

FTIR AND FT-RAMAN SPECTRAL ANALYSIS OF PACLITAXEL DRUGS

T.S. RENUGA DEVI* and S. GAYATHRI

*PG Department of Physics, Women's Christian College, Chennai-600006, India

E-mail: *devi_renuga@yahoo.com, gayakararthik0202@yahoo.com

ABSTRACT

Paclitaxel is an anti-leukemic, anti-tumor or in general an anti-neoplastic agent. It was first isolated from the bark of the pacific Yew tree, *Taxus brevifolia*. Paclitaxel drug is used to treat ovarian, breast and lung cancers. Spectroscopic investigations on Pharmaceutical samples are of importance in present. Vibrational spectral studies of many pharmaceutical drugs are extensively studied by many scientists. However the vibrational spectral analysis of polymeric nanoparticle Paclitaxel drug has not carried out before. The infrared spectrum of a compound is the superposition of the absorption bands of specific functional groups. As such, the infrared spectrum can be used as a fingerprint for identification of unknown in comparison with previously recorded reference spectra. An attempt has been made in this work to study the vibrations of the functional derivatives in polymeric nanoparticle Paclitaxel drug. By observing the position, shape and relative intensities of the vibration bands in FTIR and FT-Raman spectra of the drugs a satisfactory vibration band assignment has been made. Fourier transform infrared (FTIR) analysis was conducted to verify the occurrence of chemical bonds between the pure paclitaxel drug, castor oil-solvent based paclitaxel drug and polymeric nanoparticle paclitaxel drug. The spectral analysis indicated that the specific functional groups of the polymeric nanoparticle drug material and castor oil-solvent based paclitaxel drug have almost the same chemical characteristics of the pure paclitaxel drug. The study suggests that did not occur molecular interaction that could alter the chemical structure of the drug.

Keywords: FTIR, Paclitaxel, FT-Raman Spectroscopy

INTRODUCTION

The number of reported cases of cancer is steadily increasing in both industrialised and developing countries. In spite of the fact that significant progress has been achieved in tumor biology, molecular genetics and in the prevention, detection and treatment of cancer over the last few years, adequate therapy remains elusive due to late diagnosis, inadequate strategies for addressing aggressive metastasis, and the lack of clinical procedures overcoming multidrug resistant (MDR) cancer [1,2]. The integration of nanotechnology and medicine has the potential to uncover the structure and function of biosystems at the nanoscale level. Nanobiotechnology may provide a reliable and effective tool to treat diseases at a molecular scale. Nanobiotechnology offers an unprecedented opportunity to rationalize delivery of drugs and genes to solid tumours following systemic administration [3]. Examples of nanotechnologies applied in pharmaceutical product development include polymer-based nanoparticles, lipid-based nanoparticles (liposomes, nanoemulsions, and solid-lipid nanoparticles), self-assembling nanostructures such as micelles and dendrimers-based nanostructures among others. In recent years, much research has gone into the characterisation of nanoparticles and their biological effects and potential applications. These include bottom-up and molecular self-assembly, biological effects of naked nanoparticles and nano-safety, drug encapsulation and nanotherapeutics, and novel nanoparticles for use in microscopy, imaging and diagnostics [4].

Cancer treatment approach needs to overcome physiological barriers such as vascular endothelial pores, heterogeneous blood supply, heterogeneous architecture to name just a few [5], and it strongly depends on the method of delivery. In the past, many anticancer drugs had only limited success and had major adverse side effects [6, 7]. Nanoparticles have attracted considerable attention

worldwide because of their unique functional characters such as small particle size, high stability, lower toxicity, tuneable hydrophilic-hydrophobic balance and the ability to bear surface features for target specific localization, etc. Thus, polymeric nanoparticles constitute a versatile drug delivery system [8]. Numerous biodegradable polymeric nanoparticles made of natural polymers such as proteins or polysaccharides have been tried for drug delivery and controlled drug release. More recently the focus of such studies moved onto synthetic polymers, and much progress have been achieved in this area. Recent examples include, for example polycationic nanoparticles for encapsulation and controlled release of amphotericin B by Viera and Carmona-Ribeiro [10]; or encapsulation of curcumin for human cancer therapy by Maitra et al [11].

