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ENTHALPIMETRIC ASSAY OF THE WATER CONTENT OF SOME OILS AND FATS

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A rapid and accurate direct injection enthalpimetric (DIE) method has been developed for the determination of the water content in some oils and fats of commercial interest. The method combines the DIE and the Karl Fischer Reagent procedures and is calibrated against real samples whose water content have been determined by a standard distillation method using toluene as the immiscible solvent. The reproducibility and precision are equivalent to those of the standard method for the water content of materials such as soya bean oil. The procedure is able to determine water contents in compounds such as lard, which cannot be determined by the Dean & Stark method. Once calibrated, the proposed method can be operated routinely by semi-skilled personnel. The main advantages of the method are those of time and the cost of analysis.

Chemically, the principal roles of water in foods include: (a) solvent, (b) reaction medium, (c) reactant, (d) antioxidant, (e)pro-oxidant, (f) a component of intramolecular structure and intermolecular structure [1]. Consequently, an ability to determine the water content is vital in ascertaining stability and thermal behaviour in connection with the processing and the packaging of foodstuffs.

Many industrial food processes require reliable quantitative methods for the determination of moisture or water since water enters directly or indirectly into many chemical reactions. In response to this widespread need for reliable analysis, a wide array of methods [1-3] is presently available to cover the range of water content from parts per billion to high percentages by weight and volume, but all, even the widely used Karl-Fischer Reagent technique, suffer some limitations. Thus, a study of a new technique, such as Direct Injection Enthalpimetry is potentially of great interest to the analytical chemist.

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Physical methods, mainly gravimetric, include oven drying at ambient pressure or under vacuum, which is the oldest approach for determining water in these circumstances. In some cases water is adsorbed by a strong desiccant, such as phosphorus pentoxide or magnesium perchlorate. A rapid method based on gravimetry, which has had some use in food analysis, is based upon the reaction of calcium carbide with water to yield acetylene. It has been used for the determination of moisture in fluor, butter and fruit juices [4].

There are many chemical methods of analysis based on reproducible reactions with water, in which a product or excess of reagent is titrated. Applications of titrimetric methods, other than the Karl-Fischer Reagent procedure [5], however cover only a limited range of systems and often newer techniques have come to take their place. When water determinations are needed only occasionally, a titrimetric method may be the most convenient method available. Most of the reactions utilised involve a hydrolysis reaction, leading to the formation of a titrable species [6-7].

Another technique which is used for the determination of water is distillation. It was originally designed for the determination of moisture in cereals and cereal products and used a petroleum fraction and toluene as the immiscible distilling liquids. Many types of apparatus have been designed for this technique particularly with reference to the design of the water receivers or traps, but probably the most widely used is that due to Dean and Stark [8].

One of the few generally applicable procedures for determining water is the titrimetric Karl-Fischer technique, first introduced in 1935 [5]. The selectivity of the reagent, together with the rapidity of the analysis, has led to many variations in its use. It is the most widely applicable technique available today, following modifications designed to improve the stability of the system and the detection of the end point. Both macro and microscale determinations can be performed using various methods of end point detection. The most frequently used methods are visual, dead-stop [9-12], coulometric [13-16], and potentiometric [17-20].

Most thermal techniques available for water determination in foods are of limited use. Thermogravimetry has been reported for numerous applications [21-23], but only small solid samples can generally be used. Thermal conductivity, differential thermal analysis, and heat of reaction measurements have also been used [4, 24].

The technique of Direct Injection Enthalpimetry (DIE) [25] combined with the Karl-Fischer method provide additional sensitivity to a method for water determination in some organic solvents [26]. Binary mixtures of water and miscible organic liquids have been analysed by injection into sulphuric acid [27]. The DIE method had also been used by utilizing a temperature pulse obtained from the rapid exothermic adsorption of water by molecular sieves [28].

The present work considers the combination of DIE and the Karl-Fischer method for the determination of the water content of some foodstuffs such as oils, lard, margarines and cheeses.

Experimental

A preliminary study was done to obtain a suitable solvent for oils, fats and materials of high fat content. The solvent must be free from water and have a minimum heat of mixing with the Karl-Fischer Reagent used. Carbon tetrachloride and chloroform both as single organic solvents, and as mixtures with various proportions of methanol and pyridine were investigated. The Karl-Fischer Reagent was injected into each of the solvents and the reaction heat, released by mixing, was measured. The choice of the solvent composition CCl_4:MeOH:Pyridine = 5:5:2 was made on the basis of both heat of mixing and ease of dissolution of sample.

