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Report

Quantitative Analysis of Hydroxyurea and Urea by Proton Nuclear Magnetic Resonance (NMR) Spectroscopy

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Quantitative ^{1}H-nuclear magnetic resonance (NMR) procedures are described for measuring hydroxyurea and urea. Dimethylsulfoxide-d_6 is used as the solvent for both assays; the internal standards employed are urea and *p*-dichlorobenzene for hydroxyurea and urea, respectively. The analysis for hydroxyurea is based on the comparison of the area of the $-NH_2$ peak of hydroxyurea with the area of the urea $-NH_2$ peak area. The analysis of urea is based on the comparison of the $-NH_2$ peak of urea with the area of the *p*-dichlorobenzene singlet. The ^{1}H-NMR assays yield results that are precise and agree well with results of the more cumbersome and less specific USP procedures.

KEY WORDS: hydroxyurea; urea; quantitative ^{1}H-NMR.

INTRODUCTION

Hydroxyurea and its parent compound, urea, have widely differing clinical uses. Hydroxyurea is an effective antineoplastic agent used primarily in the treatment of chronic myeloid leukemia (1–4), its activity being attributed to its ability to inhibit the enzyme ribonucleoside diphosphate reductase (5). On the other hand, urea, first discovered in the urine of carnivores by Rouelle in 1773 (6), is used as an osmotic diuretic for various conditions and as an agent in topical moisturizers (7).

The approaches to the analysis of hydroxyurea and urea are varied. Many of the currently available methods for the analysis of hydroxyurea rely on its ability to undergo chromogenic reaction with a variety of reagents, for example, pentacyanoamine ferrate (8), nitroprusside-ferricyanide, *p*-dimethylaminobenzaldehyde (9), and picryl chloride (10,11), the resulting color intensities being measured spectrophotometrically. An alternate spectrophotometric assay relies on measurement of the change in the ultraviolet absorption of a sodium perchlorate solution after reaction with hydroxyurea or urea (12). Other methods for measurement of hydroxyurea include a quantitative paper chromatographic separation (13), gas chromatographic analyses (14), and high-performance liquid chromatographic methods (15,16).

The currently official method of analysis for hydroxyurea (17,18) is a titrimetric procedure; the compound is oxidized with excess iodine and the unreacted iodine is then titrated with thiosulfate, using starch indicator. However, since the stoichiometry of the iodine hydroxyurea reaction is not established, titration of standard hydroxyurea must be carried out to provide a quantitative basis for the determination. The USP monograph for urea includes an assay that is a Kjeldahl nitrogen determination (19).

There are two procedures proposed in this study, both utilizing quantitative ^{1}H-nuclear magnetic resonance (NMR) for the analysis of hydroxyurea and urea. Quantitative NMR is an absolute method; it is rapid and simple and involves little sample preparation. Since an internal standard other than the compound of interest is used, there is no requirements for standard-grade hydroxyurea or urea.

MATERIALS AND METHODS

A JEOLCo. 60-MHz NMR spectrometer using the field-sweep method with a sweep time of 10 min and field-sweep integration of 2.5 min was used. Rf power ranged from 20 to 30 db. Urea and hydroxyurea were obtained from J. T. Baker Chemical Co., Phillipsburg, N.J., and E. R. Squibb & Sons, New Brunswick, N.J., respectively. Urea (99+%, Gold Label, Aldrich Chemical Co., Milwaukee, Wis.) and *p*-dichlorobenzene (Eastman Organic Chemical, Rochester, N.Y.; purified by sublimation; mp, 54.0°C were used as internal standards. Dimethylsulfoxide-d_6 with 1% TMS (Sci-Graphics, Wayne, N.J.) was used as the solvent.

