Liquid and Solid Solution Interactions of Primary Certified Colorants with Pharmaceutical Gelatins

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Abstract □ The liquid and solid solution interactions of eight FD&C dyes in 1% concentration with pharmaceutical gelatins and the effects of the dyes on the disintegration behavior of gelatins were observed. In liquid solution by visible region spectroscopy, all FD&C dyes interacted with a type A gelatin. The xanthene dye, FD&C Red No. 3 (erythrosine), interacted to the greatest extent by a combination of ionic, hydrogen, and hydrophobic types of bonds. The FD&C dyes did not appear to interact with a type B gelatin. A method for simulating a gelatin capsule shell wall was devised to examine solid-state interactions by visible and IR-attenuated total reflectance spectroscopy, dye release patterns, and the effect of the dyes on gelatin disintegration. FD&C Red No. 3 and Violet No. 1 in solid solution appeared to interact to a greater extent than the other FD&C dyes. Hydrogen bonding changes in the solid solution films of both gelatins were not observable by IR-attenuated total reflectance spectroscopy until very high (1:10 to 1:5) dye-protein ratios were attained. The same two dyes (FD&C Red No. 3 and Violet No. 1) that showed the greatest spectral changes exhibited the slowest release times from both gelatins at temperatures both below and within the temperature range at which gelatins disintegrate and go into colloidal solution. In simulated gastric fluid USP without pepsin, the FD&C Red No. 3 dye greatly diminished the average disintegration rate of both gelatins. The addition of pepsin essentially eliminated the effect of FD&C Red No. 3 on gelatin disintegration. In simulated intestinal fluid USP without pancreatin, both gelatins disintegrated slower than in simulated gastric fluid without pepsin. FD&C Red No. 3 also had a detrimental effect on gelatin disintegration in simulated intestinal fluid without pancreatin. Pancreatin was not added to the simulated intestinal fluid because of its interference with visualization of disintegration.

Keyphrases □ FD&C dyes—liquid and solid solution interactions with gelatins, effect on gelatin disintegration behavior, visible and IR-attenuated total reflectance spectroscopy □ Gelatins, pharmaceutical—effect of eight FD&C dyes on disintegration behavior, liquid and solid solution interactions with dyes, visible and IR-attenuated total reflectance spectroscopy □ Dye-protein interactions—effect on gelatin disintegration behavior □ Protein-dye interactions—effect on gelatin disintegration behavior □ Colorants, eight FD&C dyes—liquid and solid solution interactions with gelatins, effect on gelatin disintegration behavior, visible and IR-attenuated total reflectance spectroscopy □ Interactions—FD&C dyes-pharmaceutical gelatins

Gelatins used for the encapsulation of medication may be colored by the single or combined use of primary certified colorants. Several primary certified colorants were shown (1) to interact with pharmaceutical adjuvants with metallic or polyfunctional character in a chelation and/or chemisorption type of mechanism. Dyes were shown to inhibit the dissolution of sulfathiazole, sulfaguanidine, diethylstilbestrol, phenobarbital, riboflavin, and thymol (2–5) by a hypothesized preferential adsorption of dye at the primary dissolution faces in the various crystals (2–4). In addition, dyes were shown to affect the disintegration and solubility of cellulose polymers by mechanisms that vary with each dye–polymer combination (5).

While the capsule dosage form has been assumed to release its contents quickly upon administration, an article appeared that may question the reliability of capsule dosage forms (6). It stated that the use of dyes and/or opaquants in the capsule shell have no effect on disintegration rate. However, the article also stated that red-opaque and black capsules require 100–200 sec. longer to disintegrate than colorless or other colored or colored-opaque capsules which disintegrated or released their contents within 60–100 sec. All capsules were filled with sodium bicarbonate. An examination of the effects of colorants on the disintegration of gelatin was deemed necessary as the logical beginning toward a better understanding of the effects of intrinsic variables on gelatin disintegration.

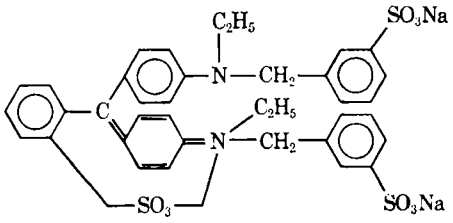
The purposes of this investigation were to define the mechanisms of dye–protein binding in the liquid and solid solution states and to relate the dye–protein interaction to its effect on the disintegration of gelatin. The objectives of this study were:

1. To prepare a solid solution film system of dye and gelatin as a simplest simulation of a capsule shell wall.
2. To examine, by spectroscopic means, interactions of dyes with both cationic and anionic gelatins in both liquid and solid solutions.
3. To observe differences in binding of dyes to the two differently charged proteins by diffusion from the protein gel at temperatures within and below the point at which gelatin "melts" and disintegrates.
4. To compare the disintegration behavior of colored with uncolored gelatins in simulated gastric and intestinal fluid environments.

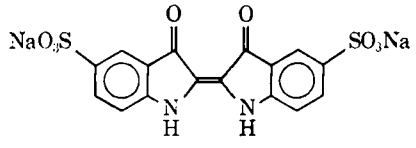
EXPERIMENTAL

Materials—The FD&C dyes (I–VIII) used in this study are listed in Table I[1], along with their synonym, color index number, and chemical class. All dyes were used as obtained. The pharmaceutical gelatins[2] utilized are presented in Table II. Gelatins were purified by the dialysis of a heterogeneous mixture of 5% gelatin and distilled water previously chilled to 5°. The gelatin served as its own dialyzing membrane. This mixture was allowed to stand overnight at 5° and was then filtered through a 20-mesh screen. The dialysis process

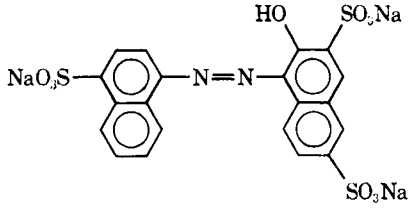
[1] Supplied by Specialty Chemicals Division, Allied Chemical Corp., Charlotte, N. C.
[2] Supplied by P. Leiner & Sons, America, Inc., St. Claire Shores, Mich.



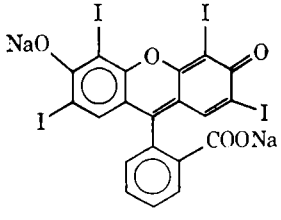
I: FD&C Blue No. 1



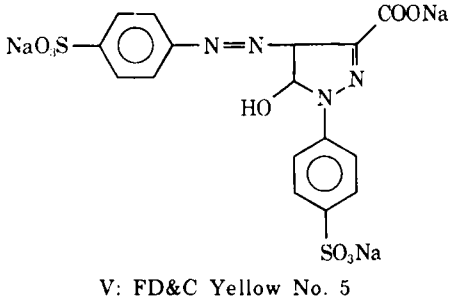
II: FD&C Blue No. 2



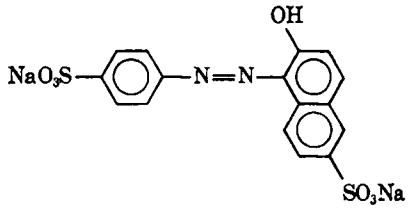
III: FD&C Red No. 2



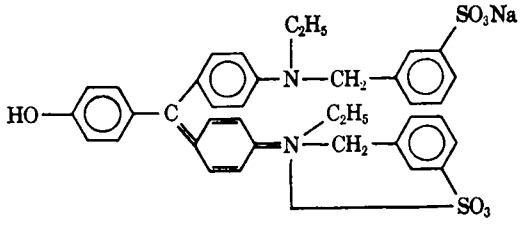
IV: FD&C Red No. 3



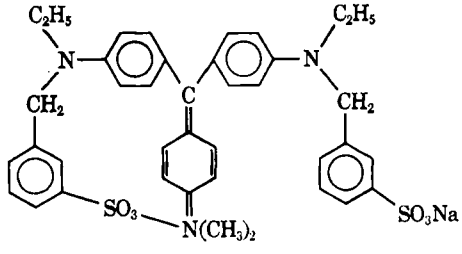
V: FD&C Yellow No. 5



VI: FD&C Yellow No. 6



VII: FD&C Green No. 3



VIII: FD&C Violet No. 1

was repeated two more times. This method appeared in the literature with little attention to details (7–9) and reportedly removes inorganic ions and polypeptide fragments with a molecular weight range from 3000 to 20,000 (9). Following the third removal of supernate, the swollen gelatin particles were again combined with distilled water and the temperature was increased to 37° to dissolve the hydrated gelatin.

