CONCLUSION

This study demonstrated a simple method that was useful for the determination of the release of certain drugs from a polymer–plasticizer combination. The drug release data indicated the usability of ethylcellulose and polyamide films. An ethylcellulose–tributyl citrate combination proved to be an excellent matrix for benzocaine. Cyclomethycaine could be incorporated into an ethylcellulose and polyamide film while methapyrilene hydrochloride was found to be incompatible with ethylcellulose.

A kinetic study of the release of benzocaine from ethylcellulose showed that the diffusion mechanism was operative according to the Higuchi diffusion-controlled model where the rate of release was inversely proportional to the concentration of drug released. The release rates were changed significantly with cetyl alcohol or tributyl citrate at different concentrations. These studies demonstrated the release of therapeutically active agents from polymeric film into the surrounding medium. The medicinal agent must be sufficiently insoluble in the film to allow for its release into the surrounding medium but not so soluble as to remain preferentially in the film.

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§ Present address: Schering Corporation, Kenilworth, NJ 07033

ˣ To whom inquiries should be directed.

NOTES

Automated Analysis of Warfarin Sodium Tablets

S. HANNA ˣ, G. DZUS, L. RASERO, and L. LACHMAN

Abstract □ An automated procedure was developed for the determination of warfarin sodium by following the steps of the manual USP procedure. The automated procedure is applicable to single tablets and composites of 20 tablets at different tablet concentrations. Sensitivity, precision, accuracy, and reproducibility are equivalent to the manual USP procedure. Sensitivity was approximately 15 μg/ml, with a coefficient of variation of 0.711%.

Keyphrases □ Warfarin sodium—automated analysis, commercial dosage forms □ Automated analyses—warfarin sodium, commercial dosage forms □ Anticoagulants—warfarin sodium, commercial dosage forms

The semiautomated method recognized officially by the Association of Official Analytical Chemists (1–3) is an adaptation of the USP compendial assay (4) for warfarin sodium tablets. This semiautomated method uses suspensions of either single tablets or portions of tablet composites equivalent to single tablets. The preparation of these suspensions requires the disintegration of individual tablets or dispersion of weighed composites in an accurately measured volume of 0.01 *N* NaOH to give a drug concentration of 0.1 mg/ml. Homogenization of the sample is achieved by using an ultrasonic generator for at least 10 min with intermittent swirling and letting the suspension stand for 1.5 hr with occasional mixing. An aliquot of the homogenized sample is then transferred to the automatic analyzer.

This report discusses an automated system that follows the manual USP procedure and saves the labor and time consumed in the semiautomated method.

EXPERIMENTAL

Apparatus—The analytical system consisted of the following modules: solid sampler II[1], programmed at 20/hr, homogenizing speed 3, and stir speed 1; proportioning pump III[1]; a continuous filter[1], speed

