CHAPTER 3

MATERIALS AND METHODS
ABSTRACT

The structure, physical and chemical properties of the molecules taken for investigations, the antibiotic Doxycycline hyclate, Human insulin actrapid, unsaturated fatty acids - linoleic acid, oleic acid and ricinoleic acid and the saturated fatty acids - stearic acid, palmitic acid and myristic acid were outlined. The properties of the solvents, water and ethyl methyl ketone were also provided in brief. The procedure adopted for the preparations of solutions and blends and the experimental procedures of the techniques were provided.
MATERIALS AND METHODS

3.1. INTRODUCTION

The present investigation is to identify the specific interactions between the antibiotic doxycycline hyclate and bio-molecules in aqueous and non-aqueous environment at room temperature and at physiological temperatures. It is preferable to know the structures and structural behaviours of these molecules before employing techniques to identify the specific interactions. The physicochemical properties influence the absorption, distribution, metabolism and some of the intra and intermolecular interactions of bio-molecules.

3.2. MATERIALS SELECTED

3.2.1. NITRO CELLULOSE (NC)

Nitrocellulose is a widely used cellulose derivative in the protective and decorative lacquer coating application. Low temperature flexibility \(^1\) can be improved by combining nitro cellulose with suitable polymers. The nitro cellulose is the only cellulose inorganic ester in which the hydroxyl group of cellulose is replaced by the radical \(-\text{NO}_2\). Industrial lacquer finishes constitute the largest market for nitrocellulose\(^2\). The regularly soluble (RS-type) type nitro cellulose which contains 11.2-12.2\% nitrogen is soluble in ketones, esters and ether-alcohol mixtures. The alcohol soluble type (AS type) nitro cellulose (with 11.3-11.7 \% nitrogen) is soluble in the same type of solvents as RS-type. The spirit-soluble (SS type) type nitro cellulose with 10.9-11.2\% nitrogen in soluble in alcohols and is more thermoplastic than the other types. The development of stable nitro cellulose with lower viscosity resulted in
nitrocellulose in lacquer coatings depend upon the solvent\[^{[3]}\]. The structure of polymer nitrocellulose is shown in figure 3.1.

![Figure 3.1. Structure of Nitrocellulose](image)

In the present investigation, the cellulose nitrate manufactured by Ashahi chemical industry, Japan is used where the nitrogen content of the material is 11.8-12.2%.

### 3.2.2. POLY (METHYL METHACRYLATE) (PMMA)

Most important among the acrylic plastics is Poly (methyl methacrylate) (PMMA) prepared by the polymerization of methyl methacrylate. This transparent thermoplastic is often preferred because of its moderate properties, easy handling and processing and low cost; it has high impact strength than of glass. But it is softer and more easily scratched than glass. It is readily miscible with some of the other polymers to employ them in some important applications. It has poor resistance to solvents, as it swells and dissolves easily.

Density -1.19 g/cm\(^3\), melting point – 130-140 °C, boiling point - 200°C and glass transition temperature - 114°C are the some of the other properties of PMMA. PMMA represented by formula as \((C_5O_2H_8)_n\) and has the molecular weight of
major and the most important homo-polymer in the series of acrylics with sufficient high glass transition temperature has been material of choice for IOL for nearly four decades \cite{51}. The structural representation is shown in figure 3.2.

\[
\begin{array}{c}
\text{CH}_3 \\
| \\
\text{C} - \text{CH}_2 \\
| \\
\text{C} - \text{CH}_2 \\
| \\
\text{C} = \text{O} \\
| \\
\text{OCH}_3 \\
\end{array}
\]

\[
\begin{array}{c}
\text{CH}_3 \\
| \\
\text{C} - \text{CH}_2 \\
| \\
\text{C} - \text{CH}_2 \\
| \\
\text{C} = \text{O} \\
| \\
\text{OCH}_3 \\
\end{array}
\]

$n$

Figure 3.2. Structure of Poly (methyl methacrylate)

Poly (methyl methacrylate) is a widely used support medium for the embedding of intact, undecalcified bone\cite{61}. In the present investigation, the pure methyl methacrylate supplied by S. D. fine chemicals, India, is polymerised to Poly (methyl methacrylate) and used.

