Iron-Adjuvant Interactions Observed with Diffuse Reflectance Spectroscopy

By MICHAEL BORNSTEIN*, JOHN L. LACH†, and BILL J. MUNDEN

Numerous adjuvants have been screened for the existence of chemical and physical interactions with iron compounds. Results indicate that calcium hydroxide, magnesium hydroxide, magnesium oxide, magnesium carbonate, magnesium citrate, zinc oxide, and potassium iodide do undergo large spectral and visual color changes on equilibration with ferrous sulfate in aqueous and nonaqueous systems.

ALTHOUGH chemical reactions of iron in solution have been previously investigated, very little information is available in the literature concerning the possible interactions of highly insoluble excipient materials with iron. In this communication, ferrous sulfate reactions have been studied with various organic and inorganic materials, using diffuse reflectance techniques.

Lach and Bornstein have recently shown that many of these insoluble adjuvants do undergo strong chemical interactions with a number of therapeutic agents (1–3) as well as certified dyes (4). Clarke (5) found that the addition of citric acid to a ferrous sulfate elixir increased its stability. Freedman (6), Uprety (7), and associates investigated the formation and stability of iron ascorbate complexes. Wagner (8) synthesized iron-gentisate complexes to be used for colorimetric assay. Lange and associates (9) studied a number of commercial preparations with respect to their ability to liberate iron from metallo-organic iron chelates and concluded that chelated iron is carried through the GI tract with less loss and lower toxicity than is ionic iron.

In view of the fact that the literature dealing with inorganic salt-iron complexes is limited and since many inorganic salts are not only integral parts of dosage forms, but may in themselves serve as the active therapeutic agent, it was felt that information concerning their solid-solid interactions would be of pharmaceutical importance.

EXPERIMENTAL

Reagents—Dried ferrous sulfate USP (Mallinckrodt Chem. Works), ferric chloride ACS (J. T. Baker Co.), sodium hydroxide pellets ACS (J. T. Baker Co.), acacia, aluminum hydroxide, aluminum stearate, calcium carbonate (dense), calcium hydroxide, cetyl alcohol, citric acid, kaolin, lactose, magnesium carbonate (light), magnesium hydroxide (heavy), magnesium citrate, magnesium stearate, Pharmasorb [colloidal (Minerals and Chemicals Philipp)], Pharmasorb [regular (Minerals and Chemicals Philipp)], polyethylene glycol 6,000, potassium iodide, sodium chloride, sodium phosphate (dibasic), sodium phosphate (tribasic), starch USP, stearic acid (flakes), stearyl alcohol (granular), Sterotex [edible peanut, cottonseed, and soybean oils (Capital City Products Co.)], tannic acid, tragacanth, distilled water, chloroform AR, and dehydrated alcohol.

Preparation of Ferric Hydroxide—Carefully weigh 50.0 g. triturated ferric chloride and 120.0 g. sodium hydroxide pellets. Place 1 l. distilled water into a 2-l. beaker and add the sodium hydroxide slowly. Stir until dissolved and add the ferric chloride. Mix for 15 min., vacuum filter, and wash the precipitate. Freeze the precipitate with the aid of a dry ice–methanol mixture, lyophilize for 24 hr., and triturate the resulting dark brown powder with a glass mortar and pestle.

Apparatus—The apparatus and technique of measurement have been reported (1–4).

Equilibration Technique—The procedure for preparing the samples and their corresponding controls has been reported (4). Thirty milliliters of aqueous or nonaqueous dispersion media has been employed in the equilibrations reported in this communication. A lyophilization technique (4) was used to remove the aqueous dispersion medium. The other nonaqueous solvents employed, including chloroform and dehydrated alcohol, were removed with the aid of ambient temperature vacuum evaporation.

RESULTS AND DISCUSSION

In view of the numerous difficulties encountered in pharmaceutical dosage forms containing iron, ferrous sulfate was selected as the inorganic material for this study of salt-adjuvant interaction. Based on DRS data obtained with respect to spectral intensity and visual color changes, it was evident that many of the interactions taking place were of a complex nature dealing with chemical and physical changes. These observations are summarized in Tables I and II.

