



GAMMA RADIOLYTIC DEGRADATION OF VITAMIN B₁ AND VITAMIN C

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ABSTRACT

Radiation chemistry has many novel applications. Earlier it was considered to have only degradation effect on the materials on which radiations were incident but the scenario has changed a lot now and several constructive applications are deliberated using high energy radiations. Gamma radiation sterilization method is a very clean procedure as it does not leave behind any chemical products. The heat labile compounds can be sterilized using these methods. This method of sterilization can be directly used after final packaging of products reducing the requirement of strict aseptic conditions at all stages of

production. In the present work we have focused on the radiation induced degradation of Vitamin B₁ and Vitamin C. Thiamine hydrochloride and two vitamin B₁ tablets, Beplex Forte and Neurobion Forte, were used. Ascorbic acid and two vitamin C tablets namely, Celin and Limcee, were used for the present study. The standards and tablets were irradiated in Gamma chamber in solid state as well as aqueous phase at the dose rate of ~0.3 kGy/hr and characterized by IR and NMR. Thiamine hydrochloride was irradiated in solid phase and its assay was checked spectrophotometrically. The assay of ascorbic acid was studied by iodometric titration. It was found from FTIR and NMR spectra that irradiation does not cause any change in the chemical structure. The radiation sterilization of these vitamins can be effectively carried out in solid state as decomposition of vitamins does not occur due to gamma irradiations in the solid state. However, aqueous solution of thiamine hydrochloride gets extensively degraded due to gamma irradiation. Aqueous solution of ascorbic acid is quite resistant to gamma radiation and does not undergo much radiation damage at low

dosages of gamma irradiation. Thus, vitamin C syrups can also be sterilized by low dose of gamma radiation.

KEYWORDS: Gamma radiation sterilization, Vitamin B₁, Vitamin C.

INTRODUCTION

Sterilization process is used for the benefit of human beings which includes the sterilization of medical supplies and pharmaceuticals. Gamma irradiation sterilization methods stand out, with numerous advantages. In many countries, it has been accepted as a method of sterilization and has become routine since it was introduced. With regards to pharmaceutical products, gamma irradiation sterilization has been used as the better method and records show the continuous development of the method for sterilization. Recent studies have focused on many different pharmaceutical products, active and auxiliary substances, active ingredients in drugs, new drug delivery systems and discuss the effects of radiation sterilization on the work.

Gamma irradiation is the most important method for the sterilization of pharmaceuticals, due to the high ability to penetrate the sterile packaging of pharmaceutical and cosmetic products. The fact that the final heat during the process did not increase their traceability or ability to deliver effectively is helpful for the heat-sensitive substances in packaging materials or operations. Gamma rays are also easier to control, secure, reliable and provide a fast process. The process does not require post-quarantine measures and the product obtained has no harmful effect on the environment.^[1]

Gamma-radiation is increasingly coming into use in place of conventional agents such as heat and ethylene oxide for sterilization of medical products including pharmaceutical preparations. However, before this technique becomes acceptable, it is absolutely essential to establish that radiation does not introduce toxic substances by transformation of either the active material or the medium in which it is present during radiation sterilization. Many pharmaceutical preparations e.g. vitamins and antibiotics are often administered in dilute aqueous media, such as syrups and suspensions. In such systems, the ionising radiation interacts almost exclusively with water to give hydrated electrons, hydrogen atoms and hydroxyl radicals and also molecular hydrogen and hydrogen peroxide. Of these, the first three are very reactive towards many functional groups present in organic molecules that constitute the active component of the pharmaceutical. Hydrogen peroxide, being capable of

acting both as an oxidising and reducing agent, can cause damage to the active component. Even in the solid state a compound can undergo radiation damage as a result of ionisation and excitation events resulting from exposure to the ionizing radiation. Therefore, during radiation sterilization, pharmaceutical preparations may not only lose their potency wholly or partly but also new substances would be produced whose effect on the organism could be entirely different from that of the parent. It is therefore very essential to first assess the extent of radiation damage to the active component and identify the products formed. Secondly, knowing the reaction pathways that lead to the damage, it should be possible, by the addition of suitable chemicals or otherwise, to find out ways and means to minimise such radiolytic transformations.^[2]

Gamma radiation is included in the sterilization process (BP 1963 (British Pharmacopoeia) and USP XVII (U.S. Pharmacopoeia)) and the proposed dose of sterilization is 25 kGy. However, the level of sterility in some products requires doses of 1–15 kGy due to the microbial load (bioburden).^[3]

Radiolysis of water soluble vitamins

A vitamin is an organic compound and a vital nutrient that an organism requires in limited amounts. An organic chemical compound (or related set of compounds) is called a vitamin when the organism cannot synthesize the compound in sufficient quantities and it must be obtained through diet. Thirteen vitamins are universally recognized at present. Vitamins are classified by their biological and chemical activity and not by their structure. Thus, each vitamin refers to a number of vitamers compounds that all show the biological activity associated with a particular vitamin. The vitamins are also grouped according to their solubility as fat soluble vitamins and water soluble vitamins. The fat soluble vitamins are vitamin A, D, E and K while the water soluble vitamins are vitamins of B complex group and vitamin C.

Vitamin B includes different vitamins such as Vitamin B₁ (Thiamine), Vitamin B₂ (Riboflavin), Vitamin B₃ (Niacine), Vitamin B₅ (Pantothenic acid), Vitamin B₆ (Pyridoxine), Vitamin B₉ (Folic acid) and Vitamin B₁₂ (Cyanocobalamin). In the present work we have focused on the radiolysis of water soluble vitamins.

Thiamine is an essential nutrient required by all tissues, including the brain. The human body itself cannot produce thiamine but must ingest it with the diet. Thiamine derivatives and

thiamine-dependent enzymes are present in all cells of the body. Thus, a thiamine deficiency would seem to adversely affect all of the organ systems. However, the nervous system is particularly sensitive to thiamine deficiency, because of its dependence on oxidative metabolism. Well-known syndromes caused by thiamine deficiency include beriberi, Wernicke-Korsakoff syndrome and optic neuropathy.

Colovos and Churchill^[4] observed thiamine, riboflavin, pyridoxine, nicotinamide and two multivitamin preparations containing Ca-pantothenate to be strong enough to be sterilised by electron irradiation. Rhee^[5] et al. studied effects of gamma irradiation on an argon-saturated solution of thiamine and found that radiolysis products increased with increasing thiamine concentration. Kishore^[6] et al. studied the radiation damage and protection of some water soluble vitamins in aqueous media. Kishore^[7-9] et al. also studied the extent of radiolytic decomposition of the B-group vitamins, thiamine, riboflavin, pyridoxin, nicotinamide, pantothenic acid and folic acid in aqueous media as a function of γ -dose both in the absence and presence of glucose as a protective additive. Thiamine hydrochloride, riboflavin, calcium pantothenate, nicotinamide, pyridoxal HCl and cyanocobalamin decay up to 10 kGy was observed in a study by Jeszka.^[10]

Ascorbic acid (vitamin C) is a water-soluble micronutrient required for multiple biological functions. Ascorbic acid is a cofactor for several enzymes participating in the post-translational hydroxylation of collagen, in the biosynthesis of carnitine, in the conversion of the neurotransmitter dopamine to norepinephrine, in peptide amidation and in tyrosine metabolism. In addition, vitamin C is an important regulator of iron uptake. It reduces ferric to ferrous ions, thus promoting dietary non-haem iron absorption from the gastrointestinal tract and stabilizes iron-binding proteins. Most animals are able to synthesise vitamin C from glucose, but humans, other primates, guinea pigs and fruit bats lack the last enzyme involved in the synthesis of vitamin C (gulonolactone oxidase) and so require the presence of the vitamin in their diet. Thus, the prolonged deprivation of vitamin C generates defects in the post-translational modification of collagen that causes scurvy and eventually death. In addition to its antiscorbutic action, vitamin C is a potent reducing agent and scavenger of free radicals in biological systems.^[11]

B. S. N. Rao^[12] carried out a study of the effect of γ -radiation on pure ascorbic acid solution and on its solution containing dissolved solutes and gases. L'ova and colleagues^[13] found

that ascorbic acid in the solid form is resistant to 25 kGy of radiation. They reported that the deterioration and decay increased significantly with increasing concentrations of beta and x-ray radiolysis of dilute solutions and also that degradation is more common in a nitrogen atmosphere.

In the present work we have carried out the radiolysis of Thiamine (Vitamin B₁) and ascorbic acid (Vitamin C) in the solid state as well as aqueous solutions. Two tablets of Vitamin B₁ and Vitamin C were also irradiated and their assay was checked. Also, their IR and NMR spectra were recorded.

Water soluble vitamins- Characteristics

Thiamine

Thiamine, thiamin or vitamin B₁ named as the "thio-vitamine" is a water soluble vitamin of the B complex. Its IUPAC name is 3-((4-Amino-2-methyl-5-pyrimidinyl)methyl)-5-(2-hydroxyethyl)-4-methylthiazolium chloride with molecular formula C₁₂H₁₇N₄OS⁺[Cl⁻] and molecular weight 265.35. The structural formula of the compound is shown in Fig. 1. Thiamine is a colourless organosulphur compound which is soluble in water, methanol and glycerol and practically insoluble in less polar organic solvents.

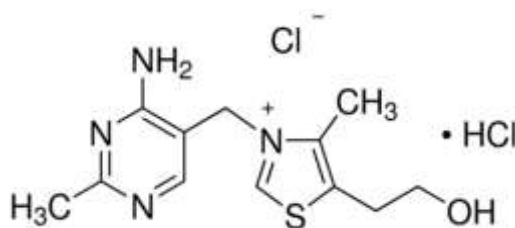


Fig 1: Thiamine hydrochloride.

Ascorbic acid

Ascorbic acid is a naturally occurring organic compound with antioxidant properties. It is one form of vitamin C. Its IUPAC name is (5R)-[(1S)-1,2-Dihydroxyethyl]-3,4-dihydroxyfuran-2(5H)-one. Its molecular formula is C₆H₈O₆ and molecular weight 176.12. The structural formula of ascorbic acid is shown in Fig. 2. It is a white solid, but impure samples can appear yellowish. It dissolves well in water to give mild acidic solutions.

