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Simple and Specific Assay of Penicillins by IR Spectrophotometry in Deuterium Oxide and Dimethyl Sulfoxide Solutions

BENITO CASU*x and PAOLO VENTURA‡

Abstract □ An IR spectrophotometric method for the qualitative and quantitative analysis of penicillins is described. The method is based on the inspection of the carbonyl region of the IR spectrum of penicillins dissolved in deuterium oxide or dimethyl sulfoxide solution and on the measurement of the absorbance of the β-lactam band at about 1760 cm^{-1}. Most common natural and synthetic penicillins are amenable to the present IR analysis in solution, without previous derivatization. The accuracy of the method is better than ±2% and is thus comparable to that usually attainable by current iodometric procedures. Since the solution spectra in the analytical region are characteristic for the individual penicillins, the described method is superior to iodometry as far as specificity is concerned. A single analysis usually requires no more than 15 min. The method allows a direct evaluation of the stability of penicillins in aqueous (deuterium oxide or deuterium chloride) solutions. Usual salts or buffers generally do not interfere with the analysis. Some qualitative and quantitative aspects of the IR spectra of penicillins in solution are discussed.

Keyphrases □ Penicillins—IR spectrophotometric analysis in deuterium oxide, deuterium chloride, or dimethyl sulfoxide □ Deuterium oxide—solvent for IR spectrophotometric analysis of penicillins □ Dimethyl sulfoxide—solvent for IR spectrophotometric analysis of penicillins □ IR spectrophotometry—analysis, penicillins, deuterium oxide, deuterium chloride, or dimethyl sulfoxide solutions

Current chemical methods for penicillin assay, such as those based on iodometric (1, 2) and acidimetric (3) titrations, are indirect and nonspecific. UV spectrophotometry is of limited applicability in penicillin analysis. The aminopenicillanic moiety of penicillins displays only end-absorption in the UV region. Only the degradation products of penicillins and a few compounds related to penicillins (including cephalosporins) display characteristic UV absorption bands (4). Because of the lack of specificity of the iodometric and acidimetric methods, it is also a common practice to characterize penicillins from their IR spectra. Such spectra, usually obtained on solid samples, are excellent "fingerprints" for individual penicillins, the elucidation of the structure of penicillins having actually been one of the oldest applications of IR spectroscopy (5).

It should be attractive to use the IR spectra for both the qualitative and quantitative analysis of penicillins. However, the IR spectra run on solid samples are not easily amenable to quantitative measurements. In fact, the absorbance of the IR bands is strongly affected by scattering and crystallinity effects (6). The logical approach to an IR quantitative analysis is to work on samples in solution. An IR assay in chloroform solution of a number of semisynthetic penicillins in the acid form was reported (7). Since most important penicillins are in the salt form, this method requires acidification and solvent extraction. Moreover, the chloroform method does not apply to ampicillins (7).

In principle, water could be taken into consider-

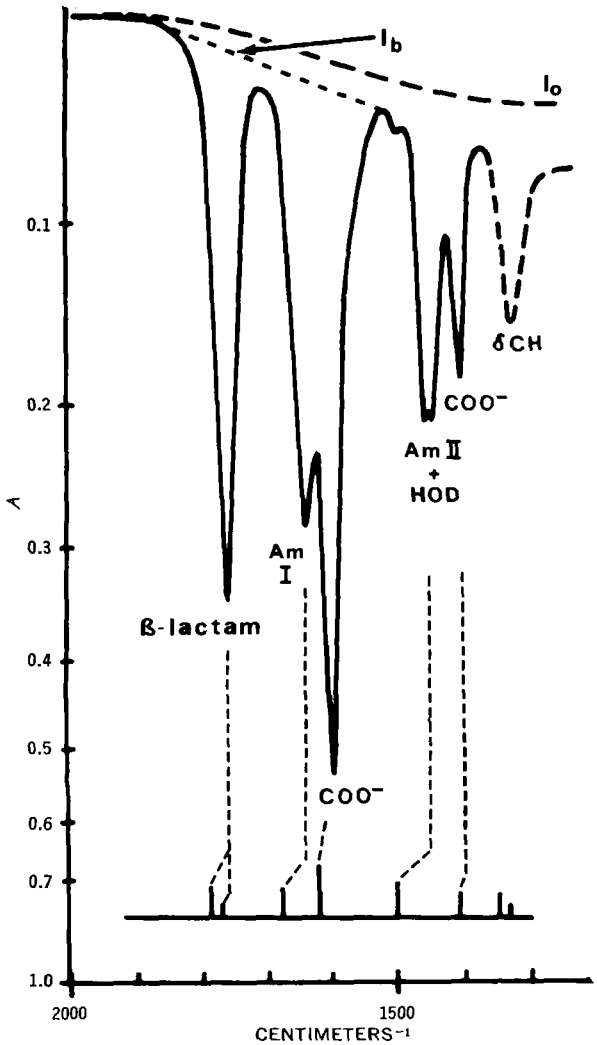


Figure 1—*IR absorption in the carbonyl region of potassium penicillin G in deuterium oxide solution (c 0.040 g/ml; l = 0.055 mm). The stick diagram in the bottom of the figure refers to band positions in the solid (KBr disk).*

ation as an IR solvent for most penicillins. However, water strongly absorbs the IR radiation except in the region between 1300 and 1000 cm^{-1} (8). On the other hand, deuterium oxide is more transparent than water in a number of analytically important regions of the IR spectrum.

The present work was undertaken to evaluate the feasibility of a direct IR analysis of penicillins in deuterium oxide solution. Such an approach looked promising because this solvent is sufficiently transparent in the carbonyl (1900–1400 cm^{-1}) region, where typical penicillin bands, including the key band of the β-lactam group, occur. The β-lactam band in a deuterium oxide–phosphate buffer was already shown (9) to be suitable for kinetic studies of the enzymic cleavage of a few penicillins. Moreover, unpublished work from these laboratories has shown that the bands of the unionized carboxyls, carboxylate ions, and amides lend themselves to the quantitative analysis of such groups in uronic acids and *N*-acetylated amino sugars.

