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Application of atmospheric pressure microwave digestion to total Kjeldahl nitrogen determination in pharmaceutical, agricultural and food products

Z. Chemat[1,*], D.E. Hadj-Boussaad[1,*] and F. Chemat[2]

[1] *Institut de Chimie Industrielle de Blida, BP. 270, route de Soumaa, 09000 Blida, Algérie*
[2] *Agrotechnological Research Institute ATO-DLO, PO Box 17, 6700 AA Wageningen, The Netherlands*

Abstract. The kinetics of Kjeldahl's digestion for nitrogen determination was compared between microwave and classical heating conditions. Various parameters (microwave power, concentrations, time) of microwave digestion were studied in order to define the optimal digestion conditions. Finally, the Kjeldahl's analysis of nitrogen using microwave digestion was applied to various pharmaceutical, agricultural and food products.

Key words. Microwave – Kjeldahl – nitrogen – digestion.

Introduction

Chemical analysis methods necessitate the introduction of samples under a liquid form. The dissolution of samples is frequently done by a prolonged heating in strong acid solution [1]. This is the case with nitrogen analysis using Kjeldahl's procedure. This method comprises three steps: digestion, distillation and titration. Whereas the latter two steps finished after only 15 minutes, digestion took at least several hours.

The Kjeldahl's method was discovered in 1883 and became a reference method of total nitrogen determination. It is applied for a large variety of samples: pharmaceutical, agricultural, food products, biological sediments and waste water matrices. The method consists of complete digestion of samples in concentrated sulphuric acid with catalysts such as copper, mercury and titanium salts at a high temperature ranging from 300 °C to 450 °C. Other additives can be introduced during the digestion in order to increase the boiling point of sulphuric acid. In these conditions of classical digestion, a substantial length of time is necessary to degrade proteinic or mineral nitrogen into ammonium ion NH_4^+ [2-4].

To remedy these constraints, various technological and chemical solutions have been proposed. In particular, the use of microwave heating for the stage of digestion. This method of electromagnetic heating is based on the direct absorption of energy by the sample. Thereby, thermal phenomena like conduction, convection or radiation play only a secondary role of temperature control and equalisation. Heat absorption in the sample is instantaneous, as a consequence, more energy is collected by the solution, decreasing the digestion time. This explains the recent interest for this new method by chemists and analysts [5-11].

The aim of this study is to show the potential of microwave heating in Kjeldahl's digestion with a critical comparison with classical heating.

In this paper, we describe the kinetic comparative study of the digestion of the arginine (amino acid taken as a model system). Before this, a study of the effect of various reaction conditions (micro-wave power, concentration, reaction time) on the microwave Kjeldahl's digestion is given.

Finally, the Kjeldahl's microwave method for nitrogen analysis is applied to various pharmaceutical, agricultural and food products. The results are compared with the French standard method AFNOR [12-15].

Material and methods

Reagents

Sulphuric acid 98%, d = 1.83 (Prolabo).

Hydrogen peroxide 33% (Prolabo).

Catalysts: potassium sulphate and selenium (Prolabo); copper sulphate (Merck).

Samples

Pharmaceutical matrices are represented by serums furnished by Institut Pasteur of Algiers (Algeria). These serums are used for blood group determination. Nitrogen concentration varies from 0.3 to 0.98 g per 100 g of sample. Agricultural and food matrices cover a range of nitrogen concentration from 0.5 to 4.75 g per 100 g of sample. Amino acids cover a higher range of nitrogen concentration from 10 to 32 g per 100 g of sample. Arginine is taken as a model system for the comparative kinetic study between microwave and classical digestion. This amino acid is present in a large variety of biological samples. It is also reputed to be difficult to

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digest because of the high nitrogen concentration 32.18 g per 100 g.

Classical procedure

According to the french standard method AFNOR [12-15], 0.2 g of the sample was added to 20 mL of sulphuric acid and 2 g of a catalyst (composed of 1 g of selenium, 10 g of $CuSO_4$ and 100 g of K_2SO_4). The digestion is the principal phase in the protocol and takes several hours. The success of the analysis depends on the efficiency of this digestion process. It must be conducted with the highest rigour and it is necessary that all the nitrogeneous forms are transformed into ammoniacal nitrogen. The digestion was carried out using an electrical heating block "Büchi 430". The distillation stage allows to shift the ammoniac from the digestion solution to a boric acid solution using a distillation block "Büchi 315". Finally, titration gave the nitrogen concentration of the analysed samples.

