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Determination of Hexamine in the *Dithane DG* Fungicide Using Capillary Isotachophoresis

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Abstract: In this article, hexamine (HX; *syn.*: urotropine, *chem.*: 1,3,5,7- tetraaza-tricyclo [3.3.1.1^{3,7}]decane, $C_6H_{12}N_4$) is the analyte of interest determined in commercially marketed fungicide (*Dithane DG*) with the aid of capillary isotachophoresis. After method development, the optimal electrolyte system comprised 0.01 M KOH + 0.02 M acetic acid + 0.1 % hydroxy-ethylcellulose (pH 4.75) as the leading electrolyte and 0.01 M acetic acid as the terminator. The calibration / detection characteristics are as follows: linearity over the concentration range of 0–200 mg.l^{-1} HX, limit of detection *ca.* 2 mg.l^{-1}, limit of quantification of *ca.* 6 mg.l^{-1} HX. Furthermore, relatively simple procedure (involving extraction as the only major sample pretreatment), low operational costs, and sufficient sensitivity are also typical attributes of the method that can be recommended for analysis of hexamine in herbicides and related samples.

Keywords: Capillary isotachophoresis, Hexamine (urotropine), *Dithane DG* fungicide; Determination.

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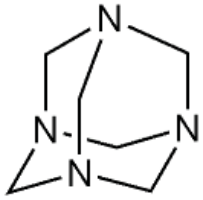
Introduction

Hexamine is a heterocyclic organic compound, also known as hexamethylenetetramine, urotropine, methenamine, formin, aminoform, 1,3,5,7-tetraazaadamantane, or 1,3,5,7-tetraaza-tricyclo [3.3.1.1^{3,7}]decane; the last version being the official name by IUPAC. The 3D- structure of this industrially and in-laboratory important compound is shown in Scheme 1.



Scheme 1:
Structural formula of hexamine, $C_6H_{12}N_4$.

Hexamine was firstly introduced in 1859 [1]. It is a white crystalline compound with very good solubility in water and can be prepared by the reaction of formaldehyde and ammonia, when the respective reaction can be performed either in gas-phase or in solution.

$$4\ NH_3 + 6\ HCHO \longrightarrow (CH_2)_6N_4 + 6\ H_2O \qquad (\mathbf{1})$$

The main use of hexamine is in organic synthesis; *e.g.*, in the production of explosives and plastics, as vulcanizing agent, the component of pesticides, *etc.* [2-6]. Under the trademark of *Urotropine*®, it was widely used as a preserving additive (item "E239") in food industry, as well as in combination with some antibiotics (between 1965 and 1990) to treat urinary infections until a toxicological finding that this substance might be harmful due to some side effects – mainly, undesirable release of highly toxic formaldehyde [7-14].

Hexamine is likely determinable using a variety of classical and instrumental measurements; among others, by alkalimetry [15,16], gasometry [17], conductometry [18], spectrophotometry [12,19-22], gas chromatography [23,24] or even HPLC in the ion-exchange-based column chromatography [13].

The aim of this paper was to develop a method for the determination of hexamine in a commercial fungicide, when using cathodic capillary isotachophoresis. To the authors knowledge, the method and the corresponding procedure has not been yet reported.

Experimental

Chemicals and Apparatus

All used chemicals were of analytical reagent grade. Sulphuric acid (96 %), potassium hydroxide (in pellets), and hexamine were purchased from Penta (Chrudim, Czech Republic), acetic acid from *Lachner* (Neratovice, Czech Rep.), hydroxyethylcellulose (HEC) from *Serva* (Heidelberg, Germany). All the remaining chemicals were then purchased from *Sigma Aldrich.*

Samples were prepared in common laboratory glassware; the appropriate volumes of water and solutions having been introduced with the aid of a set of adjustable transfer-pipettes (model "Proline"; Biohit, Helsinky, Finland). All solutions for measurements were made from deionized water. If needed, the individual solutions and samples were dissolved — when additionally deaerated — using a laboratory ultrasound bath (model "K-2"; Kraintek, Podhájska, Slovakia).

Isotachophoretic analyses were performed using a one-purpose analyzer (model "Agrofor"; *JZD Odra*, Krmelín, Czech Republic), which is an instrument in the one-column arrangement consisting of a conductivity detector and a fixed-volume internal sample loop. The analytical signal was recorded via X-line recorder (model "TZ 4620" (Laboratorní přístroje Prague, Czech Rep.).