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

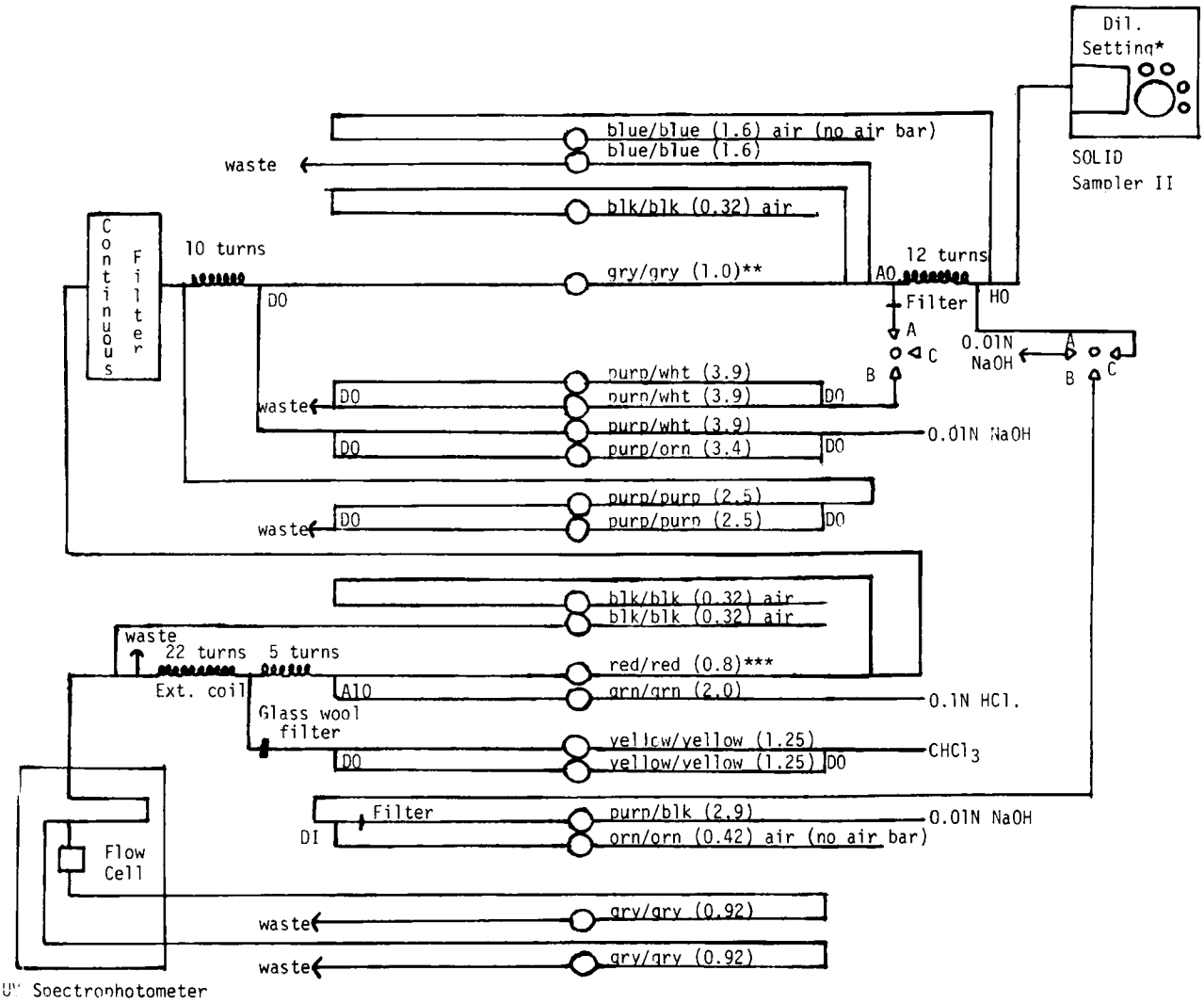

Figure 1—*Schematic diagram of the automated system for warfarin sodium analysis. * Diluent setting is adjusted to obtain absorbance between 0.4 and 0.6. ** For unit-dose analysis, remove red/red resample line and attach the gry/gry line directly to A10. *** Change to wht/wht (0.6) for warfarin sodium 10-mg tablets.*

4; a spectrophotometer[2] at a fixed wavelength of 308 nm and equipped with a 10-mm continuous flowcell[3]; and a recorder[1] with a chart speed of 45 cm/hr.

Reagents[4] and Solutions—The following were used: chloroform, spectrograde; methanol; 0.01 *N* NaOH; 0.1 *N* HCl; and USP warfarin reference standard in 0.01 *N* NaOH, 0.94 and 18.8 mg/ml.

Automated Procedure—A schematic diagram of the automated system is shown in Fig. 1. Organic solvent line tubes were pumped with methanol and chloroform, each for 5 min; then all tubes were placed in their respective solutions, and the system was allowed to equilibrate for 20–30 min. The spectrophotometer was adjusted to a baseline of 0.02 absorbance unit. Standards, baseline blanks, and samples of either individual tablets or composites of 20 tablets were placed into polystyrene cups and the system was activated.