Stock Solution Preparation—A freshly prepared 5% gelatin stock solution was maintained at 37 ± 0.5° in a constant-temperature bath[3], and samples were pipeted from the immersed flask. Stock solutions of the various dyes were prepared to contain 0.01, 0.1, 1, and 10 mg./ml. A 5 *M* stock solution of sodium chloride was prepared by diluting 29.22 g. of sodium chloride to 100 ml. with distilled water. A 5 *M* urea solution was prepared by diluting 30.0 g. of urea to 100 ml. Simulated gastric fluid USP was prepared by diluting 2.0 g. sodium chloride, 3.2 g. pepsin, and 7.0 ml. hydrochloric acid to 1 l. with water. Simulated intestinal fluid USP without pancreatin was prepared by dissolving 6.8 g. of monobasic potassium phosphate in 250 ml. water and adding 190 ml. 0.2 *N* sodium hydroxide and 400 ml. water. The resulting solution was adjusted to pH 7.5 ± 0.1 with 0.2 *N* sodium hydroxide and diluted to 1 l. with water.

[3] Model MW 1172SSA, Blue M Scientific Co.

Pancreatin could not be added due to its interference with the visualization of gelatin disintegration.

Liquid Solution Procedures—In a preliminary investigation, the effects of pH, ionic strength, and urea and dye concentration changes on the λ_{max} and absorbance of aqueous 0.001% dye solutions (0.01 mg./ml.) were observed at 25 ± 2°. The various pH solutions were prepared by diluting 0.1 mg. of each dye to 10 ml. with constant ionic strength (μ = 0.2) buffer solutions (10). The sodium chloride–dye solutions were prepared by adding sufficient sodium chloride solution to make the final solution 1.0 *M*. Similarly, the urea–dye solutions were made 1.0 *M* with respect to urea. Each dye solution was scanned through the UV–visible range from 190 to 750 nm., utilizing a distilled water blank.

For each liquid-state solution in which changes in dye λ_{max} spectra were to be observed, the dye–protein sample, in a 1:100 dye–protein weight ratio, was run *versus* a blank with a similar quantity of protein. Each experiment consisted of a sample containing 0.10 mg. dye and 10 mg. protein in 10 ml. distilled water *versus* a blank of 10 mg. protein in 10 ml. solvent. The sample was incubated for 30 min. at 25 ± 2° prior to the observation of the spectra in all liquid-state experiments (7). The effects of sodium chloride and urea were observed separately by the addition of sufficient respective stock solutions to make the sample and blank solutions 1.0 *M* in urea or 0.154 *M* (0.9%) in sodium chloride. All dye–protein *versus* protein spectra were observed in the visible range only and were reproducible within 1 nm.

In those liquid-state experiments in which new bands were sought, the dye–protein samples, in a 1:10 dye–protein weight ratio, were run *versus* a similar quantity of dye in the blank solution. In these cases, 5 mg. dye was combined with 50 mg. protein in 10 ml. distilled water and run *versus* a blank containing 5 mg. dye/10 ml. The dye–protein *versus* dye experiments were repeated using the same ratio of dye to protein but diluted by a factor of 10 to observe the effect of dilution on the new bands obtained. Samples consisted of 0.5 mg. dye and 5 mg. protein in 10 ml. distilled water *versus* a blank of 0.5 mg. dye in 10 ml. of solvent. Sodium chloride and urea effects were also noted by making the sample and blank solutions 0.154 and 1.0 *M*, respectively, in concentration of each reagent for each separate experiment. New bands were sought in the range of 190–750 nm.

Solid Solution Procedures—All solid-state solutions were prepared by thoroughly mixing an appropriate amount of 5% gelatin stock solution at 37° with dye and other reagents (sodium chloride

Table I—FD&C Dyes with Synonym, Color Index Number, and Chemical Class[a]

FD&C Dye	Synonym	Color Index Number	Chemical Class	Percent Dye[b]
Blue No. 1 (I)	Brilliant Blue FCF	42090	Triphenylmethane	91
Blue No. 2 (II)	Indigotine	73015	Indigoid	90
Red No. 2 (III)	Amaranth	16185	Monoazo	90
Red No. 3 (IV)	Erythrosine	45430	Xanthene	90
Yellow No. 5 (V)	Tartrazine	19140	Monoazo-pyrazolone	90
Yellow No. 6 (VI)	Sunset Yellow FCF	15985	Monoazo	89
Green No. 3 (VII)	Fast Green FCF	42053	Triphenylmethane	91
Violet No. 1 (VIII)	Wool Violet 5BN	42640	Triphenylmethane	91

[a] With the exception of FD&C Red No. 4, which is approved for use in maraschino cherries only. [b] Dyes may contain sodium chloride, sodium sulfate, leucoforms, intermediates, and subsidiary dyes.

Table II—Properties of Pharmaceutical Gelatins

Specification	Type A Gelatin	Type B Gelatin
Isoionic point	8.5	4.9
Gel strength, Bloom grams	170	255
Viscosity, millipoises	25.6	49.2
Water, %	9.8	10.2
Lot number	71058	4282

Table III—Liquid Solution Maxima for FD&C Dyes[a]

FD&C Dye	Wavelength, nm. UV	Visible
Blue No. 1	310	411, 630[b]
Blue No. 2	252, 284	610[b]
Red No. 2	217, 335	522[b]
Red No. 3	260, 312	526[b]
Yellow No. 5	258	428[b]
Yellow No. 6	237, 315	484[b]
Green No. 3	305	422, 625[b]
Violet No. 1	248, 307	545[b]

[a] As 0.001% unbuffered aqueous solutions at 25 ± 2°. [b] Major peak.

or urea). All films were prepared in this manner in a 13-cm. diameter, Teflon-coated pan suspended on the surface of the constant-temperature bath at 37°. The final liquid-state volume was sufficient to fill the pan bottom with an even distribution of solution (50 ml. or more in total volume). The liquid solutions were allowed to evaporate at room temperature in a laminar flow hood[4]. The resulting solid solution films were lifted from the pan, placed in a desiccator over anhydrous calcium chloride, and allowed to equilibrate to a constant weight. Then they were cut into strips suitable for spectrophotometric analysis (approximately 1 × 4 cm.). The blank film contained all chemicals used in the sample for the respective experiments except the dye. Sample films were examined for shifts in λ_{max} in the visible range and for changes in the Amide I (1625 cm.$^{-1}$) and Amide II (1520 cm.$^{-1}$) bands of the proteins in the IR study.

Films to be examined by visible and IR spectroscopy were prepared using 500 mg. protein and 5–100 mg. dye. Average film thickness was 25 μ. The addition of any amount of sodium chloride or urea tended to inhibit film formation. Nevertheless, up to 135 mg. sodium chloride or 900 mg. urea was added with variable effects on film formation depending on the dye. Higher amounts of either reagent made lifting any portion of film a practical impossibility.