An IR spectrophotometric method that has proved

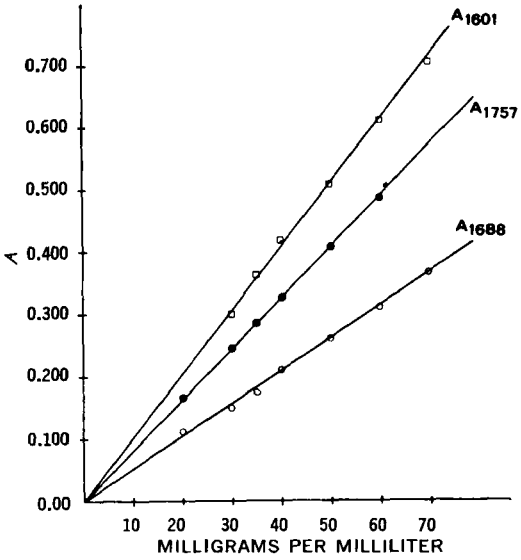


Figure 2—*Absorbance–concentration relationship for the β-lactam (1757 cm^{-1}), cyclic amide (1688 cm^{-1}), and carboxylate-ion (1601 cm^{-1}) bands of hetacillin in deuterium oxide (l = 0.055 mm, I_0 measured from a baseline).*

adequate for all water-soluble penicillins was then worked out. The water-insoluble penicillins were handled in 0.5 *N* DCl or, more conveniently, in dimethyl sulfoxide. The present method in solution appears to provide a rapid, specific, and accurate alternative to the currently used methods for penicillin assay.

EXPERIMENTAL[1]

Assay in Deuterium Oxide Solution—Forty milligrams of each penicillin sample was weighed in a ground-stoppered 5-ml flask, from a precision pipet or a self-filling microburet. Most sodium and potassium salts of penicillins easily dissolve in deuterium oxide. Part of the solution was transferred *via* an ordinary Lauer lock syringe in an IR cell of about 0.050-mm thickness, fitted with calcium fluoride windows. [The actual thickness of the sample cell used for the present work was 0.0552 mm, as measured by the interference-fringes (10) method.] A similar cell was filled with deuterium oxide. The IR spectrum of the solution was run from 2000 to 1300 cm^{-1}, using the cell filled with the solvent alone as the reference.

The absorbance of the analytical (β-lactam) band in the 1760-cm^{-1} region was measured either from a reference 100% (I_0) line, obtained by recording the spectrum with deuterium oxide in both the sample and the reference cell, or by the baseline method (10). The chosen baseline is shown in Fig. 1.

The penicillin content was calculated from the measured absorbance (*A*) of the β-lactam band by referring to a calibration curve such as that in Fig. 2, obtained using solutions of a reference sample of the same penicillin at different concentrations in deuterium oxide (2–6% w/v). Since the absorbance of the analytical band varies linearly with concentration, reference can be made to the absorbance value (A_{st}) measured for a single (4% w/v) solution of the standard sample. The percent concentration of penicillin in the sample (*c* %) can thus be obtained using the

[1] Penicillins were from different commercial sources. Their iodine consumption was 100 ± 2% of theoretical, except for sodium ampicillin which gave 95 ± 1.5%. Deuterium oxide (99.75%) and dimethyl sulfoxide were from C. Erba, Milan, Italy. Since the water content of the latter solvent did not exceed 0.5% (IR spectrophotometric analysis in the 3500-cm^{-1} region), dimethyl sulfoxide was used without any further purification. Deuterium chloride (~38%, 99.5% D) was from Ciba, Basel, Switzerland. An IR spectrophotometer (small grating), Perkin-Elmer model 337, was used.

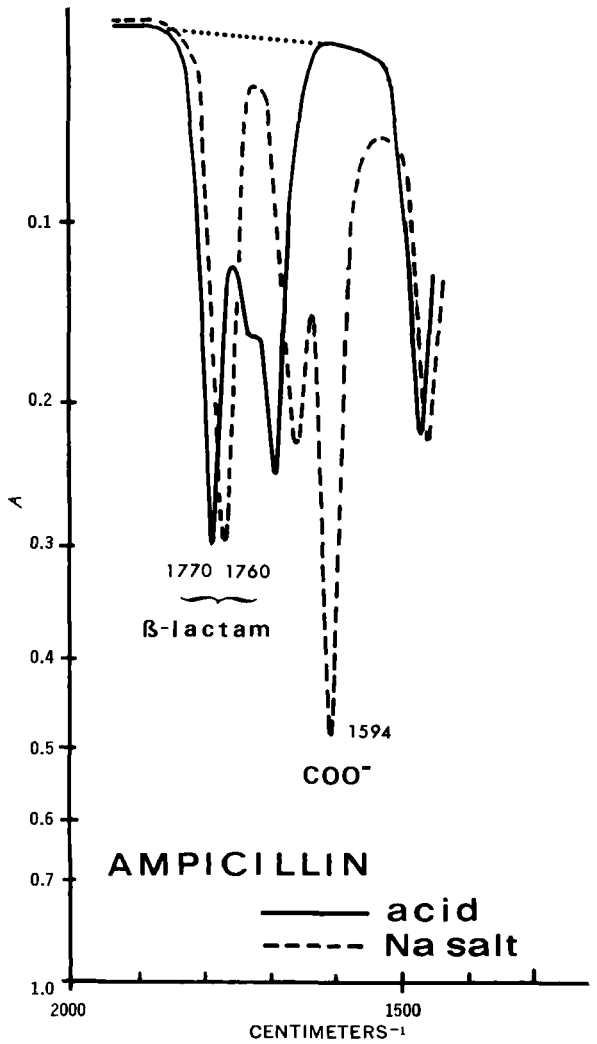


Figure 3—*IR spectrum of ampicillin (anhydrous), freshly dissolved in 0.5 N DCl, compared with the spectrum of the sodium salt in deuterium oxide (experimental conditions as in Fig. 1).*

simple relationship:

$$c\,\% = \frac{A}{A_{st}} \times 100 \qquad \text{(Eq. 1)}$$

Assay in Deuterium Chloride Solution—The penicillin samples were dissolved in deuterium chloride (approximately 0.5 *N*) prepared by diluting a concentrated (~38% w/v) deuterium chloride solution with deuterium oxide; the analyses were conducted as described for deuterium oxide solutions. Since deuterium chloride shows some background absorption in the analytical region, unbalanced solvent can shift the I_0 line. The 100% transmittance was then measured exclusively from a baseline as shown in Fig. 3 for ampicillin.

