As in the studies on dissolution rates of drugs (9, 10), the model based on convective diffusion theory is able to describe quite accurately the membrane transport rate as a function of the physical–chemical parameters of the system. The convective diffusion model should apply to membrane transport for conditions intermediate between extremely low and high liquid flow rates. For the former conditions, as the flow approaches zero, it would be more appropriate to base a model on a nonsteady-state differential equation; for the latter, the transport is "membrane controlled" and has been thoroughly discussed (1).

REFERENCES

(1) G. L. Flynn, S. H. Yalkowsky, and T. J. Roseman, *J. Pharm. Sci.*, **63**, 479 (1974).

(2) S. B. Tuwiner, "Diffusion and Membrane Technology," Reinhold, New York, N.Y., 1962.

(3) O. H. LeBland, Jr., *J. Membr. Biol.*, **4**, 227 (1971).

(4) R. G. Stehle and W. I. Higuchi, *J. Pharm. Sci.*, **61**, 1922 (1972).

(5) E. G. Lovering and D. B. Black, *ibid.*, **63**, 1399 (1974).

(6) V. G. Levich, "Physiochemical Hydrodynamics," Prentice-Hall, Englewood Cliffs, N.J., 1962.

(7) R. B. Bird, W. E. Stewart, and E. N. Lightfoot, "Transport Phenomena," Wiley, New York, N.Y., 1960.

(8) H. Kramers and P. J. Kreyger, *Chem. Eng. Sci.*, **6**, 42 (1956).

(9) K. G. Nelson and A. C. Shah, *J. Pharm. Sci.*, **64**, 610 (1975).

(10) A. C. Shah and K. G. Nelson, *ibid.*, **64**, 1518 (1975).

(11) T. J. Roseman, *ibid.*, **61**, 46 (1972).

(12) G. L. Flynn and S. H. Yalkowsky, *ibid.*, **61**, 838 (1972).

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Quick Specific Assay for Aspirin

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Abstract □ Use of the Schoenemann reaction for the assay of aspirin in pharmaceutical combinations is described.

Keyphrases □ Aspirin—colorimetric analysis using Schoenemann reaction, pharmaceutical combinations □ Colorimetry—analysis using Schoenemann reaction, aspirin in pharmaceutical combinations □ Analgesics—aspirin, colorimetric analysis using Schoenemann reaction, pharmaceutical combinations

Aspirin is often the most labile component in a combination-type analgesic compound. Therefore, its stability is often the initial concern in any formulation screening program. Preliminary screening of a large number of potential formulations can be arduous, since most current methods of analysis generally consist of several steps: extractions or column separations followed by UV, colorimetric, or GLC assay (1–4). The method proposed here is quite suited to large numbers of assays. It is straightforward and is not subject to interference from substances such as salicylic acid, caffeine, phenacetin, acetaminophen, and codeine. It is essentially a Schoenemann reaction with slight modifications (5, 6).

EXPERIMENTAL

Preparation of Sample—An amount of tablet granulation equivalent to 100 mg of aspirin is weighed into a 250-ml volumetric flask, and 200 ml of distilled water is added. The slurry is shaken for about 10 min; then the flask is diluted to volume with distilled water.

Standard—Aspirin, 0.4 mg/ml of water, is prepared freshly.

Equipment—A recording dual-beam spectrophotometer[1] was used at 455 nm with the recorder speed at 5 mm/min.

Reagents—*o*-Dianisidine hydrochloride[2] (3,3′-dimethoxybenzidine dihydrochloride) was obtained commercially[3]. Tribasic sodium phosphate, 3% hydrogen peroxide, and the various pharmaceuticals tested were all reagent or USP grade or good commercial grade. Acetone[4] was reagent spectrophotometric grade and was used without purification. Previous investigators warned that impure acetone leads to false results (5).

Assay—The reagents were mixed in the following order: 0.5 ml of *o*-dianisidine hydrochloride (0.5% aqueous solution), 3.0 ml of acetone, 1.0 ml of 3% hydrogen peroxide, 1.0 ml of standard, and 2.0 ml of 0.05 *M* tribasic sodium phosphate.

The maximum color developed was measured in a 1-cm cell against water in the reference beam of the spectrophotometer at 455 nm by recording absorbance as a function of time. The maximum always occurred within 5 min.

The entire procedure was repeated, substituting the sample solution for the standard.

The ratio of the maximum absorbances of sample and standard was used to calculate the amount of aspirin in the sample taken.

RESULTS AND DISCUSSION

Linearity—The maximum absorbance was determined for the standard over a concentration range from 0.1 to 1.6 mg/ml and found to be linear and directly proportional to concentration throughout this range.

Interferences—The specificity of the Schoenemann reaction for certain esters and anhydrides is well documented (8) and was confirmed by testing a wide range of pharmaceuticals likely to be present in aspirin formulations. Only salicylsalicylic acid interfered; it gave absorbance proportionate to its molar concentration when compared to aspirin. That is, the method should also be applicable to salicylsalicylic acid. Minor interference was noted with propylene glycol diacetate and acetylated mannitol.

Color of Blank—Initially, a reagent blank was routinely used in the reference beam during absorption scanning. However, it was unnecessary for aspirin samples, since the color change was negligible in the few minutes required to reach the maximum color of the sample.

Method Validation—The proposed method and a chromatographic method analogous to the NF procedure for aspirin, phenacetin, and caffeine tablets were used for the analysis of aspirin in stability samples of an experimental preparation containing aspirin, acetaminophen, and caffeine. The aspirin found (percent of label) with the chromatographic

[1] Cary model 15.

[2] *o*-Dianisidine is a derivative of benzidine, a common reagent used in blood analysis. Although there is evidence that it is less carcinogenic than benzidine (7), it should be handled with usual precautions against skin contact.