For the purpose of illustration and discussion,

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TABLE I—IRON-CALCIUM CARBONATE REACTIONS USING AQUEOUS AND NONAQUEOUS DISPERSION MEDIA

Iron	Concentration Iron:$CaCO_3$, g.	Dispersion Medium	DRS Change[a]	Visual Color Change[a]
Ferric hydroxide	0.100:2.00	Water	0	No change[b]
Ferric hydroxide	0.400:2.00	Water	0-S	No change
Ferric hydroxide	1.00 :2.00	Water	0-S	No change
Ferric hydroxide	0.100:2.00	Ethanol	0-S	No change
Ferric hydroxide	0.400:2.00	Ethanol	0-S	No change
Ferric hydroxide	1.00 :2.00	Ethanol	S	No change
Ferric hydroxide	0.100:2.00	Chloroform	0-S	No change
Ferric hydroxide	0.400:2.00	Chloroform	S	No change
Ferric hydroxide	1.00 :2.00	Chloroform	0	No change
Ferrous sulfate	0.700:2.00	Water	L	Off-white to brownish-green
Ferrous sulfate	0.700:2.00	Ethanol	0	No change
Ferrous sulfate	0.700:2.00	Chloroform	M-L	Off-white to golden-brown

[a] Effects observed in comparing physically mixed controls to equilibrated samples. [b] No significant visual color change apparent. 0, No significant spectral change observed (≃DRS change of less than 5 reflectance units [RU] at $\lambda_{max.}$). S, Small spectral change (≃DRS change of 5–20 RU at $\lambda_{max.}$). M, Medium spectral change (≃DRS change of 20–50 RU at $\lambda_{max.}$). L = Large spectral change (≃DRS change of 50 or more RU at $\lambda_{max.}$).

several systems are presented in greater detail since space limitation does not permit a discussion of all the systems involved.

The diffuse reflectance data of an equilibrated $FeSO_4$-$CaCO_3$ sample and control are presented in Table I and illustrated in Fig. 1. An examination of these results indicates that some change has taken place as seen by the spectral differences reported for the control and the equilibrated and lyophilized sample. These observed changes are due not only to a surface adsorption phenomenon, but also to the existence of chemical reactions. The latter is true since although the solubility of $CaCO_3$ ($K_{sp.} = 0.87 \times 10^{-8}$) is limited, it is nevertheless sufficient to result in chemical reaction of small amounts of ferrous-ferric hydroxide. Although it is recognized that ferrous salts undergo oxidation, the addition of the oxidizing agent ascorbic

TABLE II—FERROUS SULFATE-ADJUVANT REACTIONS USING AQUEOUS DISPERSION MEDIA

Adjuvant	Concentration $FeSO_4$:Adjuvant, g.	DRS Change[a]	Visual Color Change[a]
Acacia	0.700:2.00	M	Beige to deep tan
Aluminum hydroxide	0.700:6.00	M-L	Tan to brown
Aluminum stearate	0.700:2.00	S-M	Off-white to yellow
Calcium carbonate	0.700:2.00	L	Off-white to dark gold
Calcium hydroxide	0.700:2.00	M-L	Tan to brown
Cetyl alcohol	0.700:2.00	0-S	No change[b]
Citric acid	0.700:2.00	S-M	No change
Kaolin	0.700:2.00	S-M	Off-white to dull gold
Lactose	0.700:2.00	S-M	Off-white to gray
Magnesium carbonate	0.700:2.00	L	Off-white to light brown
Magnesium citrate	0.700:2.00	L	White to olive green
Magnesium hydroxide	2.00 :2.00	L	Tan to olive green
Magnesium oxide	0.700:2.00	L	Off-white to olive green
Magnesium stearate	0.700:2.00	L	Light pink to golden brown
Pharmasorb (colloidal)	0.700:2.00	S-M	Tan to dull gold
Pharmasorb (regular)	0.700:2.00	S-M	No change
Polyethylene glycol 6,000	0.700:2.00	0	No change
Potassium iodide	0.700:2.00	L	Gold to dark brown
Sodium chloride	0.700:2.00	0	No change
Sodium phosphate (dibasic)	0.700:2.00	S-M	Gray to bluish-gray
Sodium phosphate (tribasic)	0.700:2.00	S	Tan to medium brown
Starch, USP	0.700:2.00	M	Off-white to yellow
Stearic acid	0.700:2.00	0-S	No change
Stearyl alcohol	0.700:2.00	S	No change
Sterotex	0.700:2.00	S	No change
Tannic acid	0.200:4.00	0-S	Tan to gray
Tragacanth	0.700:2.00	S-M	Beige to yellow
Zinc oxide	0.700:2.00	L	Beige to olive green

