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# Diethylenetriaminepentaacetic acid (DTPA): A metal chelating agent

by

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**Abbreviations:**

- DETAPAC / DTPA : Diethylenetriaminepentaacetic acid
- EDTA: ethylenediaminetetraacetic acid
- HEDTA: hydroxyethylethyelenediaminetriacetic acid
- PAC: polyaminocarboxylate
- HPLC: high performance liquid chromatography
- IDA: iminodiacetate
- NTA: nitrilotriacetate
- k: rate constant

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**Abstract**

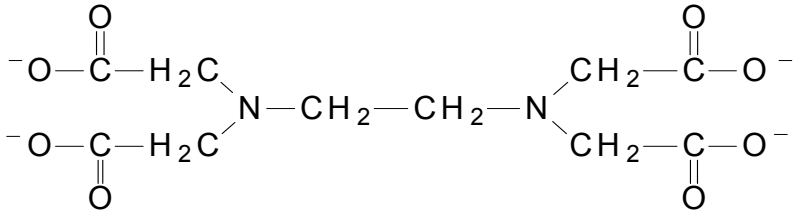
Diethylenetriaminepentaacetic acid (DTPA) is a polyaminocarboxylate (PAC) and a metal chelating agent, a compound that has niches in its ring structure for heavy metals. Many Chelating agents are commonly used in vitro and in vivo biological studies. Fahey and associates introduced DTPA for the treatment of iron storage diseases in 1961. It binds iron with great affinity, forms a colorless iron chelate, and has a pattern of distribution and excretion similar to EDTA. This paper primarily focuses on structure, characteristic reactions of DTPA and its detection with high performance liquid chromatography.

## Introduction

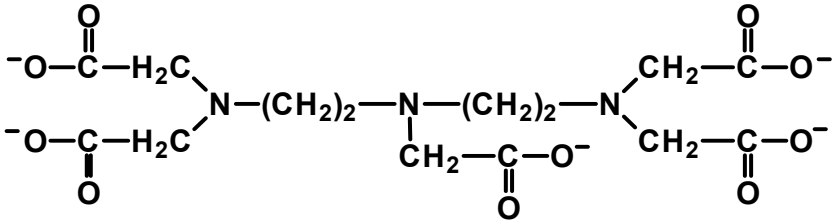
Diethylenetriaminepentaacetic acid (DTPA) is a polyaminocarboxylate (PAC) and a metal chelating agent, a compound that has niches in its ring structure for heavy metals like iron and copper. It attracts and holds the metal ions so tightly that they lose their normal characteristics, such as the ability to catalyze oxidation or form a precipitate [2]. Many Chelating agents like EDTA and its variants, DTPA, iminodiacetate (IDA), nitrilotriacetate (NTA) etc. are commonly used in vitro and in vivo biological studies. This paper primarily focuses on DTPA (DETAPAC). Fahey and associates introduced DTPA for the treatment of iron storage diseases in 1961. It binds iron with great affinity, forms a colorless iron chelate, and has a pattern of distribution and excretion similar to EDTA. DTPA binds to number of metals like $Zn^{2+}$, $Mg^{2+}$, $Mn^{2+}$, $Co^{2+}$, $Cu^{2+}$, $Pb^{2+}$, and $Ca^{2+}$, and various radionuclides [1]. DTPA has a number of uses but particularly has a predictable excretion from the glomeruli with very little tubular reabsorption. When labeled with technetium-99m (aperfusion agent) this can be detected by a gamma camera and can be used as the most popular radiopharmaceutical for diuretic renography [3].

## Structure of DTPA

DTPA has five acetate moieties linked by a molecular backbone (Figure 1) which can tightly complex a metallic ion and are more stable then complexes made by other chelating agents like EDTA [1]. It is structurally similar to EDTA except that it has an extra basic unit of $NCH_2COO^-$ and diethylene in place of ethylene in EDTA.