Spectroscopic investigations on pharmaceutical samples are of importance in the present. Vibrational spectral studies of many pharmaceutical drugs are extensively studied by many scientists. Spectroscopic studies of the Paclitaxel binding and proximity relationships with cisplatin and adriamycin are done by Lilianna Trynda-Lemiesz[12], FTIR and NMR spectroscopy studies on the interaction of paclitaxel with lipid bilayers are studied by Dhanikula Anand[13], conformation of microtubule bound paclitaxel by fluorescence spectroscopy and NMR were given by Yankun Li[14]., however the vibrational spectral analysis of polymeric nanoparticle Paclitaxel drug have not carried out before. An attempt has been made in this work to study the vibrations of the functional derivatives in the drug.

Experimental

Paclitaxel is off-white crystalline powder insoluble in water with molecular formula $C_{47}H_{51}NO_{14}$ (IUPAC name: 5 beta, 20-Epoxy-1, 2a, 4, 7 beta, 10 beta, 13 alpha-hexahydroxytax-11-en-9-one 4, 10-diacetate 2-benzoate 13-ester with (2 R, 3S)-N-benzoyl-3-phenylisoserine). The

molecular structure of the Paclitaxel drug is shown in Figure 1.

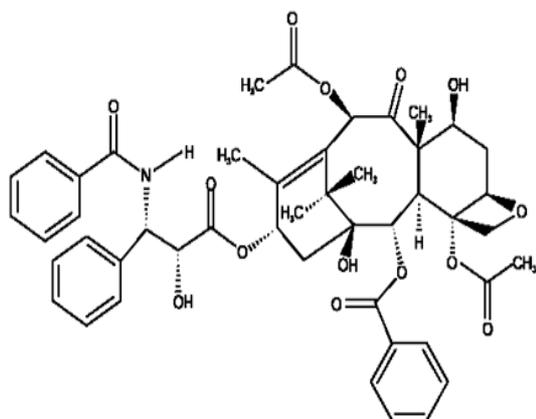


Figure 1: Molecular Structure of Paclitaxel Drug

The polymeric nanoparticle paclitaxel drug is commercially available drug as Nanoxel from Dabur pharma, India and used as such for the analysis. The FTIR spectra have been recorded using Perkin Elmer Spectrum one FTIR over the region 4000-400 cm^{-1} at Sophisticated Analytical Instrumentation Facility (SAIF), IIT, Chennai, India. The FT Raman spectra are recorded using Nexus 670 spectrophotometer in the region 4000-350 cm^{-1} at Central Instrumentation Facility (CIF), CECRI, Karaikudi, India. The FT and FTIR Raman spectra of the drug are presented in the Fig 2 and Fig 3 respectively.

Vibrational band assignment of Paclitaxel drug

The infrared spectrum of a compound is the superposition of the absorption bands of specific functional groups. As such, the infrared spectrum can be used as a finger print for identification of unknown in comparison with previously recorded reference spectra. By observing the position, shape and relative intensities of the vibrational bands in FTIR and FT Raman spectra of the drugs a satisfactory vibrational band assignment has been made. The FT and FTIR Raman spectra of the drug are presented in the fig 1 and Fig 2. The vibrational band assignments of the drugs are summarized in the table 1 and are discussed as follows.

(i) Ring vibrations:

The bands exhibited in the region around 3000 cm^{-1} can be immediately assigned to be due to aromatic C-H stretchings[15]. In this view, the vibrational frequencies exhibited at 2973-2547 cm^{-1} in the FTIR spectrum and 2971-2519 cm^{-1} in FT Raman spectrum are considered to be due to C-H stretching vibrations of the compound. The C-C ring stretching vibrations occur in the region 1652-1579 cm^{-1} in FTIR spectra and 1608-1581 cm^{-1} in FT-Raman spectra.