3.2.3. ANTIBIOTICS TETRACYCLINE

Antibiotics are microbial metabolites that in high dilution can inhibit the growth of microorganisms \cite{7}. For more than 60 years, the antibiotic tetracyclines and several of its derivatives have been widely applied. Tetracycline inhibits bacterial protein synthesis. They acts as growth inhibitors (bacteriostatic) rather than killer of the infectious agent (bacteriocidal) and are effective against multiplying the microorganisms. The basic structure of tetracycline is shown in figure 3.3.

The basic chemical structure of tetracycline consists of a hydronaphthacene nucleus containing four hexacyclic DCBA rings. Along the lower periphery, bioactive tetracyclines possess a complex series of oxygen functional groups: a phenol group on
between rings C and B, and a tertiary hydroxyl group at position 12a at the ring BA juncture. The A ring oxygen functional groups comprise a unique molecular substructure as a tri-keto-enol system, spread across carbons C1-C3, where the exocyclic carboxamide group attached at position C2 is also required for bioactivity. As a rule, concerning structure-activity relationship, most synthetic modifications along the lower peripheral region of tetracycline greatly decrease biological activity, as both antibiotics and non-antibiotics. Different intramolecular hydrogen bonds between the substituent of ring A are formed. The intramolecular hydrogen bonds are formed on the BCD fragment between O11, OH10 and OH12. Acceptors of intermolecular bonds are O1, O2 and O12a, where as N411, OH6 and OH12a act as donor groups. Since, most of the non-antibiotic targets show greater activity with 4-dedimethyl tetracycline derivatives, a first consideration in describing tetracycline chemistry is to compare the molecular structures of tetracycline with and without the 4-dimethyl amino group.

Modification of the molecular functional groups and substituent within the upper peripheral region may drastically alter the bioactivity of a tetracycline, by changing its basic physicochemical attributes such as molecular size and shape, charge development, electron density and polarity. Changes in molecular structure lead to changes in solubility in biological fluids, lipophilicity and binding affinity with its macromolecular target. Most of the non-antibiotic targets show greater activity with 4-dedimethyl tetracycline derivatives. Truly one of the most interesting and novel uses of semi synthetic tetracycline like doxycycline hyclate found recently is as inhibitors of non-antibiotic targets such as mammalian enzymes and metabolic processes [8].
Figure 3.3. Basic structure of Tetracycline

<table>
<thead>
<tr>
<th>Generic Name</th>
<th>R¹</th>
<th>R²</th>
<th>R³</th>
<th>R⁴</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetracycline</td>
<td>H</td>
<td>CH₃</td>
<td>OH</td>
<td>H</td>
</tr>
</tbody>
</table>

Figure 3.4. Structure of Doxycycline Hyclate

Figure 3.5. Tautomeric form of the amide substituent in the structure of Doxycycline hyclate
The macromolecule antibiotic doxycycline is a broad spectrum semi-synthetic, antibacterial tetracycline is manufactured from oxytetracycline. Doxycycline is available in several different salts (Hyclate, monohydrate, carrageenate). But their pharmacokinetics parameter do not significantly differ under standard condition. Doxycycline was first introduced into clinical practise in 1968 as the HCl salt called doxycycline hyclate. The structural formula of doxycycline hyclate is shown in figure 3.4. Its empirical formula is $C_{22}H_{24}N_2O_8 \cdot HCl \cdot 1/2C_2H_5OH \cdot 1/2H_2O$ and has a molecular weight of 512.94. Its chemical name is doxycycline hydrochloride hemihydrates hemiethanolate. The chemical difference between the tetracycline and semi synthetic derivative doxycycline hyclate is by the substituent of OH instead of H at $C_5$ carbon atom. Of the antibiotics, doxycycline hyclate exhibits very strong characteristic behaviour not only as a bioactive agent but also act as a chemotherapeutic drug.