EDTA Ethylenediaminetetraacetic acid anion



Diethylenetriaminepentaacetic acid anion

**Figure 1:** EDTA and DTPA [12]

**Reaction of DTPA with OH/ $O^-$**

$OH/O^-$ radicals react very efficiently with DTPA and other PACs (equation 1). The detailed mechanism is still uncertain but it is well established that OH radical attack on PACs resulting in decarboxylation [2]. The rate constants of the reactivity of OH with a number of PACs are given in Table 1. It can be seen that reactivity of DTPA is more than other PACs.

$$OH \leftrightarrow O^- + H^+ \quad (pk_1 = 11.9) \qquad (1)$$

**Table 1:** The reactivity of OH radical with PAC [2]

| Reaction: OH + PAC | pH | pk of PAC | k (x $10^9$, $M^{-1}$ $s^{-1}$ ) |
|---|---|---|---|
| IDA | 1, 7 | 1.8,2.6,9.3 | 0.05 |
| NTA | 0-9 | 0.8, 1.8, 2.5, 9.3 | 2.3 |
| EDTA | 4, 9 | 1.5, 2.0, 2.7, 6.1, 10.2 | 0.4, 0.2 |
| DTPA | 5-11 | 1.8, 2.7, 4.3, 8.5, 10.5 | 5 |

**Kinetics and thermodynamics of reaction of superoxide radical with $Fe^{3+}$DTPA**

In presence of $H_2O_2$, superoxide radical gives rise to the highly reactive hydroxyl free radical, however, addition of small amounts of iron salts to superoxide-generating systems results in the formation of $^{\bullet}OH$ and the mechanism is believed to be 'iron catalyzed Haber-Weiss reaction' (equation 2) [7].

$$O_2^{\bullet-} + Fe^{3+}L_n \rightarrow Fe^{2+}L_n + O_2 \qquad (2a)$$

$$Fe^{2+}L_n + H_2O_2 \rightarrow Fe^{3+}L_n + OH^- + OH\bullet \qquad (2b)$$

The iron-catalyzed production of hydroxyl radical by superoxide-generating systems can be inhibited by DTPA, but not by the related ligands like EDTA and HEDTA [7]. Buettner *et al* (1983) found the rate constants for equation 2a (Table 2a) for different ligands by doing the pulse radiolysis kinetic study and spin trapping of hydroxyl radical. Results from both the experiments were in conjunction suggesting that DTPA inhibits the formation of $OH^{\bullet}$ by slowing the reduction of Fe(III) to Fe(II) and not by inhibiting by Fenton reaction [5, 6].

**Table 2a:** Rate constants for reaction 2a determined by pulse radiolysis and spin trapping of $^{\bullet}OH$ at pH 7 [7]

| **Ligand** | **k / $M^{-1}$ $s^{-1}$** |
|---|---|
| EDTA | $2 \times 10^6$ |
| HEDTA | $7.6 \times 10^5$ |
| DTPA | $< 10^4$ |

On the contrary, $Fe^{2+}$DTPA reacts readily with $H_2O_2$ in a Fenton process as shown in reaction 2b. Thus, $O_2^{\bullet-}$ is not a strong enough reductant to reduce $Fe^{3+}$DTPA in the Haber-Weiss reaction, but the paraquet radical reduces $Fe^{3+}$DTPA to $Fe^{2+}$DTPA, which then forms $OH^{\bullet}$ by participating in Fenton reaction [8]. The rate constants for such a reaction is very high and thus favorable for the reaction as given in Table 2b. This suggests that the reduction of $Fe^{3+}$DTPA by $O_2^{\bullet-}$ is not restricted thermodynamically (with reduction potential of 0.03 V), but kinetically.

**Table 2b:** Rate constants reaction 2b [8]

| Ligand | $k / M^{-1} s^{-1}$ |
|---|---|
| EDTA | $2 \times 10^6$ |
| DTPA | $2 \times 10^7$ |
| ATP | $1 \times 10^6$ |

The reduction potentials of other Fe(III) complexes are tabulated in Table 2c.

