

# Pharmaceutical Applications of the Near Infrared Determination of Water

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The combination band for water at  $1.9 \mu$  has been observed in a series of solvent systems and for selected solid-state samples. Pharmaceutical applications of the measurement of this band are described.

ALTHOUGH SEVERAL STUDIES and analytical applications involving near IR water bands have been reported (1, 2), little has been developed concerning pharmaceutical systems. It is the purpose of this paper to expand pharmaceutical applications of measurements of the  $1.9\text{-}\mu$  combination band of water in the areas of (a) studies of water as a solute, (b) determinations comparable to Karl Fischer titrations of water, and (c) the direct determination of water in solids.

## EXPERIMENTAL

**Reagents**—Spectrograde acetonitrile was employed. All other solvents were reagent grade and except for chloroform were used without further purification. The chloroform employed was passed through a column of alumina to remove ethanol and used within 5 days.

**Instrumental Parameters**—The Beckman DK-2A settings employed for absorbance spectra and 0 to 100 scale percent transmittance spectra were: sensitivity, 50; time constant, 0.2; recording speed,  $18 \text{ m}\mu/\text{min.}$ ; and wavelength expansion,  $25 \text{ m}\mu/\text{cm.}$  These parameters were also employed for 100 to 90 expanded scale percent transmittance spectra with a change in time constant to 2.0 and recording speed to  $3.6 \text{ m}\mu/\text{min.}$  Solid-state samples were recorded at a maximum sensitivity of 312, time constant of 2.0, and recording speed of  $3.6 \text{ m}\mu/\text{min.}$  Wavelength calibration was on the basis of a spectrum of 1,2,4-trichlorobenzene (3).

**Spectra**—(a) Except where noted, water spectra were obtained in solution in 1 cm. near IR matching cells against the anhydrous solvents from 2,100 to  $1,700 \text{ m}\mu$ .

(b) Water in hydrophilic petrolatum was observed as a thin film between silica plates against a silica plate as the reference and under nitrogen purge from 2,150 to  $1,550 \text{ m}\mu$ . The 90 to 100% expanded transmittance range was employed with manual attenuation of the reference shutter required for hydrated ointment samples to establish a base line in this range.

(c) Solid-state absorption spectra were obtained against air and with manual attenuation of the reference shutter when required. From 25 to 40 mg. of compound was mixed with 60 to 90 mg. of pow-

dered and dried analytical grade KCl in a plastic capsule of the Wig-L-Bug<sup>1</sup> for 30 sec. Thirteen-millimeter disks were formed at 20 tons pressure for 5 min. in a specially constructed die.<sup>2</sup> Hydrated samples were pressed without vacuum while anhydrous samples were held in an evacuated die for 5 min. before being pressed for an additional 5 min. at 20 tons. Anhydrous samples and dry powdered KCl were obtained by drying these materials for 48 hr. under vacuum at  $110^\circ$ .

**Extraction Procedures**—(a) From 0.1 to 0.3 g. of insoluble hydrate samples accurately weighed were allowed to equilibrate with 5 ml. of 5% pyridine in methanol for 1 hr. at  $60^\circ$  in stoppered 10-ml. flasks. The samples were centrifuged at 1,000 r.p.m. for 10 min. and a portion of the supernatant liquid was transferred by pipet to a cell. This solution was read against a blank pyridine-methanol solution processed in the same manner and compared to standard series of water in pyridine-methanol solutions.

(b) From 10 to 15 g. of crude drug accurately weighed was extracted in an electric blender (Waring) for 7 min. with 100 ml. of methanol. The methanol solution was decanted, centrifuged, and read against an aliquot of a 100-ml. methanol blank processed in the same manner.

## RESULTS AND DISCUSSION

Greiner, Lüttke, and Mecke (4) in establishing water bands in both the IR and near IR laid the basis for the assignment of the  $1.9 \mu$  water band as a combination band of fundamental stretching and bending vibrations of water. These authors also established the effect on bond shape, intensity, and wavelength of maximum absorbance by various solvents in regard to the  $2.7\text{-}\mu$  fundamental stretching vibrations. These effects are considered hydrogen bonding phenomena (5).

Similar hydrogen bonding effects are exhibited by the  $1.9\text{-}\mu$  band of water in solvents. Figure 1 shows these variations for 0.2% v/v solutions of water in miscible solvents of interest in this investigation. Table I lists the wavelength of maximum absorbance for water in such solvents as well as for saturated water solutions in solvents in which water has only limited solubility.

These results are consistent with the recent work of O'Laughlin *et al.* (6) in their near IR study of phosphine oxides as bases in association with water in carbon tetrachloride. Their investigation in turn takes note of the report of Mohr, Wilk, and Barrow (7) which assigns OH stretching fundamental vibrations for free water, water bonded with one molecule of base (solvent),  $\text{B}\cdot\text{HOH}$ , and with water bonded to two molecules of base,  $\text{B}\cdot\text{HOH}\cdot\text{B}$ . Thus, based upon these references,

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<sup>1</sup> Crescent Dental Co., Chicago, Ill.