Determination of water content by the distillation method

A standard Dean and Stark distillation apparatus was used in the present work. The procedure followed is:

Weigh a suitable amount of sample (oils, fats = 100 grams; margarine = 10 grams; cheese = 3 grams) into a dried, distillation flask. Add an excess of toluene (for oils and fats = 150-200 ml; margarine and cheese = 50 ml) and a small amount of anti-bumping granules. Fit the receiver and the condenser, then distill the sample for two hours. Measure the condensed water is the receiver and calculate the water content in the sample. The results are presented in Table 1.

Preparation of a calibration curve

Heat pulse measurements of the heat of the reaction between water in the samples and the Karl-Fischer Reagent (ex. BDH) were carried out using a mixture of chloroform, methanol and pyridine (5:5:2 v/v) as a solvent. The apparatus used has been previously described [29].

Table 1 Water content of some commercial oils, lard, margarines and cheeses, determined by a standard distillation method

Sample	Amount of sample, g	Water content			
		*Vol., ml	%, v/w	ref., %w	
Corn oil	100	0.16	0.16	0.18	a
Grapeseed oil	100	0.24	0.24	–	b
Groundnut oil	100	0.06	0.06	0.075	a
Olive oil	100	0.10	0.10	0.095	a
Soyabean oil	100	0.17	0.17	0.185	a
Lard	100	unmeasurable	–	–	b
Soft margarine	10	1.45	14.50	max. 16	c
Sunflower oil-margarine	10	1.32	13.20	max. 16	c
Cheddar cheese	5	1.80	36.00	37	d
Cheese spread	3	1.45	48.30	46-60	d

a = Mitchell and Smith (1980) [1], b = no reference available, c = Aurand and co-workers (1987) [30], d = Egan and co-workers (1987) [31], * Average of 3 concordant results

To evaluate the water content in the sample by DIE (as a proposed method), a calibration curve can be realized by using distilled water as an analyte for the "standard compound". The procedure is as follows: To a (50 ml) of blank solution in the reaction vessel, add known amounts (2-15 μl) of distilled water. Fit the lid, thermistor and stirrer into the top of Dewar flask. Switch on the stirrer, electronic bridge and the recorder. When the temperature equilibrium has been attained (recorder reading is steady),

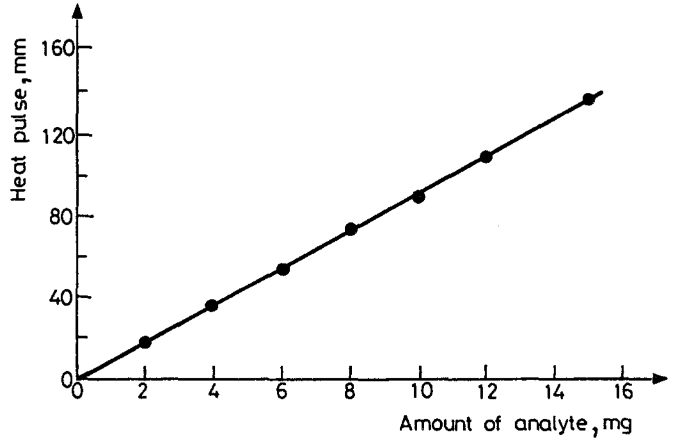

Fig. 1 Calibration curve for moisture determination

inject an aliquot (5 ml) of thermostatted Karl-Fischer Reagent. Note the heat pulse recorded. The results are presented in Fig. 1.

Determination of water content by a standard addition method

To ascertain whether the Karl-Fischer Reagent reacts with materials (other than water) present in the sample, the method of standard addition of the analyte is used. Distilled water is used as the standard compound. The procedure for water determination by this method is as follows: Dissolved a known amount of sample in the solvent, transfer an aliquot (50 ml) into the reaction vessel.

(i) Inject an aliquot (5 ml) of thermostatted Karl-Fischer Reagent and measure the heat pulse (A)

(ii) To another aliquot (50 ml) of sample, add a know amount of water. Inject Karl-Fischer Reagent (5 ml), measure the heat pulse (T).