The degree of potentiation of insulin hypoglycaemia was higher with doxycycline hyclate as compare to other derivatives of tetracycline \textsuperscript{[9]}. doxycycline hyclate is a light-yellow crystalline powder which is soluble in water. It involves in interaction with different conformations in aqueous (water) and nonaqueous (organic solvent) environments. This behavioural interaction places the doxycycline hyclate as a special and significant drug. Absorption of doxycycline hyclate is almost complete (95%) when compared to other tetracycline but its absorption is reduced due to the intake of fatty substances along with the antibiotic \textsuperscript{[10]}. doxycycline hyclate is more lipophilic than compare to other tetracycline antibiotics and is widely distributed in the tissues. High concentrations are found in renal tissue and gall bladder/bile. It is not metabolites in human \textsuperscript{[11]}. As per the literature \textsuperscript{[12]}, about 70% of the antibiotic doxycycline hyclate is excreted through faeces and urine. Doxycycline hyclate is
fairly reliable absorption and its long half-life, which permit smaller dosage. Doxycycline hyclate is much more soluble than other tetracycline, which is one of the main reasons for its more frequent use in pharmaceutical samples.\textsuperscript{[13]}

The protonated/deprotonation behaviour of tetracycline is of major importance in understanding their pharmacokinetic properties because an orally administered drug will most likely change its state of protonation. This change of protonation influences the drug's solubility in an aqueous environment and its permeability through membranes.\textsuperscript{[14]} The nonionized molecule presents a probable species for the lipid phase solubility of these derivatives as a molecular entity in the presence of organic solvent.\textsuperscript{[15]} There is also evidence in the literature that tetracycline undergo a conformational change upon deprotonation.\textsuperscript{[14]} For the present investigation, doxycycline hyclate manufactured by Yangzhou huashu winsome pharmacy Co. Ltd., China and supplied by Prime Research laboratory (India) Pvt. Ltd., Chennai is used.

3.2.5. INSULIN

Insulin is a proteinaceous hormone secreted by the β cells of the islets of langerhans of the pancreas. Insulin, as a key modulator of various functions of living cells, has been extensively studied.\textsuperscript{[16]} After secretion by pancreatic β-cells, insulin regulates carbohydrate, fat and aminoacid metabolism and stimulates protein synthesis and glycogen formation. Its chemical structure was elucidated by Sanger et al.\textsuperscript{[17]} in the early 1950s. Sanger\textsuperscript{[17]} found that the insulin molecule is composed of two polypeptide chain, an A chain, consisting of 21 amino acid residues and a B chain, containing 30 residues. The two chains are connected by two disulphide bonds and there is an additional di-sulphide linkage within A chain. The pro-insulin and active insulin structure is represented in figure 3.6 and 3.7 respectively.
Figure 3.6. Structure of Proinsulin

Figure 3.7. Structure of Active insulin
insulin exists as a monomer only at low, physiologic concentration. Insulin dimerizes at the higher concentration found in pharmaceutical preparations. Pullen and collaborators proposed a receptor-binding region within the insulin molecule. The receptor-binding region consist largely of invariant amino acids located both in the A chain, residues A1Gly, A5Gln, A19tyr, A21Asn and in the B chain, residues B24 Phe, B25 Phe, B26Tyr, B12 Val, B16Tyr \[15\]. Modification of insulin at the receptor may decrease biologic activity by reducing the hormone: receptor affinity.

Sheffer and Kaplan, 1979 found that the amino groups of insulin had unusual chemical properties \[18\]. Metabolic alterations which arise due to the deficiency of insulin can be partially controlled by the exogenous administration of insulin. The synthetic preparation of exogenous insulin’s consists primarily of insulin and they exhibit the biologic effects of insulin they do differ in their onset and duration of action into short, intermediate and long acting categories \[19\]. A somewhat shorter acting and more acceptable preparation is neutral protein hagedorn (NPH) insulin which includes metacresol as a preservative. This preparation is known as isophane insulin and commercially known as Actrapid \[20\]. Actrapid is human insulin. It is a neutral solution of human insulin (rys). It appears clear and colourless. Actrapid formulation is designed for fast/rapid onset of action, is a neutral solution containing human insulin as active ingredient and metacresol as preservative. Human insulin Actrapid (HIA) 40 IU/ml, 10ml vial, of molecular weight 508 manufactured by Novo Nordisk India private limited, India, has been used in the present study.