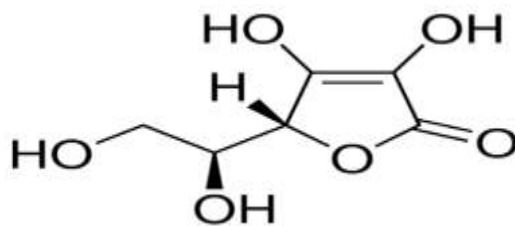


Fig 2: Ascorbic acid.

MATERIALS AND METHODS

Thiamine hydrochloride obtained from Loba Chemie was used for the present study. Two vitamin B₁ tablets were also used namely Beplex Forte and Neurobion Forte obtained from Merck. Ascorbic acid obtained from Merck and two vitamin C tablets namely, Celin (Glaxo Smithkline) and Limcee (Abbott Healthcare), were used for the present study.

Thiamine hydrochloride, Beplex Forte, Neurobion Forte, ascorbic acid, Celin and Limcee were irradiated in Gamma chamber (GC-900, housed in the Department of Chemistry, RTM Nagpur University) in solid state (as well as aqueous phase) at the dose rate of ~0.3 kGy/hr and characterized by IR and NMR. FTIR analysis was carried out on Perkin Elmer-Spectrum RX-IFTIR spectrophotometer at Central Instrumentation Laboratory, Chandigarh. ¹H NMR spectra operating at the frequency of 100 MHz was recorded on a Cryo-magnet (Bruker Avance II 400 NMR Spectrometer) instrument using tetramethylsilane (TMS) as an internal standard ($\delta = 0$ ppm) with D₂O as a solvent at SAIF, Chandigarh. Chemical shifts are reported in parts per million (ppm scale).

Assay of unirradiated and irradiated thiamine

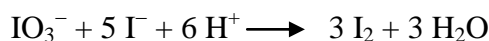
Thiamine hydrochloride was irradiated in solid phase and its assay was checked spectrophotometrically. 0.1 mM solution was prepared from irradiated solid thiamine hydrochloride for recording absorption spectra. Also, for thiamine hydrochloride solution, 0.1 mM solution (unirradiated) was prepared for gamma irradiation. The solvent required for spectrophotometric analysis of thiamine is phosphate buffer having pH 6.8. The solvent was prepared by mixing equal volumes of 0.01 M solutions of potassium dihydrogen orthophosphate and disodium hydrogen orthophosphate. The amount of vitamin decomposed after irradiation was estimated by recording absorption spectra on Elico SL 210 double beam UV-visible spectrophotometer in the UV region.

The tablets that were used for the study contained B complex vitamins i.e. thiamine, riboflavin, nicotinic acid, niacinamide, pyridoxine hydrochloride, calcium pantothenate, folic acid, cyanacobalamin and other substances. All the vitamins of B complex group have absorption maxima in the range of 200-300 nm. The absorption spectrum of thiamine was quite overlapped due to presence of other components. It was not possible to extract only thiamine from the tablets and hence the absorption spectra for the tablets could not be studied.

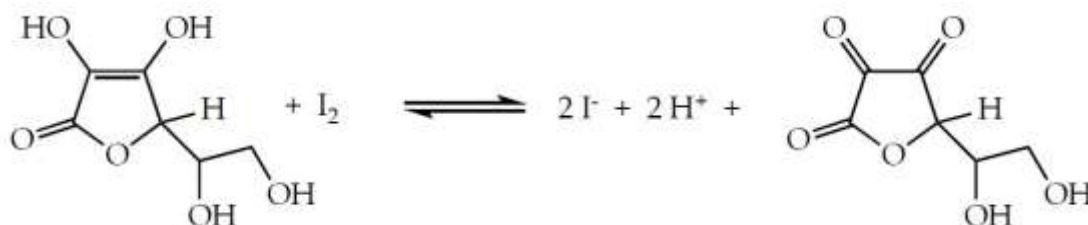
Assay of unirradiated and irradiated ascorbic acid

The assay of ascorbic acid was studied by iodometric titration. In the determination of ascorbic acid, the KIO_3 method employs a back titration of standard thiosulphate solution with I_2 generated by adding a known amount of KIO_3 solution to ascorbic acid solution.

In this titration ascorbic acid is titrated with standard potassium iodate (KIO_3) in the presence of excess potassium iodide in acidic medium. The iodate ion oxidizes iodide ion to I_2 and is itself reduced to I_2 according to the reaction,



The ascorbic acid is oxidized by the liberated iodine to dehydroascorbic acid as follows-



The excess of I_2 generated in the KIO_3 reaction is titrated against sodium thiosulphate using starch-iodine complex as indicator.

0.01 M ascorbic acid solution was used for checking the assay while four tablets were dissolved in 1 L doubly distilled water and used for subsequent radiolysis and assay determination. 15 mL of the prepared solutions were kept for irradiation in gamma chamber and amount of ascorbic acid remaining was determined after irradiation.

RESULTS AND DISCUSSION

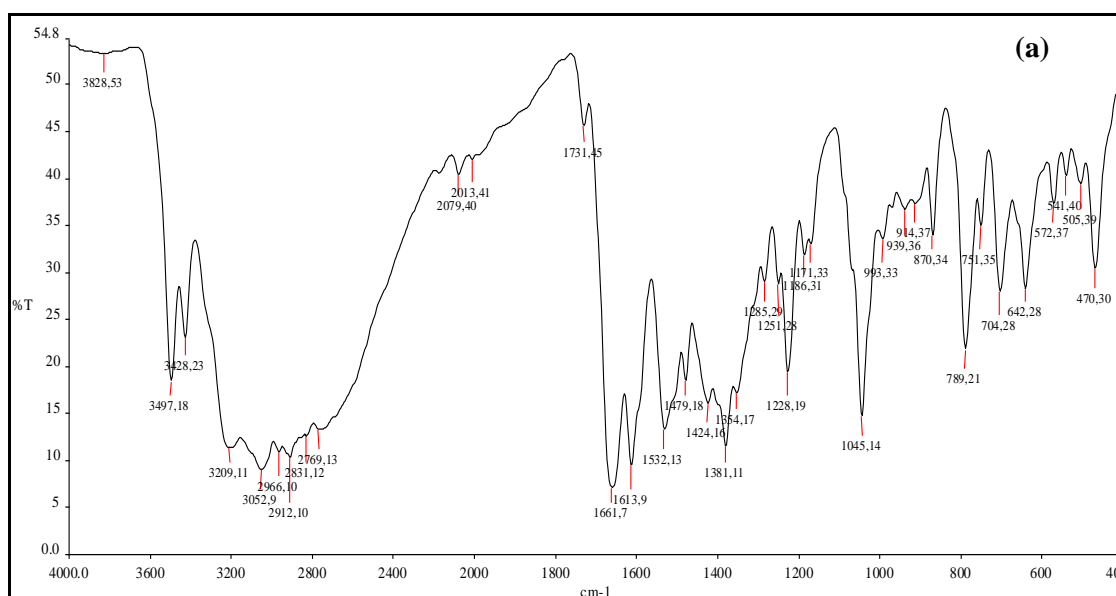
Characterization of standards and tablets by FTIR and NMR

The FTIR spectra of unirradiated and irradiated thiamine hydrochloride, Beplex Forte, Neurobion Forte, ascorbic acid, Celin and Limcee are shown in Fig. 3 (a-l).

In the IR spectrum of unirradiated and irradiated Thiamine hydrochloride^[14] (Fig. 3 (a and b)), the broad peak at 3209.11 cm^{-1} represents the primary hydroxyl OH stretching and the band at 3497.18 cm^{-1} corresponds to the primary aromatic amine group. Methyl asymmetric stretching is observed at 2966.10 cm^{-1} and 2912.10 cm^{-1} frequency is assigned to methylene asymmetric stretching frequency. The aromatic tertiary amine CN stretching is observed at 1354.17 cm^{-1} .

The FTIR spectra of ascorbic acid, unirradiated and irradiated, are shown in Fig. 3 (g and h). The spectra show OH stretching between 3526 and 3031 cm^{-1} and C–H stretching at 2916 cm^{-1} whereas O–H stretching is observed at 2744 cm^{-1} . C=O stretching is observed at 1754 cm^{-1} while C=C stretching can be seen at 1673 cm^{-1} . CH₂ scissor frequency, CH₂ wagging frequency and C-H deformation can be observed at 1456 , 1321 and 1275 cm^{-1} respectively. The peaks at 1221 and 1198 cm^{-1} are observed due to skeletal vibrations. The peaks in the range of 1076 to 1044 cm^{-1} are assigned to C–O–C stretch while peaks at 1026 and 998 cm^{-1} are possibly due to C–C stretch.^[15]

The IR spectra for unirradiated and irradiated standards and tablets are almost identical indicating that gamma irradiation does not cause any chemical change in the vitamins.