(iii) Inject an aliquot (5 ml) of thermostatted Karl-Fischer Reagent into 50 ml solvent. Measure Neat Pulse (B).

If T is the heat pulse of (sample + additional analyte), A is the heat pulse of (solvent + analyte added) and B is the heat pulse of blank., then the heat pulse of water in the sample (X) $= T - A - B$. The results are presented in Table 2.

Table 2 Water content of some commercial oils, lard, margarines and cheese, determined by standard addition enthalpimetric method

Sample	Amount of sample, g	Amount of water added, mg	Heat pulses, mm				Water content, %w
			Actual		Mean		
Blank	0	0	99	98	98	98	
	0	3	125	124	125	125	
	0	5	143	143	142	143	
Corn oil	5.00	5	220	219	219	219	0.17
Grapeseed-oil	2.00	5	187	186	186	186	0.24
Groundnut-oil	5.00	5	173	175	174	174	0.07
Olive oil	5.00	5	185	183	184	184	0.09
Soyabean-oil	5.00	5	223	223	224	223	0.18
Lard	10.00	5	154	154	154	154	0.01
Soft-matgarine	0.05	5	209	210	209	209	14.67
Sunflower oil margarine	0.05	5	204	202	203	203	13.33
Cheddar-cheese	0.03	3	223	224	223	223	36.30
Cheese spread	0.02	5	230	229	230	230	48.33

Statistical parameters of the proposed method

In separate experiments, four series of aliquots of four different amounts of soyabean oil were assayed; the first two series by DIE (as a proposed method) and the second, two series by distillation method. The results are presented in Table 3.

Table 3 Statistical results

Amount of sample used,	DIE			Distillation method		
g	*Mean	S D	RSD	*Mean	S D	RSD
2.00	3.56	0.67	2.07%		–	–
5.00	8.95	0.71	0.88%		–	–
40.00		–	–	70.48	0.018	1.61%
100.00		–	–	180.05	0.007	3.97%

Number of samples: 10 samples per series, *Mean value: - weight % water (in mg) per sample

Results and discussion

Results

All of the experiments accomplished by the DIE method for determining water of moisture content of some commercial oils, lard, margarine and cheese are carried out at 20°, with a recorder sensitivity of 100 mV for FSD, a sensitivity of the bridge of 5.0 (arbitrary units), and the chart speed of 80 mm per minute. Typical results of determining water content by the DIE technique and the distillation method are presented in the following tables.

Discussion

For both the chemical and instrumental determinations of moisture or water content of any materials, it is necessary to have a water-free organic solvent to dissolve the sample. The most favourable solvent for an enthalpimetric determination is chosen not only on its capability to dissolve the sample, but also on its heat of mixing with the reagent. Thus for an ideal solvent, the samples must be readily soluble in the solvent and the reagent blank should be zero.

Various mixtures of carbon tetrachloride, methanol and pyridine; chloroform, methanol and pyridine were investigated. The results from these mixtures indicate that the heat pulses of the blanks decreased by decreasing the proportions both of carbon tetrachloride and of chloroform, but the solubility of the samples also decreased. However, the samples still completely dissolve in mixtures of both carbon tetrachloride, methanol and pyridine (4:5:2) and chloroform, methanol and pyridine (5:5:2). The heat pulse of the blank of the second mixture is much lower than that of the first. Hence, it is considered that the mixture of chloroform, methanol and pyridine (5:5:2) showed promise as a suitable solvent for oils, fats and materials of high fat content in the present work.

Since different methods used for the determination of the water content in any materials routinely or in control laboratories produce results which are not directly comparable because they are obtained by different techniques, it is regarded as being necessary to have a comparison method to evaluate and calibrate any proposed method. The comparison of results obtained by different methods or in different laboratories must be based on a commonly accepted reference method. In the purpose of water determinations of materials, the distillation method with an immiscible solvent is one, of the reference methods widely adopted. The AOAC has adopted this method using toluene as the solvent.

From a consideration of the simplicity of the procedure and the apparatus used, this method was used to evaluate the proposed method. It can be seen from Table 1, that using the distillation method for determining water contents of commercial oils, lard, margarine and cheese, the results obtained are in agreement with the values shown in any references available.

