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DETERMINATION OF WATER IN SOLID SAMPLES USING HEADSPACE GAS CHROMATOGRAPHY

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SUMMARY

The research described herein concerns the use of the headspace technique coupled with gas chromatography for the quantitative determination of water in solid chemical compounds and pharmaceutical products. Samples were dissolved in alcohol and the water reacted with calcium carbide. The generated acetylene was measured with a flame ionization detector and related to the original amount of water in the sample. Samples were also analyzed by the Karl Fischer titration technique and the two sets of experimental data were subsequently compared.

INTRODUCTION

A method for the indirect determination of water by headspace gas chromatography (HSGC) was developed and shown to be effective for various organic solvents¹. Samples containing water were transferred to dry vials containing calcium carbide and the water reacted with the calcium carbide to produce acetylene. This acetylene was then measured by HSGC and used to calculate the original concentration of water in the sample.

This article describes an investigation into the use of the same technique for the determination of water in solid sample materials. Some minor modifications to the sample preparation procedures and solvent systems have expanded the range of the technique to include various organic solids and pharmaceutical products.

EXPERIMENTAL

Apparatus

A Hewlett-Packard Model 5880A gas chromatograph and terminal, equipped with a flame ionization detector was used for the analysis. The separation was accomplished with a 6 ft. × 0.25 in. (4 mm I.D.) glass column with a packing of 5% SE-30 on Porapak Q (80-100 mesh) (Waters Chromatography Division, Millipore, Milford, MA, U.S.A.). The headspace samples were injected manually with a gas-tight precision sampling syringe (Series A-2, 2.0 ml) available from Precision Sam-

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pling Corp. (Baton Rouge, LA, U.S.A.). The sample reaction and subsequent headspace equilibration was completed in 50 ml glass reaction vials available from Supelco (Bellefonte, PA, U.S.A.). The vials were sealed with septa of brominated butyl rubber, obtained from The West Co. (Phoenixville, PA, U.S.A.), and aluminum seals from Wheaton Scientific (Millville, NJ, U.S.A.).

Reagents

Methanol, ethanol, 2-propanol, hydrated salts, and organic acids were purchased from J. T. Baker (Phillipsburg, NJ, U.S.A.). Calcium carbide (10–40 mesh) was obtained through Fisher Scientific (Fair Lawn, NJ, U.S.A.).

Analysis of solid materials

The percent water content of the solid materials was determined initially by the headspace calcium carbide procedure and then by Karl Fischer titration. Consequently, only compounds which would not interfere in a Karl Fischer titration were selected. The results from the Karl Fischer titrations could then be compared with those obtained with the headspace technique.

For the headspace analysis (HSA), samples were transferred to dry, 15 ml vials and capped. Transfers were done in a glovebag and the sample weight determined by difference. Methanol (10 ml) was then added to the vial to dissolve the sample. Solutions of methanol spiked with water and a blank were also prepared. The headspace vials were then prepared by adding 1.0 g calcium carbide (an excess) and 10.0 ml of 2-propanol to each. A 0.20-ml aliquot of the methanol solution was then transferred to a headspace vial (already containing 10.0 ml of 2-propanol). The vial was then sealed and equilibrated for the HSA.

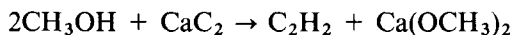
For the Karl Fischer determinations, samples were titrated directly in methanol, with the end point determined potentiometrically.

Six individual samples were analyzed by each technique.

RESULTS AND DISCUSSION

Solvent considerations

The determination of water in a solid material requires that water comes into contact with the calcium carbide in the headspace vial. Dissolution of a compound into an appropriate solvent followed by the addition of the resulting solution into the headspace vial would be the most practical means of providing the contact. Under the proper conditions, one might also be able to extract the water present in some solids into a suitable solvent. Addition of this extract to the headspace vial should also prove to be adequate. Methanol is an excellent solvent for water determinations as demonstrated by its wide utility in Karl Fischer titrations, but it was eliminated from consideration due to its non-specific reaction with calcium carbide to produce acetylene.



