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THE DETERMINATION OF UREA AND OTHER NITROGEN COMPOUNDS IN URINE BY THE DIE METHOD

P. MARIK-KORDA

Institute for General and Analytical Chemistry, Technical University Budapest, Hungary

The heat of reaction of hypobromite with the non-protein nitrogen compounds contained in urine is very high. This fact can be utilized for the determination of these compounds by the inverse DIE method. The DIE method also allows a separate determination of urea based on the specific action of the enzyme urease.

Urine is an aqueous solution of various inorganic and organic substances. Its composition is not constant: it varies with the temporary state of the organism. The degradation products of proteins secreted by the kidneys appear in urine in the form of non-protein nitrogen compounds (NPN) [1]. The urine excretion of the healthy organism is a function of its water balance. The amounts of individual substances discharged in human urine during a period of 24 hours – in the case of normal mixed nutrition and living conditions – are listed in Table 1.

Table 1

Urinary excretion of healthy adults during a period of 24 hours

Inorganic	g	Organic	g
Sodium	2–4	Nitrogen, total	10–18
Potassium	1–2	in urea	9–16
Calcium	0.2–0.4	in ammonia	0.4–0.8
Magnesium	0.2–0.4	in amino acids	0.4–1
Chloride	5–10	in uric acid	0.1–0.3
Phosphate	2–4	in creatine	0.4–0.6
Sulphate etc.	1.5–4	in urobilinogen etc.	30 mg

Depending on the liquid intake and loss of the organism, these substances are present in a total volume of 1 . . . 1.5 dm^3. Their analysis provides information on kidney functioning, on nitrogen balance and protein demand, resp., and on their relationships.

Practically all medical examinations start with routine blood and urine tests. However, the results obtained from analyzing a single urine sample are largely

accidental. For this reason, the sample to be tested is usually taken from the collected urine discharged during 24 hours. The urine collected is stored in deep-freezers, or a preserving agent is applied, to prevent eventual changes during storage.

Medical authorities agree that it would be preferable to test the samples immediately. This would, however, require 5 . . . 6 or more tests per patient per day, which – considering that NPN is determined by Kjeldahl destruction and subsequent distillation and titration, or by nesslerization and colorimetry – would overload the laboratory with work.

By the progress of thermometric methods and the increased sensitivity of both chemical and instrumental techniques, the amounts of material for the tests could substantially be reduced during the past decade, and hence the potential of biochemical applications was increased. Also, continuous analyses and computerized data processing became feasible. Following the pattern of the enzyme electrode the enzyme thermistor has been developed [2]. These up-to-date techniques have not, however, become widespread in clinical practice, but are limited to research institutes only.

A review [3] characterized the Hungarian thermometric analyzers as macro-calorimeters suited for industrial applications. The instrument we used in our work, the Silicotherm (manufacturer: MOM, Budapest) was in fact developed for the purpose of determining the silicon content in large-volume industrial materials by the DIE method, requiring samples of 100 . . . 200 cm^3 [4].

By means of a simple technique, we were able to reduce the amount of sample required substantially, and have developed several determinations which can be carried out with this instrument [5, 6].

For many decades, sodium hypobromite has been in use as oxidizing agent in quantitative analysis. Its application is based on the reaction

$$BrO^- + H_2O + 2\,e^- \rightleftharpoons Br^- + 2\,OH^- \qquad E = +0.76\ V$$

Direct titration with NaOBr solutions causes difficulties owing to the instability of the reagent and to problems in end point indication. For this reason, indirect iodometric titration was used in classical titrimetry. Potentiometry, amperometry and coulometry are all suited for instrumental end point indication.

Malinger and Malingerova [7] found that the reaction of hypobromite with numerous compounds containing amino groups is accompanied by high evolution of heat. The application in their measurements of the DIE method operating with excess reagent was obvious, due to the instability of the reagent. Trischler [8] developed a procedure to determine urea in fertilizers. It is based on oxidation performed with excess hypobromite, reduction of the excess with sodium sulfite and measurement of the heat of reaction. Halász and co-workers [11] developed a method for the joint determination of urea and ammonium salts in fertilizers, based on the fortunate coincidence that the heat of reaction for the two compounds with hypobromite is very similar:

$$\begin{matrix} & NH_2 \\ C=O & \\ & NH_2 \end{matrix} + 3\,BrO^- \rightleftharpoons N_2 + 3\,Br^- + 2\,H_2O + CO_2$$

$$\Delta H = -280.9\ \text{kJ/mol}$$

$$2\,NH_3 + 3\,BrO^- \rightleftharpoons N_2 + 3\,Br^- + 3\,H_2O$$

$$\Delta H = -270.5\ \text{kJ/mol}$$

The reaction of amino acids with hypobromite is accompanied by heat of reaction of similar value.