3.2.6. FATTY ACIDS

The fats and oils used almost universally as stored forms of energy in living organisms are derivatives of fatty acids. Fatty acids are carboxylic acids with
is fully saturated and unbranched; in others the chain contains one or more double bonds. A few contain three-carbon rings, hydroxyl groups or methyl-group branches. The nonpolar hydrocarbon chain accounts for the poor solubility of fatty acids in water. The carboxylic acid group is polar and account for the slight solubility of short-chain fatty acids in water. The solubility behaviour of the fatty acids in organic solvents is of considerable importance in many research works. According to the properties of the fatty acids it is classified as saturated and unsaturated fatty acids\textsuperscript{[21]}.

3.2.6.1. SATURATED FATTY ACIDS

Saturated fatty acids have a straight hydrocarbon chain. At room temperature, the saturated fatty acids have a dense solid phase. In the fully saturated compounds, free rotation around each carbon-carbon bond gives the hydrocarbon chain great flexibility; the most stable conformation is the fully extended form, in which the steric hindrance of neighbouring atoms is minimized. These molecules can pack together tightly in nearly crystalline arrays, with atoms all along their lengths in Vanderwaals contact with the atoms of neighbouring molecules\textsuperscript{[22, 23]}. The main source of saturated fatty acid is animal fat. The saturated fats are needed for energy, hormone production, cellular membranes and for organ padding. Some saturated fatty acids are antimicrobial and antifungal agents.

3.2.6.1.1 STEARIC ACID (STR)

Stearic acid has the highest molecular weight of 284.4 among the other saturated fatty acid used in our present investigation. Melting point 69°C, boiling point 383°C and density 0.847 g/cm\textsuperscript{3} at 70°C are the physical properties of stearic acid. It occurs in many animal and vegetable fats and oils. One important source is cocoa.
The basic structure, a hydrophobic hydrocarbon chain with a hydrophilic polar group at one end, endows fatty acids and their derivatives with distinctive properties.

### 3.2.6.1.2. PALMITIC ACID (PAL)

Palmitic acid with the molecular formula of C₁₆H₃₂O₂ has the molecular weight of 256.4 is employed in our present investigation. It has the melting point as 63°C. The major source of this fatty acid is palm oil and butter, cheese, milk and meat also contain this fatty acid[24]. The G-Protein receptors become stimulated by 16-carbon palmitic acid. The body makes palmitic acid out of excess carbohydrates and excess protein[24]. The physical properties of the palmitic acid is melting point 63-64°C, boiling point 351-352°C and density 0.853 g/cm³ at 62°C. The structural representation is shown in figure 3.9.

![Structure of Palmitic acid](image)
Myristic acid has a short 14 carbons chain. The molecular weight of the fatty acid is 228.4, melting point is 55°C, boiling point is 250.5°C and density is 0.8622 g/cm³. The myristic acid has a sufficiently high hydrophobicity to become incorporated into the fatty acyl core of the phospholipids bilayer of the plasma membrane of the eukaryotic cell. In this way, myristic acid acts as a lipid anchor in biomembranes. It is also binds with proteins to stabilize the proteins [24].

\[
\begin{align*}
\text{Methyl} & \quad \text{Carboxyl} \\
\text{Oil-Soluble} & \quad \text{Water-Soluble} \\
\text{OMEGA (\(\omega\)) END} & \quad \text{DELTA (\(\Delta\)) END}
\end{align*}
\]

Figure 3.10. Structure of Myristic acid

Besides nutmeg, myristic acid is also found in palm oil, coconut oil, butter fat, and is a minor component of many other animal fats [25]. Its molecular formula is C₁₄H₂₈O₂. The structure is represented in figure 3.10. All fats have a COOH acid at the beginning of the chain, also known as the alpha end. The opposite end is called the omega. All the carbon bonds in the middle of the chain are filled with hydrogen as shown in the above structure.