The system was run in the sequence described in Table I. The tablets were ground to a fine powder and homogenized in the solid sampler II blender with the aliquot volume of 0.01 *N* NaOH for 1.47 min (48.9% of the complete 3-min cycle). The sampling time was 16.7% and the triple rinsing time was 14.4% of the complete cycle. A continuous filter was used to filter off the undesirable, insoluble tablet excipients from the solution before mixing with solvents.

After completing the analysis, acid, base, and sampling line tubes were placed in water while organic solvent line tubes were left in their reservoirs; pumping was continued for 5 min. Then all lines were removed from solvents, and the system was pumped until dry.

Calculations—A correction was necessary to account for the volume change resulting from the use of liquid standards. The correction

Table I—Disposition of Standards[a], Baseline Blanks[b], and Samples

Baseline
Standard solution I[c], three samples
Standard solution II[c], three samples
Baseline blank, one empty cup
Standard solution, one sample
Five samples
Standard solution, one sample
Baseline blank, one empty cup

[a] Before and after every group of five samples, average the net absorbance of the two standards to obtain the average absorbance value of standard reading. [b] Before and after every group of 10 samples and two standards, average the baseline values and subtract from the samples and standards peak values to obtain their net absorbance readings. [c] Two separate standards were accurately weighed and prepared. The percent absorbance spread for each standard should be less than 3%, and average absorbance ratios of the two standards should differ by not more than 1.5%. The standard concentrations, chosen according to sample warfarin content, of 0.375–1.88 and 7.52–37.6 mg/ml are for single-tablet and 20-tablet composite analyses, respectively.

[2] Beckman DB-G, Beckman, Fullerton, Calif.
[3] Arthur H. Thomas, Philadelphia, Pa.
[4] All chemicals were analytical grade except when specified.

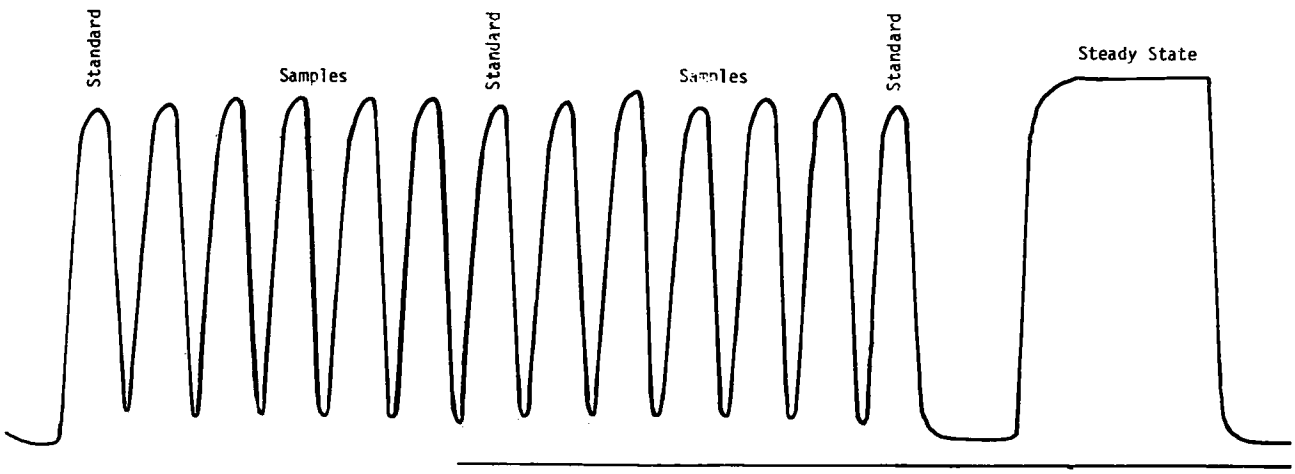

Figure 2—*Typical recordings from the automated analysis of warfarin sodium.*

Table II—Recoveries and Precision with Automated Method

Warfarin Sodium Added, mg	Warfarin Sodium Recovered[a], %
2	99.0
2.5	98.8
5	100.4
7.5	100.2
10	99.5
Average	99.58
Coefficient of variation, %	0.711

[a] Mean of 10 results.

chloroform was added and mixed in the extraction coil. Phases were separated in the phase separator fitting, and chloroform containing warfarin was debubbled. Chloroform was pumped through the continuous flowcell, where the absorbance was continuously measured and recorded on the recorder chart. A typical recording is shown in Fig. 2.

