since it is difficult to see increases in the rate of transport of substances for which the GI membrane ordinarily offers little resistance.

Based upon the increased rate of barbital absorption *in vivo*, the increased rate of barbital transport *in vitro*, the increased *in vitro* transport observed for phenolsulfonphthalein through the everted gut of alpha tocopherol-deficient animals, and the enhanced excretion of phenolsulfonphthalein after oral administration in the same animals, it appears that morphological changes in membrane structure previously observed with alpha tocopherol-deficient animals and humans result in increased membrane permeability, at least to the drugs used in this study. The significance of these changes in terms of pharmacological effect is currently under study.

REFERENCES

(1) J. A. Lucy, *Ann. N.Y. Acad. Sci.*, **203**, 4(1972).

(2) A. L. Tappel, *Vitam. Horm.*, **20**, 493(1962).

(3) L. A. Witling, *Progr. Chem. Fats Lipids*, **9**, 519(1970).

(4) M. K. Horwitt, *Fed. Proc.*, **24**, 68(1965).

(5) A. L. Tappel, in "The Fat Soluble Vitamins," H. F. DeLuca and J. W. Suttie, Eds., University of Wisconsin Press, Madison, Wis., 1969, pp. 369–373.

(6) A. L. Tappel, *Ann. N.Y. Acad. Sci.*, **203**, 12(1972).

(7) R. E. Heikkila, J. A. Mezick, and D. G. Cornwell, *Physiol. Chem. Phys.*, **3**, 93(1971).

(8) S. Gross and D. K. Melhorn, *Ann. N.Y. Acad. Sci.*, **203**, 141(1972).

(9) I. Molenaar, F. A. Hommes, W. G. Braams, and H. A. Polman, *Proc. Nat. Acad. Sci. USA*, **61**, 982(1968).

(10) R. H. Imami, S. Reiser, and P. A. Christiansen, *J. Nutr.*, **100**, 101(1970).

(11) M. M. Meshali and C. H. Nightingale, *J. Pharm. Sci.*, **63**, 1084(1974).

(12) M. Mayersohn and M. Gibaldi, *ibid.*, **60**, 225(1971).

(13) P. Trinder, *Biochem. J.*, **57**, 301(1954).

(14) B. B. Brodie, J. J. Burns, L. C. Mark, P. A. Lief, E. Bernstein, and E. M. Papper, *J. Pharmacol. Exp. Ther.*, **108**, 26(1953).

(15) "The Pharmacological Basis of Therapeutics," 4th ed., L. S. Goodman and A. Gilman, Eds., Macmillan, New York, N.Y., 1970, pp. 98–120.

(16) E. W. Maynert and H. B. Van Dyke, *J. Pharmacol. Exp. Ther.*, **98**, 184(1950).

(17) A. Dorfman and L. R. Goldbaum, *ibid.*, **90**, 330(1947).

(18) A. G. Ebert, G. K. W. Yim, and T. S. Miya, *Biochem. Pharmacol.*, **13**, 1267(1964).

(19) J. J. Burns, C. Evans, and N. Trousof, *J. Biol. Chem.*, **227**, 785(1957).

(20) P. Lous, *Acta Pharmacol. Toxicol.*, **10**, 147(1954).

(21) A. Giotti and E. W. Maynert, *J. Pharmacol. Exp. Ther.*, **101**, 296(1951).

(22) A. Goldstein, L. Aronow, and S. M. Kalman, "Principles of Drug Action," Harper and Row, New York, N.Y., 1969, pp. 106–205.

(23) N. J. Giarman, G. N. Bowers, P. G. Quie, and L. J. Hampton, *Arch. Int. Pharmacodyn. Ther.*, **97**, 473(1954).

(24) A. J. P. Martin and T. Moore, *Chem. Ind.*, **57**, 973(1938).

(25) A. J. P. Martin and T. Moore, *J. Hyg.*, **39**, 643(1939).

(26) T. Moore, *Chem. Ind.*, **58**, 651(1939).

(27) V. M. Emmel, *J. Nutr.*, **61**, 51(1957).

(28) V. M. Emmel and P. L. LaCelle, *ibid.*, **75**, 335(1961).