<sup>2</sup> Die constructed by Limit Div., Beckman Corp., Fullerton, Calif., according to the specification of Sister M. M. Stimson.

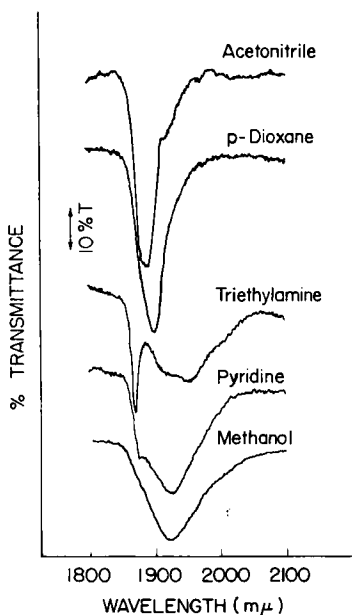


Fig. 1—Spectra of 0.5% v/v water in acetonitrile, p-dioxane, triethylamine, pyridine, and methanol.

the following assignments were made in the present investigation: (a) free water, 1.89 to 1.90  $\mu$  (values for dilute solutions in the inert solvents, carbon tetrachloride, and tetrachloroethylene); (b) free OH for water bonded at one H only, 1.90–1.92  $\mu$  (values for higher concentrations of water in intermediate basic strength solvents); and (c) bonded OH stretching, associated water and the bonded OH of both the B · HOH and B · HOH · B species, broad bands from 1.90 to 2.00  $\mu$  (values for higher concentrations of water in stronger basic solvents).

Figure 2 represents the Beer's law relationships

TABLE I—WAVELENGTH OF MAXIMUM ABSORBANCE FOR WATER IN VARIOUS SOLVENTS

Solvent	0.01% Water as Solute, $\mu$	0.2% Water and 12% Alcohol, $\mu$
n-Hexane	—	1,890; 1,932
Cyclohexane	—	1,885; 1,937
Triethylamine	1,882; 1,966 <sup>a</sup> ; 1,990 <sup>a</sup>	—
Carbon tetrachloride	1,886	1,887; 1,936
Tetrachloroethylene	1,887	1,885; 1,935
Benzene	1,891	1,897; 1,937 <sup>a</sup>
Toluene	1,891	1,897; 1,937 <sup>a</sup>
Carbon disulfide	1,892	—
Chloroform	1,893	1,891; 1,944 <sup>a</sup>
1,2-Dichloroethane	1,895	1,896; 1,945 <sup>a</sup>
Bromoform	1,896	1,898; 1,947 <sup>a</sup>
Ethyl acetate	1,897	—
Dibromomethane	1,899	1,900; 1,945
Acetonitrile	1,901	1,904
Acetone	1,903	1,903
Dioxane	1,911	1,915
Methanol	1,932	—
Ethanol <sup>b</sup>	1,887 <sup>a</sup> ; 1,935	—
Pyridine	1,943	1,894 <sup>a</sup> ; 1,939

<sup>a</sup> Wavelength reading at the center of a shoulder. <sup>b</sup> With 0.2% water.

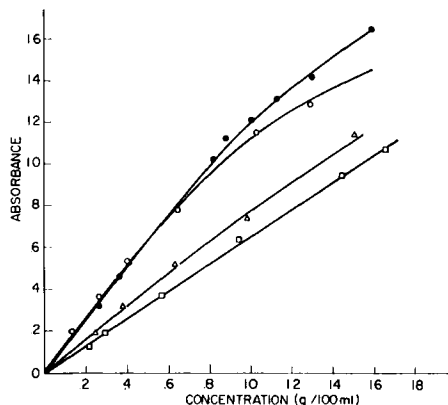


Fig. 2—Absorbance versus concentration relationship for water in p-dioxane at 1.91  $\mu$ , acetonitrile at 1.90  $\mu$ , pyridine at 1.94  $\mu$ , and methanol at 1.93  $\mu$ . Key: ●, p-dioxane; ○, acetonitrile; △, pyridine; □, methanol.

for some representative water-miscible solvents. The values plotted for the less basic solvents, acetonitrile and p-dioxane, are for the more intense peaks for these solvents at 1.9  $\mu$  while the broad bonded peaks are plotted for pyridine and methanol. Note the deviation from conformity to Beer's law at the higher concentrations for acetonitrile and p-dioxane which is consistent with the opportunity for more water to water association at higher water concentrations. This is also indicated by an increased ratio of absorbance in the 1.90 to 2.00- $\mu$  region as compared to 1.90  $\mu$  absorbance at higher water concentrations. Thus, while water can be quantitatively determined in a variety of solvents on the basis of its near infrared combination band, it is important to realize that range of conformity to Beer's law is limited in weakly basic solvents to the lower concentrations. It also follows that initial traces of water in the reagent employed has more effect in the weakly basic solvents. That is, while the absorbance of such water can be compensated in the reference cell, its contribution to increasing water associations is not compensated.