Procedure for Hydroxyurea Analysis. An accurately weighed quantity of hydroxyurea (about 170 mg) and internal standard urea (about 80 mg) was transferred to a glass-stoppered test tube, and 2 ml of DMSO-d_6 was added. The test tube was stoppered and shaken to ensure complete dissolution. An aliquot of about 0.5 ml of this solution was transferred into a standard 5-mm analytical NMR tube. The tube was placed into an NMR spectrometer, and the proton spectrum was obtained, adjusting the spin rate so that no spinning side bands interfered with the peaks of interest. The peaks at 5.5 and 6.2 ppm were integrated not fewer than five times, taking care to avoid saturation.

The amount of hydroxyurea present in the sample was calculated as follows:

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$$\text{mg of hydroxyurea} = \text{mg of urea} \times \text{purity} \times A_u/A_s \times E_u/E_s$$

where A_u is the integral value representing hydroxyurea (6.2 ppm), A_s is the integral value representing urea (5.5 ppm), E_u is the proton equivalent weight of the hydroxyurea (FW/2 = 38.03), and E_s is the proton equivalent weight of urea (FW/4 = 15.015).

Procedure for Urea Analysis. An accurately weighed quantity of urea (about 60 mg) and purified *p*-dichlorobenzene (about 150 mg) was transferred to a glass-stoppered test tube, and 2 ml of DMSO-d_6 was added. The test tube was stoppered and shaken to ensure complete dissolution. An aliquot of about 0.5 ml of this solution was transferred into a standard 5-mm analytical NMR tube. The tube was placed into a NMR spectrometer and the proton spectrum was obtained, adjusting the spin rate so that no spinning side bands interfered with peaks of interest. The peaks at 5.5 and 7.3 ppm were integrated not fewer than five times, taking care to avoid saturation.

The amount of urea present in the sample was calculated as follows:

$$\text{mg of urea} = \text{mg of } p\text{-dichlorobenzene} \times A_u/A_s \times E_u/E_s$$

where A_u is the integral value representing urea (5.5 ppm), A_s is the integral value representing *p*-dichlorobenzene (7.3 ppm), E_u is the proton equivalent weight of urea (FW/4 = 15.015), and E_s is the proton equivalent weight of *p*-dichlorobenzene (FW/4 = 36.75).

RESULTS AND DISCUSSION

Internal standards for NMR methods, as with an quantitative assay, are chosen on the basis of their compatability with the analyte and solvent and their availability as pure compounds. The presence of exchangable hydrogens in hydroxyurea presents problems in the selection of an internal standard. Preliminary experiments demonstrated that the hydroxyurea spectrum degrades in quality, in that the $-OH$ and $-NH$ resonances are very much broadened when maleic anhydride or iodoform is present. Urea, although not an ideal internal standard for hydroxyurea because of its closely related chemical structure, was chosen as the internal standard since it did not affect the hydroxyurea spectrum.

Table I. Quantitative Analysis of Hydroxyurea

Sample	Added (mg)	Found (mg)	Purity (%)
		NMR assay	
1	175.28	180.46	102.96
2	170.57	173.28	101.59
3	171.88	172.84	99.98
4	170.61	170.92	100.18
5	170.51	170.12	99.77
Mean			100.91
RSD			1.36
		USP assay	
1	50.49	52.73	104.44
2	51.73	50.32	97.27
3	48.79	50.13	102.75
4	46.31	47.73	103.07
5	47.34	46.32	97.85
Mean			101.08
RSD			3.28

