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Spectral Analysis of Drug Loaded Nanoparticles for Drug-Polymer Interactions

Gurpreet Singh*, Abdul Faruk, Preet Mohinder Singh Bedi

Department of Pharmaceutical Sciences, Guru Nanak Dev University, Amritsar, Punjab-143005, India.

ABSTRACT

PLGA [Poly (lactic-co-glycolic acid)] is a one of the widely used biodegradable polymer. The extensive use of PLGA is due to its biocompatible properties and is also approved by FDA and European Medicine Agency in parenteral drug delivery system. Chitosan (CS) is also an extensively used natural polymer in the field of medicine. It has been well documented as a potential drug carrier due to its biocompatibility. Various reports have also suggested the role of chitosan in formulation of nanoparticles to increase the drug bioavailability and efficacy. The characterizations of these systems pose interesting analytical challenges. Fourier Transform Infrared (FTIR) technique helps to analyze the adsorption of functional groups on nanoparticles and also to investigate the drug polymer interactions. X-ray powder diffraction (XRD) analysis helps in detection of crystallinity of drugs and polymers on basis of diffraction patterns. This study investigates the characterizations of methotrexate (MTX) and Fluorouracil (5-FU) loaded nanoparticles of PLGA and chitosan with help of FTIR and XRD techniques.

Keywords: PLGA, XRD, MTX, Chitosan, FTIR, 5-FU

*Address for Correspondence:

Gurpreet Singh, Department of Pharmaceutical Sciences, Guru Nanak Dev University, Amritsar Punjab-143005, India.

1. INTRODUCTION

The preformulation studies are of great importance in the design of a new drug formulation and its quality control. It helps in the assessment of compatibility between the drug and excipients[1]. In the formulation of nanoparticles various polymers are used and therefore their interaction with the drug should be assessed. The incompatibility may affect the chemical nature, stability and bioavailability of drug along with the therapeutic efficacy and safety of formulation[2]. Various techniques are used now days for the assessment of compatibility between drug and polymers. Fourier Transform Infrared (FTIR) and X-ray powder diffraction (XRD) are most widely used analytical techniques for the compatibility assessment of drug and polymers due to the hypothesis that same functional groups may change during the drug-polymer interaction. The spectrum of FTIR represents the molecular absorption and transmission, two different molecular structures cannot produce the same infrared spectrum. The information can be used for identification of unknown drugs, polymer nature and drug excipient interaction along with their quality and purity.

FTIR is preferred over dispersive or filter methods of infrared spectral analysis, as it is a non-destructive technique[3-6]. XRD technique is a non-destructive technique and required minimal sample. This technique is most commonly used for identification of crystalline material by their unique diffraction patterns of pharmaceutical solids /drugs for both scientific and drug regulatory purposes[7]. This work involves the preparation and characterization of MTX and 5-FU loaded PLGA and Chitosan nanoparticles with FTIR and XRD methods.

2. MATERIALS AND METHODS

2.1 Materials

Methotrexate (MTX) was obtained as a gift sample from Naprod Life Sciences (Mumbai), 5- Fluorouracil (5-FU) and Chitosan were purchased from Himedia, India, PLGA from Sigma Aldrich, India, Sodium Tripolyphosphate (TPP) and polysorbate-80 were procured from CDH chemicals Laboratory, New Delhi. All other reagents were of analytical grade.

2.2 Methods

2.2.1 Preparation of Chitosan (CS) nanoparticles for MTX and 5-FU

The preparation of CS nanoparticles is based on an ionic interaction between positively charged CS solution and negatively charged sodium tripolyphosphate (TPP)