Under the experimental conditions, a linear relationship existed between the absorbance and concentration of warfarin over the ±25% range of the average standard concentration chosen according to the sample warfarin content, with a linear correlation coefficient of 0.996. The precision and recoveries obtained by the automated method were checked by running placebos of the tablet formulations with standard solutions with the same active concentrations (Table II). No significant interference from the excipients was observed.

Table III—Comparison of Results, in Milligrams of Warfarin Sodium, Obtained by Automated and USP XIX Methods

Labeled Concentration, mg	Single-Tablet Analysis[a]				20-Tablet Composite Analysis[b]			
	Automated	*CV*, %[c]	USP	*CV*, %	Automated	*CV*, %	USP	*CV*, %
2 A[d]	2.03	0.57	2.05	1.58	2.02	0.77	1.97	1.3
B[e]	1.97	1.61	2.02	1.99	1.96	0.59	1.94	1.2
2.5 A	2.47	0.50	2.49	1.42	2.48	0.57	2.47	0.73
B	2.45	1.76	2.51	1.92	2.45	0.35	2.42	0.92
5 A	5.04	0.36	5.10	0.47	5.02	0.39	4.98	0.40
B	4.91	1.37	4.95	1.25	4.97	0.50	4.92	0.85
7.5 A	7.45	0.17	7.47	0.34	7.47	0.18	7.48	0.50
B	7.42	1.27	7.46	0.95	7.50	0.32	7.48	0.67
10 A	9.95	0.18	9.8	2.29	9.96	0.19	9.88	1.17
B	9.89	0.78	9.95	1.10	10.06	0.32	9.95	0.90

[a] Mean of 10 results. [b] Mean of 30 results for Sample A and mean of 10 results for Sample B. [c] Coefficient of variation. [d] Coumadin tablets, Endo Laboratories, Garden City, N.Y. [e] Panwarfin tablets, Abbott Laboratories, Chicago, Ill.

factor, *CF*, was calculated by:

$$CF = \frac{\text{diluent pump setting + sample displacement volume}}{\text{diluent pump setting + standard volume}} \quad \text{(Eq. 1)}$$

The concentration, X, of warfarin sodium per tablet was calculated by:

$$X = \frac{A_{sample}}{A_{standard}} \times 1.071 \times CF \times C \times D \quad \text{(Eq. 2)}$$

where A = net absorbance value, 1.071 = ratio of molecular weight of warfarin sodium to warfarin, C = concentration of standard in milligrams per milliliter, and D = dilution factor.

RESULTS AND DISCUSSION

Warfarin sodium samples were withdrawn, segmented with air, and acidified with 0.1 *N* HCl. The acidified solution was mixed, and

As shown in Table III, results obtained with the automated procedure compared favorably with those of the manual USP method in the analysis of 2-, 2.5-, 5-, 7.5-, and 10-mg warfarin sodium tablets. The coefficient of variation percent with each sample, single tablets or composites of 20 tablets, was significantly smaller by the automated procedure. Results obtained by the automated method appear to demonstrate reproducibility in comparison to the official manual USP procedure.

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[x] To whom inquiries should be directed.

Inhibition of *Streptococcus faecalis* by Long Chain Aliphatic Monoamines: Quantitative Structure–Activity Studies

G. E. BASS [x], LARRY J. POWERS, and E. O. DILLINGHAM

Abstract □ Primary, secondary, and tertiary long chain aliphatic amines were synthesized, and their activity against *Streptococcus faecalis* was determined. Quantitative structure–activity analyses were carried out based on the Hansch extrathermodynamic model, using partition coefficients, CMC's, quantum mechanical charges on the amine nitrogen, and the Taft steric parameter. The best correlations were obtained with the CMC. Steric properties of the ammonium head become important for tertiary amines. The structural feature of these compounds that dominates biological activity is the length of the alkyl tail. Ammonium head substituents are of only secondary importance.

