CHAPTER 5

FTIR SPECTROSCOPY OF CHARGE TRANSFER
COMPLEXES OF FIBRINOGEN, LIPOPROTEIN,
ALBUMIN AND CASEIN
5.1 Introduction

Fibrinogen is a fibrous material and is a soluble plasma glycoprotein. It is converted into fibrin during blood coagulation. It is in the form $\alpha$, $\beta$, $\gamma$, $\delta$ chains having chain structure. Because of this it is a one-dimensional system. Structural changes in plasma circulating fibrinogen are studied through spectroscopy [1]. Structure of fibrinogen is also studied through dynamic light scattering and viscosity measurements [2]. Electrophoretic, sedimentation coefficient and viscosity of fibrinogen are also studied [3]. Some physicochemical properties of human fibrinogen are also reported [4]. Conformation transition between two allosteric states in the binding of fibrinogen to platelets is studied [5]. Noadhesive properties of fibrinogen are verified through force spectroscopy [6]. Infrared spectroscopic studies of fibrinogen adsorbed to polyurethanes are also subject of study [7]. Vibrational spectroscopic studies on adsorption fibrinogen are also carried out in detail [8]. A Raman spectroscopic study of fibronectin and fibrinogen is also a subject matter of investigation [9]. Study of mechanism of contact activation of fibrinogen is carried out through scanning force microscopy [10]. A lipoprotein is a biochemical assembly that contains both proteins and lipids water bound to the proteins. Infrared studies of protein-induced perturbation of lipids in lipoproteins...
and membranes have been made [11]. Accelerated clearance of low-density and high density lipoproteins after modification of lysine residues is studied [12]. FTIR spectroscopy of lipoprotein containing both lipids and protein bands is studied [13]. Effects of o-ctreotide therapy on lipoprotein is studied [14]. Albumin is a protein and human serum albumin is a blood protein. Interaction between naproxen and human serum albumin is studied through UV-visible spectroscopy [15]. Surface properties of PDMS microfluidic chips treated with albumin have been studied through electrokinetic characteristics (such as electro-osmotic velocity, electro-osmotic mobility and zeta potential) and atomic force microscopy [16]. Globular protein such as Bovine serum Albumin leads to formation of amyloid fibrils and mechanism is studied through fluorescence techniques, light scattering circular dichroism, FTIR spectroscopy and Atomic Force Microscopy [17]. Human serum albumin under various ligand loads is studied with the help of infrared and Raman spectroscopy [18]. Optical spectroscopy is used to study the binding of herbicide glyphosate to human serum albumin [19]. Interaction of formononetin with human serum albumin is studied through fluorescence anisotropy and FTIR spectroscopy [20]. Interaction of sophoricoside with human serum albumin is studied by optical spectroscopy [21]. Optical
spectroscopy is also used in studying interaction of honokiol with human serum albumin [22]. Electrical properties such as thermally stimulated depolarization currents, d.c. electrical conductivity and current time characteristics are studied for albumin/poly(vinylalcohol) blends [23]. Interaction of a surfactant with bovin serum albumin has been studied with electron paramagnetic resonance [24]. Structure of human serum albumin is studied by UV resonance Raman spectroscopy [25]. Casein is a milk protein. Far-infrared spectra were obtained for $\kappa$-casein [26]. Casein-methylglyoxal complex is studied with electron spin resonance d.c. conductivity, microwave permittivity and electronic transference number [27]. Finally, an FTIR spectroscopic study of interaction between $\alpha_s$-casein-bound phosphoryl groups and chitosan is also carried out [28].

In the present work, FTIR spectroscopy of charge transfer complexes of fibrinogen, lipoprotein, albumin and casein with organic acceptors such as TCNQ, TCNE, DDQ, chloranil and KI-I$_2$ or iodine has been carried out.

