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Determination of nitrogen in hydrolyzed protein formulations by continuous vapour phase FTIR

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Abstract

An on-line system with vapour generation (VG) and Fourier transform infrared (FTIR) spectrometric detection has been developed for the determination of free ammonium and organic nitrogen in agrochemical formulations containing hydrolyzed proteins. Commercial samples were digested, in batch mode, with sulphuric acid and the obtained solution was alkalinized on-line to transform the NH_4^+ to NH_3 that was continuously monitored by FTIR. Free ammonium was determined in the same system after simple dilution of undigested samples with water. Different gas phase separators were assayed in order to introduce gaseous NH_3 into a home made IR gas cell of 10 cm pathlength, where the corresponding FTIR spectra were acquired by accumulating 10 scans per spectrum. The 967.0 cm^{-1} band was used for the quantification of ammonia. The figures of merit of the proposed method involve a linear range up to 100 $mg\,L^{-1}$, a limit of detection (3σ) of 1.4 $mg\,L^{-1}$ of N, a limit of quantification (10σ) of 4.8 $mg\,L^{-1}$ of N, a precision (R.S.D.) of 3.0% for 10 replicate determinations of a 10.0 $mg\,L^{-1}$ of N and a sample measurement frequency of 60 h^{-1}. The method was successfully applied to the determination of free ammonium and total N in commercial amino acid formulations and results compare well with those obtained by the Kjeldhal method.

Keywords: Total N; FTIR; Amino acid formulations; Vapour phase

1. Introduction

The presence of ammonium in a wide variety of environmental, clinical, and industrial samples has stimulated the development of a big number of methods for its determination. The most widely used methods for the determination of ammonium are spectrophotometric ones based on the Berthelot [1] or to a less extent the Nessler method [2]. A variety of instrumental procedures, such as chromatography [3–5], potenciometry [6], conductimetry [7], and chemiluminescence [8] have been developed for ammonium determination.

Most of the reported methods use expensive or unstable reagents that must be freshly prepared each day or assemblies including separation by membranes that must be periodically cleaned and performances change with time. Many of them entail prior separation of the analyte or masking of potential interferences and it makes batch processes time-consuming, especially when distillation is required.

The gas phase molecular absorption spectrometry technique introduced by Syty [9] has been applied to the determination of anions and cations in solution by conversion of the analyte to volatile molecular species followed by their molecular absorbance measurements in the gas phase in the UV–vis region [10–12].

The literature relates interesting contributions of Fourier transform infrared (FTIR) spectrometry applied to liquid and solid samples analysis based on the on-line generation of

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vapour phases by means of the simple sample volatilization or a chemical reaction [13].

Main applications involve determination of alcohols in different matrices [14], carbonates in liquid and solid samples [15,16], gasoline components [17], and paint solvents [18] or metal hydrides [19]. The advantages of the on-line vapour phase generation FTIR are (i) high transparency of gases in this region, (ii) low background values achieved, (iii) possibilities offered by using multiple-pass cells to increase the analytical sensitivity, (iv) efficient matrix removal, thus reducing spectral interferences, and (v) easy calibration using liquid or solid standards.

In spite of infrared has been used for the determination of amino compounds, such as alpha-amino group in raw sugar cane juices [20], there is only one precedent on the IR methodologies for determination of ammonia. This involved regulatory monitoring of pollutants of industrial processes, including ammonia, based on the use of an emission FTIR spectrometer configured with telescope entrance optics that allow the acquisition of naturally occurring IR radiance within a small field of view [21]. The methodology for estimating ammonia in gaseous effluents was based on the use of gravimetrically calibrated flow tubes.

However, the development of a simple procedure with a high sampling frequency, which could be suitable for routine direct determination of ammonia from liquid samples is still necessary.

The main objective of this work has been to evaluate the possibilities of the use of the tandem on line vapour phase generation FTIR spectrometry for the determination of ammonium from solutions, which allows to solve many limitations of the traditional methods. In this sense, a set-up configuration, that includes a home made gas phase separator and a low cost IR gas cell, was used to determine ammonium and organic nitrogen in commercial agrochemical products containing hydrolyzed proteins.

2. Experimental

2.1. Instrumental

A Bruker (Ettlingen, Germany) TENSOR 27 FTIR spectrometer equipped with a temperature stabilized DTGS detector, a MIR-IR source and a KBr beamsplitter was employed to carry out the IR measurements, using a low cost home made IR gas cell with a 10 cm path length, a 7 mL internal volume and equipped with two 9 mm × 2 mm circular ZnSe windows. The Spectrum OPUS software was used to control the instrument, for data acquisition and also for processing the results.

A basic scheme of the manifold, employed for the on-line vapour phase generation and FTIR measurements, is shown in Fig. 1. The solutions were pumped using a Spetec (Erding, Germany) peristaltic pump of six channels, furnished with Tygon tubes. The connection tubes were made from 0.8 mm i.d. Teflon tubing and reaction coil length was 60 cm. The gaseous phase was separated from the liquid stream in a homemade gas phase separator (GPS). The manifold incorporates a J.P. Selecta (Barcelona, Spain) heated unit in order to improve the removal of ammonia.

2.2. Reagents and samples

All chemicals used were of analytical-reagent grade and deionized water (resistivity 18.2 MΩ cm) was used for the preparation of solutions. A stock standard solution, with a concentration 1000 mg L^{-1} of N (expressed as NH_4^+), was prepared from ammonium chloride (Probus, Badalona, Spain). Working standard solutions were freshly prepared each day and diluted as appropriate directly before use. Sulphuric acid used for preparation of samples was obtained from Panreac (Barcelona, Spain) and NaOH from Scharlau (Barcelona, Spain). Samples of commercial agrochemical

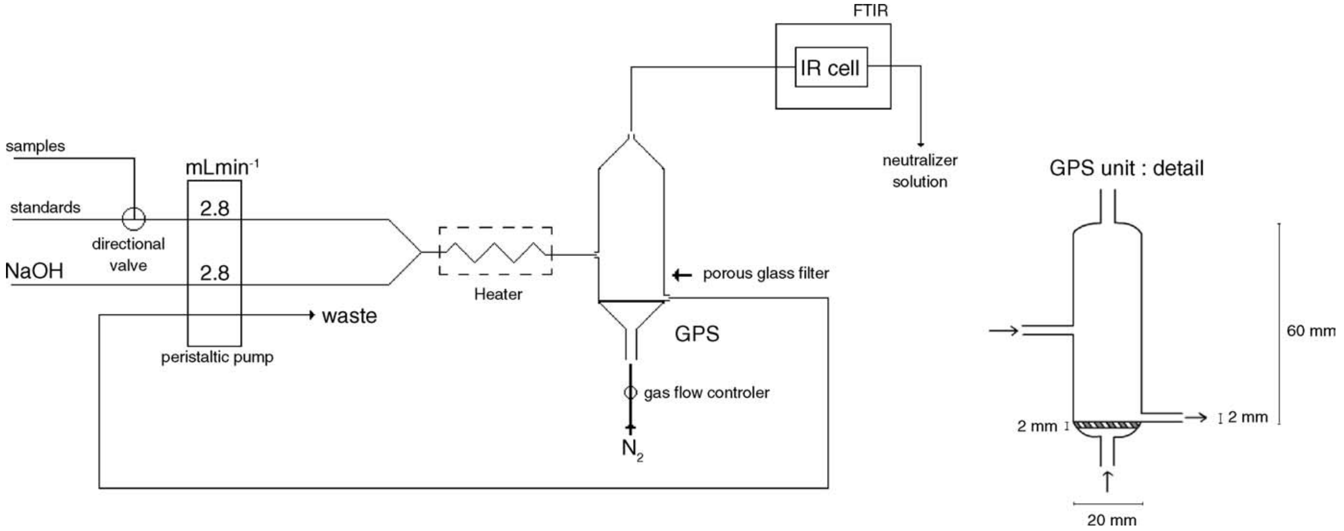

Fig. 1. Schematic diagram of the experimental set-up for the on-line vapour phase generation and FTIR determination of NH_3. GPS, gas phase separator; HU, heater unit; P, peristaltic pump. Experimental conditions: see Table 1 and text.

formulations containing hydrolyzed proteins were obtained on the local market.

2.3. General procedure

For the determination of free $NH_4{}^+$ samples were directly diluted with water. However, a sample pre-treatment with H_2SO_4 is required previously to the on-line vapour phase generation-FTIR determination of total N, including ammonium and organic nitrogen, as NH_3.

A total of 0.1 g of the commercial product was accurately weight into a 12 cm × 1.2 cm Pyrex glass tube and after adding 2.0 mL of concentrated sulphuric acid was digested at 200° C during 2 h using a metallic block heater. The digested sample was transferred to a 100 mL volumetric flask and diluted with water. This solution, previously, filtered through a Whatman 42 paper, was continuously aspirated in the manifold at 2.8 mL min^{-1} and merged with a 1.0 mol L^{-1} NaOH carrier (2.8 mL min^{-1}) and the mixture passed through a 60 cm length mixture coil heated at 95 ± 1 °C, was introduced in the gas phase separator and the generated NH_3 was transported to the gas cell using a N_2 carrier flow of 50 mL min^{-1}. The IR spectrum was recorded by accumulating 10 scans at 1 cm^{-1} resolution using a background obtained from water treated in the same conditions than sample. Nitrogen concentration was determined from the peak height measurements at 967.0 cm^{-1} corrected with a two points baseline (970.0 and 957.0 cm^{-1}) interpolated in a calibration line obtained for aqueous ammonium standards measured in the same conditions.

For free $NH_4{}^+$ determination, a 1 g of the product were diluted to 100 g with water.

3. Results and discussion

3.1. IR spectrum of ammonia in vapour phase

Fig. 2 shows the gaseous FTIR spectra obtained from a blank solution, two standard solutions of NH_4Cl and two samples of agrochemicals products containing hydrolyzed proteins, previously treated in a sulphuric medium. As it can be seen ammonia shows two intense bands at 967.0 and 930.4 cm^{-1}, and others less intense bands localized from 850.0 to 1175.0 cm^{-1}. The band at 967.0 cm^{-1} was selected for the quantitative determination of NH_3 because this band: (i) is strong, (ii) is well resolved for standard and samples, and (iii) appears in a really transparent zone of the blank.

3.2. Evaluation of the experimental conditions

The principle of the proposed method for ammonium and total nitrogen determination is the sample confluence with NaOH solution and the generation of ammonia that separates through the GPS using a N_2 stream carrier. The IR spectra were obtained in the gaseous phase and its quantitative determination based on measurements at 967 cm^{-1}.

The effect of the instrumental parameters, such as nominal resolution, number of scans, and background conditions on the quality of the analytical signal were evaluated using

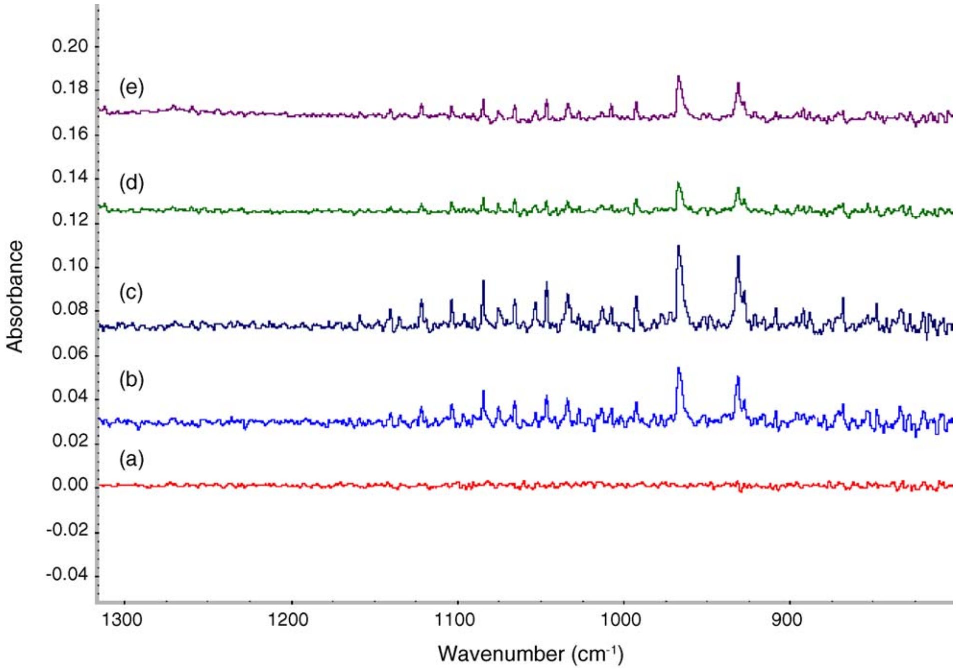

Fig. 2. Vapour phase FTIR spectra obtained for $NH_4{}^+$ standard solutions and digested protein hydrolyzed samples. (a) Blank (b) 50.0 mg L^{-1} N (as $NH_4{}^+$), (c) 100.0 mg L^{-1} N (as $NH_4{}^+$), (d) sample 1 containing 28.0 mg L^{-1}, and (e) sample 2 with 40.0 mg L^{-1}. *Note:* spectra have seen shifted to avoid overlapping.

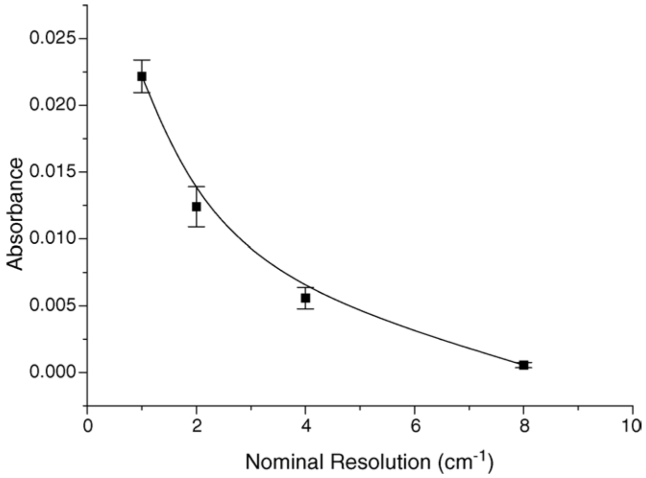

Fig. 3. Effect of the spectral resolution on the FTIR determination of ammonia. Experimental conditions: N concentration 100 mg L^{-1}; sample/carrier flow 2.8 mL min^{-1}; reaction coil length 60 cm; NaOH concentration 1.0 mol L^{-1}; temperature 95 °C; N_2 gas carrier flow 100 mL min^{-1}; number of scans 10. Peak height values indicated are the average of three consecutive spectra and error bars correspond to their standard deviations.

a solution of 100 mg L^{-1} of N prepared from NH_4Cl. The influence of the number of scans employed to establish the background and to obtain each spectrum was checked from 3 to 30 scans. 10 scans are enough to obtain a stable background and an appropriate signal-to-noise ratio. So, this number of scans was selected for further studies. Higher values than 10 do not improve significantly the quality of the analytical signal, and deteriorated the sample throughput.

The spectral resolution significantly affects the shape of the FTIR absorption peaks. Fig. 3 shows the effect of this parameter, in the range from 1 to 8 cm^{-1}, on the absorbance at 967.0 cm^{-1}. An increase of the spectral resolution causes the depression and the broadening of the analytical band. In this work, a nominal resolution of 1 cm^{-1} was selected in further studies in order to obtain the best sensitivity of the measurement with an adequate precision.

The effect of experimental parameters, such as flow rate, coil length, NaOH concentration, and temperature were investigated in order to find a compromise between sensitivity and reproducibility.

The effect of the mixing coil was evaluated from 30 to 160 cm. The analytical signal not changes on increasing the coil length, reflecting a good sample and reagent mixture, and a fast reaction. However, a reaction coil of 60 cm was chosen to improve the repeatability of measurements. On increasing NaOH concentration, from 0.1 to 2.0 mol L^{-1}, the absorbance of NH_3 at 967 cm^{-1} practically not changes. However, in order to apply the method to acid sample digests, it was chosen a 1.0 mol L^{-1} NaOH concentration.

On increasing the flow rate of both channels, from 1.0 to 4.2 mL min^{-1}, it increases the signal until to reach a maximum for flow equal or higher than 2.8 mL min^{-1} (see Fig. 4). On the other hand, an increase of the flow rate also increases the volume of the liquid phase in the GPS, thus affecting a good separation of the gaseous NH_3, and thus a carrier flow of 2.8 mL min^{-1} for each channel was selected.

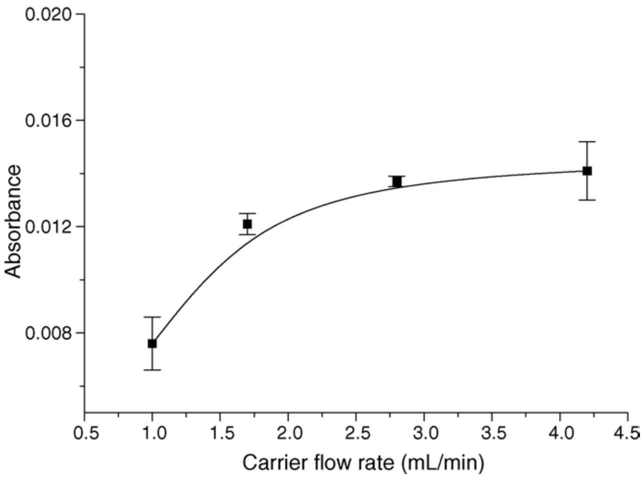

Fig. 4. Effect of the flow rate on the determination of ammonia by FTIR. Experimental conditions: reaction coil length 30 cm and temperature 65 °C. The rest of parameter values and measurement conditions were the same that indicated in Fig. 3.

The gas carrier in the proposed VG-FTIR system causes (i) the stripping of the NH_3 from the solution and (ii) its transport to the IR gas cell. For low N_2 flow values, a maximum and constant signal was obtained, being observed for N_2 flows equal or higher than 150 mL min^{-1}, a decrease of the analytical signal of NH_3 (see Fig. 5). Based on these results, gas carriers flow of 50 mL min^{-1} was selected for further studies.

In order to obtain enhance the analytical sensitivity, it was evaluated the effect of the temperature (see Fig. 6). In this case, the reaction coil was submerged inside a water batch with stabilized temperature. The increase of temperature improves the liberation of ammonia that separated on the

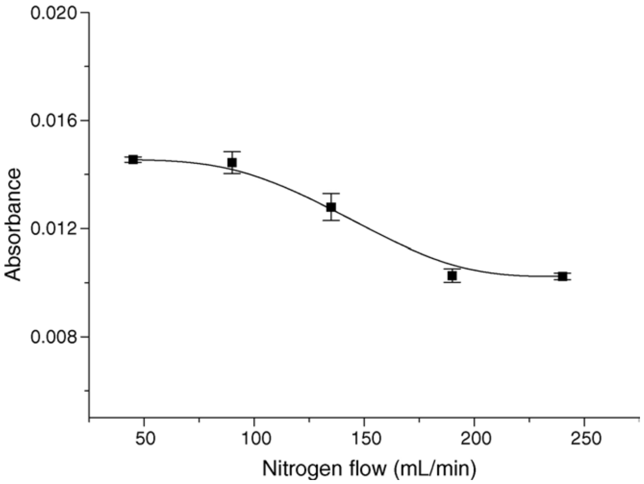

Fig. 5. Effect of the nitrogen flow on the determination of ammonia. Experimental conditions: N concentration 100 mg L^{-1}; sample/carrier flow 2.8 mL min^{-1}; reaction coil length 60 cm; NaOH concentration 1.0 mol L^{-1}; temperature 65 °C; number of scans 10. Peak height values were measured as indicated in Fig. 3.

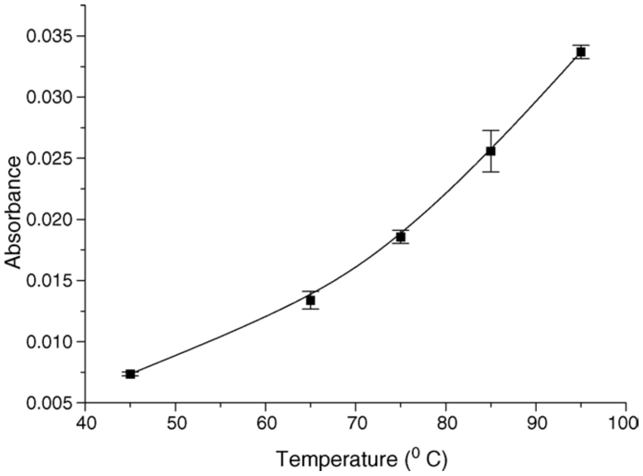

Fig. 6. Effect of the temperature on the FTIR determination of ammonia. Experimental conditions: as indicated in Fig. 5 with a N_2 gas carrier flow of 100 mL min^{-1}. Peak height values were measured as indicated in Fig. 3.