(29) C. S. Tidball, *Amer. J. Physiol.*, **206**, 243(1964).

(30) M. M. Cassidy and C. S. Tidball, *J. Cell Biol.*, **32**, 685(1967).

(31) S. Feldman, M. Salvino, and M. Gibaldi, *J. Pharm. Sci.*, **59**, 705(1970).

(32) R. R. Levine, W. F. McNary, P. J. Kornguth, and R. LeBlanc, *Eur. J. Pharmacol.*, **9**, 211(1970).

(33) M. Gibaldi and B. Grundhofer, *J. Pharm. Sci.*, **61**, 116(1972).

(34) H. Kunze and W. Vogt, *Naunyn-Schmiedebergs Arch. Pharmakol. Exp. Pathol.*, **256**, 139(1967).

(35) H. Kunze, *ibid.*, **259**, 260(1968).

(36) C. S. Tidball, *Amer. J. Physiol.*, **206**, 239(1964).

(37) C. A. M. Hogben, D. J. Tocco, B. B. Brodie, and L. S. Schanker, *J. Pharmacol. Exp. Ther.*, **125**, 275(1959).

(38) L. S. Schanker, P. A. Shore, B. B. Brodie, and C. A. M. Hogben, *ibid.*, **120**, 528(1957).

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Automated *In Vitro* Dissolution Rate Analysis of Potassium in Plastic Matrix Slow Release Tablets

ALF ENGDAHL *, BO KARLBERG ˣ, and SIDSEL THELANDER

Abstract □ A fully automated system for dissolution rate analysis of potassium in slow release tablets is described. Aliquots are removed after 1, 2, and 4 hr from six samples, and potassium is analyzed in a flame photometer at 768 nm. A complete study of six samples takes 5.5 hr. The system may be run overnight. During the time intervals between the removal of aliquots, the system can be used for the determination of the total assay of tablets.

Keyphrases □ Potassium chloride—slow release tablets, dissolution rate analysis by automated system □ Dissolution rate analysis—potassium chloride slow release tablets, automated system □ Automated analysis—dissolution rate of potassium chloride slow release tablets

Potassium chloride tablets are often administered in the form of sustained-release preparations (1, 2). The dissolution rates of 12 different potassium chloride tablets were measured using a potassium selective glass electrode (2). The selectivity for potassium ions over other ions is poor; sodium and hydrogen ions in particular interfere strongly (3). Fortunately, sodium ions are not present; in the *in vitro* methods