Keyphrases □ *Streptococcus faecalis*—inhibition by long chain aliphatic monoamines, quantitative structure–activity relationships □ Amines, long chain aliphatic—inhibition of *Streptococcus faecalis*, structure–activity relationships □ Structure–activity relationships—long chain aliphatic monoamine inhibition of *Streptococcus faecalis*

The antibacterial activity of amines carrying long aliphatic chains has been recognized (1, 2). Although the quaternary compounds have been studied the most, simple primary, secondary, and tertiary long chain aliphatic amines, $R_1R_2N—C_nH_{2n+1}$, are also potent antibacterials. Relationships between structures and biological activities of these compounds have been investigated (3–7); but in most of these studies, structure variations were limited primarily to the length of the long aliphatic chain or "tail."

Hansch and coworkers (4–6) carried out quantitative structure–activity correlations on published activity data for primary amines using the extrathermodynamic model:

$$\log(\text{biological activity}) = a(\log P)^2 + b(\log P) + c \quad \text{(Eq. 1)}$$

where P is the octanol–water partition coefficient. Biological data included antibacterial (Gram-positive and Gram-negative species) (4, 5), antifungal (6), and hemolytic (5) activities. Very good correlations were always found.

Weiner *et al.* (3) suggested that activity variations for these compounds may be explained within the framework of the Ferguson principle (8), utilizing surface activity parameters such as the critical micelle concentration (CMC) and the surface concentration. These studies involved the inhibition of *Micrococcus pyogenes* var. *aureus*, *Escherichia coli*, and *Candida albicans* by three substituted dodecylamines.

To develop a more complete understanding of the structure–activity relationships for these compounds, the effect of alkyl substitution on the ammonium head ($R_1R_2N—$) was investigated. Physicochemical properties that might play a role in, or reflect, the bioactivities of these compounds include the partition coefficient, CMC, the Taft steric parameter (E_s) for the ammonium head, and the quantum mechanical charge (Q_N) calculated for the amine nitrogen.

Long chain aliphatic amines (primary, secondary, and tertiary) were synthesized, and their ability to inhibit the growth of *Streptococcus faecalis* was determined. The physicochemical parameters noted were evaluated, and quantitative structure–activity correlations were carried out using the generalized Hansch equation:

$$\log(\text{activity}) = a(\log P)^2 + b(\log P) + c(\log \text{CMC})^2 + d(\log \text{CMC}) + eE_s + fQ_N + g \quad \text{(Eq. 2)}$$

Not all of these parameters can be used together in a single correlation. For example, log P and log CMC reflect similar properties. In the opinion of these authors, little can be gained from these empirical correlations if more than three parameters are involved.

EXPERIMENTAL[1]

The amine salts were prepared by reacting the acyl chloride of the appropriate long chain carboxylic acid with amines to obtain the corresponding amides. After recrystallization, the amides were reduced with lithium aluminum hydride to the desired amines. The amines were isolated and converted to the hydrochloride salts. Melting points and IR spectra were in agreement with available literature data.

Partition coefficients were calculated from substituent contributions obtained from the literature (5, 9, 10). The reported value of log P = 1.85 was assumed for dodecylamine. Substituent contributions for $—NH_2$ and $—N(CH_3)_2$ were −1.85 and −0.95, respectively. The value for $—NHCH_3$ was interpolated as −1.40. Methylene groups were assumed to contribute 0.50, and branching corrections

[1] Melting points were determined on a Thomas-Hoover capillary melting-point apparatus. IR spectra were determined on a Perkin-Elmer model 257 spectrophotometer. Turbidity measurements were made using a Bausch & Lomb Spectronic 20. Surface tension was determined using a Fisher surface tensiometer. Regression analyses were performed on an IBM 1130 system.