solution in the presences of polysorbate-80 (1% w/v) as re-suspending agent to prevent aggregation as reported by calvo et al., 1997[8]. Chitosan was dissolved in 3% acetic aqueous solution at concentrations (2 mg/mL) under magnetic stirring at room temperature, and TPP was dissolved in distilled water with various concentrations (0.2, 0.4, 0.6, 0.8, 1.0 mg/mL) as described in Figure 1.
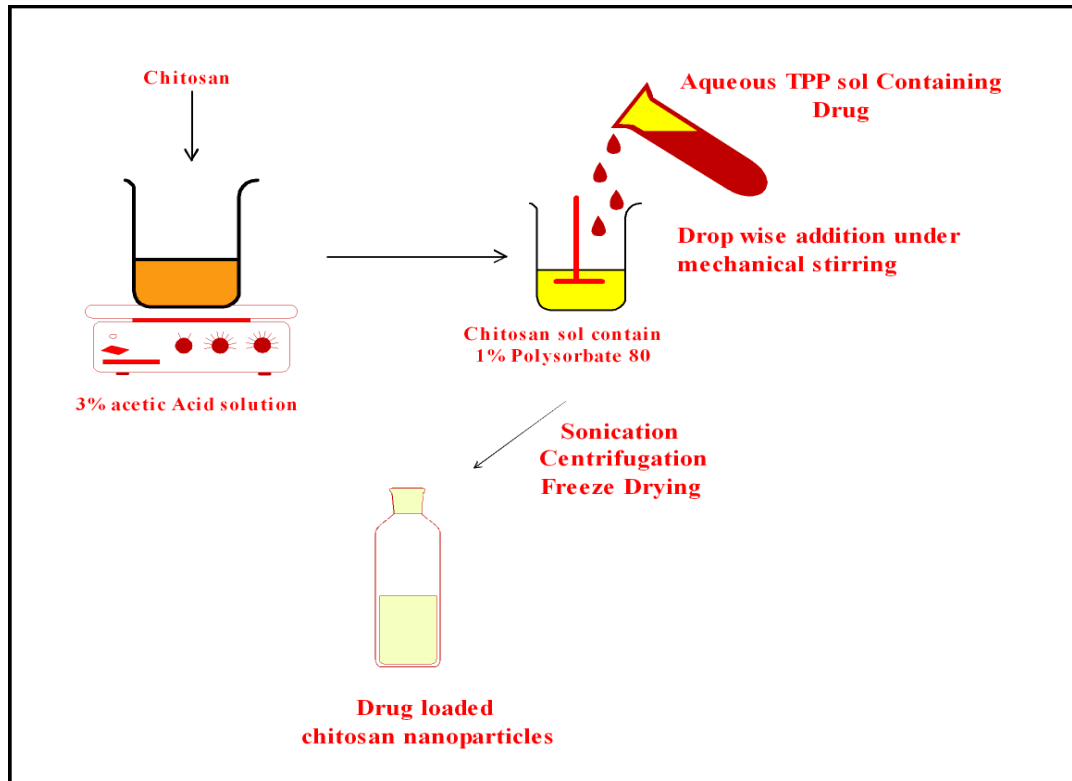

Figure 1: Schematic diagram of preparation of chitosan (CS) nanoparticles

2.2.2 Preparation of PLGA nanoparticles for MTX and 5-FU

PLGA nanoparticles were prepared by emulsification sonication-solvent evaporation method as reported by Budhian *et al.*, 2007[9]. This method involves preparation of an organic phase consisting of polymer (PLGA) and drug (MTX or 5-FU) dissolved in DCM (typical volume, 5 ml)

process represented in Figure 2. Then, resulted solution drop wise added to aqueous solution of surfactant. In presented study, Polysorbate-80, polyvinyl alcohol and poloxamer188 were investigated as stabilizers for the preparation of nanoparticles of MTX loaded PLGA nanoparticles by emulsification sonication-solvent evaporation method.
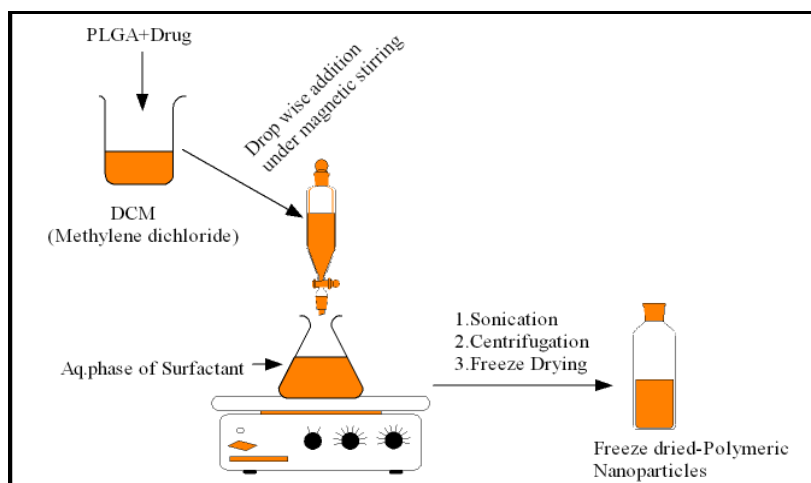

Figure 2: Schematic diagram of preparation of PLGA by emulsification sonication-solvent evaporation method

2.2.3 Fourier Transform Infrared spectroscopy (FT-IR) analysis

FT-IR spectra of drugs (MTX and 5-FU) were recorded using Shimadzu-8400, Japan FTIR spectrometer by KBr pellet method in the region 400 cm^{-1} to 4000 cm^{-1}. Samples equivalent to 2 mg of drugs were mixed with potassium bromide (about 100 mg) in a clean glass pestle and mortar and were compressed to obtain a pellet. Baseline was corrected and the samples were scanned against a blank[10].