Investigations with ethanol also indicated that this solvent reacts with the calcium carbide, but the situation is not as severe as that encountered with methanol. With the

ethanol, some of the difficulties are probably from the reaction of methanol in the alcohol (5%), used for denaturation, as well as reaction of the ethanol itself. In ethanol, these non-specific reactions take place slowly, and reaction of the methanol is incomplete. Since the reaction of methanol in ethanol was slow, we felt that the same situation might exist with solutions of 2-propanol containing methanol. If this were true, then solid materials could be dissolved in methanol and diluted with 2-propanol before addition to the headspace vials.

Solutions of 2-propanol containing 2.0, 5.0, 7.5 and 10.0% (v/v) of methanol were prepared. Using these solutions as solvents for the calcium carbide technique, both blank solutions and solutions spiked with known amounts of water were prepared and used with the procedure. Eight vials were prepared for each of the solutions. After completion of the HSA, the average peak area was calculated for the blank solution of a given methanol concentration. This blank was then subtracted from the peak areas for individual vials containing the spiked solutions. The average net peak area for each of the spiked solutions was calculated, as well as the relative standard deviation (R.S.D.) of the spike determinations. These results are listed in Table I. The results indicate that levels of up to 5% methanol in 2-propanol can be tolerated. For solutions of 7.5 and 10.0%, however, the interferences from methanol caused poor precision.

Fig. 1 illustrates a plot of the change in peak areas for three of the blank solutions as a function of time. The plot illustrates the difficulties introduced by the presence of methanol. At the 5% level there is no significant change in the level of acetylene over a 3-h period. This increase in acetylene would account for the poor precision obtained with solutions containing the higher percentages of methanol.

Analysis of organic compounds and sodium salts of organic acids

Five hydrated compounds were chosen as test materials for use with the HSGC-calcium carbide technique and comparison with Karl Fischer titrations. The compounds are listed in Table II along with their percent purity and percent water content, corrected for purity.

The results of the water determinations for all of these compounds, except *p*-toluenesulfonic acid, are also listed in Table II. Some difficulties were encountered with this compound, and these results will be discussed separately.

The results obtained with the headspace technique are in good agreement with those obtained by Karl Fischer titration. The largest discrepancy is with the sodium diethyldithiocarbamate, with a relative difference of 4.86%. The headspace results are

TABLE I
REPRODUCIBILITY STUDIES WITH 2-PROPANOL-METHANOL SOLUTIONS

<i>Methanol in 2-propanol (%)</i>	<i>Spike, water concentration (ppm)</i>	<i>R.S.D. (%)</i>
2.0	250	3.28
5.0	300	3.34
7.5	300	8.13
10.0	300	5.29

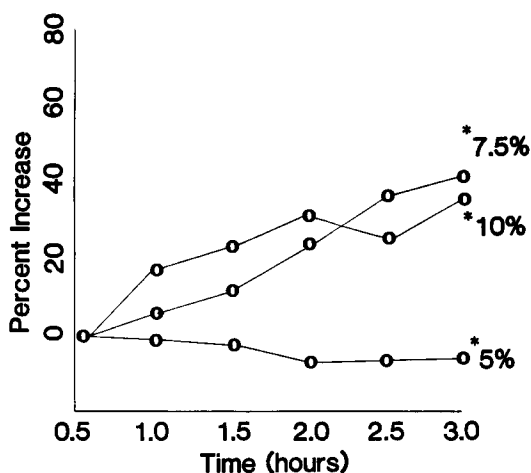


Fig. 1. Percent increase in acetylene versus time for solutions of methanol in 2-propanol. Percent methanol in 2-propanol is indicated by the asterisks.