Films prepared for dye release disintegration studies were made using 2500 mg. protein and 25 mg. dye and were equilibrated over a saturated solution of calcium chloride in a desiccator. To assess the effect of pH change, the dye–protein solutions that were to be examined by visible spectroscopy as films were adjusted to pH 7 on a pH meter[5] with dilute sodium hydroxide solution before evaporation. All films for a particular experiment were prepared simultaneously. Average thickness of films prepared for dye release and disintegration studies was 130 μ.

Dye Release Study Procedure—Disks prepared for the dye release study were made by punching out portions, approximately 1.2 cm. in diameter and 20 mg. in weight, from the 2500-mg. protein–25 mg. dye films with a No. 6 cork borer attached to a drill press. For each dye release experiment at 37°, an accurately weighed disk was placed in an NF XIII dissolution basket, which was attached by a 30-cm. stainless steel rod of 0.6-cm. diameter to a variable speed, reversible, 1/40-hp. motor[6]. The basket rotation speed was 20 r.p.m. The rotating basket plus disk was centered on a 100-ml. beaker containing 50 ml. distilled water at a temperature of 37 ± 0.5°, and the bottom edge was placed approximately 2 cm. from the bottom of the beaker after allowing the hydrated film to adhere and flatten against the bottom of the basket. In the 27° experiments, the disk was placed in the same size beaker and volume of water, and a propeller of 3.0-cm. diameter replaced the basket. The agitation speed was 40 r.p.m. but did not appear to affect dye release in the range of 20–120 r.p.m. at 27 ± 0.5°, as long as the propeller did not physically tear the swollen, freely rotating film apart.

The vessel size and volume parameters were adjusted so that an approximately 1-ml. volume could be repeatedly withdrawn at the same location and read directly within Beer's law limits of 18–82% transmittance in a 0.5-cm. microcell with a solvent blank of distilled water. The withdrawn sample was returned to the experiment within 10–15 sec. after withdrawal; the solution loss due to this process and evaporation was 2–3% over 40 min. at 37° and 1–2% at 27° over 1–200 hr. Longer experiments at 27° were sealed in aluminum foil to prevent excess evaporation and to prevent photodecomposition of the dyes. A solution of similar protein weight per unit volume (20 mg. gelatin in 50 ml. water) was scanned through the visible region in which the λ_{max} occurs and did not absorb nor scatter any portion of that spectrum when run *versus* a distilled water blank.

The study at 27° was intended to determine the relative release times of 50% of the dyes from a gelatin film in distilled water that is not simultaneously dissolving, as a relative measure of protein binding of 1% dye within the film. Gelatin films have a "melt point" of 35 ± 5°, at which point the hydrated protein passes into colloidal solution (9). The purpose of the 37° study was to determine if the differences in dye release could also be observed when polymer dissolution is occurring simultaneously with dye diffusion and dissolution and if those differences have the same rank order as when the film is merely releasing dye. All dyes, when placed in similar conditions and concentrations without protein, dissolved within 1 min. The percent dye released at various specific time intervals for each dye–protein combination from at least three separate experiments was averaged, and the 50% release times were read directly from the graphs.

Disintegration Study Procedure—Gelatin disks were prepared by pressing out portions, approximately 2 cm. in diameter and 50 mg. in weight, of the 2500-mg. protein–25-mg. dye films with a No. 14 cork borer. Each disintegration experiment consisted of placing an accurately weighed disk in the center of a round-bottom 500-ml. beaker filled with 200 ml. of simulated gastric or intestinal fluid, with or without the respective enzyme (pepsin or pancreatin), equilibrated to 37 ± 0.5°, and covering the disk with a 2.54-cm. square of 8-mesh stainless steel screen. To allow sufficient time for the gelatin to adhere firmly to the bottom of the beaker, agitation was not begun for 1 min. At 1 min. from the start of the experiment, a three-

[4] Model C 1001, Air Control, Inc.
[5] Radiometer type TTTlc.
[6] Model GT 21-18, G. K. Heller Corp.

Table IV—Protein Effect on Dye Maxima (Nanometers) and Absorbance (A)[a] from 1:100 Dye–Protein *versus* Protein Spectra in Liquid Solution at 25°

FD&C Dye	Type A Gelatin with Dye			+ Sodium Chloride		+ Urea		Type B Gelatin with Dye	
	λ_{max} Obtained	Change	A	λ_{max}	A	λ_{max}	A	λ_{max} Obtained	A
Blue No. 1	632	+2	D	630	I	NC	NC	630	NC
Blue No. 2	613	+3	D	NC	NC	NC	NC	610	NC
Red No. 2	525	+3	D	522	I	NC	NC	522	NC
Red No. 3	533	+7	D	529	I	NC	NC	526	NC
Yellow No. 5	428	0	D	—	—	—	—	428	NC
Yellow No. 6	484	0	D	—	—	—	—	484	NC
Green No. 3	626	+1	D	625	I	NC	NC	625	NC
Violet No. 1	540	−5	D	545	I	NC	NC	545	NC

[a] D = decrease, I = increase, and NC = no change.

blade, 5.0-cm. diameter, polyethylene propeller attached to a hollow rod of the same material, with a concentric 0.3-cm. diameter stainless steel rod inside, was started at 40 r.p.m. by a variable-speed motor[7] controlled by a constant-speed and torque control unit[8]. The disintegration end-point was taken to be the complete visual disappearance of the gelatin disk. All various dye–protein combinations were observed in at least three separate experiments, and their disintegration rates (as milligrams per minute) were averaged.

UV–Visible Spectroscopy Method—The visible spectra for both liquid- and solid-state solutions and the UV spectra for some liquid dye–protein *versus* dye and all dye reference solutions were determined using a spectrophotometer[9]. Matched cells with a 1-cm. path length were employed for the liquid solutions, and the various film strips (approximately 1 × 4 cm.) were placed in liquid sample holders of the spectrophotometer and held in the beam path by the clip used to secure the liquid sample cells. A scan speed of 1.6 and 3.2 nm./sec. was used in the UV and the visible region, respectively. The spectrophotometer was calibrated using a holmium oxide standard. Where absorbance and/or scattering were high, appropriate filter screens were used.

For the diffusion study, a 0.5-cm. microcell was utilized[10].

IR–Attenuated Total Reflectance Method—The IR-attenuated spectra of the various films were observed in the 2000–625-cm.$^{-1}$ region, utilizing an IR spectrophotometer[11] equipped with a attenuated total reflectance attachment[12] and a 50 × 20 × 2-mm. thallous bromide–iodide internal-reflecting crystal[13]. Film strips cut to fit the crystal dimensions were placed on both sides of the crystal. An angle of incidence of 45° and minimal plate pressure were used to obtain the spectra at a scan speed of 1 cm.$^{-1}$/sec. Since only 30–40% of the expected transmission is obtained with the KRS-5 plate (11), a variable-slit filter[14] was placed in the reference beam for all experiments. The spectrophotometer was calibrated using a polystyrene standard.

RESULTS AND DISCUSSION

UV and Visible Maxima of FD&C Dyes—Table III lists the maxima of 0.001% unbuffered (pH 6.6–7.0) aqueous dye solutions at 25 ± 2°. By varying the pH from 1 to 13 at constant ionic strength (μ = 0.2), it was found that the dyes exhibited the same maxima at pH 6–7 as in unbuffered solution. At lower and higher pH values, the dyes exhibited changes in both maxima and absorbance. Sodium chloride had no effect on unbuffered dye maxima with the exception of FD&C Violet No. 1; this dye's visible maximum wavelength shifted hypsochromically to 540 nm. Urea had no effect on unbuffered dye maxima. Both sodium chloride and urea produced a variable effect on absorbance, from no effect to less than a 5% decrease in absorbance. The spectral effect of concentration changes from 0.001 to 20% dye can be summarized with the general statement that as concentration increases the visible region bands broaden and may shift their center in a random manner.

