Article NIMHANS Journal

Absorption Spectral Characteristics of Diethyldithiocarbamate-Nickel Complex and its Analytical Application

Volume: 12 Issue: 01 January 1994 Page: 63-68

S N Nagendra ,

Reprints request

, M N Subhash, K Taranath Shetty, K Mamatha Rao &, B S Sridhara Rama Rao,

- Department of Neurochemistry, National Institute of Mental Health & Neuro Sciences, Bangalore 560 029, India

Abstract

A method for the assay of diethyldithiocarbamate and nickel in biological fluids has been described. The method is based on the principle of DDC-Ni complex formation and its selective extraction in amyl alcohol in presence of other thiols. The UV absorption spectral characteristics of diethyldithiocarbamate (DDC) with other divalent metal ions such as Cu^{2+}, Co^{2+}, Zn^{2+} and Ni^{2+} are described. DDC-Ni complex has a higher $\in = 1.4 \times 10^4$ with a λ max at 325 nm. In view of the lack of sensitive method for the assay of DDC in biological fluids, in the present study a spectrophotometric method based on DDC-Ni complex formation was developed. Though Ni^{2+} was found to react with some of the endogenous (GSH, cysteine) and exogenous (dithioerythreitol) sulfhydryl compounds with some what different absorption spectral in aqueous system, they were not extractable in amyl alcohol hence, rendering DDC-Ni complex in organic solvent as specific for DDC. The method was found to be applicable for the assay of disulfiram (DS) in pharmaceutical preparations as well as in biological specimens.

Key words -

Diethldithiocarbamate-nickel complex,
Absorption spectral characteristics,
Sulfhydryl compounds,
Disulfiram

A growing interest in the biological role of sulfhydryls and disulfides has spurred the development of techniques for measuring these compounds. Diethyldithiocarbamate (DDC), a well-known chelator of divalent metal ions known for its high affinity binding to copper, has been extensively used in the quantification of the latter [1]. As a sulfhydryl compound, DDC is known for its protective action against poisoning radiation [2] as well as immunorestorative function of T-cell dependent immune response [3], [4], [5]. Further, DDC has been often used therapeutically to detoxify heavy metal poisoning such as nickel, lead etc [6], [7]. Endogenously, DDC could be formed in the body as an intermediary metabolite of disulfiram (tetraethylthiuram disulfide), the most often used antabuse against alcoholism. A major drawback in the assay of DS and its metabolite in body fluids is the lack of specific and sensitive assay methods. Modern methods

for the determination of DS and its metabolites are mainly based on gas chromatography (GC) [8], [9] and liquid chromatography (LC) [10], [11], [12],. GC method require tedious sample pretreatment and therefore they are not suitable for monitoring of rapid metabolic reactions. Though LC allows to overcome this problem the nickel of the stainless steel components readily undergo exchange reaction with DDC- metal complexes which are relatively unstable [13].

Thus, although there are reports to implicate the metal chelation property of DDC because of its therapeutic efficacy in heavy metal poisoning, and also in the lipophilic character of DDC-Cu complex considered as responsible for its accumulation in lipid rich tissues including the nervous system [14], there is no information available about the absorption spectral characteristics of DDC-metal complexes except for DDC-Cu. The present study is an attempt to characterise some of the divalent metal complexes with respect to their UV absorption spectra, characteristics and compare with that of DDC-Cu. Based on the observations made with respect to differential lipophilic and absorption spectral characteristics, a method has been evolved to extract the complex in an organic solvent for quantification. Further, the feasibility of the proposed method used in the assay of nickel, DDC and its precursor DS in biological fluids has been carried out.

Materials and Methods

Material

Diethyldithiocarbamate sodium salt, disulfiram, reduced glutathione (GSH), dithioeythreitol (DTE), Cysteine (Cys) and Tris were purchased from Sigma Chemicals. Co., (USA). Other chemicals of analytical grade such as nickel chloride, amyl alcohol, copper sulfate etc., were purchased locally from BDH-E. Merck Ltd. (India).

Methods

Absorption spectral characteristics of DDC-divalent metal complex(s) in aqueous and organic phases:

An equimolar concentration (100μ M) of divalent metals such as Zn^{++}, Cu^{++}, Ni^{++} and Co^{++} was allowed to react with DDC (1mM) in 1 ml of 0.1 M Tris/HCI buffer, pH 7.4. After 10 minutes of incubation at room temperature DDC-metal complexes in aqueous system were scanned for absorption spectral characteristics against appropriate controls using UVIKON-810 (KON-TRON) double beam spectrophotometer. Subsequently DDC metal complexes from aqueous phases were extracted in 1.5 ml of amyl alcohol and the organic phases were scanned for UV spectral characteristics against appropriate blanks.