also in agreement with the theoretical percentage of water for all of the compounds except citric acid. There were no difficulties encountered with the Karl Fischer titration of this compound and there are no anticipated interferences. Citric acid monohydrate is characterized as a compound which will begin to lose water in an anhydrous atmosphere² so it might be possible that it lost some of its water of hydration while in storage. It is interesting to note that the results obtained by the headspace technique for sodium diethyldithiocarbamate are actually in better agreement with the theoretical percentage of water than with the Karl Fischer results. It would be difficult to determine which of the experimental techniques was more accurate in this case, however. The percent purity of the material was listed as 98.4% on the label of the bottle, but the composition of the impurities was not specified.

TABLE II

PERCENT WATER CONTENT OF HYDRATED TEST MATERIALS

Compound	Purity (%)	Water content (%)		
		Theoretical	HSGC (n = 6)	Karl Fischer (n = 6)
Citric acid monohydrate	99.9	8.56	7.75 (2.69)*	7.84 (2.28)
Oxalic acid dihydrate	100.2	28.61	28.19 (3.36)	28.48 (0.25)
Sodium acetate trihydrate	99.8	39.60	39.70 (1.42)	38.33 (0.60)
Sodium diethyl-dithiocarbamate trihydrate	98.4	23.59	23.50 (4.00)	24.70 (0.72)
p-Toluenesulfonic acid monohydrate	99.6	9.42	—	—

* R.S.D. (%)

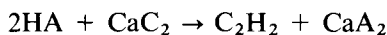
TABLE III
SPIKE RECOVERY EXPERIMENTS

Compound	Recovery (%) (<i>n</i> = 6)
Oxalic acid dihydrate	98.5 ± 3.3 (3.36)*
Sodium diethyldithiocarbamate trihydrate	98.8 ± 3.9 (3.90)

* R.S.D. (%)

Matrix spike recoveries were determined with oxalic acid and sodium diethyl dithiocarbamate. The results are listed in Table III. The quantitative recoveries obtained in both experiments would indicate that there is no change in the distribution coefficient of the acetylene in the presence of either of these compounds.

Some difficulties were encountered with the use of *p*-toluenesulfonic acid as a sample. Determination of the water content of this compound yielded results which were higher than the theoretical values and the results obtained by Karl Fischer titration. The vials containing the samples never achieved complete reaction, as the concentration of acetylene increased significantly with time, even after the usual 18-h reaction period. The *p*-toluenesulfonic acid was believed to be the cause of a non-specific reaction which resulted in the formation of acetylene. Since *p*-toluenesulfonic acid is a strong acid, there are several possible explanations which could account for the results which were obtained. One possibility would be reaction of the acid with an alcohol to produce an ester and water. Another possibility might be the neutralization of the acid with the calcium hydroxide in the system to produce the calcium salt of the acid and water. A final possibility is the direct reaction of the acid with the calcium carbide to produce acetylene.



where HA is *p*-toluenesulfonic acid. Difficulties with ester formation in the presence of a strong acid are also encountered in Karl Fischer titrations^{3,4}. To overcome this, one typically adds an amine to the solution which will increase the pH and inhibit ester formation. This same approach was applied to the HSGC-calcium carbide technique. A solution of 0.4% diethylamine in 2-propanol was prepared and tested with the calcium carbide technique. The presence of the amine caused no difficulties with the determination of water in the solvent. The solvent was then used for dissolution of the *p*-toluenesulfonic acid for water determination with the HSGC-calcium carbide technique. The results which were obtained were still significantly higher than expected. All results of the determinations of the water content of *p*-toluenesulfonic acid are listed in Table IV.