Liquid Solution Interaction—Spectrophotometric evidence of interaction between substances may take the form of a change in maximal wavelength, absorbance, or both. The results of liquid solution dye–protein *versus* protein spectra are summarized in Table IV. The pH value of all combinations of dye and protein ranged from 6.5 to 7.0. Figure 1 illustrates the spectral interaction effect with the two dyes that appeared to interact to the greatest extent with the gelatins. Since interactions between dyes and type B gelatin appeared to be minimal, sodium chloride and urea effects were not investigated.

The masking effect of sodium chloride and the lack of urea effect lend evidence that the observed liquid solution interactions were primarily electrostatic in nature. By reversal of the overall charge on the type B gelatin (to a positive sign) by changing the solution pH to 1, a type A gelatin shift occurred when the charge-reversed type B gelatin and Blue No. 1 were combined. This finding lends further support to the hypothesis that the liquid solution interactions of at least the three triphenylmethane dyes (Blue No. 1, Green No. 3, and Violet No. 1) are probably due to electrostatic considerations. However, in the case of the Red No. 3 interaction, the addition of sodium chloride did not return the wavelength to its original position (526 nm.). Even doubling the sodium chloride concentration (0.308 M) had no further effect; the wavelength remained at 529 nm. This may indicate a degree of irreversibility, binding other than hydrogen, hydrophobic, and/or electrostatic, or both. Anderson and Boyce (12) found that erythrosine (FD&C Red No. 3) interacts in equimolar aqueous solution with polyvinylpyrrolidone, a nonionic polymer, producing a greater shift (to 538 nm.). They postulated that electrostatic and van der Waals' bonds might be responsible, but they did not investigate the mechanism(s) of binding. Nevertheless, the fact that Red No. 3 does bind with a nonionic polymer does imply a capability to bind in other than an electrovalent manner. Otto (13) and Gustavson (14) considered the overlap of π-electron clouds of dye and polymer to be the primary force responsible for

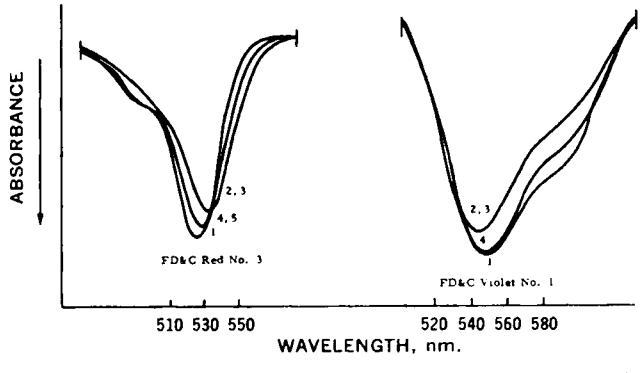


Figure 1—*Effect of type A gelatin on the absorption spectra of FD&C dyes Red No. 3 and Violet No. 1. Key: 1, dye, 0.001% in water; 2, dye with 0.1% type A gelatin; 3, dye–gelatin in 1.0 M urea; 4, dye–gelatin in 0.154 M sodium chloride; and 5, dye–gelatin in 0.308 M sodium chloride.*

[7] E. C. Motomatic model E600-013.
[8] Cole-Parmer model 4425.
[9] Perkin-Elmer model 202.
[10] With a model 9085-D Roto-Cell attachment (Arthur H. Thomas Co.). This cell holder was used in place of a standard cell holder in a Spectronic 20 photometer.
[11] Perkin-Elmer 237*b*.
[12] Model 9000, Wilks Scientific Corp.
[13] KRS-5, Wilks Scientific Corp.
[14] Barnes Engineering Co.

Table V—Bands (Nanometers) Obtained from 1:10 Dye-Protein *versus* Dye Spectra at 25° before and after 10-Fold Dilution

FD&C Dye	Before Dilution: Type A Gelatin, Dialyzed	Before Dilution: Type B Gelatin, Dialyzed	After Dilution: Type A Gelatin, Dialyzed	After Dilution: Type B Gelatin, Dialyzed
Blue No. 1	348, 430, 672	351, 432, 673	—	—
Blue No. 2	398, 408, 672	398, 408, 673	—	—
Red No. 2	590	593	—	—
Red No. 3	392, 566	380, 562	500, 547	—
Yellow No. 5	316, 497	314, 497	—	—
Yellow No. 6	542	545	—	—
Green No. 3	360, 468, 683	359, 468, 686	—	—
Violet No. 1	397, 659	400, 670	—	—

Table VI—Solid Dye-Protein Solution Visible Spectrum Maxima[a,b]

FD&C Dye	λ_{max} of Type A Gelatin Dialyzed Systems, nm.: Dye-Protein Alone	Change from Liquid Solution Dye Alone[c]	Containing: pH 7 Buffer	Containing: Sodium Chloride	Containing: Urea	λ_{max} of Type B Gelatin Dialyzed Systems, nm.: Dye-Protein Alone	Change from Liquid Solution Dye Alone[c]	Containing: pH 7 Buffer	Containing: Sodium Chloride	Containing: Urea
Blue No. 1	638	+8	630	642	647	636	+6	626	624	615
Blue No. 2	620	+10	619	613	622	617	+7	614	613	625
Red No. 2	532	+10	530	528	534	532	+10	532	557	535
Red No. 3	542	+16	539	540	543	541	+15	540	553	542
Yellow No. 5	437	+9	440	437	463	438	+10	440	490	475
Yellow No. 6	487	+3	492	487	504	487	+3	486	527	521
Green No. 3	632	+7	630	626	616	631	+5	632	620	628
Violet No. 1	557	+12	567	557	601	554	+9	554	599	600

[a] Average film thickness was 25 μ. [b] At 25°. [c] Liquid values are in Table III.

dyestuff fixation. This could lead to a hypothesis that overlapping π cloud hydrophobic bonds play a major role in binding erythrosine to type A gelatin.

Table V summarizes the band search. The purposely high (1:10) ratio of dye to protein as well as overall solution concentration of dye (0.05%) and protein (0.5%), as compared with 0.001% dye and 0.1% protein in the dye-protein *versus* protein experiments, was employed to investigate the effect of excess dye and dye aggregation on dye-gelatin interactions. As can be seen from the effect of 10-fold dilution, all bands disappeared except those with erythrosine and type A gelatin. The bands of undiluted combinations are characteristic of the dye irrespective of the gelatin type, and this could be evidence of dye-dye aggregation. Figure 2 shows the new bands obtained with the erythrosine-type A gelatin dilution combination and the effects of both urea and sodium chloride on the absorbance of the new bands. The effect of urea or sodium chloride on the 547-nm. band led to the hypothesis that the interaction represented by this new band involves both electrostatic and hydrogen bonding. The enhancement of the absorption of the 500-nm. band by sodium chloride suggests an ionic mechanism for this band, which is intensified by an increase in ionic strength.

