

THE IMPORTANCE OF KARL FISCHER IN QUALITY CONTROL FOR THE SUPPORT OF AUTOMATED LIQUID STORES

Zoe Heaton, Mark Connell, Analytical Sciences, Dr Sue Holland, Rob Hughes, Dr Rob Lively, Compound Management, Stevenage, UK

ABSTRACT

Karl Fischer is a reliable and widely used method in industry for determining water content by coulometric titration. It is widely used in the process development area but is not generally applied to sample management operations.

A number of experiments are being set up to validate the storage and environmental conditions selected for the storage of compounds in the GlaxoSmithKline Automated Liquid Storage Systems. This poster will describe how Karl Fischer titration has been used at GSK to validate storage conditions and define operational "best practices" for sample preparation.

INTRODUCTION

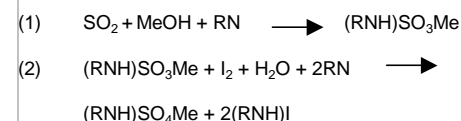
The Karl Fischer Titration (KFT) technique was introduced in 1935, for the determination of small amounts of water in a variety of organic and inorganic solid and liquid samples.

The estimation of water in a material may be significant in defining its history, its chemical composition and its stability. In compound management Karl Fischer Titration is used to measure the water content of DMSO, (a very hygroscopic solvent) and other samples dissolved in DMSO.

BACKGROUND

The Karl Fischer Titration

The Karl Fischer Titration is a two stage process. The first stage involves the reaction of sulphur dioxide with methanol to form an ester which is neutralised by a nitrogen base (RN) (1). The second stage is a rapid oxidation of the alkyl sulphite anion to alkyl sulphate by iodine - a reaction which consumes water (2).



There are two important requirements if the above reactions are to be stoichiometric. Firstly, the alcohol used must completely esterify the sulphur dioxide and secondly the base must be of suitable strength to completely neutralise the acids produced during the reaction.

TYPES OF KARL FISCHER TITRATIONS

There are essentially two types of Karl Fischer titrations; Volumetric and Coulometric.

Volumetric Karl Fischer Titration

In volumetric Karl Fischer the sample is dissolved typically in a methanol solution of SO₂ containing pyridine for example. The iodine required for the reaction is titrated into the sample solution either manually or automatically. The end-point is generally detected potentiometrically.

Coulometric Karl Fischer Titration

In a coulometric Karl Fischer method, the iodine required for the reaction is produced by the anodic oxidation of iodide. The iodine then reacts with the water that is present. According to Faraday's Law the amount of water titrated is proportional to the total current used in generating the iodine necessary to react with the water present. One mole of iodine reacts quantitatively with one mole of water. As a result, one milligram of water is equivalent to 10.71 coulombs. Based on this principle, the water content of the sample can be determined by the quantity of electricity required for electrolysis.

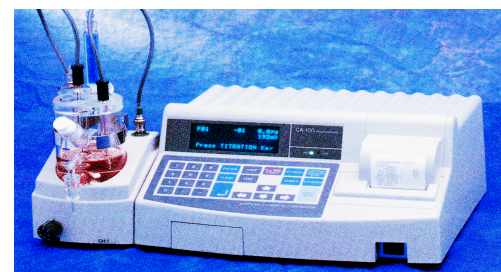
COMPARISON OF COULOMETRIC AND VOLUMETRIC METHODS

COULOMETRIC	VOLUMETRIC
Allows the determination of microgram amounts of water in samples. A major advantage for determining small concentrations of water in aprotic solvents such as DMSO, DMF, DMAc.	Allows the determination of large amounts of water in samples.
It is mainly a suitable method for liquid samples, although it can be used for solids and gases.	Suitable for both liquid and solid samples.
Coulometric reagent does not require standardisation and has the advantage of being used repeatedly.	Volumetric reagents require standardisation every day.

The coulometric Karl Fischer method was selected for work in compound management due to the sensitivity of the technique and the limited amount of sample available.

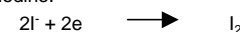
KARL FISCHER INSTRUMENT

The Karl Fischer instrument used is the Mitsubishi Coulometric moisture meter Model CA-100



TITRATION METHOD

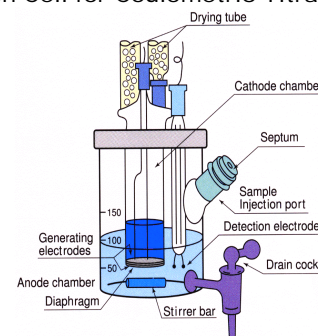
Electrolytic oxidation is carried out in the anolyte, which consists mainly of iodide, sulphur dioxide, bases, and solvents such as alcohols. Electrolytic oxidation proceeds when a sample is added to the anolyte. The Karl Fischer reaction occurs to produce iodine.



Iodine is produced in proportion to the quantity of electricity so the water content can be estimated based on the coulombs required for electrolytic oxidation.

A check solution containing a known amount of water is titrated at the beginning and the end of experiments to ensure that the KF instrument is functioning correctly.