The sensitivity of the procedure is comparable to that of Karl Fischer titrations. Most measurements were made with normal 1 cm. near IR cells of 4-ml. capacity. Under these conditions, as shown in Fig. 2, less than 1% solution of water is required to obtain significant absorbance. Microcells, particularly a 50- $\mu$ l. cell,<sup>3</sup> were also found to be of value. For example, 50  $\mu$ l. of a methanol solution, which is a relatively opaque solvent at 1.9  $\mu$ , containing 0.5% water was readily measured in this cell against a methanol blank in a similar cell.

In an effort both to increase the solubility of water in the nonpolar solvents and to increase the range of conformity to Beer's law, a limited number of ternary systems were evaluated. These involved 0.2% water and 12% alcohol in various solvents. Typical results are illustrated in Fig. 3 and are summarized in Table I. It is particularly interesting that while there is an increase in the bonded OH bands (broad absorption in the 1.9 to 2.0- $\mu$

<sup>3</sup> Cell No. 97250, Beckman Corp., Fullerton, Calif.

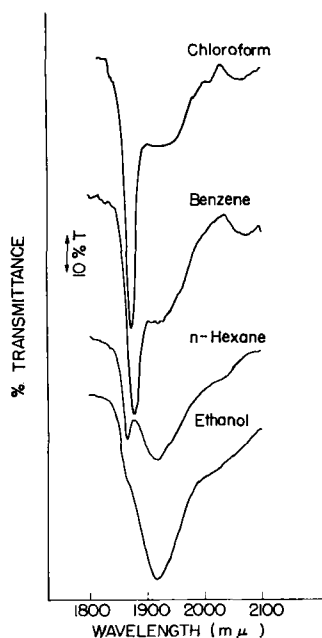


Fig. 3—Spectra of 0.2% v/v water and 12% v/v ethanol in chloroform, benzene, n-hexane as compared to 0.2% v/v water in ethanol.

region), there is also retention of essentially the same absorption of the sharp 1.9- $\mu$  bands which appear in the aprotic and the less basic solvents. It should be noted that a similar situation exists for the assignment of the lowest wavelength band in the fundamental stretching region. Errera, Gaspard, and Sack (8) pointed out in the fundamental region that low wavelength OH stretching absorption is retained in ternary systems.

Based upon conformity to Beer's law over a greater range, systems involving pyridine and methanol were evaluated for quantitative water determinations comparable to Karl Fischer determinations. Where a compound is soluble in these solvents and shows no interfering absorption in the 1.9- $\mu$  region, a direct measurement of the solution and comparison to standard water in solvent values becomes a possible moisture determination. Thus, as outlined in Table II, the water of crystallization for methylene blue and atropine sulfate was determined by solution in methanol. However, because methylene blue absorbs in the 1.9- $\mu$  region, as indicated by an atypical water in methanol spectrum, a base-line calculation method was used to obtain these results. The dehydration of a sample of cholesterol was also followed by dissolving it in pyridine and noting the decrease in absorbance at 1.92  $\mu$ . Ascorbic acid systems were of potential interest in that they cannot be determined directly in the Karl Fischer procedure. While water in presence of ascorbic acid can be determined in methanol as indicated in Table II, there again is the limitation of having to compensate for absorption by ascorbic acid at 1.92  $\mu$ .

The determination of water of hydration of stable hydrates by extraction can be illustrated by the successful results reported in Table II for ammonium oxalate and sodium tartrate. While

TABLE II—NEAR INFRARED WATER DETERMINATIONS

System	Found, %	Comparison Values, %
Solutions		
Atropine sulfate	3.96, 3.98, 3.96	3.35 <sup>a</sup>
Methylene blue	13.5, 13.4, 13.1	13.2 <sup>a</sup>
Water in the presence of ascorbic acid	1.25 2.55 3.81	1.26 <sup>b</sup> 2.52 <sup>b</sup> 3.79 <sup>b</sup>
Extracted water		
Stable hydrates		
Ammonium oxalate	12.3, 12.6, 12.3	12.68 <sup>c</sup>
Sodium tartrate	15.3, 15.7, 15.7	15.66 <sup>c</sup>
Crude drugs		
Belladonna leaves	6.04	6.62 <sup>a</sup>
Cinchona bark	6.87	6.51 <sup>a</sup>
Colchicum root	5.31	6.42 <sup>a</sup>
Podophyllum	7.54	7.27 <sup>a</sup>

