FREQUENTLY ASKED QUESTIONS ABOUT KARL FISCHER TITRATION (KFT)

Agnieszka Kossakowska (Honeywell Research Chemicals) and Michael Margreth (Metrohm) are KFT experts who share their knowledge and experience with users of this technique at seminars across the globe. No matter where the technical training is performed, they often encounter the same questions. In an effort to help KFT users world over, Agnieszka & Michael have hereby compiled the most frequently asked questions and arranged these into categories for quick reference.

INSTRUMENT PREPARATION AND HANDLING

How can I check if the electrode is working correctly?

MM: We recommend carrying out a volumetric or coulometric Karl Fischer titration using a certified standard as a sample. In volumetry, a threefold titer determination followed by a determination of a different standard is carried out and the recovery of the water content in the determination of the standard is calculated. To check a coulometric system, a threefold determination with a certified water standard is carried out and the recovery calculated. If the recovery is between 97 and 103%, the system, including the electrode, is working fine. The color of the working medium is an additional indicator whether the indication is working properly. Pale yellow is perfect, whereas dark yellow or even pale brown suggests indication problems. If this happens, the indicator electrode should be cleaned.

How long can an electrode be stored in the KF reagent?

MM: Karl Fischer electrodes are made of glass and platinum. Therefore, the KF reagent does not affect the electrode. It can be stored in the reagent as long as you want.

How often should I change the drying agents?

AK: Each type of drying agent has a water adsorption capacity limit. How fast this limit is reached depends on the humidity level in the laboratory – the higher the humidity, the more frequently the drying agent should be changed. Generally, this time should be weeks, not months. An average frequency is 4–6 weeks. Drying agents based on silica gel (e.g., HYDRANAL™-Humidity Absorber) offer the advantage of a color change when exhausted. Also, please note that these silica gel beads require a different regeneration procedure: they should be dried at 140°C until the color turns back.

Can a molecular sieve be dried and reused, or should it be replaced?

MM: Yes, of course, a molecular sieve can be dried and reused. We recommend drying for at least 24 hours at a temperature between 200 and 300°C.

Can I use HYDRANAL-Humidity Absorber instead of molecular sieves?

AK: Of course. It is perfect for drying carrier gases and on top of HYDRANAL-Composite one-component reagents. However, the choice of drying system depends on the size of the drying tube. HYDRANAL-Humidity Absorber beads are larger than molecular sieves, so they fit well into larger drying tubes. Any empty spaces inside the drying tube cause the air to pass between the drying agent beads, not through them.

How long does conditioning normally take?

MM: Conditioning of a freshly filled titration vessel normally takes around 2–4 minutes for volumetry, depending on the reaction speed (type of reagent), and around 15–30 minutes for coulometry. In combination with an oven, it might take a few minutes longer to reach a stable drift due to the constant gas flow. We recommend stabilizing the whole oven system for at least one hour.

Between single measurements in the same working medium, conditioning takes approximately 1–2 minutes. Take care that the original drift level is reached again.
When I start conditioning, there are a lot of bubbles in the coulometric cell for several minutes and a very high drift, even with fresh reagent. What could be the reason for this effect?

MM: At the anode, the generator electrode produces iodine from the iodide-containing reagent. The bubbles you see at the cathode are the result of the reduction of H+ ions to hydrogen.

After opening the titration cell or filling it with fresh reagent, the conditioning step removes the water brought into the system and avoids falsifying the water content determination of the sample. Removing the water results in an increased drift level. During conditioning, the above-mentioned hydrogen is generated. The gas bubbles are therefore completely normal and not a cause for concern. Generally, the following rule applies: the more water in the titration vessel, the higher the drift value and the quantity of hydrogen formed.

How often should I clean the Karl Fischer equipment?

MM: There is no strict rule as to when you should clean the KF equipment. The cleaning intervals strongly depend on the type and the amount of sample added to the titration cell. Poor solubility and contamination of the indicator electrode (deposition layer on its surface) or memory effects due to large amounts of sample can be a reason for cleaning the equipment. The drift can also be a good indicator. In case you observe higher and unstable drift values, I would recommend cleaning the titration cell or at least refilling the working medium.

