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Sorption Rates of Water-Soluble Dyes on Soft Gelatin Capsules

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Abstract □ A method was developed for determining sorption rates of water-soluble dyes by soft gelatin capsules. This method was used for determining the diffusivities and activation energies of five FD&C water-soluble dyes. The activation energies of these dyes were in either of two groups which had ranges of 25.1–25.7 and 34.8–36.0 kcal/mole.

Keyphrases □ Dyes, water soluble—determination of sorption rates on soft gelatin capsules □ Gelatin capsules, soft—determination of sorption rates of water-soluble dyes □ FD&C water-soluble dyes—determination of sorption rates on soft gelatin capsules □ Surface dyeing—determination of sorption rates of five FD&C water-soluble dyes on soft gelatin capsules

Several FD&C dyes were removed from the Food and Drug Administration's food additives list because they exhibited carcinogenic activity in animals (1). As a result, various methods for reducing the quantity of dyes in pharmaceutical dosage forms have become a subject of considerable interest. For soft shell gelatin capsules, such dye reductions have been achieved by surface dyeing untinted capsules instead of incorporating the dye into the gelatin shell formulation prior to preparing the capsules. The use of surface dyeing methods for this purpose is predicated upon the dyes diffusing into the capsule surface at rates slow enough to obtain a desired color and fast enough to be a reasonable processing step in the manufacture of the dosage form. A survey of the literature reveals that a method suitable for studying dyeing rates of this type of product has not been reported.

The purpose of this article is to describe a method for determining the sorption rates of dyes by intact untinted soft gelatin capsules under conditions similar to those used in production for surface dyeing. Data obtained using this method are presented for five FD&C water-soluble dyes: Yellow No. 5 (Tartrazine), Yellow No. 6 (Sunset Yellow FCF), Red No. 2 (Amaranth), Red No. 4 (Ponceau SX), and Green No. 3 (Fast Green FCF).

Table I—Diffusivities for the Sorption of FD&C Water-Soluble Dyes by Soft Gelatin Capsules

FD&C Dye	Temperature	Diffusivity $\times 10^7$, cm^2 sec^{-1}	Correlation Coefficient
Yellow No. 5	3.8°	1.11	0.994
Yellow No. 5	8.4°	1.79	0.997
Yellow No. 5	12.9°	4.70	0.997
Yellow No. 5	15.6°	6.66	0.994
Yellow No. 5	20.0°	13.6	0.999
Yellow No. 6	6.0°	1.35	0.995
Yellow No. 6	11.2°	2.88	0.999
Yellow No. 6	15.0°	4.75	0.994
Yellow No. 6	20.3°	12.6	0.999
Yellow No. 6	25.0°	24.5	0.999
Red No. 4	4.3°	0.657	0.989
Red No. 4	7.8°	1.28	0.999
Red No. 4	12.4°	2.34	0.995
Red No. 4	16.1°	4.85	0.999
Red No. 4	20.0°	7.70	0.999
Red No. 2	4.0°	0.865	0.998
Red No. 2	7.6°	1.65	0.996
Red No. 2	13.0°	5.94	0.997
Red No. 2	16.2°	12.4	0.999
Red No. 2	20.0°	25.2	0.999
Green No. 3	4.0°	0.323	0.998
Green No. 3	8.3°	0.805	0.995
Green No. 3	12.9°	2.31	0.999
Green No. 3	15.6°	5.05	0.999
Green No. 3	20.0°	10.8	0.999

EXPERIMENTAL

The capsules used were prepared with no fill material (air-fills) on a conventional soft gelatin encapsulating machine. The dried capsules contained approximately 66% gelatin[1] (125 Bloom strength), 30% glycerol, 3% water, and 1% parabens [methylparaben-propylparaben (1:3)]. All dye solutions contained 0.5% of one of the FD&C soluble dyes[2] in 50% (v/v) isopropyl alcohol and water.