Titration Cell for Coulometric Titration

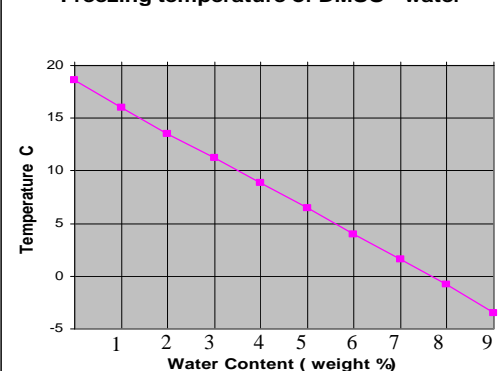


WHY MONITOR WATER UPTAKE ?

If water enters a sample which is dissolved in DMSO then various problems can occur such as:

- change in concentration
- change in solubility which could cause precipitation
- degradation of compound
- change in physical state - see graph below

Freezing temperature of DMSO - water



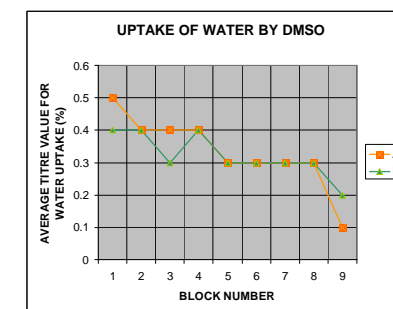
APPLICATIONS: DMSO MONITORING

The % water content of DMSO is monitored at the start of the sample dispensing process before it is used for the dilution of samples. DMSO is monitored for water content in:

- drums of DMSO upon arrival
- day storage tank
- robotic systems

Investigation of the % Water Uptake of DMSO During Manufacture of Master Stock Blocks

In the preparation of Liquid Samples, procedures are designed to minimise water uptake by DMSO. Quality control studies were carried out to measure water uptake during the process of manufacturing Master Sample Blocks and Source Blocks used in the GSK Automated Liquid Store. The study mirrored sample preparation carried out on Tecan Genesis Machines such that water uptake was monitored for the maximum time a block in preparation was unsealed.

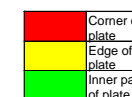


The results indicate that the entire procedure of dispensing through to storage and transferring of DMSO is a favourable process as the % water uptake is below 0.7%.

WATER UPTAKE DURING SAMPLE STORAGE

A five year study was started in Jan 2000 to determine how the storage of samples at -20°C in DMSO affects stability and water uptake. Samples were stored in master blocks at -20°C sealed with Beckman foil seal. Columns 11 and 12 were filled with 2mLs of fresh DMSO. 100ul of DMSO was tested using Karl Fischer at each time-point to monitor the % water uptake by DMSO.

	Average % water uptake		
	3 Months	6 Months	1 Year
A11	0.64	2.54	3.85
A12	1.80	4.93	8.21
B11	0.14	0.62	1.02
B12	0.57	1.77	2.79
C11	0.10	0.33	0.51
C12	0.46	1.37	2.21
D11	0.08	0.34	0.42
D12	0.45	1.57	2.03
E11	0.10	0.35	1.10
E12	0.43	1.28	3.45
F11	0.12	0.34	1.09
F12	0.41	1.18	3.62
G11	0.18	0.60	1.80
G12	0.60	1.84	5.80
H11	0.79	2.64	7.68
H12	1.79	4.75	8.89



The results demonstrate a clear correlation between the position on the plate and water uptake. DMSO on the edge of the plate, and in particular the corners, has greater water uptake than DMSO in the interior of the plate. Water uptake also increases with time.

DIFFERENT SEALING TECHNOLOGIES

Currently Master Sample Blocks and Source Blocks are sealed with an aluminium foil lid as supplied by Beckman. Various lids and seals from different manufacturers were tested to see if any offered any advantages in preventing water uptake during storage.

Master Sample Blocks were filled with 1mL of fresh DMSO in well position A1 and sealed immediately using each different method. A control Master Sample Block was set up with a lid only. The blocks were left in the laboratory at 20°C for 2 weeks. After 2 weeks, the blocks were tested for % water content.

Results

Sample Number	Supplier	Well Position	% water uptake
5	control, only lid	A1	17.2
6	CAP MAT	A1	15.3
7	COSTAR	A1	3.8
9	BECKMAN	A1	0.3
11	MATRIX	A1	2.9
12	MATRIX CLEAR	A1	18.3
13	HEAT SEAL	A1	0.5

The recommendation from these results was to continue to use foil seals from Beckman as they gave the least % water uptake after 2 weeks and to investigate the possible use of heat sealers.

Sealing technologies including septum seals, pierceable seals and other manufacturers seals have also been tested.

FURTHER WORK

Our in-house Applied Technology department are looking at the possibility of automating the KF method, possibly by using a Gilson 233XL autosampler to inject the samples and also to replace the Karl Fischer solvents when they have expired so 96 and 384 well plates can be run overnight.

REFERENCES

- Jeffery, G.H, Bassett, J, Mendham, J, *Vogels Textbook of Quantitative Chemical Analysis*, Longman Scientific & Technical, 1989.
- Willard, H. H, Merritt, L. L, Dean, J. A, Settle, F. A, *Instrumental Methods of Analysis*, Wadsworth, USA, 1988.