How do I clean the Karl Fischer equipment?

MM: For a connected titration vessel, it can be as simple as rinsing with alcohol. For an intense cleaning, the vessel should be disconnected from the titrator. Water, solvents like alcohols, or cleaning agents are fine to clean the KF equipment. Even concentrated nitric acid can be used as an oxidizing agent, for example, in case of contaminated indicator electrodes or coulometric generator electrodes. All these options are acceptable, but please keep in mind that the last cleaning step should always be rinsing with alcohol followed by proper drying.

Ketones should never be used to clean Karl Fischer equipment as they react with methanol. This reaction releases water. If there are still traces of ketones left in the titration cell after cleaning, those ketone traces react with the methanol in the KF reagent and might cause the drift to be too high to start a titration.

Can I also use a cleaning agent like “CIF” or toothpaste to clean the double Pt electrode?

MM: Normally, rinsing with alcoholic solvents and polishing with paper tissue should be sufficient. To clean the electrode, you may also use detergents or toothpaste. Just make sure you rinse the electrode properly after the cleaning to remove all traces of the cleaning agent before using the electrode again.

How do I clean a generator electrode with diaphragm?

MM: After removing the generator electrode from the titration vessel, dispose of the catholyte solution. Next, rinse the electrode with water. Place the generator electrode upright (for example, in an Erlenmeyer flask) and cover the connector with the protection cap. Fill the generator electrode with some milliliters of concentrated nitric acid and let the acid flow through the diaphragm. Then fill the cathode compartment with water and again allow the liquid to flow through the diaphragm. Repeat the rinsing step with water several times to make sure that all traces of nitric acid are washed out of the diaphragm. Please note that the nitric acid treatment can be left out if the level of contamination does not require it. Finally, fill some methanol into the generator electrode to remove the water. Repeat this step a few times to remove all water. The last step is proper drying. After this cleaning procedure, the electrode is as good as new and can be used for measurements again.

TITRATION PROCESS

In case the drift value is zero, does this mean that the titration cell is over-titrated?

MM: A drift of zero can be a sign that the cell might be over-titrated. In combination with the mV signal (lower than end-point criteria) and the color of the working medium (darker yellow than usual), it is a clear indicator of over-titrination. However, volumetric titrators sometimes show zero drift for a short time without being over-titrated.

If you have a real excess of iodine in the titration cell, the result of the next determination will most probably be wrong. Therefore, over-titrination should be avoided. There are various possible reasons for over-titration, such as the sample itself (oxidizing agents generate iodine from the working medium), the electrode (covering or invisible depositions), the reagent, and method parameters (titration rate too high), to name a few.

When the over-titration period causes a noticeable change in color, does this mean I should discard the Karl Fischer reagent and use a fresh one immediately, or does it not affect the result in any way?

MM: Different factors can cause over-titration. The reagent is not always the reason for over-titration. The indicator electrode can also be the reason for overshooting the endpoint. In this case, cleaning the electrode can prevent over-titration. A low stirring speed also increases the risk of over-titration. Make sure the solution is mixed well. Depending on the type of reagent, the parameters of the titration need to be adjusted. Especially if you use two-component reagents, we recommend decreasing the speed of the titrant addition to avoid over-titration. Over-titration has an influence on the result, especially if the degree of over-titration changes from one determination to the next. Therefore, over-titration should be avoided to guarantee correct results.

I always subtract the drift from the final water content. What if the blank is higher than the water content found, will I get a negative water content? What does a negative water content mean?

MM: We recommend using the drift correction in coulometric KF titration only. You can also use it in volumetric titration, but here the drift level is normally not absolutely stable. This can result in variations in the results. A long stabilization time can reduce such an effect. Compared to absolute water amounts in volumetry, the influence of drift is usually negligible. Back to the coulometric technique: Negative values do occur if you have a high start drift and a sample with a very low water content. If possible, use a larger sample size to increase the amount of water added to the titration cell with the sample. Furthermore, you should try to reduce the drift value in general. The molecular sieve or septum may also need to be replaced. You can also use additional stabilizing time to make sure the drift is stable before analyzing the sample.
Is the volume of KF solution used for volumetric titration determined by measuring the titration time? How is it measured – with a chronometer or another instrument?