The pH value of the leading electrolyte was controlled by combined pH-electrode (model "HI-1131"; Hanna Instruments Czech, Prague, Czech Rep.) connected to a portable pH meter (model "GRYF 208 L"; Elektronické přístroje Gryf, Havlíčkův Brod, Czech Rep.).

The Sample

Dithane DG (Neotec) is a commercially available product (Lovela, Terezín, Czech Rep.; see Fig. 1, upper image) marketed as contact fungicide against broad spectrum of fungal diseases of field crops, vegetables, fruit tree species, grape-vine, ornamental plants, as well as some forest-tree species, when the usual dosing form of this fungicide are fine microgranules (see Fig. 1, lower photo).

Regarding hexamine (HX), this compound is added in as the so-called auxiliary agent, whereas *Mancozeb* (*i.e.,* ethylene bis-dithiocarbamate) is the proper substance of action. The content of hexamine in *Dithane DG* is less than 1%, which is also declared by the manufacturer itself.

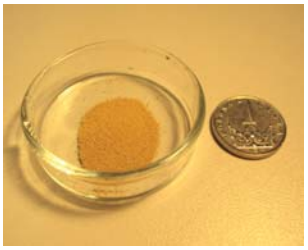


Fig. 1:
Commercial packaging of 'Dithane DG-Neotec' fungicide (**above**)*, a specimen of micro-granules* (**below**).

Sample Treatment

A small portion (approximately 0.7 g) of the real sample was weighted with analytical precision and pulverized in a mortar, together with small amount of deionized water. (The amount of pesticide taken for analysis was chosen to have less than 140 $mg.l^{-1}$ HX in the solution; hence, the concentration should be within the expected calibration range.) Pulverized sample was transferred into 50 ml volumetric flask with the aid of a small piece of folded filter paper and finally filled with deionized water up to the mark.

Experimental Conditions and Instrumental Parameters

Hexamine was determined under following conditions (unless otherwise stated): Leading electrolyte consisted of 0.01 M KOH + 0.02 mol.l^{-1} CH_3COOH + 0.1 % HEC (pH 4.75), where the lastly named served for reducing the electroosmotic flow. As the terminating electrolyte, the 0.01 mol.l^{-1} solution of acetic acid was used (with H^+ as terminating ion). Analysis was performed at a driving current of 60 μA. Two signals were recorded: (i) conductivity of solution leading through the detector and (ii) the absolute value of conductivity derivation. The 0.01 mol.l^{-1} fresh stock solution of hexamine (HX) was prepared daily. Model samples of HX were prepared by diluting the freshly made stock solution.

Results and Discussion

Hexamine accept proton in weak acidic media (see Fig. 2) hence become positively charged ion (with $pK_a = 4.9$ [25]):

$$(CH_2)_6N_4 + H^+ \longleftrightarrow (CH_2)_6N_4H^+ \qquad (\mathbf{2})$$

Such a reaction is often used in analytical chemistry in order to set the acidity of solutions to a mild value of about pH 5.

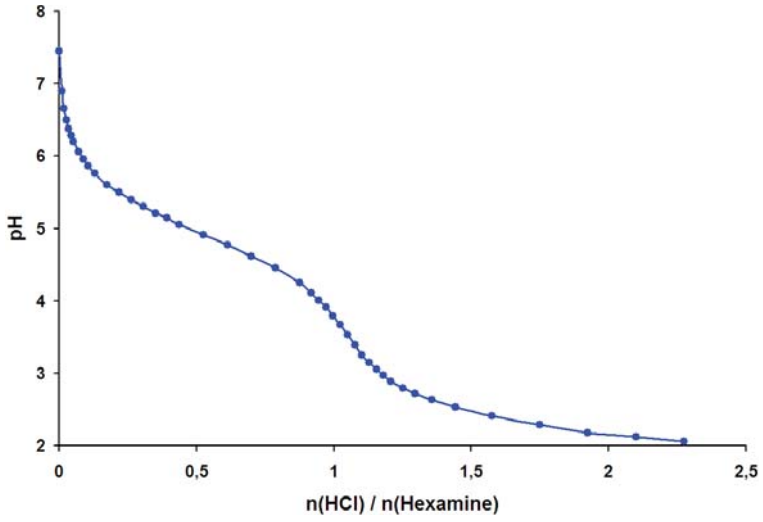


Fig. 2: *Titration of hexamine by the standard solution of 0.1 M HCl: A typical titration curve.* c = 75 mg HX / 50 ml H_2O (From authors' archives)

And it should be added that, within the pH 4–6, the ion $(CH_2)_6N_4H^+$ is sufficiently stable and therefore, it is possible to use it for isotachophoretic determination of hexamine (HX) as described and explained below.