[a] Effects observed in comparing physically mixed controls to equilibrated samples. [b] No significant visual color change apparent. 0, No significant spectral change observed (≃DRS change of less than 5 reflectance units [RU] at $\lambda_{max.}$). S, Small spectral change (≃DRS change of 5–20 RU at $\lambda_{max.}$). M, Medium spectral change (≃DRS change of 20–50 RU at $\lambda_{max.}$). L, Large spectral change (≃DRS change of 50 or more RU at $\lambda_{max.}$).

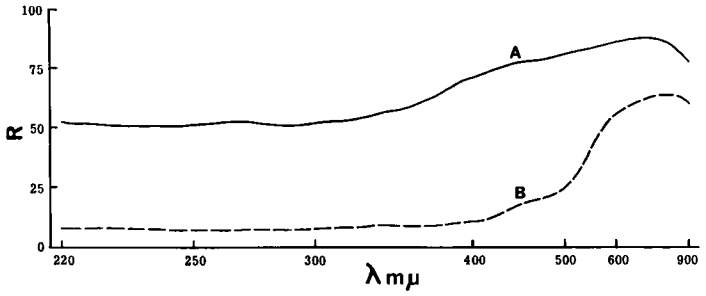

Fig. 1—DRS of dried ferrous sulfate (700 mg.) and calcium carbonate (2.00 g.). Key: A, control; B, water-equilibrated sample.

acid to the $FeSO_4$-$CaCO_3$ system did not alter the spectral characteristics to any appreciable extent.

The possibility that the spectral changes observed are due primarily to iron hydroxide formation was investigated and the results are presented in Table I and Fig. 2. The equilibration of a freshly prepared sample of $Fe(OH)_3$ with $CaCO_3$ at various iron concentrations, as shown in Fig. 2, indicates that the spectral changes observed here do not totally account for differences seen in the previous system. An examination of this $Fe(OH)_3$-$CaCO_3$ system indicates only minor spectral changes in comparing the control with the equilibrated sample. Only an intensity change, due to greater amounts of iron, is observed. The latter is due to a concentration effect (1, 3). Comparable spectra, Table I, were also obtained for this $Fe(OH)_3$-$CaCO_3$ equilibration when using chloroform or dehydrated alcohol as the dispersion medium. These results suggest that surface adsorption effects were minor in this $Fe(OH)_3$-$CaCO_3$ system since there was little spectral change between the controls and equilibrated samples in both aqueous and nonaqueous dispersion media.

Spectral changes observed in the $FeSO_4$-$CaCO_3$ aqueous system not only represent changes resulting from iron hydroxide formation, but also possibly due to the presence of iron oxides as well as spectral changes brought about by chemisorption of the various ionic species onto the insoluble calcium carbonate. It is significant to point out here that although the chances of iron hydroxide formation from ferrous sulfate in a chloroform medium are highly remote, the spectral changes obtained on equilibrating $FeSO_4$ wih $CaCO_3$ in this nonaqueous system did nevertheless produce significant spectral changes. Not only were these changes large, but they were also accompanied by important visual color changes. For example, the control, represented by Curve 3A (Fig. 3), is off-white in color as compared to a golden-brown chloroform equilibrated sample observed in Curve 3C. This is interesting in that the equilibration of ferrous sulfate with calcium carbon-

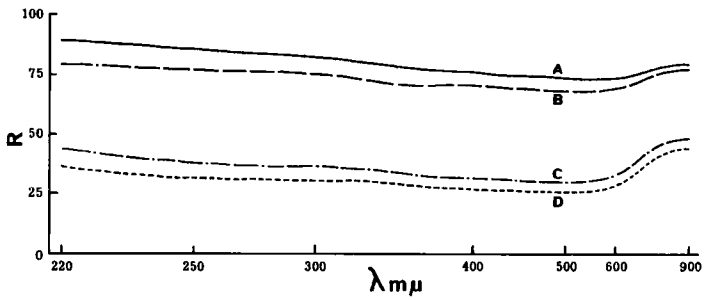

Fig. 2—DRS showing the effects of varying iron hydroxide concentrations in water-equilibrated samples, using 2.00 g. calcium carbonate as the adsorbent. Key: A, control, 100 mg. ferric hydroxide; B, sample, 100 mg. ferric hydroxide; C, control, 1.00 g. ferric hydroxide; D, sample, 1.00 g. ferric hydroxide.

