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PHOTOMETRIC DETERMINATION OF BIURET IN MELTED UREA USING FLOW INJECTION ANALYSIS

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ABSTRACT. A sensitive, rapid and automatable flow injection fotocolorimetric analysis is described for the determination of biuret in melted urea as blue-violet $[CuBt(OH)_2]^{2-}$ complex in strongly basic medium (pH>13). The automatic system consists of a refractometer and a double beam automated photometer equipped with a green filter (530-570 nm). The sample and reagents ($CuSO_4$ $5H_2O$ 15 g l^{-1} and an alkaline solution of tartrate 50 g l^{-1} in NaOH 40 g l^{-1}) are mixed in a 4:1:1 volumetric ratio. The kinetic study and the absorption spectra have shown the development of $[CuBt(OH)_2]^{2-}$ complex in the system urea-biuret-Cu-tartrate as a result of the decomposition of tartric complex according to a 1:1 stoechiometry. The experimental conditions were optimised at 555 nm where the best limit of detection, calibration and analytical sensitivities are obtained. For a detection limit of 0.05 % biuret in urea (m/m), the method allows the quantitation of biuret in the range 0.25-2.5% with an RSD lower than 10%. This approach offers significant advantages in terms of speed (5 samples/hour), automation and precision compared with existing manual procedures.

INTRODUCTION

Urea is the most important nitrogenous fertiliser with the best agrochemical properties. Urea losses are a real problem in the process of concentration of urea solution by evaporation. Ostrogovici *et all*. have studied the successive processes involved in the urea thermolysis and the mechanism of biuret development [1]. They indicated that the loss of urea by evaporation is due to both hydrolysis and formation of biuret from urea condensation (eqns. 1,2):

$$(NH_2)_2CO + H_2O = 2\ NH_3 + CO_2 \quad (1)$$

$$2(NH_2)_2CO = NH_2 - CO - NH - CO - NH_2 + NH_3 \quad (2)$$

While hydrolysis results in losses in urea quantity, the resulted biuret is an impurity in the final product and depreciates the fertiliser quality as it has a toxic action on plants and animals. The two $-NH_2$ groups in the molecule are responsible for the slightly basic character of the biuret whose corresponding stepwise dissociation constants are $K_1=10^{-9.2}$ and $K_2=10^{-12.15}$ [2]. Biuret is slightly soluble in water, but its solubility increases in basic media (NaOH, KOH, Na_2CO_3) [3] as well as in the presence of urea [4]. Generally, the biuret content in granulated urea is in the range of 0.4-2% and below 0.1% in crystallised urea. The biuret formation could be avoided if evaporation occurs at as low as possible temperature and pressure with short time maintenance in the evaporator.

In order to discourage the biuret formation it is necessarily to continuously determine the biuret content in melted urea. The knowledge of this content makes possible the intervention in the thermal stage of the urea concentration process.

Among the methods used to determine biuret, the most common are those based on the total nitrogen determination (Kjeldahl method) [5], the photocolorimetric determination of complex developed between biuret and different cations (Ni^{2+}, Cu^{2+}, Co^{2+}) [6-13], potentiometric titration with Cu^{2+} solution [14], amperometric titration [15] and polarographic determination [16].

The colorimetric method is best suited to be automatabled. Thus, an automated Technicon Autoanalyser was achieved [17]. An other automated apparatus based on a different photocolorimetric method was reported [18, 19].

This paper presents a sensitive and rapid flow injection photocolorimetric analysis for the determination of biuret as Cu-biuret complex in melted urea in the range 0.25-2.5%. The study and optimisation of the method in order to adapt it to flow injection analysis is presented. Thus the physical, chemical and time parameters of Cu-biuret complex development, the spectra of biuret-Cu-tartrate and urea-biuret-Cu-tartrate systems, the optimum experimental conditions using synthetic solution of urea-biuret were under study.

EXPERIMENTAL

Reagents. Biuret stock solution (2 g l^{-1}) was prepared by dissolving 2 g biuret and 4 g NaOH in 1 l solution. Urea stock solution (7.8 % m/v) was prepared by dissolving urea in distilled water. Copper sulphate (15 g $CuSO_4 \cdot 5H_2O$ g l^{-1}) solution and alkaline solution of sodium potassium tartrate tetrahidrate (50 g l^{-1} and 40 g l^{-1} NaOH) were also prepared. All reagents were of analytical grade (Flucka). At the same time, standard solutions of urea-biuret were prepared of 20 ml urea, 0…2 ml of biuret, 5 ml of tartrate and 5 ml of $CuSO_4$ stock solutions. All dilutions were made with distilled water to 50 ml. The standard solutions prepared as shown above have a biuret level in technical urea (urea+biuret) up to 2.5%. Standard solutions containing only biuret (without urea) were similarly prepared.

