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^{13}C-n.m.r. as a convenient aid to the differentiation of triethylenetetraamine (TETA) dihydrochloride from related impurities

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The use of TETA[$(H_2NCH_2CH_2NHCH_2)_2$] as an alternative chelating agent for copper in patients suffering from Wilson's disease and intolerant to penicillamine (Walshe 1969; Dixon et al 1972) has posed problems of quality control. The required base is the chief component of mixtures of amines, obtained by polymerizing ethylenimine, which are used as industrial solvents. The quality of the dihydrochloride of TETA, the preferred therapeutic form (separated from the acidified mixture of bases by fractional crystallization), is not readily established since many of its properties are not well defined from those of impurities such as tri(2-aminoethyl)amine[TREN,-$(H_2NCH_2CH_2)_3N$], N-substituted piperazines and other polyamines (Spell 1969). ^{13}C n.m.r. spectroscopy offers an easy means of differentiating these amines and establishing the content of TETA base in commercial samples of polymerized ethylenimine.

Thus the proton-noise decoupled spectrum of recrystallized TETA dihydrochloride in deuterium oxide displayed the correct number of lines required by the three non-equivalent carbons in this structure (Table 1), while that of TREN trihydrochloride showed one less signal consistent with two non-equivalent carbons. Neither spectrum showed further lines of significant intensity, a result indicative of the purity of the salts. Three commercial samples of TETA base each gave a spectrum with three major lines with chemical shifts close to those of the spectrum of TETA dihydrochloride made alkaline with potash (Fig. 1). Exact correspondence of chemical shifts cannot be expected because TETA, a strong base, competes with hydroxyl for protons even at high pH; *N*-protonation moves the chemical shifts of carbons α- and β- to nitrogen upfield (Stothers 1972; Jones & Hassan 1972). A fourth commercial sample, believed from g.l.c. evidence to be diethylenetriamine (DETA), had a spectrum with two lines only, confirming it to be the lower molecular weight material. The spectra of the commercial samples 1–3 displayed 9 additional signals of similar chemical shifts and intensities relative to the main lines. Two of the lines (−13·4, −29·2 ppm) were close in chemical shift to those of TREN base; other signals must be due to piperazine derivatives and other impurities. From relative line intensities it is clear that TETA base is the *main* component of these mixtures. The tetrahydrochloride may be differentiated from the dihydrochloride of TETA by the higher field chemical shifts of its three carbon resonances in accord with anticipated protonation shifts (Table 1); the spectrum of a commercial sample of the tetrahydrochloride showed several lines of minor intensities indicative of impurities.

Table 1. ^{13}C-chemical shifts for triethylenetetramine (TETA) and related amines in deuterium oxide with dioxane as internal reference[a]

Sample	Form	chemical shifts (upfield of dioxane)[b]		
TETA	2HCl	$-20{\cdot}6_3$	$-21{\cdot}6_7$	$-29{\cdot}1_4$
	base[c]	$-18{\cdot}4_2$	$-19{\cdot}7_2$	$-27{\cdot}6_8$
	base[d]	$-16{\cdot}6_8$	$-19{\cdot}2_3$	$-26{\cdot}9_2$
	base-1[e]	$-15{\cdot}6_6$	$-18{\cdot}8_0$	$-26{\cdot}5_5$
	base-2	$-15{\cdot}7_1$	$-18{\cdot}8_5$	$-26{\cdot}5_5$
	base-3	$-15{\cdot}7_6$	$-18{\cdot}8_5$	$-26{\cdot}6_0$
	base-4[f]	$-15{\cdot}8_1$	$-26{\cdot}5_3$	
	4HCl	$-21{\cdot}8_9$	$-23{\cdot}2_4$	$-31{\cdot}2_0$
TREN	3HCl	$-16{\cdot}6_3$	$-29{\cdot}9_6$	
	base[c]	$-13{\cdot}2_2$	$-29{\cdot}0_4$	

[a] Proton-noise and off-resonance decoupled ^{13}C spectra were measured on a Jeol FX 90-Q n.m.r. spectrometer operating at 23·5 MHz. Samples were prepared in 5 mm o.d. tubes in approximately 10% solution in deuterium oxide with dioxane as internal reference (chemical shift 67·4 ppm relative to tetramethylsilane). The deuterium of the solvent provided the lock signal. Spectra were recorded with 8 K data points at a probe temperature of 23 °C. For an average spectral width of 5000 Hz, a 4 μs pulse corresponding to a tilt angle of 30 ° was employed with a 1·819 s interval between pulses. Data from at least 1500 pulses were accumulated per spectrum.

