The production of pharmaceutical grade trientine dihydrochloride for the treatment of Wilson's disease: a personal account

Introduction

The news in November 2015 of a steep rise in the price of trientine dihydrochloride capsules imposed by the manufacturer of the drug, Univar, has caused dismay in the Wilson's disease community in the United Kingdom. Patients are worried about a potential dislocation to their treatment, and hospital pharmacists and other health professionals view the financial impact of the price increase on health service budgets with great concern. For me personally, the increase in cost is a big disappointment. Trientine dihydrochloride is a relatively cheap drug to manufacture, and by building on the experimental and clinical work of Dr Hal Dixon and Dr John Walshe, respectively, I played a part, in the 1970s, in securing the supply of pharmaceutical grade trientine for Wilson's disease patients. No one denies that business is driven by the need to make a profit, but the checks and balances of society demand a degree of reasonableness between suppliers and consumers. Let us hope that Univar's current distortion of the business model is temporary, and one day trientine will be readily available worldwide at a price all countries can afford.

I have previously written three notes on trientine: (i) a summary of some experimental work on the purification of technical grade triethylenetetramine, which was published in the *Journal of Chemical Research*, 2005, 233-235; (ii) an account of the discovery of trientine (and other drugs) for treating Wilson's disease, which appeared in *Science Progress*, 2013, **96**, 19-32; (iii) a description of Hal Dixon's suggestion that triethylenetetramine might be used to treat Wilson's disease (available on the WDSG-UK website http://www.wilsonsdisease.org.uk/documents/March2010Extract.pdf).

In this additional note, I should like to explain a little more of the chemistry of triethylenetetramine and why trientine is relatively inexpensive to manufacture. I also wish to recount Hal Dixon's role in establishing trientine dihydrochloride as the preferred salt for therapeutic use.

Triethylenetetramine and trientine

By selecting *tri*, *en*, *t*, and *ine* from *tri*ethyl*ene*t*e*tram*ine*, the British Pharmacopoeia Commission chose trientine in 1978 as the British Approved Name for pharmaceutical grade triethylenetetramine. In all countries except the USA, the dihydrochloride salt of triethylenetetramine is named trientine dihydrochloride. Confusingly, the US Approved Name for the same compound is trientine hydrochloride.

Triethylenetetramine, a polyamine and a liquid organic base, was first characterised by the German chemist A. W. Hofmann in 1860-1862 whilst he was researching amine chemistry at the Royal College of Chemistry in London. Hofmann's early work on the synthesis of polyamines has been summarised by F. G. Mann, *Journal of the Chemical Society*, 1934, 461-466.

Triethylenetetramine evolved from a laboratory curiosity into an industrial chemical produced on a large scale during the 1930s. Utilising Hofmann's aminolysis chemistry, technical grade triethylenetetramine is manufactured from the reaction of ethylene dichloride (EDC) and ammonia. A mixture of linear, branched and cyclic amines is obtained from this reaction. The triethylenetetramine content of the mixture is about 75%. A glance at the Dow website for triethylenetetramine (http://www.dow.com/amines/prod/ethyl-teta.htm) reveals the very many commercial uses of technical grade triethylenetetramine. The manufacture of epoxy curing agents, fabric softeners, lube oil and fuel additives, asphalt additives and paper wet-strength resins are just some of the many applications for this versatile polyamine mixture.

Consider the cheapness of the two ingredients used to make technical grade triethylenetetramine – EDC and ammonia. Ethylene dichloride results from the combination of ethylene with chlorine. Ammonia is made from hydrogen and atmospheric nitrogen by the Haber process. Ethylene is manufactured by cracking petroleum fractions, and chlorine can be obtained by the electrolysis of brine (aqueous sodium chloride). By manipulating the most readily available and cheapest chemicals on the planet – air, water, evaporated seawater, and hydrocarbons – chemists can produce tonne quantities of technical grade triethylenetetramine with only energy as the meaningful cost! (One tonne = 1,000 kilograms). A current quotation from the laboratory chemicals

supplier Sigma-Aldrich UK for 18 kg technical grade triethylenetetramine is £474.50 ≡ 2.6 pence per gram. The unit cost from one of the manufacturers of technical grade triethylenetetramine (e.g. Huntsman or Dow) would be considerably cheaper.