The absorption spectra for biuret-Cu-tartrate and urea-biuret-Cu-tartrate solutions were read against distilled water as blank. In the kinetic study and calibration curves construction, the blanks were Cu-tartrate and urea solution, respectively.

Instrumentation. The photocolorimetric measurements were carried out using the double beam photometer FEK-56M, the UV-VIS spectrometer M40 Karl Zeiss Jena (Germany) and the Automated Batchmeter of Biuret in Urea (ADAB-01, Research Institute for Analytical Instrumentation, Cluj-Napoca, Romania). The schematic diagram of the ADAB-01 is depicted in Fig. 1.

The ADAB-01 previously described is designed as a three-modular system consisting of a reagents and urea sample batchmeter, a refractometer and a double-beam automated photometer equipped with two photocells as

optical detectors [18,19]. The sample is continuously prepared from the melted urea resulted from the technological process further diluted to a concentration of 7-10%. The resulting solution passes through the refractometer in order to determine the accurate concentration in urea. The solution is then passed through an automated dosing system to the photometer for the determination of the biuret level. Than, the urea solution is mechanically mixed with reagents (copper sulphate and tartrate solutions) in a 3 cm-cell and after 10 min it is photometrated against urea solution as blank using a green-filter (530-570 nm). The mixing ratio urea sample: copper sulphate: tartrate is 4:1:1 (v/v/v). The controller sets out the biuret level in the original melted urea within an accuracy of ±5%. Five such determination sequences are possible in an hour.

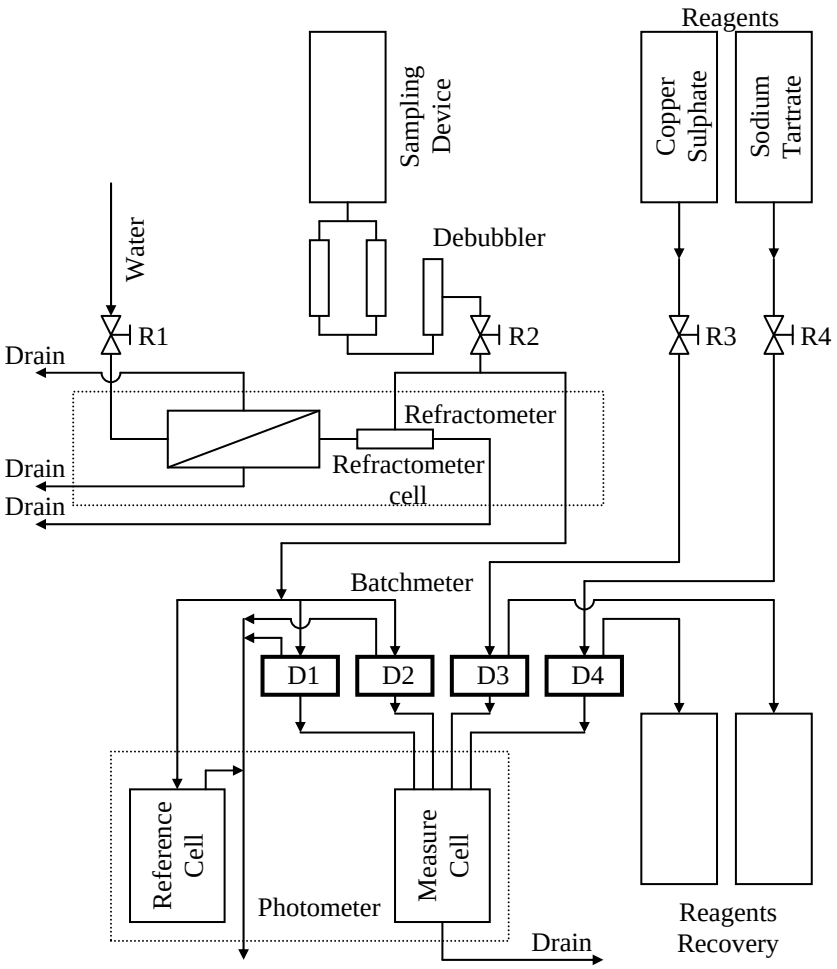

Fig.1. Bloc diagram for the Automated Batchmeter of Biuret in Urea ADAB 01

RESULTS AND DISCUSSION

Kinetic study. In the kinetic study of Cu-biuret complex development the absorbance change versus time for standard solutions of biuret-Cu-tartrate with constant content of reagents and different levels of biuret was followed. After adding the reagents, the absorbance was read at different times against the corresponding blank using a FEK-56M photocolorimeter, on the green filter (530-570 nm) in a 2-cm cell. The kinetic curves are presented in Fig. 2. As it can be seen, the absorbance changes significantly in the first two minutes after the addition of the reagents and remains almost constant after 8-12 minutes for all solutions under study. Consequently, all further measurements were performed 10 minutes after the addition of reagents.