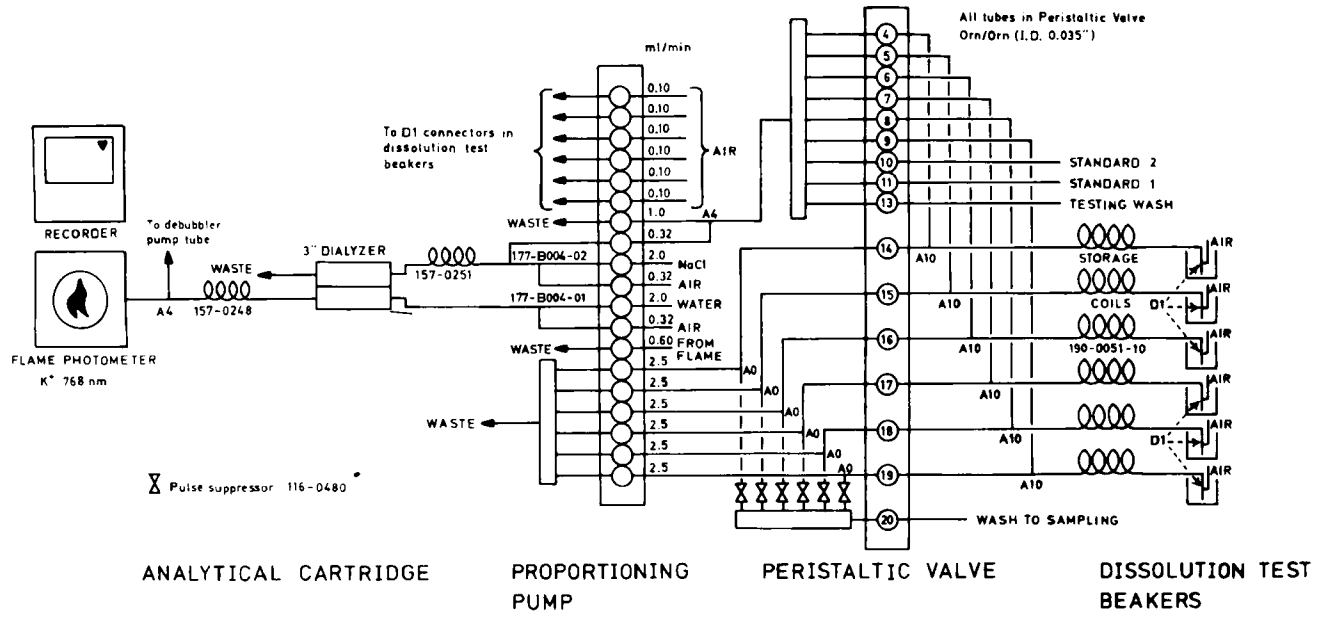

Figure 1—*Hydraulic flow diagram for determination of potassium in slow release tablets.*

applied, pure water was used as the dissolution medium. Sink conditions may be applied for the dissolution of potassium chloride into water at 37° (4).

The absorption of potassium in humans has been studied (1). Tablets of the insoluble plastic matrix type (5–7) were compared with three other sustained-release tablets. The *in vitro* method used was a modification of the beaker method of Levy and Hayes (8). The modified method has been approved for quality control in this laboratory. Manually, this method is time consuming and tedious. Therefore, automation was desirable since potassium chloride tablets frequently occur in the quality control schedule.

Several automated apparatus for dissolution studies have been developed (9–12).

EXPERIMENTAL

Reagents—Sodium chloride, reagent grade (1.0 g/liter in distilled water containing 1.5 ml of a wetting agent[1]), and distilled water containing 1.0 ml of a wetting agent[1]/liter were used.

Apparatus—The automated system for programmed sampling consists of the following: six 600-ml dissolution test beakers with stirrers placed in a thermostatically controlled water bath at 37°

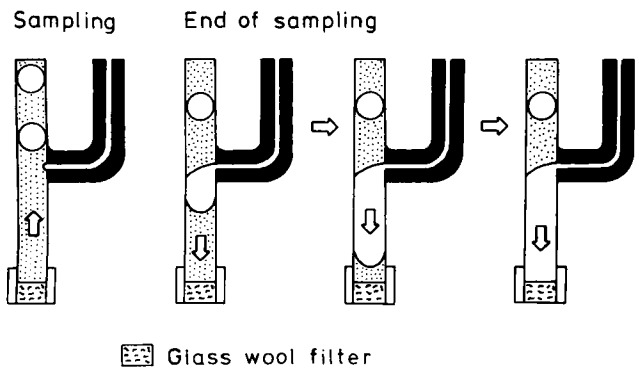

Figure 2—*Function of sampling probe with filter device.*

and a sample acquisition system for dissolution rate analysis consisting of a peristaltic valve[2], a programmer[2], and a proportioning pump III[2] with manifold.

The analytical system connected to the sample acquisition system consisted of an analytical cartridge for determination of potassium including a 7.6-cm (3-in.) dialyzer, a flame photometer IV[2], and a one-channel strip-chart recorder[2].

Standard Preparation—Standards of potassium chloride corresponding to 25, 50, and 75% of tablet specification were prepared. For dissolution rate analysis, six tablets were taken and 500 ml of distilled water was added. The tablet specification was 0.75 g in this case. The 50% standard thus contained 4.5 g of potassium chloride/liter. When 1.0 g of potassium chloride was specified, five tablets were taken for each run. When using the same standards, conversion of the percentages gave 22.5, 45, and 67.5, respectively.

Sample Preparation—Representative tablets from each batch were placed on a wire net of stainless steel 1.5 cm above the bottom of a 600-ml beaker. A total of six tablets was taken if the specification was 0.75 g, and five tablets were used if the specification was 1.0 g. A volume of 500 ml of distilled water at 37° was added to each beaker, and the temperature was kept at this value by keeping the beakers in a thermostatically controlled bath. Stirring was carried out with a steel blade (45 × 15 mm) at a rate of 40 rpm. If three batches or less were to be investigated, duplicate analyses were readily performed.

