ROLE OF METAL FERROCYANIDES IN CONVERSION OF CYSTEINE TO CYSTINE

A DISSERTATION

Submitted in partial fulfillment of the requirements for the award of the degree of

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in

ADVANCED CHEMICAL ANALYSIS

By

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CERTIFICATE

It is certified that the dissertation report entitled "**ROLE OF METAL FERROCYANIDES IN CONVERSION OF CYSTEINE TO CYSTINE**" is the result of work carried out during the period of July, 2011 to June, 2012, by Umesh Chandra Sharma, M.Tech student in Advanced Chemical Analysis, Department of Chemistry, Indian Institute of Technology Roorkee. This work is done by him under my supervision and neither this report nor any part of it has been submitted for any degree / diploma or any other academic award any where before.

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CANDIDATE'S DECLARATION

I hereby declare that the work which is being presented in the project entitled "**ROLE OF METAL FERROCYANIDES IN CONVERSION OF CYSTEINE TO CYSTINE**" in partial fulfillment of the requirements for the award of the degree of Masters of Technology in Advanced Chemical Analysis submitted in the Department of Chemistry, IIT Roorkee is an authentic record of my own work carried out during the period from July, 2011 to June, 2012 under the supervision and guidance of Dr. Kamaluddin.

The matter embodied in this project work has not been submitted for the award of any other degree.

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ABSTRACT

Minerals adsorb more amino acids with charged R-groups than amino acids with uncharged R-groups. Thus, the peptides that form from the condensation of amino acids on the surface of minerals should be composed of amino acid residues that are more charged than uncharged. However, most of the amino acids (74%) in today's proteins have an uncharged R-group. One mechanism with which to solve this problem is the use of metal-ferrocyanides over the two pH values (pH-3and pH-7) used in these experiments, the R-group of is positive for cysteine (cys) at pH-3 and negative at pH-7.

Transition metal-ferrocyanides are important materials due to their wide application in the area of catalysis, magnetic material etc. These metal-ferrocyanides were prepared by the double decomposition method under specific conditions. Their characterization was done using XRD, TGA-DTA, and FTIR analysis.

Present study involves the adsorption of cysteine on metal ferrocyanides (iron-, cobalt-, nickel-, copper-, and zinc-ferrocyanides) and their study using FTIR-spectroscopy and X-ray diffractometry. The two main findings of study are: - First, after the cysteine adsorption, the FTIR spectroscopy and X-ray diffractometry data showed the formation of cystine. Second, when compared to other metal-ferrocyanides, iron-ferrocyanide adsorbed significantly more cysteine.

The FT-IR spectroscopy results showed that cystine remains adsorbed on the surface of the metal-ferrocyanides and the amine and carboxylic groups are involved in this interaction. X-ray diffractometry showed no changes on metal-ferrocayides mineralogy and the following precipitated substances were found along with the metal-ferrocyanides after drying the samples: cysteine and cystine.

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CHAPTER-1

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction:

Amino acids serve as the building blocks of proteins, which are linear chains of amino acids. Amino acids can be linked together in varying sequences to form a vast variety of proteins[1].The sulfur containing amino acid, cysteine (abbreviated as cys or C) [2] is essential for the entire biological kingdom because of their prominent tasks in primary and secondary metabolism [3-5]. The lowest oxidation state (-2) of sulfur in cys is the fundamental chemical and physical state of these organosulfur compounds that account for their biochemical functions [6]. In proteins, Cys residues are important for stabilization of tertiary and quaternary protein conformation through disulfide bridges. In the living beings of today, amino acids are very important since they are involved in most of life's reactions. Thus, the study of their production and reactions are very important issues in Chemistry [7].

Cysteine is an α-amino acid with the chemical formula $HO_2CCH(NH_2)CH_2SH$. It is a semi-essential amino acid, which means that it can be biosynthesized in humans [8]. Cysteine was considered to be a hydrophilic amino acid based on the belief that the thiol group interacts well with water. The thiol side chain often participates in enzymatic reactions, serving as a nucleophile. The thiol is susceptible to oxidation to give the disulfide derivative cystine, which serves an important structural role in many proteins [9-11].

Cystine is a dimeric amino acid formed by the oxidation of two cysteine residues that covalently link to make a disulfide bond. This organosulfur-compound has the formula $(SCH_2CH(NH_2)CO_2H)_2$[12]. Cystine crystals are six sided and come in various sizes. They can be found in both human and mammal urine under a light microscope. The presence of cystine crystals is often indicative of amino acid re-absorption defects. In humans the presence of cystine crystals is indicative of cystinosis, a rare genetic disease.

1.2 Industrial sources of cysteine:

The majority of L-Cysteine was once obtained industrially by hydrolysis of human hair, but in recent years 80% is produced from duck feathers. Due to marketing restraints with Jewish Kosher and Muslim Halal however, it is now possible to get synthetically produced material, albeit at a higher price [13]. The synthetic route involves fermentation utilizing a mutant of *E. coli*. Wacker Chemie introduced a route from substituted thioazolines [14]. Following this technology, L-cysteine is produced by the hydrolysis of racemic 2-amino-Δ^2-thiazoline-4-carboxylic acid using *Pseudomonas thiazolinophilum*[15].

1.3 Biological functions of cysteine and cystine:

Cysteine has thiol group which is nucleophile and can be easily oxidized. The reactivity is enhanced when the thiol is ionized, and cysteine residues in proteins have pK_a values close to neutrality, so are often in their reactive thiolate form in the cell [16]. Because of its high reactivity, the thiol group of cysteine has numerous biological functions.

1.3.1 Disulfide bonds:

Disulfide bonds play an important role in the folding and stability of some proteins, usually proteins secreted to the extracellular medium [17]. Since most cellular compartments have reducing environments, disulfide bonds are generally unstable in the cytosol with some exceptions as noted below.

Disulfide bonds in proteins are formed by oxidation of the thiol groups of cysteine residues. The other sulfur-containing amino acid, methionine cannot form disulfide bonds. More aggressive oxidants convert cysteine to the corresponding sulfinic acid and sulfonic acid. Cysteine residues play a valuable role by cross-linking proteins, which increases the rigidity of proteins and also functions to confer proteolytic resistance (since protein export is a costly process, minimizing its necessity is advantageous). Inside the cell, disulfide bridges between cysteine residues within a polypeptide support the protein's tertiary structure. Insulin is an example of a protein with cystine cross-linking; wherein two separate peptide chains are connected by a pair of disulfide bonds.

Protein disulfide isomerases [18, 19] catalyze the proper formation of disulfide bonds; the cell transfers dehydroascorbic-acidto the endoplasmic reticulum, which oxidises the environment. In this environment, cysteines are generally oxidized to cystine and are no longer functional as nucleophiles.

1.3.2 Metal ion binding:

Beyond the iron-sulfur proteins, many other metal cofactors in enzymes are bound to the thiolate substituent of cysteinyl residues. Examples include zinc in zinc-fingers and alcohol dehydrogenase, copper in the blue copper proteins, iron in cytochrome P450, and nickel in the [NiFe]-hydrogenases [20]. The thiol group also has a high affinity for heavy metals, so that proteins containing cysteine, such as mettalothionin, will bind metals such as mercury, lead, and cadmium tightly [21].

1.3.3 Post-translational modifications:

Other than its oxidation to cystine, cysteine participates in numerous posttranslational modifications. The nucleophilic thiol group allows cysteine to conjugate to other groups, e.g., in prenylation. Ubiquitin ligases transfer ubiquitin to its pendant, proteins, and caspases, which engage in proteolysis in the apoptotic cycle. These roles are typically limited to the intracellular milieu, where the environment is reducing, and cysteine is not oxidized to cystine.

1.4 Industrial applications:

Cysteine, in the form of L-Cysteine, is a precursor in the food, pharmaceutical, and personal care industries. One of the largest applications is the production of flavours [22]. For example, the reaction of cysteine with sugars in a Maillard reaction yields meat flavours. L-cysteine is also used as a processing aid for baking [23].

In the field of personal care, cysteine is used for permanent wave applications predominantly in Asia. Again the cysteine is used for breaking up the disulfide bonds in the hair's keratin.

1.5 Reducing toxic effects of alcohol:

Cysteine has been proposed as a preventative or antidote for some of the negative effects of alcohol, including liver damage and hangover. It counteracts the poisonous effects of acetaldehyde, which is the major by-product of alcohol metabolism and is responsible for most of the negative effects and long-term damage associated with alcohol use (but not the immediate effects of drunkenness). Cysteine supports the next step in metabolism, which turns acetaldehyde into the relatively harmless acetic acid. In a rat study, test animals received an LD_{50} dose of acetaldehyde (the amount that normally kills half of all animals). Those that received cysteine had an 80% survival rate; when both cysteine and thiamine were administered, all animals survived. There is not yet direct evidence for or against its effectiveness in humans who consume alcohol at normal levels [24].

The sulfhydryl group in the cysteine is highly reactive and plays a key role in protein shaping by disulfide bonds. On a noble metal such as a Au (110) surface, the cysteine molecules make a short-range ordering which is believed as a consequence of homochiral dimer interaction and unidirectional molecular growth [25,26] on a transition metal surface like Cu (110). However, cysteine makes a long-range ordered pattern, [27] which might hint at a different interaction on the molecule/metal interface. Furthermore, simple alkanethiols decompose easily to thiolates on the Cu (110) surface by S-H bond

scission even at low temperatures,[28] whereas those are usually intact on a Au (111) surface [25,26].

1.6 Review of literature:

Bernal [29] showed that minerals could play an important role in origin of life on primeval Earth, because they participated in the concentration of bio-molecules from dilute solution as well as in the formation of biopolymers, several experiments have been carried out to test this hypothesis. The adsorption of amino acids on minerals is an important issue from prebiotic chemistry point of view since most of the reactions of the living beings involve amino acids/peptides/proteins [30]. Several good reviews related to the adsorption of amino acids on minerals appear in the literature [31-33].

As reviewed by Zaia et al. [35] cysteine could be synthesized under conditions that simulate prebiotic atmospheres and hydrothermal vents. Iron is the fourth most abundant element in the crust of Earth. Thus, the reactions and the compounds of this metal are very important in several fields of science and technology such as metallurgy, pure chemistry, medicine, industrial chemistry, soil science and environmental science. For all iron oxides the basic structural unit is octahedron, where each Fe atom is surrounded by six O or OH ions [36]. Thus, cysteine and metal-ferrocyandes are substances that could be easily found on the prebiotic Earth.

The adsorption of cysteine on several minerals has been studied such as pyrite [36], clays [38,39], silica [40,41] and metals [42-44]. In general, the adsorption of cysteine on minerals is due to the interactions of cysteine/metals. Besides, cysteine has three functional groups (thiol, amine, carboxylic) that can interact with minerals. The thiol group involved in these

interactions can be easily identified by FT-IR, where the stretching S–H band at 2,562 cm^{-1} vanishes [37,38,44]. However, in some cases the amine and/or carboxylic groups may also be involved [38,39,42].

Several authors have studied the role of cysteine as well as thioglycolic acid in the dissolution [45-47] or formation [48,49] processes of iron oxides. The dissolution of iron oxides by cysteine is a complex mechanism that involves the formation of cystine as well as the participation of free radicals [46,47], cysteine prevents the formation of magnetite. Cohen et al. (2008) described the synthesis of Fe_3O_4 nanoparticles capped with DL-cysteine as well as the formation of cystine [48].

Several simulated experiments have been performed to trace out the possible pathways of polymerisation of amino acids, peptides and polypeptides. Few studies are focused about how long polypeptide chains could have complexes to secondary proteins and further complexes to tertiary proteins. However, it seems that long polypeptide chains could have coiled itself and / or coupled with other polypeptide chain giving rise to α-helix / β-sheet. Further, S-H group of secondary-proteins (α-helix / β-sheet) containing cystein residues must have been transformed into disulphide group between two cysteine residues resulting the formation of tertiary-proteins.

1.7 Metal-ferrocyanides:

The molecular formula of metal-ferrocyanide was found to be $M_2[Fe(CN)_6]$ .nH2O, where M = Fe, Co, Ni, Cu, and Zn respectively and n=2, 2, 3, 7, and 3 respectively. $[Fe(CN)_6]^{4-}$ species in metal ferrocyanides exist in octahedral geometry, where the central

ferrous ion is surrounded by six CN^- ligands [50]. Because of strong field behaviour of CN^- ligands, all six electrons became paired to give the electronic configuration t^6_{2g}. Although CN^- ligands are bonded with Fe^{2+} ions through σ donation, there is sufficient back bonding from Fe^{2+} ions to CN^- ligands through the donation of π electrons of $d\pi$ orbital of Fe^{2+} ions to the anti-bonding $p\pi$ orbital of CN^-. Transition metal-ferrocyanide complexes generally have a polymeric lattice structure with $[Fe(CN)_6]^{4-}$ anions and the outer transition metal ion coordinated through the nitrogen atom of the cyanide ligand. Attempts are being made to solve the structural problem of metal ferrocyanides. Recently, Gomez and Reguera (2001) [51] characterized the structure of $Cd_2[Fe(CN)_6].8H_2O$. Chromium and manganese ferrocyanides have been found to exhibit linkage isomerism by electron transfer mechanism [52].

We propose that metal ferrocyanides would have catalysed the oxidation of thiol group of cystein residue into disulphide group under suitable conditions. Thus metal ferrocyanides might have played an important role in complexation of secondary proteins to tertiary proteins.

1.8 Problem statement:

Since cyanide ion is strong field ligand, it is, therefore, reasonable to assume that cyanide ions might have formed a number of insoluble and soluble cyanocomplexes (metal ferrocyanides) with transition metal ions. It is assumed that these metal ferrocyanides could have catalysed several reactions like polymerisation and condensation of bio-monomers.

From the literature, it becomes clear that several clay minerals have been participated for adsorption and condensation of bio-monomers like amino acids, nucleotides, monosaccharide, pyrimidines and purines. There are many detailed studies related to adsorption of amino acids (like tryptophan, phenylalanine, cysteine) on metal oxides and clays.

The present work involves the interaction of cysteine on metal ferrocyanides (iron-, cobalt-, nickel-, copper-, and zinc-ferrocyanide) at two different pHs (pH-3 and pH- 7) values. The interaction of cysteine with iron-, cobalt-, nickel-, copper-, and zinc-ferrocyanide was studied using FT-IR and X-ray diffractometry.

It should be pointed out that as far as we know there are no studies related to the interaction of cysteine on metal-ferrocyanides.

Thus, present problem "The adsorption and conversion of cysteine to cystine by metal ferrocyanides (iron-, cobalt-, nickel-, copper-, and zinc-ferrocyanide)" was undertaken keeping in view the structural simplicity of metal-ferrocyanides and their probable catalytic activity.

CHAPTER-2

EXPERIMENTAL

The present work has been carried out systematically, in the following sequence:

1. Synthesis of metal-ferrocyanides (iron-, cobalt-, nickel-, copper-, and zinc-ferrocyanide).
2. Characterization of metal-ferrocyanides by XRD analysis, FT-IR spectroscopy, and TGA-DTA-analysis.
3. Conversion of cysteine to cystine using above synthesized metal-ferrocyanides.
4. IR-Spectroscopy and XRD-analysis of adduct obtained after adsorption.

2.1 Materials required:

Following chemicals of analytical grade were used:

- Potassium ferrocyanide (Merck).
- Ferric nitrate (Merck).
- Cobalt nitrate (Merck).
- Nickel nitrate (Merck).
- Copper nitrate (Merck).

- Zinc nitrate (Merck).
- Cysteine (Sisco India).
- Cystine (Sisco India).
- p-benzoquinone (Merck).

All these chemicals used in this synthesis were used as received without further purification.

2.2 Equipments used:

- Bruker D8 Advance X-Ray diffractometer at Institute Instrumentation Centre IIT Roorkee with following parameters :

Target	Copper (λ-1.54 A°)
Filter	Nickel
Scanning	5°-90°
Current	20 mA
Range	2 KC/S
Voltage	35 KV
Chart Speed	1cm/min.
Goniometer Speed	2°/min.

The peak location and calculated d-values were compared with X-Ray Diffraction database of JCPDA for identification. The d-value is calculated using formula:-

$d = \lambda / 2 \sin \theta$

Where, λ= X-Ray wavelength (1.5418A°)

θ = Bragg's angle

- Perkin Elmer (Pyris Diamond) TGA-DTA high temperature-115 instrument at Institute Instrumentation Centre IIT Roorkee with following parameters :

Heating Rate	10 °C/min.
Atmosphere	Air
Chart Speed	10 cm/hr.
Flow Rate	200 mL/min.
Reference	Alumina Powder
Weight of Sample	8-12 mg

- NEXUS (FT-IR) Thermo-Nicolet spectrophotometer.
- R-8C Lab Centrifuge (REMI) was used to separate metal-ferrocyanide from cysteine solution.

- Metal-ferrocyanides and cysteine solution were shaken on exp. Shaker.
- UV-VIS spectrophotometer (Simadzu).

2.3 Synthesis of metal-ferrocyanides :

Zinc ferrocyanides was synthesised by Kourim's method [58]. A solution of potassium ferrocyanide (167 ml, 0.1 M) was added slowly to the solution of zinc nitrate (500 ml, 0.1 M) with constant stirring. An excess amount of zinc nitrate solution is used so that precipitate gets coagulated properly. The reaction mixture is allowed to heat at 60°C for 2-3 hours and kept as such for 24 hours at room temperature. Now the precipitate was filtered under vacuum and washed several times with distilled water. It was dried in oven at 60 °C. The dried compound was ground and sieved to 100 mesh sizes.

The exact similar procedure as stated above for zinc-ferrocyanide was applied for the synthesis of iron-, cobalt-, nickel-, and copper-ferrocyanides.

2.4 Sample preparation:

Cysteine was dissolved in millipore water at concentrations of 0.72 and 24.2 mg/ml. Each metal-ferrocyanides (iron iron-, cobalt-, nickel-, copper-, and zinc-ferrocyanide) was processed as follows: to five different sets of three tubes (15 ml) containing 100 mg of metal ferrocyanides (iron-, cobalt-, nickel-, copper-, and zinc-ferrocyanide) were added:

(1) 5.00 ml of double-distilled water, (2) 5.00 ml of double-distilled water with 0.72 mg/ml of cysteine and (3) 5.00 ml double-distilled water with 24.2 mg/ml of cysteine. The

pH was adjusted to 3.00 and 7 with HCl or NaOH. The tubes were mixed for 24 h, after which they were spun for 15 min at 2,000 rpm.

The aqueous phase of the tubes with concentration of 0.72 mg/ml of cysteine was used for the measurement of the amount of cysteine adsorbed on metal-ferrocyanides and the solid was discharged. The solid phase of 24.2 mg/ml of cysteine was dried in an oven at 60 °C for 24 h. A portion of dried solids was used for FT-IR and X-ray diffractometry.

2.5 Infrared spectroscopic analysis:

The IR spectra were recorded with NEXUS (FT-IR) Thermo-Nicolet Spectrophotometer using pressed KBr disks and a spectral resolution of 4 cm^{-1}, and each spectrum was obtained after acquiring 85 spectra. FT-IR analysis was carried out with metal-ferrocyanides samples, with and without cysteine adsorption. About 10 mg of iron oxide plus 200 mg of KBr were weighed and ground in an agate mortar with a pestle until a homogeneous mixture was obtained. Disc pellets were prepared and spectra were recorded from 400 to 4,000 cm^{-1}. FT-IR spectra were analyzed by the Origin 8 software.

2.6 X-Ray diffractometry analysis:

The X-ray diffractograms were obtained in an Bruker D8 Advance X-Ray diffractometer, using Cu K_{α}, a monochromator, and the scanning parameters were set as mentioned above. The powder samples were placed on a glass slide. X-Ray diffractometry analysis was carried out with metal-ferrocyanides samples, with and without cysteine adsorption X-ray diffractograms data were analyzed by Origin 8 software.

2.7 UV-Visible spectrophotometric Method :

Cysteine was determined using the p-benzoquinone method using UV-VIS Spectrophotometer (Simadzu) as described by Zaia et al.[35]

2.8 Adsorption of cysteine on metal-ferrocyanides:

Adsorption studies were done by adding 5ml of amino acid solution of different concentration to 100mg of each metal-ferrocyanide separately each time. This suspension was shaken for 20 minutes and then allowed to stand for 24 hours to exist equilibrium at room temperature (27°C). Now these suspensions were centrifuged after 24 hours and then their concentrations were determined spectrophotometrically by taking absorbance at λmax (500 nm) of cysteine by p-benzoquinone method.

The difference between initial concentration and concentration after adsorption is equal to the amount of cysteine adsorbed on metal-ferrocyanide. It was determined with the help of a Calibration curve. Calibration curve of cysteine has been shown in Fig. 1.

CHAPTER-3

RESULT AND DISCUSSION

The synthesis of cysteine under conditions that simulate prebiotic atmospheres has been proposed by Zaia et al. (2008) [35]. Also the presence of metal-ferrocyanides on primitive earth has been proposed by Amirbahman et al. [46] .It is assumed that metal-ferrocyanides effectively catalysed general prebiotic reactions such as condensation, polymerisation and oxidation etc. Biomolecules could have accumulated on surface of metal-ferrocyanides through adsorption process. Selective adsorption properties of metal-ferrocyanides might have played a significant role in synthesis of several important pre-biological compounds. Adsorptions of different amino acids, nucleotides and nucleoside [48-50] have been studied and its application in chemical evolution has been highlighted. The present study involves the interaction of thiol group containing amino acid namely cysteine at pH-3 and pH-7.

