contribution of vitamin A to the degree of rearrangement of the trimethylsilane derivatives. No such effect was noted in this study using the propionate esters. However, some difficulty was encountered with high proportions of vitamin A causing a large "solvent front" in the region where the internal standard was eluting. This was readily overcome by using a higher level of internal standard which allowed a higher attenuation in the region of interest and a consequent reduction of the "front" magnitude.

The response of vitamin D relative to trioctanoin was determined over the range of weight ratios used by plotting the mean ratios of the peak areas against the weight ratios and calculating the equations of the lines by the method of least squares (Figs. 3 and 4). The lines passed through the origin and had standard deviations of 0.011 and 0.013 for vitamin D_2 and vitamin D_3, respectively.

Column overloading was observed when injections of the internal standard exceeded 4 mcg.

At the start of each day's run, the chromatogram of the first injection was discarded because its value was sometimes erroneous.

The precision attained by this method is indicated by the data in Tables II and III.

Blends for accuracy analysis (Tables III and IV) contained sufficient γ-tocopherol to produce a peak height equivalent to pyrovitamin D and vitamin A in excess of the amount normally encountered in multivitamin preparations.

For comparison, assays of a variety of samples which had recently been assayed by the procedure given in USP XVII are given in Table V. The "oil," "beaded," and "tablet" samples are multivitamin preparations of various potencies.

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X-Ray Diffraction Analysis for Identification of Kaolin NF and Bentonite USP

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Abstract □ The identities of kaolin NF and bentonite USP were tested by using the identification test stated in the NF XIII monograph on kaolin. The results were inconclusive because both clays gave positive results to the test. Since kaolin NF and bentonite USP are crystalline in nature, X-ray diffraction analysis could be used for their identification. Because of its accuracy, X-ray diffraction analysis is recommended to be included in the NF and USP monographs on kaolin and bentonite, respectively, as an alternative test.

Keyphrases □ Kaolin—identification □ Bentonite—identification □ X-ray diffractometry—identification, kaolin, bentonite

Kaolin was first introduced into the USP VIII in 1905 as a constituent of Cataplasma Kaolini. Later, it was deleted from the USP IX and was introduced into the NF IV in 1916. Bentonite was first introduced into the USP XII in 1942. The NF XIII monograph on kaolin includes a specific identification test, which is merely a test for the presence of aluminum. On the other hand, the USP XVIII monograph on bentonite does not include a specific identification test but relies on tests such as gel formation and swelling power.

Since both clays are chemically similar and contain aluminum, it was obvious that methods other than the chemical analysis for aluminum needed to be included in the NF monograph on kaolin in order to distinguish between kaolin NF and bentonite USP. Furthermore, it was felt that a specific identification test should be included in the USP monograph on bentonite. The objective of this study was to show the importance of the inclusion of X-ray diffraction analysis in the NF and USP monographs on kaolin and bentonite, respectively. Since both clays are crystalline in nature (1), it was possible to utilize X-ray diffraction analysis to measure the angles of diffraction of the X-ray from the atomic planes of the crystal.

EXPERIMENTAL

NF XIII Identification Test for Kaolin—The NF XIII identification test for kaolin was conducted on kaolin NF and bentonite USP. Both clays responded positively to the test for aluminum, *i.e.*, a gelatinous, white precipitate was produced.

X-Ray Diffraction Analysis—Two types of samples were prepared. An unoriented powder sample was prepared by packing the powder in an aluminum planchet. The powder was smoothed by

Table I—Basal (001) and Higher Order X-Ray Diffraction Spacings of Official Clays[a]

Kaolin NF	Bentonite USP	Glycolated Bentonite USP
7.1091	12.2670	16.2910
4.4445	4.4357	8.3544
4.3496	3.1163	5.6041
4.1639		4.4226
3.8176		4.2108
3.7169		3.3706
3.5587		
3.3731		

[a] Lattice spacings in angstroms.