<sup>a</sup> Water content based upon loss on drying. <sup>b</sup> Percentage of water actually added. <sup>c</sup> Amount present in pure crystal.

such water could not be efficiently determined with pyridine as the solvent, quantitative extraction was accomplished after 2 hr. at 60° with methanol. The addition of 5% pyridine permitted a reduction of the extraction time to 1 hr. Centrifugation was found to be a satisfactory method to remove traces of suspended solids without extended exposure to atmospheric moisture. In general, the stronger hydrogen bonding solvents were the most useful for quantitative measurements not only based upon their greater miscibility with water and conformity to Beer's law over greater range, but also because of their greater capacity to extract bonded water.

The Waring blender extraction procedure used by Mary (9) in the gas chromatography determination of water in crude drugs was applied for the assay of their moisture content by near IR measurements. It was found necessary to run a blank methanol determination in the electric blender in order to obtain consistent results in agreement with the NF (10) oven drying procedure. The results of these determinations are listed in Table II. The oven dehydrated drugs were also extracted and evaluated by the same procedure. Only the extract of the dried cinchona bark showed absorbance equivalent to water of about 0.5% of the hydrated drug.

The near IR moisture procedure was applied in connection with studies in progress<sup>4</sup> on the water content of the micellar system: Dioctyl sodium sulfosuccinate (Aerosol OT) (0.1413 g.), *n*-octane (6.925 g.), water (0.04 and 0.08 g.). Water could be determined directly in such a system against a reference anhydrous system and was not significantly different than water as a neat film. The quantitative comparison of water at the two levels (1:2) by direct measure of these systems was somewhat inhibited (1:1.93) by a nonlinear base line caused by light diffusion of the

<sup>4</sup> Samples provided by Drs. G. Zografis and S. Frank, University of Michigan.

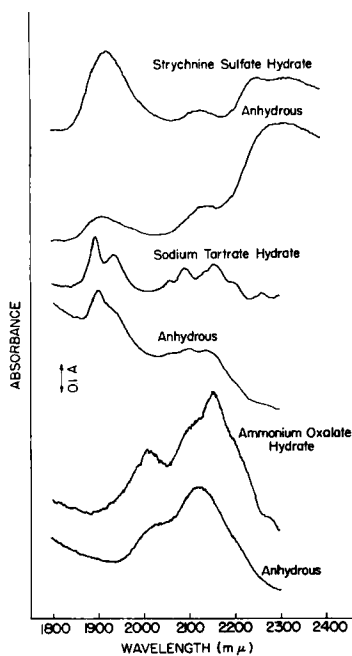


Fig. 4—Solid-state absorbance spectra for: (a) strychnine sulfate hydrate (0.0323 to 0.0793 g. KCl to form 0.1154-g. disk) compared to strychnine sulfate anhydrous (0.0356 to 0.0891 g. KCl to form 0.1154-g. disk); (b) sodium tartrate hydrate (0.0355 to 0.0681 g. KCl to form 0.0654-g. disk) compared to sodium tartrate anhydrous (0.0412 to 0.0925 g. KCl to form 0.1162-g. disk); and (c) ammonium oxalate hydrate (0.0272 to 0.0619 g. KCl to form 0.0776-g. disk) compared to ammonium oxalate anhydrous (0.0266 to 0.0797 g. KCl to form 0.0546-g. disk).

system. A quantitative ratio of absorbance was obtained, however, by quantitatively diluting 1 ml. of the micellar system with 4 ml. of methanol and determining the water content as compared to a standard water in methanol solution.

Water could also be readily observed in a hydrophilic petrolatum emulsion. Samples of ointment and water film were determined against a reference silica plate. While quantitative measurements of the water content of such an ointment were attempted using the intensity of CH overtone at 1.7  $\mu$  as an internal standard, these were only partially

successful. Both diffusion and lack of stability at higher water content interfered with quantitative results.

The opportunity to observe water directly in solid-state samples is an attractive feature of near infrared measurements. In Fig. 4 is shown a comparison of three pairs of hydrated *versus* anhydrous samples. While changes in absorbance in the 1.9- and 2.0- $\mu$  region were expected, the changes above 2.1  $\mu$  as found in sodium tartrate and ammonium oxalate were not anticipated. As 100-mg. KCl disks were prepared containing at least 25 mg. of compound, it was difficult to control diffusion and to obtain quantitative results.

In common with other spectral procedures, the major limitation of the near IR water measurements is the necessity to account for background and reference absorbance. However, the ease of obtaining data, the potential for microdetermination, and the ability to examine redox compounds make this technique a valuable addition to the Karl Fischer method.

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#### Keyphrases

Water determination  
Solvents, solids—water determination  
IR spectrophotometry, near—analysis