The NPN contained in urine consists of compounds oxidizable with hypobromite, so that this reagent appears suited for the determination.

The separate determination of urea with the usual methods is very cumbersome, and has the further disadvantage of being indirect. It is therefore expedient to perform the thermometric determination with the enzyme urease which is specific for urea.

Determination of NPN

Experimental

Apparatus: Silicotherm (MOM) Fig 1/a, Fig. 1/b
Recorder Radelkis OH-814

Reagents: 1 *M* NaOBr solution
0.5 *M* urea stock solution
0.1 *M* urea stock solution
0.1 *M* ammonia stock solution
0.1 *M* glycine stock solution

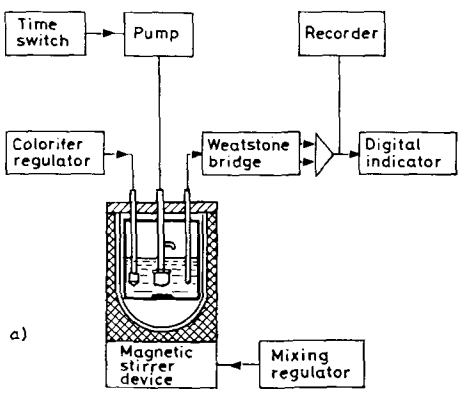

Fig. 1/a. Measuring cell and block diagram of Silicotherm apparatus

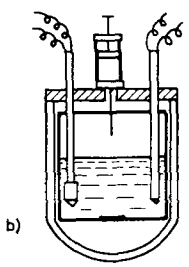

Fig. 1/b. Measuring cell (without immersion pipette) with syringe

Calibration graph: 2, 4, 6, 8 and 10 cm^3 of the three different 0.1*M* stock solutions are made up to 100 cm^3 each. These sets of calibrating solutions are introduced into the plastic cup of the instrument, while 5 cm^3 of the 1*M* NaOBr reagent are introduced into the submerged pipette. After the temperature equilibrium is established, the reagent is administered to the sample at a sensitivity of 200 mV. The total deflection of the recorder corresponds to $\Delta T \approx 0.8°$.

Determination: This was carried out in two manners. In direct determination, the urine specimen was diluted by a factor of 50 or 100, and three parallel determinations were performed with 100 cm^3 of this solution, similarly to the above-described procedure. The other mode, the inverse determination is carried out as follows: 200 cm^3 of an 0.01 *M* NaOBr solution at ambient temperature were introduced into the plastic cup of the instrument, the submerged pipette was disconnected, and 0.2 cm^3 of the 0.5*M* urea stock solution were injected three times consecutively from a hypodermic syringe into the reagent, at a sensitivity of 50 mV. Subsequently 0.2 cm^3 of a known volume of the urine to be tested were measured in the same manner.

Results and discussion

Figure 2 represents recorder deflection *versus* nitrogen (mg) in the reaction chamber for all three calibrating solutions. Straight lines tending towards the origo and practically coinciding were obtained.

As demonstrated by Fig. 3, the reaction takes place instantaneously. It is therefore more convenient and fast to proceed as follows: instead of the usual individual measurements, the calibrating solution and the specimen (three parallels each) are successively administered into the high thermal capacity reagent, without opening the reaction chamber (Fig. 4).

In the direct determination, the NPN content is calculated from the value found on the calibration line, in the knowledge of the volume and dilution of the urine. In the inverse determination the NPN concentration is obtained from the

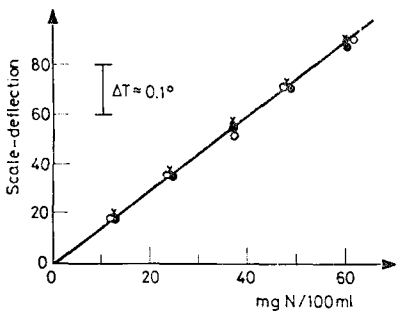

Fig. 2 Calibration curve of nitrogen (recorder scale-deflection as a function of the nitrogen content in 100 ml solution)

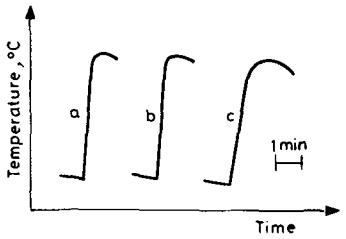

Fig. 3. Enthalpograms of 0.1 *M* stock solutions: a) urea, b) ammonia, c) glycine

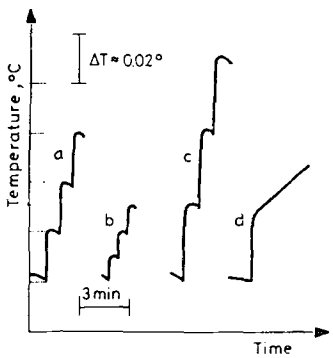

Fig. 4. Enthalpograms of a) 0.5 *M* urea stock solution, b) urine specimen with low NPN content, c) urine specimen with high NPN content (three parallels each, succesively), d) urine specimen with protein content

ratio of the deflections caused by the identical volumes of urea stock solution and urine specimen added to the reagent.