3.2.6.2. UNSATURATED FATTY ACIDS

The physical properties of the fatty acids, and of compounds that contain them, are largely determined by the length and degree of unsaturation of the hydrocarbon chain. Thus the unsaturated fatty acids are having lower melting point than that of saturated one, this is seen through the oily nature of the unsaturated fatty acids and also because unsaturated fats have kink or bend, the molecules do not stack together easily.
membrane is mostly of flexible type unsaturated fatty acids \cite{22, 23}.

### 3.2.6.2.1. OLEIC ACID (OLE)

Oleic acid is a common fatty acid found in most animal and vegetable fats. It is a monounsaturated fat, which means it has only one double bond. It has the molecular weight of 280.45, the melting point of 13°C, the boiling point of 360°C and the density of 0.895g/ml. One of the sources of oleic acid is olive oil.

![Structure of Oleic acid](image)

Figure 3.11. Structure of Oleic acid

It also present in the membranes of red blood cell in the human body and it helps to boost memory. It has the molecular formula as C\textsubscript{18}H\textsubscript{34}O\textsubscript{2}. The structural representation of oleic acid is given in figure 3.11.

### 3.2.6.2.2 LINOLEIC ACID (LIN)

Linoleic acid is one of the essential poly unsaturated fatty acid with more than one double bond in its structure. One of it is in ninth position and other in the 12\textsuperscript{th} position. It find in the lipids of cell membrane to give the permeability to cell membrane.

![Structure of Linoleic acid](image)

Figure 3.12. Structure of Linoleic acid

It is abundant in many vegetable oils, especially poppy seed, safflower and sunflower oils. The molecular weight of linoleic acid is 280.45, the
molecular formula of this essential fatty acid is \( \text{C}_{18}\text{H}_{32}\text{O}_2 \). The structural representation of oleic acid is given in figure 3.12.

3.2.6.2.3. RICINOLEIC ACID (RIC)

Ricinoleic acid, a hydroxy monounsaturated fatty acid and it is abundant in castor oil (90%). Its Molecular formula and molecular weight is \( \text{C}_{18}\text{H}_{34}\text{O}_3 \) and 298.46 respectively. This fatty acid has high medicinal importance, shown in figure 3.13.

![Figure 3.13. Structure of Ricinoleic acid](image)

Ricinoleic acid is a common C18 fatty acid with a cis-configured double bond in the ninth position and unusual hydroxyl group in the 12th position. The hydroxyl group of ricinoleic acid imparts a polar character to the fatty acid. For the interest the three unsaturated straight chain fatty acids; oleic, linoleic and ricinoleic acid supplied by merck company limited, India and the three saturated fatty acids; stearic, palmitic and myristic supplied by s-d chemicals private limited, India, are the preferred solute molecules for the present investigations. The fatty acids selected were AR grade and they were 99% pure.

3.2.7. SOLVENTS

3.2.7.1. WATER

Water used in the present study is double distilled water of high purity it is obtained by redistilling the distilled water over alkaline potassium permanganate in an all glass quick-fit distillation set up. It was stored in amber coloured bottle.
The choice of a suitable solvent for a given solute molecules plays an important role in deciding the end use \cite{26}. For the present investigations, in order to provide insight into the structural consequences of intermolecular interactions, the solvent ethyl methyl ketone (EMK) is particularly chosen because EMK belongs to a class of dipolar-aprotic solvents. In pure state, there is no tendency to associate through dipole-dipole interactions. This behaviour is similar to that of observed in N-N-Dimethyl formamide (DMF) and Dimethyl sulphur oxide (DMSO) \cite{27}.

\[
\text{CH}_3\text{C} \equiv \text{CH}_2\text{CH}_3
\]

\text{O}

\text{Figure 3.14. Structure of Ethyl methyl ketone}

The empirical formula of ethyl methyl ketone is \( \text{C}_4\text{H}_9\text{O} \) or structurally shown in figure 3.14. The carbonyl compounds such as aldehydes and ketones contain polar molecules. These molecules can interact with non-polar molecules through dipole-induced dipole interactions and polar molecules through dipole-dipole interactions. The aliphatic ketone, the methyl ethyl ketone is less polar in character \cite{28} when compared to aromatic carbonyl compounds. In our present study the ethyl methyl ketone used is of molecular weight 77. The solvent used is of 99% pure GR grade. It is supplied by merck company limited, India.