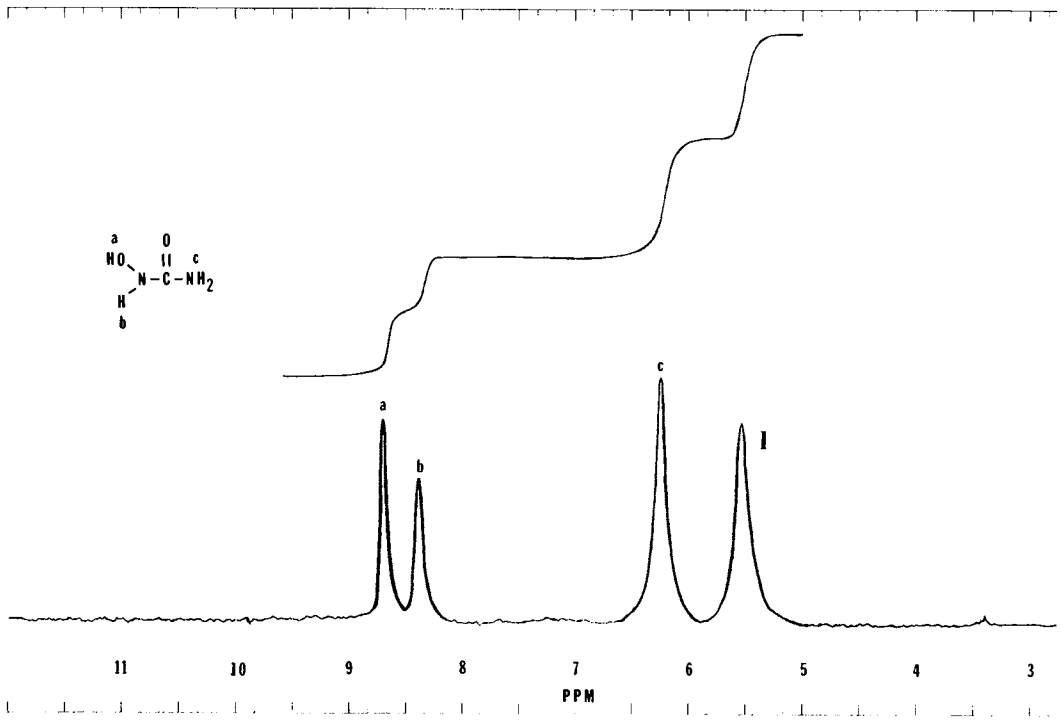

Fig. 1. ^{1}H-NMR spectrum of hydroxyurea in dimethylsulfoxide-d_6. a–c, hydroxyurea (analyte); I, urea (internal standard).