2.2.4 X-Ray Diffraction (XRD) analysis

X-ray powder diffraction measurements were carried out on drugs using a diffractometer (FOCUS D 8, Bruker, USA). The results were recorded over a range of 5–50° (2θ)[11].

3 RESULTS AND DISCUSSION

3.1 Preparation of PLGA and Chitosan nanoparticles for MTX and 5-FU

MTX and 5-FU loaded Chitosan nanoparticles were prepared by Ionotropic gelation. The preparation of nanoparticles by this technique, particle morphology can be modified by selecting different variables such as agitation speed, polymer concentration and sonication time. In preliminary experiments, MTX and 5-FU loaded chitosan nanoparticles were prepared by using different concentrations of TPP (0.2, 0.4, 0.6, 0.8, 1.0 mg/mL) as gelling counter ion agent. The size of nanoparticles depended upon concentration of polymer and stabilizer. Various formulations were prepared using these variables and evaluated in terms of stability of nanoparticles during storages. Those combination of formulations were rejected which showed instability and trace of aggregation of particles during storage. Only stable formulations were evaluated for FTIR and XRD studies. Selected formulation have particle size ranges from 282.6± 13.14 to 289.± 12.13 in case of MTX and 219.6± 13.14 to 327.8± 17.42 in case of

5-FU. While in case of MTX and 5-FU PLGA loaded nanoparticles prepared by emulsification sonication-solvent evaporation method particle size ranges from 160.43 ± 33.34 to 290.21 ± 15.11 for MTX and 189.43 ± 14.34 to 310.21 ± 14.11. The morphology of particles depends upon type and concentration of surfactant used for preparation of nanoparticles.

3.2 Fourier Transform Infrared Spectroscopy (FTIR)

Infrared spectroscopy is an essential and crucial characterization technique to elucidate the structure chemical composition and the bonding arrangement of constituents in polymeric materials. IR spectrum of distinct functional groups of MTX, 5-FU, PLGA, Chitosan, physical mixture and drug loaded nanoparticles exhibits molecular vibrations of functional groups as shown in Figures 3a-3c as illustrated in Table 1 and main functional groups listed in Table 2a-2d. The FTIR spectra of MTX characteristic absorption band appeared at wave numbers 3390.63 cm^{-1} (-NH stretch), 1681.81 cm^{-1} (-COOH), 1647.1 cm^{-1} (-CONH), 854.2 cm^{-1} (Aromatic stretch out- of- plane bend). While in case of 5-FU absorption bands in the region of 3000-2900 cm^{-1} represents C-H stretching, bands in the region 1429-1660 cm^{-1} represents the C=N and C=C ring stretching vibrations. The bands at about 1348.15 cm^{-1} were vibration of the pyrimidine compound; bands at 1182.28 cm^{-1} and 1245.15 cm^{-1} were assigned to the C-O and C-N vibrations, respectively. Other absorption peaks includes 3137.97 cm^{-1} (-NH Stretch), 1722.31 cm^{-1} and 1658.67 cm^{-1} (-C=O Stretch), 1245.15 cm^{-1} (CH in Plane deformation), 813.9 cm^{-1} (CH out of plane deformation).

In case of chitosan, the characteristic absorption band appeared at 1589.23 cm^{-1}, which is represented the stretching vibration of amino group of chitosan. Another band at 3413.77 cm^{-1} is due to amine NH symmetric vibration.

Table 1: Summarize peaks of FTIR spectra of Drugs (MTX, 5-FU), polymer (PLGA, Chitosan) and nanoparticles formulations