The molecular formula of metal-ferrocyanide was found to be $M_2[Fe(CN)_6]\ .nH_2O$, where M = Zn , Ni, Co, and Cu respectively and n=2, 2, 3, 7, and 3 respectively. $[Fe(CN)_6]^{4-}$ species in metal-ferrocyanides exist in octahedral geometry, where the central ferrous ion is surrounded by six CN^- ligands [50]. Because of strong field behaviour of CN^- ligands, all six electrons became paired to give the electronic configuration t^6_{2g}. Although CN^- ligands are bonded with Fe^{2+} ions through σ donation, there is sufficient back bonding from Fe^{2+} ions to CN^- ligands through the donation of π electrons of $d\pi$ orbital of Fe^{2+} ions to the anti-bonding $p\pi$ orbital of CN^- . Transition metal-ferrocyanide complexes generally

have a polymeric lattice structure with $[Fe(CN)_6]^{4-}$ anions and the outer transition metal ion coordinated through the nitrogen atom of the cyanide ligand. Attempts are being made to solve the structural problem of metal-ferrocyanides. Recently, Gomez and Reguera (2001) [51] characterized the structure of $Cd_2[Fe(CN)_6].8H_2O$. Chromium-, and manganese-ferrocyanides have been found to exhibit linkage isomerism by electron transfer mechanism[52].

The adsorption of cystiene on various metal ferrocyanides (iron-, cobalt-, nickel-, copper-, and zinc-ferrocyanide) at two different pHs i.e. pH-3 and pH-7 gives the idea about the adsorption and conversion (cyteine to cystine) efficiency of metal-ferrocyanides at room temperature.

3.1 X-Ray diffraction analysis:

The different X-Ray diffractograms of different double metal-ferrocyanides before and after adsorption of cysteine are shown in the (Fig.2-6) and (Fig.7-11) respectively.

The X-Ray diffraction data for each metal-ferrocyanides, cysteine and cystine are given in (Tables 4-8). And all the observed d-values are matched with the reported d-values which confirmed the formation of double metal-ferrocyanides.

(Fig.2-6) shows X-ray diffractograms of metal-ferrocyanides (iron-, cobalt-, nickel-, copper-, and zinc-ferrocyanide) without adsorption of cysteine. Peak positions and crystalographical attributes for all metal-ferrocyanides (iron-, cobalt-, nickel-, copper-, and zinc-ferrocyanide) were preserved. After adsorption of cysteine, the X-ray diffractograms of the samples showed peaks of both cysteine and cystine (Fig.7-11). Presence of cysteine

on X-ray diffractograms is an artefact of the samples treatment after the adsorption experiments and there is no evidence of any chemical relation between the metal-ferrocyanides and these precipitates.

(Fig.7-11) in the region 5°-30° showed the strongest peak for corresponding d-values of cysteine at d= 3.67 and d=2.64 and of cystine are d=3.15 and d=4.67. Presence of cystine peaks on X-ray diffractograms confirms the conversion of some amount of cyteine into cystine.

(Fig.14-18) shows the conversion of cysteine to cystine by different metal-ferrocyanides.

3.2 FT-IR analysis:

The metal ferrocyanides adsorbed with cysteine were washed several times with water and dried. Infrared spectra of adduct was noted. Comparison of the main characteristic peaks before and after adsorption has been done. Results are shown in (Fig.19-30).

Infrared spectra of iron-, cobalt-, nickel-, copper-, and zinc-ferrocyanide in KBr disc before adsorption of cysteine showed following characteristic peaks which is same as reported in literature. A broad band in range of 3400-3700 cm^{-1} found in case of all metal-ferrocyanides was due to interstitial water molecules. Two sharp bands, one at 2080^{+}- 20 cm^{-1} characteristic of cyanide stretching and other at 590^{+}-10 cm^{-1} due to Fe-C stretching were observed.

Infrared absorption peak of metal-ferrocyanide after adsorption of cysteine showed remarkable change in $^{\delta}$Fe-CN peaks and ν_{CN} peaks was not affected much. It seems that cysteine was attached through strong chemical forces between cysteine and divalent metal ions present in the lattice of ferrocyanides. More careful studies are required to decide interaction sites of cysteine with metal-ferrocyanids.

Interaction between cysteine and the metal-ferrocyanides (iron-, cobalt-, nickel-, copper-, and zinc-ferrocyanide) has been studied by IR spectroscopy. (Fig.19-23) show the FT-IR spectra of pure solids, cysteine (Fig.24) and cystine (Fig.25), and solids mixed for 24 h with cysteine (Fig.26-30).

When the FT-IR spectrum of pure solid cysteine (Fig.24) was compared to the FT-IR spectra of cysteine adsorbed on iron-ferrocyanides (Fig.19), cobalt-ferrocyanide (Fig.20), nickel-ferrocyanide (Fig.21), copper-ferrocyanide (Fig.22) and zinc-ferrocyanide (Fig.23), the vanishing or shifting of several bands was observed. The bands at 1,140, 1,202, 1,223 and 1,742 cm^{-1} can be attributed to the NH_3^+ and CH_2 deformations, C–C(O)–O axial deformation and C=O stretching, respectively[39,42,53,54]. The metal-ferrocyanides samples with cysteine adsorbed on did not show the 2,562 cm^{-1} band attributed to the SH stretching vibration of cysteine.Several authors also observed the vanishing of the 2,562 cm^{-1} band when they studied the interaction of cysteine with different minerals[38,44,53].

The FT-IR spectrum of cystine showed bands at 539 and 650–672 cm^{-1} attribute to S–S and C–S stretching, respectively[54,55] and the same bands were observed for the samples of cysteine adsorbed on the metal-ferrocyanides. It should be pointed out that FT-IR spectra of the cystine and cysteine adsorbed on metal-ferrocyanides are very similar (Fig.24-30).

3.3 TGA-DTA Analysis:

The TGA–DTA analysis curve (Fig.31-35) of the metal-ferrocyanides (iron-, cobalt-, nickel-, copper-, and zinc-ferrocyanide) showed a continuous loss of mass (about 10%) up to 120°C, which may be due to the removal of the water of crystallization (Ali et al.)[57] And DTA tells about the exothermic nature of the reaction.

From the analysis of TGA-DTA data the number interstitial water molecules was found as follows $M_2[Fe(CN)_6].nH_2O$, where M = Zn , Ni, Co, and Cu respectively and n=2, 2, 3, 7, and 3 respectively

A further mass loss between may be due to decomposition of the Cyanide part. The total loss of weight is around 35-45% up to 1000°C indicating that the material is somehow stable.

3.4 Adsorption and conversion of cysteine into cystine:

Solid surfaces have been proposed to play an important role in concentrating the bio-monomers from their dilute aqueous solutions in primeval seas during the course of chemical evolution. Clays have a specific distribution of surface charge, a property that can be associated with exchangeable metal ions and variable adsorption capacity for different organic compounds.

(Table-2-3) shows the amount of cysteine adsorbed on metal-ferrocyanides (iron-, cobalt-, nickel-, copper-, and zinc-ferrocyanide) at pH-7 and pH-3 respectively. Based on pH values and isoelectric point of cysteine (pI=5.07), at lower pH range (pH 3) the surface functional groups are protonated and attribute positive charge to metal-ferrocyanides. For

the pH values above 5, the carboxylic surface functional group (pKa & 5.0) become more negative while the thiol and amino groups (pKa 9.0) remain protonated and attribute positive charge to the metal-ferrocyanides. So the adsorption of cysteine at pH-7 is more than pH-3.

Iron-ferrocyanide showed the highest and cobalt-ferrocyanide showed least adsorption of cysteine when compared to other metal-ferrocyanides (Table 2-3) at both pH-3 and pH-7 which can be explained on the basis of specific surface area (SSA m^2/g). The surface area of metal-ferrocyanides was determined using the BET method (Vladimir et al., 1974), which involves the physical adsorption of N2 gas at its boiling temperature. The calculated surface areas of zinc-, nickel-, cobalt-, and copper-ferrocyanides were determined to be 412.40, 302.00, 20.00, and 44.77 m^2/g , respectively. Since iron-ferrocyanide has the largest SSA value and cobalt-ferrocyanide has the least SSA value [57].

From (Table 2-3) it can be explained that all metal-ferrocyanide adsorb more cysteine at pH-7 than at pH-3.

Order of adsorption at pH-3

iron-ferrocyanide > zinc-ferrocyanide > nickel ferrocyanide > copper-ferrocyanide > cobalt-ferrocyanide.

Order of adsorption at pH-7

iron-ferrocyanide > zinc-ferrocyanide > nickel ferrocyanide > copper-ferrocyanide > cobalt-ferrocyanide.

Matrajt and Blanot (2004) [56] also observed same results when they studied the adsorption of aspartic and glutamic acids (negative net charge) on ferrihydrite (positive net

charge). According to the authors, those amino acids were totally adsorbed on ferrihydrite but other amino acids not. Amirbahman et al. (1997) and Doong and Schink (2002) [46,47] observed that pH had an effect on adsorption of cysteine on Fe^{3+}(hydroxyl) oxides.

Otherwise, Benetoli et al. (2007)[38] studied the adsorption of cysteine on kaolinite and bentonite in three different range of pH (3.00, 6.00, 8.00), and observed no effect on the adsorption of cysteine on both minerals. Iron-ferrocyanide showed the highest adsorption of cysteine compared to other metal-ferrocyanides.(Table 2-3). This higher adsorption is probably due to the highest specific surface area(SSA) of iron-ferrocyanide compared to other metal-ferrocyanide.

Brigatti et al. (1999) [39] observed that the amount of cysteine adsorbed on sometimes depends on the cation in the interlayer of the mineral. Basiuk and Gromovoy (1996) and Basiuk (2002) [40,41] studied the adsorption of several amino acids on silica. According to them, the adsorption of cysteine on silica was higher than other amino acids because the sulfide group of cysteine noticeably decreases adsorption. However, Benetoli et al. (2007) [38] did not observe variation of the amount of cysteine adsorbed on bentonite and kaolinite.

CHAPTER-4

CONCLUSION

Our study involves the synthesis of several metal-ferrocyanides (iron-, cobalt-, nickel-, copper-, and zinc-ferrocyanide) and the adsorption of cysteine on the surface of these metal-ferrocyanides which involves the conversion of cysteine to cysine due to oxidation of cysteine. Analysis of product was done using X-ray diffractrometry and FTIR-specroscopy.

In general minerals, adsorb more amino acids with charged R-groups than amino acids with uncharged R-groups. However, most of the amino acids (74%) in the proteins consist of amino acids with uncharged R-group. Cysteine was adsorbed in the highest amount by iron-ferrocyanide and least by cobalt-ferrocyanide. In our experiments, the R-group of cysteine is positively charged at pH-3 and negatively charged at pH-7 owing to Isoelectric point of cysteine (pI=5.07).

X-ray diffractometry showed no change on the metal-ferrocyanide mineralogy but the formation of cystine from the oxidation of cysteine in all five synthetic metal-ferrocyanide. Cystine synthesized remains adsorbed on the surface of metal-ferrocyanides confirmed by the XRD-Analysis and FTIR Analysis. It is also explained that amine and carboxylic group are involved in this interaction.

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Table-1: Absorbance of cysteine for calibration curve.

S.No.	Concentration of cysteine (µg/ml)	Absorbance
1	0	0
2	7.5	0.109
3	15	0.202
4	22.5	0.303
5	30	0.391

Table-2: Amount of cysteine (μg) adsorbed on 100 mg of metal-ferrocyanides iron-, cobalt-, nickel-, copper-, and zinc-ferrocyanide) at pH-7.

S.No.	Metal-ferrocyanide	Absorbance after adsorption	Concentration of cysteine after adsorption(C_f) μg/ml	Amount adsorbed (720-C_f) * 5 (μg)
1	Iron-ferrocyanide	0.020	1.0914	3594.54 (4)
2	Cobalt-ferrocyanide	0.4945	37.563	3412.19 (4)
3	Nickel-ferrocyanide	0.0549	3.774	3581.13 (4)
4	Copper-ferrocyanide	0.0945	6.818	3565.91 (4)
5	Zinc-ferrocyanide	0.0386	2.5211	3582.39 (4)

Table-3 : Amount of cysteine (μg) adsorbed on 100 mg of metal-ferrocyanides iron-, cobalt-, nickel-, copper-, and zinc-ferrocyanide) at pH-3.

S.No.	Metal-ferrocyanide	Absorbance after adsorption	Concentration of cysteine after adsorption (C_f) μg/ml	Amount adsorbed (720-C_f) * 5 (μg)
1	Iron-ferrocyanide	0.035	2.224	3588.78 (4)
2	Cobalt-ferrocyanide	1.97	150.976	2845.12 (4)
3	Nickel-ferrocyanide	0.4731	35.918	3420.01 (4)
4	Copper-ferrocyanide	1.6112	123.391	2983.02 (4)
5	Zinc-ferrocyanide	0.1202	8.793	3556.03 (4)

Table - 4: X-Ray diffraction data for iron-ferrocyanide.

S.No.	2θ	θ	sin θ	2 sin θ	$d_{observed}$(A°)	$d_{reporetd}$(A°)	$(I/I_j)*100\%$
1	17.54	8.73	0.1516	0.3033	5.08	5.10	100.00
2	24.86	12.43	0.2152	0.4304	3.58	3.60	32
3	35.09	17.54	0.3011	0.6079	2.56	2.55	40
4	39.67	19.84	0.3393	0.6786	2.27	2.28	32
5	43.20	2.60	0.3681	0.7362	2.09	2.07	3

Table -5: X-Ray diffraction data for cobalt-ferrocyanide.

S.No.	2θ	θ	sin θ	2 sin θ	$d_{observed}$(A°)	$d_{reporetd}$(A°)	$(I/I_j)*100\%$
1	17.92	8.96	0.1557	0.3114	4.95	5.06	70.83
2	25.37	12.68	0.2185	0.4370	3.52	3.57	100.00
3	36.12	18.06	0.3100	0.6200	2.48	2.52	59.72
4	44.95	22.47	0.3822	0.7640	2.02	2.05	11.11
5	51.56	29.17	0.4874	0.9748	1.77	1.60	12.50

Table - 6: X-Ray diffraction data for nickel-ferrocyanide.

S.No.	2θ	θ	sin θ	2 sin θ	$d_{observed}$(A°)	$d_{reporetd}$(A°)	$(I/I_j)*100\%$
1	17.57	8.78	0.1526	0.3052	5.05	5.00	84.75
2	24.99	12,49	0.2162	0.4324	3.56	3.53	100.00
3	35.74	17.87	0.3063	0.6126	2.51	2.50	61.86
4	40.03	20.01	0.3422	0.6845	2.25	2.26	21.19
5	43.89	21.94	0.3737	0.7474	2.06	2.06	19.49

Table - 7: X-Ray diffraction data for copper-ferrocyanide.

S.No.	2θ	θ	sin θ	2 sin θ	$d_{observed}$(A°)	$d_{reporetd}$(A°)	$(I/I_j)*100\%$
1	17.93	8.96	0.1558	0.2116	4.94	4.99	100.00
2	25.37	12.69	0.2196	0.4392	3.51	3.52	75.18
3	36.15	18.07	0.3102	0.6205	2.48	2.50	40.07
4	40.05	20.25	0.3462	0.6924	2.23	2.25	18.44

Table – 8: X-Ray diffraction data for zinc-ferrocyanide.

S.No.	2θ	θ	sin θ	2 sin θ	$d_{observed}$(A°)	$d_{reporetd}$(A°)	$(I/I_j)*100\%$
1	16.46	8.23	0.1430	0.286	5.38	5.40	77.91
2	19.79	9.89	0.1718	0.3437	4.48	4.51	40.11
3	21.73	10.86	0.1884	0.3769	4.08	4.08	100.00
4	24.62	12.31	0.2131	0.4263	3.62	3.64	36.05

Table - 9: X-Ray diffraction data for cysteine.

S.No.	2θ	θ	sin θ	2 sin θ	$d_{observed}$(A°)
1	24.26	12.13	0.2101	0.4202	3.67
2	33.90	16.95	0.291	0.5830	2.64
3	36.50	18.25	0.3131	0.6263	2.46
4	39.83	19.91	0.3406	0.6812	2.26

Table -10: X-Ray diffraction data for cystine.

S.No.	2θ	θ	sin θ	2 sin θ	$d_{observed}$(A°)
1	28.29	14.15	0.2443	0.4887	3.15
2	19.01	9.51	0.1651	0.3300	4.67
3	33.11	16.55	0.2849	0.5698	2.70
4	34.25	17.15	0.2944	0.5889	2.61

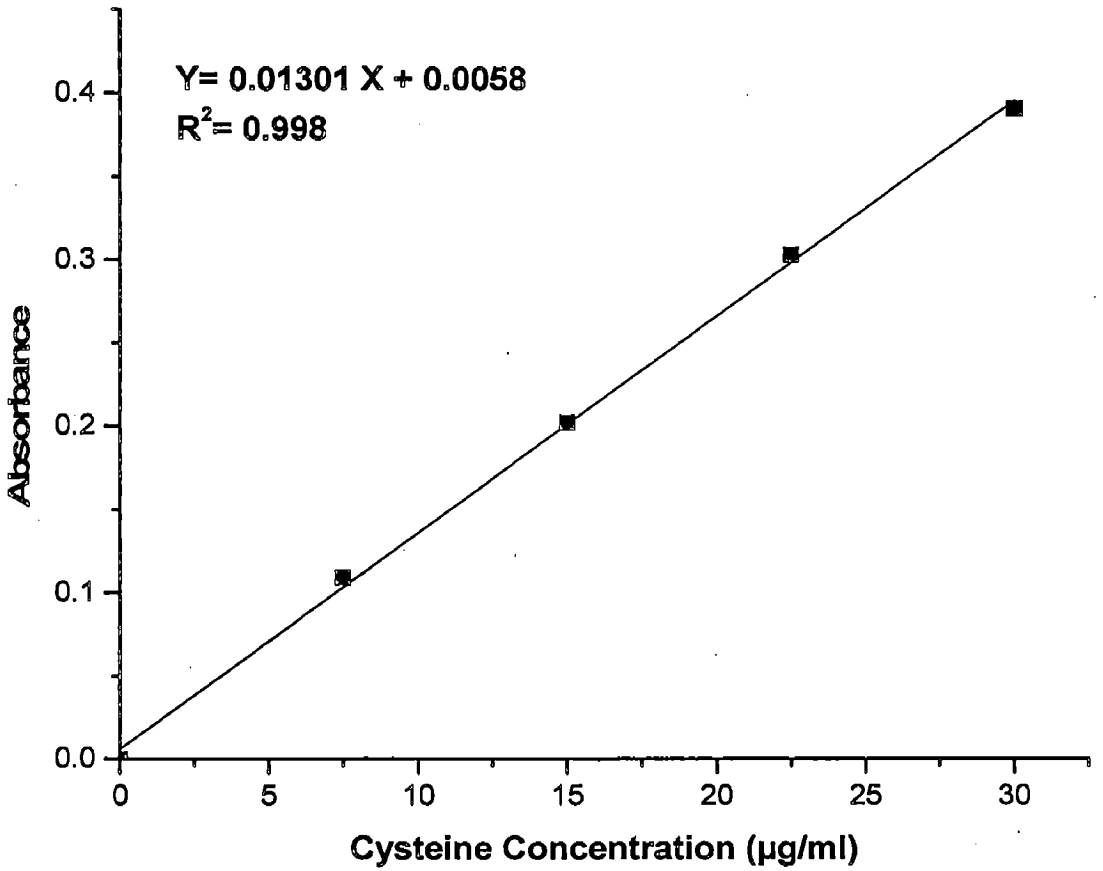

Figure -1: Calibration curve for estimation of cysteine.

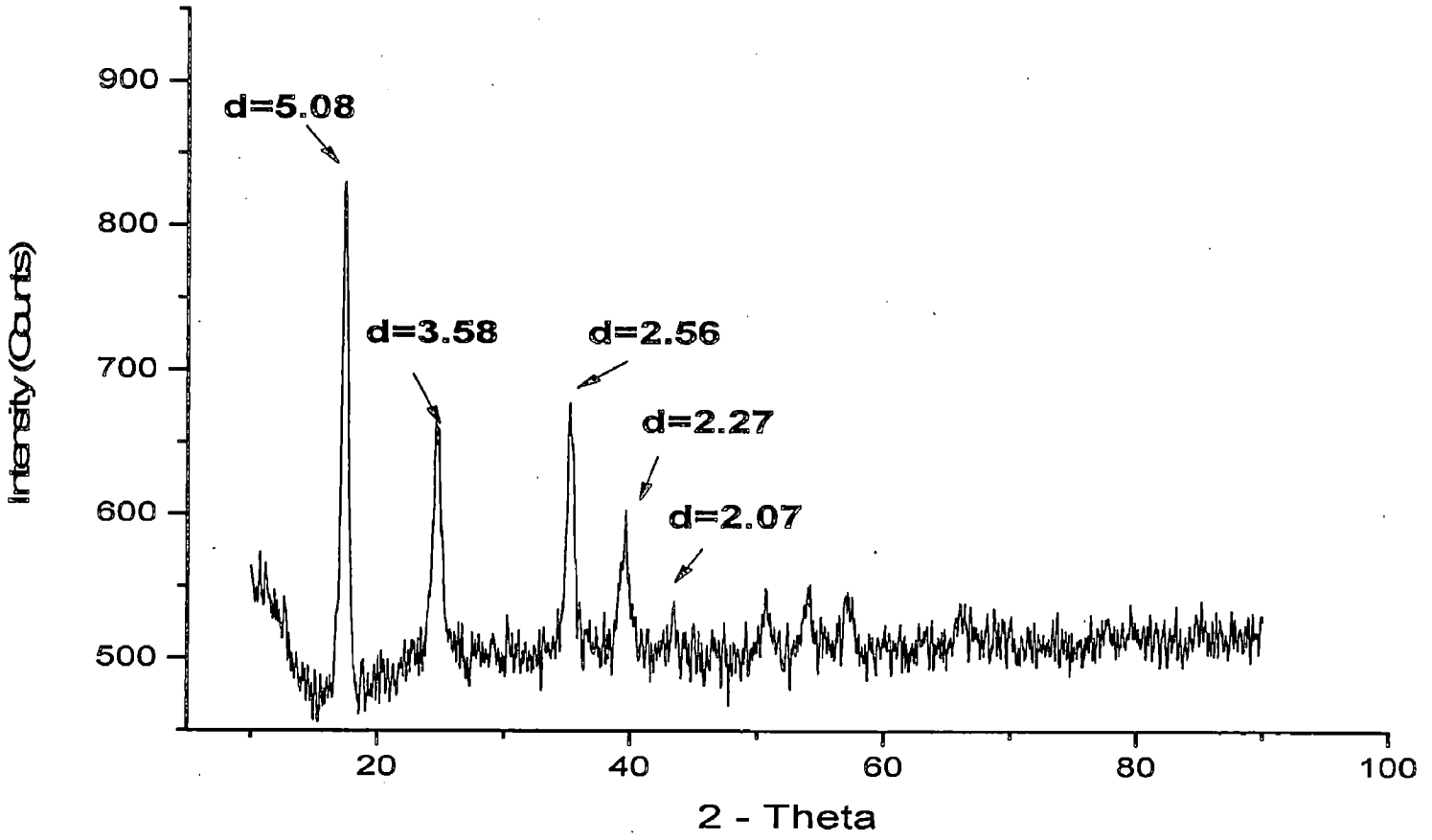

Figure -2:XRD graph of iron-ferrocyanide.