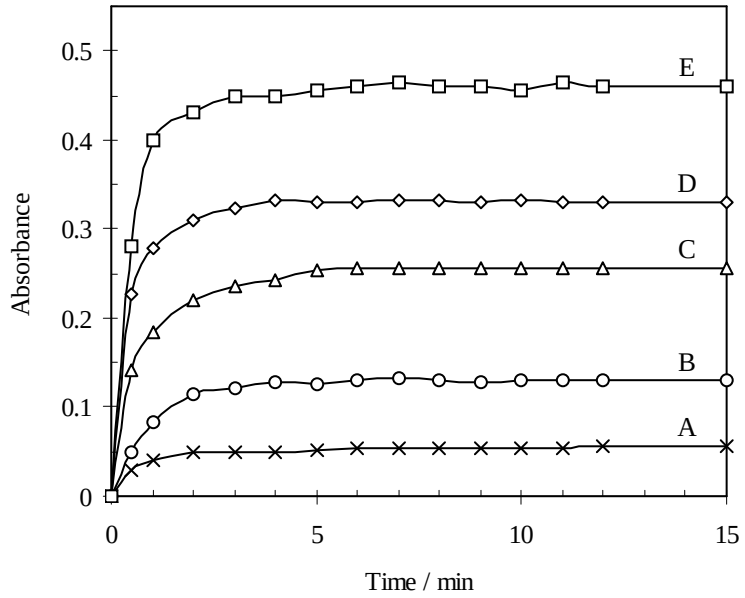

Fig. 2 Kinetic curves as absorbance-time variation for 0.006 (A); 0.02 (B); 0.03 (C); 0.04 (D) and 0.06 % (E) biuret in solution (m/v). Green filter (530-570 nm), 2-cm cell.

Absorption spectra and calibration curves. Copper ions develop differently co-ordinated complexes with biuret according to the pH range. Among these, the red-violet $[CuBt_2]^{2-}$ and the blue-violet $[CuBt(OH)_2]^{2-}$ intern complexes with maximum absorbances at 505 nm and 564 nm, respectively, are mentioned. The first complex develops generally in the range 10-12 pH, while the second at pH higher than 13. The structures of these complexes are presented in Fig. 3:

The recording of absorption spectra of Cu-biuret complexes was necessarily in order to study the mechanism of their development and the influence of urea on the complex absorbance. With this purpose, two sets of spectra of Cu-tartrate and Cu-biuret complexes developed both in the presence and in the absence of 3.12 % urea (m/v) and different biuret contents were

recorded. In both sets of solutions the biuret content was in the range 0.008-0.08 % (m/v) that corresponded to 0.25-2.5% biuret in solid urea. Spectra obtained with a Specord UV/VIS spectrophotometer in the range 450-800 nm are shown in Fig 4a,b. Unlike the Cu-biuret complexes, copper-tartrate complex exhibits a maximum absorption at 680 nm both in the presence and in the absence of urea and does not show a significant absorbance in the range 500-580 nm.The presence of urea in solution does not have an impact on the development of Cu-biuret complexes as it suggests the similitude of spectra presented in Fig. 4a,b. As the biuret is added in Cu-tartrate solution, the maximum absorbance moves from 680 nm to 580 nm, that indicates the formation of an other complex, Cu-biuret. The isosbestic point at 640 nm demonstrates the formation of Cu-biuret complex as a result of the decomposition of Cu-tartrate complex according to 1:1 stoechiometric equilibrium.

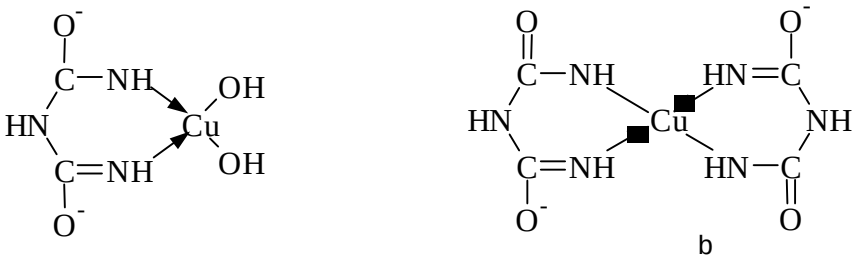

Fig. 3. a. Blue-violet $[CuBt(OH)_2]^{2-}$ complex (absorption maximum at 564 nm);
b. Structure of red-violet $[CuBt_2]^{2-}$ complex (absorption maximum at 505 nm)