Three capsules were mounted near the end of each of five sample holders (8 × 1 × 0.1 cm) with a space of approximately 1.0 cm left between capsules. Each capsule was mounted perpendicular to the holder with a small amount of silicon rubber[3]. The

[1] Vyse and Co., Chicago, Ill.
[2] H. Kohnstamm and Co., Inc., New York, N.Y.
[3] RTV 102 General Electric.

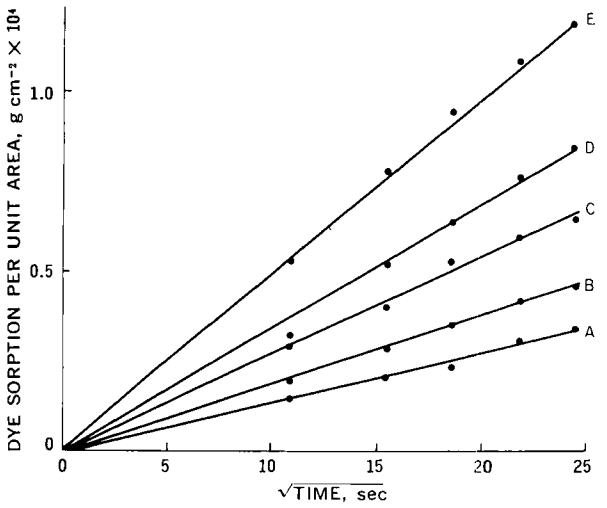

Figure 1—*Temperature dependency of the sorption of FD&C Red No. 4 by soft gelatin capsules as a function of the square root of time. Key: A, 4.3°; B, 7.8°; C, 12.4°; D, 16.1°; and E, 20.0°.*

sample holders were mounted near the top of a 1.0-liter water-jacketed beaker located on a magnetic stirrer. The temperature was controlled (±0.1°) by connecting the jacketed beaker to a circulating water bath. The holders were positioned around the inside of this beaker in a manner that would maintain the capsules below the surface of 500 ml of dye solution. The opposite ends of the holders projected from the water-jacketed beaker for easy removal. The magnetic stirrer was started and set at a predetermined rate for moderate stirring of the dye solution. Five hundred milliliters of dye solution was added and a stopwatch was started. Samples (holders) were withdrawn at 2, 4, 6, 8, and 10 min. Upon withdrawal, each sample was immediately dipped into isopropyl alcohol and water (50:50 v/v) and then into isopropyl alcohol.

The samples were dried for 2 hr to reestablish the original moisture content of the capsules. A 54.5-mm² disk was cut from one surface of each capsule. The three disks obtained from the capsules for each sample time were dissolved in sufficient water to provide a volume of 100 ml. These solutions were assayed spectrophotometically at the appropriate wavelengths.

Table II—Activation Energies for the Sorption of FD&C Water-Soluble Dyes by Soft Gelatin Capsules

FD&C Dye	Activation Energy, kcal/mole	Sulfonate Groups per Dye Molecule	Correlation Coefficient
Yellow No. 5	25.7	2	0.994
Yellow No. 6	25.1	2	0.998
Red No. 4	25.4	2	0.998
Red No. 2	34.8	3	0.999
Green No. 3	36.0	3	0.999

RESULTS AND DISCUSSION

Diffusivities were reported (2) for the rates of dye uptake by gelatin films using the following equation, which is an approximate solution for Fick's law:

$$x = 2C_0(kt/\pi)^{1/2} \qquad \text{(Eq. 1)}$$

where x is the amount of dye sorbed per unit area of surface at any time t, C_0 is the initial concentration of the dyeing solution, and k is the diffusivity. Several reports (3–5) indicated that good agreement is usually obtained with this equation when it is used for describing diffusion rates into infinite solids for low time periods.