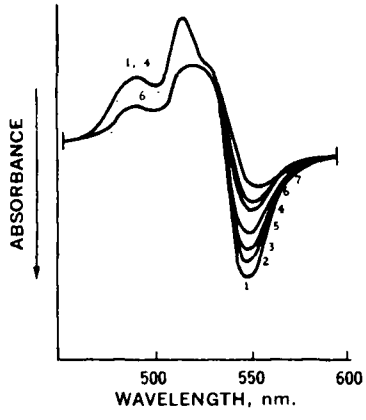


Figure 2—*Effect of sodium chloride and urea on Red No. 3–type A gelatin* versus *Red No. 3 spectra. Key: 1, 0.005% dye–0.05% protein in water* versus *0.005% dye; 2, dye-protein in 0.2* M *urea; 3, dye-protein in 0.5* M *urea; 4, dye-protein in 1.0* M *urea; 5, dye-protein in 0.077* M *sodium chloride; 6, dye-protein in 0.154* M *sodium chloride; and 7, dye-protein in 0.308* M *sodium chloride.*

Visible Spectrum Solid Solution Maxima—The visible absorption bands obtained from dyed films are shown in Table VI. The similarity of shifts with either protein suggests that the shifts may be due to solid-state dye in solution characteristics rather than solely an interaction phenomenon. While the solid solution bands for the dye-protein films without buffer, sodium chloride, or urea are as sharp as liquid solution bands, the bands obtained from dye protein films that had buffer solution, sodium chloride, or urea added before evaporation to films show variable width in their band characteristics. In addition, it should be reemphasized that any additive to the dye-protein solution seriously affected film structural integrity. Figure 3 shows bands obtained from the erythrosine-protein series with and without additional reagents and the spectra obtained for solid-state erythrosine recrystallized on a cell face.

The range of total shift from liquid solution dye without protein to solid protein-dye solution showed wide variability between dyes. Sunset yellow exhibited the least shift (+3 nm.) and erythrosine the greatest shift with either protein (+16 nm. with type A and +15 nm. with type B gelatins).

Some investigators (1, 15–18) studied solid-solid interactions of dyes and various drugs with metallic and nonmetallic adjuvants using diffuse reflectance spectroscopy. Spectra are broader and less intense in diffuse reflectance spectroscopy than solution transmittance spectra. They stated that spectral changes in diffuse reflectance spectroscopy in the order of 5–10 nm. are usually associated with physical adsorption processes. Chemisorption changes are several times this range in magnitude (18). Although the authors (1, 15–18) refer to the spectral changes observed as solid-state surface interactions, most drug-adjuvant combinations are equilibrated in aqueous suspension to facilitate maximal interaction. However, the interactions appear to occur in the solid unequilibrated states in the presence of moisture (17). The bearing that these findings have on the dye-protein solid solution spectra is debatable due to differences in spectroscopic method, method of preparation, physical state (solid particle surface interaction *versus* solid solution in this study), and substances combined with the dyes or drugs. There is basis for the belief from the results (1, 15–18) that the shifts observed in this study on the transition of dye-protein from liquid to solid solution are

Table VII—Results of IR-Attenuated Total Reflectance Study[a] at 25° as Changes in Amide I (1625 cm.$^{-1}$) and/or Amide II (1520 cm.$^{-1}$)[b]

FD&C Dye	Gelatin Type	Dye Concentration 1% I	1% II	2% I	2% II	5% I	5% II	10% I	10% II	20% I	20% II
Blue No. 1	A	NC	NC	NC	NC	+4	NC	+8	−10	+10	+7
	B	NC	NC	NC	NC	−6	+13	−5	−8	−8	−10
Blue No. 2	A	NC	+8	+8	NC	−8	−4	+5	−5	NC	+10
	B	NC	NC	NC	NC	NC	NC	−13	−12	−8	−5
Red No. 2	A	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
	B	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
Red No. 3	A	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
	B	−10	−13	NC	−16	−5	+5	−9	−6	−10	−12
Yellow No. 5	A	NC	NC	NC	NC	−5	−5	+5	+3	+12	+13
	B	NC	NC	NC	NC	NC	NC	NC	NC	−5	NC
Yellow No. 6	A	NC	−6	+13	+10	−9	−8	+8	+7	+5	+11
	B	NC	NC	NC	NC	NC	+8	−12	NC	−8	−18
Green No. 3	A	NC	NC	NC	NC	NC	NC	+5	−7	+11	−5
	B	NC	NC	NC	NC	−5	NC	−7	−7	−8	−5
Violet No. 1	A	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
	B	NC	NC	NC	NC	−6	NC	−7	−5	−17	−16

[a] NC = no change. [b] All changes are in cm.$^{-1}$.

merely due to change in state. This is further substantiated in Table VI by the fact that individual bands of each dye with either protein (without added buffer, sodium chloride, or urea) are no more than 3 nm. apart.

Carroll (19), in a discussion of sensitizing dyes used in photography, stated that dye spectra in aqueous photographic gelatin-silver halide suspensions become more complex as a result of molecular interaction and dye aggregation in liquid solution, emulsion, and dried emulsion (film). Generally, the sensitizing dye absorption bands are shifted bathochromically in emulsion as compared to a solution of the dye by itself. Polymethine dyes commonly used as sensitizing dyes may shift from 0 to 190 nm. However, to be sensitized the dye has to be first adsorbed on the surface of silver halide crystals in an aqueous suspension. Furthermore, most polymethine dyes are cationic, whereas all FD&C dyes are anionic.

IR-Attenuated Total Reflectance Study—The effects of dye concentrations (on a gelatin weight basis) of from 1 to 20% on the Amide I and Amide II bands of the protein are summarized in Table VII. Changes in hydrogen bonding between molecules are usually indicated by shifts in the area of 3100–3600 cm.$^{-1}$ (8), but since many of the dyes absorb strongly in that area also due to O—H and N—H bonds present in their molecules, the Amide I (1625 cm.$^{-1}$) and Amide II (1520 cm.$^{-1}$) bands can be used to show hydrogen bonding changes. The Amide I band is characteristic of the C=O stretching vibrations, whereas the Amide II band is characteristic of the N—H deformation mode and may be coupled with the C—N stretching mode of the peptide group (8). The shifts observed in hydrogen bonding changes may be in either direction as is evidenced in Table VII (8). Figure 4 shows typical examples of shifts (or the

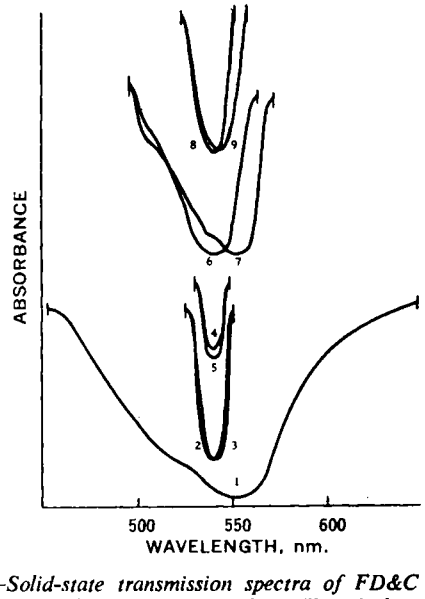


Figure 3—*Solid-state transmission spectra of FD&C Red No. 3 (1%) in type A gelatin and type B gelatin. Key: 1, dye recrystallized on cell face; 2, dye–type A gelatin dialyzed; 3, dye–type B gelatin dialyzed; 4, dye–type A gelatin dialyzed with pH 7 buffer; 5, dye–type B gelatin dialyzed with pH 7 buffer; 6, dye–type B gelatin dialyzed with urea; 7, dye–type B gelatin dialyzed with sodium chloride; 8, dye–type A gelatin dialyzed with sodium chloride; and 9, dye–type A gelatin dialyzed with urea.*