MM: The steps of the motor used to dose the titrant determine the volume of the added titrant. The motors for dosing in Metrohm instruments offer between 10,000 and 100,000 steps. The determination time has no influence on the volume of titrant dosed.

Is pH important in KF titration?

AK: The Karl Fischer reaction runs quickly and stoichiometrically in the ideal pH range of 5–7.5. At higher pH values, a side reaction occurs that consumes iodine. In stronger acidic conditions, the reaction constant of the KF reaction decreases. Even the KF reaction itself produces acids that must be neutralized. Hydralan reagents contain an excess of imidazole derivatives as the base to neutralize these acids and buffer the titration system. The pH is thus stabilized around the ideal value of 5–6. The pH balance of the titration can be damaged by large amounts of strong acids or bases introduced as a sample. Those must be neutralized by adding an appropriate weak base or acid (imidazole, salicylic acid, benzoic acid) or a buffer solution (HYDRANAL-Buffer for Acids, HYDRANAL-Buffer for Bases) to the working medium.

What are the possible reasons for not obtaining repeatable results with the same sample when all conditioning is taken care of?

AK: Key influencing factors for the reliability and the accuracy of the results include: Reactivity of the reagents (titration time, endpoint stability), conditions of the titration vessel (drift, tightness, diaphragm), matrix of the sample (side reaction, solubility, pH effects), electrode condition, accuracy of the titer determination and its stability, titer decrease speed, water capacity limit of the reagent reached, activity of drying agents, and various errors during sample handling.

KARL FISCHER OVEN

What is the highest possible water content I can measure with a Karl Fischer oven?

MM: Typically the oven is used in combination with a coulometric device. The coulometric titration cell used in an oven system is filled with 150 mL of reagent. Theoretically, this amount of reagent allows for the determination of 1500 mg of water. However, this amount is too high to be determined in one titration and it would lead to very long titration times and negative effects on the results. We recommend that the water content of a single sample should not be higher than 10 mg, ideally around 1000–2000 µg. For samples with water content in the higher percentage range, you should consider the combination of a KF oven with a volumetric titrator instead.

How do I find the optimal oven temperature for water extraction?

MM: Depending on the instrument, you can run a temperature gradient of 2°C/min. This means you heat a sample from 50 to 250°C within 100 min. The software will then display a curve of water release against temperature. From this curve, you can determine the optimal temperature. Different peaks may show blank, adherent water, different kinds of bound water, or even decomposition of the sample. Generally, you should choose a temperature after the last water release peak (where the drift came back to the base level) but approximately 20°C below decomposition temperature. Decomposition can be recognized by increasing drift, smoke, or a color change of the sample.

In case the instrument you use does not offer the option to run a temperature gradient, you can manually increase the temperature and measure the sample at different temperatures. In an Excel spreadsheet, you can display the curve (released water against temperature). If there is a plateau (i.e., a temperature range where you find reproducible water contents), you have found the optimal oven temperature.

Can all types of samples be analyzed with the oven method?

MM: Theoretically, many samples can be analyzed with the oven. Whether an application actually works for a sample strongly depends on the sample itself. Of course, there are samples that are not suitable for the oven method. For example, samples that decompose before releasing the water or release their water at higher temperatures than the maximum oven temperature.

What is the maximum sample size that can be used with the oven?

If I use too much sample, will the needle be blocked?

MM: The standard vial for the oven method has a volume of approximately 9 mL. However, we do not recommend filling the vial completely. Do not fill more than 5–6 mL of sample in a vial.

For liquid samples, we recommend using a long needle to lead the gas through the sample. Solid samples, and especially samples that melt during analysis, require a short needle. The tip of the needle is positioned above the sample material to avoid needle blockage.

Additionally, you should use a “relative blank value” (i.e., taking only the remaining air volume into account for blank subtraction).
What is the detection limit of the oven method and how much sample is required to analyze a sample with 10 ppm water content?