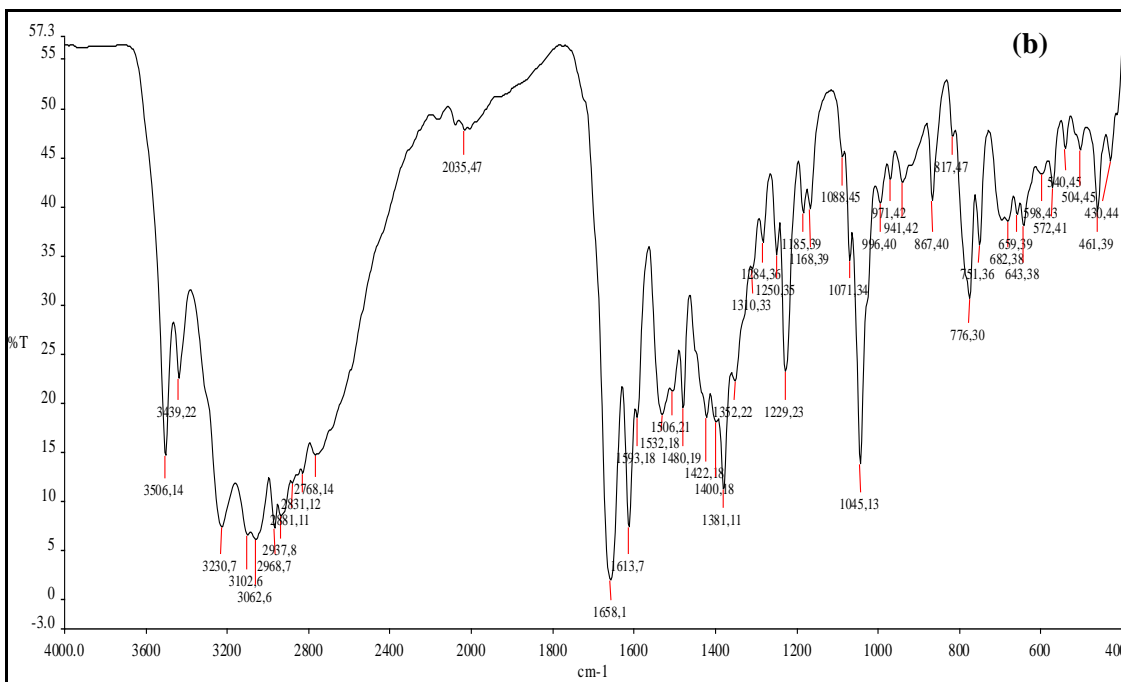
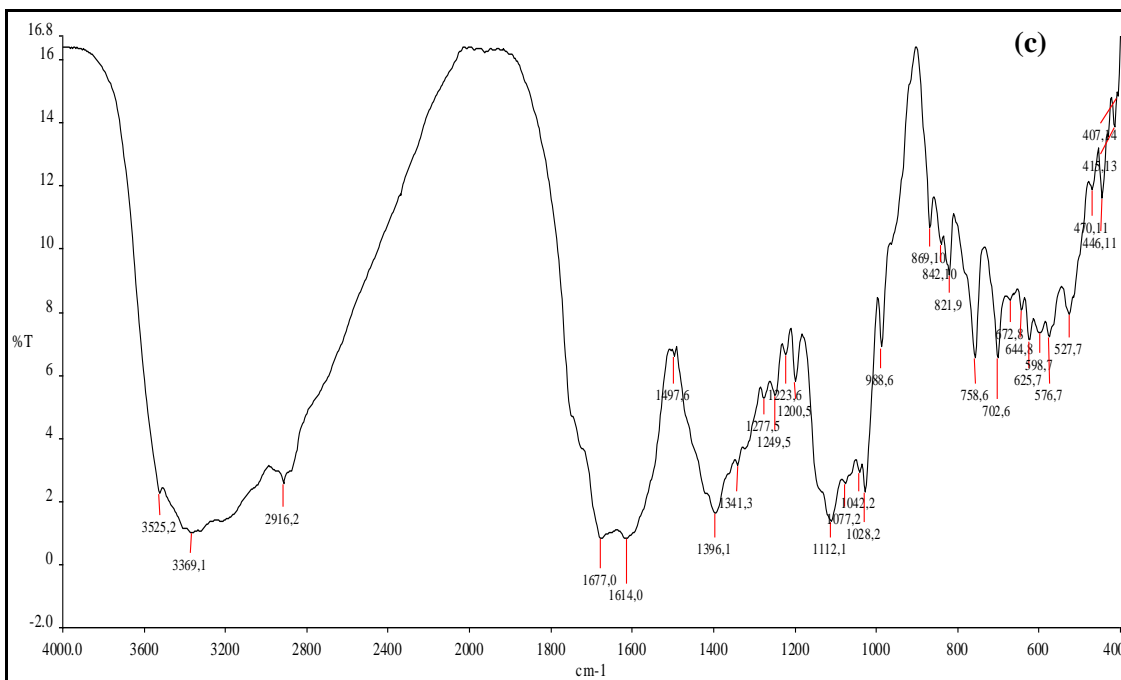
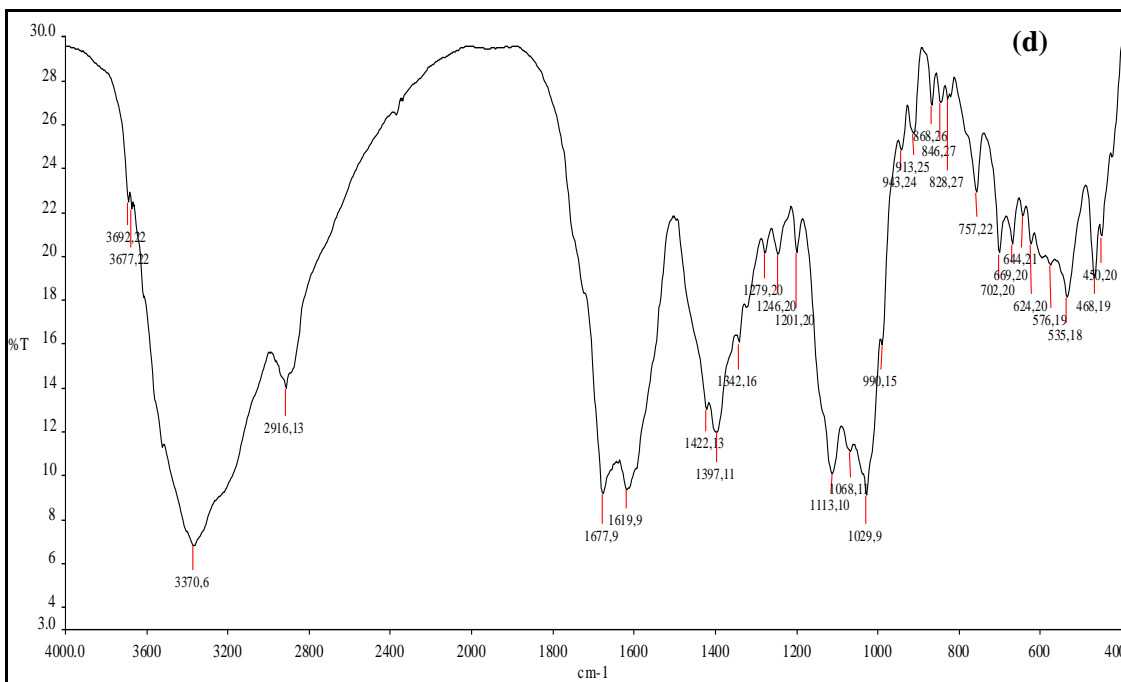
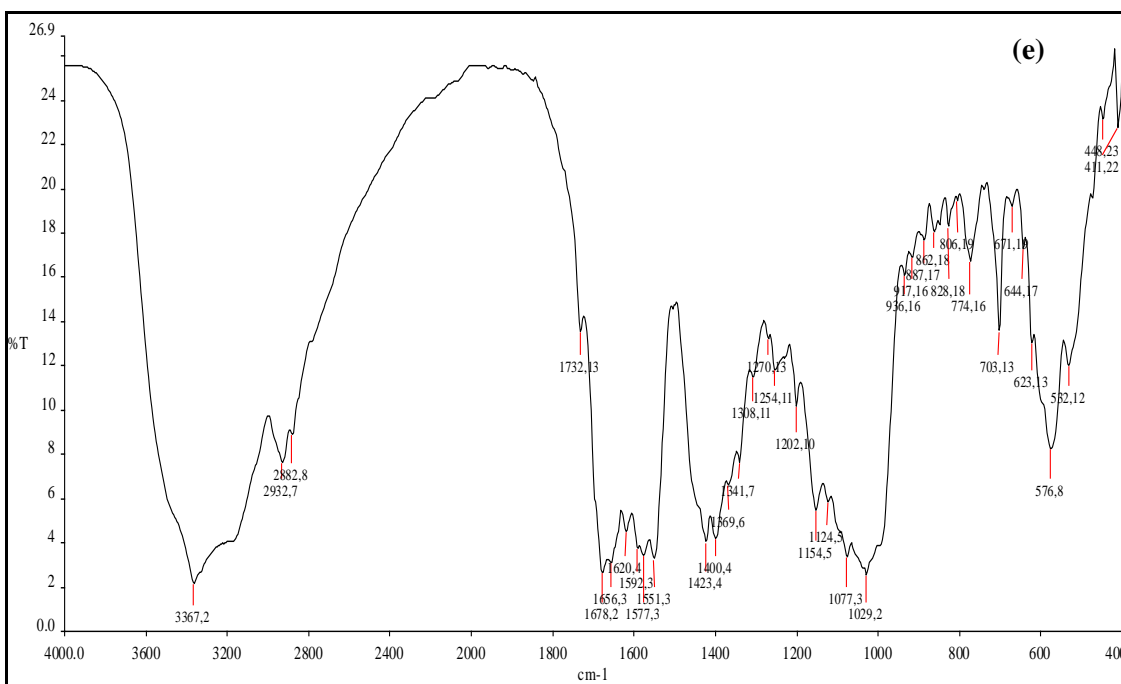


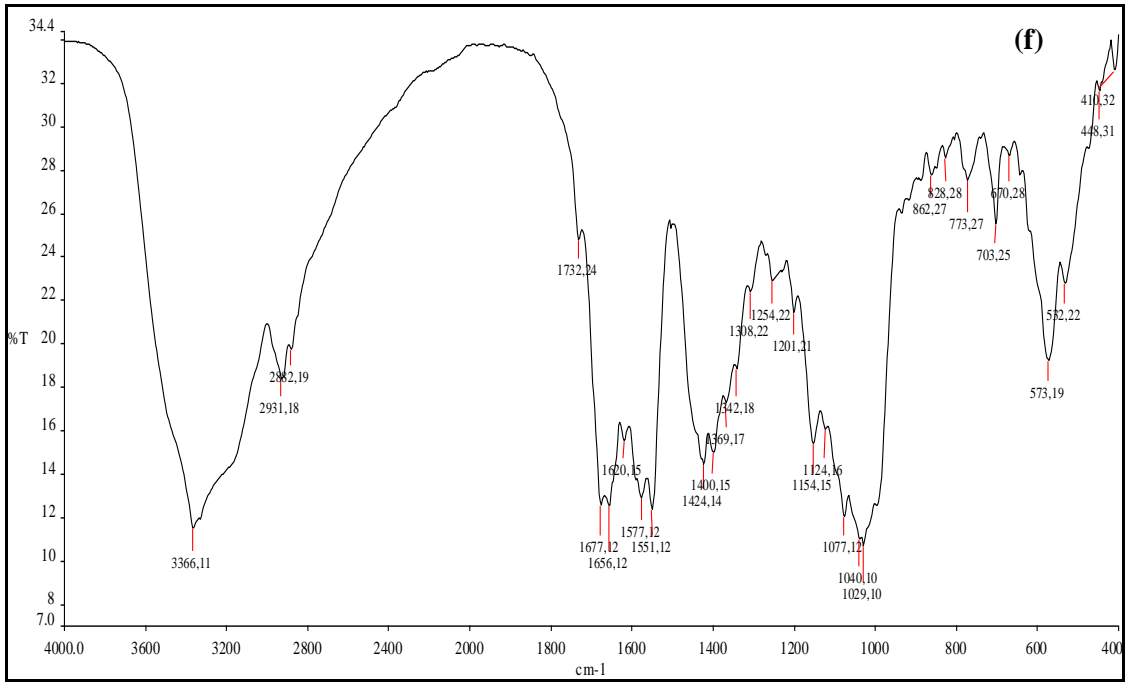
Fig. 3 FTIR spectra for (a) Thiamine hydrochloride (unirradiated) (b) Thiamine hydrochloride (irradiated)