Calibration

In the DIE method of analysis, it is usual to have a calibration curve using the pure analyte in the materials being determined. In this instance the "standard compound" was distilled water.

Figure 1 shows that using distilled water as a standard compound, a linear relationship between the amount of analyte and the heat pulse of its reaction with Karl-Fischer Reagent can be obtained. In the present work, the amounts of analyte used were added by using a micro-syringe.

By using the calibration curve, any evaluation of water content of some samples can be easily accomplished. It must be emphesised that any change in the enthalpimetric system, such as reaction temperature, thermal sensor sensitivity of the bridge or the recorder, will necessitate re-calibration.

In order to ascertain the frequency of calibration required, over a period of 10 working days, a series of approximately 200 samples was assayed by the proposed method and every 20th sample was also assayed by the distillation method. The results indicate that it is not necessary to calibrate the DIE method more than once every 5 working days or whenever the type of sample is altered. It is recognised that if frequent recalibration is required, it would, to some extent, detract from the advantages of the proposed method.

Although the standard addition method is widely used, the results of this investigation show that the Karl-Fischer Reagent does not react with the matrix of any of the sample considered. Thus it is not necessary to "spike" samples for routine analysis by the proposed method.

It can be seen in the data, that a wide range of water content (0.012-48.30%) can be monitored by the proposed method using the same sensitivity of the system. The use of a recorder sensitivity of 100 mV for FSD and sensitivity of the bridge of 5.0 (arbitrary units) are relatively low sensitivities for the system used. It is possible to increase either sensitivity to achieve a lower limit of determination. However, the heat pulse of the blank will increase by increasing the sensitivity of the system.

Relatively high heat pulses of the blank were obtained. Several factors such as the heat of dilution and of mixing of the reagents may contribute to the heat of the system. Another possibility is the relatively high water content of the blank solution used, and therefore more heat will be released on injecting the Karl-Fischer Reagent into the system. Although all chemicals used in the present work are of HPLC grade and their water contents are so small (chloroform is less than 0.01% methanol and pyridine are less than 0.05%, toluene is less than 0.03%)(Aldrich catalogue, 1989), the contribution of these to the blank is, possibly, still significantly high. In real situations, it is impossible to obtain the blank absolutly dry. The problem mentioned is frequently encountered in the use of an enthalpimetric technique. Theoretically, the problem can be minimised by compensating the blank using an endothermic blank solution, however, in many cases no endothermic blank solution is available.

The results presented in Table 3 indicate that on statistical grounds, the enthalpimetric method is at least as acceptable as is the distillation method, moreover the relative standard deviation (RSD) results are lower than that of the distillation method.

Distillation and oven drying methods that are usually used as standard methods for determining water or moisture content of any materials specify a long process time. A long analysis time is not convenient in the rapid control of continuous plant operations or in routine analysis. The proposed method takes less than 10 minutes per sample. The DIE method has other advantages when considered for routine use in quality control and quality assurance laboratories involved in the moisture determination.

Using the proposed procedure with a disposable polystyrene reaction vessel and having samples determined in triplicate with a blank determination every 10 samples, 20-25 samples per working day can be determined. The time between receiving the sample and the calculation of the results is approximately 15 minutes. This is in contrast to the distillation method which takes approximately 2 hours and even more time is required for the oven drying method.

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Zusammenfassung — Zur Bestimmung des Wassergehaltes einiger kommerziell interessanter Öle und Fette wurde eine schnelle und genaue enthalpimetrische Methode mit Direkteinspritzung (DIE) entwickelt. Dieses Verfahren vereint DIE und die Methode nach Karl Fischer und wurde anhand von reellen Proben kalibriert, deren Wassergehalt durch die Standard-Destillationsmethode mit Toluol als nicht mischbares Lösungsmittel bestimmt wurde. Reproduzierbarkeit und Genauigkeit stimmen mit denen der Standardmethoden zur Wasserbestimmung von Stoffen wie z.B. Sojabohnenöl überein. Mit diesem Verfahren kann auch der Wassergehalt von Verbindungen wie Schweinefett bestimmt werden, der mit dem Verfahren nach Dean und Stark nicht ermittelt werden kann. Nach Kalibrieren der Methode kann sie routinehaft auch von angelerntem Personal durchgeführt werden. Die Hauptvorteile bestehen in dem geringen Zeitbedarf und in den Kosten der Analyse.