Microwave procedure

Microwave digestion was reached at atmospheric pressure in a Prolabo Maxidigest 350 monomode microwave oven cavity operating at 2.45 GHz with a power range from 0 to 300 watts. The borosilicate reactor has a capacity of 20 to 150 mL. The incident microwave power is controlled with a programmer which allows to work with one or several steps of microwave incident power according to the duration expressed in minutes [1].

A microwave digestion protocol was adopted from several scientific works in this field [8-10]. First, the sample was mixed with 20 mL of sulphuric acid without heating. Then, microwave carbonisation of the organic matrix was carried out. The carbonisation duration represents 80 to 90% of the digestion time. After that, hydrogen peroxide was added without heating. Finally, microwave oxidation permits the destruction of the most resistant molecules. This step represents only 20% of the total duration.

Microwave digestion was conducted without a catalyst or antifoaming agent, to avoid pollution problems and to reduce chemical reagents. The hydrogen peroxide was selected as an oxidant agent to complete the digestion [3,10]. At the end, the solution was distilled then proportioned according to the standard method AFNOR. The degree of nitrogen reported in this study is given from the average of three identical tests.

Results and discussion

Kinetic study of the arginine digestion

This kinetic study has been realised following the degree of nitrogen during the arginine digestion. This study allows a comparison to be made between the standard method AFNOR [12-15] and the Kjeldahl's microwave method.

In practice, it is difficult to sample from the acidic solution during the digestion procedure because of the presence of the fuming sulphuric acid and various oxidants. In order to rectify this problem, various digestions were prepared under the two methods at varying times. The kinetic results are presented in figure 1.

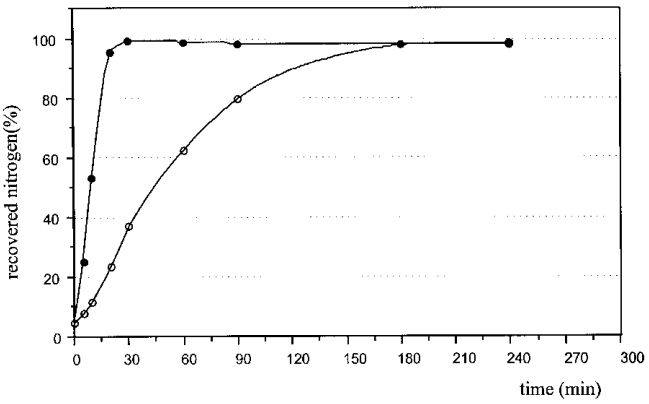

Fig. 1. Comparative kinetic study for Kjeldahl's nitrogen determination between the classical standard method AFNOR ❍ and the microwave method ●.

The digestion rate with the microwave method is six times faster than the classical process described by AFNOR [12-15]. Under microwave heating, the totality of arginine is digested in only 30 minutes, whereas, the classical method took 3 hours. After an infinite time, the nitrogen recovered yields for the two processes became the same.

At the first contact of arginine with sulphuric acid, the digestion starts instantaneously even without heating. In fact, 4.78% of the initial present nitrogen in arginine was recovered at t = 0 min. Before the heating step, the mineralization yields are practically identical whether using sulphuric acid alone or with a catalyst.

The digestion kinetic curves differ enormously between the microwave and classical method. Microwave digestion seems to be a first order reaction whereas the conventional method gives a second order kinetic curve.

Influence of the use of the oxidant or the catalyst in the digestion process

The kinetic study revealed the existence of microwave activation in the digestion process. In order to dissociate the effect of microwave heating from the oxidative effect of hydrogen peroxide, kinetic studies were carried out, under carefully monitored conditions, at different reaction conditions of digestion. The kinetic results of four digestions are illustrated in table I.