3.3. POLYMERIZATION PROCEDURE OF POLY (METHYL METHACRYLATE)

The polymerization of methyl methacrylate was carried out in a homogenous medium using recrystallised Benzoyl Peroxide (BPO) as initiator. Polymerization was conducted in sealed glass ampoules, under nitrogen atmosphere. Specified amount of
dissolved in xylene to get homogenous phase and slow stream of purified nitrogen was passed through the solution and the reaction was carried out at 70°C for 6 hours. The ampoules were sealed and thermostated immediately. After the required polymerization time, the reaction tube was removed from the thermostat, immediately cooled in ice to arrest the reaction. The contents of the reactions tube were poured into excess of methanol to precipitate the polymers. The reaction tube was washed with pure solvent in order to recover completely the adhering polymers and the washing were added to the polymer suspension were kept overnight in a refrigerator to allow the polymer to settle down after which it was filtered and washed in a weighed sintered glass crucible. The gross polymer, obtained was dried in vacuum at 55°C to a constant weight.

3.4. PREPARATION OF STOCK SOLUTION

The method of preparation of the solutions for investigation through different techniques is discussed.

3.4.1. PREPARATION OF SOLUTIONS OF PURE COMPONENT

Nitrocellulose and Poly (Methyl methacrylate) of 1gm each were dissolved in 100ml ethyl methyl ketone separately to give one per cent solutions. These solutions were stirred well at room temperature for 15 minutes using remi cutter type mechanical stirrer at a rate of 100 rpm. doxycycline hyclate and Insulin of 0.2gm each were dissolved in 100ml double distilled water separately to give 0.2 percent solutions. These solutions were prepared for the investigation of molecular interaction between the doxycycline hyclate and Insulin molecules.

Doxycycline hyclate, Saturated fatty acids - palmitic acid, stearic acid and myristic acid and the unsaturated fatty acids – linoleic acid, oleic acid and ricinoleic
give 0.1 percent solutions. These solutions were employed in the investigation of interaction of doxycycline hyclate with fatty acids.

### 3.4.2. PREPARATION OF BLENDS

Different percentage solutions were prepared as said earlier, for 1%w/v, 0.2%w/v and 0.1%w/v concentration with common solvents. The 1%w/v solutions of PMMA and NC were mixed at different compositions 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8 to form PMMA/NC blend solutions. Similarly doxycycline/Insulin actrapid, doxycycline/stearic acid, doxycycline/palmitic acid, doxycycline/myristic acid, doxycycline/oleic acid, doxycycline/linoleic acid and doxycycline/ricinoleic acid were prepared at different compositions 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8 and 1:9 solutions. The prepared pure component solutions and the blend solutions were employed in dilute solution viscometric, ultrasonics, refractometric, FTIR, and UV-VIS techniques for analysis.

### 3.5. EXPERIMENTAL PROCEDURE

The experimental procedures that are adopted in the present investigation for the techniques, dilute solution viscometry, ultrasonics, refractometric, FTIR and UV-VIS are outlined.

#### 3.5.1. DILUTE SOLUTION VISCOMETRY (DSV)

For the present investigation by Dilute Solution Viscometric studies an ubbelohde suspended level viscometer has been employed. Viscometric measurements were carried out at different temperatures of 303K, 310K and 313K by keeping the viscometer immersed in a constant temperature bath. The temperature was measured by a thermometer with an accuracy of $\pm 0.01^\circ C$. The flow time was measured using a
the viscometer for all experiments. The absolute viscosity of the polymer solutions $\eta_2$ was calculated from the formula

$$\eta_2 = \eta_1 \left( \frac{\rho_2 t_2}{\rho_1 t_1} \right) \quad (3.1)$$

Where $\rho_1, \rho_2$ are the densities of solvent and solution and $t_1, t_2$ are the flow time in seconds for solvent and polymer solution respectively. $\eta_1$ is the viscosity of the solvent. The flow time of solvent/solution was determined three times and the mean value was taken. A derivation of $\pm 0.01$ Sec was allowed.