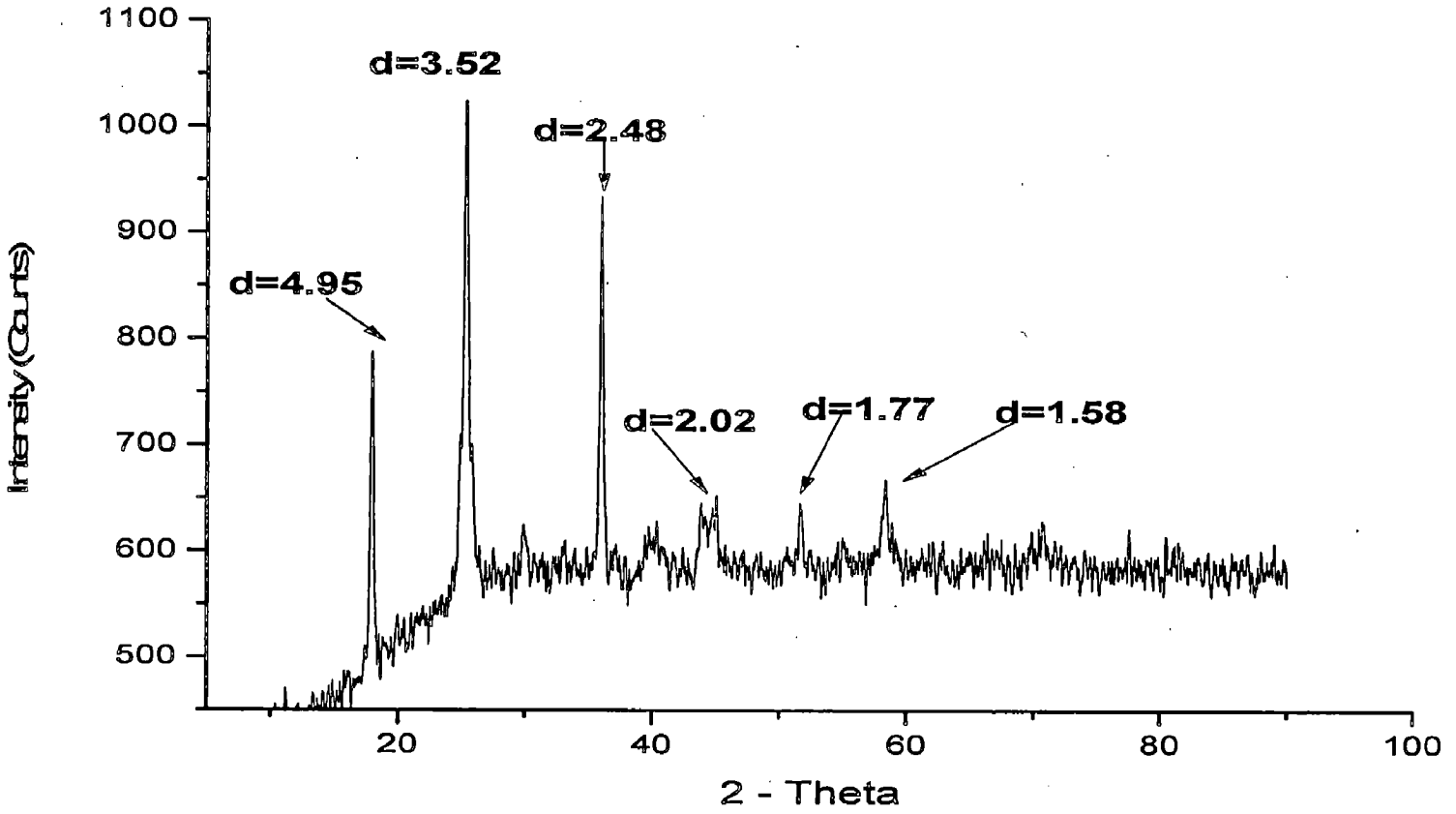

Figure -3: XRD graph of cobalt-ferrocyanide.

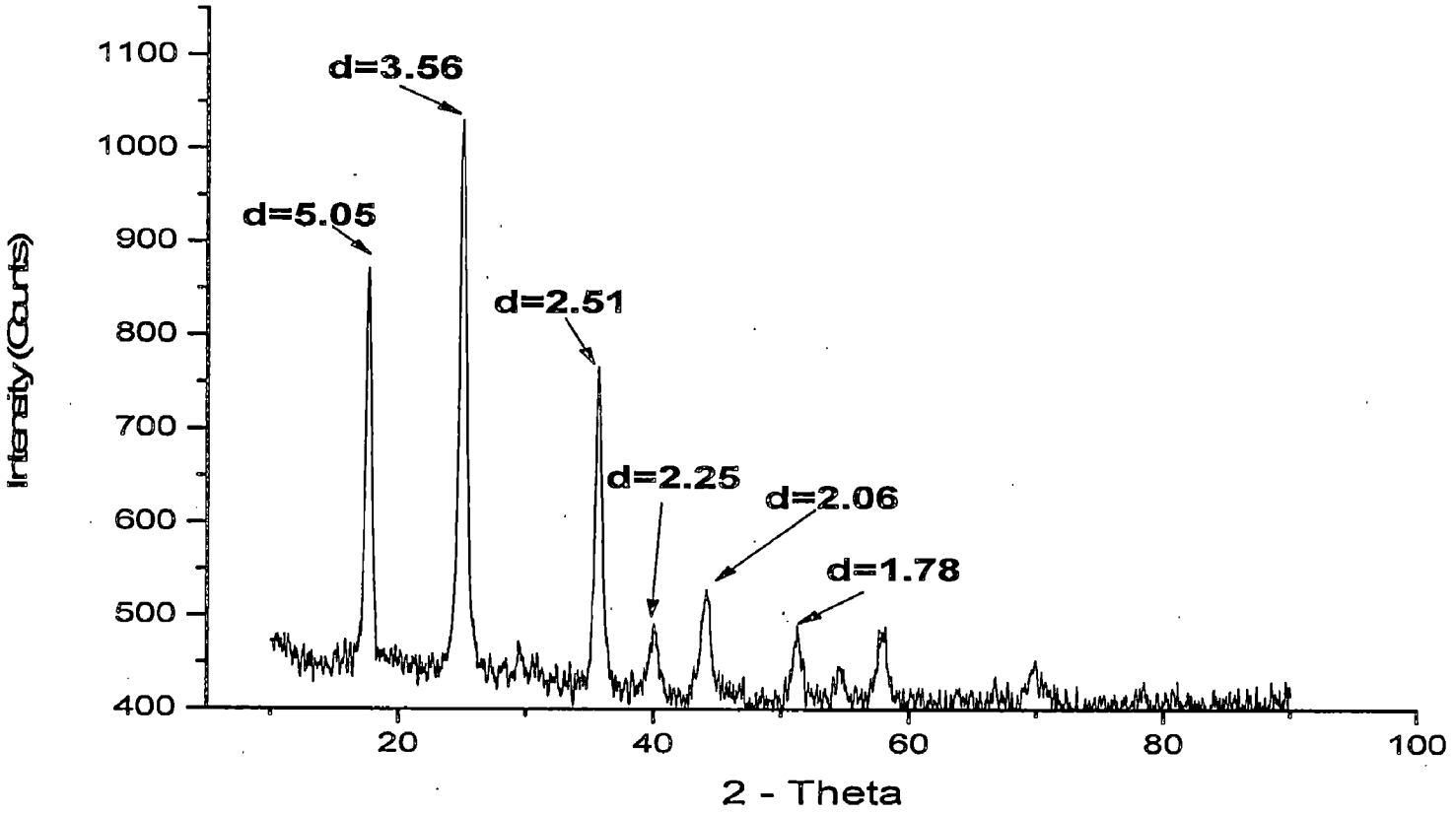

Figure -4:XRD graph of nickel-ferrocyanide.

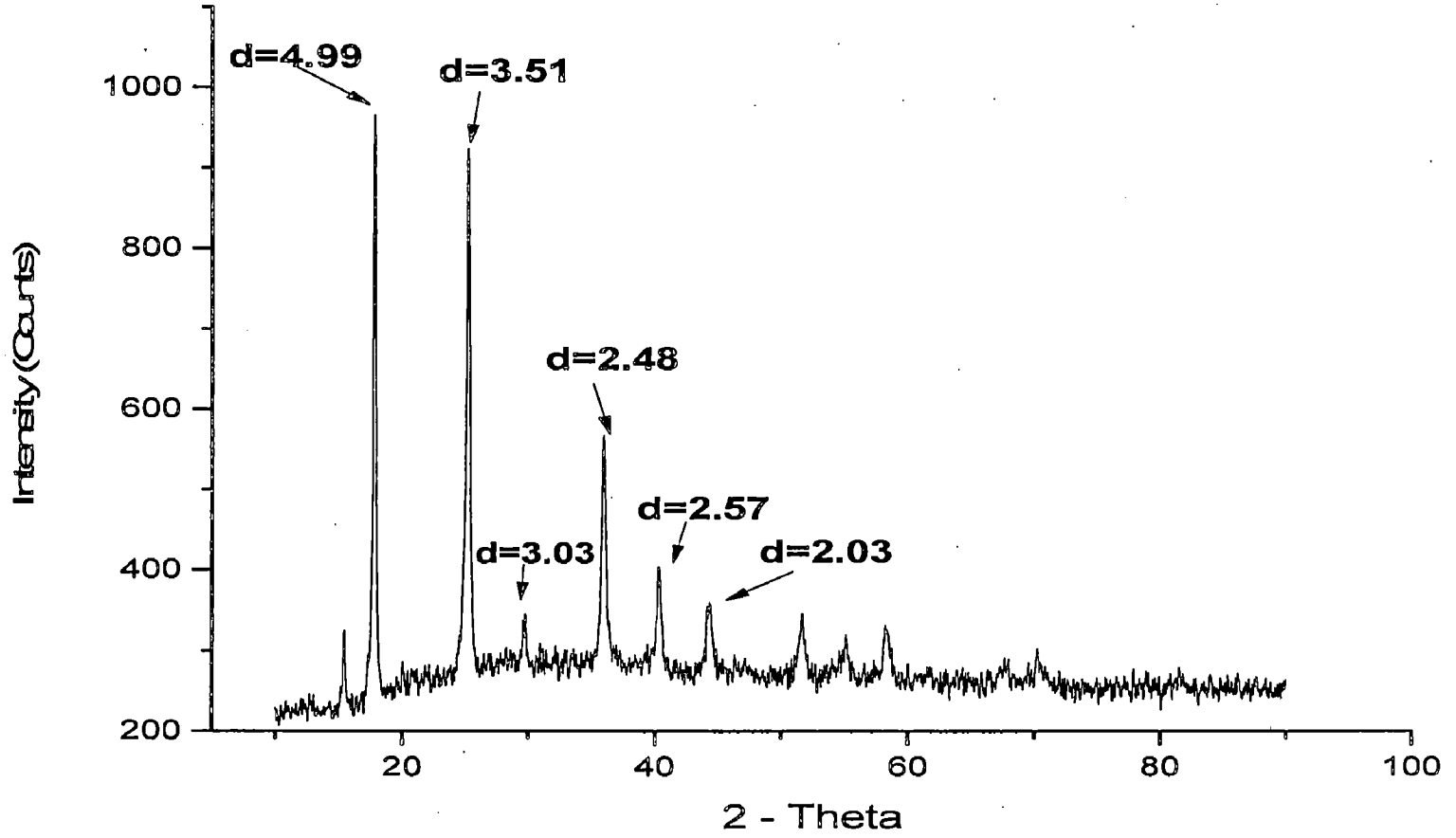

Figure -5: XRD graph of copper-ferrocyanide.

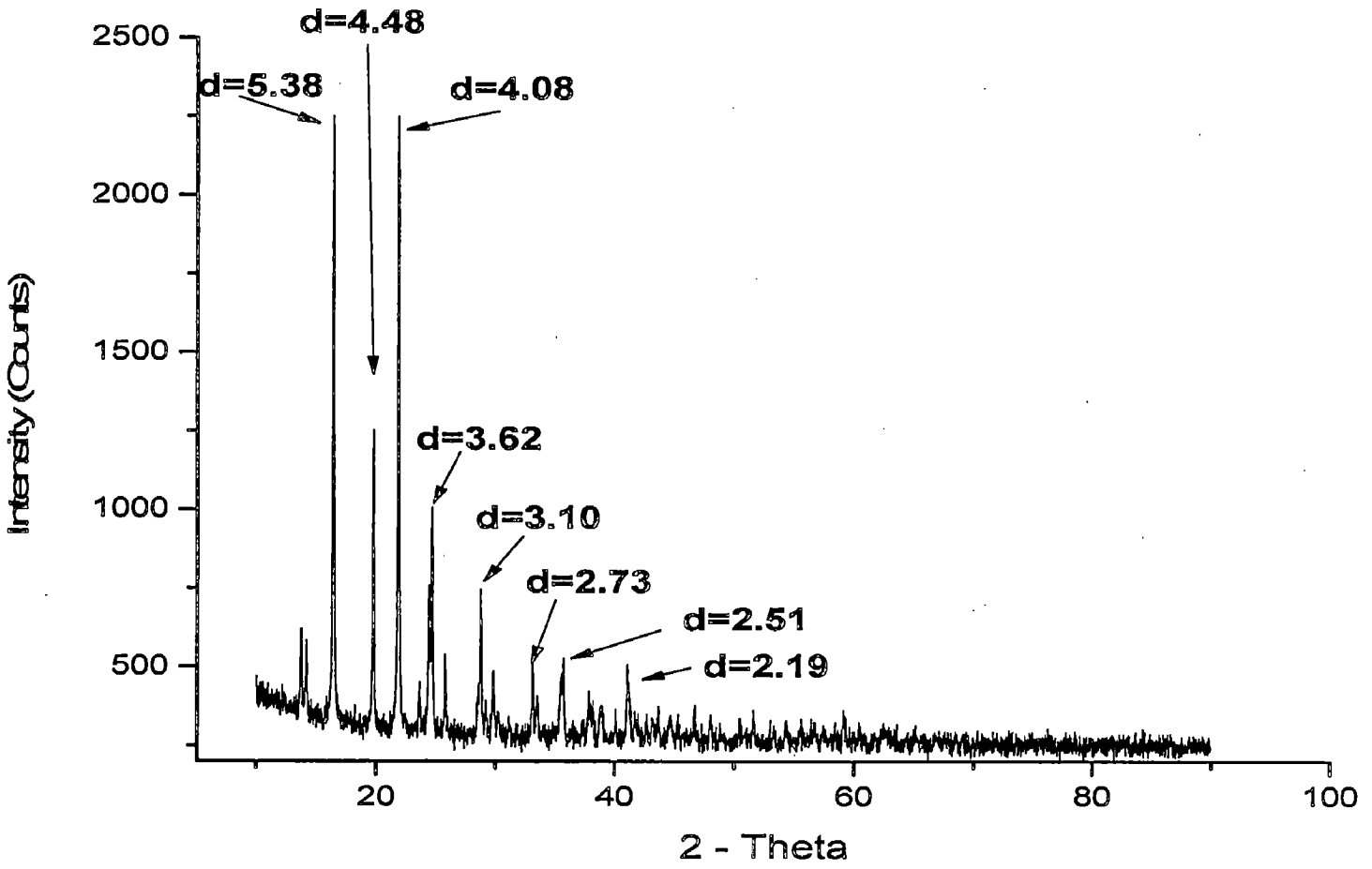

Figure -6: XRD graph of zinc-ferrocyanide.

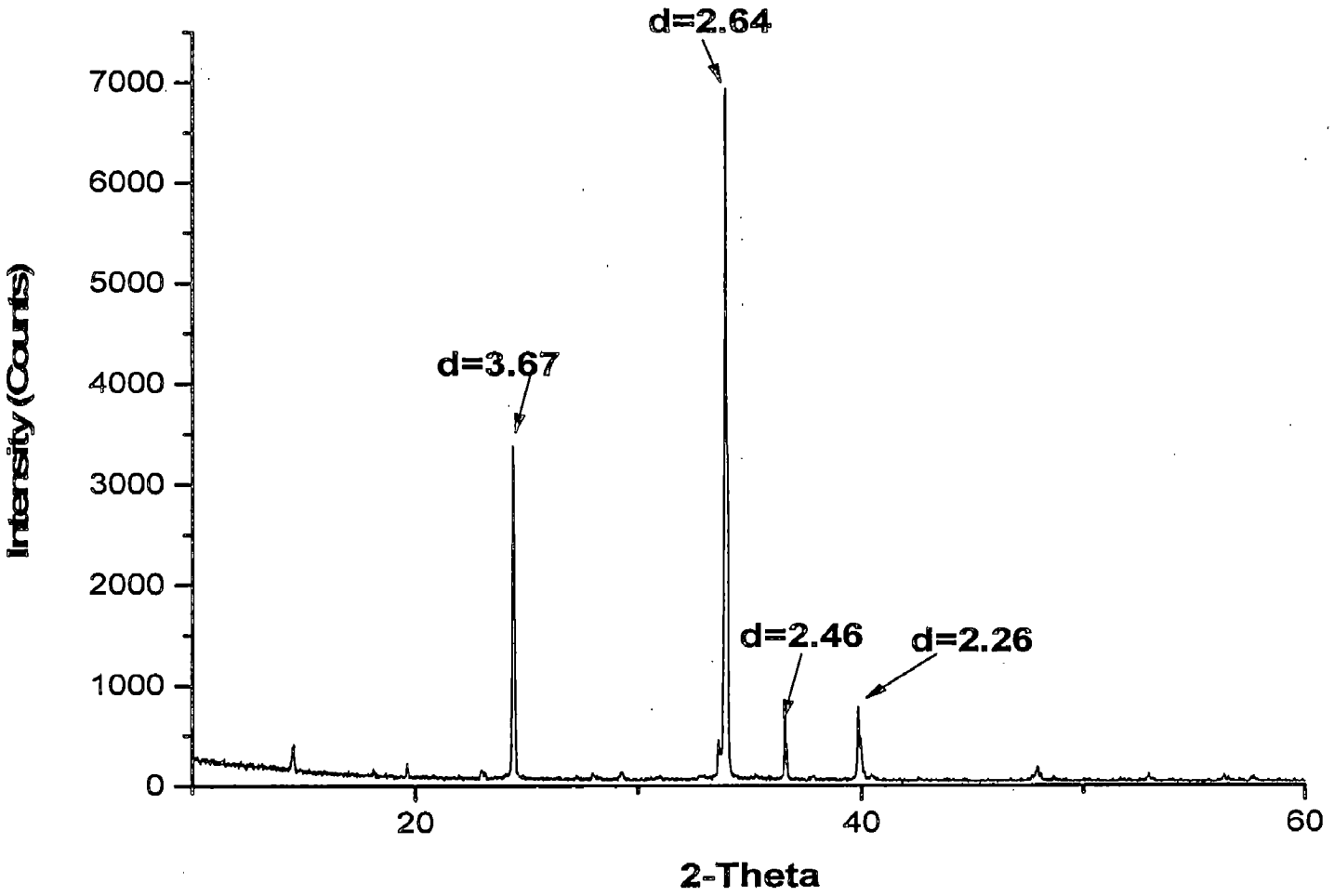

Figure -7: XRD graph of cysteine.