Table I. Influence of the use of the oxidant or the catalyst in Kjeldahl's digestion of arginine by classical and microwave methods.

	recovered nitrogen (%)			
t (min)	*H_2SO_4 + oxidant H_2O_2*		*H_2SO_4 + cata. AFNOR*	
	Microwave	*Classical*	*Microwave*	*Classical*
0	4.78	4.89	5.12	4.78
5	25.32	23.58	18.60	7.98
10	53.60	41.59	39.15	12.12
20	95.71	66.57	59.33	23.61
30	**99.13**	86.33	81.72	36.97
60	98.91	94.22	92.60	62.46
120	98.35	**99.39**	**97.57**	79.86
180	98.35	98.51	97.72	**98.19**
240	98.11	99.07	98.60	98.8

Digestion (1) was made with sulphuric acid and hydrogen peroxide under microwave heating. A sample of 0.2 g of arginine was introduced into the reactor. In the carbonisation step, 20 mL of sulphuric acid was added and the microwave power was 180 watts for 27 min. In the oxidative step, 7 mL of hydrogen peroxide was used and the microwave power was 290 watts for 3 min.

Digestion (2) was made with sulphuric acid and hydrogen peroxide under classical heating conditions using "Büchi 430". The monitored reaction conditions of experiment 1 were applied.

Digestion (3) was made with sulphuric acid and a catalyst according to the standard method AFNOR, but using microwave heating. A sample of 0.2 g of arginine was introduced into the reactor with 20 mL of sulphuric acid and 2 g of a catalyst. The digestion was performed in one step by microwave heating at an average power of 220 watts. This power setting was applied in order to sufficiently heat without degradation of organic and ammoniacal nitrogen to an elementary state.

Digestion (4) was made according to the standard method AFNOR with classical heating.

The fastest digestion (30 min) was achieved with microwave heating using sulphuric acid and hydrogen peroxide. The other digestions were complete after 2 or 3 reaction hours. It appears that the addition of hydrogen peroxide instead of the catalyst is more efficient for the digestion process. After an infinite time, the recovered nitrogen rates are identical under the four digestion processes. The activation effect observed in this kinetic study is principally due to microwave heating and not only to the addition of hydrogen peroxide.

Optimisation of the microwave digestion conditions

Classical Kjeldahl's digestion dates back to more than one hundred years. Throughout this century, hundreds of scientific studies [2-4] were made to improve the efficiency and the duration of this method. Microwave Kjeldahl's digestion dates back only a few years and publications in this field are scarce [5-11]. The aim of this part is to precisely determine the optimum reaction conditions needed for the proposal of a reliable, reproducible, economic and fast microwave Kjeldahl's digestion method. Therefore, six variables have been studied and optimised, and in each case, the other parameters remain unchanged.

Digestion time

Nine microwave digestions of arginine at different reaction times from 5 to 240 minutes were carried out. Microwave protocol takes place in two steps. In the first carbonisation step, a mixture of arginine and sulphuric acid was heated under microwave heating with a power range of 180 watts.

The carbonisation time represents 80 to 90% of the digestion time. In the oxidative step, 7 mL of hydrogen peroxide was added to the mixture and microwave heated at a power of 290 watts. The oxidation time represents 10 to 20% of the digestion time. This distribution time has been optimised by Feinberg et al. [8].

The digestion of arginine accomplished a yield of 95% after only 20 minutes (Fig. 1). The maximum nitrogen recovered was approximately 31.92 g/100 g (yield = 99%) after 30 minutes. It was not necessary to continue the digestion after this duration, due to a risk of degrading the ammoniacal nitrogen into an elementary form which cannot be detected by titration.

For future reference, the total digestion time for the optimal protocol will be 30 minutes. The carbonisation time was fixed to 27 minutes. The oxidation time at an average of 3 minutes is left to the discretion of the manipulator. The oxidation step was complete when the mineralized solution became clear.

Volume of sulphuric acid

Sulphuric acid plays an important role in Kjeldah's digestion. It transforms the organic matrix by carbonisation and fixes the free nitrogen under the form of ammonia sulphate. The volume (20 to 30 mL) and the concentration of sulphuric acid (98%) have been fixed by standard methods AFNOR [12-15]. Six microwave digestions of arginine were carried out with different quantities of sulphuric acid from 5 to 30 mL. The results are given as a percentage of recovered nitrogen over reaction time (Fig. 2). The optimum yield was obtained from a volume of 15 mL of sulphuric acid. Beyond this, the digestion is total and the recovered nitrogen is identical. The optimum volume required is therefore 20 mL and will be sufficient for difficult molecules.