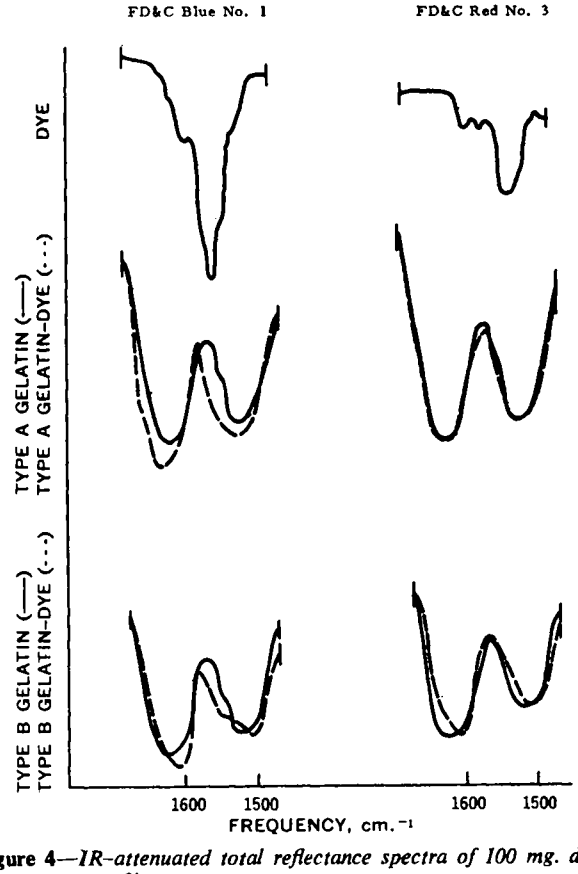


Figure 4—*IR-attenuated total reflectance spectra of 100 mg. dye–500 mg. protein films.*

Table VIII—Dye 50% Release Time (min.) into Distilled Water at 27 and 37° from Type A and Type B Gelatins[a,b]

FD&C Dye	27° Gelatin Type A	27° Gelatin Type B	37° Gelatin Type A	37° Gelatin Type B
Blue No. 1	744	10.8	15.8	6.3
Blue No. 2	792	7.5	—	—
Red No. 2	840	19.8	13.0	5.8
Red No. 3	9600	103	19.2	10.0
Yellow No. 5	1380	4.6	14.6	4.9
Yellow No. 6	588	6.8	13.8	4.8
Green No. 3	1092	18.0	12.7	7.2
Violet No. 1	6900	115	12.9	8.5

[a] Each value represents the average of three experiments. [b] Average film thickness was 130 μ; average disk weight was 20 mg.

lack of same) observed with the 20% dye spectra. Noteworthy is the lack of effect of Red No. 3 on type A gelatin hydrogen bonding. This lends support to the hypothesis that the Red No. 3-type A gelatin interaction involves primarily ionic and possibly hydrophobic types of bonding.

Nelson (20) investigated the photoelectric properties of dyed gelatin films and stated that the addition of dye to gelatin greatly increases the yield of charge carriers in a gelatin film. Charge carriers are able to mediate redox processes, which may enhance gelatin cross-linking. Erythrosine enhanced the irreversible UV "hardening" effect on gelatin in Nelson's work, but only after heavy exposures to UV light. Furthermore, Nelson's films were dehydrated by immersion in isopropanol before photoemission was observed.

From the data presented in Table VII, it can be seen that some dyes affect hydrogen bonding between polypeptide chains, but the effect is detectable for the most part only at higher ratios of dye to protein. Films of 0.1 mg. dye to 100 mg. gelatin were also examined for hydrogen bonding changes, but none was detectable.

Artemova and Usova (21) found that indigotine, tartrazine, and amaranth in 0.02% concentration in 3 and 6% gelatin gels hinder structure formation in gelation. Idson and Braswell proposed (22) that hydrogen bonding is the primary force in gel formation. Iijima and Sekido (23) found that, in the dyeing of protein, all of the *p*-hydroxy dyes and those *o*-hydroxy azo dyes, in which the OH group is not involved in an intramolecular hydrogen bond are adsorbed to the protein by a hydrogen bonding mechanism in addition to the electrostatic attraction between the sulfonic acid groups and amino groups of the protein. This has bearing on the finding that the *o*-hydroxy azo dye, amaranth, showed no effect on either gelatin's intermolecular hydrogen bonding at all concentrations. In the case of the other *o*-hydroxy dyes, tartrazine and sunset yellow,

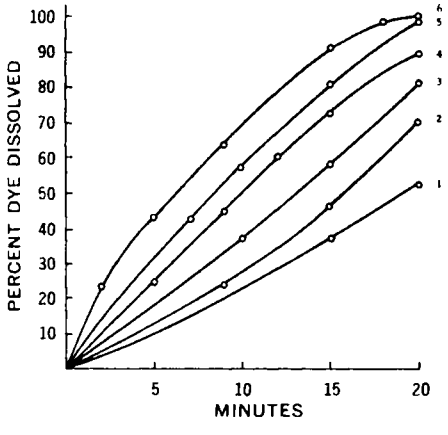


Figure 5—*Partial release curves for selected dyes from both proteins at 37°, 20 r.p.m., in NF XIII basket. Key: 1, type A gelatin dialyzed-Red No. 3; 2, type A gelatin dialyzed-Blue No. 1; 3, type A gelatin dialyzed-Violet No. 1; 4, type B gelatin dialyzed-Red No. 3; 5, type B gelatin dialyzed-Violet No. 1; 6, type B gelatin dialyzed-Blue No. 1. Each point represents the average of three experiments.*

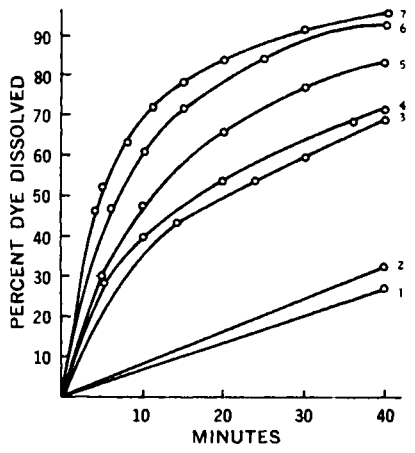


Figure 6—*Partial release curves for selected dyes from type B gelatin at 27°, 40 r.p.m. Key: 1, type B gelatin dialyzed-Violet No. 1; 2, type B gelatin dialyzed-Red No. 3; 3, type B gelatin dialyzed-Red No. 2; 4, type B gelatin dialyzed-Green No. 3; 5, type B gelatin dialyzed-Blue No. 1; 6, type B gelatin dialyzed-Yellow No. 6; and 7, type B gelatin dialyzed-Yellow No. 5. Each point represents the average of three experiments.*

effects on the hydrogen bonding of both proteins were seen. The *o*-hydroxy group of amaranth may be tied up in an intramolecular hydrogen bond, whereas the hydroxyls of the other two dyes apparently participate in intermolecular hydrogen bonding. Indigotine appeared to interact with hydrogen bonds of either type gelatin. The triphenylmethanes, with the exception of the Violet No. 1-type A gelatin combinations, appear to affect hydrogen bonding in either protein at primarily higher dye to protein ratios.

Other workers (7, 8) used IR-attenuated total reflectance to detect solid-state hydrogen bonding changes in gelatins combined with various carbohydrates.

Dye Release Results—Table VIII summarizes the times required for 50% dye release into distilled water from both proteins at 27 and 37°. Figures 5–7 illustrate representative examples of the release curves from which the data were obtained. The rank orders observed differed both between the two differently charged proteins and at the two different temperatures. Generally, the anionic FD&C dyes appeared to be more strongly bound to a cationic type A gelatin (Bloom No. 170, viscosity 25.6 millipoises) of lower Bloom strength and viscosity than to an anionic type B gelatin (Bloom No. 255, viscosity 49.2 millipoises) of higher Bloom strength and viscosity, at temperatures both within and below the "melt point" of gelatin.