### 5.2 Experimental detail

Fibrinogen, lipoprotein, albumin and casein were obtained in pure forms from Sigma-Aldrich chemical company, USA and were used
without any chemical treatment. Fibrinogen, the fibrous material, was added TCNQ (7, 7, 8, 8-tetracyano-\textit{p}-quinodimethane), TCNE(tetracyano-\textit{p}-ethylene) and chloranil (2,3,5,6-tetrachloro-\textit{p}-benzoquinone) and mixtures were grinded using an agate mortar with a pastle. Equal volume proportions of fibrinogen, and organic acceptors were taken, i.e. fibrinogen was heavily doped with acceptors. Similarly, lipoprotein made of lipids and proteins was also mixed with TCNQ, TCNE, chloranil, DDQ (2, 3-dichloro-5, 6-dicyano-\textit{p}-benzoquinone) and KI-I\textsubscript{2} and the mixture were grinded to form fine homogeneous powder. Thus lipoprotein was also heavily doped with acceptors. Albumin powder was also mixed with TCNQ, TCNE, chloranil, DDQ and KI-I\textsubscript{2} and fine powders of charge transfer complexes in 1:1 molecular weight proportions were formed. Casein the milk protein was also mixed with TCNQ, TCNE, chloranil and iodine in 1:1 molecular weight proportions and CTCs were prepared in the form of homogeneous powders. The above CTCs of fibrinogen, lipoprotein, albumin and casein were further mixed with 95% anhydrous spectrograde KBr powders and circular pellets were made using a manually operated compressing machine. These pellets were placed in a dark chamber of standard spectrophotometer.
The spectra in the range 400-4000 cm\(^{-1}\) were recorded using GXFTIR single beam spectrophotometer manufactured by Perkin-Elmer Co. USA, having a resolution of 0.15 cm\(^{-1}\), a scan range of 15,600-30 cm\(^{-1}\), a scan time of 20 scan/s, and OPD velocity of 0.20 cm/s and MIRTGS and FIRTGS detectors. A beam splitter of opt-KBr type was used having a range of 7800-370 cm\(^{-1}\). The spectra were recorded in purge mode.

The three dimensional structure of fibrinogen, lipoprotein, and albumin are shown in figure 5.1.

![Figure 5.1 three dimensional structure of fibrinogen, lipoprotein, and albumin](image)

**5.3 Results and discussion**

The FTIR spectrum of fibrinogen is shown in figure 5.2a. This contains a flat peak in transmission in the range 2970-1650 cm\(^{-1}\) which can be fitted a half-power beta density as shown in figure 5.2 b. This is associated with polaron hopping along one-dimensional chain of fibrous fibrinogen. A nature of transition having \(E_g \approx 0.125\text{eV}\) is also revealed in the spectra. This can be related with dissociation of bipolarons in free polarons. Finally, a low-frequency gaussian band around 600 cm\(^{-1}\) is
observed in low-frequency range which is also fitted as shown in figure 5.2.

Figure 5.2a The FTIR spectrum of fibrinogen only

Figure 5.2b Half-power beta density fitted for fibrinogen
The FTIR spectrum of fibrinogen-TCNQ complex is shown in figure 5.3a. As the wave-number (frequency) decreases, transmission rather than absorption decreases. This shows that absorption can be replaced by transmission in nature of transition. This is true for highly conducting material. The nature of transition is fitted as in figure 5.3b and reveals semi-metallic behavior. Two gaussian function- one in mid-IR range around 1500cm\(^{-1}\) and one in low frequency range around 620cm\(^{-1}\) are also observed and are fitted by plotting lnA vs \((k-k_0)^2\) as shown in figure 5.3c and 3d respectively. These two gaussian bends show continuous motion of polarons in a band.
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Figure 5.3a The FTIR spectrum of fibrinogen-TCNQ

Figure 5.3b Indirect transition showing semimetallic behavior in fibrinogen-TCNQ CTC

Figure 5.3c Gaussian band fitted around 1500 cm$^{-1}$ in fibrinogen-TCNQ CTC
The FTIR spectrum of fibrinogen-TCNE complex is shown in figure 5.4a. This also contains a half power beta density as flat peak in transmission which plotted as in figure 5.4b and in lesser range a semi-metallic behavior. A semiconducting nature of transition is found with $E_g \approx 0.125\text{eV}$ as found in fibrinogen only. A gaussian band around 600 cm$^{-1}$ is also fitted as shown in figure 5.4c.