MTX	5-FU	CS	PLGA	MTX PLGA NPs	MTX PLGA Physical Mix	5-FU PLGA NPs	5-FU PLGA Physical Mix	CS-MTX NPs	CS-MTX Physical Mix	CS-FU NPs	CS-FU Mix
3411.84	**3137.97**	3436.91	3541.06	**2935.46**	3460.06	3411.12	2829.38	**3402.27**	2351.23	3400.27	1986.54
3390.63	3066.61	**3413.77**	2997.17	**2360.71**	**3066.61**	3390.11	1986.54	1733.89	1681.1	3284.55	1893.97
3363.62	**3028.6**	**2875.67**	2948.96	**1674.1**	**2918.1**	**2829.2**	1893.97	**1647.1**	**1645.17**	2725.23	1724.24
2935.46	**2999.1**	2362.64	2117	**1647.1**	**2358.78**	2362.56	1650.95	1419.51	1600.81	2339.49	750.26
2358.78	**2931.6**	1670.24	1758	1463.87	**2329.85**	**1681.81**	1504.37	1282.57	1494.73	1737.74	642.25
1681.81	2885.31	**1589.23**	1625	1456.16	2262.35	**1647.1**	**1448.44**	1080.06	1207.36	**1650.95**	**1658.67**
1647.1	2829.38	**1157.21**	1456.16	1085.85	**1683.74**	1602.74	**1429.15**	1018.34	1099.35	1417.58	1245.93
1600.81	1986.54	**1024.13**	1394.44	1022.2	**1647.1**	1541.2	1429.15	929.63	**831.26**	**1348.15**	1429.15
1496.66	**1722.31**	989.41	1384.79	931.55	1600.81	1496.66	**1348.15**	881.41	767.62	1280.65	**1348.15**
1207.36	**1670.24**	**892.98**	1276.79	889.12	1496.66	**1448.44**	**1245.93**	**864.05**	578.6	1259.43	995.2
854.2	**1658.67**	669.25	1182.28	**873.69**	1404.08	1330.79	1224.71	705.9		1080.06	813.9
831.26	**1504.37**		1091	715.54	1207.36	1249.79	955.2	630.68		881.41	
	1448.44		754	626.82	1099.35	1207.36	879.48			630.68	
	1431.08				853.03	1099.35	813.9				
	1348.15				831.26	831.26	750.26				
	1245.15				767.62	767.62	642.25				
	1182.28				580.53	578.6	551.6				
	813.9										

MTX-Methotrexate, 5-FU-:5-Fluorouracil, PLGA-:Poly (lactide-co-glycolide), CS-Chitosan, Physical Mix-Physical mixture, NPs: Nanoparticles

The peak of 2875.67 cm-1 is typical C-H vibration. The peaks around 892.98 and 1157.21 cm⁻¹ correspond to saccharide structure of chitosan. The broad peak at 1024.13 indicates C-O stretching vibration similar result reported in literature[12-13]. In FTIR spectra of PLGA intense bands observed in the region between 1770 and 1750 cm⁻¹, are attributed to the stretching vibration of the carbonyl groups present in the two monomers. Medium intensity bands between 1300 and 1150 cm⁻¹ were attributed to asymmetric and symmetric C-C(=O)-O stretches

respectively. The bands in these regions are useful in the characterization of esters. Bands at 3500 cm⁻¹ and 3450 cm⁻¹ in the FTIR spectra for lactide and glycolide are attributed to stretching vibrations of OH group[14]. The characteristics peaks of drugs in both the methods of polymeric nanoparticles were diminished or shifted when compared with pure drug peak at same wave number but not in physical mixture. This indicated that the drug is interacting with polymer.
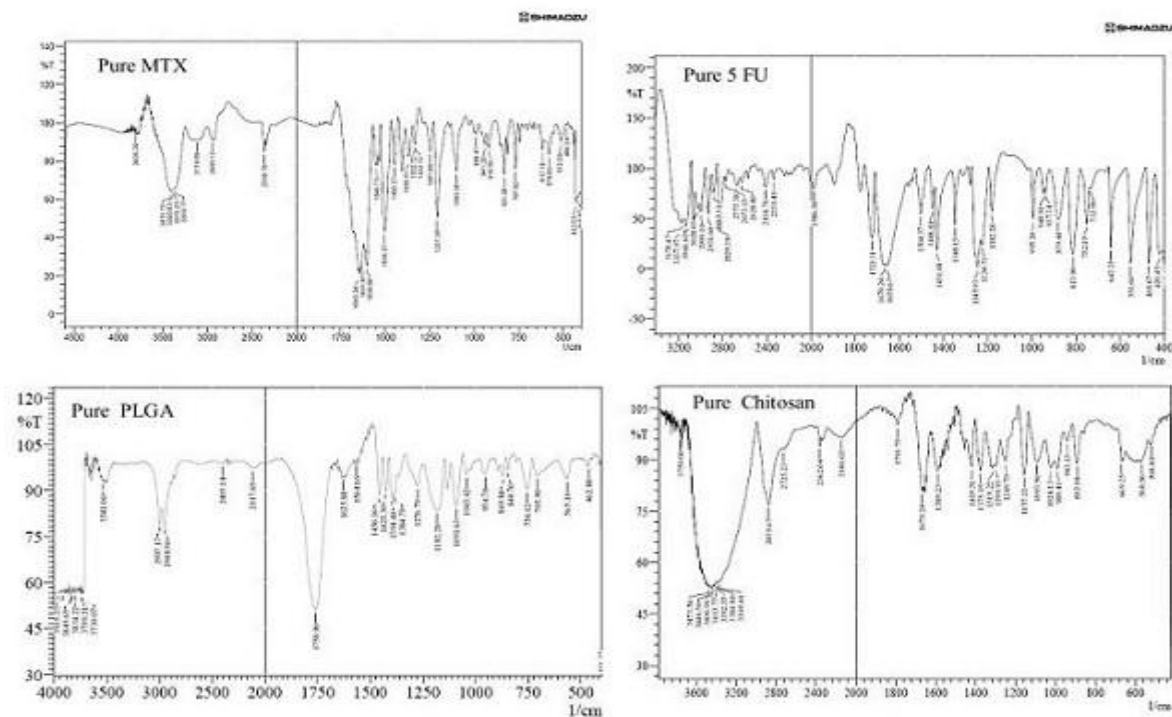