The cheapness and multiple applications of technical grade triethylenetetramine made by the EDC route have two consequences. First, despite its longevity as a chemical process, and the many patented alternatives, the EDC method is still in use for manufacturing technical grade triethylenetetramine. Secondly, the cheapest and only realistic option for preparing pharmaceutical grade triethylenetetramine for the treatment of Wilson's disease is by purifying the technical grade material.

Coordination chemistry of triethylenetetramine

The characteristic blue colour (**Figure 1**) formed on mixing cupric sulfate with triethylenetetramine, synthesised in the laboratory, was reported by D. H. Peacock, *Journal of the Chemical Society*, 1936, 1518-1520. With the ready availability of commercial triethylenetetramine from the EDC process, the coordination chemistry of this ligand (L) with copper and other metals (M) could be explored more fully. By the early 1950s, triethylenetetramine was characterised as a quadridentate ligand, which formed a 1:1 complex ML (a 'chelate') with cupric ions, M + L ⇌ ML. The stability constant for the complex ML is the equilibrium constant K_{ML} where K_{ML} = [ML]/[M][L].

Figure 1: The blue colour of Cu[trientine]$^{2+}$, obtained by adding a 300 mg trientine dihydrochloride capsule to aqueous copper sulfate.

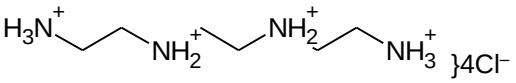
A large stability constant indicates the formation of a stable complex. Stability constants are usually quoted as $\log_{10}K_{ML}$. For example, for the chelation of cupric ions with trientine, Cu^{2+} + trientine ⇌ $[Cu(trientine)]^{2+}$, K_{ML} = $[Cu(trientine)]^{2+}/[Cu^{2+}][trientine] = 10^{20.1}$ mol^{-1} dm^3, i.e. $\log_{10} K_{ML}$ = 20.1 (a typical experimental value). The definitive study of the aqueous chemistry of trientine and cupric ions is by S. H. Laurie and B. Sarkar, *Journal of the Chemical Society, Dalton Transactions*, 1977, 1822-1827. Trientine can chelate other essential transition (e.g. manganese, iron, cobalt, nickel) and non-transition (e.g. zinc) metal ions.

Trientine, Dr Hal Dixon and Wilson's Disease

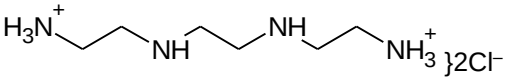
The stage was set to link the copper chelating properties of triethylenetetramine with Wilson's disease. The happy coincidence of Dr John Walshe, the clinician who had introduced the first chelating agent, D-penicillamine, for the treatment of Wilson's disease, and Hal Dixon, an erudite biochemist, both working at the University of Cambridge in the 1960s, led to the introduction of trientine dihydrochloride as the second oral drug for the treatment of Wilson's disease in 1969 (J. M. Walshe, *Lancet*, 1969, **294**, 1401-1402).

Apart from its strong affinity for cupric ions, Hal Dixon recommended triethylenetetramine as a candidate drug because its structural similarity to the naturally occurring polyamines, spermine and spermidine, suggested triethylenetetramine was unlikely to be toxic in humans. A recent account of the acute toxicity of trientine in man supports this view: A. Hashim and N. Parnell, 'A case of trientine overdose', *Toxicology International*, 2015, **22**, 158-159.