Figure 5.4a The FTIR spectrum of fibrinogen-TCNE CTC
The FTIR spectrum of fibrinogen-chloranil complex is shown in figure 5.5a which shows semiconducting nature of transition with \( \omega_g \approx 1900\text{cm}^{-1} \). Nature of transition is fitted as forbidden indirect transition with \( E_g \approx 0.236 \text{ eV} \) as shown in figure 5.5b. A gaussian band around...
1400cm\(^{-1}\) is also fitted as shown in figure 5.5c. A low frequency triangular distribution associated with internal Fraz-Keldysh effect with fitted valence and conduction bands providing a triangular potential barrier is observed around 608cm\(^{-1}\).

![Figure 5.5a The FTIR spectrum of fibrinogen-chloranil CTC](image1)

![Figure 5.5b Forbidden indirect transition in fibrinogen-chloranil CTC](image2)
The FTIR spectrum of pure lipoprotein is shown in figure 5.6a. The range of nature of transition between 1800cm\(^{-1}\) and 2800cm\(^{-1}\) show a semimetallic behavior is denoted as in figure 5.6b. A low frequency half power beta density between 980cm\(^{-1}\) and 620cm\(^{-1}\) is also observed. This corresponds to polaron hopping in one-dimension.
Figure 5.6b Indirect transition showing semimetallic behavior in lipoprotein

The FTIR spectrum of lipoprotein-TCNQ complex as shown in figure 5.7a also shows a region of semi-metallic behavior with $(\tau \nu)^{1/3}$ vs $\nu h$ being a rectilinear plot as in figure 5.7b. There is also a mid-IR gaussian band showing continuous band motion of small polarons as in figure 5.7c.

Figure 5.7a The FTIR spectrum of lipoprotein-TCNQ CTC
The spectrum of lipoprotein-TCNE complex is shown in figure 5.8a. There is a half-power beta density between 2900\,cm\(^{-1}\) to 1728\,cm\(^{-1}\) is shown in figure 5.8b and there is a gaussian band around 1400\,cm\(^{-1}\) as shown in figure 5.8c.
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Figure 5.8a The FTIR spectrum of lipoprotein-TCNE CTC

Figure 5.8b Half-power beta density fitted for lipoprotein-TCNE CTC

Figure 5.8c Gaussian band around 1400cm\(^{-1}\) in lipoprotein-TCNE CTC
The FTIR spectrum of lipoprotein-chloranil shows a different feature which shown in figure 5.9a. There is a semiconducting nature of transition fitting an allowed indirect transition but along with the presence of emission and absorption of excitons as shown in figure 5.9b. Rests in the mid-IR and low-IR range are oscillations of the density of states along chloranil stacks as found in many chloranil based CTCs.

Figure 5.9a The FTIR spectrum of lipoprotein-chloranil CTC

Figure 5.9b Allowed indirect transition observed in lipoprotein-chloranil CTC
In the spectrum of lipoprotein-DDQ, there is a range of nature of transition which fits a forbidden indirect transition as shown in figure 5.10a (spectrum of CTC) and 5.10b (transition). In the mid-IR range between 1800\textsuperscript{cm}\textsuperscript{-1} and 1000\textsuperscript{cm}\textsuperscript{-1} there is a half-density revealing hopping of polarons as shown in figure 5.10c.

Figure 5.10a The FTIR spectrum of lipoprotein-DDQ CTC

Figure 5.10b Forbidden indirect transition fitted in lipoprotein-DDQ
A semi-metallic behavior is again observed in the spectrum of lipoprotein-KI-I\textsubscript{2} as shown in figure 5.11a but transition is found to be allowed direct type as shown in figure 5.11b. The negative band gap is around 0.21eV. A half-power beta density is observed between 976cm\textsuperscript{-1} and 618cm\textsuperscript{-1}.