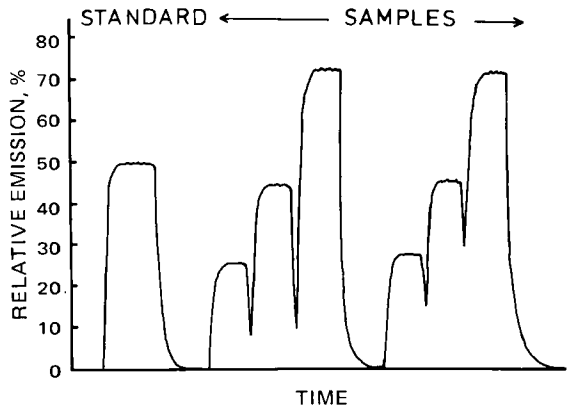

Figure 3—*Typical curves for potassium standard (50% of tablet specification) and dissolution rate analyses of two samples.*

[1] Brij 35, Atlas Chemical Industries.

[2] Technicon Corp., Tarrytown, N.Y.

Table I—Tape Program and Corresponding Activities of the System

Roller	Roller Time, min	Total Time, min	Activity of System
1	58.5	58.5	Wash to testing
2	1.5	60.0	First sampling
3	58.5	118.5	Introduction of Standard 1
2	1.5	120.0	Second sampling
3	118.5	238.5	Introduction of Standard 1
4	1.5	240.0	Third sampling
5	12.0	252.0	Emptying of coil 1
6	12.0	264.0	Emptying of coil 2
7	12.0	276.0	Emptying of coil 3
8	12.0	288.0	Emptying of coil 4
9	12.0	300.0	Emptying of coil 5
10	12.0	312.0	Emptying of coil 6
11	5.0	317.0	Introduction of Standard 2
12	5.0	322.0	Wash to testing

To determine the total assay, 15–20 tablets were pulverized in an electrical mill. An amount corresponding to exactly 1.5 times the average tablet weight was taken, dissolved in water, diluted to a volume of 250 ml, and filtered. The solution was introduced in the flame photometer alternately with the 50% standard solution. A total assay of 100% of tablet specification corresponded exactly to this standard for the 0.75-g tablets. A conversion factor was used for the 1.0-g tablets.

Procedure—A schematic diagram of the automated system is shown in Fig. 1. The system controls both the sampling and testing cycles of the dissolution study according to the tape program (Table I). Forward and reverse indexing of the peristaltic valve is achieved by means of signals from the programmer. One sampling cycle consists of three 3.6-ml aliquots taken simultaneously after 1, 2, and 4 hr from the six dissolution vessels.

The aliquots are filtered through a glass wool filter and immediately air segmented in the dissolution vessel by a connector (Fig. 2). Air also is used to prevent contamination by the solution in the vessel between the sampling periods. At the conclusion of the sampling cycle, each storage coil thus contains aliquots taken at three different times. The total volume of each coil is 14 ml.

The contents of the storage coils are then introduced in turn into the analytical system for quantitation. The sample is diluted with an air-segmented stream of sodium chloride solution and dialyzed into distilled water (Fig. 1). The emission of potassium is measured in the flame photometer; the sodium serves as an internal standard. The system is calibrated by a standard solution of potassium chloride, which is introduced when rollers 3 and 11 are in position, *i.e.*, before and after the test cycle.

When roller 3 is in position between the second and third samplings, 2 hr is available for determining total assays of potassium tablets. This is done by connecting line 11 (Standard 1 in Fig. 1) to a sampler or by direct immersion into the test solutions.