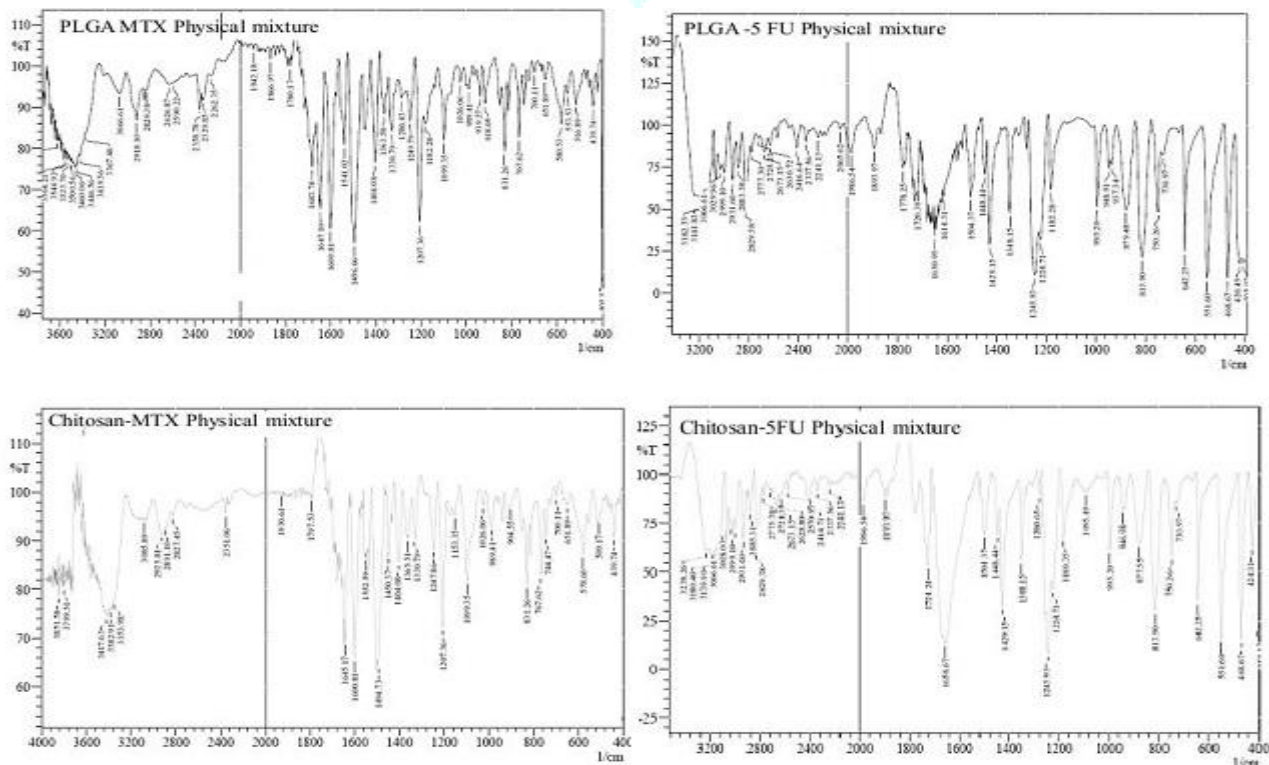Figure 3a: FTIR spectrum of Pure MTX, 5-FU, PLGA and Chitosan