On the basis of Cu-biuret-tartrate and Cu-urea-biuret-tartrate spectra, the chemical echilibria established in solution could be explained. In weakly alkaline medium, copper ion develops the $[Cu(OH)_4]^{2-}$ easily soluble complex with the stability constant of 1.3 10^{18} [20]. In strongly basic medium, copper ion could precipitate. To avoid this, a complexation reagent such as tartrate is added, which develops with copper ion mainly the $[CuT(OH)_2]^{2-}$ and $[CuT_2]^{2-}$ complexes with the stability constants of 1.4 10^{21} and $10^{5.4}$ [20, 21] (eqns. 3-6).

$$Cu^{2+} + 2OH^- \leftrightarrow Cu(OH)_2$$

$$Cu(OH)_2 + 2OH^- \leftrightarrow [Cu(OH)_4]^{2-} \quad (3)$$

$$[Cu(OH)_4]^{2-} + T^{2-} \leftrightarrow [CuT(OH)_2]^{2-} + 2OH^- \quad (4)$$

$$2Cu^{2+} + OH^- + 3T^{2-} \leftrightarrow [Cu_2T_3(OH)]^{3-} \leftrightarrow [CuT_2]^{2-} + Cu^{2+} + T^{2-} + OH^- \quad (5)$$

(6)

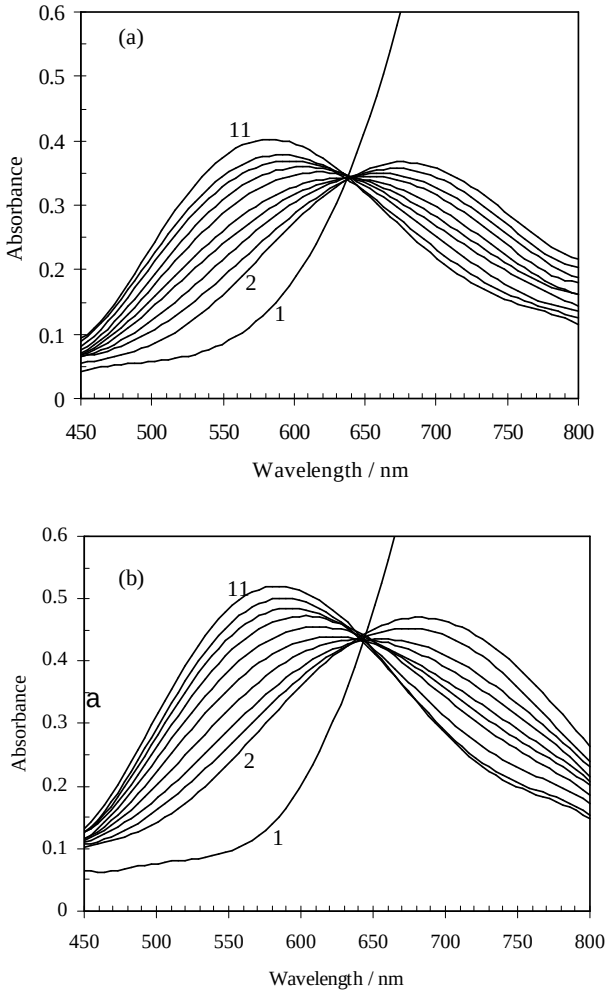

Fig. 4. Absorption spectra of copper-biuret and copper tartrate complexes (pH>13) in the absence (a) and in the presence of 3.12 % (m/v) urea content (b). Biuret content (m/v) in solutions: 0.008 % (curve 2) - 0.08% (curve 11). Curve (1): absorption spectra of copper-tartrate complex in the absence (a) and in the presence (b) of urea.

As biuret is added to the Cu-tartrate solution, the Cu-biuret complex develops as a result of the decomposition of tartric complexes according to a 1:1 stoechiometric equilibrium (eqns. 7-9).

$$\left[CuT(OH)_2\right]^{2-} + Bt^{2-} \leftrightarrow \left[CuBt(OH)_2\right]^{2-} + T^{2-} \quad (7)$$

$$\left[CuT_2\right]^{2-} + Bt^{2-} + 2OH^- \leftrightarrow \left[CuBt(OH)_2\right]^{2-} + 2T^{2-} \quad (8)$$

$$\left[CuT_2\right]^{2-} + 2Bt^{2-} \leftrightarrow \left[CuBt_2\right]^{2-} + 2T^{2-} \quad (9)$$