(ii) N-H /O-H vibrations:

As solids or liquids, primary aliphatic amines absorb in the region 3450-3250 cm^{-1} and exhibit a broad band of medium intensity. In dilute solution in non-polar solvents, two bands are observed for primary amines due to N-H asymmetric and symmetric vibration in the range 3550-3250 cm^{-1} .

The relative intensity of the band due to the hydroxyl stretching decreases with the increase in concentration with additional to broader bands appearing at lower frequencies 3580-3200 cm^{-1} . In aminobenzoic acid the hydroxyl stretching occurs at 3364 cm^{-1} in FTIR and 3363 cm^{-1} in FT Raman respectively [16]. From the above band assignments, the sample under study shows a very strong band at 3339 cm^{-1} in FTIR spectrum and weak intensity band at 3319 cm^{-1} in FT Raman spectrum due to N-H and O-H stretching vibrations.

(iii) C=O vibrations:

The carbonyl group exhibits a strong absorption band due to C=O stretching vibration and is observed in the region 1850-1550 cm^{-1} . In fluorouracil, the bands observed at 1621 cm^{-1} in IR and 1617 cm^{-1} in Raman are assigned to C=O symmetry stretching. The band observed at 1657 cm^{-1} in IR and 1660 cm^{-1} in Raman assigned to C=O asymmetry stretching vibration [17]. A strong band at 1712 cm^{-1} is assigned for C=O carbonyl stretching of nalidixic acid [18]. The band at 1684 cm^{-1} , which is close to the literature range with due characteristics is assigned for C=O stretching [19] in benzocaine. Keeping this in mind, the sharp band present in the expected region being at 1727,1720 cm^{-1} in the FTIR spectrum and 1727,1714 cm^{-1} in FT Raman spectrum are allotted to be due to C=O stretching vibrations.

(iv) C-O/C-N vibrations:

C-N stretching absorption of primary aliphatic amines is weak and occurs in the region 1090-1020 cm^{-1} . Secondary aliphatic amines have bands of medium intensity at 1190-1170 cm^{-1} . The band in the region 1222 cm^{-1} in IR and 1226 cm^{-1} in Raman have assigned to C-N symmetry stretching of the compound fluorouracil [17]. In this analogy the bands at 1274 cm^{-1} in FTIR and 1264 cm^{-1} in FT Raman spectrum of the drug sample are assigned due to C-N vibrations.

The bands due to C-O stretching vibrations are strong and occur in the region 1260-1000 cm^{-1} . In aminobenzoic acid the strong band near 1071 cm^{-1} in the IR and 1070 cm^{-1} in FT Raman are assigned to C-O stretching vibration [16]. In benzocaine, the very strong and sharp peak at 1281 cm^{-1} has been assigned to the C-O stretching. Taking the above band assignments, the band at 1090, 1049 cm^{-1} in FTIR and 1093, 1050 cm^{-1} in FT Raman spectrum of the sample under study are assigned due to C-O vibrations.

(v) Deformation vibration:

A number of C-H in plane deformation bands occur in region 1290-1000 cm^{-1} , the bands being sharp but weak to medium intensity. However, these bands are not normally of importance for interpretation purpose although they can be used. The aromatic C-H out of plane deformation bands occurs below 700 cm^{-1} . The bending vibrations are generally found at lower wave numbers. The frequencies observed at 765, 722, 660 and 613 cm^{-1} are assigned to be due to O=C-C, O=C-N, C=C-N and C=C-C bendings of the pyrimidine ring in the FTIR spectra of Xanthine and C-N-C bending vibrations are assigned at 498 and 428 cm^{-1} [19, 20]. Using the above analogy, the bands at 941-803 cm^{-1} in FTIR spectrum and 944-804 cm^{-1} in FT Raman

spectrum are due to C-H in plane deformation. The bands at 689 cm^{-1} in FTIR spectrum and $566\text{-}250\text{ cm}^{-1}$ in FT

Raman spectrum are due to C-H out of plane deformation/C-C=O deformation.

Figure 2: FT Raman spectra of Polymeric Nanoparticle Paclitaxel Drug

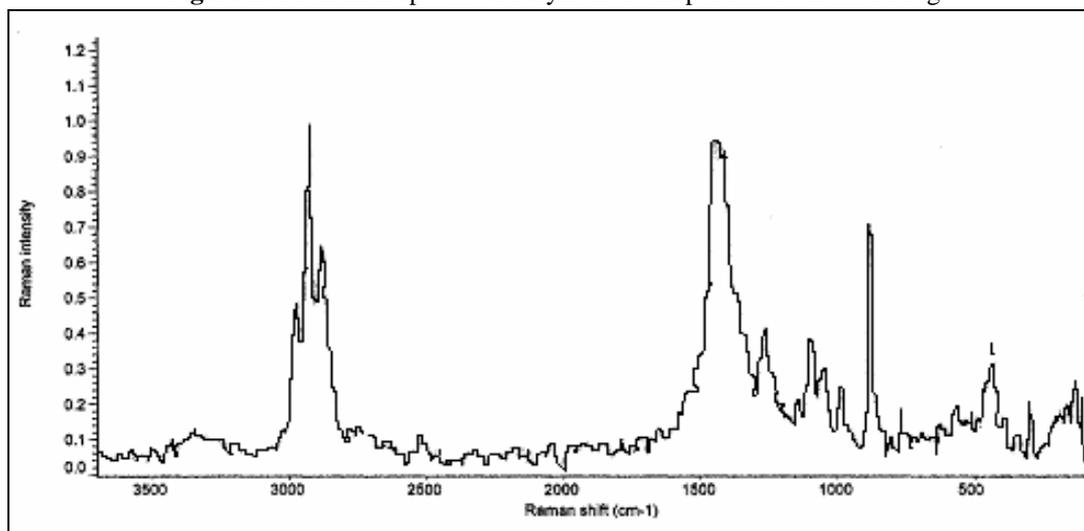


Figure 3: FTIR spectra of Polymeric Nanoparticle Paclitaxel Drug

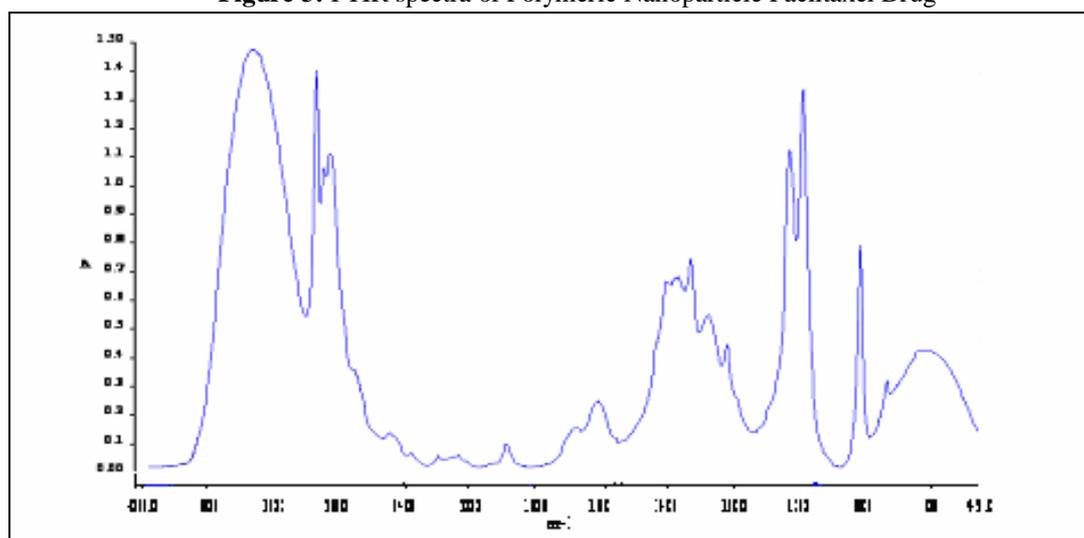


Table 1: Vibrational band assignments of Nanoparticle Paclitaxel drug

Frequency in cm-1		Assignments
FTIR	FT Raman	
3339	3319	N-H/O-H stretching
2973-2541	2971-2519	CH ₃ /C-H stretching
1727-1720	1727-1714	C=O stretching
1652-1579	1608-1581	C-C stretching
1380-1330	1428-1336	CH ₃ deformation
1274	1264	C-N stretching
1090-1049	1093-1050	C-O stretching
941-803	944-804	C-H in-plane deformation
689	566-250	C-H out-of-plane/C-C=O deformation

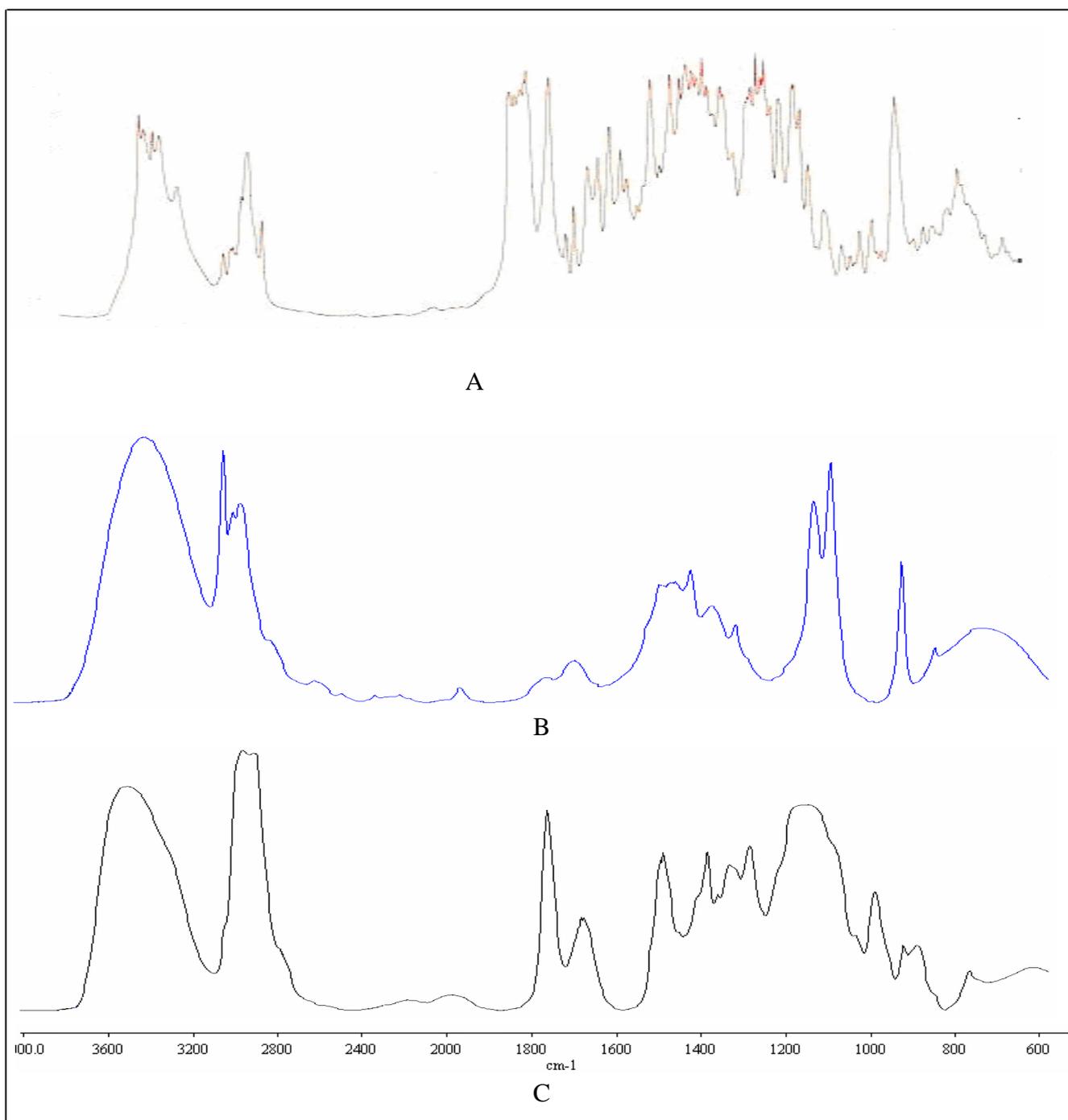


Figure 4: (A) Pure Paclitaxel drug (B) polymeric nanoparticle Paclitaxel drug (C) solvent based Paclitaxel drug

MATERIALS AND METHOD

Characterization of polymeric nanoparticle drug and solvent based cremophor paclitaxel drug using FTIR analysis

Paclitaxel is a off-white crystalline powder procured from Dabur Pharma, India whereas polymeric nanoparticle Paclitaxel drug is available in market as Nanoxel® from Dabur Pharma and castor oil-solvent based Paclitaxel is also commercially available as oncotaxel from Sun Pharmaceuticals.

FTIR spectra were obtained for pure, polymeric nanoparticle drug and solvent based paclitaxel drug.

Spectra of Paclitaxel was obtained using KBr disc method while spectra for polymeric nanoparticle drug and castor oil-solvent based paclitaxel drug of pharmaceutical formulation were obtained directly using Perkin Elmer FTIR Spectrometer in the region 4000-400 cm^{-1} .

RESULTS AND DISCUSSION

FTIR analysis measures the selective absorption of light by the vibration modes of specific chemical bonds in the sample. The observation of vibration spectrum of polymer nanoparticle drug allows evaluating the kind of interaction occurring between the drug and polymer, because the vibrations of the atoms involved in this interaction can

suffer alterations in frequency and intensity (Silverstein et al., 1994). Fourier transform infrared analysis was conducted to verify the occurrence of chemical bonds between drug and polymer. Fig 4a shows the FTIR spectrum of pure Paclitaxel, Fig 4b for polymeric nanoparticle drug and 4c for castor oil-solvent based paclitaxel drug. The spectral