These results would indicate that the calcium carbide technique is not a very viable alternative for the determination of water in the presence of *p*-toluenesulfonic acid. Two other acidic compounds, oxalic acid ($\text{p}K_a = 12.27$ in water)⁵ and citric acid ($\text{p}K_a = 3.13$ in water)⁵ did not interfere with the determination of water, so certain acidic compounds can be handled by the technique. Thus, the success or failure

TABLE IV
DETERMINATION OF WATER IN *p*-TOLUENESULFONIC ACID

Solvent	Water content (%)		
	HSGC	Theoretical	Karl Fischer
2% Methanol in 2-propanol	15.82 (<i>n</i> = 3)	9.42	9.75
2-Propanol	13.21 (<i>n</i> = 3)	9.42	9.75
2% Methanol, 0.4% diethylamine in 2-propanol	13.76 (<i>n</i> = 6)	9.42	9.75

apparently is dependent on the strength of the acidic compounds present. If the pK_a , of oxalic acid in water were used as a general guide for the strength of acids which could be handled, this would leave a large range of organic acids which do not interfere with water determinations. There is no pK_a value for *p*-toluenesulfonic acid because aromatic sulfonic acids are strong acids and completely ionized in water⁶.

Determination of water of hydration in transition metal salt

Seven hydrated transition metal salts were obtained for use as test materials. Most of the compounds were difficult to work with as received. They came in the form of fairly large chunks ($1/4 \times 1/2$ in.). To facilitate ease of handling, most of the compounds were ground to a smaller particle size with a mortar and pestle and then placed in an oven at some predetermined temperature. Many of the higher hydrates of the transition metal salts are characterized as losing some of their water of hydration at a certain temperature², resulting in the formation of a stable, lower hydrate. To prepare a compound, a temperature was selected at which a stable, lower hydrate would form.

Three compounds were prepared in this manner and then subjected to thermogravimetric analysis (TGA) for determination of percent water content. The results of the TGA are shown in Table V, along with the theoretical percentage of water for each of the compounds. TGA of $Zn(NO_3)_2 \cdot 6H_2O$ was attempted, but this compound never reached constant mass. The melting point of this compound is listed as $36^\circ C$, but no reference is made as to the formation of the anhydrous salt above $36^\circ C$.

These same compounds were then used as sample materials with the HSGC-calcium carbide technique. Some serious difficulties were encountered in the application of the HSGC procedure to the determination of water in these compounds, however. The first compound investigated was cobalt dichloride dihydrate. The results obtained by the HSGC procedure were significantly higher than those expected from the information listed in Table V. Karl Fischer titration of the salt (in duplicate) yielded results which agreed with the data listed in Table V. The results are listed in Table VI.

There are two possible explanations for the discrepancy in the results obtained. One is that the cobalt dichloride changes the activity coefficient of the acetylene in the solvent, thereby changing the distribution coefficient of the acetylene in the system.

TABLE V
THERMOGRAVIMETRIC ANALYSIS

Compound	Water content (%)	
	TGA	Theoretical
MnCl ₂ ·H ₂ O	12.48	12.51
Cd(CH ₃ COO) ₂ ·2H ₂ O	13.51	13.97
CoCl ₂ ·2H ₂ O	21.82	21.71

Since there was no cobalt dichloride present in the calibration solutions used in the analysis, a change in the distribution coefficient of acetylene caused by cobalt dichloride in the sample would result in an inaccurate quantitative determination of water in the sample. The other possibility is that some non-specific reaction through which acetylene is formed is taking place when the cobalt dichloride is present.

To investigate the possibility of a change in the distribution coefficient of the acetylene in the presence of a salt, some spike recovery experiments were performed. The sample matrices investigated consisted of cobalt dichloride dihydrate, and the anhydrous forms of cobalt dichloride, manganese dichloride and cadmium acetate.