using the flat side of a spatula to obtain a uniform level suitable to the X-ray beam. A sample of an oriented aggregate was prepared as follows: a 5% (w/w) aqueous clay suspension was prepared by sprinkling the clay over the surface of the water. The mixture was allowed to hydrate for 2 hr. and then was passed through a manual homogenizer. By means of a pipet, 2 ml. of the resultant suspension was withdrawn and allowed to drain onto a glass microscopic slide (27 × 46 mm.) which was completely covered with suspension. The slide was allowed to stand undisturbed at 22.2° (72°F.) and at a relative humidity of 52–55% for 72 hr. to permit air drying.

A Norelco X-ray diffractometer was used. Each sample was placed in the diffractometer and was exposed to a monochromatic X-ray beam generated by using a copper target X-ray tube and nickel filter operated at 40 kv. and 30 mamp. Scanning was begun at 2° (2θ) at the rate of 1°/min. The X-ray diffractogram obtained was recorded by a chart recorder. The sample was then transferred to a desiccator, in which an evaporating dish filled with ethylene glycol was placed. After exposing the sample to the ethylene glycol vapor for 24 hr., a second X-ray diffractogram was obtained as before. Table I shows the basal (001) spacings as well as the spacings of higher order X-ray reflections of each sample.

RESULTS AND DISCUSSION

The NF XIII defines kaolin as a native hydrated aluminum silicate; the USP XVIII defines bentonite as a native colloidal, hydrated aluminum silicate. Thus, the primary elements of kaolin and bentonite composition are aluminum and silicon. Because they contain aluminum in their structure, both clays gave positive results to the identification test of the NF XIII monograph on kaolin, which is basically a test for the presence of aluminum. This finding indicates that the identification test of the NF XIII is inconclusive. To distinguish between kaolin and bentonite, one needs to check other specifications in the NF and USP. The USP XVIII monograph on bentonite does not include a specific identification test.

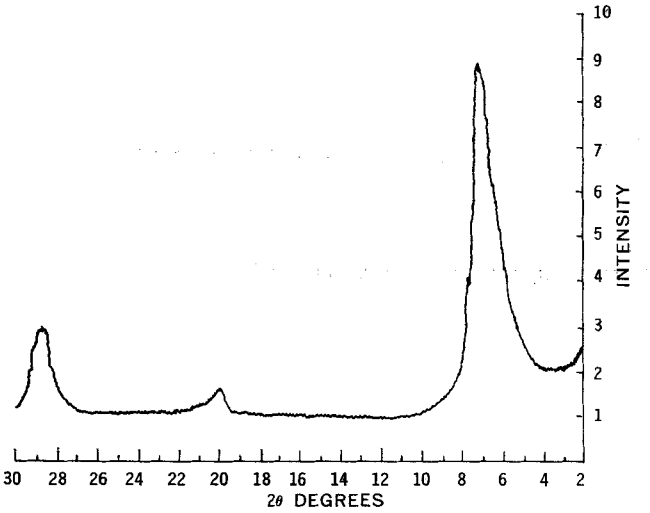

Figure 2—*X-ray diffractogram for bentonite USP.*

Although chemically similar, kaolin NF and bentonite USP differ mainly in their crystal structure. The unit cell structure of kaolin has two layers, stacked one over the other, with their flat sides together. One layer is composed of Al_2O_3 molecules and the other of SiO_2 molecules. The unit cell structure of bentonite is composed of three layers: two layers of SiO_2 molecules with one layer of Al_2O_3 molecules lying between them. This dissimilar assembly of the atoms permitted distinct identification of kaolin and bentonite by means of X-ray diffraction analysis. X-ray diffractograms for kaolin NF and bentonite USP are shown in Figs. 1 and 2, respectively. It is evident that these diffractograms differ and that each clay exhibits a characteristic series of X-ray diffraction peaks.