The administration of triethylenetetramine required the conversion of the liquid base into a solid form suitable for drug encapsulation and administration to patients. The salts of amines with simple inorganic (e.g. hydrochloric acid, sulfuric) and organic (e.g. maleic, succinic) acids are invariably solid compounds. Triethylenetetramine tetrahydrochloride had been prepared as a crystalline solid by R. G. Fargher (*Journal of the Chemical Society, Transactions*, 1920, **117**, 1351-1356). Instead of making the tetrahydrochloride, Hal Dixon chose to prepare triethylenetetramine dihydrochloride, previously characterised in aqueous solution (S. Lapanje et *al*, *J. Amer. Chem. Soc*., 1961, **83**, 1590-1598), but which had not been hitherto isolated. One of the reasons for not using the tetrahydrochloride therapeutically was as follows. In trientine.4HCl, all four (primary and secondary) amino groups are protonated:



In trientine.2HCl, only the two terminal (primary) amino groups are protonated:



The molecular weight of trientine.2HCl is 219.2 (i.e. 66.7% trientine free base). The molecular weight of trientine.4HCl is 292.08 (50.1% trientine free base). (The molecular weight of trientine free base is 146.2). Therefore for a 300 mg capsule 300 mg trientine.2HCl ≡ 200.1 mg free base; 300 mg trientine.4HCl ≡ 150.2 mg free base. Therefore a daily dose of 1200 mg trientine free base ≡ 6 capsules trientine.2HCl ≡ 8 capsules trientine.4HCl.

The preparation of pharmaceutical grade trientine dihydrochloride

Components of technical grade triethylenetetramine: Although conventional synthetic routes to trientine have been published (e.g. United States Patent Application Publication, US 2015/0057466), the cheapness and availability of technical grade triethylenetetramine mean this substance is the only economically viable starting material for preparing the pharmaceutical grade material. As mentioned previously, technical grade triethylenetetramine is a mixture of amines – predominantly a linear amine (75% triethylenetetramine) plus three impurities: a branched amine (5% tris(2-aminoethyl)amine) and two cyclic amines (20% substituted piperazine derivatives).

Removal of the impurities in technical grade triethylenetetramine: Preparation of pharmaceutical grade trientine dihydrochloride requires the complete removal of the three major, bioactive, amine impurities from technical grade triethylenetetramine. This cannot be achieved (without a drastic reduction in the percentage yield) by the addition of hydrochloric acid to technical grade triethylenetetramine followed by fractional recrystallisation of the resulting mixture of hydrochlorides. Technical grade triethylenetetramine is an azeotropic mixture, so neither can the individual amine components be separated easily by fractional distillation.

Preliminary purification of technical grade triethylenetetramine before the addition of hydrochloric acid: In order to achieve an efficient production route for trientine dihydrochloride, a preliminary purification of technical grade triethylenetetramine is needed before its conversion into the dihydrochloride. A literature search using *Chemical Abstracts* revealed a way of accomplishing this additional step for the preparation. With a fortuitous choice of solvent (R. Purchase, *Journal of Chemical Research*, 2005, *loc. cit.*), successful laboratory and pilot plant preparations of trientine dihydrochloride were developed between 1977 and 1979. Incorporation of the additional purification step is achieved with minimal expense, and, overall, the cost of converting technical grade triethylenetetramine into pharmaceutical grade trientine dihydrochloride is not influenced by the price of the chemicals or by the complexity of the chemistry for making this drug.

Conclusion

The starting material for the production of pharmaceutical grade trientine dihydrochloride is technical grade triethylenetetramine, a bulk chemical commodity available at a very low unit cost. Although the large-scale production of trientine dihydrochloride, based on the initial laboratory preparations of this drug, is accomplished with considerable skill by process chemists and chemical plant operatives, the unit cost of manufacturing trientine dihydrochloride is relatively low.

In the 1970s, much goodwill was extended to Dr Walshe by the UK Department of Health (in particular Mr John Sloggem, DHSS Supply Division), the Laboratory of the Government Chemist, and others, to facilitate the availability of encapsulated trientine dihydrochloride for the treatment of Wilson's disease. Let us hope that some of this goodwill returns to those responsible for the current hiatus.