GPS and permits its detection. The best results were found at 95 °C. Higher temperatures than that is not recommended due to the accumulation of water vapour in the GPS that can be reach the IR cell. Table 1 summarizes both instrumental and experimental conditions selected.

3.3. Analytical figures of merit

The limit of detection based on the 3σ/slope criterion (99.6% confidence level) for 10 measurements of a blank solution was 1.4 mg L^{-1} as N and the quantification limit (10σ/slope criterion) was 4.8 mg L^{-1} N. For the selected experimental conditions: flow rate of 2.8 mL min^{-1}, reaction coil length of 60 cm, NaOH concentration of 1.0 mol L^{-1}, temperature of 95 °C, and N_2 flow 50 mL min^{-1}, the sample measurement frequency was around 60 samples h^{-1}. The relative standard deviation of 10 replicate determinations of 10.0 mg L^{-1} of N was 3.0%. A typical calibration equation for ammonium standard solutions was $\text{Abs} = 0.0002C_N + 0.0012$ (where Abs is the peak height absorbance value at 967.0 cm^{-1} corrected with a baseline at 970.0 and 957.0 cm^{-1} and C_N the NH_4^+ concentration expressed in mg L^{-1}). A correlation coefficient of 0.9975 was obtained. The sample volume required was 8.5 mL (for three replicates) and the NaOH solution consume was 168 mL h^{-1}.

Table 1
The VG-FTIR conditions for ammonia determination

Parameter	Studied range	Selected conditions
Instrumental		
Nominal resolution (cm^{-1})	1.0–8.0	1.0
Number of scans accumulated for background/spectrum	3–30	10
Flow analysis system		
Sample and NaOH flow rate (mL min^{-1})	1.0–4.2	2.8
Reaction coil length (cm)	30–160	60
NaOH concentration (mol L^{-1})	0.1–2.0	1.0
Temperature (°C)	45–95	95
N_2 gas carrier flow (mL min^{-1})	50–250	50

Table 2
Determination of free NH_4^+ and total N in commercial amino acids formulations (results expressed as N %, w/w)

Sample	Vapour phase-FTIR[a]			Kjeldahl method
	Free NH_4^+	Organic N	Total N	
AlgasMar	<0.01	8.0 ± 0.3	8.0 ± 0.3	8 ± 1
Lysodin	0.5	6.9 ± 0.3	7.4 ± 0.3	8 ± 1
Aminar-20	<0.01	3.2 ± 0.3	3.2 ± 0.3	3.1 ± 0.5
Isabión	0.8	9.5 ± 0.6	10.3 ± 0.6	12 ± 1
Siapton	0.6	8.7 ± 0.5	9.3 ± 0.5	10 ± 1

[a] $N = 5$, confidence level 95%.

3.4. Analysis of agrochemical samples

The developed procedure has been applied to the analysis of different samples of agrochemical products containing protein hydrolyzates and used as organic correction for soils or in traps for insects attraction. Samples were previously digested with concentrated sulphuric acid at 200° C during 2 h in order to transform all N to NH_4^+. Table 2 presents results obtained using the vapour phase-FTIR approach and those obtained by the reference Kjeldahl method also indicating the free NH_4^+ concentration obtained by direct measurement of samples merged with NaOH without a previous acid digestion. As can be seen the N (%, w/w) contents obtained by FTIR are statistically (judged by *t*-test at 95% confidence interval) comparable with those found by Kjeldahl.

4. Conclusion

The continuous on-line vapour phase FTIR procedure developed in this work for the determination of N as ammonia in hydrolyzed protein preparations could be employed an interesting alternative to those procedures using spectrophotometric and/or classical methods of analysis. As can be seen, the proposed method is very favourable for rapid and precise quantification of ammonium and organic nitrogen in commercial agrochemical. Additional advantages are the relatively inexpensive set-up and the low consumption of reagents and the absence of use of toxic organic reagents, which is a common practice in the FTIR analysis.

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