 31, and 32 were performed with previously used membranes. Significantly higher steady-state rates were observed (average 0.1558 A/min.) relative to runs using fresh membranes (average 0.1211 A/min.). Our observations indicate that this result is not so much due to deterioration of the membrane but to the presence of noneluted *p*-aminoacetophenone in the membrane. This produced a "reservoir effect," making it difficult to assess both slope and intercept for a given run. This complication is specific to the experimental situation as the runs were only followed to an absorbance of 1.0.

Placing Support Screen in Diffusional Pathway—Table III summarizes the results of runs performed with and without the membrane supporting disk. Surprisingly, these rates are not significantly different, suggesting that the stirring was sufficiently forceful to sweep solvent under the support screen. Therefore, the membrane area available for diffusion was not appreciably altered.

Reproducibility—A statistical treatment of data from Runs 3–7, 9, 19–21, 29, 38, 40, and 41 produces a mean value for the 126-μ membrane of 0.1211, with 95% confidence limits of $0.1167 \leq x \leq 0.1254$. The standard sample error is 0.0020, and the percent coefficient of variation is 6.29%. Better precision was expected. However, the results are sufficiently reproducible to make conclusions drawn therefrom valid and more reproducible than those reported by Garrett and Chemburkar (7) using a similar system. Possible causes are variation in individual membrane thicknesses and/or variation in the receptor compartmental volume from run to run (air pockets). These factors will be checked in future experiments.

REFERENCES

(1) B. J. Poulsen, *Brit. J. Dermatol., Suppl. 6*, **82**, 49(1970).

(2) A. J. Aguiar and M. A. Weiner, *J. Pharm. Sci.*, **58**, 210 (1969).

(3) J. Scala, D. E. McOsker, and H. H. Reller, *J. Invest. Dermatol.*, **50**, 371(1968).

(4) R. J. Scheuplein, *ibid.*, **43**, 334(1965).

(5) R. T. Tregear, "Physical Functions of Skin," Academic, New York, N. Y., 1966, p. 2.
(6) J. Haleblian, R. Runkel, N. Mueller, J. Christopherson, and K. Ng, to be published.
(7) E. R. Garrett and P. B. Chemburkar, *J. Pharm. Sci.*, **57**, 944(1968).
(8) H. B. Kostenbauder, H. G. Boxenbaum, and P. L. Deluca, *ibid.*, **58**, 753(1969).
(9) C. F. Most, *J. Appl. Polym. Sci.*, **11**, 1019(1970).
(10) G. L. Flynn and R. W. Smith, *J. Pharm. Sci.*, to be published.
(11) L. S. Shenouda and A. M. Mattocks, *ibid.*, **56**, 464(1967).
(12) A. L. Misra, A. Hunger, and H. Keberle, *J. Pharm. Pharmacol.*, **18**, 531(1966).
(13) C. L. Olson, T. D. Sokoloski, S. N. Pagay, and D. Michaels, *Anal. Chem.*, **41**, 865(1969).
(14) H. A. Daynes, *Proc. Roy. Soc. Ser. A*, **97**, 286(1920).
(15) R. M. Barrer, *Trans. Faraday Soc.*, **35**, 628(1939).
(16) G. L. Flynn and T. J. Roseman, *J. Pharm. Sci.*, to be published.

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Semiautomated, Simultaneous Assay of Thiamine, Riboflavin, Pyridoxine, and Niacinamide in Multivitamin Preparations

RHYS BRYANT, F. J. BURGER, R. L. HENRY, and F. B. TRENK

Abstract □ A semiautomated analytical system was developed for the simultaneous determination of thiamine (B_1), riboflavin (B_2), pyridoxine (B_6), and niacinamide in multivitamin preparations. The automated analytical methods are similar in principle to the USP manual procedures.

Keyphrases □ Multivitamin preparations—semiautomated, simultaneous analysis □ Vitamin analysis—thiamine, riboflavin, pyridoxine, and niacinamide in multivitamin preparations

A semiautomated method was developed for the simultaneous analysis of four vitamins (niacinamide, pyridoxine, thiamine, and riboflavin) in multivitamin formulations; it is similar in principle to the USP (1) manual procedures. The method is a significant extension of the work by Khoury (2, 3) and Albright and Degner (4), who developed methods for simultaneous determination of two vitamins in multivitamin formulations.

EXPERIMENTAL

Equipment—The analytical train consisted of the following Technicon[1] modules: Liquid Sampler II, three proportioning pumps (model I), a dialyzer (model I), two colorimeters (model I) with 15-mm. flow cells, two Fluorometer I units, two double-pen recorders[2], and an autoclave[3].

Reagents—*Niacinamide Buffer (pH 6.8)*—Prepare by dissolving 206.5 g. sodium phosphate dibasic (A.R.) and 57.3 g. citric acid (A.R.) in 2 l. of distilled water. Before use, dilute 200 ml. buffer and 2 ml. polysorbate 80[4] to 1 l.

Pyridoxine Buffer—Prepare a 20% (w/v) sodium acetate (A.R.) solution (Solution A) and a second solution (Solution B) consisting of 470 g. ammonium chloride (A.R.) and 470 ml. ammonium hydroxide (A.R.) (58%) dissolved in water and diluted to 2 l. with water. Mix equal portions of Solutions A and B plus 4 ml. of polysorbate 80/l. before use.

Buffer (pH 4.0)—Prepare by dissolving 219 g. sodium phosphate dibasic (A.R.) and 258 g. citric acid (A.R.) in water and diluting to 2 l. with water. Before use, dilute 200 ml. buffer plus 10 g. potassium chloride (A.R.) to 1 l. with water.

Cyanogen Bromide[5]—Use 7% (w/v) in distilled water.

Sulfanilic Acid—Disperse 50 g. of sulfanilic acid[5] in approximately 800 ml. water. Add ammonium hydroxide (58%) slowly until dissolution of the sulfanilic acid is achieved. Adjust to pH 4.5 with concentrated hydrochloric acid (A.R.), and dilute to 1 l. with water.

N, *2,6-Trichloro-p-benzoquinoneimine Solution*—Dissolve 200 mg. of *N*,2,6-trichloro-*p*-benzoquinoneimine[5] in 500 ml. isopropyl alcohol.

Table I—Relative Standard Deviations of Three Types of Vitamin Preparations[a]

	Thiamine		Riboflavin		Pyridoxine		Niacinamide	
	Automated	Manual	Automated	Manual	Automated	Manual	Automated	Manual
Decavitamin drops	1.2	2.7	1.9	3.5	1.7	1.9	3.2	3.8
Vitamin premix for nutritional products	2.1	0.8	1.1	9.1	2.1	3.2	3.4	5.2
Decavitamin tablet granulations	2.1	1.2	2.0	3.4	0.6	4.9	4.2	5.1

[a] Values are given as percentages.

[1] AutoAnalyzer, Technicon, Tarrytown, NY 10591

[2] Model 67A-(TC)-2PHPH570-00.
[3] American Sterilizer Co., model 57CR.
[4] Atlas Chemical.
[5] Eastman Kodak.