[b] Chemical shift of dioxane taken as zero; each line formed a triplet in off-resonance spectra.

[c] Spectra run in D_2O-KOH-H_2O.

[d] Further addition of KOH-H_2O.

[e] Base-1 to base-4 are commercial samples; only principal chemical shifts are given, Fig. 1 shows a complete spectrum typical of samples 1–3.

[f] Base-4 is diethylenetriamine (DETA), judged pure by its c.m.r. spectrum.

A measure of the levels of impurities detectable in TETA by ^{13}C-n.m.r. is obtained from the observation that spectra of TETA dihydrochloride containing 5% of either TREN trihydrochloride or DETA base showed clearly defined impurity resonances after 300 scans. In the case of the TETA–TREN mixture the intensities of the five resonances, in order of increasing field strength, were approximately 6:112·5:75·5:65:5·5 after 1000 scans (intensity variations of the TETA signals reflect the well known lack of correlation of ^{13}C-resonance intensities with number of nuclei in the absence of special instrumental precautions). Levels of individual

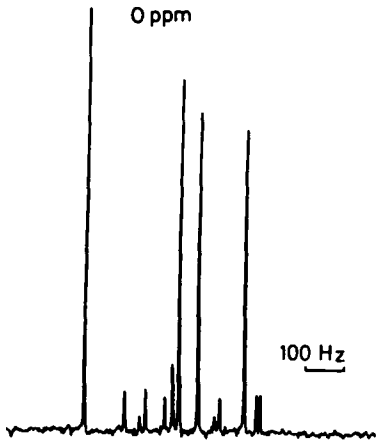

FIG. 1. C.m.r. spectrum of a commercial sample of TETA base in deuterium oxide (details as footnote[a] of Table 1, dioxane signal marked 0 ppm).

impurities of 5% and probably lower are thus capable of detection by this n.m.r. procedure.

The sample of TETA dihydrochloride used in this study was recrystallized several times from ethanol, and melted at 58°–60 °C by capillary and 123°–125 °C by hot-stage (Reichart) procedures. The salt is probably a dihydrate as judged by microanalytical data (Found: C, 28·12; H, 9·09; N, 22·02; Cl, 28·36. Calc. for $C_6H_{20}N_4Cl_2.2H_2O$; C, 28·24; H, 9·4; N, 21·96; Cl, 27·84%) and its infrared spectrum (paraffin mull, probable assignments in parentheses) which showed absorption bands at 3200 (NH), 3420 and 1640 (H_2O) and 2110 cm^{-1} ($^+NH_3$).

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Enhanced rectal absorption of insulin and heparin in rats in the presence of non-surfactant adjuvants

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There are a number of problems associated with current insulin therapy. These include the inconvenience of the injection dosage forms and the antigenicity of parenterally administered foreign polypeptides. Both polypeptide and mucopolysaccharide drug substances are normally either degraded or poorly absorbed after oral administration. The lack of alternative, non-parenteral dosage forms for drugs such as insulin and heparin has limited their use to clinical or semi-clinical situations.

To alleviate these problems considerable effort has been directed to the development of new insulin or heparin dosage forms suitable for oral or rectal absorption. Research involving the use of surfactants as adjuvants for rectal administration has been reported recently (Touitou et al 1978; Shichiri et al 1978; Ichikawa et al 1980; Bakth et al 1980). Some studies have indicated that the use of certain surfactants to enhance rectal delivery of insulin and other drugs results in rectal bleeding or other damage to the rectal mucosa (Nishioka & Kawamura 1978). In an earlier report (Nishihata et al 1980), the use of sodium salicylate to enhance the rectal absorption of theophylline and lidocaine was described. Salicylate appears to enhance rectal absorption by a different mechanism than surfactants and without the lasting changes in the membrane observed with some surfactants (Nishihata et al 1981). This communication reports the use of non-surfactant molecules including sodium salicylate, sodium 5-methoxysalicylate, sodium 3-methoxysalicylate and sodium homovanillate as adjuvants for the rectal absorption of insulin and heparin.

By means of a microenema technique, insulin (Lilly, Regular Iletin, 100 U) was administered to male, Sprague-Dawley rats, 275–300 g. The microenema was prepared with a 0·2 M phosphate buffer, pH 5·0. A volume of 0·3 ml was delivered rectally. Blood samples were taken from a jugular vein at designated time intervals. Plasma concentrations of glucose were then measured using the *o*-toluidine method (Nishihata et al 1978) and insulin concentrations were measured by an enzyme immuno assay (Nakagawa et al 1981) using a kit supplied by the Toyo Jozo Co., Ltd., Japan.

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