Rupert Purchase, D.Phil., C.Chem., FRSC

Chair, WDSG-UK, 2015-2016

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An addendum to 'The production of pharmaceutical grade trientine dihydrochloride for the treatment of Wilson's disease: a personal account' an article published in the *WDSG-UK Newsletter*, April 2016

The above article was written in March 2016 and was prompted by several unexpected rises in the price of trientine (triethylenetetramine) dihydrochloride capsules imposed by a USA-based chemical distribution company, Univar (rebranded Univar Solutions, 2019). Since 2016, Univar Solutions have relocated their trientine business to Rotterdam, adopted a trade name, Cufence, for the capsules, and crossed a number of regulatory hurdles to gain acceptance of the product by the European Medicines Agency (EMA). In the intervening years, an alternative formulation of trientine, trientine tetrahydrochloride (trade name, Cuprior), sold as tablets by the Paris-based company Orphalan SA, has also been approved by the EMA for the treatment of Wilson's disease (WD). In the UK, the cost of trientine has had repercussions for the accessibility of the drug for newly diagnosed Wilson's disease patients[1], and reports of the treatment of Wilson's disease in several continents often lament the cost and therefore the unavailability of trientine for treating patients[2]. Other companies are showing an interest in marketing their versions of trientine, but at present (May 2022), Univar and Orphalan remain the principal suppliers of trientine in the UK.[3]

The emergence of two competing drugs, Cufence and Cuprior, might have been expected to result in a decrease in price, but there appears to be a reluctance to seize this opportunity. A British National Formulary website reveals that in May 2022 one Cufence capsule (200 mg trientine base/capsule) costs £30.75 and one Cuprior tablet costs £37.85 (150 mg trientine base/tablet)[4].

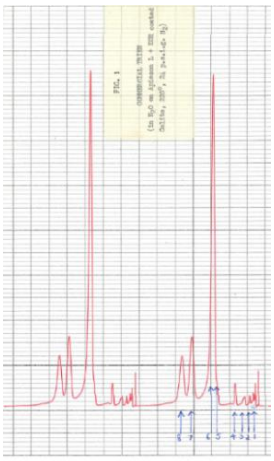

Figure 1

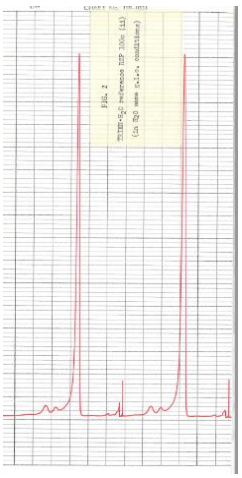

Figure 2

In the 2016 article, I explained that the manufacture of technical grade triethylenetetramine offered a cheap way to prepare pharmaceutical grade trientine dihydrochloride, and I referenced a paper published in 2005[5] for achieving this. The cyclic and linear polyamine impurities in technical grade triethylenetetramine produced by the EDC route (*vide supra*) can be removed by forming a hydrate of triethylenetetramine[5] and then washing the hydrate with an ether, tetrahydrofuran[5]. Gas-liquid chromatogram (GLC) profiles illustrate the nearly complete removal of the polyamine impurities in technical grade triethylenetetramine (**Figure 1**) by the formation of triethylenetetramine hydrate triturated with tetrahydrofuran (**Figure 2**). The main peak in each chromatogram is triethylenetetramine, the minor peaks are polyamine impurities[6].

By adjusting the pH of a solution of purified triethylenetetramine hydrate to 7.8, pure triethylenetetramine dihydrochloride may be isolated and recrystallised. (Later it was found that the optimum pH for acidification is 8.2). The work published in 2005 was suitable for kilogram preparations of pharmaceutical grade triethylenetetramine dihydrochloride in a laboratory, but was impractical for pilot plant production of the drug. In order to scale-up the laboratory route for trientine dihydrochloride, a production chemist at Humphreys & Glasgow, Dr Williamson, made a crucial change to the preparation and purification of triethylenetetramine hydrate. (Humphreys & Glasgow, a UK consultancy firm, specialising in petrochemical/gas engineering, operated a pilot plant for the production of organic chemicals in Billericay, Essex. This pilot plant was closed in the early 1980s. Humphreys & Glasgow produced four batches (80 to 90 kilograms in total) of trientine dihydrochloride at Billericay for Cambrian Chemicals/K&K Greeff between 1978 and 1980).