[3] Eastman Kodak Co.

[4] Matheson, Coleman and Bell.

and proposed methods was 97.4 and 96.7%, respectively, for undegraded aspirin and 42.9 and 35.2%, respectively, for aspirin subjected to stress conditions. The comparison is satisfactory, especially if the method is used for screening purposes in formulation work.

REFERENCES

(1) "The National Formulary," 13th ed. Mack Publishing Co., Easton, Pa., 1970, p. 66.

(2) K. T. Koshy, *J. Pharm. Sci.*, **53**, 1280 (1964).

(3) J. Levine and J. D. Weber, *ibid.*, **57**, 631 (1968).

(4) C. A. Kelly, *ibid.*, **59**, 1053 (1970).

(5) B. Gehauf, J. Epstein, G. B. Wilson, B. Witten, S. Sass, V. E. Bauer, and W. H. C. Rueggeberg, *Anal. Chem.*, **29**, 278 (1957).

(6) J. S. Hanker, A. Gelberg, and B. Witten, *J. Am. Pharm. Assoc., Sci. Ed.*, **47**, 728 (1958).

(7) R. C. Garner, A. L. Wallpole, and F. L. Rose, *Cancer Lett.*, **1**, 39 (1975).

(8) D. J. Marsh and E. Neale, *J. Appl. Chem.*, **8**, 394 (June 1958).

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Single-Dose Assay of Ferrous Ion in Pharmaceuticals

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Abstract □ A method for the assay of the ferrous ion in hematinics or multivitamin tablets is described. The method is based on the spectrophotometric measurement of the chromophore produced by reacting the ferrous ion with the highly specific chelating agent ferrozine. The method is proposed as an alternative to the redox methods in the USP and NF.

Keyphrases □ Ferrous ion—spectrophotometric analysis, pharmaceutical formulations □ Iron—ferrous ion, spectrophotometric analysis, pharmaceutical formulations □ Spectrophotometry—analysis, ferrous ion in pharmaceutical formulations □ Hematinics—ferrous ion, spectrophotometric analysis in pharmaceutical formulations

The presence of the ferrous ion in many pharmaceutical preparations necessitates a rapid, economical, and sensitive method for its determination in pharmaceuticals. The official USP and NF methods primarily rely on the classical redox conversion of the ferrous to the ferric ion (1, 2). These redox methods are tedious and inaccurate due to difficult end-point visualization.

Other methods are available for the assay of iron-containing products, *e.g.*, atomic absorption spectrophotometry and X-ray emission spectrometry. However, these methods require involved sample preparation as well as sophisticated instrumentation (3, 4). This report describes a simple colorimetric procedure applicable to ferrous-containing pharmaceuticals such as hematinics and multiple-vitamin preparations with iron. The sensitivity is such that the method is ideal for single-dose assays.

The present method utilizes the reagent ferrozine, 3-(2-pyridyl)-5,6-bis(4-phenylsulfonic acid)-1,2,4-triazine disodium salt, a chelating agent highly specific for the ferrous ion. Although this compound is similar to the well-known chelating agent 2,4,6-tripyridyltriazine, previously used for iron assays of pharmaceuticals, it has the advantages of being significantly more water soluble, more sensitive, and possibly more economically feasible for repeated analyses (4, 5).

Ferrozine has been shown to react with the ferrous ion to yield a magenta-colored tris complex, *i.e.*, Fe-(ferrozine)$_3^{2+}$. This species forms completely in aqueous solution between pH 4 and 9 and exhibits a sharp peak with maximum absorbance at 562 nm (5). Interference studies showed that only monovalent copper and divalent cobalt formed colored species with ferrozine (5). Ferrozine was used in the determination of iron in municipal waters (5) and serum iron levels (6).

Table I—Stability of Iron–Ferrozine Complex

Hours after Mixing	Absorbance[a]	Absorbance[b]	Absorbance[c]
0.17	0.492	0.615	0.520
0.50	0.492	0.615	0.520
1.00	0.492	0.615	0.520
2.00	0.492	0.615	0.520
8.00	0.496	0.615	0.518
24.00	0.496	0.611	0.518
48.00	0.496	0.611	0.511

[a] Obtained from assay of ferrous fumarate tablet (Feostat, Westerfield Co.). [b] Obtained from assay of ferrous sulfate tablet (Feosol, Smith Kline and French). [c] Obtained from assay of analytical grade ferrous ammonium sulfate.

EXPERIMENTAL

Instrumentation—A double-beam spectrophotometer[1] and a pH meter[2] were used.

Reagents and Chemicals—The following ACS grade chemicals were used: sodium phosphate monohydrate, ascorbic acid, ferrous ammonium sulfate hexahydrate, acetic acid, and ferrozine[3]. Deionized water was obtained from a commercial deionizer[4] and was shown by conductance to contain not more than 0.25 ppm of dissolved ionic solids.

Color Reagent—Ferrozine, 125 mg, was placed in a 25-ml volumetric flask, and deionized water was added to volume. The solution should be protected from light.

Buffer Solution—Sodium phosphate, 6.25 g, and 12 mg of ascorbic acid were dissolved in 100 ml of deionized water. The pH of the resultant solution was adjusted to 5.3 by the dropwise addition of 10% sodium hydroxide.

Stock Solution of Ferrous Ammonium Sulfate—Ferrous ammonium sulfate, 615 mg, was placed in a 100-ml volumetric flask and brought to volume with deionized water.

Working Solution of Ferrous Ammonium Sulfate—For the working

[1] Beckman model 25 spectrophotometer.
[2] Coleman Metrion IV.
[3] Nutritional Biochemical Corp.
[4] Culligan deionizer.