In strongly acidic media, hexamine is decomposed to formaldehyde and ammonium:

$$(CH_2)_6N_4H^+ + 6\,H_2O + 3\,H^+ \longrightarrow 6\,HCHO + 4\,NH_4^+ \qquad (\mathbf{3})$$

The proper mechanism of the reaction (3) is rather complicated due to the presence of further species in the solution [26] and the respective kinetics, rate of decomposition, is mainly dependent on the acidity of solution, whereas the time of exposure in acidic media is less important. After acidification, the part of hexamine is immediately decomposed, but the continuing decomposition is rather slow and limited (see data in Table I).

Table I: *Effect of sulphuric acid concentration on decomposition of hexamine*

Concentration of H_2SO_4 ($mmol.l^{-1}$)	**Zone length** (cm) fresh solutions	**Decrease** [a] (%)	**Zone length** (cm) after 24 h	**Decrease** [b] (%)
0	7	-	6.95	0.71
2.5	6.3	10.00	5.24	16.83
5	3.01	56.86	2.47	17.74
10	1.57	75.71	0.99	36.6

Legend: a) the value is relative to the zone length for non-acidified hexamine solution, b) the value is relative to the zone length of zone for fresh hexamine solution; initial concentration was 0.001 M HX in all cases; the results are average of three measurements (n = 3).

Hexamine is slowly decomposed even in weak acidic media and neutral solutions therefore the fresh stock solution was prepared every day.

Optimization of Electrolyte System

When considering isotachophoretic determination of hexamine, the actual pH of leading electrolyte is the most crucial parameter in. The adjustment to pH < 4 is unsuitable because too acidic media fully protonize the HX molecules and the effective mobility of the respective ion, $(CH_2)_6N_4H^+$, reaches the maximum; however, decomposition during analysis may occur.

For pH > 6 (*i.e.*, in situation when one has: $pH_i > pK_a + 1$, where index "i" specifies an actual pH value), is also inappropriate because the molecules of HX are protonized into low extent and the respective ion manifests quite a low mobility. And hand in hand with length of the corresponding zone(s), the sensitivity of such determination under inappropriately adjusted conditions is markedly decreased, too.

The above-discussed considerations have resulted from potentiometric titrations of HX with the solution of a strong acid (see Fig. 2), which is just a very rough estimation, because the pH of individual zones in cationic isotachophoresis is different as having dropped down from the leading to the terminating electrolyte [27].

The mobility of protonated hexamine is relatively low and being 36.9 x 10^{-9} m^2 V^{-1} s^{-1} [25]. Hence, the proton H^+ as the terminator had been the ion of choice whose effective mobility is possible to set via the pH value of the leading electrolyte. Fig. 3 shows a dependency of the relative effective mobilities of H^+ and $(CH_2)_6N_4H^+$ upon pH of leading electrolyte containing acetate buffer.