Flow time of each blend compositions was determined by the serial dilution technique. 15ml of solution/solvent fed into the viscometer, allowed to attain the constant temperature and then, the flow time was determined. The concentration of the solution was altered by adding 1ml of solvent/solution was in the viscometer and shaken well for thorough mixing. The mixture was then allowed to attain the bath temperature and the flow time was determined. The experiment was repeated with five different concentrations by adding 1ml of solution or solvent. From the flow time measurement for each blend composition the specific viscosities ($\eta_{spc}$) and reduced viscosities ($\eta_{spc}/C$) were calculated. Finally, the intrinsic viscosity $[\eta]$ was determined by plotting the Huggins plots $\eta_{spc}/C$ against the concentration $C$.

3.5.2. ULTRASONICS

The acoustic interferometer is a simple and direct device to determine the ultrasonic velocity in solutions. It has become the standard instrument since it allows determination of sound velocity with high precision. The ultrasonic velocities of 1%w/v polymer solutions of NC and PMMA in the presence of solvent ethyl methyl
solvent double distilled water and the 0.1%w/v solutions of doxycycline hyclate and preferred fatty acids in solvent ethyl methyl ketone and blend solutions at compositions were determined. The ultrasonic interferometer operating at frequency 2 MHz with an accuracy of ±0.1% supplied by Mittal Enterprises, New Delhi is employed for the determination of velocities. The temperatures 303, 310 and 313K are maintained with electronically operated thermostatic bath. The density of the solutions is found using standard specific gravity bottle.

3.5.3. REFRACTOMETRIC

High-precision measurements of the refractive index \( \mu \) were made in the reflection mode using a bench-top Abbe's Refractometer with a temperature controlled prism. While taking the measurement light enters the measuring prism directly through an aperture. A drop of the experimental solution was placed on the prism. At the face of contact, between the prism and the experimental solution, part of the light was reflected and showed as a bright field in the microscope. The microscope was adjusted in such a way that one half of the field of view appeared brighter than the other. The colour fringe formed near the line of demarcation was removed using a compensator screw. After adjusting for the bright field the refractive index measurements were made from the scale which is inscribed in the microscope. The accuracy in the measurement of refractive index is ±0.0001. The experiment was carried out for all the blend solutions at 303, 310 and 313K by circulating thermostatically controlled water.

3.5.4. FTIR SPECTROSCOPY

The Nicolet Impact 400 FT-IR is a very high end optical bench. It is useful for product characterization, component qualitative analysis, product ID and product QC
spectroscopy is useful for the identification of both organic and inorganic compounds. Aggregates of atoms (or functional groups) such as C=O, -NO₂, -C-N, and -C-F; just to name a few, are all associated with characteristic infrared absorptions. Thus, infrared spectroscopy is ideal for the identification of functional groups present within a sample. In the present study Nicolet Impact 400 instrument is used to record the spectra of the cast films of polymer blends in the region 400-4000 cm⁻¹.

3.5.5. UV-VISIBLE SPECTROSCOPY

For recording the UV-Visible spectra of the liquid samples, Perkin Elmer Lambda 35 UV/VIS Spectrophotometer has been employed in the present investigation. The Lambda 35 is used for measurements on liquids, solids, pastes and powder samples, also regulatory tests requiring variable resolution. The variable bandwidth operation (0.5-4 nm), allows sensitive measurements with accessories such as integrating spheres and fiberoptics probes, extending the range (190-1100nm) of samples that can be analyzed. The operation is true double beam operation. The modes of operation are scanning, wavelength program, time-drive, rate, quant, and scanning quant. The spectrometer has the features of very high stability, high accuracy and reproducibility. So that, this UV-VIS spectrometer best matches for the present study. For recording the UV-VIS spectra the pure component solutions of doxycycline hyclate and insulin in the presence of solvent water and the blends of doxycycline hyclate/insulin were prepared. The spectra of the sample is recorded in the region 190-500nm.
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