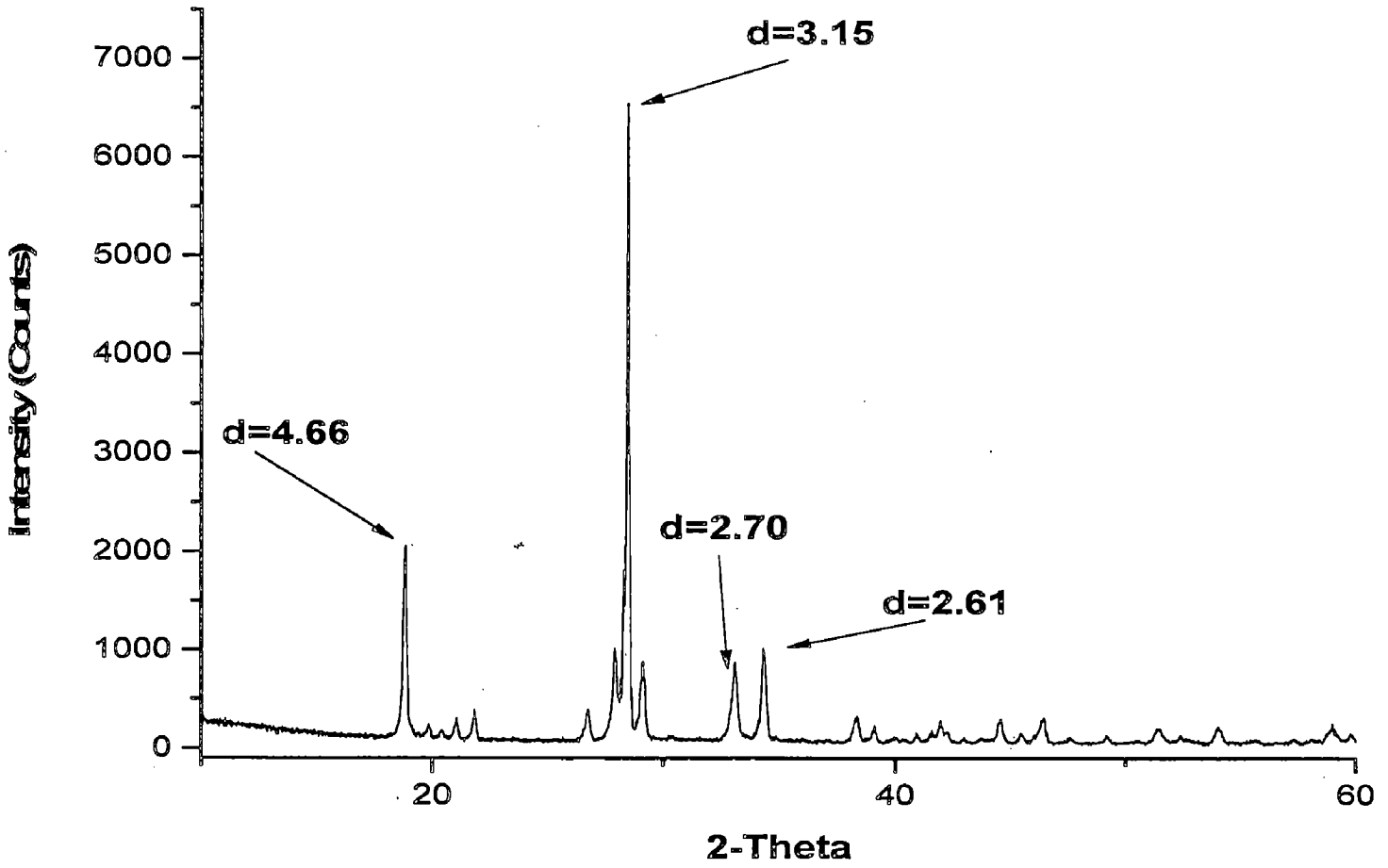

Figure -8: XRD graph of cystine.

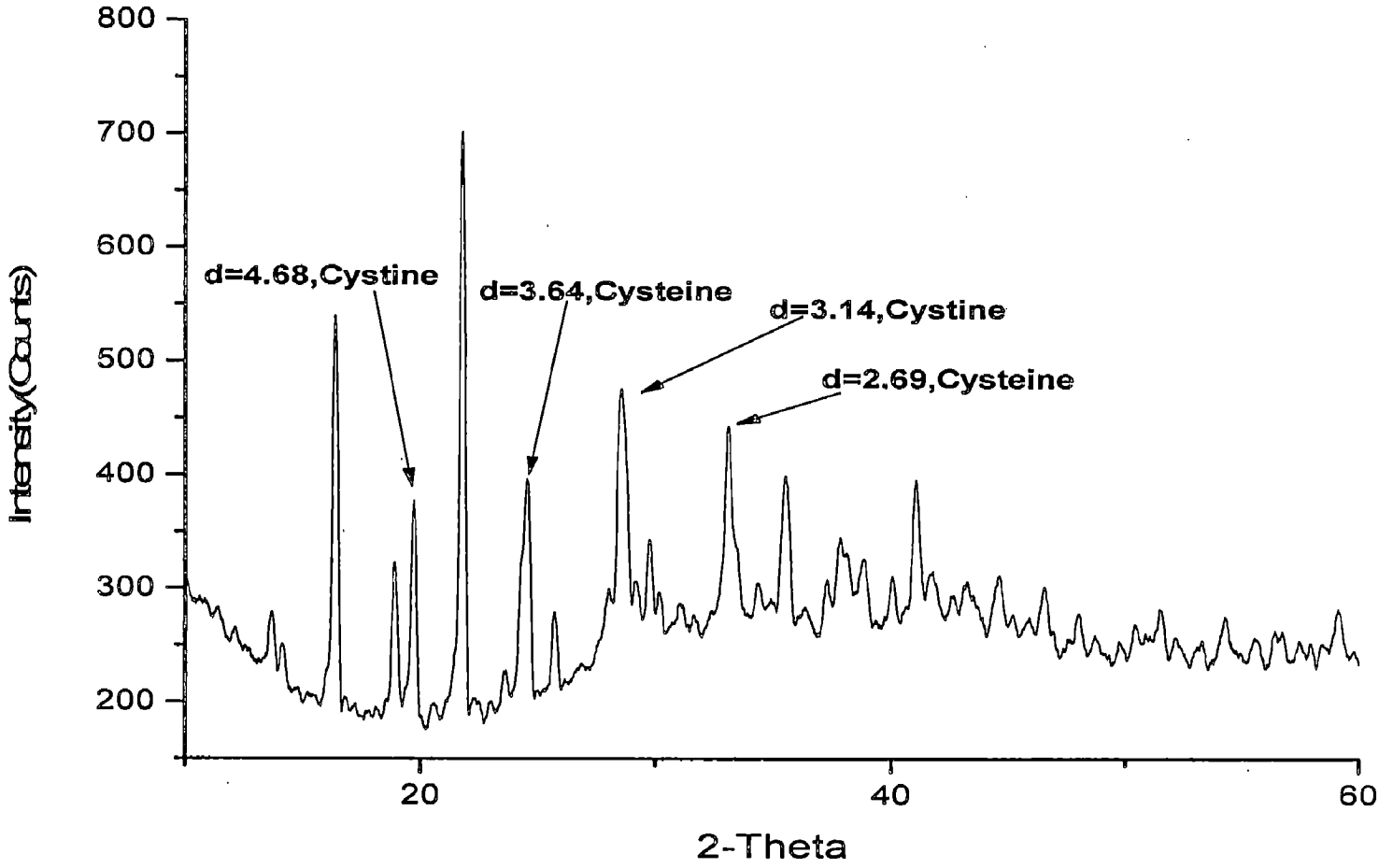

Figure -9: XRD graph of iron-ferrocyanide after adsorption of cysteine, showing the conversion of cysteine to cystine.

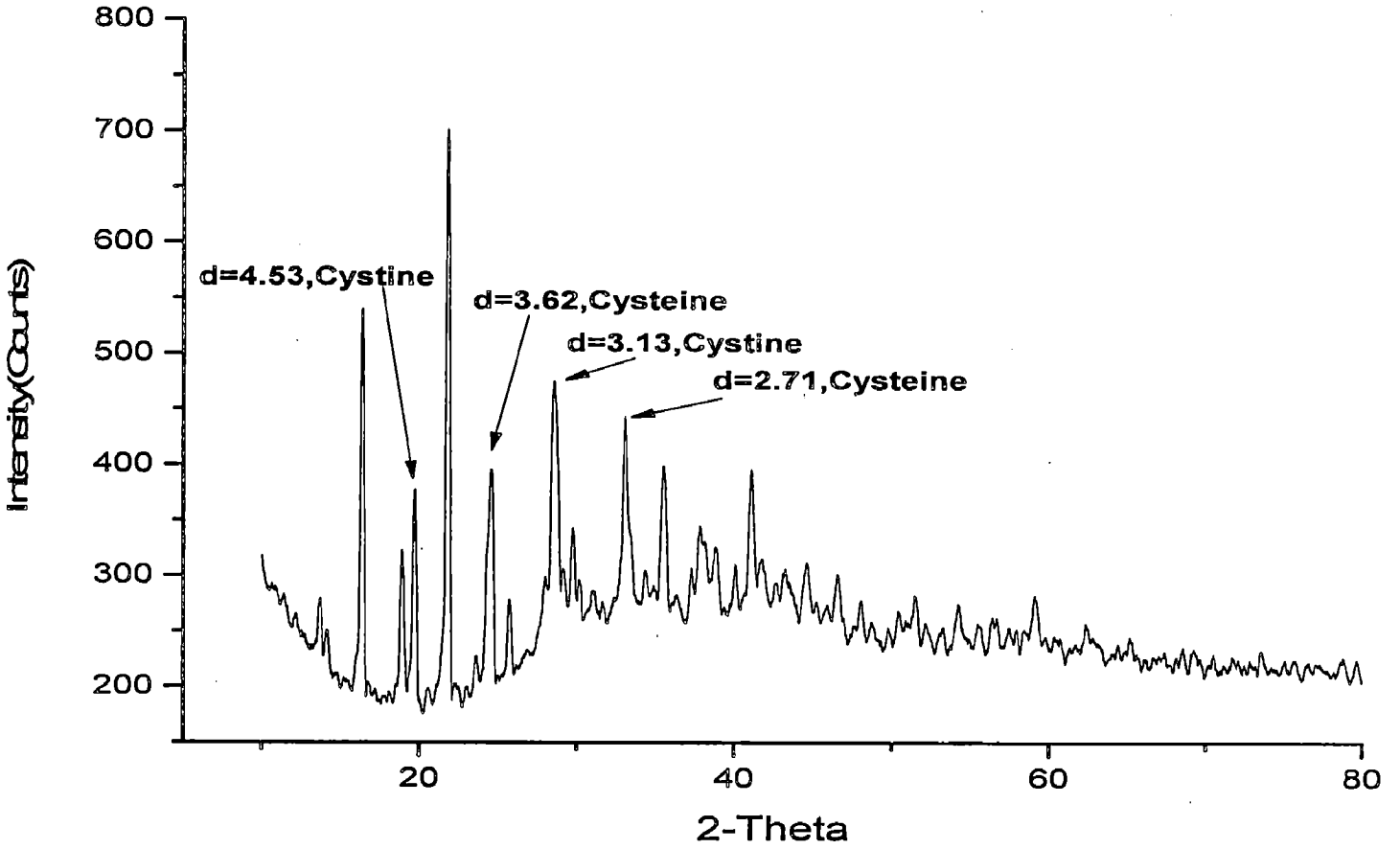

Figure -10: XRD graph of cobalt-ferrocyanide after adsorption of cysteine, showing the conversion of cysteine to cystine.

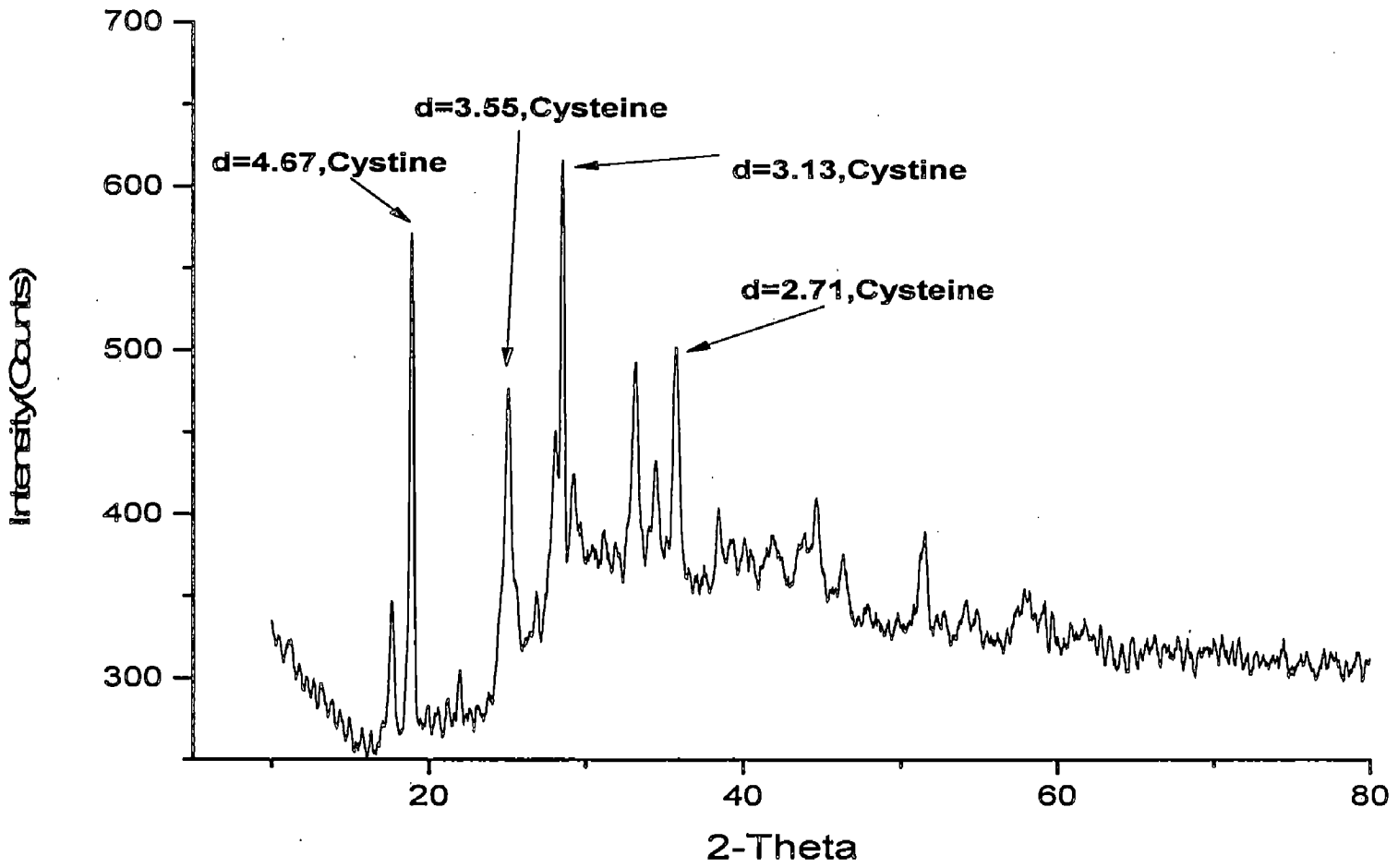

Figure -11: XRD graph of nickel-ferrocyanide after adsorption of cysteine, showing the conversion of cysteine to cystine.

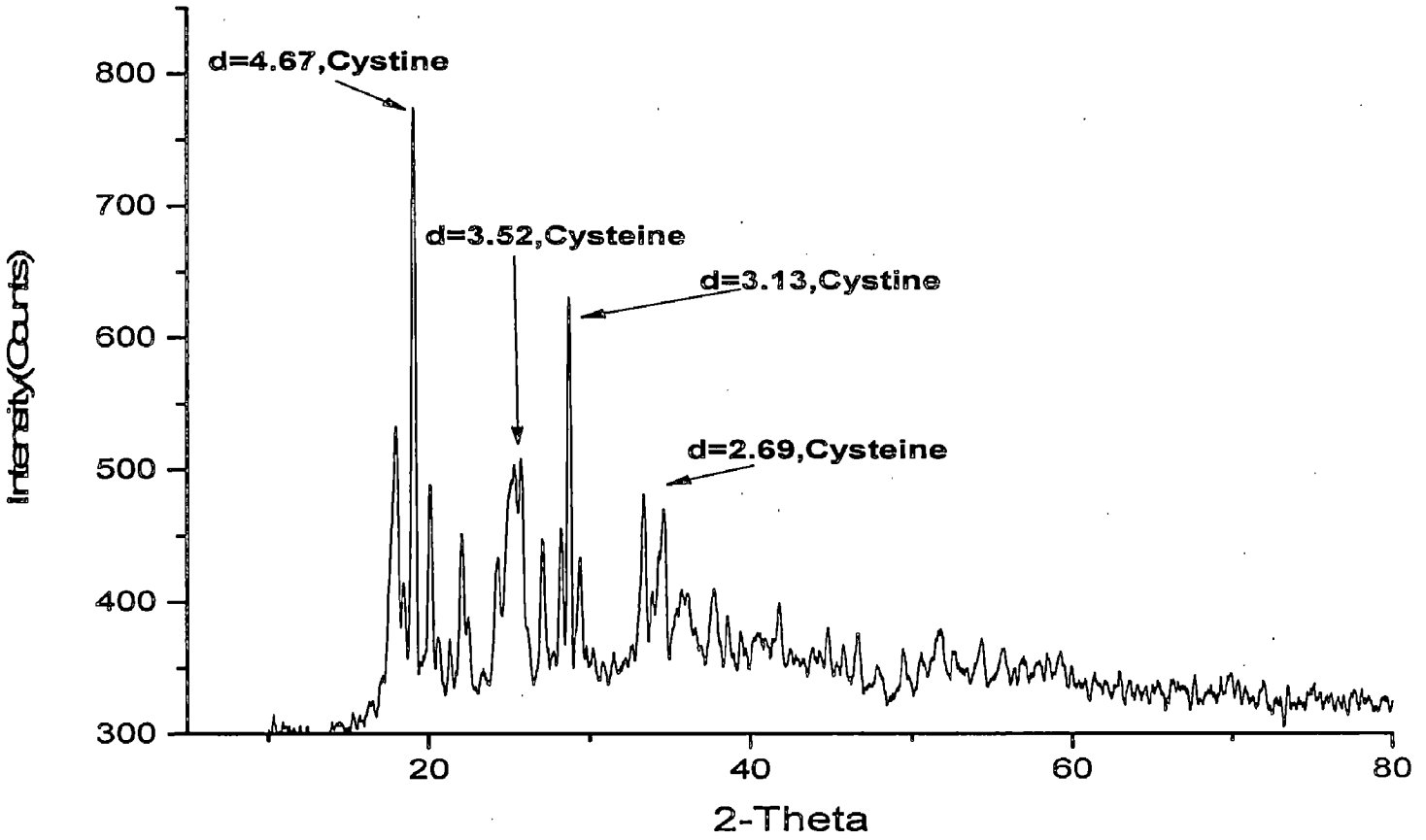

Figure -12:XRD graph of copper-ferrocyanide after adsorption of cysteine, showing the conversion of cysteine to cystine.

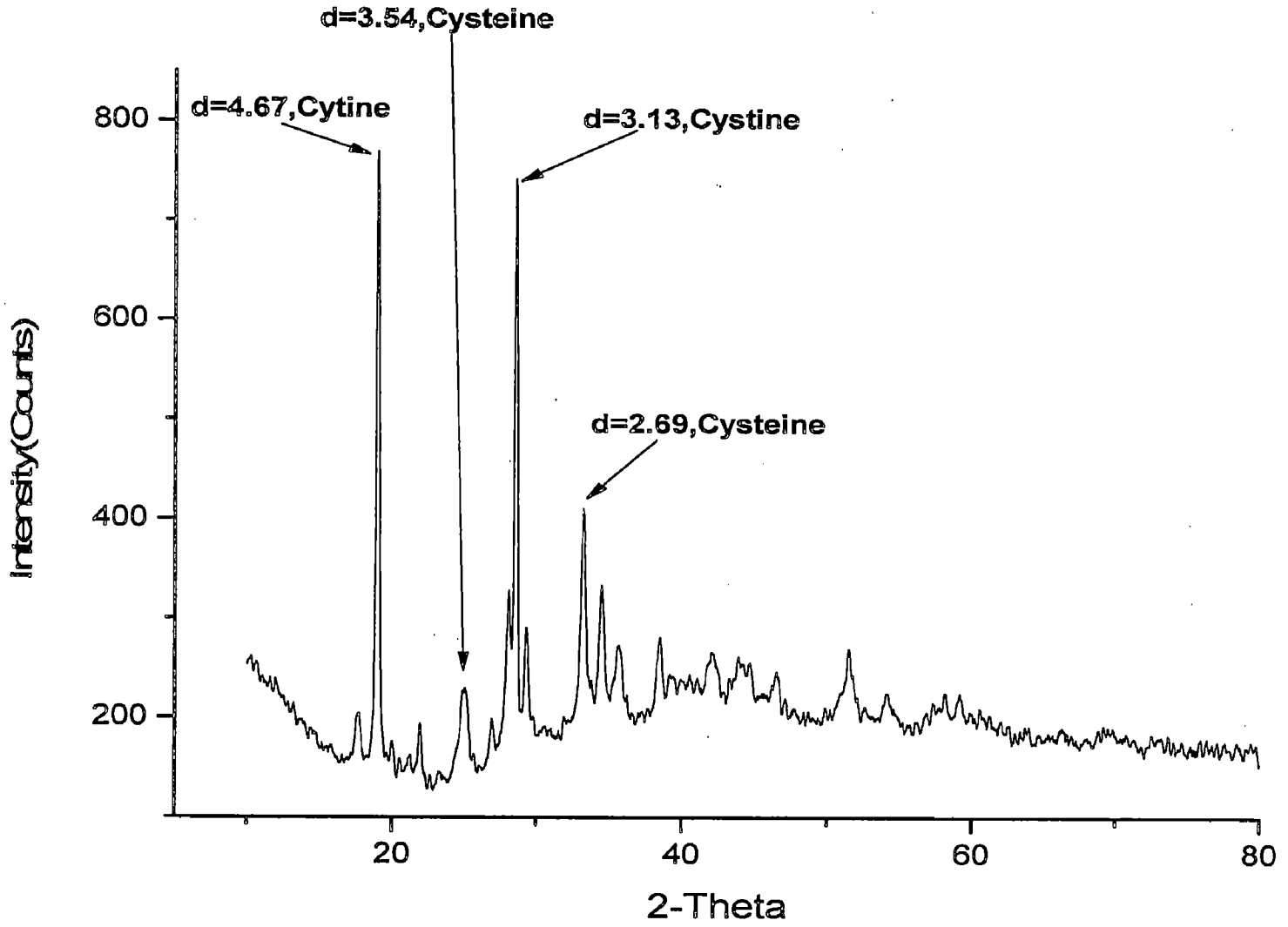

Figure -13: XRD graph of zinc-ferrocyanide after adsorption of cysteine, showing the conversion of cysteine to cystine.

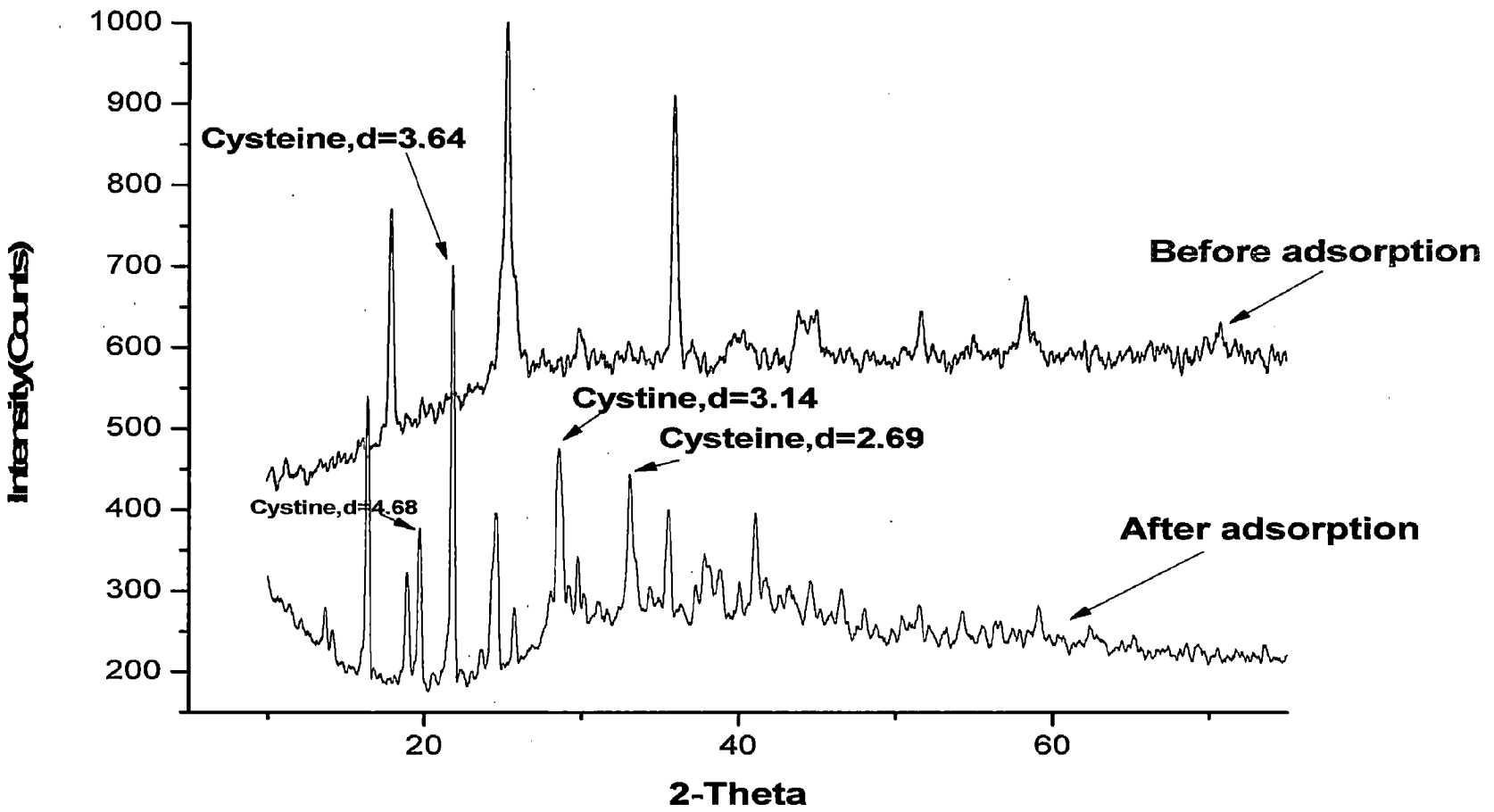

Figure-14: XRD-graph of iron-ferrocyanide before and after adsorption of cysteine, showing the conversion of cysteine to cystine.

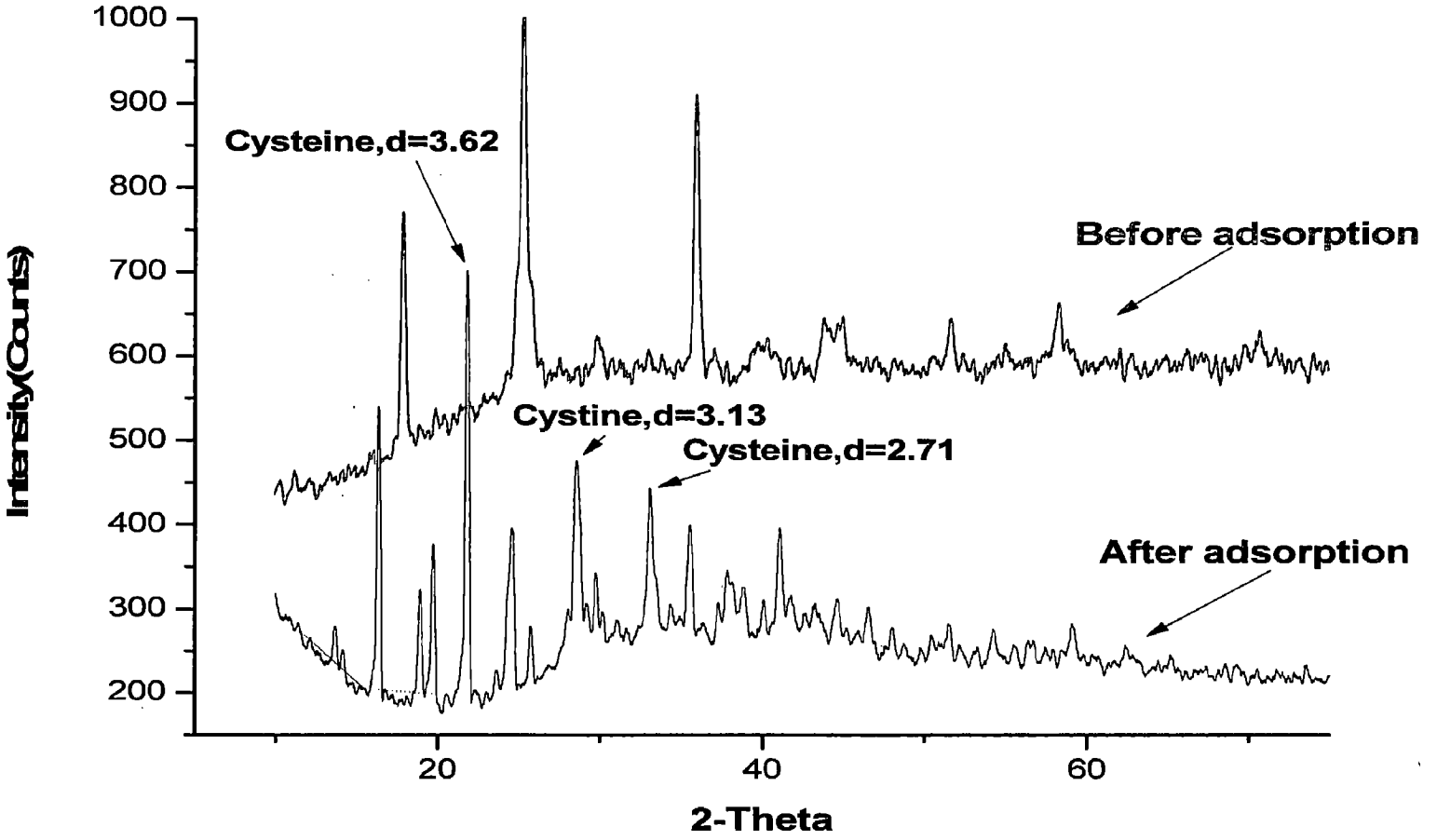

Figure-15: XRD-graph of cobalt-ferrocyanide before and after adsorption of cysteine, showing the conversion of cysteine to cystine.

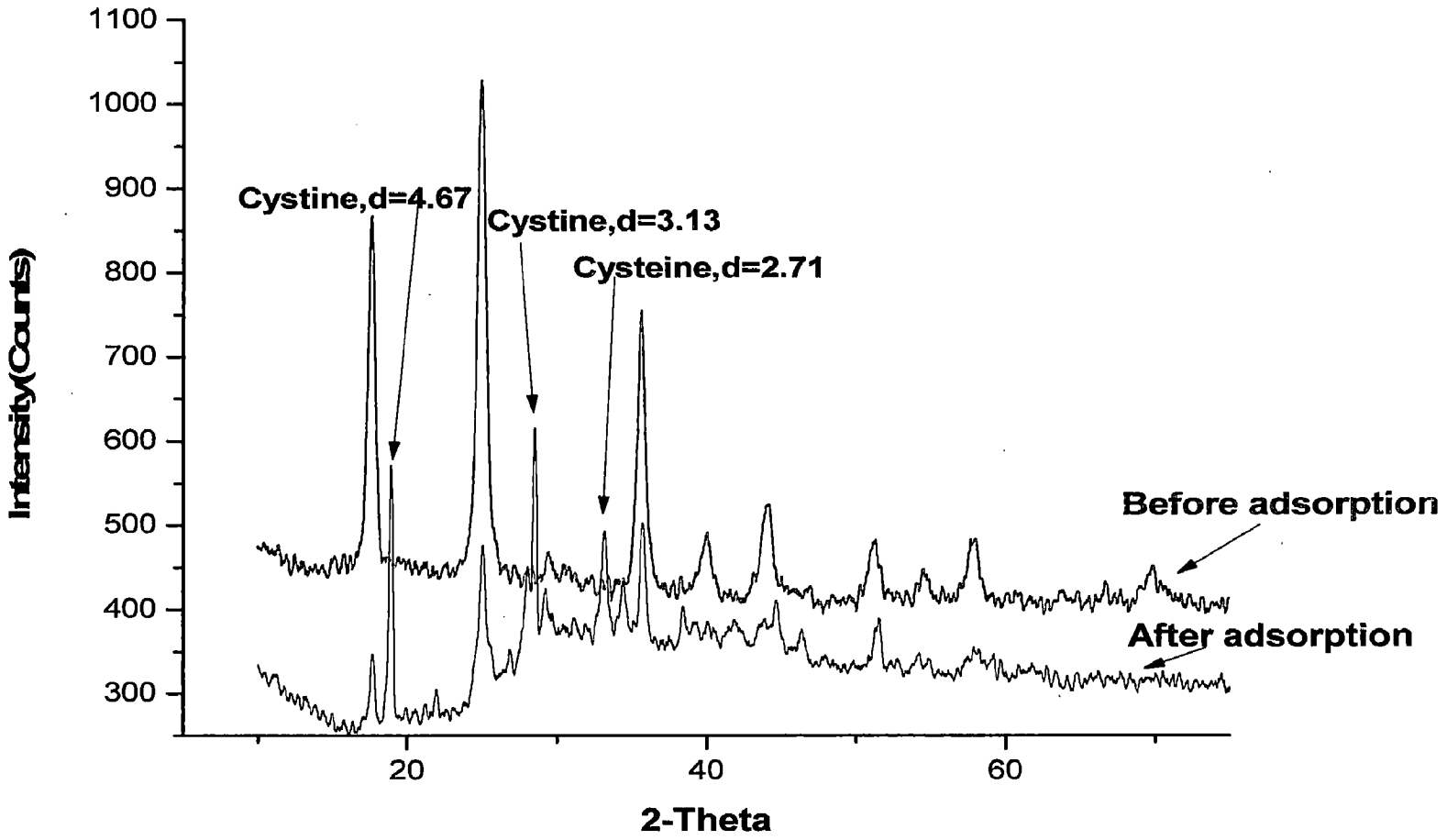

Figure-16: XRD-graph of nickel-ferrocyanide before and after adsorption of cysteine, showing the conversion of cysteine to cystine.

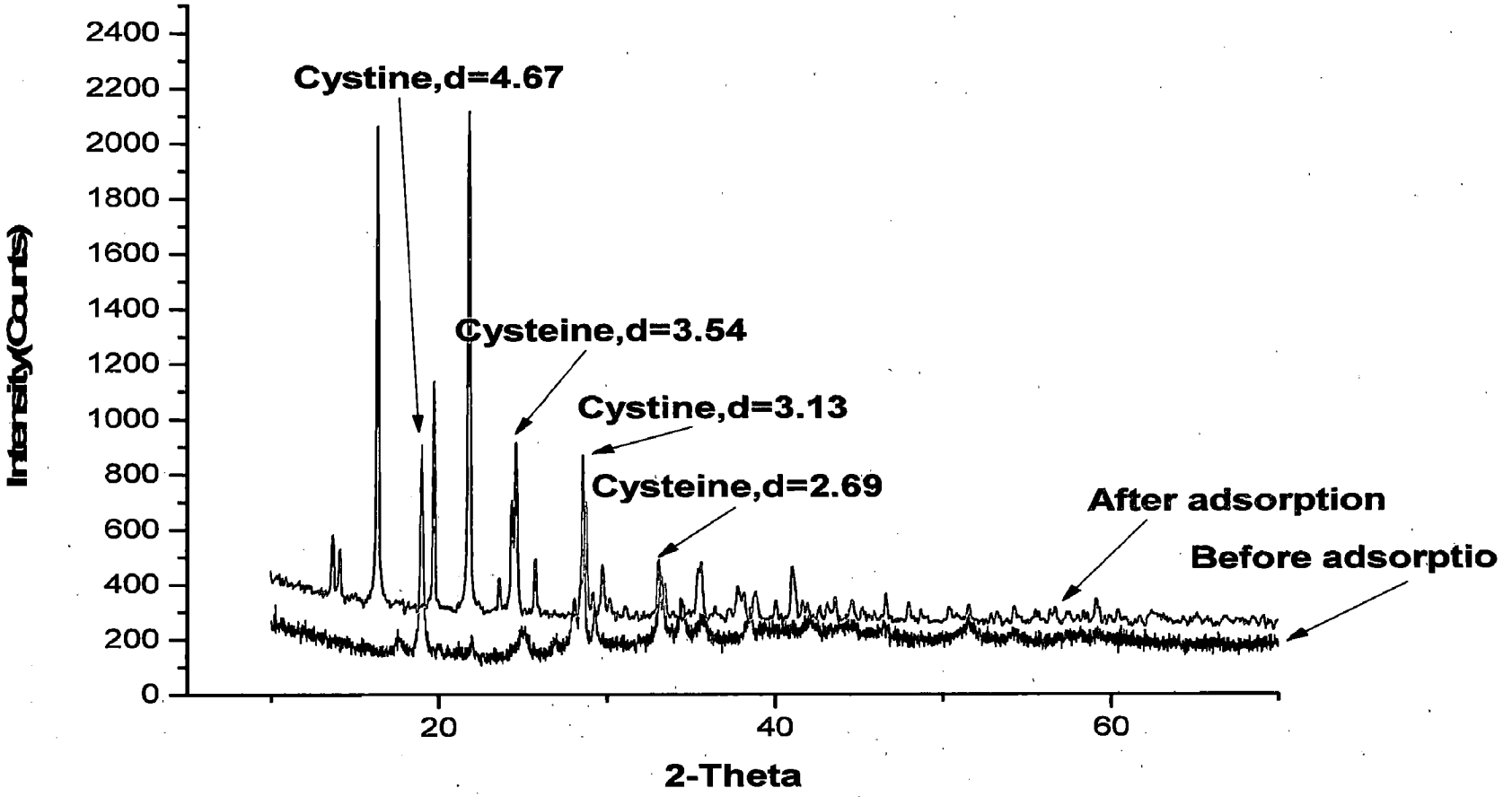

Figure-17: XRD-graph of copper-ferrocyanide before and after adsorption of cysteine, showing the conversion of cysteine to cystine.

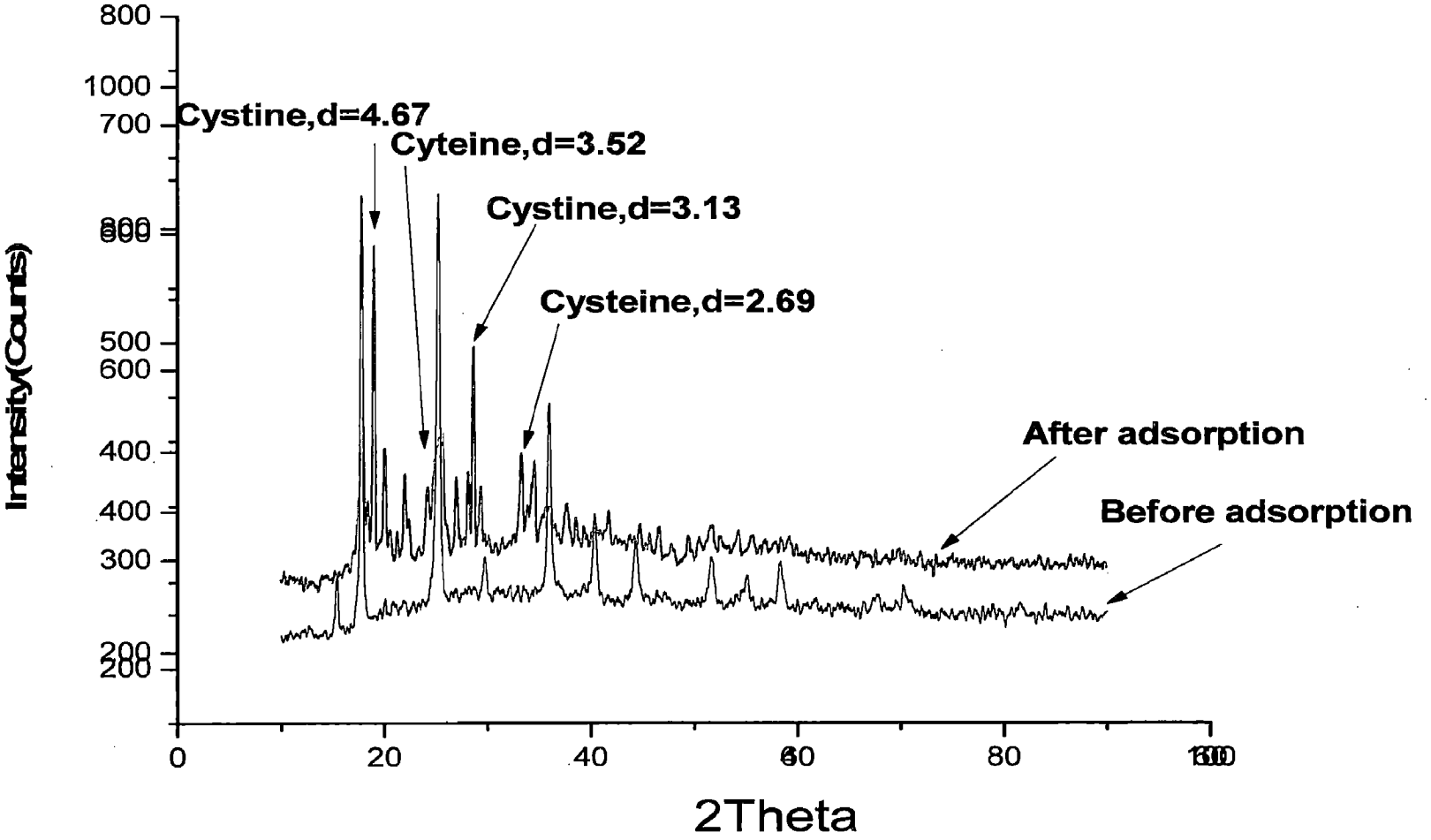

Figure-18: XRD-graph of zinc-ferrocyanide before and after adsorption of cysteine, showing the conversion of cysteine to cystine.