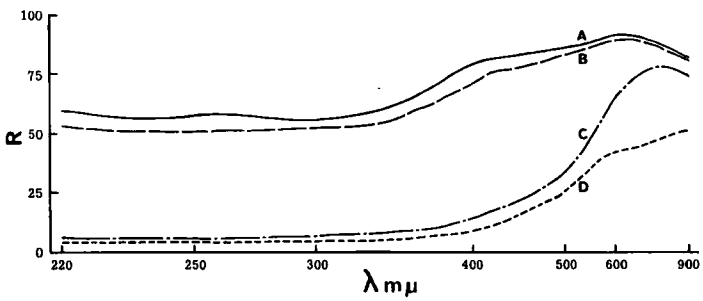

Fig. 3—DRS showing the effects of various solvents on equilibrating dried ferrous sulfate (700 mg.) with calcium carbonate (2.00 g.). Key: A, control; B, sample, equilibrated in 30 ml. dehydrated ethanol; C, sample, equilibrated in 30 ml. chloroform; D, sample, equilibrated in 30 ml. distilled water.

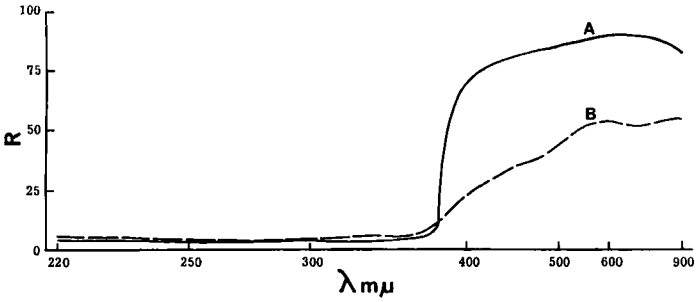

Fig. 4—DRS of dried ferrous sulfate (700 mg.) and zinc oxide (2.00 g.). Key: A, control; B, water-equilibrated sample.

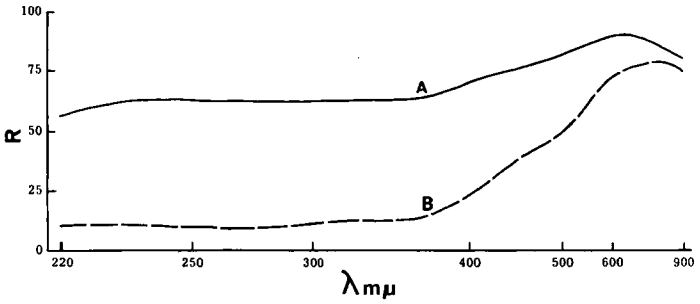

Fig. 5—DRS of dried ferrous sulfate (700 mg.) and magnesium stearate (2.00 g.). Key: A, control; B, water-equilibrated sample.

ate in a chloroform medium was expected to produce no significant changes since chemical reaction of these two inorganic salts in this medium is unlikely. Curve 3D, representing an aqueous equilibrated sample, which was brownish-green in color, indicates the presence of more interaction than the latter chloroform system. This greater reaction difference in the pressence of water is attributed to ionic reaction species. The $FeSO_4$-MgO, -$MgCO_3$, and ZnO systems presented in Table II exhibit spectral changes comparable to those observed for the $FeSO_4$-$CaCO_3$ equilibration, indicative of strong chemical and physical interaction. Another sample of this interaction is further illustrated in Fig. 4.

Although there is no evident reaction of ferrous sulfate with stearic acid, equilibration of ferrous sulfate with magnesium stearate does, however, produce a significant change as seen in Fig. 5. It is interesting to note the spectral changes brought about by the reaction of ferrous sulfate with citric acid and magnesium citrate presented in Table II as well as Figs. 6 and 7. Interaction of citric acid and its magnesium salt with ferrous sulfate was expected to result in spectral changes, in that iron-citrate complexes are known (1). It is of further interest to point out that the larger spectral changes observed with the magnesium citrate system were to be expected in view of the greater availability of the free citrate ion in solution.

