CHROMIUM (VI) COMPLEXES OF GLUTAMINE – STUDY OF SYNTHESIS AND CHARACTERIZATION OF ITS PROPERTIES FOR VIABILITY AS FOOD SUPPLEMENT

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Abstract

Studies were carried out to determine the properties of the complexes of Glutamine with Ditertiary butyl chromate (TBC). The oxidation of amino acids in general is very difficult. Under microwave irradiation condition also, it takes many hours of irradiation and standing for any fruitful reaction to take place. The oxidation of glutamine by TBC has been taken up for two purposes - firstly to prepare some variants of Cr(III) which may function as supplement for chromium deficiency and secondly to find out some ways to remove toxic Cr (VI) from the body and natural habitats. The complexes were prepared by allowing aqueous solutions of glutamine to react with Ditertiary butyl chromate in different molar ratios for a better comparison. These products were characterised by elemental analysis, FT-IR studies and DTA-TGA mass loss pattern.

Keywords: Ditertiary butyl chromate, Glutamine, Microwave irradiation, Oxidizing agent.

Introduction

Glutamine (L-glutamine) is 2-Amino-4-carbamoylbutanoic acid (C5H10N2O3). It is the most abundant free amino acid in human blood with a concentration of about 500-900µ-mol/L1. Glutamine is synthesized by the enzyme glutamine synthetase mainly in the muscle tissues. During metabolic processes, it is converted into glutamate, aspartate, pyruvate, lactate, alanine, citrate etc. and has an important role to play in the maintenance of TCA cycle[2].

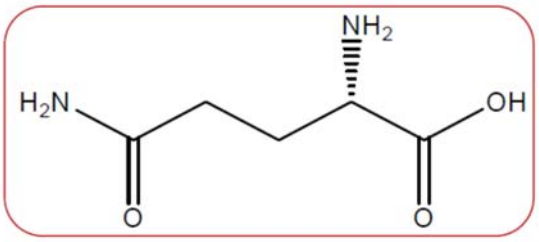

Figure 1: Structure of glutamine

Chromium is a metal that exists in several oxidation states, ranging from Cr(-II) to Cr(+VI)of which trivalent chromium Cr(III) and hexavalent chromium Cr(VI) are the common forms. Chromium(III) has capacity to form complexes with natural organic matter in the environment3. It was estimated that 60% of the total dissolved chromium in the Sea of Japan exists as its organic complexes4. Chromium(III) is biologically active and found in food. It is known to enhance the action of insulin5, a harmone critical to metabolism and storage of carbohydrate, fat and protein in the body. Cr (III) is an essential trace metal nutrient, also improves lipid profile and glucose tolerance by increasing the level of high density lipoprotein cholesterol and decreasing total serum cholesterol6. Chromium deficiency can be improved by taking it from different sources of food or dietary supplements such as chromium picolinate and chromium polynicotinate. Z. Luo et. al. made a detailed study of relative sorption of organic complexes of Cr(III) like glutamate, oxalate, citrate, pyruvate etc7.The interaction of heavy metals such as hexavalent chromium, Cr (VI) with the environment drastically influences living organisms leading to an ecological

imbalance. Chromium (VI) is recognized as an environmental and occupational carcinogen as well as mutagen causing DNA damage24. It is highly toxic and constitutes a major environmental problem. The toxicity of Cr(VI) stems from its higher aqueous solubility and greater ability in comparison to Cr(III) to penetrate across the cell membrane8,9. Breathing high levels of hexavalent chromium can damage and irritate nose, lungs, stomach, and intestine25-27. Cr(VI) as an environmental pollutant and health hazard has been reported21. The studies of methods of preparation and characterization of Cr(III) and Cr(VI) complexes with biologically significant organic compounds like amino acids may lead to the understanding of the role of Cr(III) in biological processes and more importantly it can give a clue to the methods of removing toxic Cr(VI) from the body and from the natural habitat. The formation of various chromium complexes with amino acids as ligands have been reported10,11. A large number of Cr(VI) complexes acting as potent oxidising agents like Pyridinium dichromate (PDC)[12], Pyridinium chlorochromate (PCC)[13],Pyridinium fluorochromate (PFC)[14], 2,2'-Bipyridinium chlorochromate (BIPCC)[15], Tetramethyl ammonium fluorochromate (MBAFC)[16], Quinolinium chlorochromate[17], Ditertiary butyl chromate (TBC)[18], Ditertiary amyl chromate (TAC)[19] etc. have been developed for the organic synthetic work. The last two i.e. TBC and TAC, of late, have been found to be highly efficient and easy to handle oxidants for organic substrates. The oxidation of chromium from (III) to (VI) state is an important environmental process because Cr(VI) is easily taken up by cells and subsequently reduced to the trivalent state. The formation of Cr(III) or other intermediate oxidation states such as Cr(V) or Cr(IV) is believed to play a role in the adverse biological effects of Cr(VI) compounds[20].

The detailed studies of the redox processes involving Cr(VI) based oxidants on the one hand and biologically significant organic substrates like water soluble amino acid glutamine on the other, may be helpful in the preparation of chromium supplements if chromium is reduced to (III) state and in finding out the ways to get rid of toxic Cr(VI) from the body or from the natural habitats which may otherwise be a source of environmental pollution.