Nickel-Thiol(s) complex formation and spectral characteristics:

An equimolar concentration (100μ M) of Ni^{++} was allowed to react with different thiols such as DTE, GSH and Cysteine in 1 ml of 0.1 M Tris/HCI buffer, pH 7.4 in different test tubes. After 10 minutes of incubation at room temperature they were scanned for UV spectral characteristics against corresponding blanks in aqueous system. Nickel thiol complexes were extracted in 1.5 ml of amyl alcohol and the organic phase was scanned for UV absorption spectral characteristics.

By maintaining DDC concentration (50μ M) constant and varying the Ni^{++} concentration (10-200μ M) and subsequently extracting the complex in amyl alcohol, the stoichiometry of DDC to nickel in DDC-Ni complex was established. Similarly by keeping the Nickel concentration (50μ M) constant and varying DDC concentration (5 - 100μ M), the stoichiometric relationship between the metal chelator and ligand was re-established.

Extinction coefficient of DDC-Ni complex:

A standard curve was plotted by adding different amounts of DDC (10-100μ M) to Nickel (200μ M) in an assay volume of 1 ml containing 0.1 M of Tris/HCI buffer pH 7.4.The resulting DDC-Ni complex was extracted in 1.5 ml of amyl alcohol and absorption spectrum and standard curve were plotted and molar extinction coefficient factor ($\in$) of DDC-Ni complex was established.

Applicatin of the method for DDC and Nickel assay in biological fluids:

Experiments were carried out to find out if the principle of DDC metal complex formation could be used for the assay of DDC or Ni^{++} in biological fluids. For this purpose both urine and plasma were spiked with a known amount of either DDC or nickel (10μ M) and made to react with (100μ M) metal ligand (Ni^{++}) or the chelator (DDC) respectively. The complex formed was extracted in amyl alcohol as described earlier and quantified.

Extension of the assay method for DS quantification:

The ability of reduced glutathione (GSH) to reduce DS through sulfhydryl group exchange reaction [15] was made use of in the proposed method of DS estimation by DDC - Ni complex formation and its subsequent quantification. Thus, 1 ml of urine sample (human) was spiked with various concentrations of DS (10 - 50μ M) and treated with 0.1 ml of GSH (0.5 mM) in presence of nickel chloride (1 mM) and allowed to react at room temperature for 10 minutes. The DDC-Ni complex formed was extracted in amyl alcohol. The organic phase was separated by centrifugation and absorbance measured at 325 nm on a spectrophotometer against reagent blank containing DS. GSH in 1 ml of urine similarly treated. The co-efficient of extinction was established from the standard graph obtained by using different concentrations of DDC and Ni. The amount of DDC in urine was calculated using (and a standard graph. Considering the fact that stoichiometric relationship between DS and GSH (1:2), the quantity of parent compound (DS) was calculated by dividing the amount of DDC formed by two. By employing this method, the DS content of pharmaceutical commercial preparations of antabuse tablets could be assayed.

Results

It was observed that DDC reacted with divalent metal ions such as Zn^{++}, CU^{++}, Co^{++}, and Ni^{++} resulting in a slight turbid complex which was insoluble in water. While DDC-Cu complex was found to be slightly yellowish brown in aqueous system, the other three metals gave rise to slightly whitish turbid complex which were extractable in organic phase (amyl alcohol). However, absorption spectral characteristics of DDC mental complexes in aqueous and organic phases were found to be different (Figure I). Further, it was also noticed that for an equimolar concentration of DDC, DDC-Ni complex was found to have a high extinction coefficient value ($\in 325 = 1.4 \times 10^4$) (Figure II) as compared to DDC-Cu complex($\in 432 = 0.63 \times 10^4$), whereas, DDC-Zn complex was not found to be extractable in amyl alcohol.