MM: We recommend having at least 50 μg of absolute water in the sample, if analyzed with coulometry. However, if conditions are absolutely perfect (i.e., very low and stable drift plus perfect blank determination), it is possible to determine even lower water contents — down to 20 μg of absolute water. For a sample with a water content of <10 ppm, this would correspond to a sample size of at least 2 g.

WATER STANDARDS

Why are water standards preferred over pure water for titer standardization and system checks?

AK: The challenge with pure water is the low amount required (e.g., 1–50 mg for volumetry and 0.1–1 mg for coulometry), which is difficult to weigh and handle accurately. Those problems can be easily overcome by using water standards, available in both liquid and solid forms.

Which water standard should be used for each technique?

AK: Liquid water standards are freely mixable with the common KF solvent systems and can therefore be used easily for titer determination, general system checks, or for verification of the results between individual samples. Due to the low water concentration, the amount that needs to be handled for one determination is in grams and not milligrams as with pure water; thereby providing higher reliability. Generally, water standards with higher concentration are recommended for volumetry, and water standards with lower concentration are recommended for coulometry.

Solid water standards can be used for volumetry or for the control of indirect KF oven systems. They are not recommended for direct use in coulometry, as solid samples should be avoided in that technique. Due to the limited solubility of sodium tartrate dihydrate in volumetric media, solid standards cannot be used for bigger sample sizes, as this influences the reliability of the results.

Why are plastic syringes not recommended for handling liquid water standards?

AK: After an ampoule is opened, the water content can be altered by air humidity. Depending on the laboratory conditions (high or low humidity), the water content in the standard can increase or decrease, influencing the accuracy of the results. Proper handling by using proper labware can help minimize the influence of ambient moisture. Especially for the standards with low water content, we recommend using gas-tight glass syringes. Please note that the volume of the syringe used should fit the whole volume of the standard ampoule (after rinsing the syringe). Detailed instructions on liquid water standards handling are included in each package.

How should I treat ranges stated on Reports of Analysis / Certificates of Analysis?

AK: The amount of one ampoule of water standard is sufficient for rinsing the syringe followed by a triple determination of the water content. The recovery rate calculated should ideally be within the expanded measurement uncertainty stated on the Report of Analysis / Certificate of Analysis supplied with each pack. However, users can set their own rules based on internal requirements and industry needs. The acceptable limit should depend on the amount of water in the sample. We recommend using general limits given in documents such as Ph. Eur.: for 10.0 mg water ±0.2 mg (±2%), for 1000 μg water ±25 μg (±2.5%), for 100 μg water max ±10 μg (±1%).

How should solid water standards be stored? What is their shelf life after they have been opened?

AK: Correct storage conditions are ambient air conditions. That means you can store at ambient lab temperature around 20°C and in summer up to around 30°C. The most important point is to close the cap properly after each single use. If the standard is stored under the recommended conditions, opening does not reduce the general shelf life. However, we can guarantee the shelf life only for the originally closed bottle.

KF REAGENTS HANDLING

What is the difference between using one-component and two-component systems? How should I choose which system to use?

AK: In two-component reagents, the substrates for the Karl Fischer reaction are divided into two bottles: Titrant and Solvent. Titrant contains the iodine in methanol or ethanol. The second reagent, Solvent, contains sulfur dioxide and a base dissolved in the same alcohol. The advantages of the two-component system are: high titration speed (due to higher pH), high accuracy, high buffer capacity, and high titer stability. However, currently, one-component reagents are more popular because they are more convenient to use and more universal. In this case, all substrates for the Karl Fischer reaction are in one bottle and, moreover, they are based on DEGEE (diethylene glycol monoethylether), so they don’t contain methanol. All the necessary ingredients are already in the titrating agent; the working medium used can be easily adapted to the sample properties. We can use different types of solvents, mixtures of solvents, and even special reagents for ketones and aldehydes, while keeping at least 25% of any acceptable alcohol. Although in standard combinations one-component reagents are slower due to the lower pH, they have unlimited water capacity and are widely used mainly due to their high flexibility in working medium selection. You can also greatly improve titration speed in one-component systems to be as fast as in two-component systems if you use HYDRANAL-Methanol Rapid instead of normal dry methanol together with HYDRANAL-Composite.