Because the tests of the official compendia are time consuming and obviously not conclusive, X-ray diffraction analysis was chosen as a methodology which could be conveniently and rapidly used for the identification of kaolin and bentonite. A total time of approximately 40 min. is needed to prepare an unoriented powder sample, obtain an X-ray diffractogram, and identify a sample. The purpose of exposing the clay samples to ethylene glycol is to provide added evidence as to the identity of bentonite and to distinguish it from kaolin. Bentonite is characterized by an expanding lattice. It was shown by MacEwan (2, 3) and MacKenzie (4) that ethylene glycol is adsorbed on the basal plane surface of bentonite and that the C-axis spacing is increased. Ethylene glycol, however, did not affect the nonexpanding lattice of kaolin and the basal plane remained unchanged. The basal plane of glycolated bentonite USP expanded from 7.20° (2θ) to 5.42° (2θ); kaolin NF, when glycolated, remained unchanged at 12.44° (2θ) (Figs. 1–3).

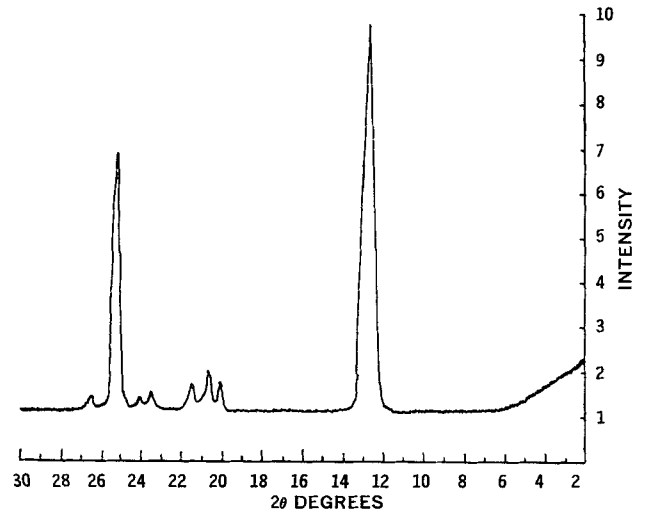

Figure 1—*X-ray diffractogram for kaolin NF.*

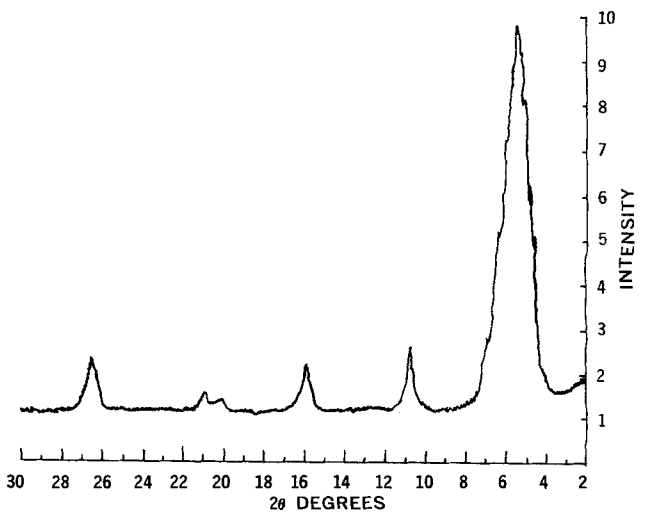

Figure 3—*X-ray diffractogram for glycolated bentonite USP.*

In conclusion, for identification of kaolin and bentonite, X-ray diffraction analysis was found to be more accurate, dependable, conclusive, and much less time consuming than the methods of identification presently employed in the NF XIII and USP XVIII monographs on kaolin and bentonite, respectively. Consequently, it is recommended that these monographs should be supplemented by X-ray diffraction analysis.