.Absorption spectral characteristics of DDC complexes with (A) Zn^{++}; (B) Cu^{++}; (C) Co^{++} and (D) Ni^{++} in aqueous (----) and organic (___) phase. DDC-metal complex in aqueous phase was extracted in 1.5 ml of amyl alcohol as described and both were scanned in a spectrophotometer

Absorption spectral characteristics of (A) and calibration curve (B) of DDC-Ni complex in amyl alcohol. DDC-Ni complex was formed as described with 500 nmoles of NiCl<2 and indicated amount of DDC and extracted on amyl alcohol. The organic phase was scanned in a spectrophotometer

The studies with respect to the possible interference by other sulfyhdryl compounds in DDC assay, indicated that DTE also forms a reddish chromogen with Ni^{++}, but with a distinctly different absorption spectrum in aqueous phase. However, the DTE-Ni complex unlike DDC-Ni complex was not extractable in amyl alcohol (Figure III) On the other hand, the endogenous SH compounds, such as GSH and cysteine, were found to have no interaction with Ni^{++} under the assay condition as revealed by the UV absorption spectrum (Figure III). The high absorbance of DDC-Ni renders it as an ideal method for the assay of DDC. The method was successfully employed to assay the DDC content of liver and brain tissues in rats treated with DS.

Absorption spectral characteristics of complexes of DDC (• - • - •); DTE (Δ - Δ - Δ) and GSH (• - • - •) with nickel in aqueous (closed) and amyl alcohol (open) phases. The complexes were formed as described and scanned in a spectrophotometer

DDC-Ni complex method developed would as well be employed for the assay of DS in pharmacological preparations. The fact that GSH could reduce DS quantitatively through -SH group exchange reaction, was exploited in the present study to extend the DDC-Ni method to assay the active ingredient of antabuse preparation. With appropriate recovery experiments, it was confirmed that the method could be employed for the assay of DS. Though, the method is quite sensitive to detect as little as 5µ M of DDC (=2.5µ M DS), being a fast metabolizing drug DS could not be detected in biological fluids. However, by spiking with as little as 2.5µ M of DS into urine sample (human) followed by its reduction to DDC it was quantitatively assayed with the recovery ranging from 90-100%.

Discussion

DDC, a sulfhydryl compound has several potential applications with respect to analytical, therapeutic aspects and as pesticide as well as fungicide. Further, endogenously formed DDC in the body is an intermediary metabolite of DS, which is reduced by GSH through a -SH group exchange reaction [15]. Though the metal chelation property of DDC has been extensively used from analytical point of view with respect to copper estimation [1] and detoxification of heavy metal poisoning such as nickel, lead etc. [7] there are no reports with reference to physicochemical properties of these complexes. The lipophilic character of these DDC metal complexes makes them easily accessible to lipid rich tissues particularly the brain [14].

Though the metal chelation property of DDC has been extensively used from analytical point of view with respect to copper estimation [1] and detoxification of heavy metal poisoning such as nickel, lead etc., [7].surprisingly there are no reports with reference to physicochemical properties of these complexes. In the present study it has been clearly demonstrated that DDC could interact with various divalent metal cations giving rise to DDC-metal complexes with different physicochemical properties with respect to their differential solvent extractability and absorption spectrum. Thus it was observed that Zn^{++}, Cu^{++}, Ni^{++} and Co^{++} could give rise to water insoluble DDC metal complexes. Further, except for DDC-Zn complex others were extractable in amyl alcohol and different metal complexes were found to have different metal complexes were found to have different absorption spectral

characteristics (Figure I). Interestingly, both DDC-Cu and DDC-Ni complexes were found to have different absorption spectrum with $\in=0.63 \times 10^4$ (λ max. 432nm) and $\in=1.5 \times 10^4$ (λ max. 325nm) respectively. As DDC-Ni complex is having high absorption spectrum for a given amount of DDC, this could be preferably used to quantify DDC in tissues, which is more often used to estimate copper. The very fact that DDC being a sulfhydryl compound, it is likely that other sulfhydryl compounds may interfere in the assay. However, findings on this aspect has clearly indicated that endogenous -SH compounds such as GSH and cysteine do not interfered in the assay of DDC as they lack UV absorption spectrum characteristic of DDC-Ni complex. On the otherhand, an exogenous sulfhydryl compound such as dithioerythreitol (DTE) was found to have an entirely different absorption spectrum for DTE-Ni chromogen (slightly reddish in colour) in aqueous phase as compared to DDC-Ni complex was not extractable in amyl alcohol, hence whatsoever the slight chance of it affecting the assay of DDC-Ni complex in the method developed is ruled out. Thus the principle of DDC-Ni complex formation and its selective extraction in amyl alcohol as adopted in the present study proved to be specific for DDC estimation.