Figure 3b: FTIR spectrum of Physical mixture of MTX, 5-FU, PLGA and Chitosan
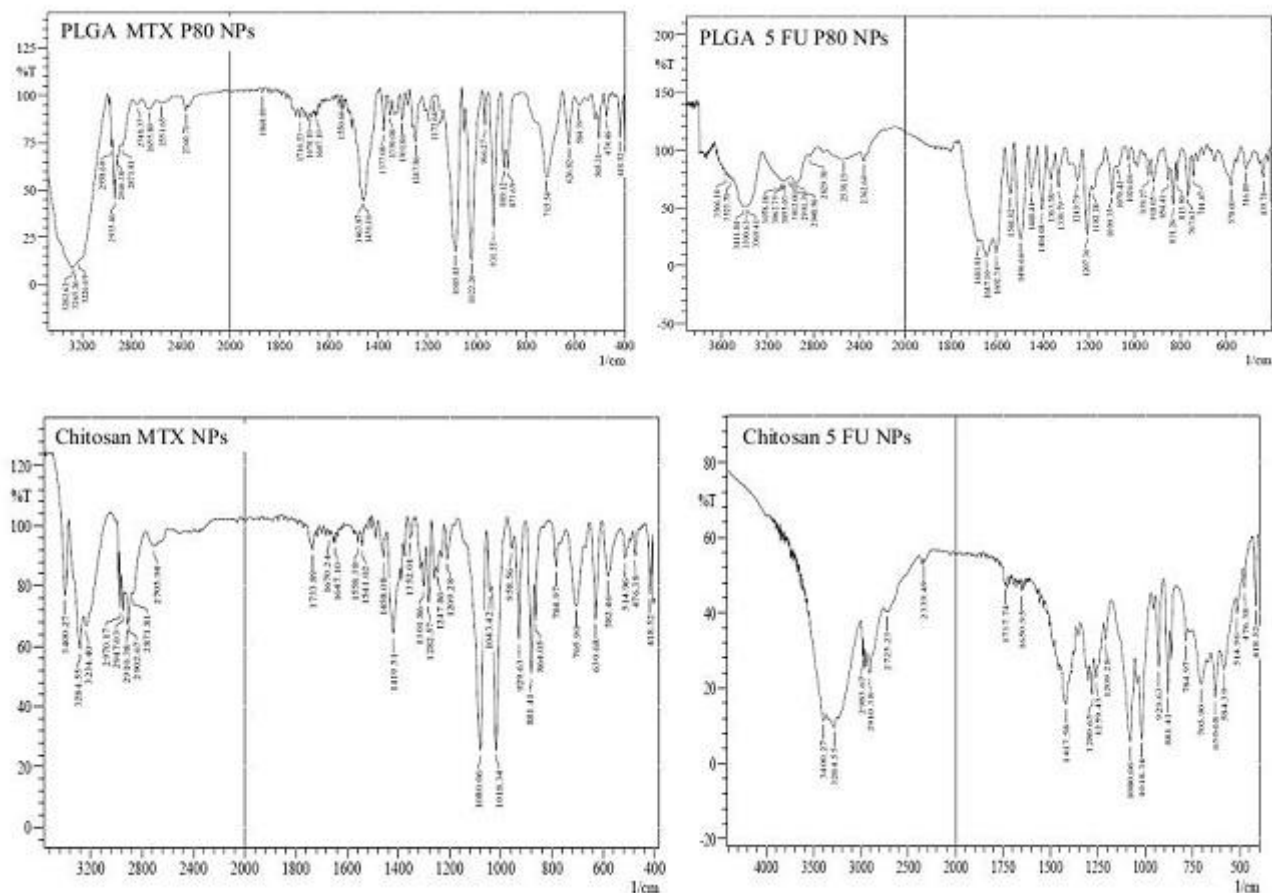
Figure 3c: FTIR spectrum of PLGA and Chitosan NPs of MTX and 5-FU

Table 2a: FTIR peaks of functional groups of MTX

Functional group	Corresponding bands (cm^{-1})
-NH stretch	3464.82
-COOH	1683.4
-CONH	1645.79
Aromatic stretch out- of- plane bend	853.588

Table 2b: FTIR peaks of functional groups of 5-FU

Functional group	Corresponding bands (cm^{-1})
C-H stretching	3000-2900
C= N and C =C ring stretching vibrations	1429-1660
vibration of the pyrimidine compound	1348
C-O	1180
C-N	1246
C=O Stretch	1716 cm^{-1} and 1657 cm^{-1}
CH in Plane deformation	1245
CH out of plane deformation	813

Table 2c: FTIR peaks of functional groups of PLGA

Functional group	Corresponding bands (cm^{-1})
OH end group	3450-3500
C-H stretches	2885- 3010
C=O stretch	1762.6
C-O stretch	1186-1089
C-H Bends	1450-850

Table 2d: FTIR peaks of functional groups of Chitosan

Functional group	Corresponding bands (cm^{-1})
Vibration of amino group	1589.23
Amine NH symmetric vibration and H bonded O–H group	3413.77
C-H vibration	2875.67
O-H, NH$_2$	3400–3800 cm
Saccharide antisymetric C-O stretching	900–1200