Diffusivities were determined for all five dyes at various temperatures between 3.8 and 25.0° by linear regression analysis of this equation (Table I). A typical plot of the dye uptake per unit area as a function of the square root of time is presented for FD&C Red No. 4 at several temperatures in Fig. 1. Inspection of the correlation coefficients listed in Table I and the lines plotted in Fig. 1 indicates that data collected using this method fit this equation. Activation energies, obtained from the linear regression analysis of the Arrhenius equation, were calculated from the diffusivities for each dye studied (Table II). Arrhenius plots for FD&C Red No. 4, Yellow No. 5, and Yellow No. 6 are presented in Fig. 2. Similar plots for Red No. 2 and Green No. 3 are presented in Fig. 3.

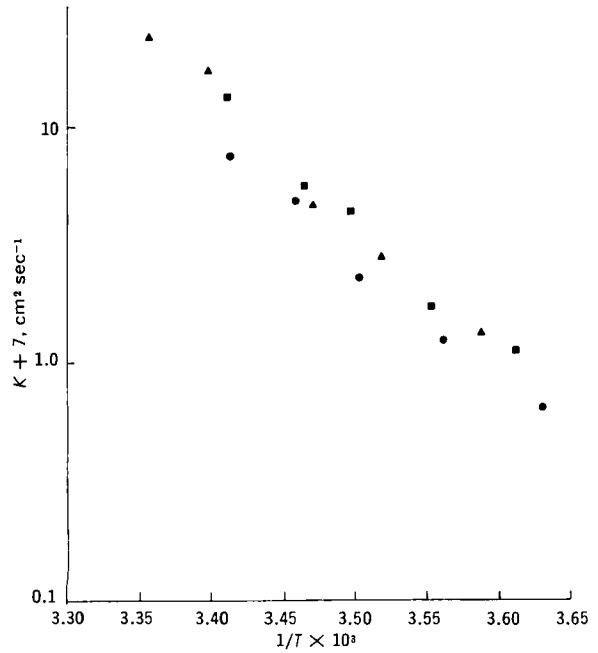

Figure 2—*Arrhenius-type plots for sorption rates of water-soluble dyes with two sulfonate groups per dye molecule. Key: ■, FD&C Yellow No. 5; ▲, FD&C Yellow No. 6; and ●, FD&C Red No. 4.*

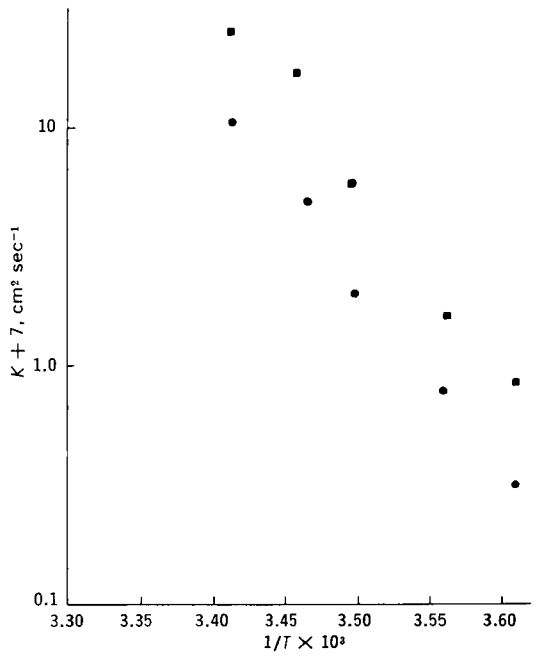

Figure 3—*Arrhenius-type plots for sorption rates of water-soluble dyes with three sulfonate groups per dye molecule. Key: ■, FD&C Red No. 2; and ●, FD&C Green No. 3.*

The method reported here is simple and reliable and could be easily modified for studying all gelatin capsule dosage forms. The diffusivities and activation energies calculated from the data obtained by this method are consistent with those obtained from diffusion studies of similar systems (6, 7). The activation energies calculated for the five water-soluble dyes studied fall into two distinct groups (Table II). The first group includes Yellow No. 5, Yellow No. 6, and Red No. 4, which have similar activation energies ranging from 25.1 to 25.7 kcal/mole. The energies for these three dyes were found to be statistically indistinguishable by testing the homogeneity of the regression coefficients for their Arrhenius slopes. The second group includes Red No. 2 and Green No. 3 which have activation energies of 34.8 and 36.0 kcal/mole, respectively. The activation energies for these two dyes were also statistically indistinguishable.