At 27°, the FD&C Red No. 3 and FD&C Violet No. 1 appeared to be relatively tightly bound to both proteins. At 37°, the Red No. 3 appeared to be much more bound than any other dye. Reliable

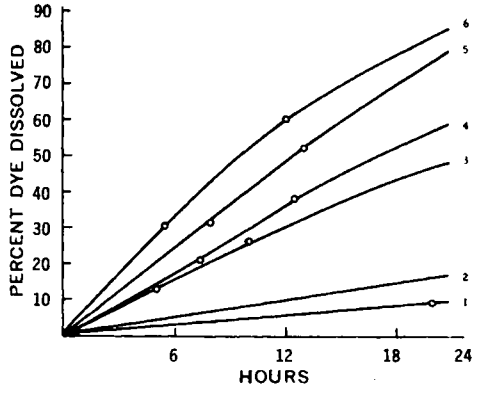


Figure 7—*Partial diffusion curves for selected dyes from type A gelatin at 27°, 40 r.p.m. Key: 1, type A gelatin dialyzed-Violet No. 1; 2, type A gelatin dialyzed-Red No. 3; 3, type A gelatin dialyzed-Yellow No. 5; 4, type A gelatin dialyzed-Green No. 3; 5, type A gelatin dialyzed-Blue No. 1; and 6, type A gelatin dialyzed-Yellow No. 6. Each point represents the average of three experiments.*

Table IX—Dyed and Undyed Gelatin Disk Average Disintegration Rates at 37°, 40 r.p.m., in 200 ml. of Simulated Milieu with or without Enzyme[a,b]

FD&C Dye	Average Disintegration Rate, mg./min.						
	Simulated Gastric Fluid				Micro-equivalents of Sodium/0.5 mg. of Dye	Simulated Intestinal Fluid —without Pancreatin—	
	Pepsin						
	Type A Gelatin, Dialyzed		Type B Gelatin, Dialyzed			Type A Gelatin, Dialyzed	Type B Gelatin, Dialyzed
	Without	With	Without	With			
Undyed	1.05	1.03	1.37	1.47	—	0.42	0.78
Blue No. 1	1.11	1.29	1.35	1.43	1.27	0.66	0.96
Blue No. 2	1.06	1.32	1.33	1.41	2.14	0.65	0.94
Red No. 2	1.07	1.44	1.34	1.44	2.48	0.61	0.87
Red No. 3	0.57	1.07	1.07	1.42	0.57	0.18	0.70
Yellow No. 5	1.09	1.37	1.34	1.52	2.81	0.66	1.02
Yellow No. 6	1.09	1.45	1.35	1.52	2.07	0.66	0.96
Green No. 3	1.06	1.37	1.35	1.28	1.23	0.65	0.93
Violet No. 1	1.09	1.17	1.36	1.34	0.68	0.60	0.83

[a] Each value represents the average of three experiments. [b] Average weight 50 mg.; average film thickness 130 μ.

spectrophotometric determination of FD&C Blue No. 2 at 37° was not possible. Others (24) found FD&C Blue No. 2 to be very unstable. Due to differences in method, revolutions per minute, and gelatin melting, comparison between the two temperatures should be approached with caution.

Disintegration Study Results—The effects of 1% dye concentrations, media, and the presence of pepsin on the average disintegration rates of both type gelatins are presented in Table IX. Although the end-point of these experiments was the disappearance of any trace of gelatin on the bottom of the beaker, the visual end-point can only be assumed to be the disintegration, dispersion, and beginning of the dissolution process or pseudodissolution rather than complete dissolution. That this was the case was confirmed by preliminary dissolution studies of undyed gelatin disks using a pH-stat[15] at pH values of 3 and 9 with a nitrogen flush to eliminate carbon dioxide alteration of the titration. Figure 8 shows that even after the gelatin had dispersed into the media, the titration of hydrated charged groups continued, indicating that the dissolution process was incomplete even though the disk was no longer visible. As a result, automation of the observation of the process would serve no useful purpose because the end-point would have to be observed visually in all experiments.

Overall, the gelatins disintegrated faster in simulated gastric fluid at pH 1.2 with or without pepsin than in simulated intestinal fluid without pancreatin at pH 7.5.

The xanthene dye FD&C Red No. 3, as might be expected from spectral and diffusion data previously given, had the most deleterious effect on average disintegration in the absence of pepsin in simulated gastric fluid or in simulated intestinal fluid. Even when pepsin was present, the type A–Red No. 3 disks in simulated gastric fluid did not disintegrate any faster than the undyed disks; *i.e.*, it appeared that pepsin did not accelerate the disintegration process as it had with other dyed disks. Xanthene dyes have been shown to inhibit the tryptic hydrolysis of casein (25). Perhaps erythrosine inhibited not only the intrinsic disintegration process by its presence in the gelatin matrix but, in addition, the diffused dye interfered with peptic action on the type A gelatin disk by complexation with enzyme. This did not appear to be the case with the type B gelatin–Red No. 3 disks in simulated gastric fluid with pepsin. However, a xanthene dye, rose bengal, has been shown to complex with trypsin (25).

The data indicate that pepsin appeared to have some effect on all disintegration rates in simulated gastric fluid with the exception of undyed type A gelatin. It was also observed that the inclusion of pepsin in simulated gastric fluid caused areas of the disk surface to be denuded within 2–5 min. into the experiment. These bare areas were irregularly shaped holes, which ranged from an estimated 20 to 50% of the disk surface. However, within several more minutes the hydrated protein reassumed a continuous circular surface with a slight convexity which increased as the experiment proceeded and the disk became smaller in diameter. Pepsin is an endopeptidase, *i.e.*, cleaves a polypeptide at peptide bonds within the chain (26). This would relieve restraints to hydration and swelling due to the intertwining of peptide chains. In addition, it would liberate progressively smaller and smaller peptide chains for the dissolution process. Results obtained in this investigation seem to agree with Czetsch-Lindenwald (27), who stated that pepsin accelerates gelatin capsule disintegration to the extent of only about 25%; type A gelatin–Red No. 2 disk disintegration rate was the most accelerated (35%) of all dye–protein combinations. On the other hand, pepsin seemed not to affect, or slightly decreased, the disintegration of undyed type A– or type B–Green No. 3 and type A gelatin–Violet No. 1 combinations.

The presence of dyes other than Red No. 3 in 1% concentration in type A gelatin in simulated gastric fluid with pepsin and both type A and type B gelatin in simulated intestinal fluid without pancreatin appeared to increase disintegration rates. This effect may be partially due to the number of microequivalents (μeq.) of sodium that are contained in the 0.5 mg. of dye in each 50-mg. disk. As can be seen in Table IX, the number of sodium microequivalents contained in each dye seems to divide the dyes into three distinct groups: those dyes with less than 1 μeq., those dyes with more than 1 and less than 2 μeq., and those dyes with greater than 2 μeq. of sodium/0.5 mg. While not absolutely correlatable, those dyes with higher microequivalent values may aid in the protein hydration process by not only pulling in water at a greater rate than those dyes with lower microequivalent values but also by diffusing outward at a greater rate (Table VII) and, in effect, allowing osmotic swelling to proceed at an increased rate. The effect may also be referred to as an internal "salting in" of the protein (28), whereby low concentrations of salts (0.05 *M* or less) solubilize the protein by breaking salt bridges and partially masking charges. The dye effect is particularly noteworthy in both type A and type B gelatins in simulated intestinal fluid. The type A gelatin disk disintegration is increased by as much as 57% by the presence of dyes; the type B gelatin disk disintegra-