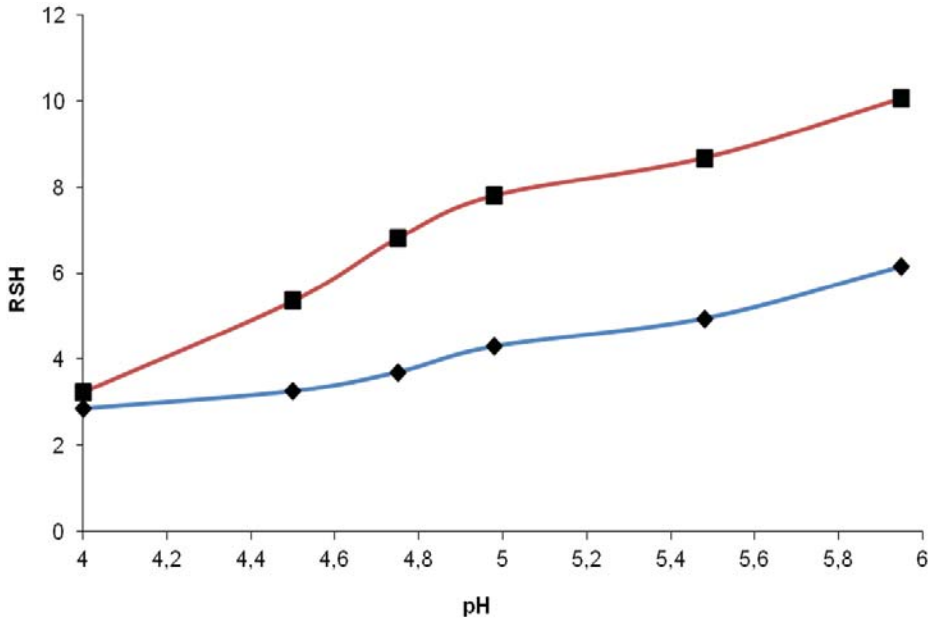


Fig. 3: *Dependence of the RSH parameter upon pH of leading electrolyte*. Legend: -♦- ratio of zones heights of $(CH_2)_6N_4H^+$ to Na^+; -■- ratio of zones heights of H^+ (terminating ion) to Na^+. Experimental conditions: c(Hx) = 1 x 10^{-3} mol.l^{-1}; isotachophoretic system: leading electrolyte: 0.01 M KOH + 0.1 % HEC (pH 4.75, corrected by adding some droplets of mineral acid), terminating electrolyte: 0.01 M CH_3COOH.

Specifically, the figure shows the dependence of a RSH parameter — the so-called relative step heights — on pH of leading electrolyte. The RSH parameter is a ratio between the zones heights for H^+ or $(CH_2)_6N_4H^+$, respectively and the zone height(s) of selected reference ion; in our case, monovalent sodium, Na^+, whose mobility is not dependent on pH. (An alternate choice of a leading species could also be the K^+ ion whose mobility is also independent on pH [27].) Under such conditions, the value of the RSH parameter is relative to the ratio between the conductivities of selected ion(s) and the reference ion; otherwise, it is relative to a ration of the effective mobility of the reference ion with respect to the studied one.

As can also be seen in Fig. 3, the use of the leading electrolyte at pH 4 has resulted in the effective mobility of H^+ ions similar to that of protonized hexamine. Within increase of pH, the difference of both effective mobilities is also rising up, while reaching the maximum at pH 5; beyond remaining constant.

Also, the increasing pH had caused a drop of the effective mobility of both H^+ and $(CH_2)_6N_4H^+$ ions, which resulted in an increase of the separation voltage / potential, reflected in the worsened reproducibility of measurements. Finally, as the optimum, the value of pH 4.75 for leading electrolyte has been the ultimate choice; being, coincidentally, the same value as the $pK_a(CH_3COOH)$.

Analysis of Model Samples

Calibration of Hexamine at the Sub-Millimolar Level. A calibration curve in Fig. 4 confirms a fine linearity of the dependence of interest – the zone length vs. concentration of HX. As seen, even low concentrations of the analyte were stable and showing on evidence for the above-discussed decomposition of HX during the entire analytical procedure.

Limit of Detection and Limit of Quantification (LOD and LOQ). Minimum detectable zone length is about one second; the respective detection limit being 14 $\mu mol.l^{-1}$ which is 2 $mg.l^{-1}$. The limit of determination has been evaluated as a triple value of detection limit, which is about 40 $\mu mol.l^{-1}$ and 6 $mg.l^{-1}$ respectively.

Reproducibility of the Analytical Signal. Relative deviation of 10-times repeated injection of standard solution at concentration 5×10^{-4} M HX was 0.77 %. The reproducibility of determination evaluated due to the less repetitions based on Dean-Dixon test was ±2.4% (for calibration graph) and ±2.2% (for standard addition).