Furthermore, although the spectral changes

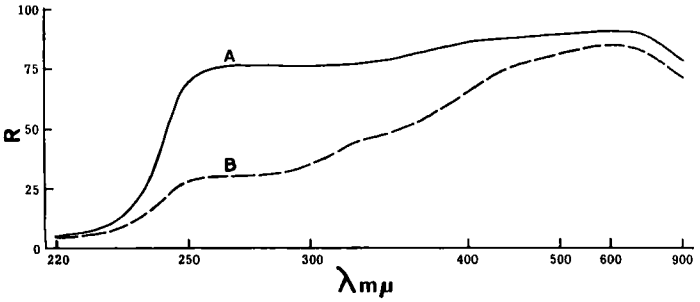

Fig. 6—DRS of dried ferrous sulfate (700 mg.) and citric acid (2.00 g.). Key: A, control; B, water-equilibrated sample.

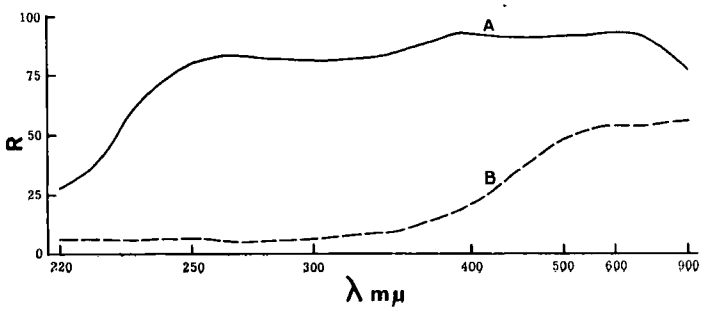

Fig. 7—DRS of dried ferrous sulfate (700 mg.) and magnesium citrate (2.00 g.). Key: A, control; B, water-equilibrated sample.

observed with other excipients studied in this investigation exhibit smaller changes, the spectral and color differences observed were indicative of some interaction. The diversity of the excipient or other type materials found in Table II also indicates that this ferrous sulfate interaction is widespread.

It should therefore be recognized that although analytical data with respect to a dosage form may substantiate the presence of the labeled amount of metallic ion, the form in which it exists in this product may certainly be one that is not available for a therapeutic response (11). Based on this ferrous sulfate-excipient interaction data, it does not seem unreasonable that other iron salts as well as salts used for other therapeutic purposes would also undergo such a phenomenon. A consideration of this possibility is therefore necessary in the development of pharmaceutical products.

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Keyphrases

Iron—adjuvant interactions
Ferrous sulfate-adjuvant reactions—aqueous dispersion
Diffuse reflectance spectroscopy—analysis

Tumor Localizing Agents VI

Radioiodinated Analogs of Dichlorodiphenyldichloroethane (DDD)

By R. E. COUNSELL, V. V. RANADE, P. POCHA, R. E. WILLETTE, and W. DiGUILIO

The preparation of 1,1-dichloro-2-(*p*-chlorophenyl)-2-(*o*-iodophenyl-^{125}I)ethane and 1,1-dichloro-2-(*p*-chlorophenyl)-2-(*m*-iodophenyl-^{125}I)ethane is reported. These compounds were prepared by chlorination of the appropriate iodoacetophenone, reduction to the corresponding carbinol, followed by acid-catalyzed condensation with chlorobenzene. Radioiodination was accomplished by isotope exchange with iodide-125. Preliminary studies in rats indicate that the position of the radioiodine has little influence on the predilection of these agents for adrenal tissue.

Wolf and Tubis (1) recently reviewed the rapidly expanding field of radiopharmaceuticals. As noted by these workers, various gamma-emitting radionuclides are now employed to externally scan many organs and major parts of the body.

Scanning has become a valuable diagnostic technique and permits the visualization of an internal organ by outlining the distribution of a radionuclide concentrating within an organ or tumor. With this technique, iodine-131 has been used to localize tumors of the thyroid, and chlormerodrin labeled with mercury-197 has served to delineate brain and kidney tumors. To date, however, no radiopharmaceutical is available for photoscanning the adrenal gland, although several laboratories are currently in the process of attempting to develop such an agent.

Nagai *et al.* (2) have reported that ^{131}I-labeled stigmasterol was useful in some cases for adrenal scanning. The agent was not regularly successful, however, because of the uptake in the liver and the excretion of free ^{131}I in the stomach. Similarly, because human adrenal glands con-

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