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Spectrophotometric Determination of Fe(II), Fe(III), and Total Fe

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Abstract □ The orderly use of two known reactions [Fe(II) with α,α'-dipyridyl and Fe(III) with ascorbic acid] allowed to take place successively in the same aliquot sample solution provides a procedure for the quantitative determination of Fe(II), Fe(III), and total Fe. The procedure is simple, rapid, and sensitive enough to cope with samples containing small amounts of iron or with samples of limited solubility. From a pharmaceutical point of view, the method is useful for the control of both hematinic raw materials or finished products, and it is especially useful for stability and content uniformity studies.

Keyphrases □ Fe(II), Fe(III), total Fe in aqueous solution—determination □ α,α'-Dipyridyl—color formation, Fe(II) determination □ Ascorbic acid—color formation, Fe(III) determination □ Hematinic salts—determination of iron content □ Colorimetric analysis—spectrophotometer

A simple procedure is presented for the simultaneous determination of Fe(II), Fe(III), and total Fe in a single aliquot from an aqueous solution of these components. This procedure can be readily adapted to content uniformity (1) and shelflife stability studies (2) on both hematinic raw materials and finished pharmaceutical products, especially those labeled to contain iron in its Fe(II) state. The titrimetric methods described by the USP (3) and NF (4) for total Fe on *finished products* are not feasible for single tablet or capsule analysis. Furthermore, these sources do not as yet provide methods for Fe(III) on finished products. These needs are met by the present procedure.

The procedure uses two well-known reactions [Fe(III) *versus* ascorbic acid to yield Fe(II), and Fe(II) *versus* α,α'-dipyridyl to yield a red complex] taking place in succession in the same aliquot solution. Initially, the intensity of the color produced by the reaction product of Fe(II) with α,α'-dipyridyl is measured against the blank to yield the Fe(II) content. Then solid ascorbic acid (10–15 mg.) is added to reduce any Fe(III) to additional Fe(II); the latter, in turn, reacts with the existing excess of α,α'-dipyridyl to produce an increase in color intensity. This intensity is then measured twice: once against the first colored solution yielding the Fe(III) content, and once against the blank yielding the total Fe content. Because of the small amount of solid ascorbic acid added, the volume increase of the final solution is negligible.

EXPERIMENTAL

Reagents and Instruments—The following were used: (*a*) α,α'-dipyridyl 0.1% solution in water; (*b*) buffer (pH 4.5) solution of 83 g. sodium acetate and 120 ml. acetic acid in water to a total volume of 1 l.; (*c*) ascorbic acid USP, crystalline powder; (*d*) Beckman DB-G double-beam spectrophotometer; (*e*) Beckman 25.4-cm. (10-in.) recorder for spectrophotometers; and (*f*) three matched cells, 1-cm. lightpath, marked as No. 1, No. 2, and No. 3.

Procedure—Using approximately 0.1 *N* HCl, dilute the soluble iron sample to an expected concentration of 10–300 mcg. Fe/ml. In the case of solid dosage forms, crush and powder the sample in a mortar, dissolve the iron salts with 0.1 *N* HCl, filter, and dilute as above. Transfer an aliquot, estimated to contain about 300–350

Table I—Analysis of Mixtures of Known Composition

Standard Mixtures[a]	Total Fe, —mcg./ml.— Taken	Found	Fe(II), —mcg./ml.— Taken	Found	Fe(III), —mcg./ml.— Taken	Found
Preparation A	2.60	2.58	0.81	0.82	1.79	1.76
Preparation B	2.83	2.84	1.63	1.62	1.20	1.22
Preparation C	3.19	3.16	2.26	2.21	0.93	0.95
Preparation D	2.94	2.97	1.69	1.70	1.25	1.27
Preparation E	2.43	2.45	2.43	2.44	0.00	0.01
Preparation F	2.33	2.36	0.00	0.02	2.33	2.34

[a] Prepared by dissolving six pairs of weighed amounts of ferrous and ferric ammonium sulfate. Single aliquots were then taken and diluted to indicated final concentration.