**Table 2c:** Reduction potential of Fe(III) complexes [6, 9]

| Ligand | $E^{o'}$ / mV |
|---|---|
| EDTA | +120 |
| HEDTA | +110 |
| DTPA | +30 |
| Transferrin | -400 |
| Desferal | -450 |

**Reaction of DTPA with hydrogen peroxide**

Rush *et al*. (1987) investigated the reactions of Fe(II)EDTA, Fe(II)DTPA, and Fe(II)HEDTA with hydrogen peroxide near neutral pH. All these reactions have been assumed to proceed through an active intermediate, $I_1$, where $L_n$ is one of the three PACs [4]. $I_1$ ($HO^\bullet$ radical or an iron complex) reacts with ethanol, formate, and other scavengers at rates relative to $k_2$ (equation 3), with the exception of *tert*-butanol and benzoate, similar to those expected for the $HO^\bullet$ radical.

$$Fe^{2+}L_n + H_2O_2 \rightarrow I_1 \quad (k_1) \quad (3a)$$

$$I_1 + Fe^{2+}L_n \rightarrow 2Fe^{3+}L_n + 2OH^- \quad (k_2) \quad (3b)$$

They also found the relative rates of the reactions between scavengers and intermediates of $Fe(II)/H_2O_2$ compared with hydroxyl radical, which are shown in Table 3. The variation of the ligand on the iron complex is expected to have consequences for the reactivity of $I_1$, if it is an iron complex. On the other hand, a hydroxyl radical intermediate is unaffected.

**Table 3:** Relative rates of the reactions between scavengers and intermediates of Fe(II)/$H_2O_2$ compared with hydroxyl radical [4]

| System | Scavenger | $k_{rel}$(exp)[a] | $\left[\frac{\Delta Fe(III)}{\Delta H_2O_2}\right]$ | $k_{rel}$(lit)[b] | $\left[\frac{\Delta Fe(III)}{\Delta H_2O_2}\right]^b$ |
|---|---|---|---|---|---|
| $Fe^{2+}/H_2O_2$ | *t*-Butanol | 1.2 ± 0.2 | 1 | 1.33 | 1 |
| | Ethanol | 6.3 | 0 | 6.3 | 0 |
| $FeEDTA^{2-}/H_2O_2$ | Ethanol | 0.18 | 0 | 0.38 | 0 |
| | Propanol | ~0.2 | 0 | 0.42 | 0 |
| | Formate | 0.7 | 0 | 0.54 | 0 |
| | Imidazole | 1.9 | 0.3 | 1.8 | — |
| | DMSO | 1.36 | 1.5 | 1.3 | — |
| | *t*-Butanol | — | 1.8 | ~0.08 | 1 |
| | Benzoate | 0.66 | 1.75 | 1.2 | 1 |
| $FeDTPA^{3-}/H_2O_2$ | Ethanol | 0.24 | 0 | *c* | 0 |
| | Ethanol[d] | 0.24 | 0 | *c* | 0 |
| | Formate | 0.6 | 0 | *c* | 0 |
| | Formate[e] | 0.8 | 0 | *c* | 0 |
| | *t*-Butanol | — | 1.8 | *c* | 1 |
| $FeHEDTA^{-}/H_2O_2$ | Ethanol | 0.124 | 0 | *c* | 0 |

Where, $k_{rel}$(exp) is experimentally determined;
$k_{rel}$(lit) is as expected for $HO^{\bullet}$

**Detection of DTPA**

Several spectrophotometric methods for the quantitative determination of DTPA in aqueous solutions based on metal–DTPA complexes have been described, e.g., the decrease of the absorption of an iron thiocyanate complex or the direct photometric UV detection with bismuth or lead complexes [11]. However, a sensitive, accurate and simple HPLC method for the determination of DTPA in biological fluids was still lacking until Weber *et al* developed a HPLC method for the quantitative detection of DTPA in biological fluids by preparing a calibration curve showing linearity between a concentration range of 10 mg/l to 1000 mg/l. It is based on the fact that DTPA forms a highly stable complex with lead (II) with an increased absorption coefficient and a bathochromic shift of the absorption maximum compared to pure DTPA [10].