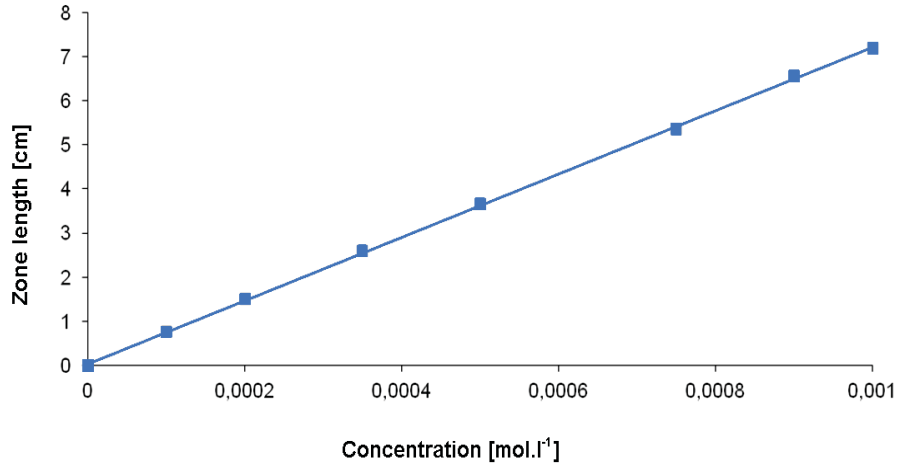


Fig. 4: *Calibration of hexamine in the concentration range of 0.1-1.0 mmol.l^{-1}.* Experimental conditions: leading electrolyte: 0.01 M KOH + 0.02 M CH_3COOH + 0.1 % HEC (pH 4.75); terminating electrolyte: 0.01 M CH_3COOH. (Note: A point plotted for zero concentration corresponds to a value recorded for blank.)

Analysis of Real Sample

The content of hexamine in commercial product, *Dithane DG* fungicide, was determined by the calibration curve method, as well as using standard addition method. The determination was made in five replicates. Both methods have offered the identical result of 0.47 % HX, which corresponds well to the value declared by manufacturer (*ca.* 0.5 %).

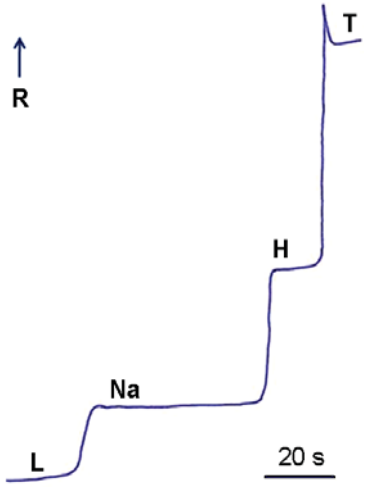


Fig. 5: *Typical isotachophoretic record / curve obtained by analyzing the real specimen of fungicide (Dithane DG®*; for specification, see ***Experimental***). Legend: **L** ... leading ion, K^+, **Na** ... sodium cation, **H** ... protonated hexamine, **T** ... terminating ion, H^+. Exp. conditions: leading electrolyte: 0.01 M KOH + 0.02 M CH_3COOH + 0.1 % HEC (pH 4.75); terminator electrolyte: 0.01 M CH_3COOH; **R** ... the actual response of conductometric detector.

Conclusions

In the above sections, a simple and rapid determination of hexamine has been presented based on the isotachophoretic separation with conductometric detection permitted by the protonation of the substance of interest – the formation of the hexamine cation, $CH_2)_6N_4H^+$. The leading electrolyte of choice was K^+ ion (alternatively replaceable with Na^+), whilst the terminating ion was proton, H^+. The electrolyte system has utilized the leading electrolyte consisting of 0.01 M KOH (possibly also NaOH) + 0.02 M CH_3COOH + 0.1 % hydroxy-ethylcellulose (pH 4.75) and the terminating electrolyte, a solution of 0.01 M CH_3COOH (with pH ≡ pK_a).

The newly proposed method has been tested on practical analysis of a commercial fungicide, *Dithane DG*, containing hexamine as auxiliary (stabilizing and homogenizing) agent, when the analyte has been determined by means of two methods of quantitative analysis, (i) calibration curve- and (ii) standard addition method, both giving the equal result, 0.47 %.

It can be stated that isotachophoretic determination of hexamine offers a number of advantages compared to other techniques; mainly, minimal requirements on sample treatment prior to analysis, inactivity and insensitivity against non-ionic species in the sample matrix, as well as a very small amount of the sample needed. At present, the method is in further development [28] and some preliminary observations have revealed that the procedure as such seems to be applicable also to other samples.

Acknowledgements

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