Assay in Dimethyl Sulfoxide Solution—The penicillin samples were dissolved directly in anhydrous dimethyl sulfoxide, and the analyses were conducted as described for deuterium oxide solutions. The absorbance of the β-lactam band was measured either from the usual I_0 line (solvent alone) or from a baseline as shown in Fig. 4.

RESULTS

Spectra of Penicillin Salts in Deuterium Oxide—The carbonyl region of the IR spectrum of potassium penicillin G is shown in Fig. 1. The reported band assignment is based on established spectra-structure correlations (11). The comparison of the spectrum of penicillin G in deuterium oxide with the corresponding spectrum in a potassium bromide disk (Fig. 1) shows that most bands shift toward lower frequencies on going from the solid to deuterium oxide solution. The largest shift (about 60 cm^{-1}), shown by the amide II band, is associated with the substitution of the amide hydrogen with deuterium. The hydrogen-deuterium (H-D) exchange of amides usually occurs within a few minutes, leading to the species —COND— (12). Since such an exchange also produces HOD, which absorbs at about 1460 cm^{-1}, and since the absorbance of the composite band shown by penicillins near the same frequency does not increase with time after the first run, it was assumed that the H-D exchange of the amide hydrogens of penicillins was complete before recording the spectrum (*i.e.*, before less than 10 min after dissolution of the sample in deuterium oxide).

The partial IR spectra in deuterium oxide of a number of water-soluble penicillins are shown in Fig. 5. The spectra share with that of penicillin G a β-lactam band at about 1760 cm^{-1}, but the spectra differ to some extent from each other and can be used as fingerprints for the individual penicillins. The β-lactam band is well resolved from the other carbonyl bands at lower frequencies. Only penicillin derivatives having ester groups (such as pivampicillin, Fig. 6) show a band partially superimposed on that of β-lactam. As can be seen in Fig. 1 for penicillin G, the characteristic splitting observed for the β-lactam band in solid-phase spectra of most penicillin salts (9) is no longer shown in deuterium oxide solution.

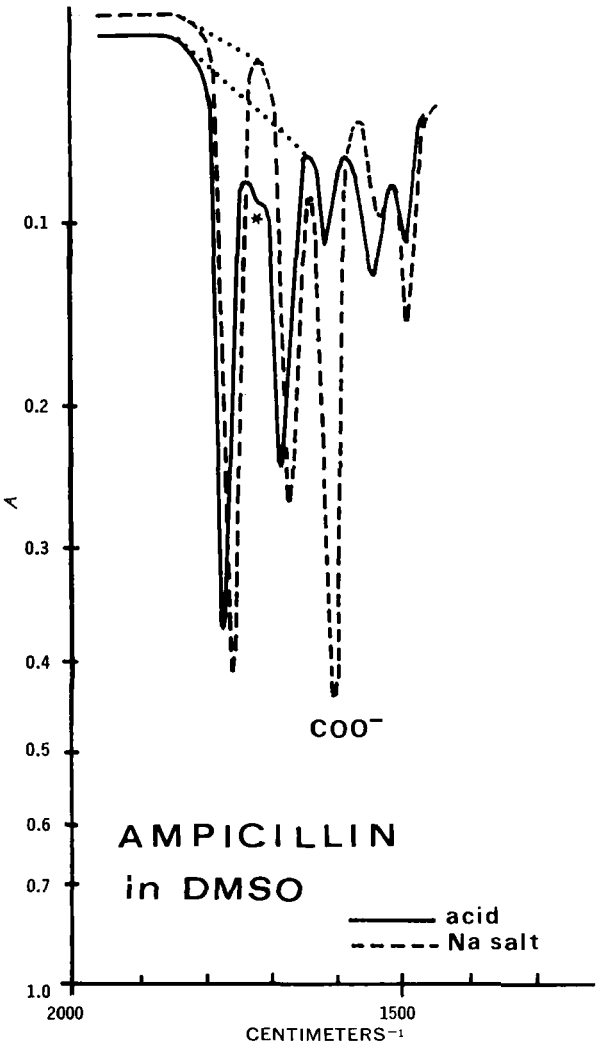


Figure 4—*IR spectra in dimethyl sulfoxide (DMSO) of ampicillin (acid, anhydrous) and its sodium salt (experimental conditions as in Fig. 1). The band of the unionized carboxyl groups is labeled with an asterisk.*