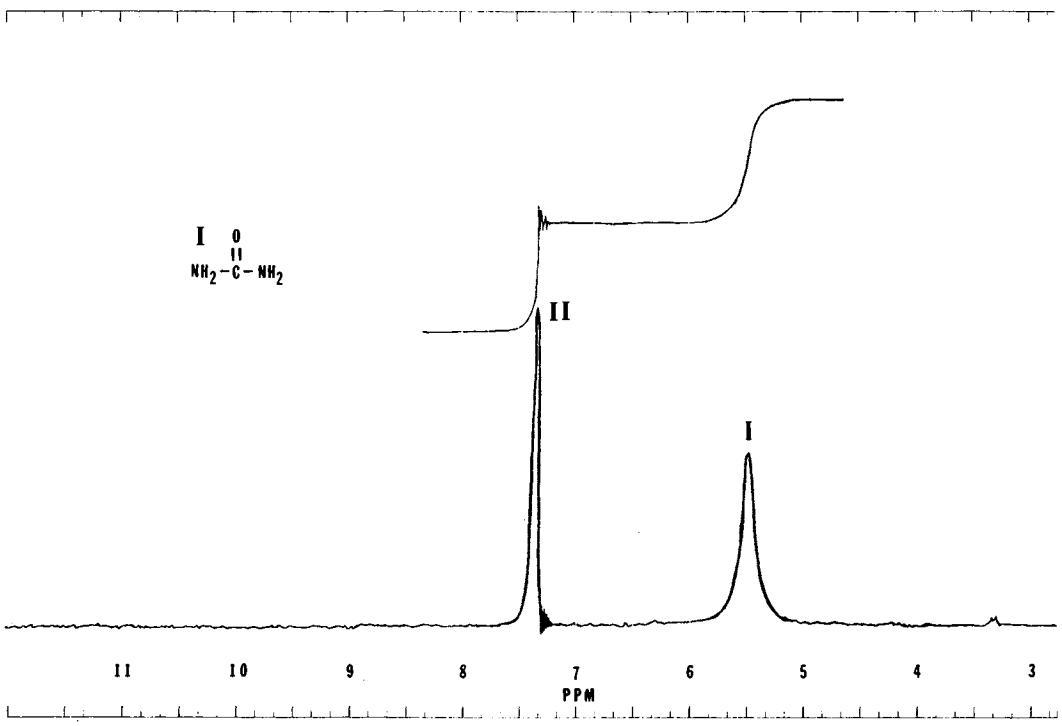

Fig. 2. ^{1}H-NMR spectrum of urea in dimethylsulfoxide-d_6. I, urea (analyte); II, *p*-dichlorobenzene (internal standard).

Optimization of a resonance signal was achieved by systematic increases in Rf power level until saturation of that signal was noted. Rf power levels employed in this experiment fall below these maximum values.

Figure 1 is a NMR spectrum of hydroxyurea and urea (I) in DMSO-d_6. The three types of hydroxyurea protons are found at 6.2 ppm, (c, $-NH_2$), 8.3 ppm, (b, $-NH$), and 8.6 ppm (a, $-OH$). The urea proton resonance is seen at 5.5 ppm. The quantitative analysis of hydroxyurea is based on the comparison of the integral area of the $-NH_2$ proton resonance (c) with the integral area measured for the four amide protons of urea.

Since urea is the internal standard, it is important to establish that no urea is present in the hydroxyurea which is to be analyzed. To ensure that no urea is initially present in the hydroxyurea itself, the spectrum of hydroxyurea without added internal standard was scanned to establish that no resonance was apparent at 5.5 ppm. The spectra showed no evidence of urea in the hydroxyurea sampled. The presence of urea may be detected to levels as low as 0.5–1% of the overall sample concentration by this means. The USP XXI monograph for hydroxyurea contains a limit test for urea, despite the fact that hydroxyurea neither is synthesized from a urea (20) nor decomposes to form urea (21).

A summary of the analysis of hydroxyurea may be found in Table I. The results indicate a precise assay, with a RSD of 1.36%, as well as a good agreement with the USP analysis, which demonstrates a RSD of over 3%. The NMR assay requires little sample preparation and excludes the need for reference material hydroxyurea as required in the official assay.

Figure 2 illustrates the spectrum of urea with added internal standard, *p*-dichlorobenzene, in DMSO-d_6. *p*-Dichlorobenzene was chosen as the internal standard for this assay because it is compatible in solution with urea, it is easily purified by sublimation, and in a 60-MHz magnetic field it displays a single resonance peak. The NMR analysis of urea relies on the comparison of the integration of this singlet arising from the four aromatic protons of the internal standard (7.3 ppm) with that of the urea peak (5.5 ppm).

Table II. Quantitative Analysis of Urea

Sample	Added (mg)	Found (mg)	Purity (%)
	NMR assay, lot 1		
1	58.17	57.94	99.60
2	60.71	58.91	97.04
3	53.91	53.81	99.81
4	64.97	64.60	99.43
5	74.33	74.03	99.60
Mean			99.10
RSD			1.16
Assay[a]			99+
	NMR assay, lot 2		
1	59.37	57.22	96.38
2	61.37	59.74	97.34
3	65.22	63.17	96.86
4	55.31	54.89	99.24
5	56.27	54.96	97.67
Mean			97.50
RSD			1.09
Assay[b]			98.50

[a] Aldrich Gold Label Claim.
[b] J. T. Baker assay.

Table II lists the results of two sets of quantitative NMR assays of urea; these results, with a demonstrated RSD of less than 1.2%, agree well with the commercial assay values. The specificity and ease of measurement make this NMR assay preferable to the traditional elemental analysis required by the USP.

To conclude, the quantitative NMR methods proposed in this paper are simple, rapid, precise, and accurate techniques for measurement of hydroxyurea and urea. The chemical shifts of the proton resonances make the measurement very specific, as is true in all quantitative NMR assays. It is felt that the procedures presented in this report are superior to the official methods of analysis.

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