Table II—Correlation between Manual and Automated Sampling Techniques

Lot	Hours	Manual Method, % Specification	Automated Method, % Specification
1	1	27	26
	2	46	46
	4	74	73
2	1	34	33
	2	54	54
	4	80	80
3	1	35	33
	2	53	53
	4	77	77
4	1	29	28
	2	47	47
	4	73	73

Table III—Dissolution Rate Analysis of Six Batches of Potassium Chloride Plastic Matrix Tablets

Batch	Hours	Manual Method, % Specification	Automated Method, % Specification	
			Run 1	Run 2
1	1	29	31	29
	2	47	50	49
	4	73	74	75
2	1	33	32	31
	2	53	53	51
	4	79	79	77
3	1	31	28	31
	2	50	50	51
	4	78	77	77
4	1	29	28	28
	2	48	46	47
	4	74	72	72
5	1	28	28	29
	2	47	45	46
	4	73	69	71
6	1	30	29	32
	2	50	50	52
	4	78	77	78

RESULTS AND DISCUSSION

Typical curves from dissolution rate analyses of two batches of potassium chloride tablets are given in Fig. 3. A standard corresponding to 50% dissolved potassium chloride of the tablet specification was run prior to the analyses to calibrate the readings. This arrangement allows a direct reading on the chart paper of the amount dissolved as a percentage of the tablet specification.

In Table II, analytical results are presented from manual and automated sample removal carried out simultaneously. Samples taken manually were analyzed on an atomic absorption spectrophotometer[3]. The values after 1 hr were slightly higher for the manual method than for the automated one. This difference may be due to a certain time delay in the manual process, since it is almost impossible to synchronize both processes fully. Furthermore, the initial part of the dissolution curve was steep in this range, making the time parameter critical.

Results from two different automated runs on six batches are shown in Table III together with results from one manually performed test. The discrepancies obtained reflect tablet variations rather than variations in the method, since removal of aliquots from *one* beaker into two separate coils gave identical analytical results.

During the development of the automated system, some minor difficulties appeared.

1. Initially, a 0.5-g/liter sodium chloride solution was used as the diluent. This concentration gave too weak a reference signal compared with the sample signal, resulting in a nonlinear calibration curve. This problem was overcome by increasing the concentration of the sodium chloride to 1.0 g/liter.

2. A source of extra peaks was detected. When the coils were emptied for analysis one by one, simultaneous filling of the sample solution occurred if the sample probes were not removed from the beakers. If the entire system was not rinsed after an analysis, potassium-containing solution thus remained in the sample coils and tubings. Therefore, a special "rinsing program" was developed so that the system could be completely washed automatically.

3. The mechanical tenacity of the tape to the programmer decreased considerably after punching. Cracks and other damages caused disturbances and wrong indexing of the peristaltic valve. This malfunction was avoided by punching the tape at the midpoint between two sprocket holes throughout. Furthermore, reverse indexing of the peristaltic valve failed if the punch holes were too small.

The system is capable of carrying out 12 complete dissolution rate studies of the type described in 24 hr. Each run with the automated system requires approximately 5 hr less work than with the manually performed tests, corresponding to a timesaving of about 60%.

[3] Perkin-Elmer 403.

REFERENCES

(1) C. Graffner and J. Sjögren, *Acta Pharm. Suecica*, **8**, 13(1971).

(2) W. H. Thomas, *J. Pharm. Pharmacol.*, **25**, 27(1973).

(3) G. Eisenman, "Glass Electrodes for Hydrogen and other Cations," Dekker, New York, N.Y., 1967.

(4) J. V. Fee, D. J. W. Grant, and J. M. Newton, *J. Pharm. Pharmacol., Suppl.*, **25**, 148P(1973).

(5) L. E. Fryklöf, E. Sandell, and I. Östholm, British pat. 808,014 (1959).

(6) J. Sjögren and L. E. Fryklöf, *Farm. Revy*, **59**, 171(1960).

(7) J. Sjögren, *Acta Pharm. Suecica*, **8**, 153(1971).

(8) G. Levy and B. A. Hayes, *N. Engl. J. Med.*, **262**, 1053(1960).

(9) A. Richter, B. Myhre, and S. C. Khanna, *J. Pharm. Pharmacol.*, **21**, 409(1969).

(10) R. Fatton, D. Fielding, S. C. Khanna, and A. Richter, *Pharm. Acta Helv.*, **47**, 487(1972).

(11) A. C. Shah, C. B. Peot, and J. F. Ochs, *J. Pharm. Sci.*, **62**, 671(1973).