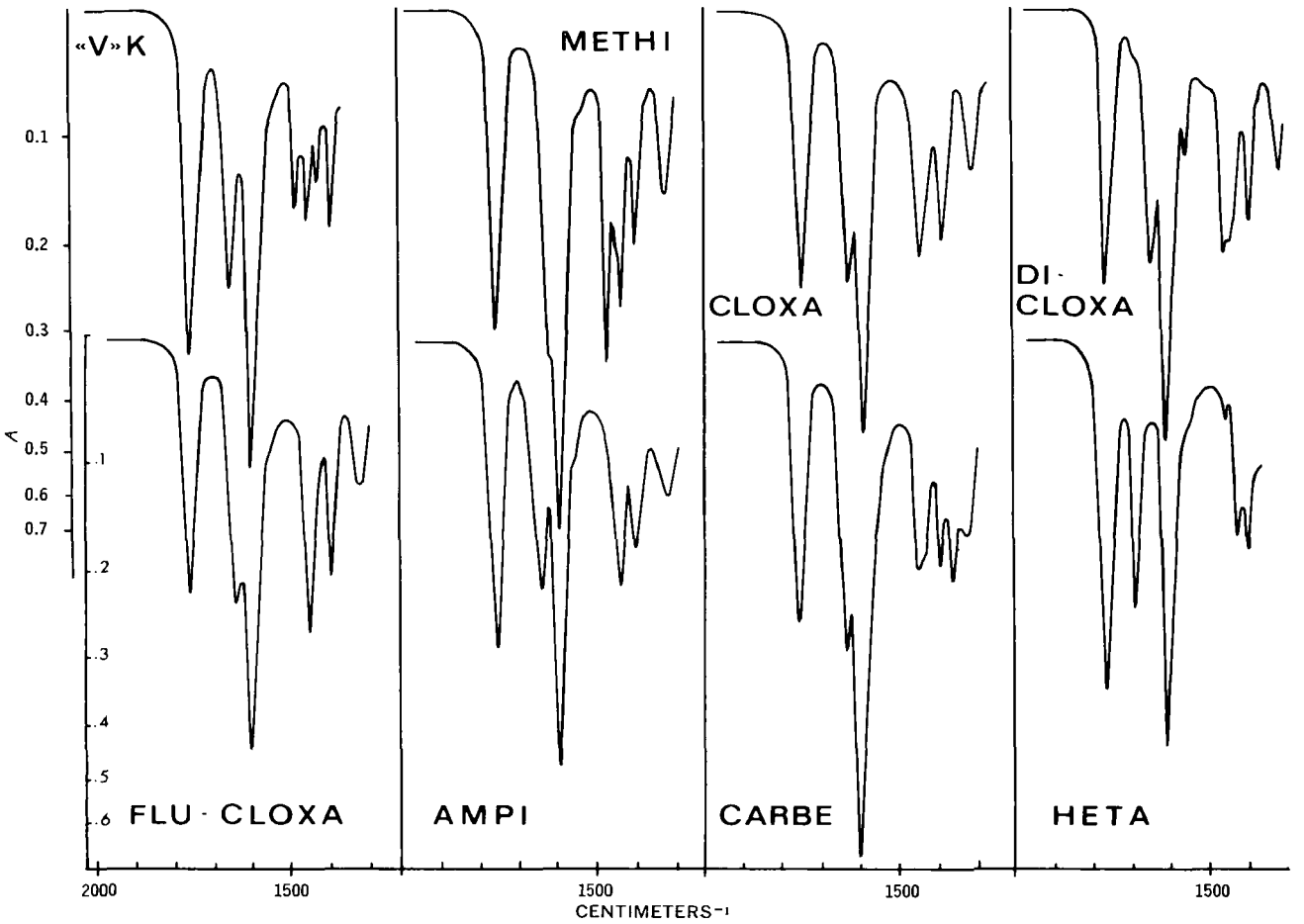


Figure 5—*IR absorption in the carbonyl region of alkaline metal salts of some common penicillins in deuterium oxide solution (concentration and cell thickness as in Fig. 1).*

The frequency and the absorptivity data for the β-lactam, amide, and carboxylate bands for a number of common penicillins in deuterium oxide are given in Table I. Data for 6-aminopenicillanic acid are included for comparison purposes. Except for the atypical 6-aminopenicillanic acid, the β-lactam band is displayed at fairly constant frequency (1760 ± 2 cm^{-1}). The amide group bands are more sensitive to the molecular environment. The amide I frequencies are in the 1670–1625-cm^{-1} range, the highest value being shown by pivampicillin and the lowest one by methycillin. The amide (γ-lactam) group of hetacillin absorbs at fairly high frequency (1688 cm^{-1}). The assignment of the amide II band frequency is uncertain because of overlapping HOD and CH_3 bands. The absorption frequency of the carboxylate ion is quite constant (asymmetrical stretching frequency, ν_{as}, = 1601–1595 cm^{-1}; symmetrical stretching frequency, ν_s, = 1405–1397 cm^{-1}).

To a certain extent also, the absorptivity of the carbonyl bands is dependent on the molecular environment of the groups. The absorptivity value for a well-resolved β-lactam band deviates from its average value (545 $mole^{-1} \cdot cm^{-1}$) up to about ±10%, the largest values found with phenoxymethyl penicillin and the lowest one found with ampicillin[2]. The absorptivity values for hetacillin and pivampicillin are consistently higher than the average, mainly because of interference by adjacent bands (due to the γ-lactam and the ester groups, respectively).

As shown in Fig. 2 for hetacillin, the main carbonyl bands of penicillins in deuterium oxide follow Beer's law, and each of them can be used for the direct quantitative analysis of individual penicillins. The 1760-cm^{-1} band, being common to all penicillins and associated with intact β-lactam groups, was chosen as the analytical band. Most functional groups of common penicillins give bands at lower frequencies and were not expected to interfere with the β-lactam band.

The reproducibility of the absorptivity data, as evaluated in a number of tests for the investigated penicillins, was well within 2%. The present IR method was also tested for accuracy against established iodometric methods, using conversion factors experimentally determined on USP or BP standards. As shown by the data in Table II referring to a sample of cloxacillin, the accuracy and precision of the IR method are of the same order of magnitude as those of the iodometric method. The absorptivity values calculated for cloxacillin samples of different purity (as evaluated iodometrically) were essentially the same, showing that impurities do not interfere with the IR analysis.

The absorbance of the β-lactam band does not vary within the time (~10 min) required for the measurements. No appreciable decrease in intensity was observed within 2 hr, unless the solution was acidified. On standing more than 2 hr, most penicillins undergo decomposition even in neutral solution, with a corresponding decrease of intensity of the β-lactam band. After 2 days, the absorbance at 1760 cm^{-1} decreased 20–60%, depending on the individual penicillin.

Spectra in Deuterium Chloride—In an attempt to extend the IR analysis to water-insoluble penicillins and related compounds in aqueous solution, the acid forms of ampicillins and cephalexin were run in acidic (deuterium chloride) solution. A 0.5 *N* acid concentration was chosen as a reasonable compromise between dissolving power of the acid and convenience of handling of solvent and solutions. Since deuterium chloride has some background absorption in the analytical region, a stronger concentration of acid (which would have decreased the instrument response below acceptable limits for quantitative work) was avoided. A reduced

[2] The absorptivity value obtained for penicillin G (566) is significantly lower than the one (614) derived by Zugaza and Hidalgo (9) in connection with their kinetic study. However, their spectrum seems superimposed on a pattern of interference fringes, a drawback that usually prevents the measurement of *absolute* absorbance values.

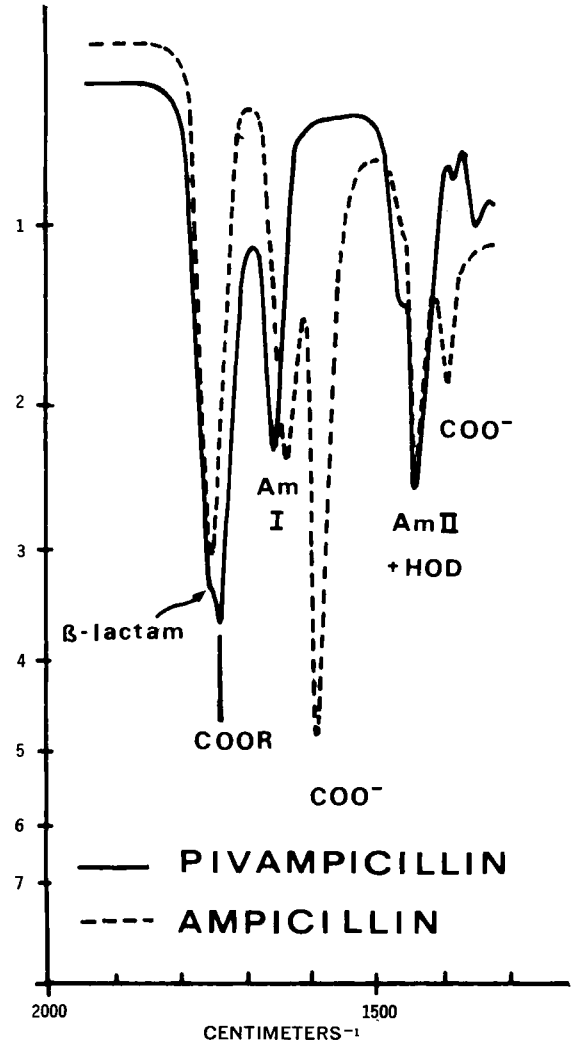


Figure 6—*Comparison of the spectrum in deuterium oxide of pivampicillin hydrochloride with that of sodium ampicillin (same experimental conditions as in Fig. 1).*

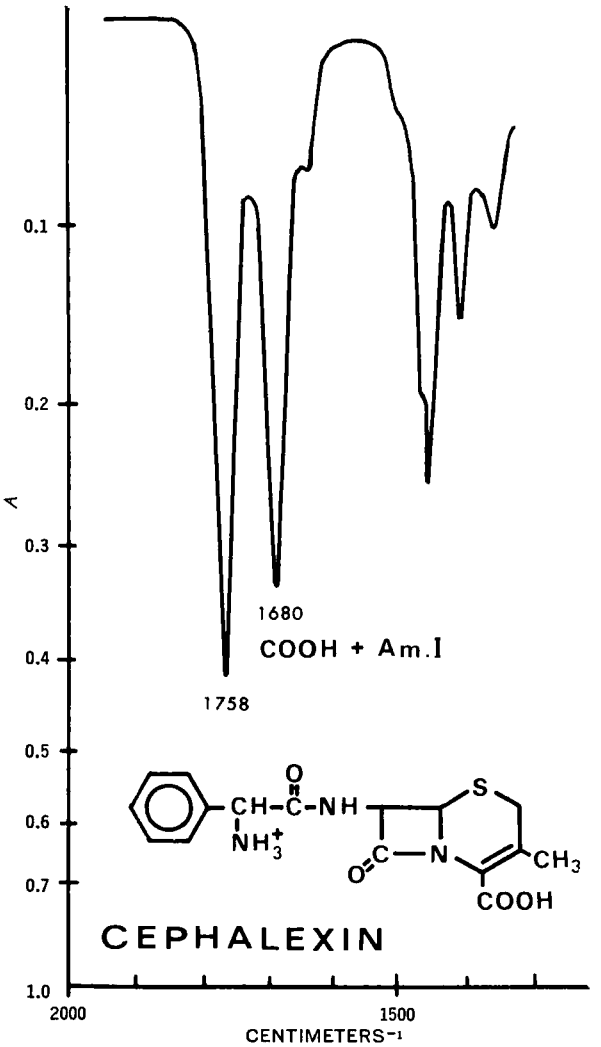


Figure 7—*IR spectrum of cephalexin in 0.5 N DCl (experimental conditions as in Fig. 1).*

acidity usually also reduces the rate of decomposition that most penicillins undergo in acidic solution (4).

Figure 3 shows the spectrum of a sample of ampicillin (anhydrous) freshly dissolved in 0.5 *N* DCl. By comparison with the superimposed spectrum of the corresponding sodium salt in neutral solution, it can be seen that the β-lactam and the amide bands shift toward higher frequencies on acidification. The most important difference between the spectra in acid and in neutral solution is the complete disappearance, in deuterium chloride solution, of the bands of the ionized carboxyl; these bands are replaced by the less intense band of the unionized carboxyl in the 1710-cm^{-1} region. The spectrum of ampicillin trihydrate in 0.5 *N* DCl differs from that of anhydrous ampicillin in the same solvent only by an increased HOD absorption in the 1460-cm^{-1} region.

Due to rapid decomposition in acidic solution, the spectra of ampicillins do not lend themselves to a direct quantitative analysis, which is possible for the more stable cephalexin (Fig. 7).

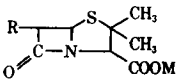
Figure 8 shows the spectral changes undergone by ampicillin on standing up to 40 hr in 0.5 *N* DCl solution. The β-lactam band decreases in intensity until completely disappearing, with a corresponding increase in intensity of the unionized carboxyl bands in the 1710-cm^{-1} region. The increase of the carboxyl band is compatible with the proposed formation of carboxylated products on acid degradation of ampicillin (13, 14).

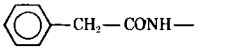
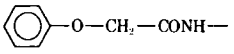
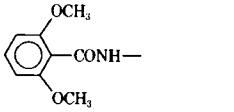
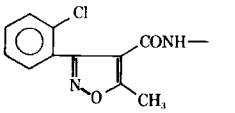
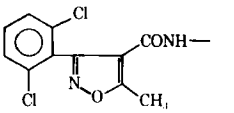
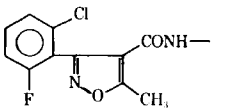
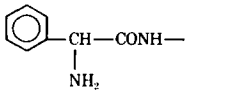
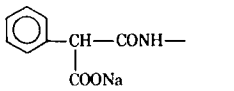
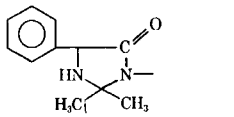
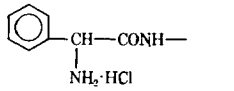
As shown in Fig. 9, extrapolation to zero time of a set of absorbance data obtained within less than 30 min allows one to obtain a "zero-time" absorbance value which can be used in a conventional way to calculate the ampicillin content in the original sample. Although in the present work such an approach was not thoroughly evaluated for its analytical potential, the accuracy of the quantitative data appears of the same order of magnitude as that obtained in deuterium oxide solution for water-soluble penicillins.

Spectra in Dimethyl Sulfoxide—A direct assay of most water-insoluble penicillins, as well as of a number of water-soluble penicillins, turned out to be possible using dimethyl sulfoxide as a solvent. Such a solvent is relatively transparent in the carbonyl region. Since water dissolved in dimethyl sulfoxide absorbs at 1655 cm^{-1} (8), care must be taken to avoid excessive exposure to atmospheric water vapor. However, since the water band is rather apart from the analytically important region for penicillins, the solvent can be conveniently handled, even in the open, without introducing interferences that could not be eliminated using the baseline technique.

Among the investigated penicillins, the acid form of ampicillin (anhydrous and trihydrated) and the cloxacillin salts almost instantaneously dissolve in dimethyl sulfoxide in the analytical concentration range (1–8% w/v). Water-soluble sodium and potassium salts of a few penicillins require some time to dissolve in dimethyl sulfoxide at room temperature (sodium ampicillin, 15 min; carbenicillin, 30 min; and potassium penicillin G, 12 hr). Potassium phenoxymethyl penicillin, potassium hetacillin, and cephalexin are practically insoluble in dimethyl sulfoxide. Figure 10 shows the spectrum in dimethyl sulfoxide of sodium cloxacillin, compared with that in deuterium oxide. The spectrum in dimethyl sulfoxide, being that of the nondeuterated species, is more reminiscent of the solid-phase spectrum than that in deuterium oxide.

Table I—Frequency ($\bar{\nu}$, cm^{-1}) and Absorptivity (a, $mole^{-1} \cdot cm^{-1}$) of the β-Lactam, Amide, and Carboxyl Bands of Some Penicillins (Sodium or Potassium Salts) in Deuterium Oxide[a]



Compound	R	M	Molecular Weight, Anhydrous	β-Lactam ν	β-Lactam a	Amide I, ν	Amide II a	Amide II ν	Carboxylate Ion $\nu_{as}COO^-$ ν	Carboxylate Ion $\nu_{as}COO^-$ a	Carboxylate Ion ν_sCOO^-, ν
6-Aminopenicillanic acid	$-NH_2$	K	254.3	1747	—[b]	—	—	—	1599	—[b]	1405
Penicillin G		K	372.5	1761	566	1639	450	1445	1601	868	1402
Phenoxymethyl penicillin		K	388.5	1760	585	1656	412	1435	1599	953	1400
Methicillin		Na	402.4	1762	566	1625 sh	617	1439	1600	1368	1401
Cloxacillin		Na	457.9	1761	551	1638	475	—[c]	1601	987	1402
Dicloxacillin[d]		Na	593.3	1760	568	1641	477	—[c]	1600	1082	1397
Flucloxacillin		Na	476.9	1760	536	1638	490	—[c]	1600	1002	1400
Ampicillin		Na	371.4	1760	527	1640	367	1445	1595	843	1403
Carbenicillin		Na	422.4	1758	537	1638	541	—[c]	1597	1459	1403
Hetacillin		Na	427.6	1757	648	1688 (γ-lactam)	440	—	1601	813	1401
Pivampicillin		—[e]	500.0	1760	714	1676	423	—[c]	—	—	—

[a] The absorptivity data were measured from the I_0 line (deuterium oxide *versus* deuterium oxide). The values are expressed on an anhydrous basis and were normalized to 100% when purity (as determined by iodometric assay) was less than 100%. Quantitative data are not given for the amide II and ν_sCOO^- bands because of interferences by HOD and CH_3 bands. [b] Data not given because of uncertain purity of the compound. [c] Uncertain assignment. [d] Contains some unionized carboxyl. [e] M = $-CH_2OC(=O)C(CH_3)_3$.

A comparison of the spectrum of ampicillin in the acid form in dimethyl sulfoxide and that of the corresponding sodium salt is shown in Fig. 4. The spectrum of the acid shows bands attributable to both an unionized carboxyl and a carboxylate ion. In the solid state, ampicillin is thought to be in a zwitterionic form (15), and its solid-phase IR spectrum (KBr disk) actually shows bands in the 1620–1560-cm^{-1} region, suggesting an ionic bond (intra- or intermolecular) between the carboxyl and the amino groups (11). The spectrum of ampicillin in dimethyl sulfoxide suggests that these ionic bonds are partially broken by the solvent, most likely by formation of a hydrogen bond between the carboxyl and dimethyl sulfoxide. On addition of a few drops of deuterium oxide to the dimethyl sulfoxide solution of ampicillin, the band of the unionized carboxyl disappeared while that of the carboxylate ion increased in intensity.

Since the carboxyl–dimethyl sulfoxide association is expected to be broken by water, the observed changes on addition of deuterium oxide support this contention.

The frequency and absorptivity of the β-lactam, amide, and carboxyl bands of some penicillins in dimethyl sulfoxide are reported in Table III. The β-lactam group and the carboxylate ion absorb at somewhat higher frequencies (~10 cm^{-1}) than in deuterium oxide. The amide I band of the sodium salt of ampicillin displays an even higher high/frequency shift (+46 cm^{-1}) relative to deuterium oxide.

An interesting feature of the spectra in dimethyl sulfoxide is that the bands are narrower than in deuterium oxide (and deuterium chloride). The absorptivity values are correspondingly higher, providing a better sensitivity for quantitative analysis.

The β-lactam band of the investigated penicillins in dimethyl sulfoxide follows Beer's law and lends itself to quantitative applications. Reproducibility of the absorbance values was within 1.5%. Precision (evaluated for a cloxacillin sample by comparison with a reference sample titrated iodometrically) was better than ±2%. Such a precision may be lowered with increasing water content of the solvent. In fact, the β-lactam band shifts toward lower frequency and broadens on addition of water to the solution. Such an effect, illustrated by the shift of the β-lactam band of hydrat-

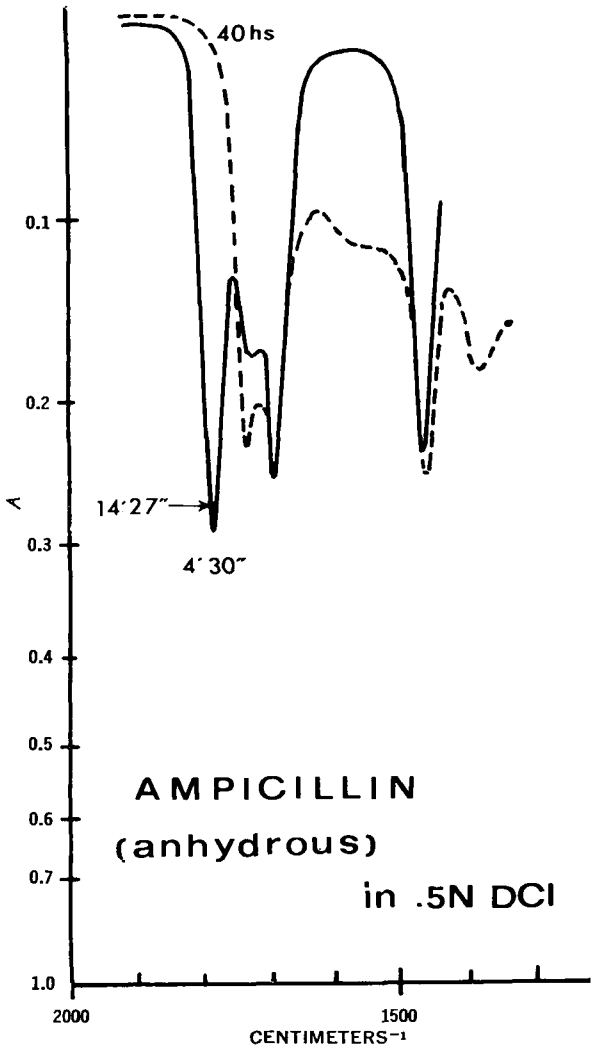


Figure 8—*IR spectra of ampicillin (anhydrous) in 0.5 N DCl at various times after dissolution. For clarity, only the absorbance of the β-lactam band is indicated for the spectrum after 14 min 27 sec (experimental conditions as in Fig. 1).*

ed ampicillin relative to anhydrous ampicillin (Table III), implies that the absorptivity of the analytical band is different for different water contents in the solution. From a practical point of view, satisfactory analyses can be performed whenever the water content is essentially the same in the sample and standard solutions.

DISCUSSION AND CONCLUSIONS

The present work has shown that the assay of most common penicillins can be performed in a very simple way by IR spectro-

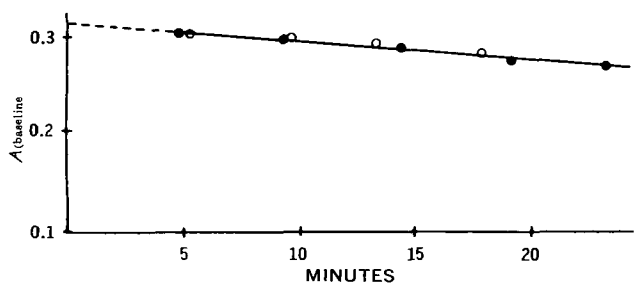


Figure 9—*Absorbance at 1770 cm^{-1}* versus *time data for ampicillin (anhydrous) in 0.5 N DCl (experimental conditions as in Fig. 1). Data refer to sets of data taken on two samples of the same ampicillin.*

Table II—Comparison of Quantitative Data Obtained for a Sample of Cloxacillin Using the IR and the Iodometric Methods[a]

Test	IR	Δ%	Iodometry	Δ%
1	98.20	−0.03	97.61	−0.68
2	97.41	−0.82	97.81	−0.48
3	98.59	+0.39	98.99	+0.70
4	97.81	−0.39	98.10	−0.19
5	97.13	−1.10	98.50	+0.21
6	98.20	−0.03	99.18	+0.89
7	98.99	+0.76	97.61	−0.68
8	99.18	+0.95	98.10	−0.19
9	98.59	+0.36	98.70	+0.41
Average	98.23		98.29	
Standard deviation		0.69		0.59
Standard error		±0.23		±0.22

[a] Percentages, relative to an international standard (BP) of cloxacillin.

photometry in deuterium oxide or dimethyl sulfoxide solution. All that is required is weighing the sample, dissolving it in the appropriate solvent, running the spectrum in the carbonyl region, and converting the absorbance of the β-lactam band to concentration values. This conversion can be done by reference to a calibration curve or to a single value from a standard sample. A complete analysis can usually be carried out in less than 15 min.