The complex shows a maximum absorption at 246 nm and a specific absorption coefficient of about 190%$^{-1}$ cm$^{-1}$. A typical chromatogram of the DTPA–lead complex with a total retention time of 5.3 min is shown in Figure 2.

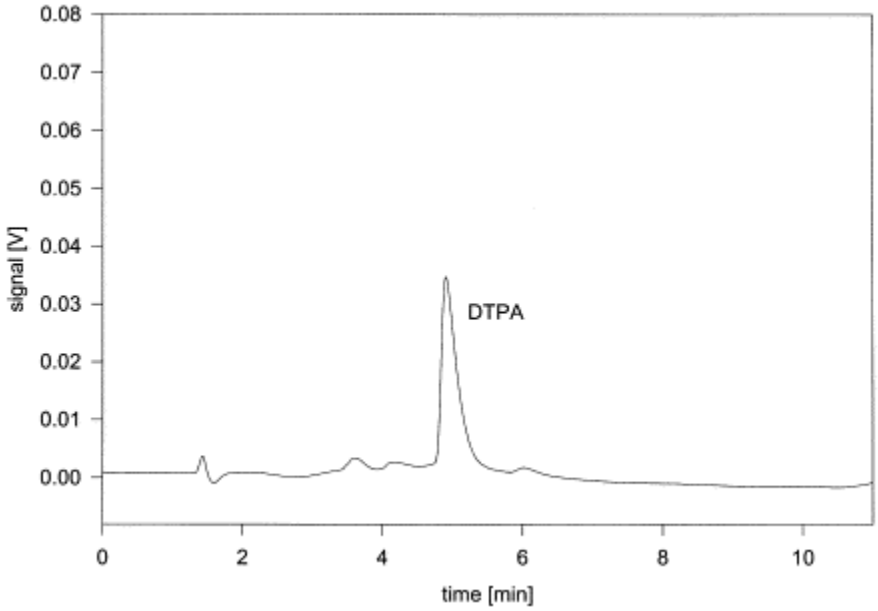



**Figure 2:** A typical DTPA chromatogram at a concentration of 200mg l$^{-1}$ DTPA [10]

**Summary**

Fahey and associates introduced DTPA for the treatment of iron storage diseases in 1961[1]. Structurally similar to EDTA, DTPA binds iron with a greater affinity, forms a colorless iron chelate, and has a pattern of distribution and excretion similar to EDTA. The detailed mechanism of reaction of DTPA and other PACs with OH/O$^{-}$ radicals is still uncertain but it is well established that OH radical attack on PACs resulting in decarboxylation and the reaction is very efficient with rate constants of the order of $10^9$ [2]. The iron-catalyzed production of hydroxyl radical by superoxide-generating systems can be inhibited by DTPA, but not by the related ligands like EDTA and HEDTA [7]. Results from the experiments done by Buettner *et al* suggests that DTPA inhibits the formation of OH$^{\bullet}$ in the iron-catalyzed Haber-Weiss reaction by

slowing the reduction of Fe(III) to Fe(II) and not by inhibiting by Fenton reaction [5,6]. Also, the reduction of $Fe^{3+}$DTPA by $O_2^{\bullet-}$ is not restricted thermodynamically, but kinetically [8]. Weber *et al* developed a HPLC method for the quantitative detection of DTPA in biological fluids which is based on the fact that DTPA forms a highly stable complex with lead (II) with an increased absorption coefficient and a bathochromic shift of the absorption maximum compared to pure DTPA [10]. The complex shows a maximum absorption at 246 nm and a specific absorption coefficient of about $190\%^{-1}$ $cm^{-1}$.

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12. A kind gift from Dr. Buettner. (2003) Professor and assistant director, free radical and radiation biology, University of Iowa, Iowa City, Iowa 52242-1181, USA.