3.3 X-Ray Diffraction

X-ray diffraction was carried out to evaluate the crystalline character of MTX, 5-FU, PLGA, Chitosan and prepared nanopartilces by both methods. XRD analysis of drug, polymer and drug loaded nanoparticles were performed and illustrated in Figures 4a and 4b respectively. The presence of sharp and intense peaks in the diffractogram of Pure 5-FU indicated its crystalline nature while in case of MTX no sharp peaks, indicated about its amorphous in nature. The physical mixture of drug and drug loaded NPs resulted in a relatively less crystalline form and exhibited less intense peaks at Pure MTX shown the characteristic intense peaks at 2θ of approximately at 9.38, 15.6, 19.48, 22.48, 27.02 and 32.82 and Pure 5-FU shown the characteristic intense peaks at 2θ of approximately at 16.42, 20.74, 22.02, 28.82, 33 and 34.24. It was clear that physical mixture showed partially sharp crystalline peaks, representative of the characteristics of a molecular compound with some crystallinity, whereas a broad peak was presented in polymeric NPs, indicating that NPs were amorphous and lack crystalline peaks. A decrease in the intensity of the peaks was explained by a lower loading compared to pure drugs. Results indicating that the drugs were encapsulated within the NPs and suggesting that MTX and 5-FU in the NP matrix was molecularly dispersed or in the amorphous form.
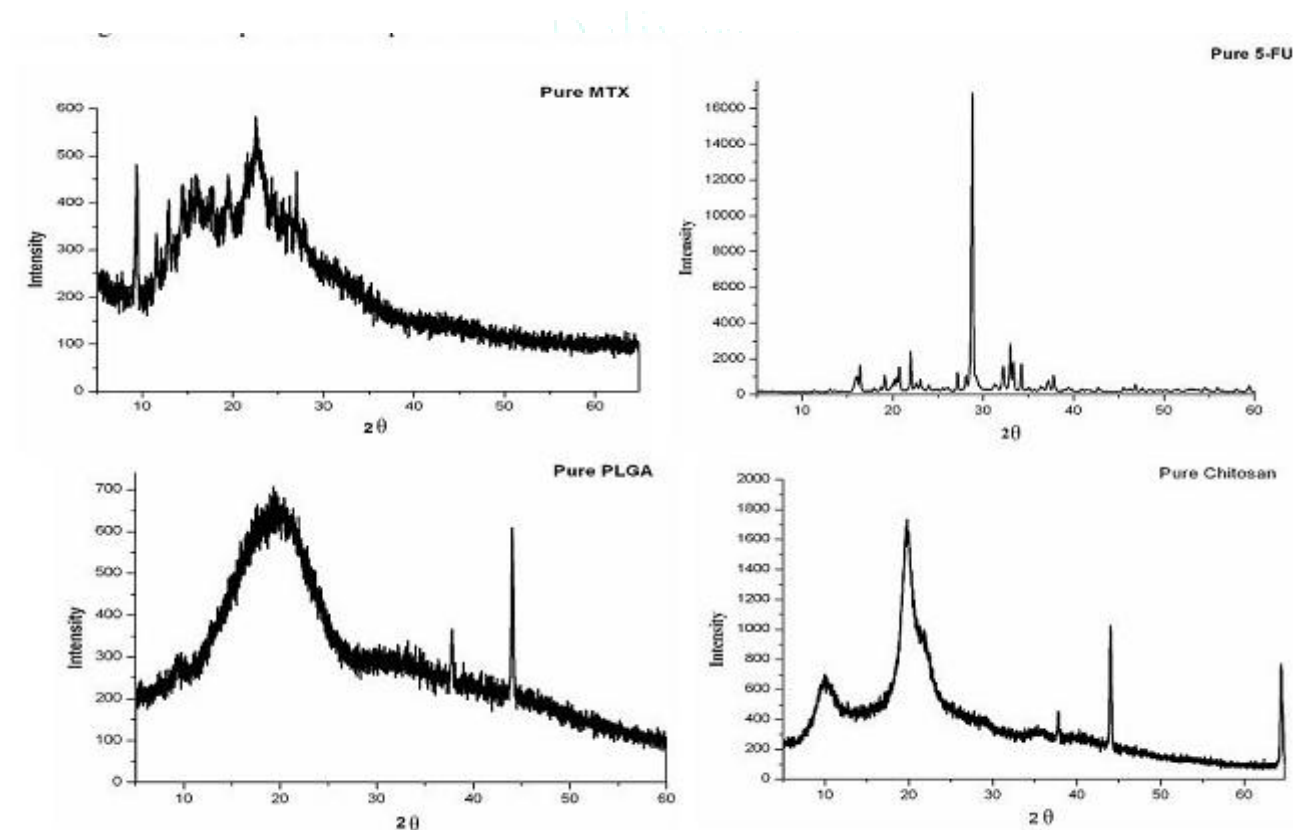