The dyes in the first group, with lower activation energies, contain two sulfonate groups per molecule; those in the second group, with higher activation energies, contain three sulfonate groups per molecule. It will be necessary, however, to conduct various additional studies on a larger number of dyes before attempting to establish a relationship between the activation energies of these compounds and their structural variations.

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Differential Pulse Polarographic Analysis of Everninomicin Complex in Fermentation Broth

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Abstract □ A direct, sensitive, and fast (15 min) method for following the laboratory fermentation course of everninomicins is described. It replaces the 20-hr elapsed-time agar-diffusion bioassay method.

Keyphrases □ Everninomicin complex—differential pulse polarographic analysis in fermentation broth □ Polarography, differential pulse—analysis, everninomicin complex in fermentation broth □ Fermentation broths—differential pulse polarographic analysis of everninomicin complex

The production of everninomicin complexes by fermentation was described previously (1, 2). To optimize the yield, a fast, reliable analytical method should be used to follow the course of fermentation. Since the components of an everninomicin complex (*e.g.*, B, C, and D) possess an electroactive group, the nitro group (3), they are amenable to polarographic analysis (4). A more recent advance, differential pulse polarography, would be ideally suited for this purpose due to its sensitivity (5) and larger linear range.

EXPERIMENTAL

The broth (5.00 ml) was transferred to a 50-ml volumetric flask and diluted to volume with acetonitrile. The suspension was mixed well and left for a few minutes to allow the insoluble matter to settle, thus permitting the antibiotic to be separated from the mycelium. The supernate (5.00 ml) was mixed with an equal volume of 0.01 *M* phosphate buffer of pH 7.0. This solution was placed in the sample compartment of a small polarographic H-cell along with the dropping mercury electrode; in the other compartment was a normal calomel electrode, used both as a reference and as a counter-electrode. The cell compartments were separated by an agar plug and a fritted-glass diaphragm. The chloride ion in both the calomel cell and the agar plug was furnished by 1 *N* tetramethylammonium chloride[1]. Both compartments of the H-cell were sealed with rubber stoppers. A small annulus was made around the dropping mercury electrode to allow the capillary to move to dislodge the mercury drops and to permit the escape of nitrogen.

The solution to be analyzed was deoxygenated using nitrogen. Before being passed through the cell, the nitrogen was passed through a gas washing bottle containing a solvent similar to that of the solution. While polarograms[2] were being run, nitrogen was passed over the solution.

A drop time of 1 sec, a scan rate of 2 mv/sec, and a modulation amplitude of 100 mv were kept constant throughout the analysis. Potentials were scanned from −0.6 to −1.4 v (normal calomel electrode). The current scale was 2 μamp.

RESULTS AND DISCUSSION

A typical differential pulse polarogram of the broth yielded peaks at a potential corresponding to the reduction of everninomicin D. No other peak was observed in the vicinity (Fig. 1). The peak currents of the broth samples as a function of the fermentation time are shown in Fig. 2. These results are in good agreement with the everninomicin production curve shown in Fig. 4 of Ref. 2, which was determined by the bioassay method.

This assay lacks specificity since it is essentially a functional group assay responding to the nitro group present in everninomicin complex. This lack of specificity is also present in the bioas-

[1] Southwestern Analytical Chemicals, Austin, TX 78767

[2] A Princeton Applied Research model 170 electrochemistry system in conjunction with a model 172A drop timer (Princeton Applied Research Corp., Princeton, NJ 08540) was used to obtain the differential pulse polarograms.