In recent years, the increasing application of microwave as a source of clean energy has prompted not only its use in the new organic synthetic work but in the revision of the old methods too. This is in accordance with the principles of clean and green chemistry[22]. The use of water as reaction medium is an important challenge for organic chemist because it is non-flammable, non-toxic, non-volatile and inexpensive "green solvent". Recently, water has been employed as a solvent in many organic reactions[28-30]. In the present paper, we have reported the synthesis and characterization of products formed by the interaction of chromium (VI) as oxidant with glutamine using water as a solvent and explored their viability as a chromium supplement.

Materials and Methods

All chemicals used were of AR grade, and were used without further purification. Reactions were monitored by TLC, performed on silica gel glass plates and visualization on TLC was achieved by UV light or iodine indicator. The experimental work mainly consists of preparation of solutions of the substrates and oxidant, their reactions and characterization of the products formed.

Preparation of tertiary butyl chromate : Pure, dry and powdered chromium trioxide (CrO_3) was weighed and dissolved in 10 ml of tertiary butyl alcohol at room temperature to prepare the oxidant. The bigger pieces of CrO_3 were avoided as they may catch fire.

Preparation of solution of glutamine : Accurately weighed amounts of pure glutamine required for a particular substrate : oxidant molar ratio (S : O ratio) was dissolved in 50 ml of water in each case. The S:O ratio which led to positive results were 1:2, 1:1, 2:1 and 3:1.

Reaction between TBC and glutamine solution : The solutions so prepared as above were mixed carefully with constant stirring. The mixture was subjected to microwave irradiation followed by standing for several days. The details of reaction conditions are tabulated in **Table-1.** The solid products so obtained were washed several times with acetone to remove any impurities and collected in air tight bottles as samples GL-1, GL-2, GL-3 and GL-4.

Characterization of the products : The chromium content of the products was determined titrimetrically whereas the percentage of other elements were obtained

from 'Elemental analyser-Heraeus Vario EL III Carlo Erba 1108". The FTIR curves were obtained by 'Fourier Transform Infrared Spectrometer – Shimadzu 8201PC. DTA-TGA thermogravimetric mass loss pattern were recorded on NETZSCH STA409 CICO instrument. The colour and solubility of the products in water was also tested.

Table-1

Sample Code	Solvent	S:O ratio	Stirring (in min)	Irradiation	Left(in days) in dark
GL-1	Distilled Water	1:2	15	17min(230 W) + 3min(560W)	30
GL-2	''	1:1	30	12 min(230W) + 15min(560W)	60
GL-3	''	2:1	60	25 min(230W)+15 min(450 W)+6 min(560W)	90
GL-4	''	3:1	90	27 min(300W) + 8 min(560 W)	120

Results and discussion

The reaction of glutamine with the oxidant TBC is very difficult even under drastic conditions as is evident from Table-1. Under microwave irradiation conditions also, it takes many hours of irradiation and many days of standing for any fruitful reaction to take place. The same has been observed in cases of other amino acids23. Major advantage of the reaction is that it is carried out in water as a green solvent. On observing table-2 many important generalizations can be drawn. For instance, Cr(VI) is reduced to Cr(III) in all the cases except GL-4. The oxidation rate increased with raising temperature. Also, the extent of degradation increases as the ratio of oxidant increases. This is supported by the decreasing number of carbon atoms in the biggest ligands in the products i.e.5 in GL-4 and GL-3, 4 in GL-2 and 3 in GL-1. Glycine is formed in all the products except GL-4. It is observed that the solubility of the products increases from GL-1 to GL-4. The product GL-2 and GL-3 are soluble in cold water and contains common ligands like water and glycine in addition to asparatic acid in GL-2 and glutamic acid in GL-3. On the basis of above observation, this is quite reasonable to expect that GL-2 and GL-3 have the potentiality to be explored as food supplement for chromium like popular chromium picolinate and chromium polynicotinate. GL-3 is much better in the respect that it is soluble in cold water and contains glutamic acid as ligand.

Table - 2

Sample Code	Color	Solubility in water	Emperical formula	Formulation
GL-1	Pale brown	Partially soluble in cold water	$C_5H_{16}O_{13}N_2Cr_2$	$Cr_2O_3.HOOC.CH.(NH_2)CH_2COOH.4H_2O$
GL-2	Dark Brown	Partially soluble in cold water	$C_{10}H_{28}O_{19}N_3Cr_2$	$Cr_2O_3.2[HOOC.CH_2CH.(NH_2)CH_2COOH].$ $NH_2CH_2COOH\ 6H_2O$
GL-3	Deep brown	Soluble in cold water	$C_{27}H_{55}O_{27}N_6Cr_2$	$Cr_2O_3.5[HOOC.CH_2CH_2\ CH.(NH_2)\ COOH].$ $NH_2CH_2COOH.\ 2H_2O$
GL-4	Deep brown	Soluble in cold water	$C_{25}H_{62}O_{14}N_7Cr_2$	$Cr_2O_3.5[CH_3CH_2CH_2\ CH.(NH_2)\ COOH]$ $2NH_3.\ H_2O$

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