Karl Fischer Titration of Foods with

AQUASTAR®

Pyridine-free Reagents

Introduction

Water content can have major impact on shelf life, texture, taste, and packaging requirements for commercial foods and related products, such as food additives, beverages, pet foods, animal feed, and natural products. Therefore, accurate water content measurement has always been of interest to the food and beverage industry.

Since its invention by Dr. Karl Fischer in 1935, Karl Fischer (KF) analysis has progressed from an esoteric laboratory procedure to a widely accepted instrumental method routinely used in a variety of industries. In the food industry, traditional methods of water content determination have historically been based on various thermal techniques, such as "loss on drying" (LOD), which measure loss in sample mass under the particular conditions applied. However, over time it became clear that mass loss produced using such methods was caused not only by the sample's water content, but also by all the other volatile substances either initially present in the original sample or produced by the heating process itself under the particular drying conditions. As a result, KF has emerged as a more accurate and reproducible method for water content determination in foods. This is reflected, for example, in recent AOAC[1] Official Methods 2001.12 (Water/Dry Matter (Moisture) in Animal Feed, Grain, and Forage (Plant Tissue), 991.02 (Moisture in Soft-Moist and Semi-Moist Pet Foods, and 977.10 (Moisture in Cacao Products).

Additionally, many recent advances in titrator instrumentation have further improved the accuracy and reproducibility of KF analyses, and enabled true automation by use of such devices as sample changers, ovens, and homogenizers. Yet, despite the development of a variety of state-of-the-art instrumentation, the key to successful KF analysis remains the fundamental understanding of the interaction between test samples and KF reagents.

Challenges Posed by Food Samples

Foodstuffs encompass a rather diverse group of products. Carbohydrates, sugars, fats, oils, milk, and complex natural matrices present different challenges for the analyst, and require appropriate techniques, reagents, and, in some cases, auxiliary instrumentation to ensure trouble-free analysis.

Fatty and oily food products, including mayonnaise, sunflower oil, margarine, and butter, have poor solubility in methanol, which is typically used a solvent in volumetric KF, or as part of many coulometric KF reagents' formulations.

Cereals and starchy products, such as wheat flour, pastas, or rice, contain very strongly entrapped water in their cellular structures, which is difficult to extract using conventional KF reagents.

Due to its high water content, milk does not present a particular challenge to KF analysis, provided that it is properly homogenized immediately prior to sample injection. However, certain milk products, like cheese, anhydrous milk fat, and powdered milk require special techniques.

Vegetables and other natural products do not release water easily from their cellular matrices

Strategies for Successful KF of Food Samples

The challenges presented by food products described above may be overcome by using various specialty reagents and titration aides, performing titrations at elevated temperatures, or by employing auxiliary equipment. As illustrated below, these techniques may be combined for maximum effect.

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For oils and fats, co-solvents such as hexanol, decanol, chloroform, and xylene are frequently used to increase the solvent capacity of conventional KF reagents in the titration cell. Alternatively, specially pre-formulated KF reagents incorporating one or several of these solvents are commercially available for both volumetric and coulometric KF analysis. For example, products such as peanut oil, butter, mayonnaise, fish oil, and margarine can be analyzed using such reagents.

Certain other food products will not dissolve in any co-solvents suitable for KF, yet will release all of their water content in the presence of the titration aide, formamide, at room temperature. In such cases, a 3:2 mixture of methanol and formamide is used as a solvent in volumetric KF. In extreme cases, a 1:1 mixture can be used. With coulometric analysis, the use of formamide is possible only with fritless cells (i.e., cells without a diaphragm). Here, a 4:1 mixture of the coulometric fritless cell reagent (e.g., AQUASTAR® CombiCoulomat Fritless, Cat. No. 1.09257.0500) and formamide should be used. In all cases, the formamide used should be as dry as possible, and the mixtures must be created fresh daily. Examples of products that can be analyzed at room temperature with the help of formamide include fructose, sorbitol, mannitol, marzipan, and many others.

Either in conjunction with use of formamide, as described above, or as a stand-alone technique, titration at elevated temperature using a special jacked titration vessel can also be used for samples which are difficult to dissolve or extract moisture from at room temperature. This technique is applicable to volumetric KF only. The maximum temperature in the titration cell should not exceed 50°C. Examples of products that can be analyzed at elevated temperature with the help of formamide include molasses, gelatin, saccharose (total water), liquorice, wine gums, ground almonds, and a number of others. Below is an illustration of a commercially available jacketed titration cell.

Moisture content of substances that do not allow for effective extraction using formamide or direct analysis at elevated temperatures in the titration cell can be determined by using an auxiliary instrument, known as a KF Oven or “tube furnace”, coupled with either a volumetric or coulometric KF titrator. Here, moisture is released from the sample in the KF Oven, at temperatures between 100-300°C, and is then carried into the titration cell using a dry inert carrier gas, such as nitrogen. Care must be exercised to select a temperature which will liberate the water content without breaking down the sample matrix. Suitable products for this technique include ground coffee (105°C), rice (110°C), lactose (150°C), table salt (250°C), etc. Below is an illustration of a commercially available KF Oven connected to a KF titrator.

Another technique that facilitates extracting the water entrapped in cellular, capillary, or crystalline matrices involves the use of a high-speed homogenizer that is inserted directly into the KF instrument's titration cell, as illustrated below. The homogenizer finely fractionates the problematic sample matrices, thus liberating their water content. Homogenizer-based methods can in some cases entirely replace the use of hazardous co-solvents and titration aides, such as chloroform and formamide. Top KF titrator manufacturers typically offer simple and convenient adapter kits that allow for commercially available homogenizers to be easily connected to their instruments. Homogenizer-based methods have been published for cheese, jams, candies, chocolate, powdered milk, and almonds.[2]

[2] *Application Brochure 27: Karl Fischer Titration with a Homogenizer, Mettler-Toledo GmbH, Greifensee, Switzerland (1999).*

Other, more esoteric techniques, such as titration in boiling methanol,[3] also exist, but are in many cases too cumbersome or impractical for routine daily use.

Examples

The tables below provide examples of reagents and techniques used to analyze several food products by volumetric and coulometric KF, respectively. Detailed procedures for analysis of these and other food products can be found in the **AQUASTAR® Karl Fischer Application Resource CD** available free of charge from EMD Chemicals.

Table I – Volumetric KF Analysis

Sample	Titrant	Solvent System	Extr. Time	Temp.	Sample Size
Peanut oil	CombiTitrant 5	CombiSolvent Fats – 50 mL	1 min	R/T	5.0 g
Fructose	CombiTitrant 2	CombiMethanol – 30 mL Formamide – 20 mL	3 min	R/T	2.0 g
Molasses	CombiTitrant 5 Keto	CombiMethanol – 30 mL Formamide – 20 mL	3-5 min	50°C (cell)	0.1 g
Lactose monohydrate	CombiTitrant 5	CombiMethanol – 50 mL	1 min	150°C (oven)	0.5 g

Table II – Coulometric KF Analysis

Sample	Cell Type	Anolyte	Catholyte	Extr. Time	Temp.	Sample Size
Maltose monohydrate	Fritted	CombiCoulomat Frit – 100 mL	CombiCoulomat Frit – 5 mL	N/A	R/T	0.1 g
Ground coffee	Fritless	CombiCoulomat Fritless – 100 mL	N/A	10 min	105°C (oven	0.1 g
Almond oil	Fritless	CombiCoulomat Fritless – 80 mL Decanol – 20 mL	N/A	N/A	R/T	1.0 g
Table salt	Fritless	CombiCoulomat Fritless – 100 mL	N/A	5 min	250°C (oven)	1.0 g

For additional **AQUASTAR®** product Technical and Applications Support, or to request a FREE copy of the **AQUASTAR® Karl Fischer Application Resource CD**, please call: **1-800-222-0342**

[3] *AOAC Official Method 2001.12 – Method II (Boiling Methanol Extraction Alternative)*