How often should the titer value be determined?

AK: The frequency of the titer control depends mainly on the choice of the titrating agent used and how tightly sealed the equipment is against permeation by atmospheric moisture. The stability of Hydranal titrating agents is exceptionally good, and a weekly control of its titer is sufficient. Please note that fluctuations in the room temperature in the laboratory can cause fluctuations in the titer due to solvent volume expansion. A temperature increase of 1°C results in a titer decrease of about 0.1%. The determined titer must always be compared with the previous results to ensure it does not vary in value outside the normal, expected range. A control chart is advisable.

What is an acceptable titer decline?

AK: Many users are uncertain to which extent a modified titer is still acceptable, that is, how long a reagent may be used. Recommendations for acceptable titer declines are:

• For a titer of 5 mg/mL a decrease to 3.5 mg/mL
• For a titer of 2 mg/mL a decrease to 1.4 mg/mL
• For a titer of 1 mg/mL a decrease to 0.6 mg/mL
As pure alcoholic iodine solutions, the titration media of two-component systems (HYDRANAL-Titran) are very stable. However, the one-component reagent HYDRANAL-Composite, with its complex composition is subject to a weak “decomposition”, which can vary somewhat depending on the storage conditions. At a slightly elevated room temperature, the maximum decline is around 5% per year. This corresponds to a maximum 0.1% per week and is therefore negligible in the weekly routine.

**Do I need to re-determine the titer when changing the working medium?**

**AK:** The titer of a KF titration medium is identical when combined with any of the Hydranal KF media. With these media, the accuracy of the stoichiometry of the KF reaction is guaranteed. For example, if the titer of HYDRANAL-Composite is determined in methanol, it does not need to be re-determined when the titration medium is switched to HYDRANAL-Medium K. This statement remains valid if the Hydranal reagent for the titration vessel is used in its original quality. Additions of external solvents are only possible to a limited extent.

**Can anolyte be used as catholyte?**

**AK:** Using any anolyte as catholyte generally works, but not so well. The instrument works and results are obtained but since the anolyte contains more water than the catholyte it can result in a higher drift when used as a catholyte. Moreover, side reactions with sulfur compounds can occur as anolytes contain more sulfur dioxide. That is why catholytes are recommended in the cathodic compartment, as their composition matches the process at the cathode and they are very dry because they are packed in 5 mL ampoules (the KF reaction does not take place in the cathodic compartment, so moisture from the reagent will not be eliminated during conditioning).

**My coulometric anolyte has a brown color – is that normal?**

**AK:** The brown color indicates that an excess of iodine is present in the reagent, which indicates that the endpoint of the titration has been reached. In some cases, this excess of iodine is already present in the fresh reagent from the bottle. The iodine indicates the endpoint, and the titrator does not start. However, the light brown color does not influence the product quality. It is caused by a very weak oxidation in the reagent itself. Over a longer time period, this oxidation forms free iodine from the iodide in the reagent. A freshly produced reagent is adjusted to a water content of approximately 20–50 ppm, which makes the reagent colorless. These trace amounts of water are eliminated by the first pre-titrations in the KF equipment. In bottles that have been stored longer, this humidity is consumed by the formed iodine. When all water is consumed, the reagent starts turning brown. This color disappears by itself when filling the reagent into the titration vessel through contact with humid air. If the air contact is not sufficient, the addition of a few drops of humid solvent (e.g., chloroform) will help. Another approach would be shaking the reagent for a few seconds in a humid environment in the opened bottle or an open beaker before filling it into the titrator. Please do not add pure water for that purpose.

**Why are some coulometric anolytes dedicated to only one type of titration cell (with diaphragm)?**

**AK:** In the coulometric cell, the generator electrode may be a version with or without diaphragm. Many laboratories use cells without diaphragm, because they are much more convenient; however, they should be used only with standard composition reagents. If you need to use any additional solvent, besides methanol or ethanol, to dissolve your sample such as chloroform, xylene, toluene, or long-chain alcohols, you should switch to a cell with diaphragm. Also, if you need to use special composition methanol-free reagents to block side reactions with ketones, you should use them in a cell with diaphragm. And generally, cells with diaphragm are much more accurate, so even if you have a sample which dissolves well in methanol or ethanol, but you have a very low water content to measure, at ppm level, we recommend using a cell with diaphragm. Please note that the combination of non-standard composition reagents and a cell without diaphragm results in higher water content results obtained.