The recoveries from the experiments (Table VII) are all close to 100%, which would substantiate the premise that there is no change in the distribution coefficient of the acetylene in the various matrices investigated. Closer examination of the data reveals some other difficulties, however. For the three experiments with the anhydrous salts, the acetylene peak areas for the blank salt solutions are noticeably larger than the acetylene peak areas for the blank calibration solutions, indicating a higher than expected water concentration. This problem is the same as that encountered in the initial work with cobalt dichloride (Table VI). Upon examination of the individual peak areas obtained for each of the vials containing a particular salt solution, one also finds a distinct increase in the acetylene peak area with time (after overnight reaction), a situation that was not encountered with the vials containing no salt. If there is no change in the distribution coefficient of the acetylene in the presence of these transition metal salts, then the only other explanation for the higher than expected results would be that some non-specific reaction is taking place which also produces acetylene.

It seems unlikely that the transition metal salts would react directly with the calcium carbide to produce acetylene. In fact, reaction of cobalt dichloride or manganese dichloride in this manner would be impossible, since these salts do not possess any hydrogen atoms. Cadmium acetate does contain hydrogen, but these are methyl

TABLE VI
DETERMINATION OF WATER IN COBALT DICHLORIDE DIHYDRATE

	Water content (%)
HSGC ($n = 6$)	33.49 (2.74)*
Theoretical	21.71
Karl Fischer ($n = 2$)	21.41

* R.S.D. (%)

TABLE VII
SPIKE RECOVERIES FROM TRANSITION METAL SALT MATRICES

<i>Matrix compound</i>	<i>Recovery (%)</i>
CoCl ₂ ·2H ₂ O	102
CoCl ₂	98
Cd(CH ₃ COO) ₂	97
MnCl ₂	106

TABLE VIII
DETERMINATION OF WATER IN PHARMACEUTICAL PRODUCTS

<i>Pharmaceutical product</i>	<i>Water content (%)</i>	
	<i>HSGC (n = 6)</i>	<i>Karl Fischer (n = 6)</i>
Cogentin*	1.70 (4.85)***	1.49 (10.78)
Elavil* (uncoated tablets)	1.72 (4.33)	1.58 (3.91)
Vasotec* (10 mg enalapril maleate)	4.83 (2.75)	4.15 (21.08)
Aldomet** (granulation)	8.68 (4.35)	8.75 (4.01)
Vasotec** (20/50)	4.06 (4.91)	4.01 (1.93)
Elavil** (coated tablets)	1.69 (2.08)	1.66 (1.70)
Ivermectin**	5.07 (2.67)	5.08 (1.74)
Vasotec** (40 mg enalapril)	5.04 (2.90)	5.27 (1.71)
Aldomet** (oral tablets)	4.21 (2.12)	4.17 (3.91)
Vasotec** (5 mg enalapril maleate)	5.32 (3.49)	5.34 (0.76)
Vasotec** (2.5 mg enalapril maleate)	5.15 (4.50)	5.19 (3.98)

* 30 min extraction time.

** 45 min extraction time.

*** R.S.D. (%)

hydrogens and would have to be considered non-reactive. The alternative to the possibility of the salt itself reacting with the calcium carbide would then be that the presence of the salt is causing one of the other components of the system (*i.e.*, the alcohols) to react.

With the transition metals in the system, the possibility of an oxidation-reduction reaction does exist. Alcohols can be oxidized with a suitable oxidizing agent and

the proper conditions. But the cobalt(II), manganese(II) and cadmium(II) are all in stable oxidation states, and reduction to a lower oxidation level with the conditions used in these experiments would probably not occur. Perhaps a more likely alternative to an oxidation–reduction reaction would be the alcohols being coordinated around the transition metal atoms in solution, with the electronegative oxygen atom of the alcohol moiety drawn in towards the positively charged cation. This, in turn, could make the hydrogen of the alcohol more acidic and more susceptible to reaction with the calcium carbide, resulting in the production of acetylene.

These difficulties encountered with the transition metal compounds indicated that the reliable determination of water in their presence would not be possible with the techniques used in this study.