Volume of hydrogen peroxide

The final digestion step is the destruction of tenacious organic molecules with hydrogen peroxide (33%). A short period of microwave heating increases the oxidative effect. With the microwave digestion method, the optimal recovered nitrogen can be obtained by using 7 mL of hydrogen peroxide. Beyond this volume, the mineralization is total and the recovered nitrogen is identical.

Microwave power

Six microwave carbonisations of arginine were performed by varying microwave power from 0 to 240 watts. The results

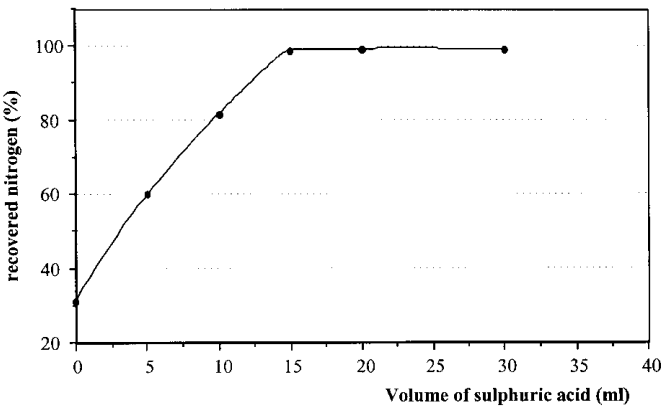

Fig. 2. Effect of sulphuric acid's volume on the Kjeldahl's microwave digestion method ($P_{carb.}$ = 180 w; $P_{oxyd.}$ = 290 w; $V_{H_2O_2}$ = 7 mL; m = 0.2 g arginine; time tot. = 30 min).

are represented in figure 3. Beyond a microwave power of 100 watts, the totality of nitrogen is recovered. At high microwave power, the recovered nitrogen yield decreases. This is due to the drastic carbonisation which degrades ammonical nitrogen into molecular nitrogen state. The optimum carbonisation microwave power required is 170 Watts. It is sufficient for heating and to avoid the degradation of ammonia. The oxidation step is added in order to achieve the digestion. Microwave power of 290 Watts allows a total oxidation. Use of the maximal microwave power (300 Watts) may damage the magnetron in the long term.

Sample mass

According to experience and the standard methods AFNOR, the mass of product is mainly as a function of its nitrogen concentration. For poor nitrogen products, it is necessary to introduce more substantial quantities of the sample.

Generalisation of the microwave Kjeldahl's method

The Kjeldahl's microwave method has been applied to nitrogen analysis of serums used for the determination of blood groups, to agricultural products (powdered milk, cow's milk, rice, corn, flour, beef, corned beef and chickpea) and to various amino acids and organic nitrogeneous products. The products cover a large range of nitrogen concentrations from 0.3 to 32 g per 100 g of the product. The same optimal microwave digestion protocol, shown in table II, is used for all the organic and biological products. Samples were taken according to AFNOR recommendations taken in function of the nitrogen content in the product: 1 g for cow's milk, rice, corn, flour and serums; 0.5 g for meat, chickpea and powdered milk; 0.2 g for amino acids. The results are represented in tables III, IV and V showing a comparative Kjeldahl's analysis between the classical standard method and the microwave method. The recovered nitrogen is comparable using the two digestion procedures: classical standard method AFNOR [12-15] and the microwave method.

It can be seen, microwave Kjeldahl's digestion is as efficient and reliable as the standard method. Furthermore, microwave digestion was complete within 30 minutes whereas the classical standard method took more than 3 hours. The microwave Kjeldahl's method appears to be efficient for liquid, viscous and solid products. It is also available for a large range of nitrogen concentration which can vary from tenth of grams to several tens of grams per 100 g of product.