Protein starvation and overfeeding, resp., cause a shift of the ratio ammonia to urea. None the less, the method yields satisfactory results for NPN in this case too, owing to the similar values of the heats of reaction. The accuracy of the results significantly exceeds the accuracy required in medical practice.

Separate determination of urea

In certain cases, e.g. in the examination of kidney diseases, the separate determination of urea is also necessary. For this purpose, the hydrolysis of urea by the enzyme urease is utilizable, since it is specific from the thermometric view, too.

$$\begin{matrix} & NH_2 \\ C=O & \\ & NH_2 \end{matrix} + 2\,H_2O \xrightarrow{\text{urease}} 2\,NH_4^+ + CO_3^{2-}.$$

A problem is caused by the very low value of the heat of reaction. We therefore applied the buffer amplification principle [3]: by letting the reaction proceed in an acid medium, the neutralization heat of ammonia will be measured together with the heat of reaction of the enzymatic hydrolysis.

Experimental

Apparatus: the above-mentioned instrument and recorder
Reagents: 0.2*M* sodium acetate solution
0.2*M* acetic acid solution
0.5*M* urea stock solution
urease, having a declared activity of 5 μ/mg at 35°.

Determination

About 0.1 g urease is added to 200 cm^3 acetate buffer solution (pH 5.6), which is then introduced into the plastics cup. 1.0 cm^3 urea solution is administered to the reagent from the submerged pipette, after the temperature equilibrium is established, at a sensitivity of 50 mV. Subsequently the determination is repeated with 1.0 cm^3 of the urine to be tested.

Results and discussion

The temperature change *versus* the reaction time plot (Fig. 5) demonstrates that notwithstanding the vast excess of enzyme, the process is slow, the decomposition is complete after about 6 minutes only. After 5 to 6 measurements, the process becomes even more protracted. For the unsatisfactorily insulated aniso-thermal calorimeter at our disposal, this process is much too slow, the ratio signal to noise is inadequate. Owing to the slow increase of thermal capacity, ΔT noticeably decreases in the parallel determinations. With the changing grade of the enzyme and changing ambient temperature, the reproducibility of the results is unsatisfactory.

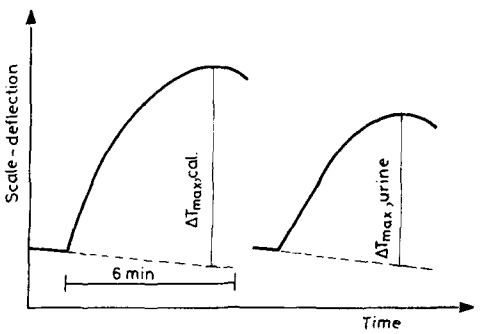

Fig. 5. Enthalpograms of enzymatic hydrolysis of the urea stock solution and urine specimen, respectively

We attempted this determination with 1 cm^3 of blood serum too, since, according to the literature [1] the clearance test (its result is a value proportional to the ratio of urine urea excreted under strictly specified test conditions to the blood urea concentration) is at present the most widespread screening test for kidney functioning. Due to the limited number of blood samples available, the different methods of the pre-treatment used, the reproducibility was found be rather poor.

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By describing the abowe two determinations, we wished to present the applicability of the inexpensive Hungarian instruments for fast and simple determinations in the biochemical field.

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ZUSAMMENFASSUNG — Die Reaktionswärme von Hypobromit mit den im Urin enthaltenen nicht-Protein Stickstoffverbindungen ist sehr hoch. Dieser Umstand kann zur Bestimmung dieser Verbindungen durch die inverse DIE-Methode verwendet werden. Die DIE-Methode gestattet auch die separate Bestimmung von Karbamid aufgrund der spezifischen Wirkung des Enzyms Urease.

Резюме — Теплота реакции гипобромита с непротеиновыми азотистыми соединениями, содержащиеся в моче, очень высокая. Этот факт использовался для определения таких соединений с помощью обратного ДИЕ-метода. Этот метод также позволяет раздел ное определение мочевинных оснований, на основе специфического действия мочевинного фермента.

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This paper completes the material of the Hungarian Symposium on Thermal Analysis