Accuracy and precision of the IR method in solution compare

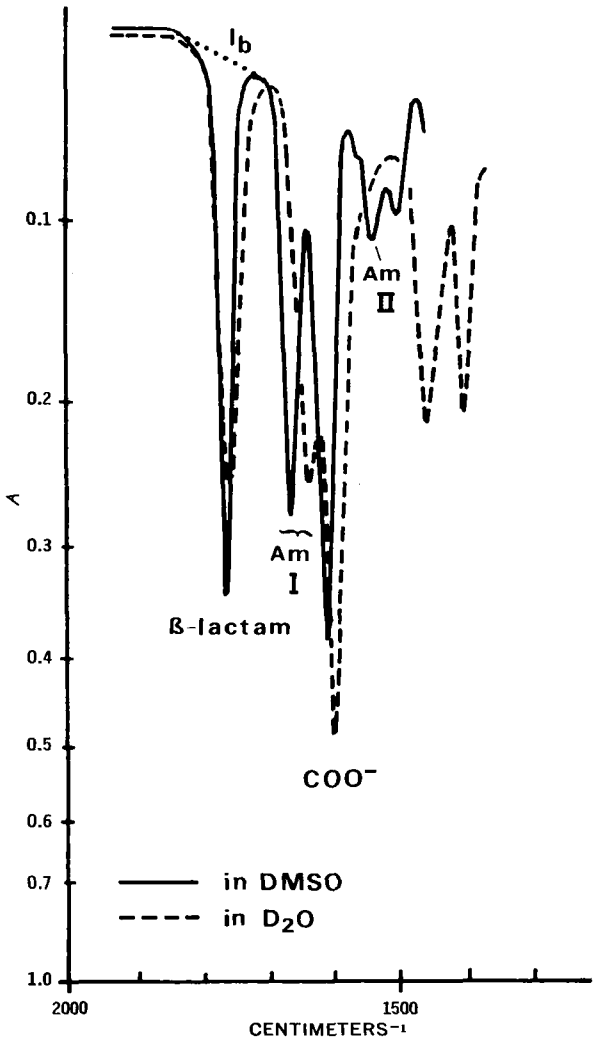
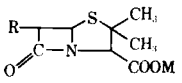
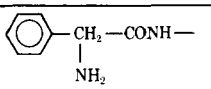
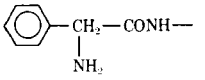
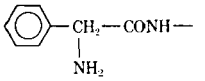
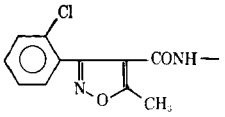


Figure 10—*IR spectra of sodium cloxacillin in dimethyl sulfoxide (DMSO) and in deuterium oxide (experimental conditions as in Fig. 1).*

Table III—Frequency ($\bar{\nu}$, cm^{-1}) and Absorptivity (a, $mole^{-1} \cdot cm^{-1}$) of β-Lactam, Amide, and Carboxyl Bands of Some Penicillins in Dimethyl Sulfoxide Solution



Compound	R	M	Molecular Weight	β-Lactam		Amide I		Carboxylate (ν_{as})	
				$\bar{\nu}$	a	$\bar{\nu}$	a	$\bar{\nu}$	a
Ampicillin[a] trihydrate		H	349.4	1771	696	1685	—[b]	1618	294
Ampicillin		H	349.4	1775	708	1690	413	1618	190
Ampicillin		Na	371.4	1774	755	1686	489	1621	793
Cloxacillin[a]		Na	457.8	1764	820	1664	661	1611	928

[a] Data calculated on the anhydrous basis. [b] Data not obtained because of interference by water.

well with those of current iodometric procedures. Due to the different absorption patterns of the amide and carboxylate groups of different penicillins in the carbonyl region of the spectrum, the specificity of the present method is definitely superior to that of the iodometric one. A further advantage of the IR method is that usually its precision is not lowered by common penicillin impurities. The precision of the iodometric method decreases with decreasing purity of the penicillin sample (16). A further advantage of the IR assay is that it lends itself to a direct investigation of the stability of penicillins in solution. Such an approach is of special interest in the case of aqueous solutions, since, apart from small isotope effects, penicillins in deuterium oxide and deuterated acids are expected to behave as in water and protonated acids. The kinetic measurements can be performed by either repetitive scanning of the spectrum or working at a fixed wavelength, by the "time-drive" technique.

Since the solvent and the cells are relatively transparent in the analytical region, the analysis can be done using an IR instrument of any class.

Most inorganic salts and organic compounds that are usually associated with penicillins in pharmaceutical preparations do not interfere with the assay on the β-lactam band, unless they possess carbonyl groups (such as ester groups) absorbing near the 1760–1670-cm^{-1} region. Glucose and inorganic phosphates are among the noninterfering partners of penicillins. A large excess of water in the sample can increase the background absorption on the low frequency side of the analytical region, both in deuterium oxide (HOD band at about 1460 cm^{-1}) and in dimethyl sulfoxide (water band at 1655 cm^{-1}). However, the baseline technique of measuring the absorbance of the β-lactam band usually allows for these potential interferences.

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