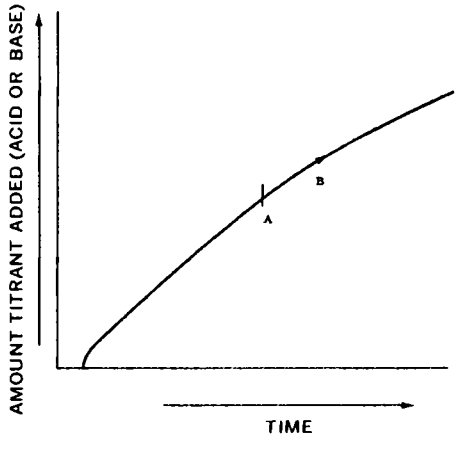


Figure 8—*Typical titration curve for type A or type B gelatin disks at pH-stat setting of 3 or 9 with acid or base titrant. Key: A, point at which disk disappeared completely and titrant continued to be added; and B, titrant may be added up to several hours beyond point A.*

[15] Radiometer, Copenhagen.

tion rate is increased by up to 31% by one dye. The pKa of sulfonic acid groups in most dyes is close to a value of 3; at a simulated intestinal fluid pH value of 7.5, the ionized form of the dyes would be predominant (23). At pH 1.2 in simulated gastric fluid, the dyes' sulfonic acid groups would be essentially (1%) unionized. In addition, the pKa of the carboxyl group of erythrosine is 4.95 (29). While approximately 1% of the dyes with sulfonic acid groups may be ionized at pH 1.2, only approximately 0.01% of the erythrosine could be ionized at pH 1.2. In addition, Red No. 3 is less soluble (9.0 g./100 ml. water, 25°) than the rest of the FD&C dyes (19.0–20.0 g./100 ml.), with the exception of Blue No. 2 (1.6 g./100 ml.) (30). At pH values of 5 or below, Red No. 3 is stated to be insoluble (30).

In summary, it appears that the effect of FD&C Red No. 3 on gelatin disk disintegration may be due to liquid and solid solution interaction with the gelatin, possible interaction with pepsin if present, and very low percent ionization and inherent insolubility at low pH values.

In vitro availability tests of capsule dosage forms, the shell of which is colored by erythrosine, may exhibit a delayed formulation release pattern as well as dye-hindered drug dissolution, particularly in acidic test media in which pepsin may have purposely been left out due to its interference with spectrophotometric determination of drug present in solution. *In vivo* effects would naturally depend on variables in stomach contents, emptying time, and drug absorption sites. Further studies on *in vitro* and *in vivo* effects with erythrosine and other xanthene dyes and dye classes are in progress.

SUMMARY AND CONCLUSIONS

1. A simple model of a gelatin shell wall was fabricated to examine dye–polymer interaction characteristics and disintegration behavior.

2. The model agreed to some extent with respect to previous findings of pepsin effect on disintegration of capsule shells.

3. In liquid solution, the anionic FD&C dyes tended to interact to a greater extent with a cationic type A gelatin than with an anionic type B gelatin.

4. All dyes interacted with both proteins in the solid state simply by virtue of the characteristics of the solid state; some dyes did affect the intermolecular hydrogen bonding of both proteins, but such effect was detectable only at relatively high concentrations.

5. The xanthene dye, FD&C Red No. 3 (erythrosine), interacted in both states of solution to the greatest extent of any FD&C class or type dye; the interaction may involve ionic, hydrogen, hydrophobic, and van der Waals' bonding, some of which may lead to a degree of irreversibility, even in the transition from solid to liquid solution state.

6. The triphenylmethane dye, FD&C Violet No. 1 (Wool Violet 5BN), interacted to some extent with the gelatins but primarily in an ionic manner that was reversible in liquid solution.

7. The FD&C dyes in 1% concentration were released at different rates from both types of proteins at temperatures both below and within the melt point of gelatins.

8. The dyes that exhibited the greatest spectral change activity (Red No. 3 and Violet No. 1) had the longest release times and adversely affected the disintegration of gelatin disks in either simulated gastric or simulated intestinal fluids; conversely, the dyes that exhibited the least spectral change activity generally appeared to be released quicker and actually may have enhanced disintegration, particularly those dyes with higher sodium microequivalent values when observed in simulated intestinal fluid.

9. It appears that, for a series of dye compounds, a spectral examination of their interactions with a polymer can yield some indication of their respective binding in liquid and solid solution. This observed indication coupled with a calculation of relative cation equivalents may lead to a presumptive hypothesis of an effect on both diffusion of the dye and the effect of the dye on the polymer's disintegration.

REFERENCES

(1) M. Bornstein, J. P. Walsh, B. J. Munden, and J. L. Lach, *J. Pharm. Sci.*, **56**, 1410(1967).

(2) J. Piccolo and R. Tawashi, *ibid.*, **59**, 56(1970).

(3) *Ibid.*, **60**, 59(1971).

(4) *Ibid.*, **60**, 1818(1971).

(5) E. B. Prillig, *J. Pharm. Sci.*, **58**, 1245(1969).

(6) H. Czetsch-Lindenwald, "Pure Gelatin Capsules," Monograph 2, S. A. Capsugel N. V., Basel, Switzerland, 1966, p. 2.

(7) E. E. Woodside, G. F. Trott, R. J. Doyle, and C. W. Fishel, *Carbohyd. Res.*, **6**, 449(1968).

(8) G. F. Trott and E. E. Woodside, *J. Colloid Interface Sci.*, **36**, 40(1971).

(9) B. Idson and E. Braswell, *Advan. Food Res.*, **7**, 297(1957).

(10) "Handbook of Chemistry and Physics," R. C. Weast, Ed., Chemical Rubber Co., Cleveland, Ohio, 1965, p. D-73.

(11) "Internal Reflection Spectroscopy," vol. 1, Wilks Scientific Corp., 1965, p. 5.

(12) J. C. Anderson and G. M. Boyce, *J. Pharm. Sci.*, **58**, 1425 (1969).

(13) G. Otto, *Das Leder*, **4**, 193(1953).

(14) K. H. Gustavson, "The Chemistry and Reactivity of Collagen," Academic, New York, N. Y., 1956, p. 313.

(15) J. L. Lach and M. Bornstein, *J. Pharm. Sci.*, **54**, 1730(1965).

(16) *Ibid.*, **55**, 1033(1966).

(17) *Ibid.*, **55**, 1040(1966).

(18) J. L. Lach and L. D. Bighley, *J. Pharm. Sci.*, **59**, 1261 (1970).

(19) B. H. Carroll, in "Scientific Photography," H. Sauvenier, Ed., Macmillan, New York, N. Y., 1962, p. 428.

(20) R. Nelson, *J. Chem. Phys.*, **39**, 112(1963).

(21) V. M. Artemova and E. M. Usova, *Izv. Vysshikh. Ucheb. Zaved., Pishch. Tekhnol.*, **2**, 46(1964); through *Chem. Abstr.*, **64**, 2659F(1966).

(22) B. Idson and E. Braswell, *Advan. Food Res.*, **7**, 308(1957).

(23) T. Iijima and M. Sekido, *Seni-i-Gakkaishi*, **16**, 406(1960); through *Chem. Abstr.*, **61**, 1992e(1964).

(24) C. A. Brownley and L. Lachman, *J. Pharm. Sci.*, **52**, 86 (1963).

(25) I. Ito, H. Ikezawa, and O. Ishizaka, *J. Hyg. Chem.*, **15**, 253 (1969).

(26) P. Karlson, "Modern Biochemistry," 2nd ed., Academic, New York, N. Y., 1965, p. 147.

(27) H. Czetsch-Lindenwald, *Pharm. Ind.*, **24**, 110(1962).

(28) H. Huettenrauch, *Pharmazie*, **2**, 107(1971).

(29) Z. Vodrazka, *Collect. Czech. Chem. Commun.*, **25**, 410 (1960).

(30) "FD&C Colors," Allied Chemical Corp., 1970, p. 7.

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