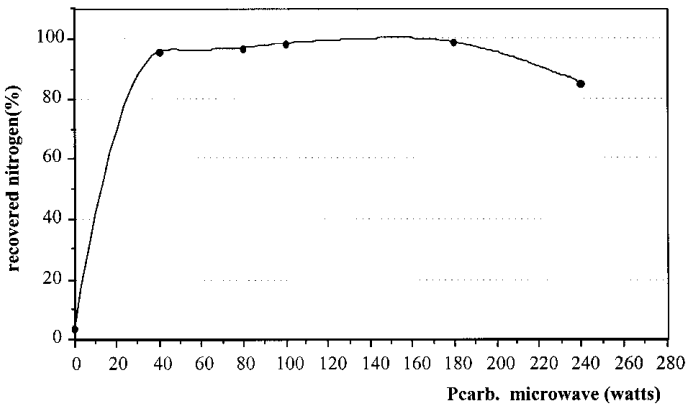

Fig. 3. Effect of microwave power during the carbonisation stage of the Kjeldahl's microwave digestion ($V_{H_2SO_4}$= 20 mL; $P_{oxyd.}$= 290 w; $V_{H_2O_2}$ = 7 mL; *m* = 0.2 g arginine; time tot. = 30 min).

Table II. Kjeldahl's microwave digestion procedure for total nitrogen analysis.

Step	*Power MW (watts)*	*time (min)*	$V_{H_2SO_4}$ *(mL)*	$V_{H_2O_2}$ *(mL)*
0	0	0	20	0
1	180	25	0	0
2	0	2	0	7
4	290	3	0	0

Table III. Comparative Kjeldahl's nitrogen determination in food and agricultural products between microwave and classical standard methods.

Agro-cultural and food products	*Classical Kjeldahl's method*		*Microwave Kjedahl's method*		*Standard*
	g % N	*t (min)*	*g % N*	*t (min)*	*g % N*
cow's milk	0.5	180	0.48	30	0.56
rice	1.04	180	1.18	30	1.16
corn	1.12	180	1.09	30	1.28
flour	1.57	180	1.72	30	1.92
beef	2.67	180	2.81	30	2.88
corned beef	3.69	180	3.61	30	3.52
chickpea	3.20	180	3.35	30	3.52
powdered milk	4.75	180	4.82	30	4.5–5.5

Table IV. Comparative Kjeldahl's nitrogen determination of serum products between microwave and classical standard methods.

Serum products	*Classical Kjeldahl's method*		*Microwave Kjedahl's method*	
	g % N	*t (min)*	*g % N*	*t (min)*
A + B N°2	/	180	0.42	30
Anti A N°3	0.45	180	0.49	30
Anti B N°6	0.48	180	0.35	30
Anti B N°8	0.34	180	0.36	30
Anti D N°7	0.95	180	0.89	30
Anti D N°10	0.96	180	0.92	30
Anti A + B N°9	0.46	180	0.39	30

Table V. Comparative Kjeldahl's nitrogen determination of amino acids between microwave and classical standard methods.

Produits	*Classical Kjeldahl's method*		*Microwave Kjedahl's method*		*Standard*
	g % N	*t (min)*	*g % N*	*t (min)*	*g % N*
Acetanilide	10.73	180	10.82	30	10.77
Casein	13.42	180	13.56	30	13.70
Glycine	16.35	180	16.82	30	17.00
Arginine	30.26	180	31.92	30	32.18

Conclusion

Since 1883, the Kjeldahl's method has been subject of a number of scientific studies especially with reference to the addition of new catalysts and salts to decrease the digestion time and to improve the carbonisation action of sulphuric acid. In the past decade, the microwave oven has been introduced to the chemical laboratory as a new way of conducting chemical reactions. In addition, the use of the couple sulphuric acid-hydrogen peroxide in the digestion reduces the Kjeldahl's digestion time and the chemical reagents needed.

This electromagnetic heating is based on the direct absorption of energy by the sample. As a consequence, more energy is collected by the solution, decreasing the digestion time. The kinetic comparative study between classical and microwave heating revealed the existence of microwave activation of the digestion process. This activation is principally due to the original electromagnetic heating, and not to the catalyst or the oxidant alone.

In order to generalise the microwave Kjeldahl's method, the digestion parameters (digestion time, microwave power, volume of reagents, sample mass) have been optimised. A microwave protocol has been adopted and applied to agricultural, pharmaceutical, food and chemical products. The recovered nitrogen by microwave (30 min) and classical (2 to 3 hours) methods are comparable as well as the given values by the French standard methods AFNOR. The microwave Kjeldahl's method shows a high reproducibility [10] of results and it seems economically profitable in comparison with the classical method. This study confirms the use of this original method as a routine analysis in chemical and food industries and laboratories for analysis and quality control.

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