**SAMPLE HANDLING**

**How is the sample size properly calculated based on the expected water content value?**

**AK:** Ideally, the sample size in the volumetric KF titration should be selected in such a way that, depending on the expected water content and the titer of the reagent used, about half of the burette volume is consumed. The sample size for a coulometric determination should be such that it contains approximately 100–5000 µg water to achieve a high degree of accuracy.

In any case, the sample size depends on the composition, solubility, and availability of the respective sample material. An individual adjustment is often necessary. Sample size tables serving as a guide for selecting the optimum sample size for both techniques are available on request.

**Is calculating sample weight also necessary for oven systems?**

**AK:** The KF oven is used to release and transfer water from the sample to the titration cell. The sample size needs to fit the titration technique – volumetry or coulometry. Sample size calculations for each technique, with or without the oven, are the same.

**How should I handle lyophilized (freeze-dried) samples without transferring these to the oven vials?**

**AK:** For volumetric titration, the substance is dissolved by injecting the proper solvent into the vial through the lyophilization stopper. The entire solvent (with the dissolved sample) is then injected into the volumetric cell with pre-titrated medium and the water content is determined according to standard procedure. A correction factor for the water content of the solvent used (blank) is necessary, preferably by simulating the extraction in a vial without sample.

For coulometric titration, the same process can be used but a common alternative is using a dried anolyte for sample transfer. The coulometric cell is filled and dried in the usual manner. Then approximately 5 mL of anolyte is removed from the cell using a long-needle syringe (plastic or ideally glass) and immediately returned to the anodic compartment. The coulometer indicates the moisture adhering to the syringe. The moisture is removed from the syringe by repeated purging of the syringe with anolyte until no drift increase can be observed. Using the dry syringe, 5 mL of anolyte is removed from the cell and injected into the sample vial through the lyophilization stopper. The substance dissolves (or suspends) in the anolyte upon shaking (vibrating shaking may be necessary). The whole sample is then injected into the anodic compartment of the coulometric cell using the same syringe. The water content is determined according to standard procedure. The blank value of the solvent can be approximated to be zero. However, the blank value of the handling should still be considered.

Newer models of Karl Fischer ovens allow for different size vials to be heated and analyzed directly with the help of different adapters.
Agnieszka and Michael recently presented a webinar about important factors in KFT which are often overlooked. This opportunity was also used for conducting a survey among KFT users to gauge their awareness of the technique. We asked our experts to comment on the results of the survey.

**Which type of Karl Fischer titration do you use in your lab? (319 responses)**

AK/MM: As expected, volumetric titration is most popular. It is good that a significant portion of users understand the need for using coulometric cells with diaphragm for some types of samples. Also, the KF oven method is becoming increasingly popular.

**How many burette sizes for KF titration do you have in your lab? (164 responses)**

AK/MM: The number of burette sizes required in the lab depends on the range of water content in the sample being analyzed. Following the rule of “half burette volume consumption per one titration” can usually be achieved by using the proper titer value and adjusting the sample size. However, in some cases a change in burette volume is needed.

**How often do you change molecular sieves in drying tubes? (152 responses)**

AK/MM: The humidity level determines how often the drying agent should be changed. However, many labs do not change agents frequently enough. Molecular sieves should be changed every few weeks.
What do you use for titer determination and system checks?  
(171 responses)

AK/MM: Here we see a positive indication that users are switching from using pure water to water standards as is recommended. Awareness of the difference in accuracy is increasing. Liquid standards in bottles are recommended only for system checks due to the increase in water content after few openings.

How do you select the heating temperature for your sample?  
(161 responses)

AK/MM: Awareness regarding proper temperature selection is increasing with users adopting temperature ramps or even manual checks.

How often do you clean the titration cell?  
(156 responses)

AK/MM: Regular cleaning of the cell helps prevent many problems and should be one of the first steps in troubleshooting.