Determination of water in pharmaceutical products

Several pharmaceutical products were obtained for use as test samples with the HSGC–calcium carbide technique. These products came from the Pharmaceutical Research and Development Section of Merck Sharpe & Dohme's Research Labs., where their water contents are determined routinely by Karl Fischer titration. The water content of the products was determined using both techniques, so that a comparison of the results could be made.

For the investigations, six 1-g samples of the product were weighed to the nearest 0.1 mg and transferred to centrifuge tubes. The water in the samples was extracted with 10.00 ml of methanol and determined in the extract with an automatic coulometric Karl Fischer titrator.

Headspace vials were then prepared, with calcium carbide and 10.0 ml of anhydrous 2-propanol added to each. Aliquots of 200 μ l of the methanol extracts were then transferred to the vials. The vials were equilibrated overnight and the HSA completed the following day. When the analyses were complete (Karl Fischer and HSA) the average percent water content of the product was calculated. The results for the analyses are listed in Table VIII.

For the first three analyses, the Karl Fischer results are significantly lower than the results of the headspace technique. The precision of the titration is also unexpectedly poor. The cause of these problems was suspected to be incomplete extraction of the water from the samples, and the order in which the two analyses were performed bears this out. The titrations were always done first, and a substantial amount of time (2 h) elapsed between the titrations and the preparation of the samples for the HSA. Apparently, this additional time was sufficient for the complete extraction of the water, as seen with the higher headspace results. The extraction times were increased (as noted in Table VIII), and the results of the two techniques were in better agreement. In general, the results indicated that the HSGC–calcium carbide technique was a very viable alternative for applications with these sample materials.

CONCLUSIONS

The calcium carbide technique was shown to be effective for the determination of water in certain types of solid sample materials. The water content of several hydrated organic acids and the sodium salts of organic acids was determined with

both the calcium carbide technique and Karl Fischer titration. The results of the determinations with the calcium carbide technique were within $\pm 5\%$ (a relative value) of the Karl Fischer results. The relative standard deviations, obtained from six individual measurements for each of the compounds, were all less than 5%. Eleven commercially available pharmaceutical products were also analyzed by both the calcium carbide technique and Karl Fischer titration. The accuracy and precision of these determinations were also within the range of 5%.

Several compounds were incompatible with the calcium carbide technique. Hydrated transition metal salts and *p*-toluenesulfonic acid yielded results which were significantly higher than expected. The difficulty with these compounds was suspected to be a non-specific reaction of calcium carbide with one of the components (others than water) of the prepared sample, resulting in the formation of additional acetylene. The water content of two transition metal salts was successfully measured after some modifications to the technique. It was concluded, however, that the calcium carbide technique, even with the modifications, was not a very feasible alternative for other techniques which can easily handle these compounds.

From a practical standpoint, there are also several advantages and disadvantages of the calcium carbide technique which should be discussed. The calcium carbide technique can measure levels of water reliably down to the 60-ppm level in liquid samples¹. There are other techniques which have significantly lower detection limits (*i.e.*, Karl Fischer titration), but most GC techniques are limited to the 60-ppm level or higher. The preparation time needed for six samples was approximately the same for the calcium carbide technique and Karl Fischer titration. For a large number of samples, an automated headspace sampler (capacity of 60–100 samples) could be used with the indirect method. This would mean that an analyst spends time only on the preparation of samples. The acetylene determinations would be completely automated. But for a laboratory which receives only one or two samples for water determinations a week, the calcium carbide technique would not be very practical.

Future investigations with the calcium carbide technique will focus on sample materials which are incompatible with the Karl Fischer titration (*i.e.*, carbonates, bicarbonates, etc.). An automated headspace sampler will be tested with the technique. These samplers demonstrate significantly improved injection reproducibility over that of a manual gas injection. This improved reproducibility would result in lower detection limits for the calcium carbide technique. And finally, some research into the problems associated with the transition metal salts might lead to a modification which would allow these materials, and other to be handled by the calcium carbide technique.

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