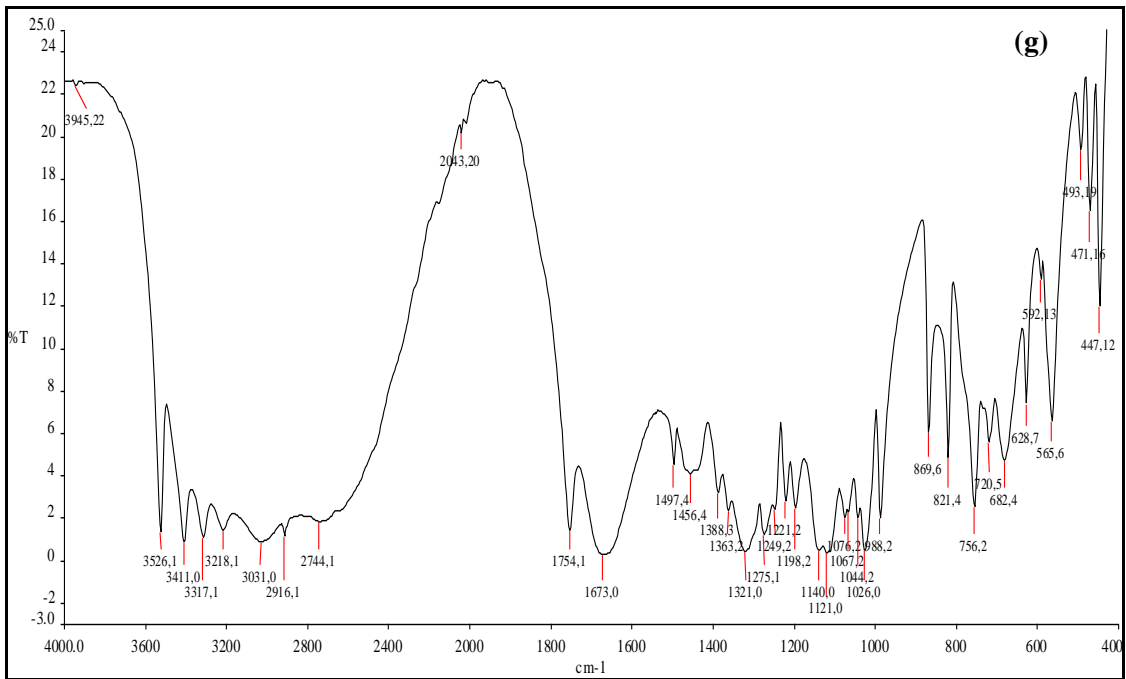


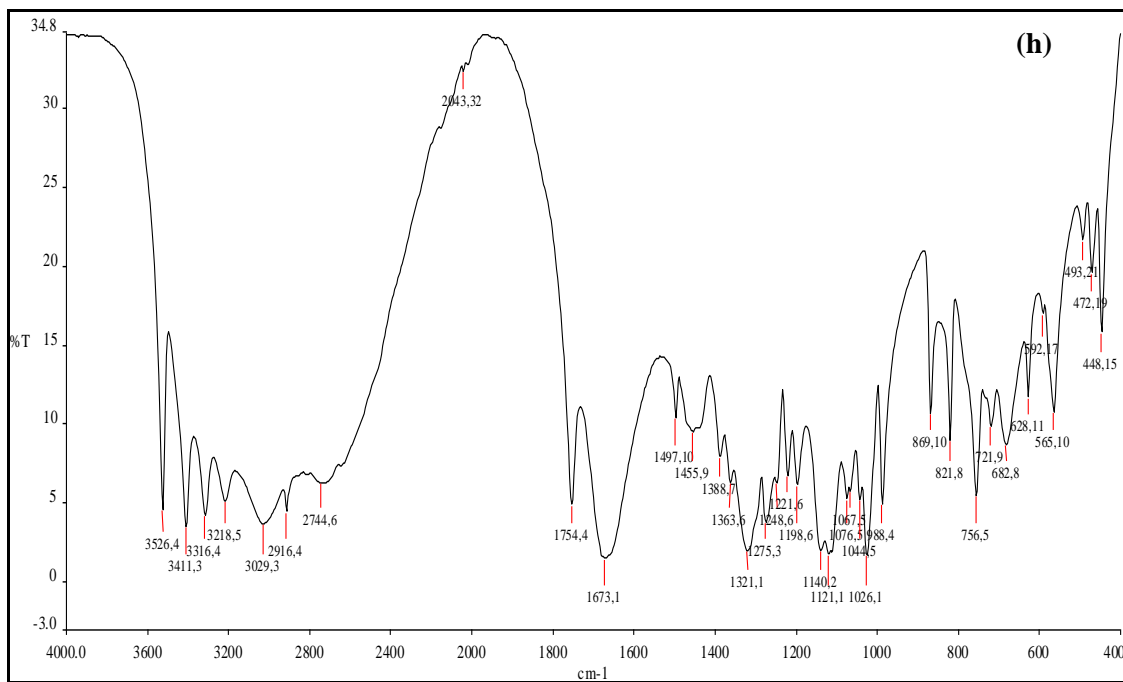
**Fig. 3 FTIR spectra for (c) Bplex Forte (unirradiated)
(d) Bplex Forte (irradiated)**