Figure 4a: X-ray diffraction pattern of Pure MTX, 5-FU, PLGA and Chitosan
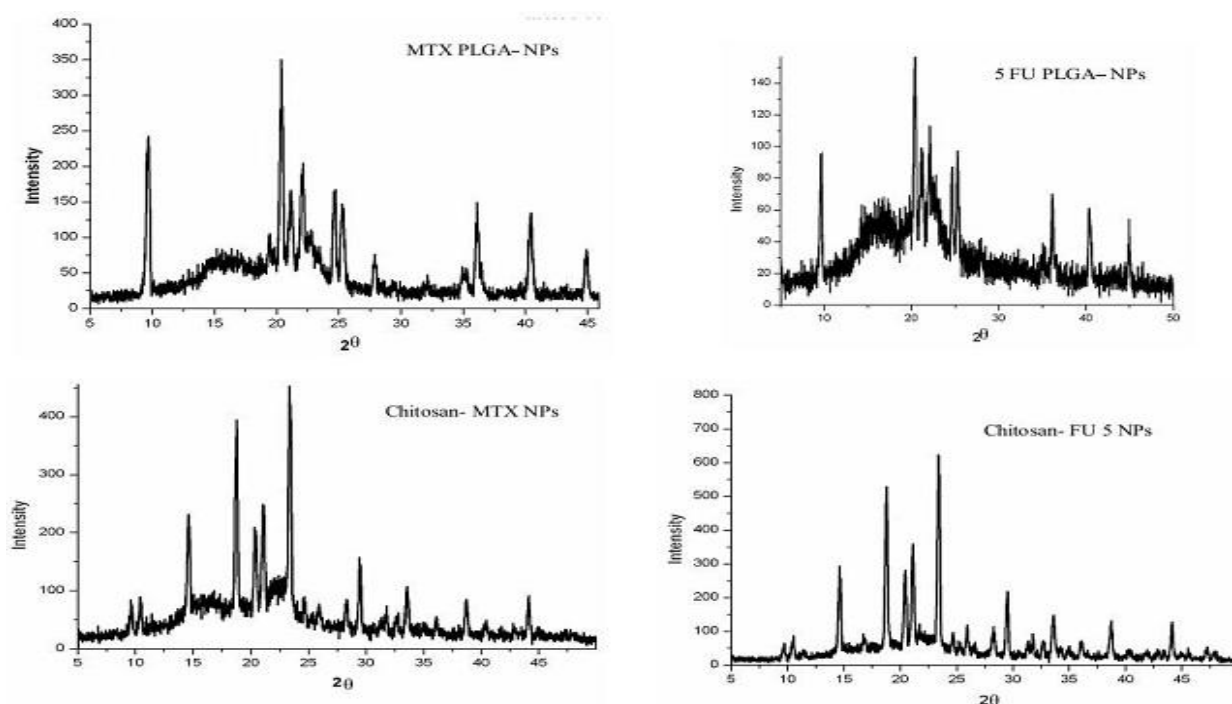
Figure 4b: X-ray diffraction pattern of PLGA and Chitosan NPs of MTX and 5-FU

4 CONCLUSION

Drug polymer characterization studies were carried out by FTIR and XRD techniques for PLGA and 5-FU loaded nanoparticles of MTX or 5-FU. All the characteristic peaks and band values of FTIR studies of pure MTX or 5-FU compared with, physical mixture and formulated nanoparticles to confirm integrity of peaks. Results confirmed that the characteristic peaks of FTIR spectra of MTX and 5-FU disappeared in nanoparticles formulations and similar results obtained in XRD studies in which less intense peaks appeared for both drugs as compare to XRD of pure drugs. The intense peaks indicative drugs existed as the crystalline form which could be observed from spectra of alone drugs. While in case of NPs loaded formulations the characteristic diffraction peaks of both drugs disappeared, which was also revealed with results obtained from FTIR studies.

CONFLICTS OF INTEREST: Nil

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Author's Detail:

Gurpreet Singh

Department of Pharmaceutical Sciences, Guru Nanak Dev University, Amritsar Punjab-143005, India.
E-mail: gurpreet_pharma85@yahoo.com
ORCID ID: 0000-0001-5436-2697
Researcher ID: D-9909-2014
Scopus Author ID: 56166600300
Mobile: +91-9814085601

Dr. Abdul Faruk

Presently, Head, Department of Pharmaceutical Sciences, HNB Garhwal University (A Central University) Chauras Campus, P.O. Kilkeleshwar, Via Kirtinagar Distt. Tehri Garhwal-249161 Uttrakhand
E-mail: abdul_faruk@yahoo.com, Mobile: +91-9412079188, +91-9456348123

Prof. Preet Mohinder Singh Bedi

Head, Department of Pharmaceutical Sciences, Guru Nanak Dev University, Amritsar, Punjab 143005, India
E-mail: bedi_preet@yahoo.com, Mobile: +91-9815698249