analysis indicated that the specific functional groups of polymeric nanoparticle drug material (fig 4b) and solvent based paclitaxel drug (fig 4c) have almost the same chemical characteristics of the pure drug (fig 4a) and shows their main characteristics peaks. The study suggests that did not occur molecular interactions that could alter the chemical structure of the drug. Hence the study indicates that, the chemical structure of the drug is likely to be unaffected due to the addition of excipients of the drug.

CONCLUSION

Paclitaxel is an anti-leukemic, anti-tumor or in general an anti-neoplastic agent. An attempt has been made in this work to study the vibrations of the functional derivatives in polymeric nanoparticle Paclitaxel drug. By observing the position, shape and relative intensities of the vibration bands in FTIR and FT-Raman spectra of the drugs a satisfactory vibration band assignment has been made. Fourier transform infrared (FTIR) analysis was conducted to verify the occurrence of chemical bonds between the pure paclitaxel drug, castor oil solvent-based paclitaxel drug and polymeric nanoparticle paclitaxel drug. The spectral analysis indicated that the specific functional groups of the polymeric nanoparticle drug material and solvent based paclitaxel drug have almost the same chemical characteristics of the pure paclitaxel drug. The studies suggest that did not occur molecular interaction that could alter the chemical structure of the drug. The stability study indicates that, the chemical structure of the drug is likely to be unaffected due to the excipients of the drug.

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