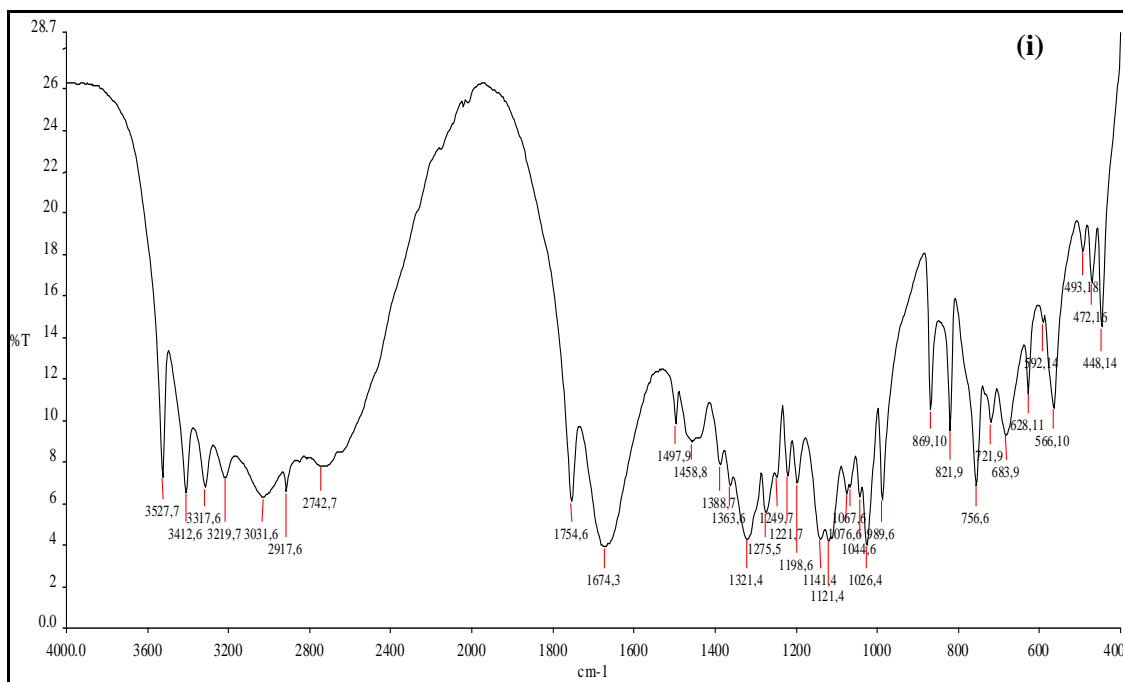


**Fig. 3 FTIR spectra for (e) Neurobion Forte (unirradiated)
(f) Neurobion Forte (irradiated)**





**Fig. 3 FTIR spectra for (g) Ascorbic acid (unirradiated)
(h) Ascorbic acid (irradiated)**



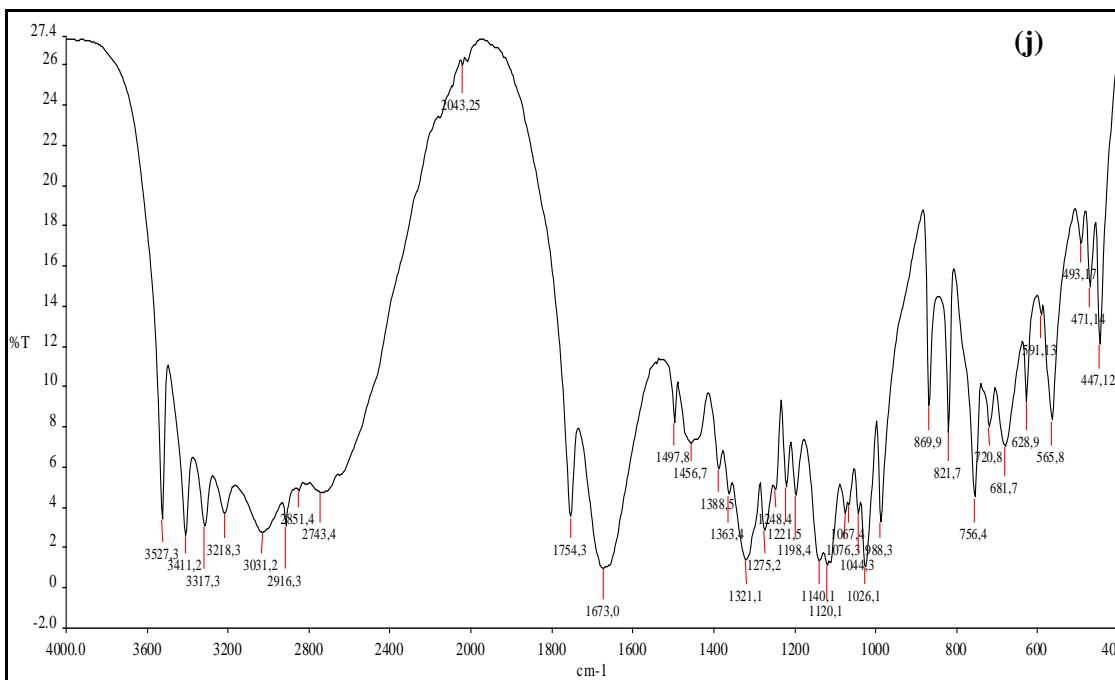
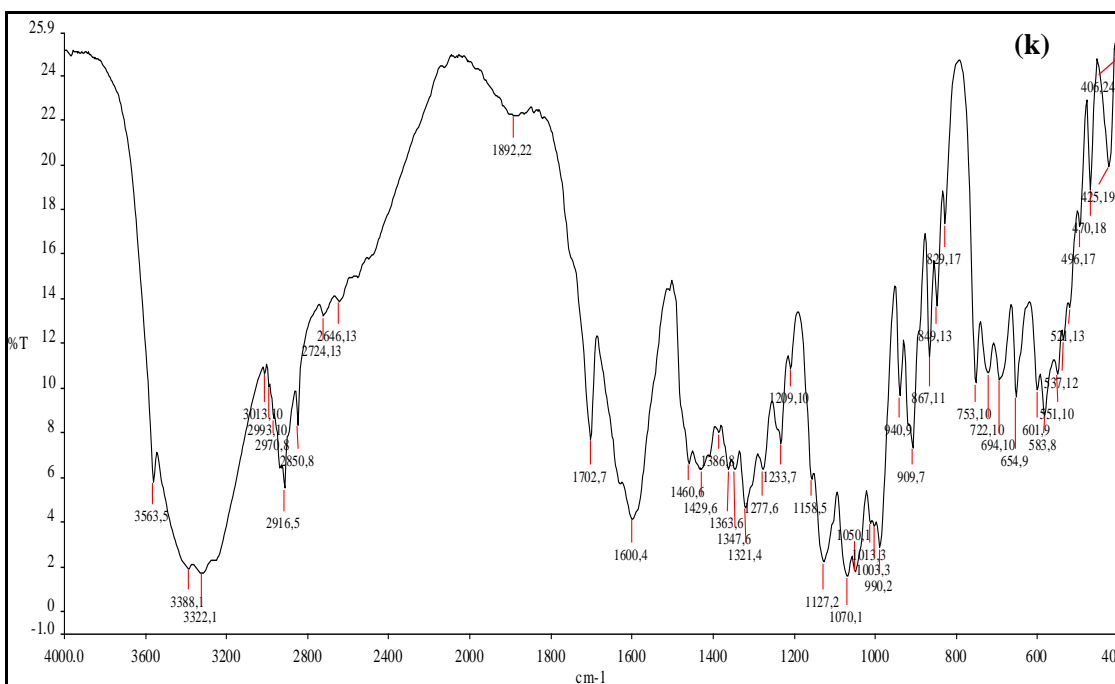


Fig. 3 FTIR spectra for (i) Celin (unirradiated) (j) Celin (irradiated)



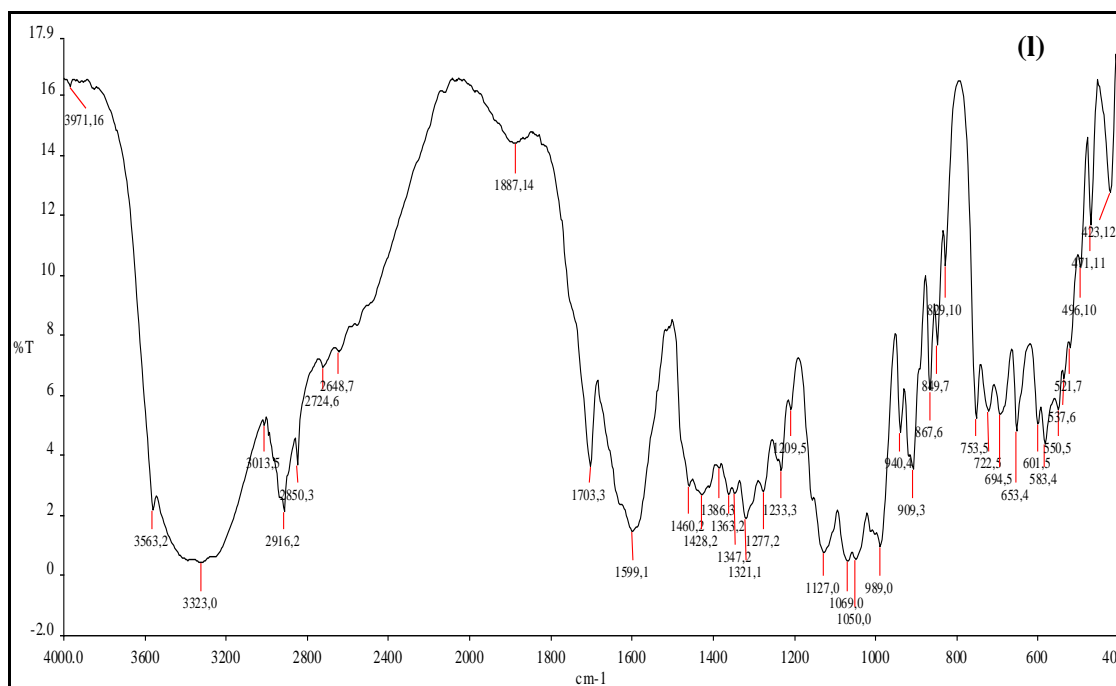


Fig. 3 FTIR spectra for (k) Limcee (unirradiated) (l) Limcee (irradiated)

The NMR spectra of unirradiated and irradiated Thiamine hydrochloride, Beplex Forte, Neurobion Forte, ascorbic acid, Celin and Limcee are shown in Fig. 4 (a-l).

In the NMR spectra of thiamine hydrochloride (Fig. 4 a and b) two singlets are observed at δ 2.4801 and δ 2.546 ppm, each having an intensity 3 corresponding to the two methyl groups at C13 and C18 position in thiamine. The triplets observed at δ 3.1212 and δ 3.809 ppm correspond to C14 and C15 CH₂ protons respectively having intensity approximately 2. The singlet peak at δ 5.5091 represents the CH₂ protons at C6 position with intensity 2 and this confirms that there is no deformation or splitting in the structure due to gamma radiation. The highly deshielded peaks observed at δ 7.9781 and δ 9.607 ppm correspond to the aromatic protons and the singlet at δ 4.6992 ppm corresponds to the hydroxyl proton.