The method devised by Dr Williamson for the large-scale production of trientine dihydrochloride has been adapted by the drug company Orphalan for the manufacture of trientine tetrahydrochloride (Cuprior). Details of Orphalan's triethylenetetramine hydrate method have appeared in some recent patents, for example a World Intellectual Property Organization (WIPO) patent[7] and a USA patent[8]. Tetrahydrofuran is substituted by another ether, *tert*-butyl methyl ether (2-methoxy-2-methylpropane), which is reputed to be less prone to peroxidation than tetrahydrofuran. The tetrahydrochloride is isolated after acidifying an aqueous solution of

triethylenetetramine hydrate to less than pH 1. Clearly, by terminating the acidification at pH 8.2, Orphalan's route would yield trientine dihydrochloride.

Conventional multi-step organic syntheses of trientine dihydrochloride continue to be published[9] but because of the cheapness and relative simplicity of the triethylenetetramine hydrate route, it is difficult to see how these more complex syntheses could be competitive. Equally, it remains difficult to justify the high cost of Cufence and Cuprior for Wilson's disease patients for drugs which can both be manufactured on a large scale with a starting material costing a few UK pence or less per gram[10].

References

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2. Eve A. Roberts, 'Trientine for Wilson Disease: Contemporary Issues'. In *Wilson Disease: Pathogenesis, Molecular Mechanisms, Diagnosis, Treatment and Monitoring*, Edited by Karl Heinz Weiss and Michael Schilsky, Academic Press, London 2019; ISBN: 978-0-12-811077-5; Chapter 17, pp 187-195. "Trientine is not uniformly available in all countries, and it has been subject to disproportionate price increases in North America and Europe".
3. 'Treatment of Wilson's Disease for Patients in the United Kingdom': WDSG-UK :: Medication (wilsonsdisease.org.uk); accessed 7th May 2022.
4. British National Formulary website: TRIENTINE | Medicinal forms | BNF content published by NICE; accessed 6th May 2022.
5. R. Purchase, 'The purification of triethylenetetramine and its dihydrochloride for the treatment of Wilson's disease', *Journal of Chemical Research*, 2005, 233-235. [The study of amine hydrates is part of the backbone of organic chemistry, e.g. see S. U. Pickering, "The Hydrate Theory of Solutions. Some Compounds of the Alkylamines and Ammonia with Water", *J. Chem. Soc., Trans.*, 1893, **63**, 141-195].
6. GLC was carried out (in 1977) on a Pye instrument (series 104) with a glass column (6 ft × 3 mm i.d.) packed with 20% Apiezon L plus 5% KOH on Celite W (80–100 mesh) operated at 220 °C, with nitrogen as carrier gas (60 ml min^{-1}) and flame ionisation detection. The GLC profile shown in Figure 1 is for Dow's technical grade triethylenetetramine manufactured in the 1970s by the EDC method. Other manufacturing routes for triethylenetetramine may be found in the patent literature, and these products will have different impurity profiles, but, in principle, purification *via* the hydrate route should be achievable.
7. World Intellectual Property Organization (WIPO) Patent WO 2019 211 464, "Crystalline Form of Triethylenetetramine Tetrahydrochloride and its Pharmaceutical Use", published 7th November 2019. Applicant: GMP-Orphan SA, Paris. (GMP-Orphan was renamed Orphalan in 2020). See pp 38 to 40.
8. United States Patent US 2021 163 398, "Crystalline Form of Triethylenetetramine Tetrahydrochloride and its Pharmaceutical Use", published 3rd June 2021. Applicant: Orphalan S. A., Paris, France. See paragraphs [0159] to [0185].
9. World Intellectual Property Organization (WIPO) Patent WO 2021 195 671, "Process for preparing trientine dihydrochloride, published 30th September 2021. Applicant: Johnson Matthey plc.
10. Sigma-Aldrich / Merck KGaA online catalogue Triethylenetetramine | Sigma-Aldrich (sigmaaldrich.com); accessed 7th May 2022.

Rupert Purchase, May 2022

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