The DDC-Ni complex method was successfully employed in the present study to assay the DDC content of tissue extracts from liver and brain of rats administered with DS. Further, the same method was adopted for assaying DS as well, by reducing the DS to DDC by treating with reducing agents such as ;GSH or Na BH4. While from sensitivity and specificity point of view, as little as 2.5 nmoles of DS could be assayed, DS could not be detected in biological fluids because, it happens to be one of the fastest metabolising drugs. It undergoes nonenzymatic reduction mediated by a number of endogenous sulfhydryl compounds in general, GSH in particular.

Thus, as stated earlier, the ability of GSH to convert DS to DDC by a sulfhydryl group exchange reaction could be successfully employed for the assay of DS by quantification of DDC-Ni complex formation. Further, the same principle could be employed to assay DDC protein mixed disulfide complexes wherein, GSH or NaBH4 could release the bound DDC from the DDC-protein mixed disulfide complex. In conclusion, the present study has described the procedure for the assay of DDC or Ni in biological fluids such as human urine or plasma. The assay has proven usefullness for the determination of DDC or its parent compound DS in biological specimen, especially following pharmacological treatment with disulfiram.

Acknowledgements

The authors are grateful to Prof. Channabasavanna S M, Director, NIMHANS, for his constant encouragement and support. One of the authors (SNN) is grateful to the authorities of AIT, Chikmagalur for deputing to work at NIMHANS.

1.Aaseth J, Soli N E & Forre O,   Increased brain uptake of copper and zinc in mice caused by diethyldithiocarbamate
Acta Pharamcology & Toxicology   Page: 45: 41-44, 1979
2.Stromme J H & Eldjarn L,   Distribution and chemical forms of diethyldithocarbamate and tetraethylthiuram disulfide (disulfiram) in mice in relation to radio protection
Biochemical Pharmacology   Page: 15: 287-297, 1966
3.Brewton G W, Hersh E M, Rios A, Mansell P W A, Hollinger B & Reuben J M,   A pilot study of

diethyldithiocarbamate in patients with acquired immune deficiency syndrome (AIDS) and the AIDS-related complex
Life Sciences Page: 45: 2509-2520, 1989
4.Renoux R J, Immunopharmacologie et pharmocologie du diethyldithiocarbamate (DTC)
Journal of Personality Page: 13 (suppl.): 95-134, 1982
5.Hersh E M, Brewton G, Abrams D, et al, Dithiocarb sodium (diethyldithiocarbamate) therapy in patients with symptomatic HIV infection and AIDS
Journal of American Medical Association Page: 265: 1538-1544, 1991
6.Sunderman F W, Efficacy of sodium diethyldithiocarbamate (dithiocarb) in acute nickel carbonyl poisoning
Annals of Clinical & Laboratory Science Page: 9: 1-10, 1979
7.Gale G R, Smith A B & Walker E M, Further evaluation of diethyldithiocarbamate as an antagonist of cisplatin toxicity
Annals of Clinical & Laboratory Science Page: 11: 476-483, 1981
8.Cobby J, Mayersohn M & Selliah S, The rapid reduction of disulfiram in blood in plasma
Journal of Pharmacology & Experimental Therapy Page: 202: 724, 1977
9.Sauter A M & Von Wartburg J P, Quantitative analysis of disulfiram and its metabolites in human blood by gas liquid; chromatography
Journal of Chromatography Page: 133: 167, 1977
10.Johansson B, Rapid and sensitive online precolumn purification and high performance liquid chromatographic assay for disulfiram and its metabolites
Journal of Chromatography Page: 378: 419-429, 1986
11.Jensen J C & Faiman M C, Determination of disulfiram and its metabolites from biological fluids by high performance liquid chromatography
Journal of Chromatography Page: 181: 407-416, 1980
12.Masso P D & Kramer P A, Simultaneous determination of disulfiram and two of its dithiocarbamate metabolites in human plasma by reversed phase liquid chromatography
Journal of Chromatography Page: 224: 457, 1981
13.Hutchins S R, Haddad P R & Dilli S, Reversed phase high performance liquid chromatography of diethyldithiocarbamate complexes using radial compression columns
Journal of Chromatography Page: 15: 185-192, 1982
14.Allian P & Krari N, Diethyldithiocarbamate, copper and neurological disorders
Life Sciences Page: 48: 291-299, 1990
15.Nagendra S N, Shetty K T, Subhash M N & Guru S C, Role of glutathione reductase system in disulfiram conversion to diethyldithiocarbamate
Life Sciences Page: 49: 23-28, 1991