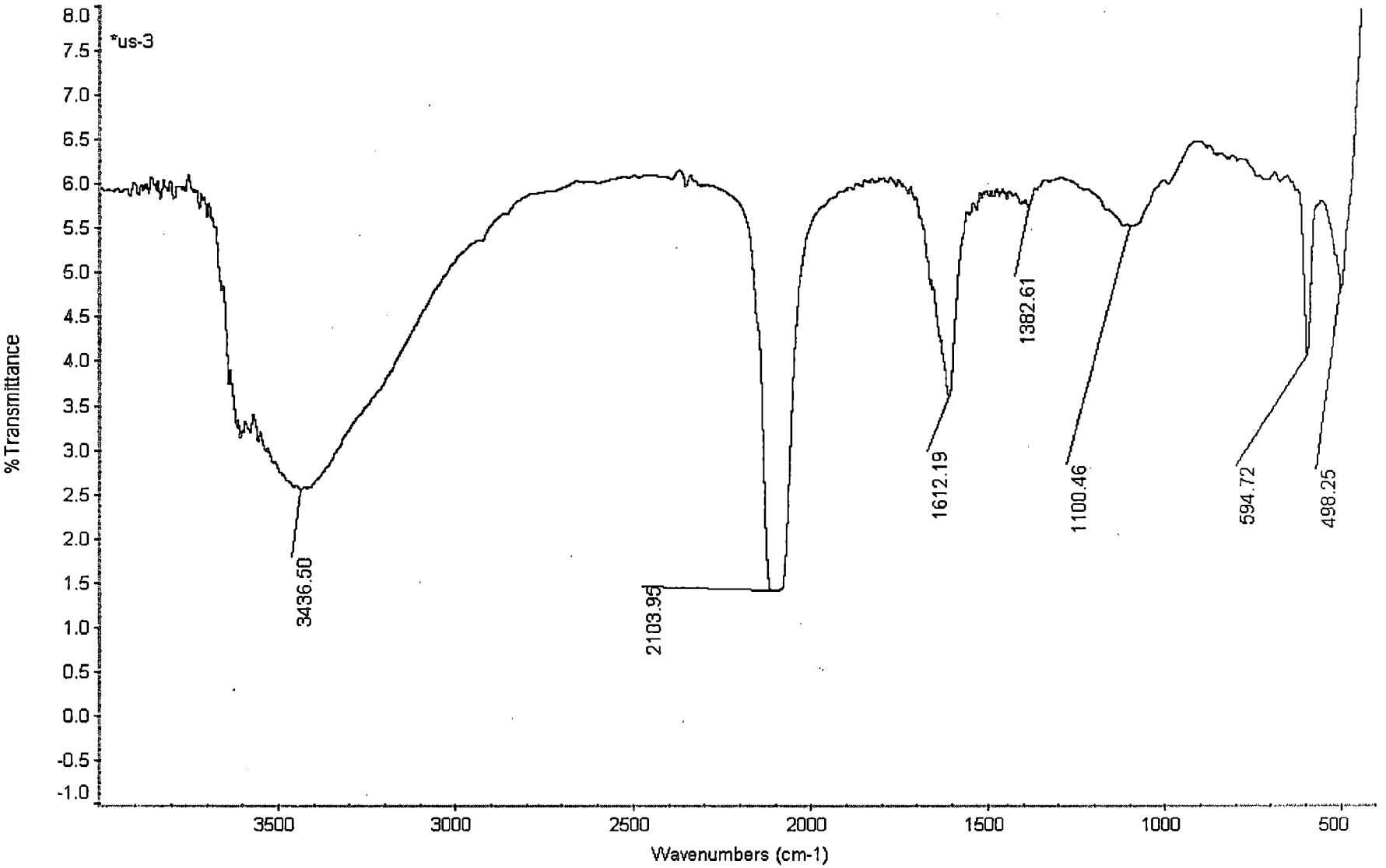

Figure -19: IR-spectrum of iron-ferrocyanide.

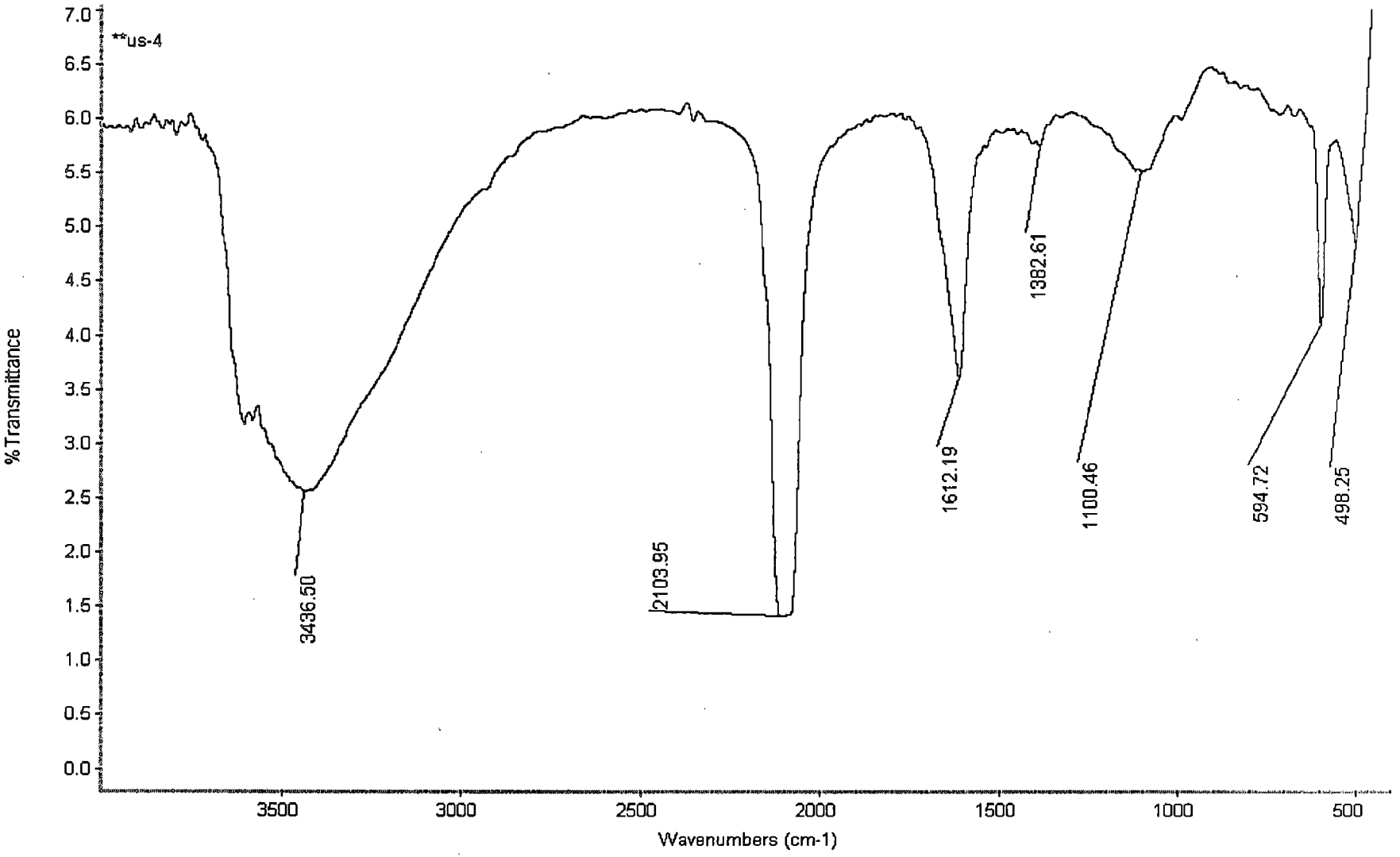

Figure -20: IR-spectrum of cobalt-ferrocyanide.

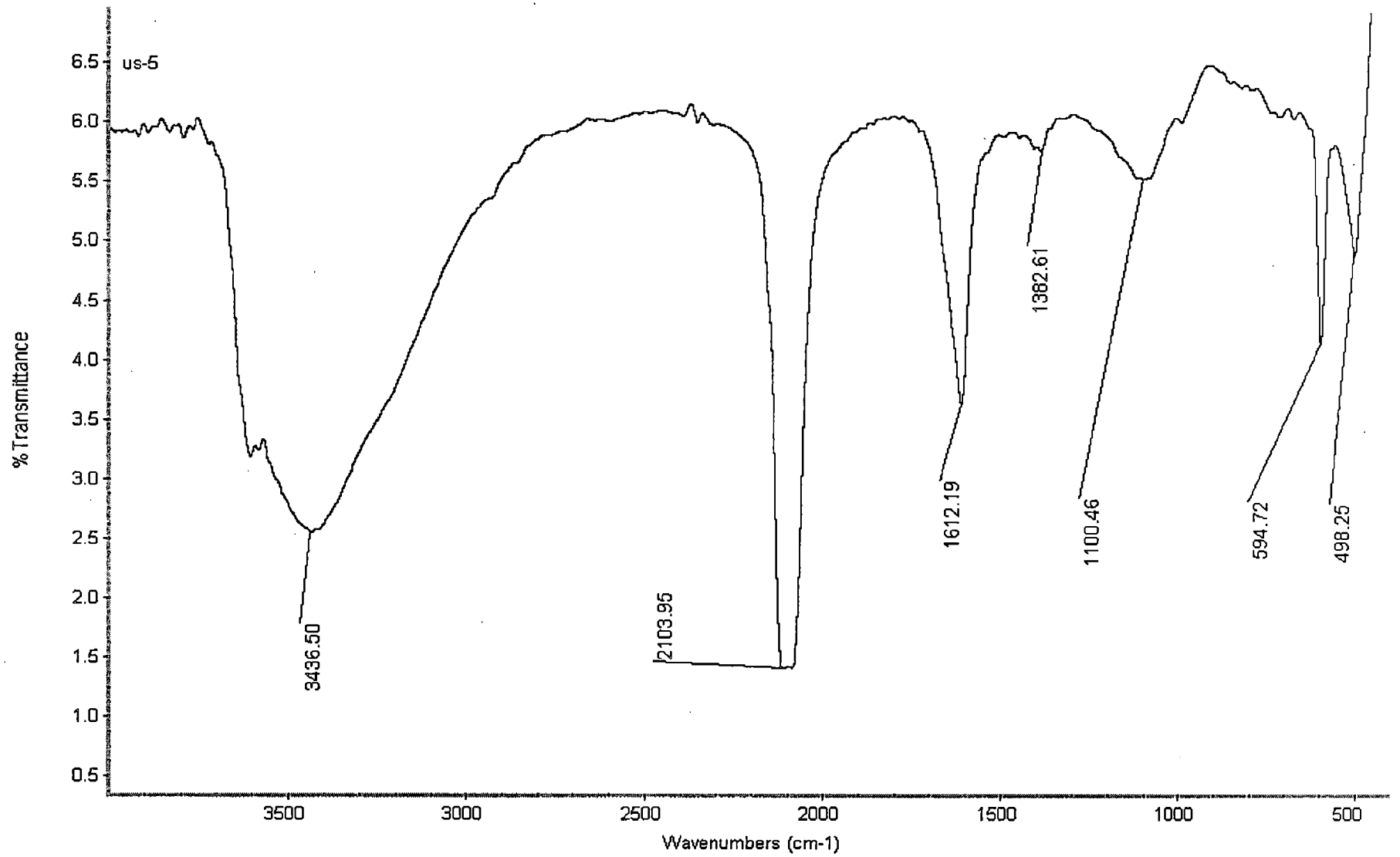

Figure -21: IR-spectrum of nickel-ferrocyanide.

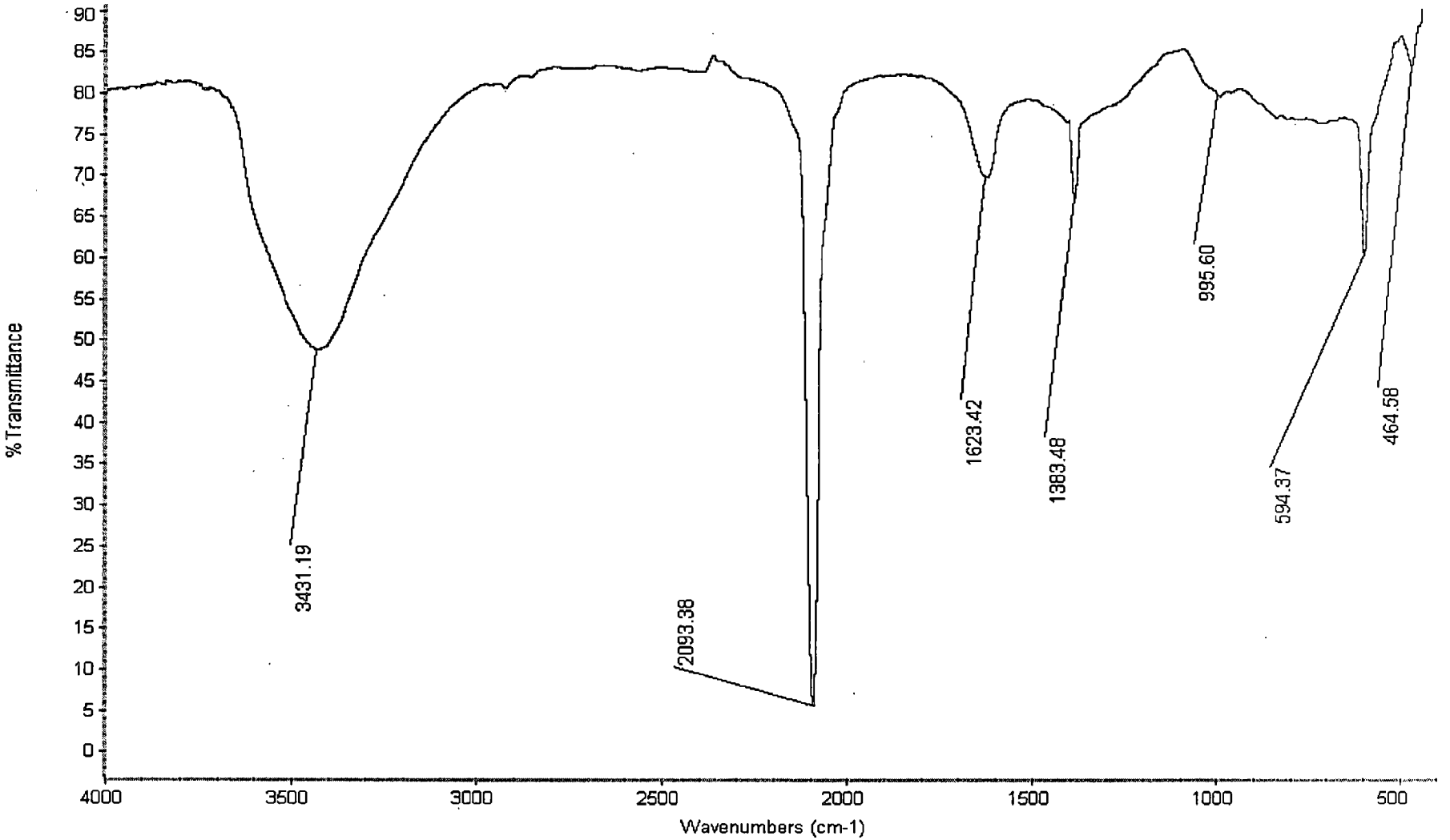

Figure-22: IR-spectrum of copper-ferrocyanide.

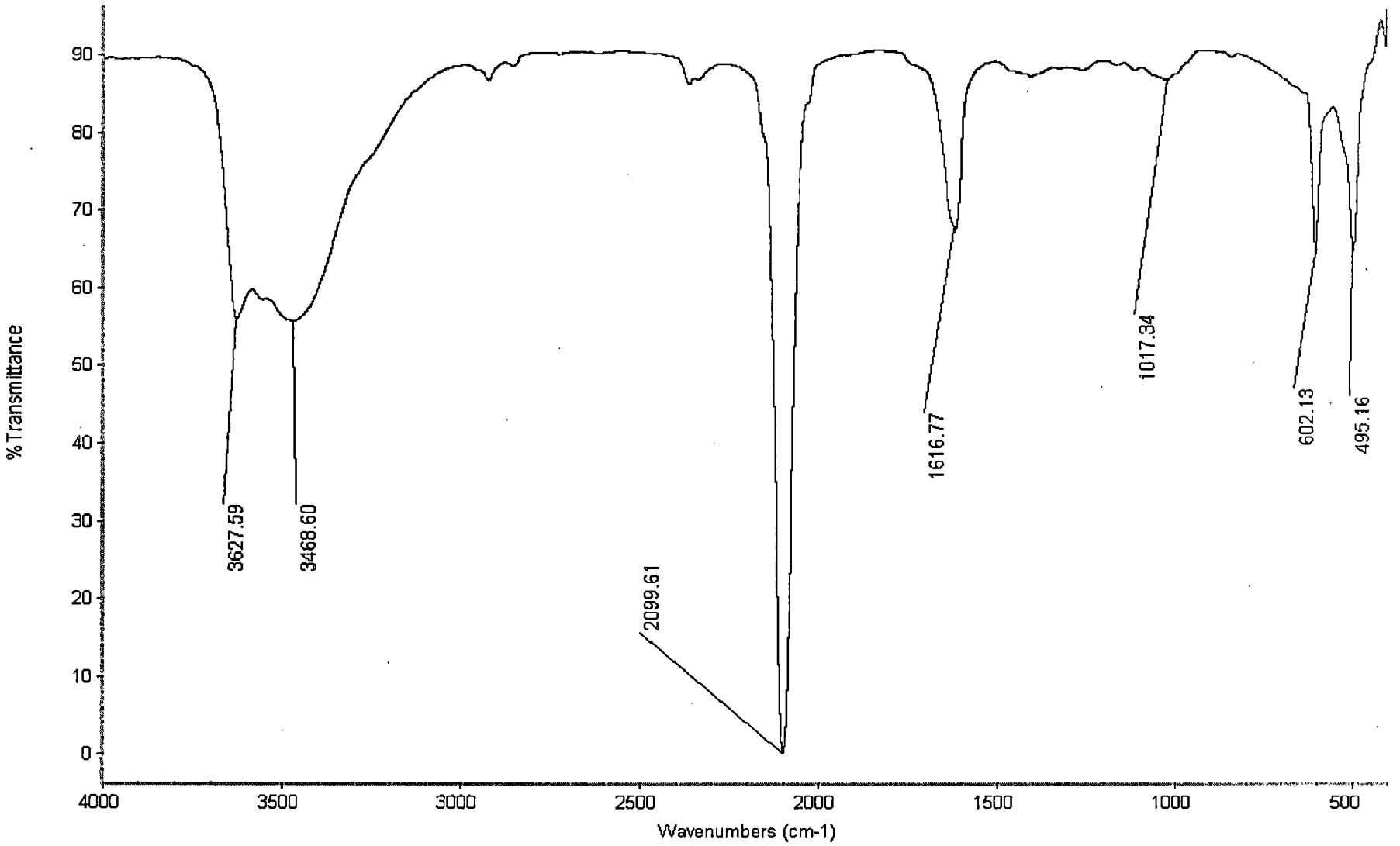

Figure -23: IR-spectrum of zinc-ferrocyanide.

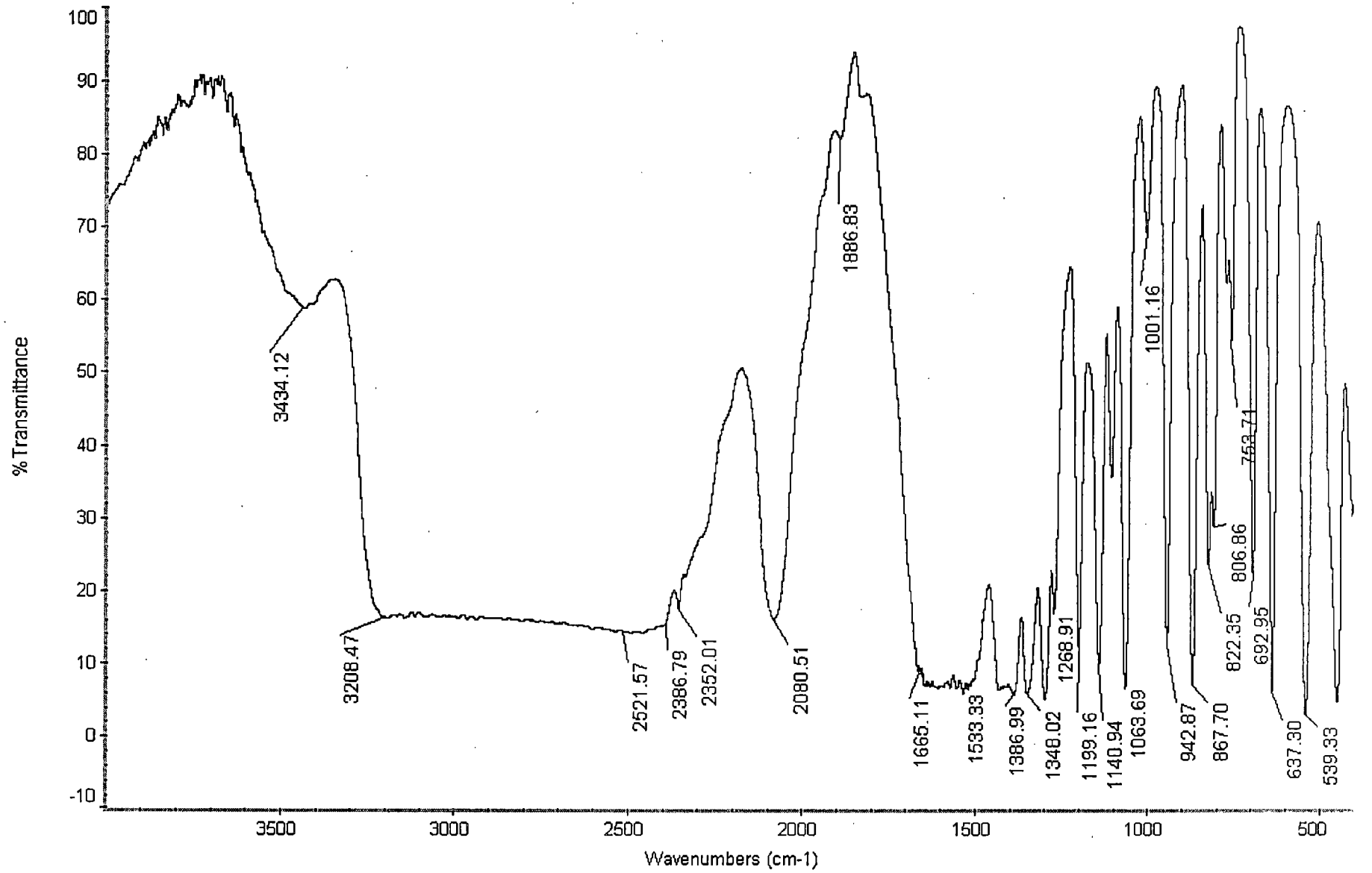

Figure -24: IR-spectrum of pure cysteine.

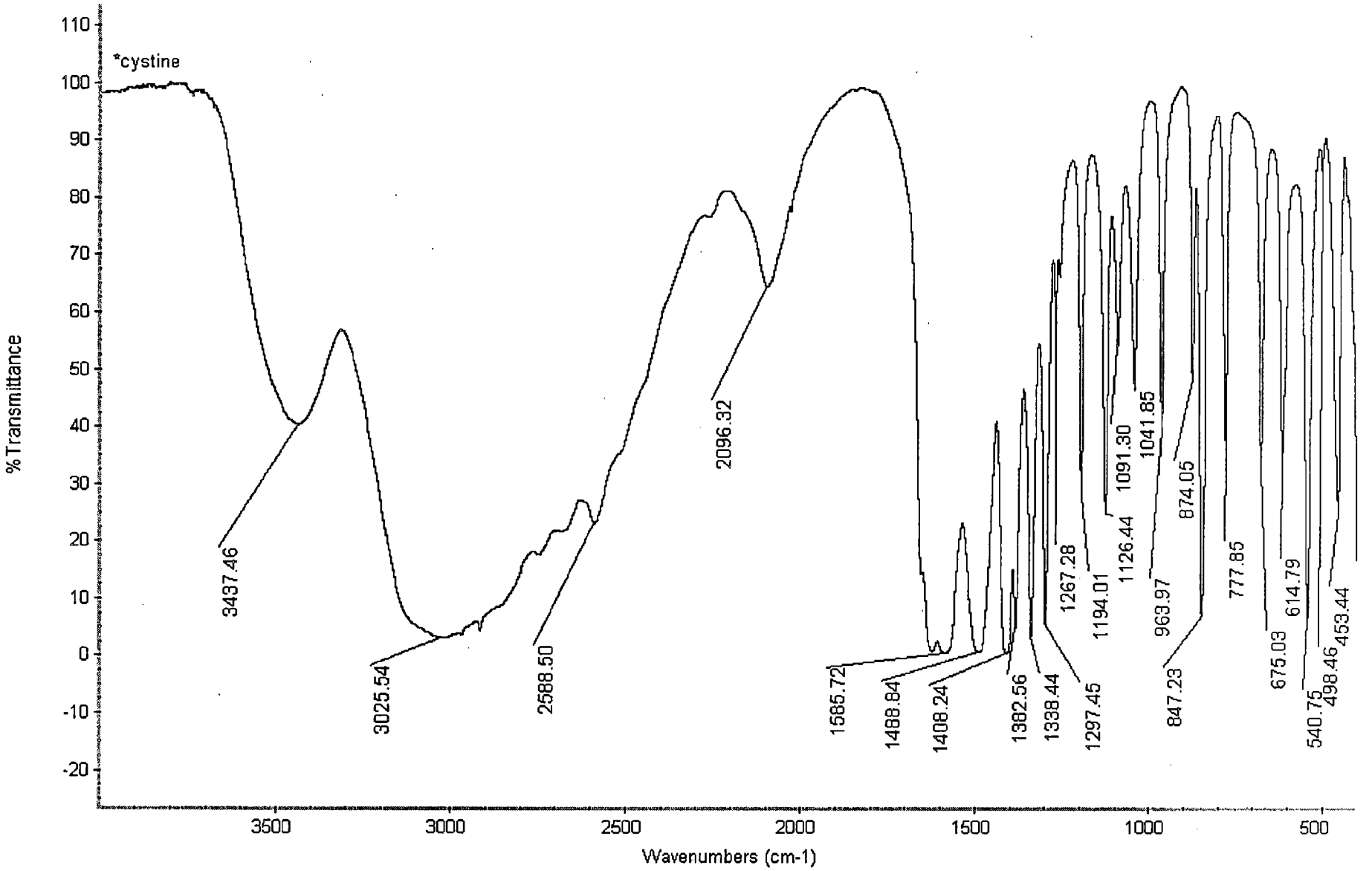

Figure -25: IR-spectrum of pure cystine.

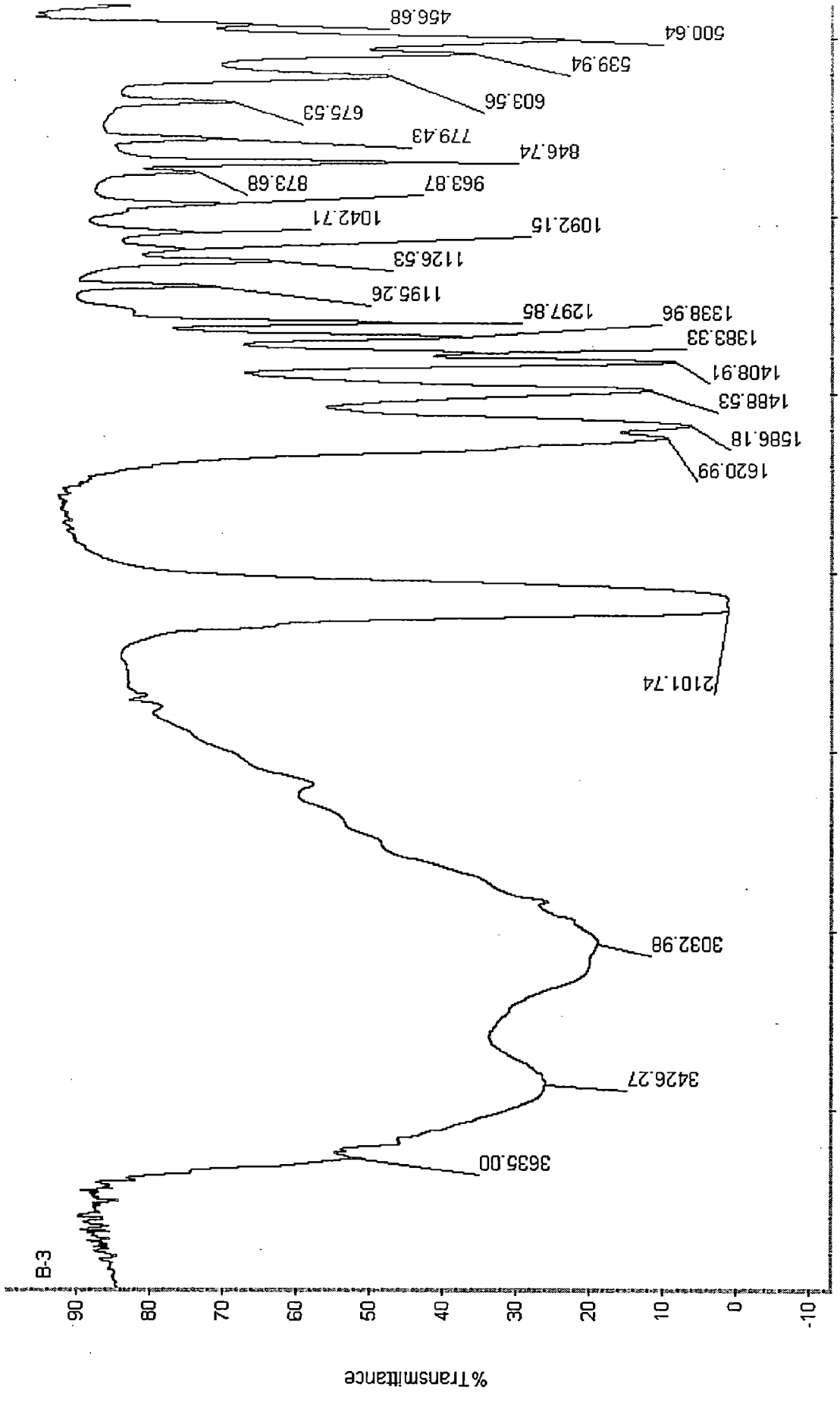

Figure -26: IR-spectrum of iron-ferrocyanide after adsorption of cysteine, showing the conversion of cysteine to cystine.

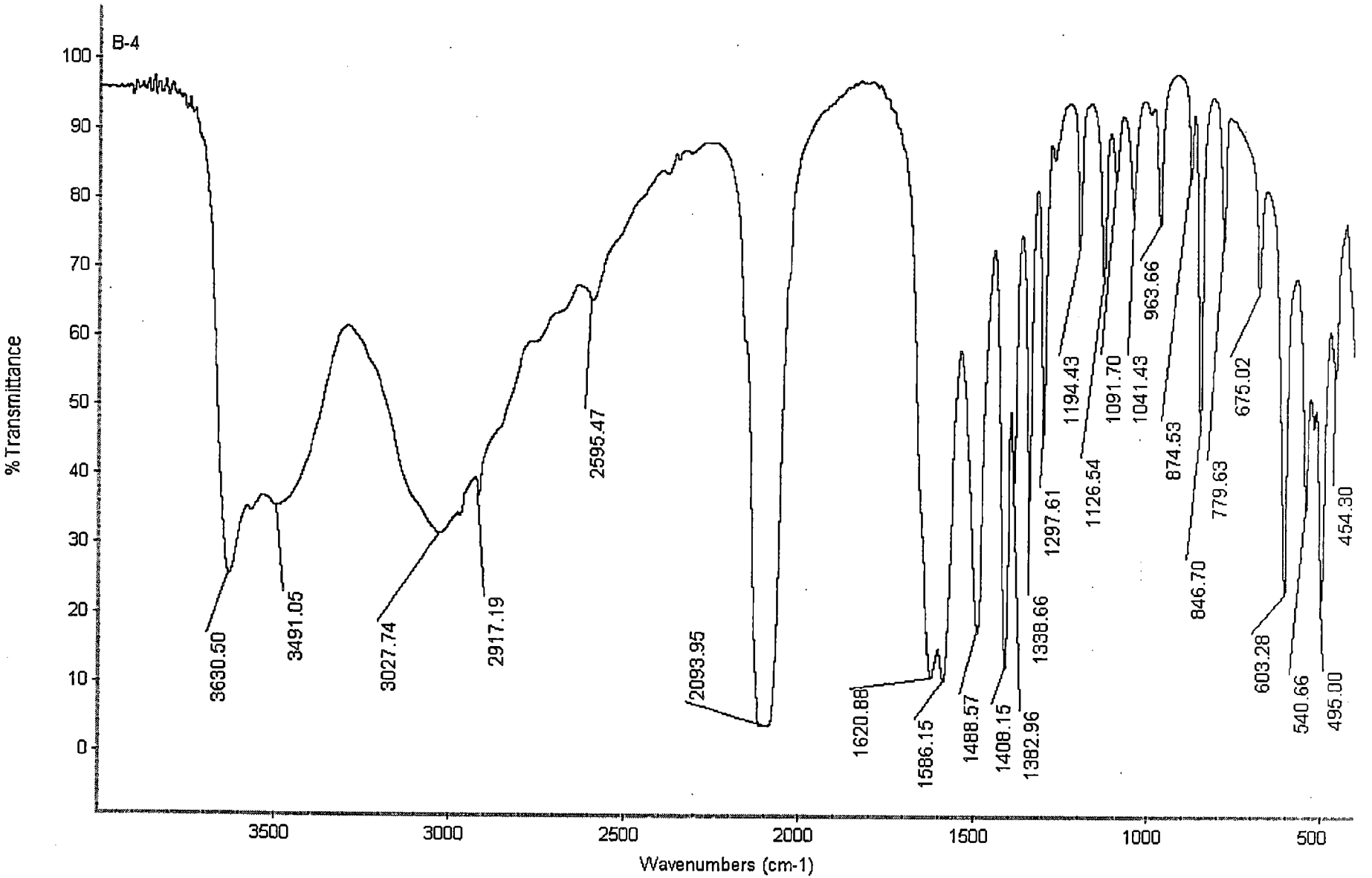

Figure -27: IR-spectrum of cobalt-ferrocyanide after adsorption of cysteine, showing the conversion of cysteine to cystine.

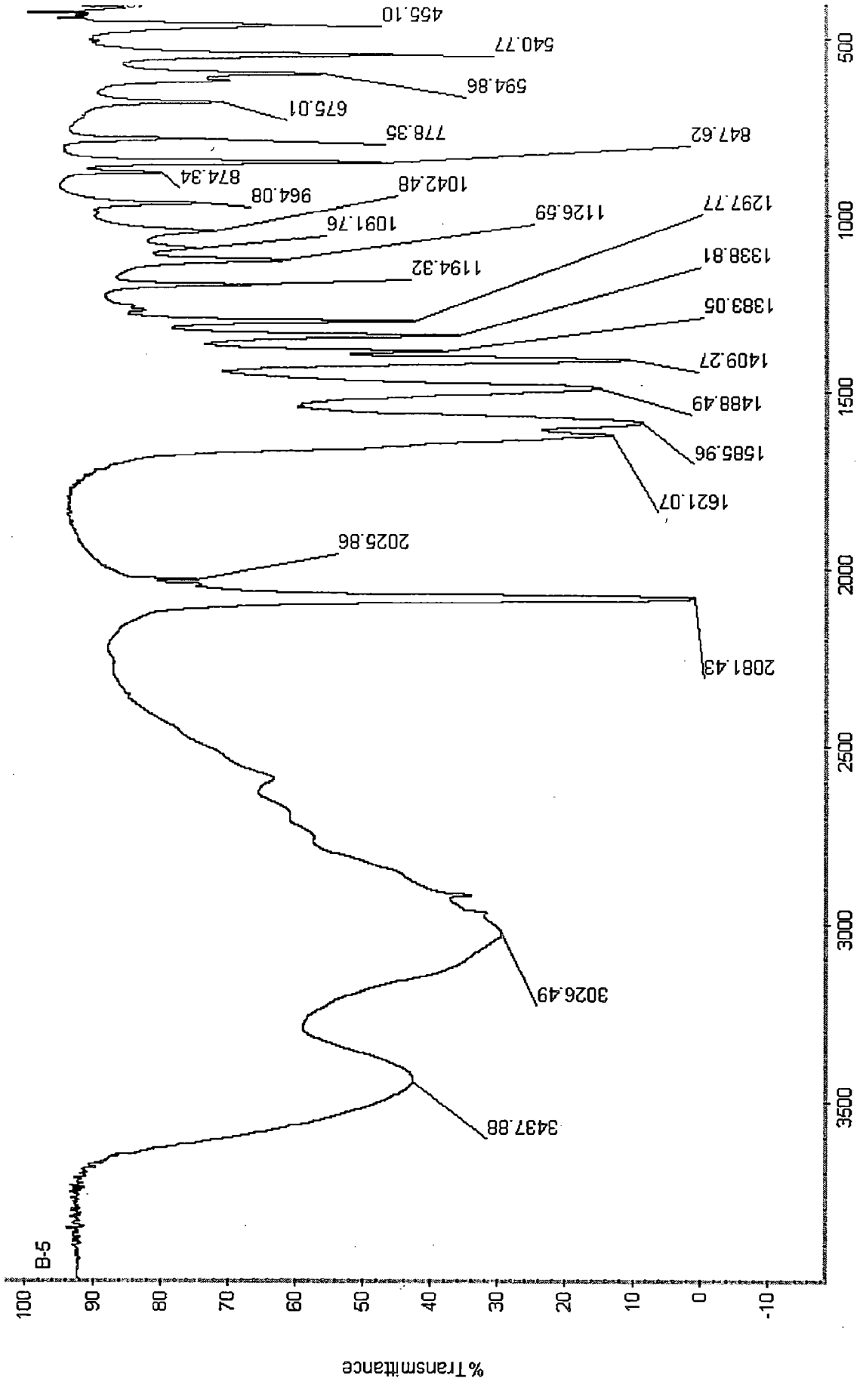

Figure -28: IR-spectrum of nickel-ferrocyanide after adsorption of cysteine, showing the conversion of cysteine to cystine.

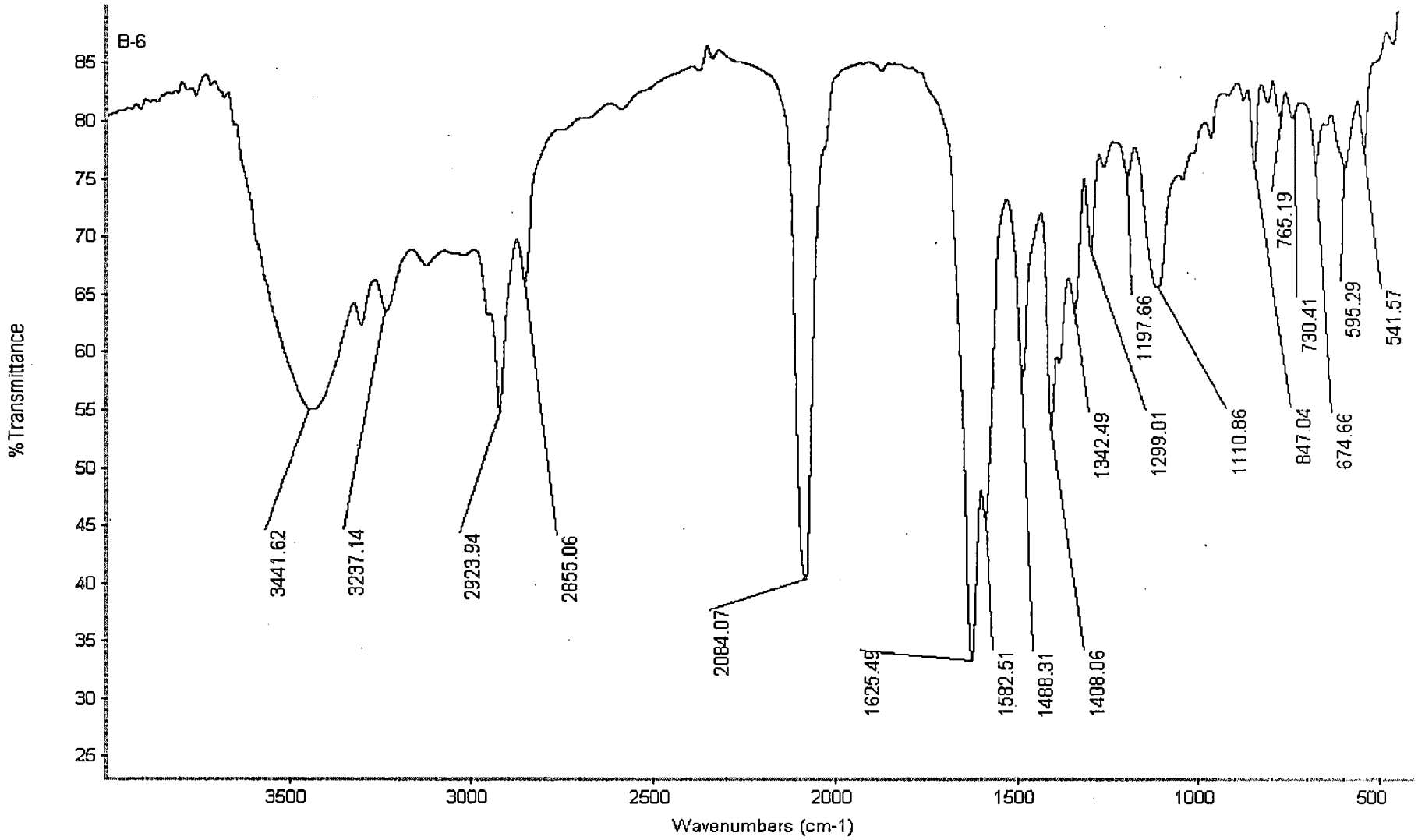

Figure -29: IR-spectrum of copper-ferrocyanide after adsorption of cysteine, showing the conversion of cysteine to cystine.

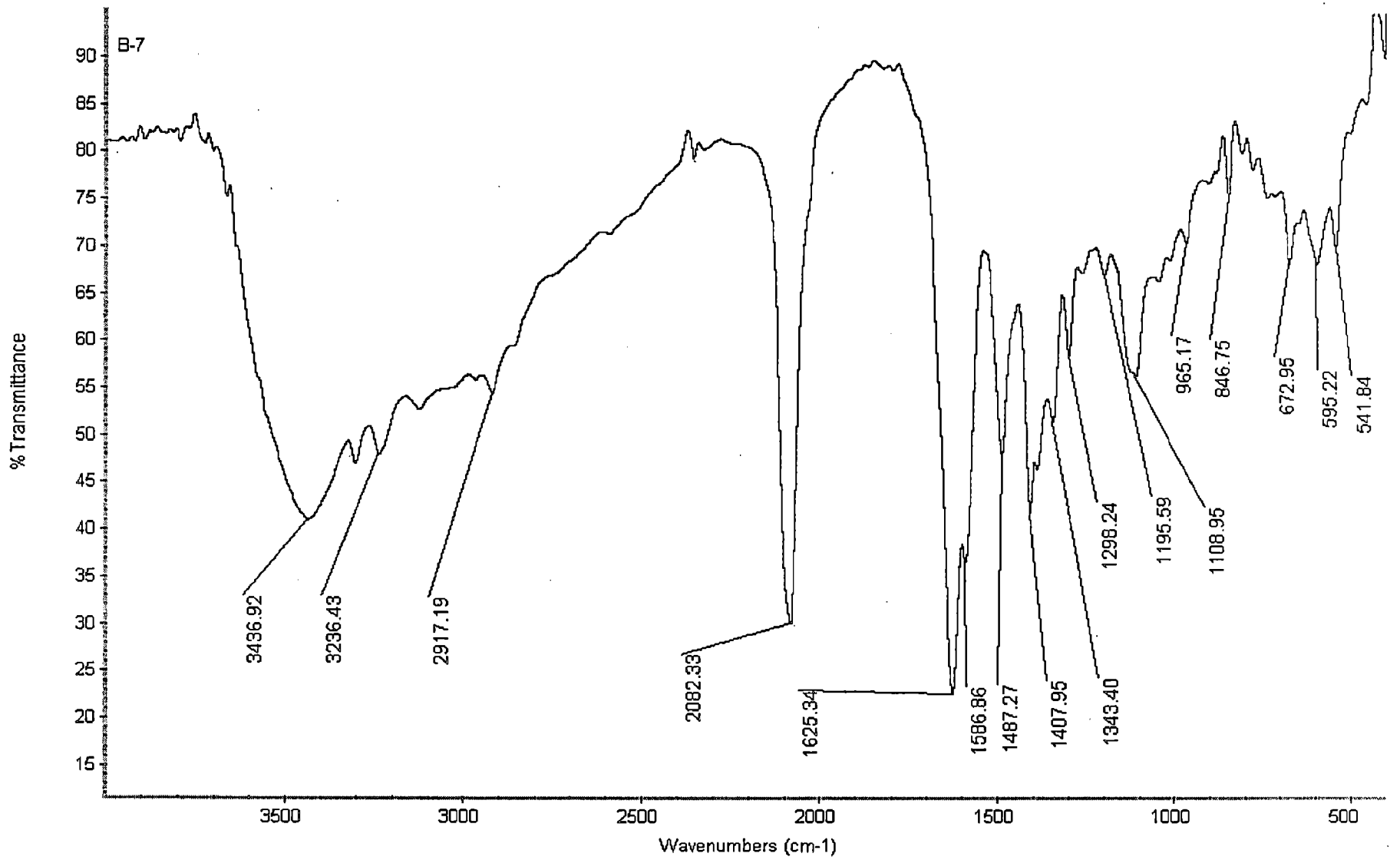

Figure -30: IR-spectrum of zinc-ferrocyanide after adsorption of cysteine, showing the conversion of cysteine to cystine.

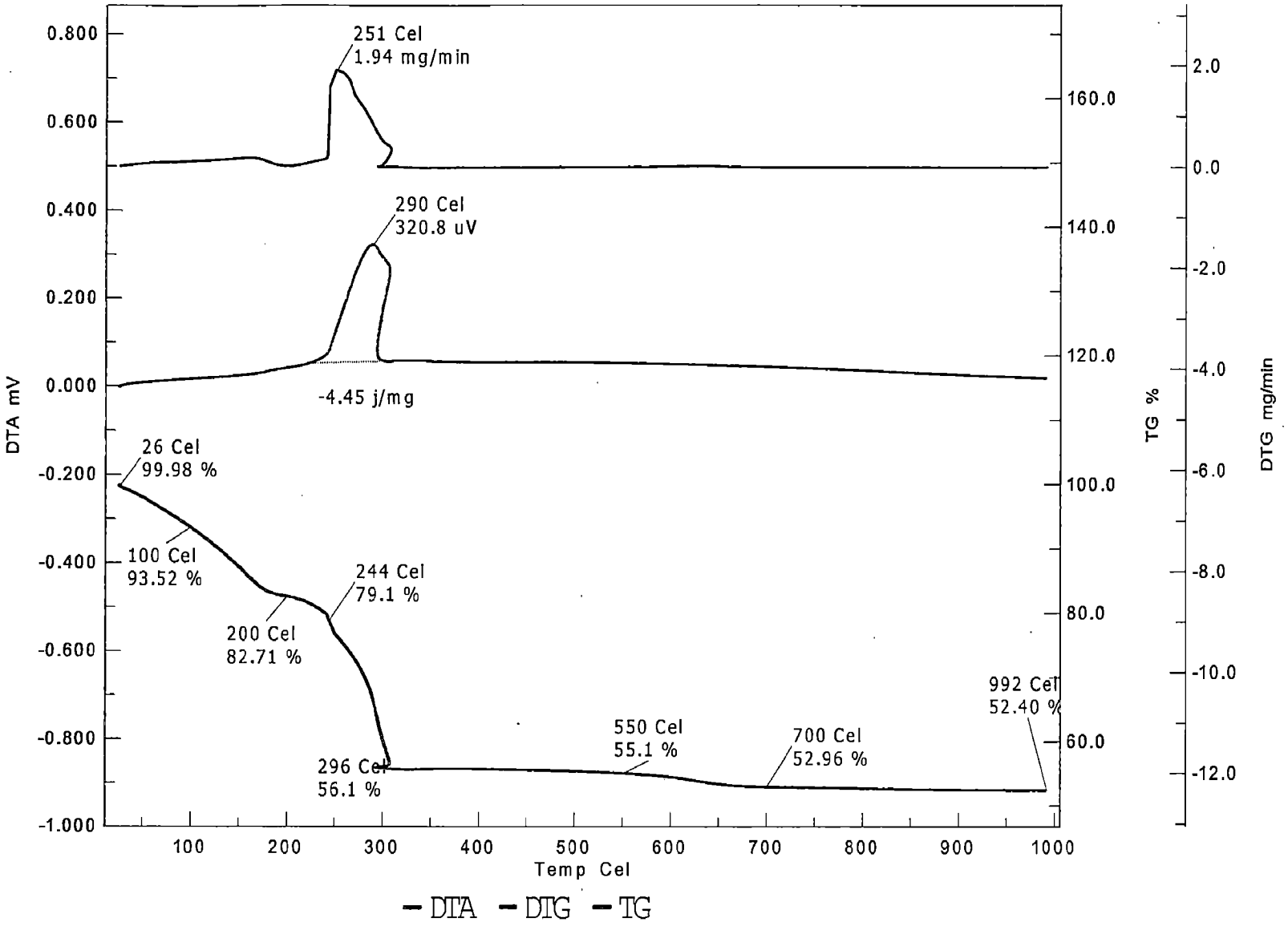

Figure -31: TGA-DTA graph of iron-ferrocyanide

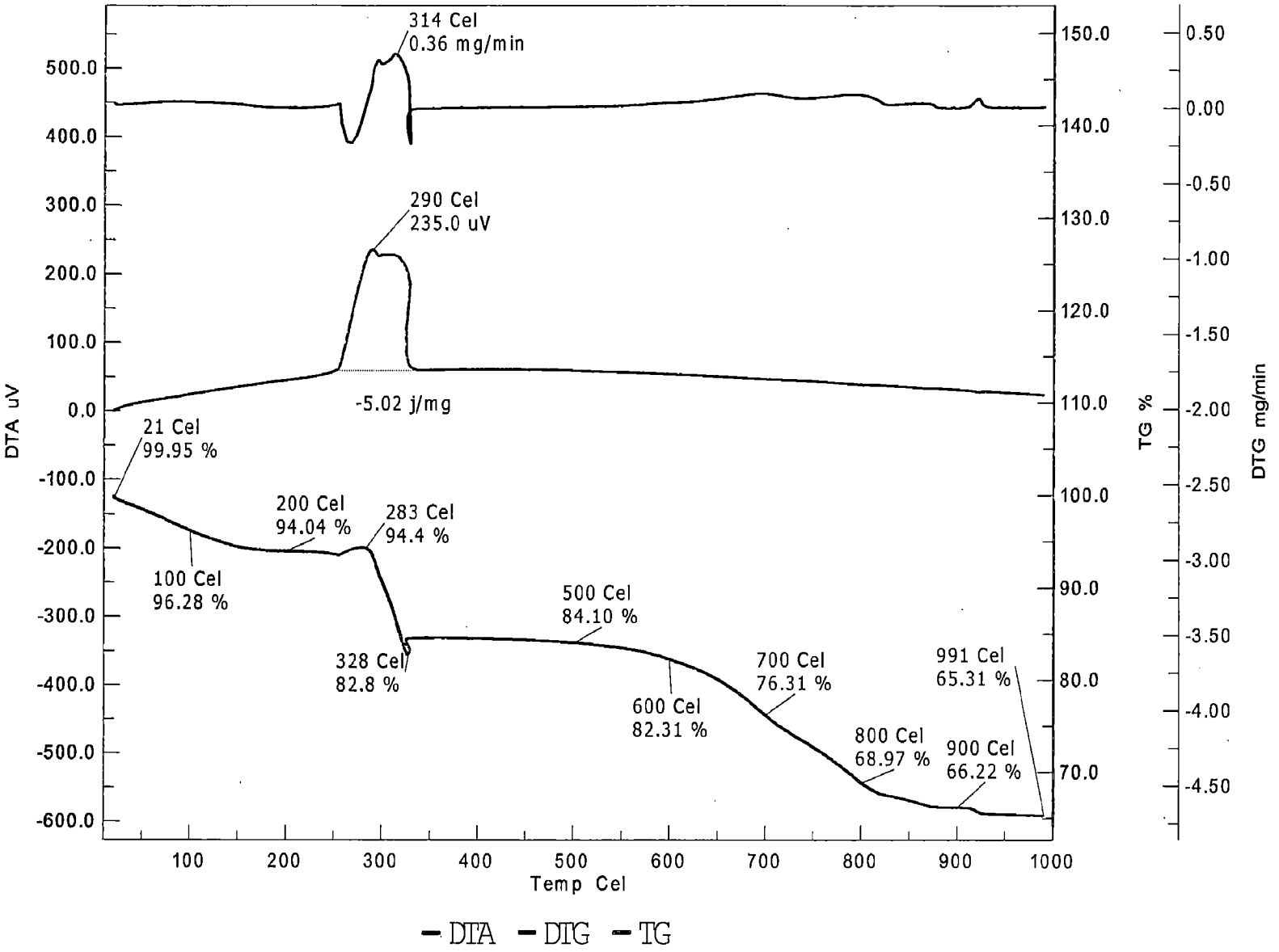

Figure -32: TGA-DTA graph of cobalt-ferrocyanide

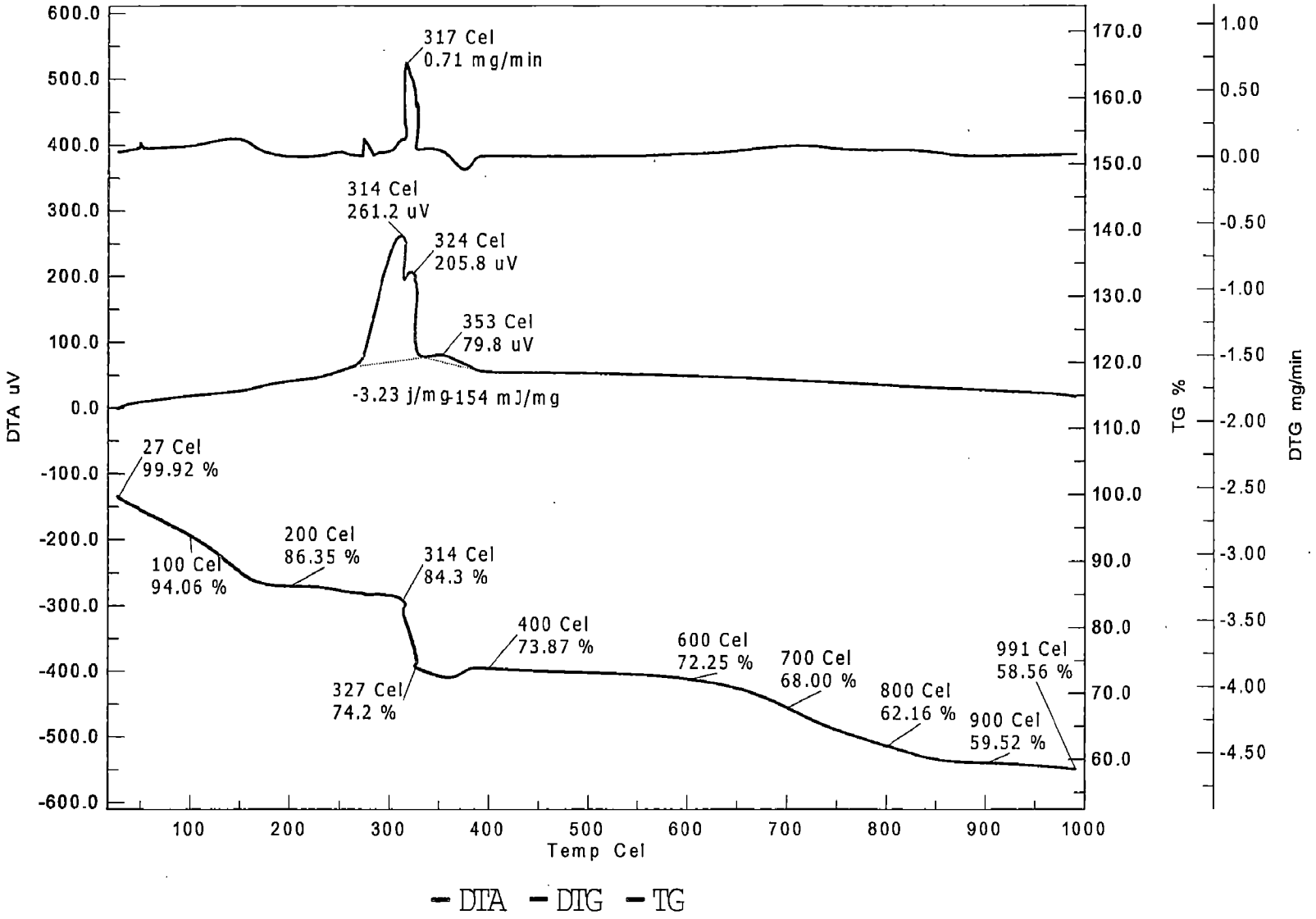

Figure -33: TGA-DTA graph of nickel-ferrocyanide

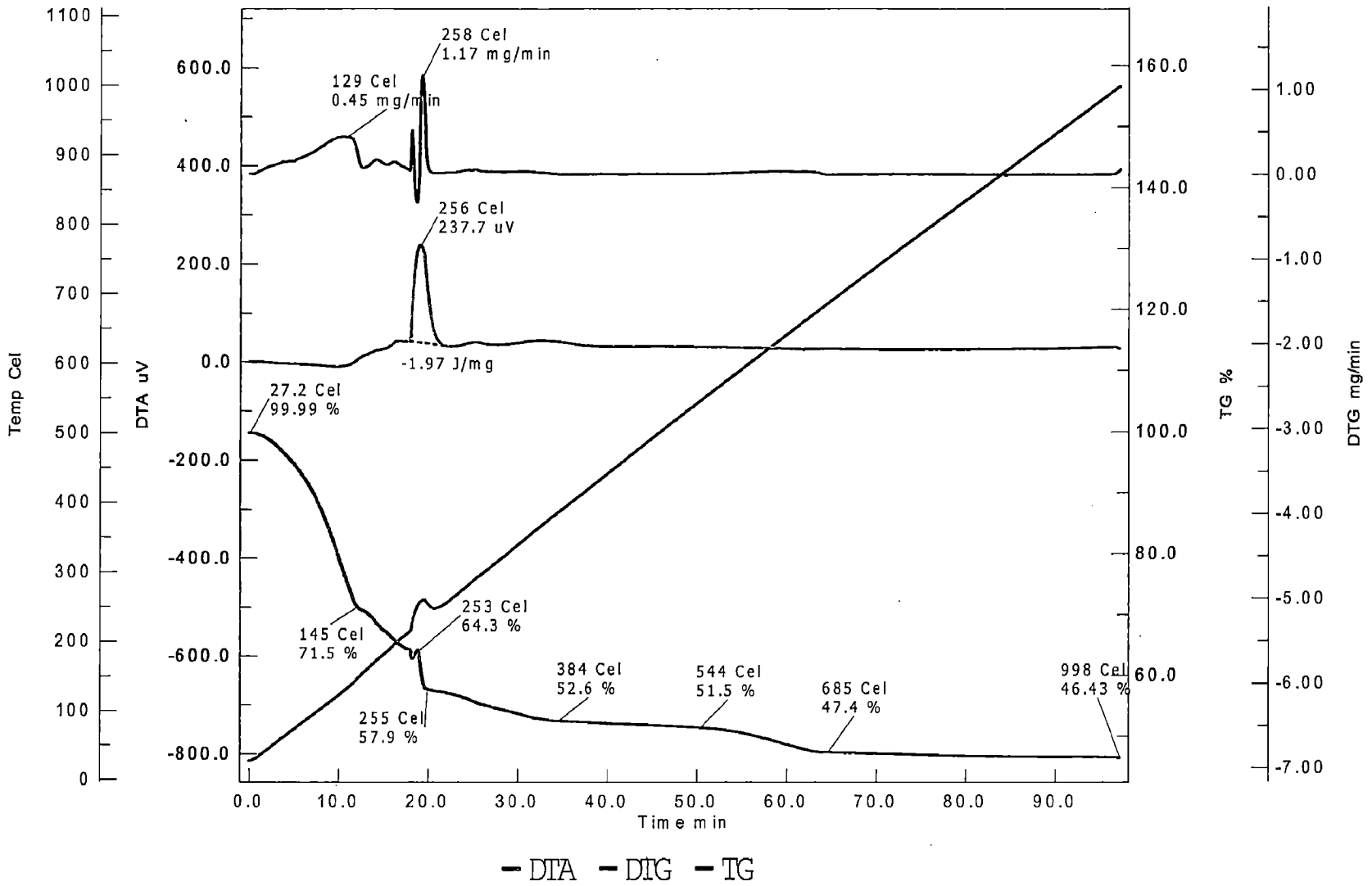

Figure -34: TGA-DTA graph of copper-ferrocyanide

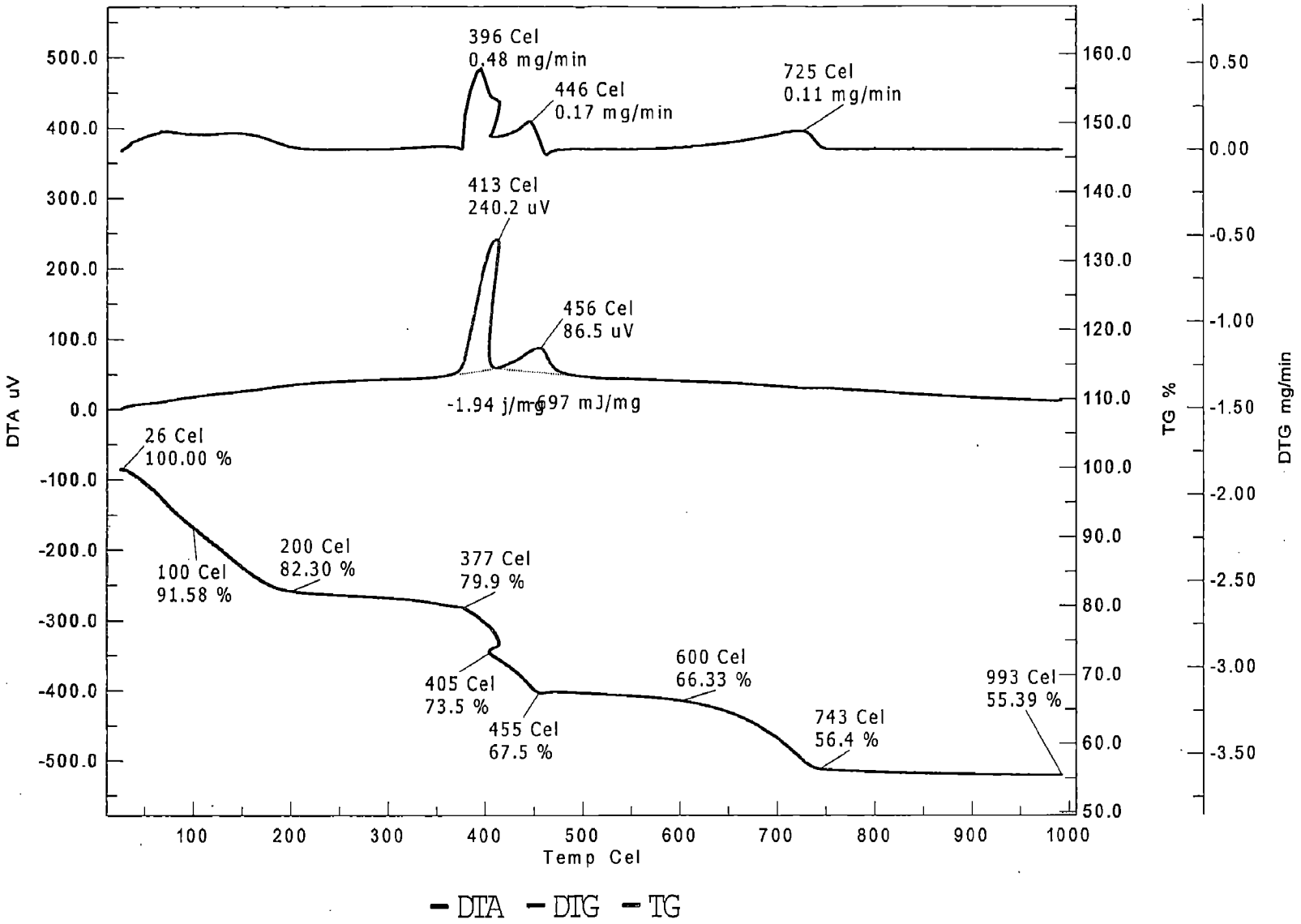

Figure -35: TGA-DTA graph of zinc-ferrocyanide