In the range of 10-12 pH the existence of the red-violet $[CuB_2]^{2-}$ complex with a maximum absorbance at 505 nm prevails, while in strongly basic medium (pH>13) the blue-violet $[CuBt(OH)_2]^{2-}$ complex with a maximum absorption at 564 nm is predominant. As compared with $[CuBt(OH)_2]^{2-}$, the $[CuBt_2]^{2-}$ complex is much more stable ($K= 10^{22.78}$) [21] but exhibits a lower molar extinction coefficient. At pH above 13, the development of the $[CuBt(OH)_2]^{2-}$ complex with a higher extinction coefficient is advantaged. Thus, to improve the sensitivity of the method as compared with other photometric procedures, the determination of biuret as $[CuBt(OH)_2]^{2-}$ at pH>13 was chosen.

In order to select the optimum wavelength for the photometric determination of biuret using ADAB-01, calibration curves in the range 0.25 – 2.5% biuret in urea at 500; 527; 555; 580 nm were constructed. The statistical data for the calibration curves of $[CuBt(OH)_2]^{2-}$ complex in urea-biuret-Cu-tartrate as well as the analytical performances are shown in Table 1.

Table 1.

Calibration results and analytical performances

	Wavelength (nm)			
Parameter	500	527	555	580
Linear range [a]/ %	0.25-2.5	0.25-2.5	0.25-2.5	0.25-2.5
R [b]	0.9991	0.9991	0.9992	0.9985
$(h\pm s_h)$ [c]	$3.2\ 10^{-4}\pm2.1\ 10^{-3}$	$2.7\ 10^{-4}\pm2.3\ 10^{-3}$	$4.6\ 10^{-4}\pm1.6\ 10^{-3}$	$1.0\ 10^{-3}\pm2.7\ 10^{-3}$
$(m\pm s_m)$ [d]	$76\ 10^{-3}\pm1.6\ 10^{-3}$	$103\ 10^{-3}\pm1.4\ 10^{-3}$	$114\ 10^{-3}\pm1.110^{-3}$	$98\ 10^{-3}\pm1.8\ 10^{-3}$
s_s [e]	$4.2\ 10^{-3}$	$3.6\ 10^{-3}$	$2.7\ 10^{-3}$	$4.8\ 10^{-3}$
γ [f], $\%^{-1}$	18	29	42	20
LOD [g], %	0.10	0.08	0.05	0.10
LOQ [h], %	0.50	0.40	0.25	0.50
m_c [i], %	0.06	0.04	0.04	0.05

[a] The number of data for each calibration curve corresponds to ten different biuret concentration (0.008…0.08% m/v); urea concentration: 3.12% m/v

[b] Correlation coefficient

[c] h and s_h - intercept and standard deviation, respectively

[d] m and s_m - slope and standard deviation, respectively. The slope of the calibration curve m is the calibration sensitivity according to IUPAC [22].

[e] s_s -standard deviation of the regression residual [23].

[f] Analytical sensitivity, $\gamma=m/s_s$ [22].

[g] Limit of detection calculated as percent biuret in urea; $(3/m)[s_b^2+s_h^2+(h/m)^2s_m^2]^{1/2}$ where s_b, s_h and s_m are the standard deviations of the blank, intercept and slope, respectively [24].

[h] Limit of quantitation calculated as five times limit of detection

[i] Characteristic mass calculated as percent biuret in urea; $m_c= 0.004343/m$ [22]

Statistical data from Table 1 show a good reproducibility in all measurements. The highest calibration and analytical sensitivity (m and γ) and the standard deviations of slope and intercept are the best at 555 nm. At this experimental wavelength the lowest limit of detection (0.05% biuret in urea) was obtained which allows a quantitation of five times the limit of detection

(0.25% m/m biuret in urea) with a relative standard deviation of 10%. Consequently, the green filter (530-570 nm) with the nominal wavelength at 555 nm was used as wavelength selector for ADAB-01. A simple formula allowed the percentage of biuret in urea to be calculated from the calibration curve. For a synthetic sample with 1% biuret in urea, the percentage standard deviation is up to 5% (5 successive measurements). Thus, for a real urea sample, the determined concentration level of biuret was 0.80±0.04 %.

CONCLUSIONS

This approach is designed for a near-continuous monitoring of biuret in urea (0.25-2.5% m/m) using a flow injection analysis system and offers significant advantages in terms of speed, automation and precision compared with existing manual procedures. The time sequence allows the analysis of five urea samples/hour. The method was satisfactorily applied to the quantitation of biuret in urea with a RSD_s down 10%.

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