The infrared spectrum of albumin is shown in figure 5.12a. It shows a transmitting range between 2873cm\textsuperscript{-1}-1654cm\textsuperscript{-1}. There is an asymmetric triangular distribution tailing towards low frequency side which is most probably related with imperfect nesting in mid-IR range. There is a low frequency gaussian band which is fitted as shown in figure 5.12b.
Figure 5.11a The FTIR spectrum of lipoprotein-KI-I

Figure 5.11b Allowed direct transition showing semimetallic behavior

Figure 5.12a The FTIR spectrum of albumin only
In the FTIR spectrum of albumin-TCNQ there is a transmitting range between 2850cm\(^{-1}\) and 1760cm\(^{-1}\) which shown in figure 5.13a. There are two gaussian bands-one in mid-IR range around 1350cm\(^{-1}\) and one in low frequency range around 600cm\(^{-1}\) which are fitted as shown in figure 5.13b and 5.13c. The presence of two gaussian bands rather than any half-power beta density indicates that albumin-TCNQ is a two-dimensional system rather than a one-dimensional system. This situation in similar to elastin which is two-dimensional. Similar is the spectrum of albumin-TCNE is shown in figure 5.14a and two gaussian profiles are fitted as in figure 5.14b and 5.14c.
Figure 5.13a The FTIR spectrum of albumin-TCNQ CTC

Figure 5.13b The Gaussian band around 1350 cm$^{-1}$ in albumin-TCNQ CTC

Figure 5.13c The Gaussian band around 600 cm$^{-1}$ in albumin-TCNQ CTC
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Figure 5.13b The FTIR spectrum of albumin-TCNE CTC

Figure 5.14b The Gaussian band in mid-IR range for albumin-TCNQ CTC

Figure 5.14c The Gaussian band around a low frequency in albumin-TCNQ CTC
The FTIR spectrum of albumin-chloranil shows two band transport revealed by two transitions in the infrared range as shown in figure 5.15a. These two natures of transitions are analyzed as forbidden indirect transitions having $E_g \approx 0.29\text{eV}$ and $E_g \approx 0.158\text{eV}$ figure 5.15b and 5.15c. Two band transport is related with presence of two conduction bands, as found in some ternary CTCs. The spectrum of DDQ complex also reveals two band transport as shown in figure 5.16a. These two nature of transitions are also forbidden indirect type having $E_g \approx 0.29\text{eV}$ and $E_g \approx 0.12\text{eV}$ are fitted as shown in figure 5.16b and 5.16c but the lower band showing also presence of indirect excitons. Excitons are emitted and absorbed (created and annihilated) in this process. Finally, a low frequency gaussian band around 604cm$^{-1}$ is also analyzed and fitted as shown in figure 5.16d.

![Figure 5.15a The FTIR spectrum of albumin-chloranil CTC](image)
Figure 5.15b Nature of transition with $E_g=0.29\text{eV}$

Figure 5.15c Nature of transition with $E_g=0.16\text{eV}$

Figure 5.16a The FTIR spectrum of albumin-DDQ CTC
Figure 5.16b Nature of transition with $E_g = 0.29\text{eV}$ in albumin-DDQ CTC

Figure 5.16c Nature of transition with $E_g = 0.16\text{eV}$ in albumin-DDQ CTC

Figure 5.16d Low-frequency Gaussian band fitted in albumin-DDQ CTC
Finally, the FTIR spectrum of albumin-KI-I$_2$ also reveals two band transport having two conduction bands as shown in figure 5.17a. The two transitions are analyzed as forbidden indirect and allowed direct type at $E_g \approx 0.225$ eV and $E_g \approx 0.12$ eV, respectively. (Figure 5.17b and 5.17c) The higher band gap transition also shows presence of indirect excitons which are created and annihilated. Lastly, a low-frequency gaussian band centered around 600 cm$^{-1}$ is analyzed as in figure 5.17d.

![Figure 5.17a The FTIR spectrum of albumin-KI-I$_2$ CTC](image)

![Figure 5.17b Nature of transition with $E_g=0.225$eV in albumin-KI-I$_2$ CTC](image)
Casein is a milk protein and its FTIR spectrum is shown in figure 5.18a. It is a small-band gap semiconductors having \( \omega_g \approx 1950\, \text{cm}^{-1} \) (0.244eV) which seems to be a Hubbard gap. There are two Gaussian bands—one in mid-IR range around 1500cm\(^{-1}\) and other in low-frequency range around 600cm\(^{-1}\). These Gaussian bands are fitted as shown in figure 5.18b and 5.18c which indicate that casein is a two-dimensional
semiconductor similar to elastin. Gaussian bands rather than half-power beta density reveals extra degree of freedom along which charge carriers move.