(12) J. B. Johnson, P. G. Kennedy, and S. H. Rubin, *ibid.*, **63**, 1931(1974).

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Structure and Toxicity of Alkaloids and Amino Acids of *Sophora secundiflora*

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Abstract □ Seeds from *Sophora secundiflora* were extracted into three major fractions: lipids, alkaloids, and amino acids. Most of the lipid material was composed of steroid esters. These esters were hydrolyzed, and the fatty acid compositions were determined. The major alkaloid component was cytisine, with *N*-methylcytisine, anagyrine, and termopsine in lower concentration. The major free ninhydrin-positive compound was γ-glutamyltyrosine. In addition to several common amino acids, pipecolic acid and 4-hydroxypipecolic acid were identified. Both the amino acid fraction and the alkaloid fractions caused minor pharmacological disorder when injected into rats. However, when both fractions were simultaneously administered, they were lethal.

Keyphrases □ *Sophora secundiflora*—seeds, alkaloids and amino acids isolated and identified, toxicity investigated in rats □ Alkaloids—isolated and identified, *Sophora secundiflora* seeds, toxicity, rats □ Amino acids—isolated and identified, *Sophora secundiflora* seeds, toxicity, rats

The toxicity of *Sophora secundiflora* (Ort.) DC to humans and domestic animals has been recognized for some time (1–3). The ingestion of the leaves was reported to lead to the production of poisonous milk (1), and *S. secundiflora* seeds (mescal beans) are known for their hallucinogenic effects among Indian tribes in the Southwestern United States and Mexico (4). Although the hallucinogenic effects of these seeds have been attributed to the presence of a high concentration of the alkaloid cytisine (4, 5), several species of *Laburnum, Ulex, Cytisus,* and *Sophora* containing this alkaloid are not hallucinogenic (6).

The presence of toxic amino acids in various plants has been documented (7, 8), and the toxic and/or hallucinogenic effects of *S. secundiflora* possibly may be due to nonalkaloid materials. Furthermore, atypical fatty acids and steroids are known to cause some neuropathies (9). Therefore, it was of interest to isolate and characterize these constituents and to determine which component(s) are responsible for the toxicity of these seeds.

EXPERIMENTAL

Extraction of Seeds—Mature seeds of *S. secundiflora*[1] were obtained from Mt. Bonnell, Austin, Tex. The hulled seeds (200 g) were machine ground and extracted with 500 ml of petroleum ether (lipid fraction). The residue was then extracted (soxhlet) with 800 ml of chloroform containing 0.5 ml of ammonium hydroxide (alkaloid fraction), and this residue was then extracted with 1000 ml of 50% ethanol (amino acid fraction). In some cases, the ground seeds were directly extracted with acidic chloroform from a methanolic extraction (soxhlet) of the ground seeds.

Fractionation and Analysis of Amino Acids—The amino acid fraction was reduced to 100 ml and applied to a 3.8 × 45-cm column of ion-exchange resin[2] (ammonium form). After washing with water, the column was eluted with 0.5 *N* ammonium hydroxide, and the ninhydrin-positive fractions were combined and reduced to dryness *in vacuo* to yield 3.8 g of solids. This mixture was then applied to a 2.8 × 45-cm column of ion-exchange resin[3] (acetate form) and washed with water to elute the neutral and basic amino acids. Subsequent elution with 0.5 *N* acetic acid resulted in the isolation of aspartic and glutamic acids and 350 mg of the unknown dipeptide.

The mixture of neutral and basic amino acids was then applied to a 2.2 × 100-cm column of ion-exchange resin[4] (hydrogen form), and the neutral amino acids were eluted with water. This fraction was applied to a 5.2 × 75-cm column of ion-exchange resin[3] (hydrogen form) and washed with water. Subsequent elution with 0.2 *N* HCl led to the isolation of 4-hydroxypipecolic acid and pipecolic acid.

[1] The plant material was identified as *Sophora secundiflora* L. by Dr. M. Aboul-Ela, Department of Biology, Texas Women's University. A voucher specimen (5-175) is available for inspection at the Herbarium of the Department of Biological Sciences, North Texas State University.

[2] Amberlite CG 120.

[3] Amberlite CG 400.

[4] Amberlite IR 50.