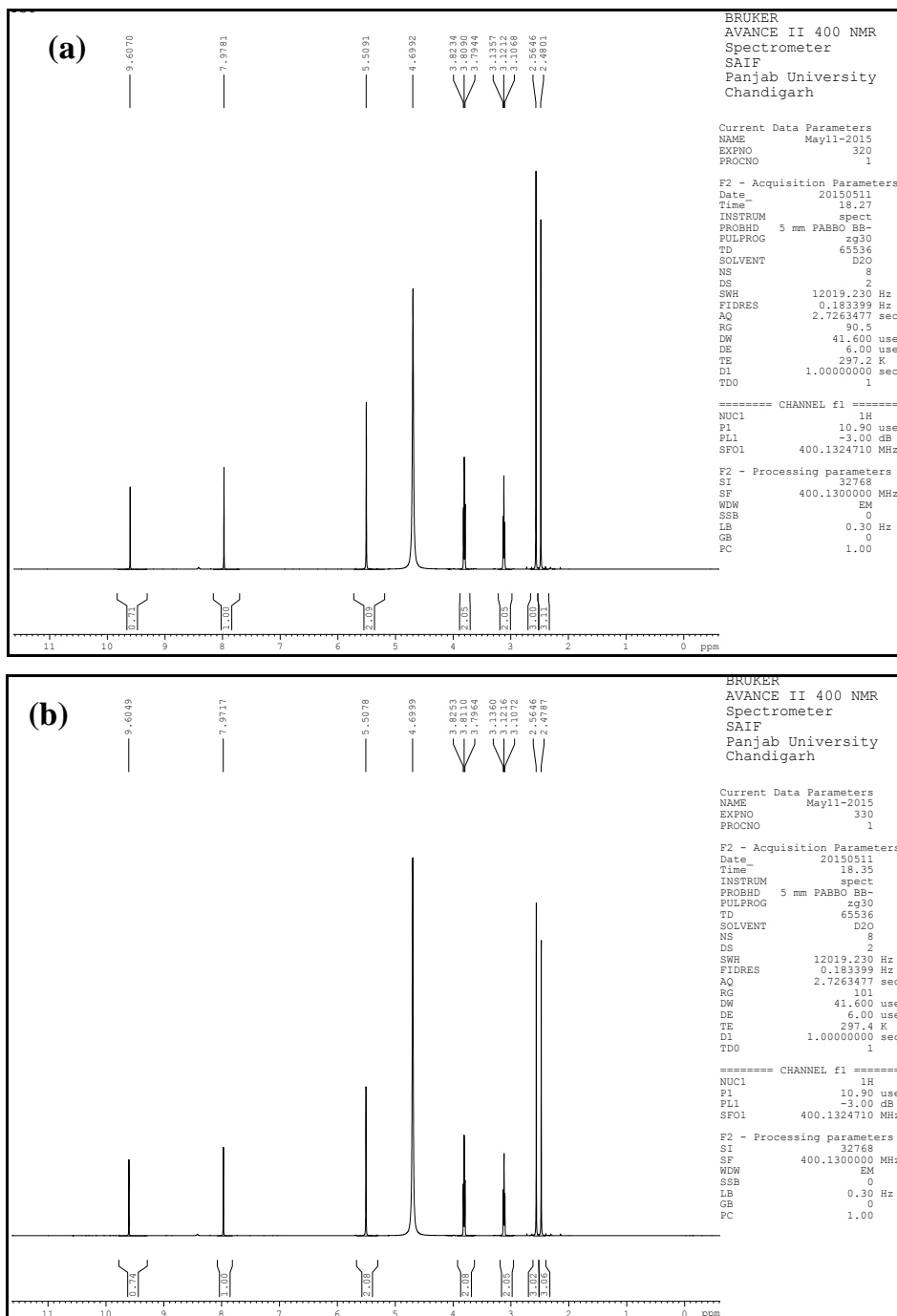
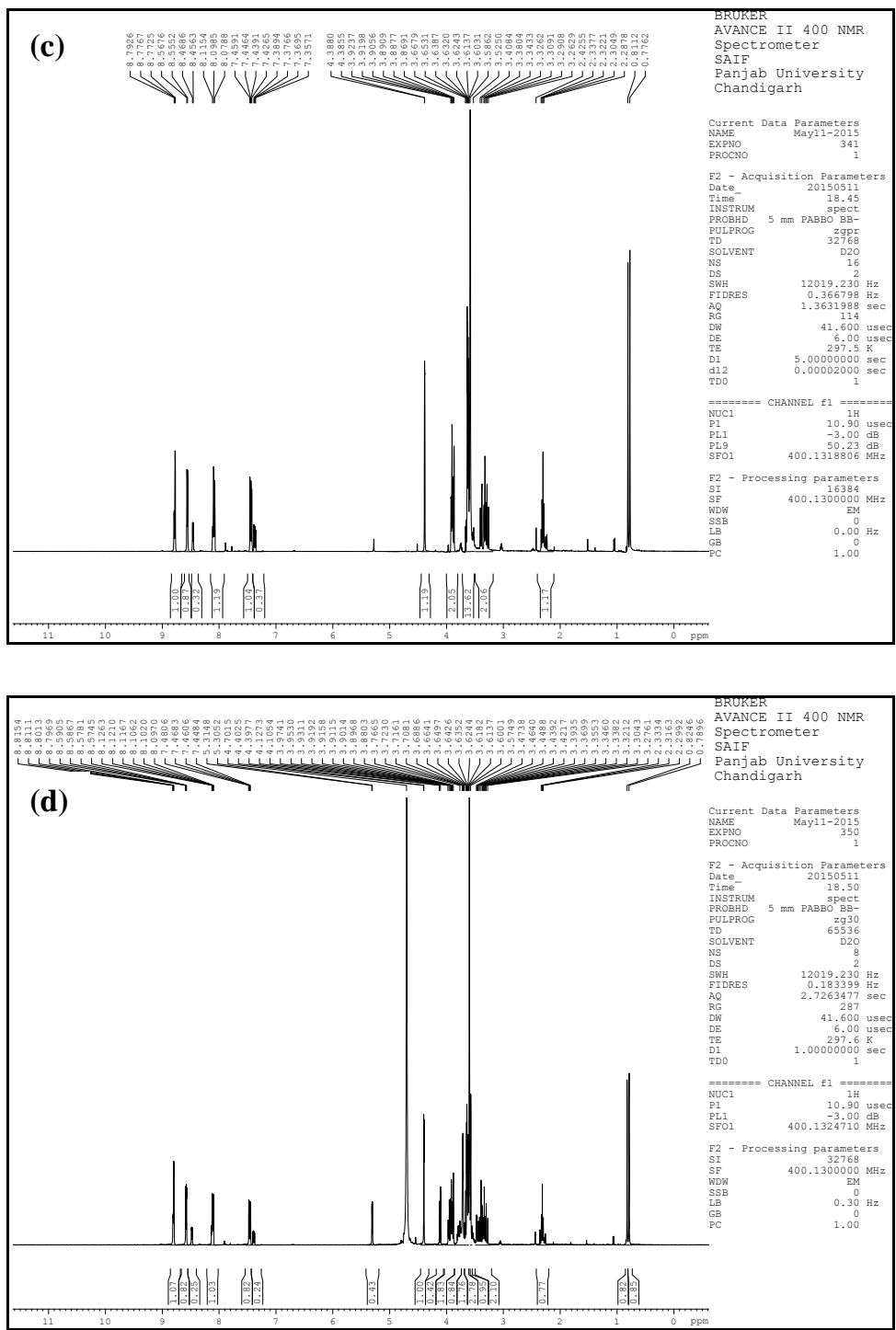
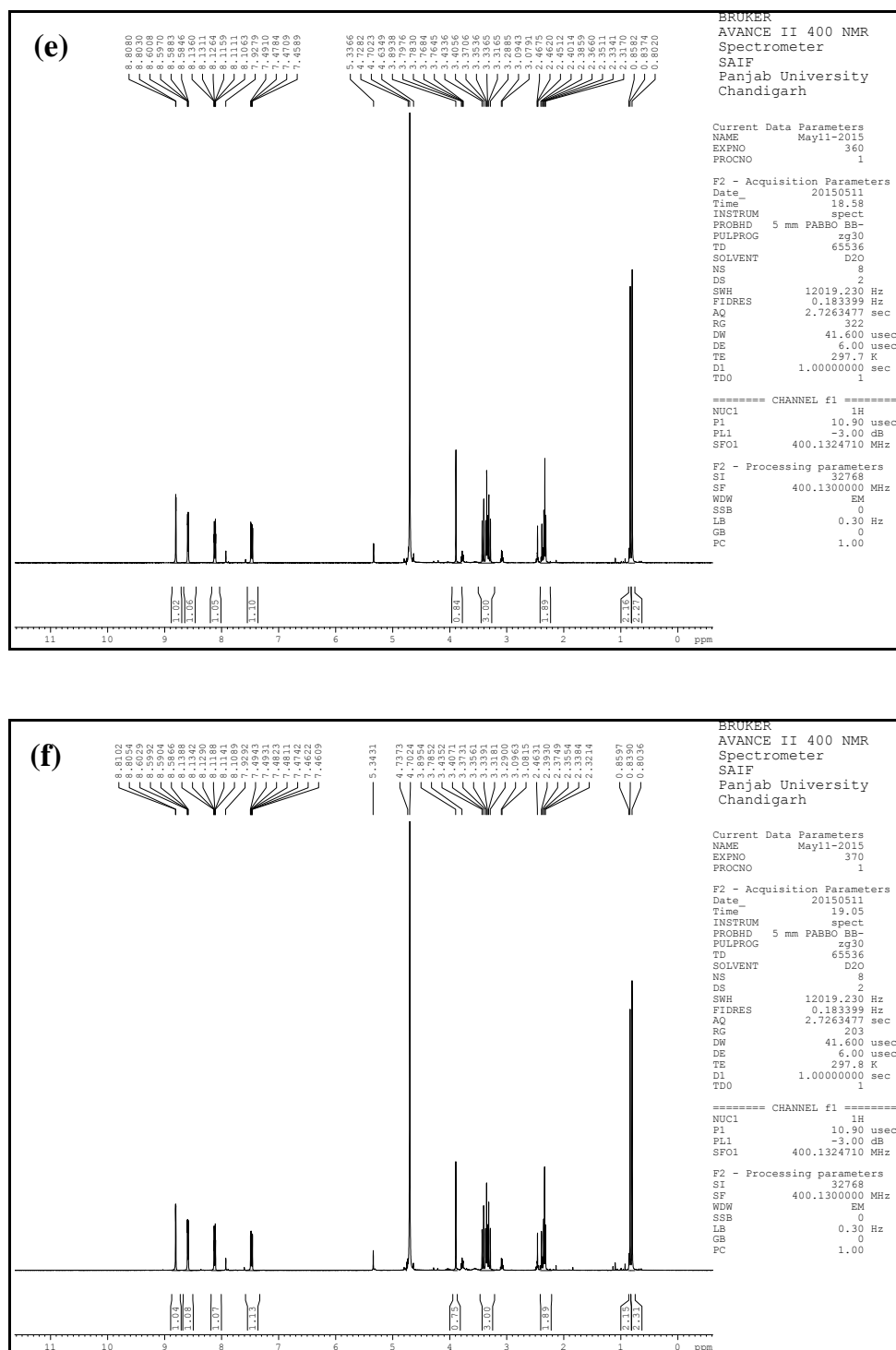


Fig. 4 NMR spectra for (a) Thiamine hydrochloride (unirradiated) (b) Thiamine hydrochloride (irradiated)



**Fig. 4 NMR spectra for (c) Beplex Forte (unirradiated)
 (d) Beplex Forte (irradiated)**



**Fig. 4 NMR spectra for (e) Neurobion Forte (unirradiated)
 (f) Neurobion Forte (irradiated)**

In the NMR spectra of ascorbic acid (Fig. 4 g and h) the methylene CH_2 protons are found in the most deshielded region of NMR at δ 3.619 ppm with intensity 2. The methyne CH proton gives multiplet split at δ 3.9417 ppm and the aromatic methyne proton gives singlet at δ 4.7107 ppm. The aliphatic hydroxyl proton is observed at δ 4.8334 ppm.

The NMR spectra for unirradiated and irradiated standards and tablets are almost identical indicating that gamma irradiation does not cause any chemical change in the vitamins.

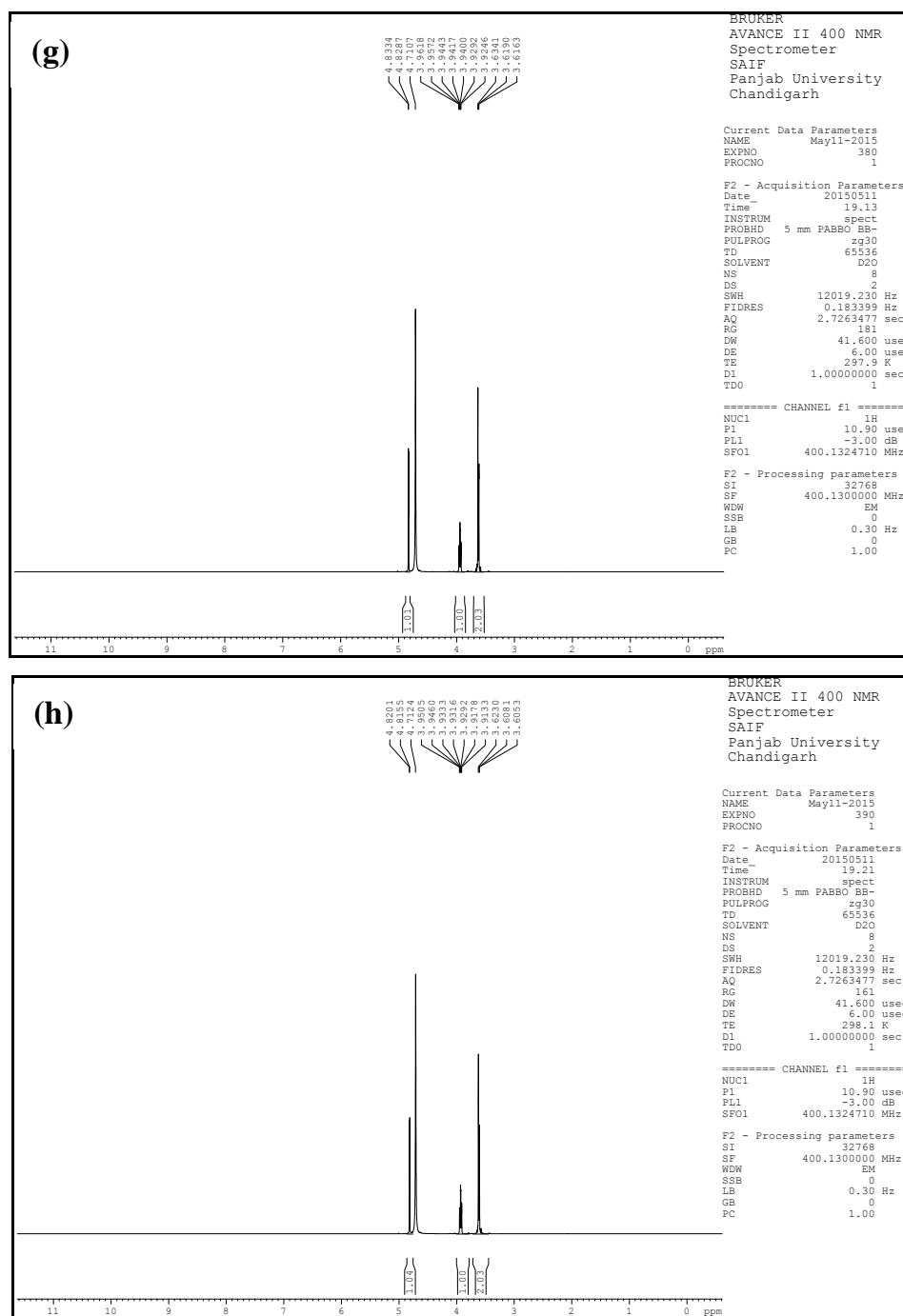


Fig 4: NMR spectra for (g) Ascorbic acid (unirradiated) (h) Ascorbic acid (irradiated).

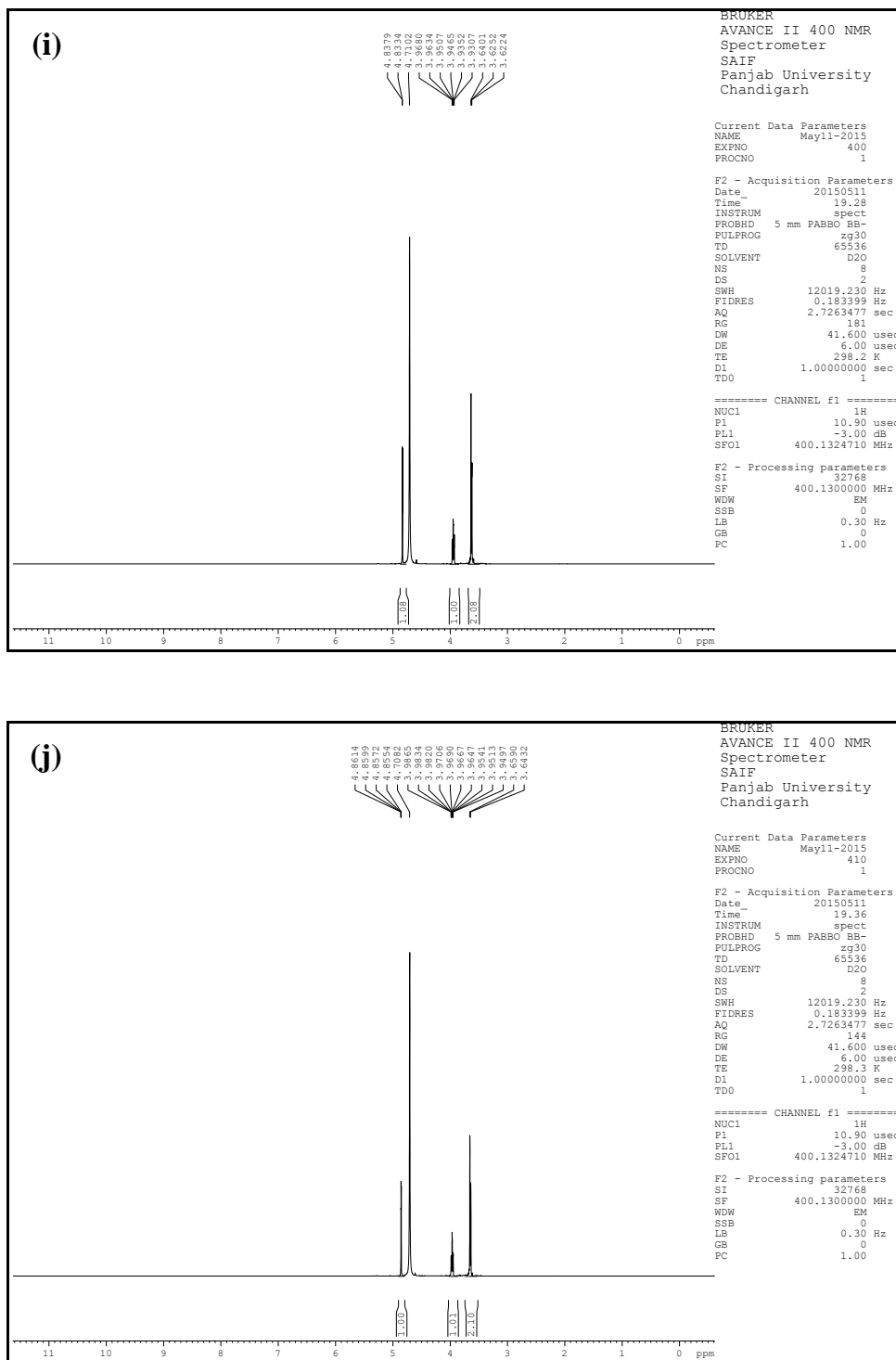


Fig. 4 NMR spectra for (i) Celin (unirradiated) (j) Celin (irradiated)

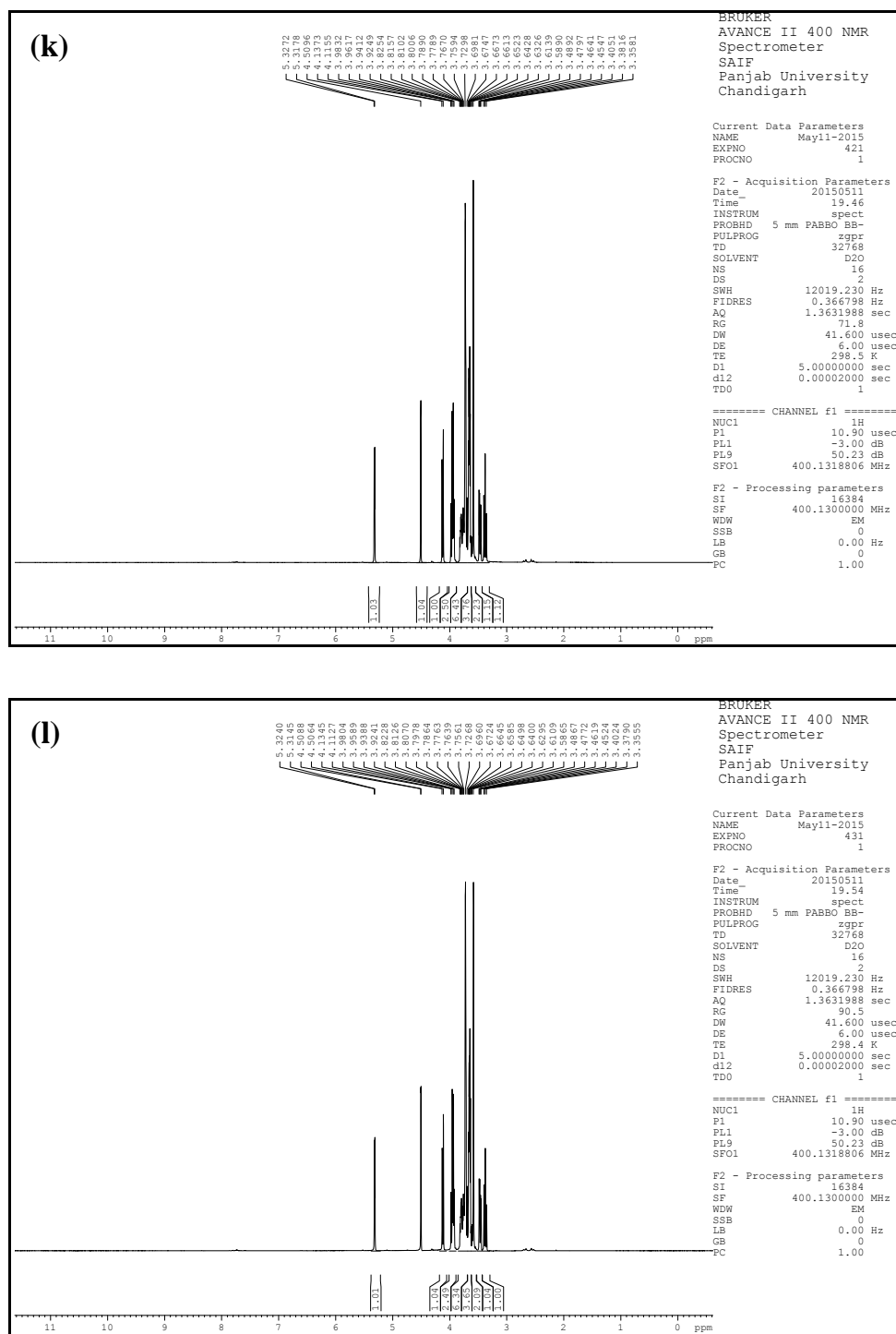


Fig. 4 NMR spectra for (k) Limcee (unirradiated) (l) Limcee (irradiated)

Radiolysis of thiamine hydrochloride

The absorption spectra of irradiated thiamine hydrochloride solution at pH 6.8 with characteristic absorption maxima at 232 and 266 nm, as recorded on Elico SL-210 double beam UV-visible spectrophotometer, are shown in Fig. 5.

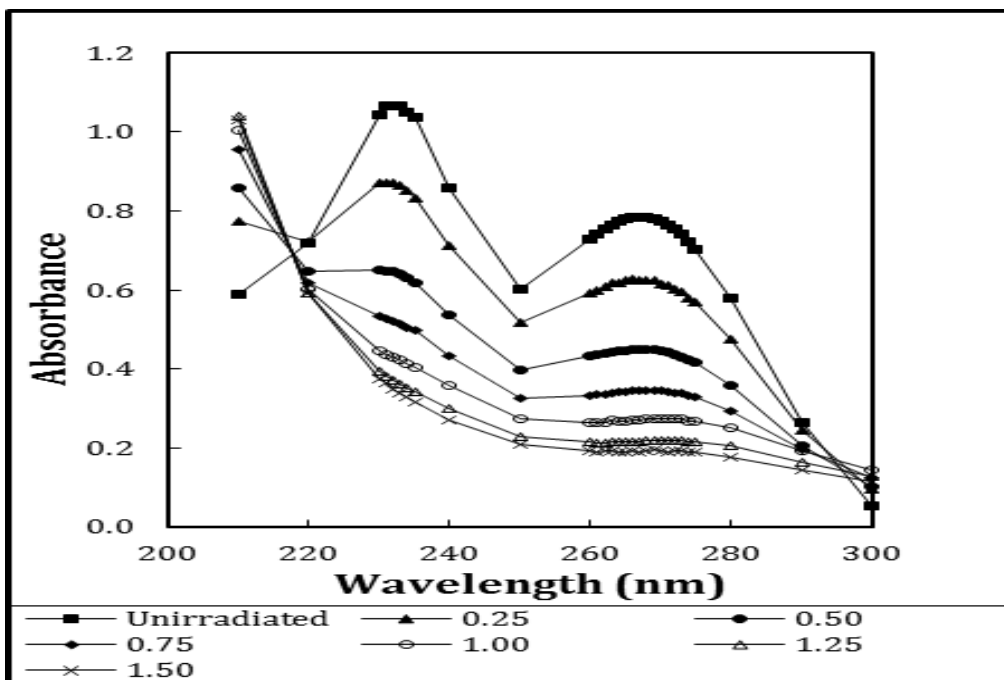


Fig. 5 Absorption spectra of thiamine hydrochloride aqueous solution.

Fig. 6 shows a plot of G(-TH) against the absorbed dose for aqueous thiamine hydrochloride solution.

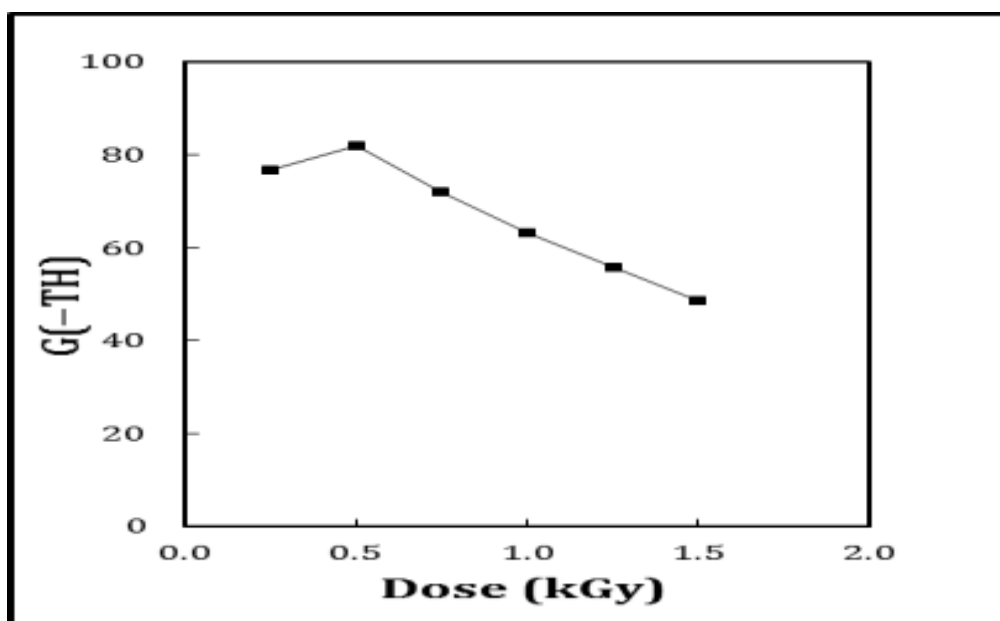


Fig. 6 A plot of G(-TH) against absorbed dose for aqueous thiamine hydrochloride solution.

Thiamine hydrochloride was found to be resistant to gamma radiations in the solid state as hardly any change was observed in the absorbance after irradiation till 50 kGy. However, it undergoes extensive degradation in aqueous state and gets completely degraded after 1 kGy.

Radiolysis of ascorbic acid

Ascorbic acid in the solid state was highly resistant to gamma radiations and there was no variation in the amount of ascorbic acid in the tablets after irradiation till 50 kGy. Also, in aqueous solution, ascorbic acid was found to be resistant to much higher gamma dose as compared to thiamine.

Fig. 7 (a and b) shows the amount of ascorbic acid remaining and G(-AA) against the absorbed dose. The results of tablets, Celin and Limcee, have been compared with standard ascorbic acid.

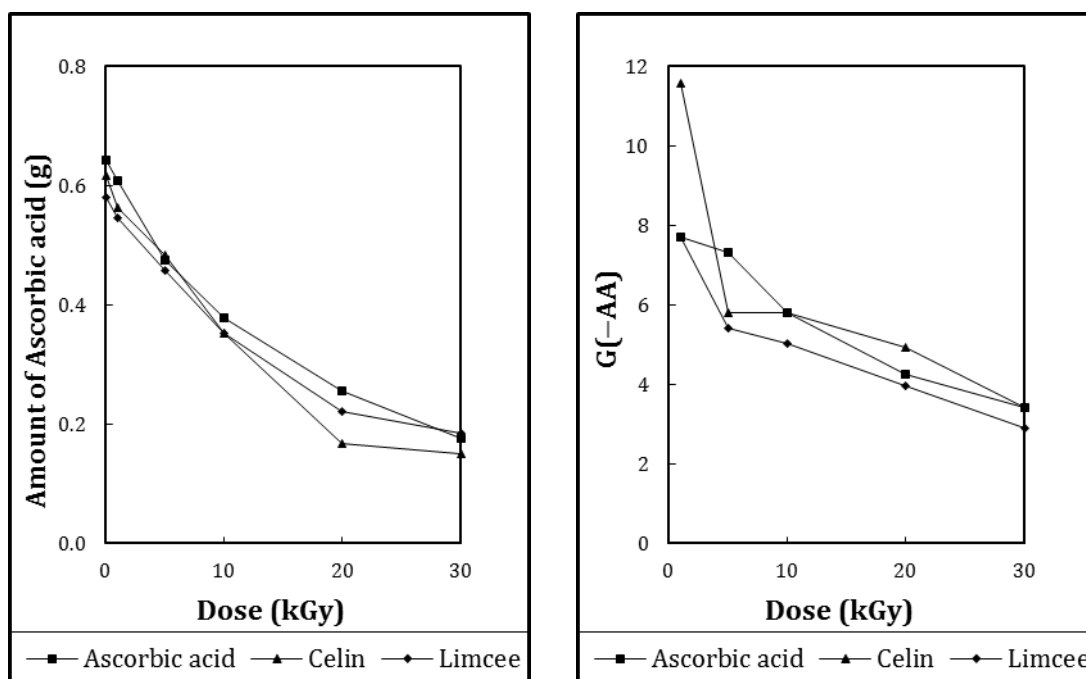


Fig. 7 (a) A plot of amount of ascorbic acid remaining

(b) G(-AA) against absorbed dose for ascorbic acid and tablets, Celin and Limcee

CONCLUSION

- i) Radiation sterilization of water soluble vitamins can be effectively carried out in solid state as decomposition of vitamins does not occur due to gamma irradiations in the solid state.
- ii) Irradiation does not cause any change in the chemical structure too, as is evident from FTIR and NMR spectra.
- iii) Aqueous solution of thiamine hydrochloride undergoes extensive damage due to gamma irradiation.

- iv) Aqueous solution of ascorbic acid is quite resistant to gamma radiation and does not undergo much radiation damage at low dosages of gamma irradiation. Thus, vitamin C syrups can also be sterilized by low dose of gamma radiation.

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