Figure 5.18a The FTIR spectrum of casein only

![FTIR spectrum of casein only](image)

Figure 5.18b Gaussian band fitted around 1500cm\(^{-1}\) in casein only

![Gaussian band fitted](image)
Casein-TCNQ shows a half-power beta density between $2800\text{cm}^{-1}$ and $1670\text{cm}^{-1}$ in its FTIR spectrum as shown in figure 5.19a. This can be ascribed to polaron hopping along TCNQ stacks. There are two Gaussian bands around $1540\text{cm}^{-1}$ and $600\text{cm}^{-1}$ which are fitted as shown in figure 5.19b and 5.19c. These Gaussian bands reveal two-dimensional conduction across casein molecules whose molecular plan is perpendicular to TCNQ stacking direction. The FTIR spectrum of casein-TCNE is shown figure 5.20a. It contains a half-power beta density in the range between $2870\text{cm}^{-1}$ and $1466\text{cm}^{-1}$ which is fitted in figure 5.20b. The beta density is associated with polaron hopping along one-dimension. The special feature of spectrum is the nature of transition with $E_g\approx0.065\text{eV}$ rather than two Gaussian bands. This transition is found to be forbidden indirect type fitted in figure 5.20c.
Figure 5.19a The FTIR spectrum of casein-TCNQ CTC

Figure 5.19b Gaussian band fitted around 1540cm⁻¹ in casein-TCNQ CTC
Figure 5.19c Gaussian band fitted around 600 cm$^{-1}$ in casein-TCNQ CTC

Figure 5.20a The FTIR spectrum of casein-TCNE CTC
The FTIR spectrum of casein-DDQ is shown in figure 5.21a. Spectrum shows a nature of transition with $E_g \approx 0.24 \text{eV}$. This transition is found to be forbidden indirect type which is fitted as in figure 5.21b. The Gaussian bands indicate that casein molecule are two dimensionally ordered which are fitted as in figure 5.21c and 5.21d respectively.
Figure 5.21a The FTIR spectrum of casein-DDQ CTC

Figure 5.21b Nature of transition with $E_g=0.24\text{eV}$ in casein-DDQ CTC

Figure 5.21c Gaussian band fitted in mid-IR range in casein-DDQ CTC
The FTIR spectrum of casein-chloranil is unique and is of different type which is shown in figure 5.22a. The nature of transition between 3360 cm\(^{-1}\) and 1800 cm\(^{-1}\) with \(E_g = 0.24\) eV also indicates presence of indirect exciton with emission threshold lower than absorption threshold as fitted in figure 5.22b.
Casein-iodine spectrum shows a half-power beta density in the range between 2850\,cm\(^{-1}\) and 1630\,cm\(^{-1}\) as in figure 5.23a which is fitted as in figure 5.23b. In almost the same range, a nature of transition showing a forbidden indirect transition with \(E_g = 0.27\,\text{eV}\) is present and shown in figure 5.23c. Finally, a Gaussian band around 606\,cm\(^{-1}\) is observed which is fitted as in figure 5.23d.
Figure 5.23b Half-power beta density in casein-iodine CTC

Figure 5.23c Nature of transition with $E_g=0.27\text{eV}$ fitted in casein-iodine CTC

Figure 5.23d Gaussian band around $606\text{cm}^{-1}$ in casein-iodine CTC
5.4 Conclusions

The FTIR spectra of CTCs of fibrinogen, lipoprotein, albumin and casein are studied in the present work. Two complexes of fibrinogen and two complexes of lipoprotein are found to be semimetallic. Lipoprotein itself was also found to be semi-metallic. Rests of the complexes are found to be small band gap semiconductors with band gap lying in the IR range. Albumin and casein are found to be two-dimensional (layered) semiconductors.

Reference:


