CHAPTER 8
SYNCHRONOUS LUMINESCENCE SPECTROSCOPIC
CHARACTERISATION OF ACETONE EXTRACTED
FORMED ELEMENTS

8.1 INTRODUCTION

The Synchronous Luminescence Spectroscopy (SLS) of acetone extracted blood formed elements of normal subjects and cervical cancer patients were recorded as already mentioned in the chapter 2. The SLS is recorded as discussed in the chapter 7.

8.2 SLS CHARACTERIZATION OF ACETONE EXTRACTED
BLOOD FORMED ELEMENTS OF NORMAL SUBJECTS
AND CERVICAL CANCER PATIENTS.

The averaged SL spectra of acetone extracted blood formed elements of normal subjects and cervical patients are shown in the Figure 8.1(a). From the Figure 8.1(a), it is observed that both the normal subjects and cervical cancer patients shows similar spectral profile. On the other hand, it is found that the fluorescence intensity of cervical cancer patients is two fold higher than that of normal subjects.

The measured SL spectra were also normalized and the normalized spectra for the acetone extracted blood formed elements of normal subjects and cervical cancer patients are shown in the Figure 8.1(b). Acetone extracted
blood formed elements of normal subjects showed peaks at 355, 420, 450, 468 and 525 nm and a valley at 390 nm between the peaks at 355 and 420 nm. From these peaks, it is observed that there may be some contribution of enzymes such as FAD, NADH / NADPH, FAD, bilirubin, vitamins, riboflavin and porphyrines in the acetone extracted blood formed elements of normal subjects. It is also observed that the acetone extracted blood formed elements of cervical cancer patients show similar spectral profile, however the peaks at 355 nm is red shifted to 10 nm with respect to normal subjects. Further it is also observed that the peak at 420 nm is completely absent in cervical cancer patients, and also the intensity of the peak at 525 nm is higher for cervical cancer patients than normal subjects. This may be attributed that FAD and its derivatives are more in acetone extracted blood formed elements of cervical cancer patients than the normal subjects.

To clearly understand the spectral signatures of acetone extracted blood formed elements of normal and cervical cancer patients, the difference spectrum was computed by subtracting the spectral signatures of acetone extracted blood formed elements of cervical cancer patients from normal subjects and it is shown in the Figure 8.1(c). From the difference spectrum it is observed that there are two sharp positive peaks at 335 and 418 nm. From the positive peaks, we can understand that the contribution of amino acids tryptophan and other amino acids are more in the acetone extracted blood formed elements of normal subjects than that of cervical cancer patients. Further, it is also observed that the spectrum shows a sharp negative peak at 523 nm and two broad negative peaks centered at 485 and 585 nm. From the negative peaks it is attributed that the formed elements of cervical cancer patients may have higher concentration of vitamins, riboflavin in the acetone extracted blood formed elements of cervical cancer patients than the normal subjects.
Figure 8.1 SL spectra of (a) Averaged, (b) Normalized spectra and (c) difference spectrum of normal subjects and cervical cancer patients acetone extracted blood formed elements
8.3 SLS CHARACTERIZATION OF ACETONE EXTRACTED BLOOD FORMED ELEMENTS OF NORMAL SUBJECTS, EARLY AND ADVANCED CERVICAL CANCER PATIENTS

The averaged SL spectra of acetone extracted blood formed elements of normal subjects, early and advanced cervical patients are shown in the Figure 8.2(a). From the Figure 8.2(a) it is observed that the normal subjects, early and advanced cervical cancer patients shows similar spectral profile except the wavelength between 416 nm to 506 nm and beyond 543 nm the intensity is lesser for advanced cervical cancer than early cervical cancer. Acetone extracted blood formed elements of normal subjects as well as early and advanced cervical cancer patients showed peaks at 365, 450, and 523 nm. From these peaks it is observed that the contribution of enzymes NADH / NADPH, FAD, and lipids like phospholipids, lipofuscin and ceroid, bilirubin vitamins, riboflavin and porphyrines in the acetone extracted blood formed elements of normal subjects. It is also noted that the peak intensities for acetone extracted blood formed elements of advanced cervical cancer patients exhibits higher intensity than that of normal subjects and the order of intensity is advanced > early > normal.

The measured SL spectra were normalized and the normalized spectra for the acetone extracted blood formed elements of normal subjects, early and advanced cervical cancer patients are shown in the Figure 8.2(b). From the normalized spectra it is observed that the spectrum of normal subjects is blue shifted with respect to early and advanced cervical cancer. Acetone extracted blood formed elements of normal subjects showed peaks at 350, 397, 420, 448 and 520 nm and one valley at 388 nm between the peaks at 350 and 397 nm. It is observed that the normalized spectra of the early and advanced cervical cancer patients showed the similar spectral profile as that of normal subjects except the following observation. The sharp peak at 350 nm is red shifted to 10 nm and 8 nm respectively for both early and advanced
cervical cancers with respect to normal subjects. Further, it is also observed that the intensity of the peak at 523 nm, for advanced cervical cancer is higher than that of the early cervical cancer patients and normal subjects. The SL spectrum below 444 nm, the intensities of different peaks are in the order of advance > normal > early, beyond 444 nm it is advanced > early > normal.

To clearly understand the spectral signatures of acetone extracted blood formed elements of normal, early and advanced cervical cancer patients, the difference spectrum was computed by subtracting the spectral signatures of acetone extracted blood formed elements of early cervical cancer patients from normal subjects, advanced cervical cancer patients from normal subjects and shown in the Figure 8.2(c). From the difference spectrum of early and normal subjects, it is observed that there are three sharp positive peaks at 338, and 416 nm and 465 nm. The positive peaks may be attributed to enzymes NADH / NADPH, structural proteins such as elastin, bilirubin, and porphyrins and they are more in the acetone extracted blood formed elements of normal subjects than that of early cervical cancer patients. The difference spectrum between the advanced cervical cancer patients and normal subjects also shows similar spectral profile, however the peak at 338 nm in normal subjects minus advanced cervical cancer is blue shifted to 5 nm with respect to the normal minus early cervical cancer. The negative peak at 524 nm in the normal subjects minus advanced cervical cancer is absent with respect to normal subjects minus early cervical cancer. Further, it is also observed that the overall intensity of normal subjects minus advanced cervical cancer is lesser than normal subjects minus early cervical cancer on the positive side of the difference spectrum and it is vice-versa on the negative side of the spectrum. This clearly indicates that the photophysical characteristic of acetone extracted blood formed elements not only differs between normal subjects and cancer, but also with respect to stage of the diseases.
Figure 8.2  SL spectra of (a) Averaged (b) Normalized spectra of acetone extracted blood formed elements of normal, early and advanced cervical cancer patients and (c) Difference spectra.
8.3 SLS CHARACTERIZATION OF ACETONE EXTRACTED BLOOD FORMED ELEMENTS OF NORMAL SUBJECTS MDSCC, WDSCC AND PDSCC OF CERVICAL CANCER PATIENTS

The average fluorescence emission spectra of acetone extracted blood formed elements of normal subjects and cervical cancer patients MDSCC, WDSCC and PDSCC conditions are shown in Figure 8.3 (a). The averaged fluorescence emission spectra show considerable differences between the normal and the different histopathological conditions of cervical cancer patients. From the Figure 8.3(a), it is observed that PDSCC acetone extracted blood formed elements exhibits overall higher fluorescence emission intensity than the normal subjects as well as the other histopathologically cervical cancer patients. The order of fluorescence intensity is PDSCC > WDSCC > MDSCC > normal.

The normalized emission spectra of acetone extracted blood formed elements of normal subjects, MDSCC, WDSCC and PDSCC of cervical cancer patients are shown in Figure 8.3(b). From the normalized spectra it is observed that the spectra of normal as well as different histopathologically classified cervical cancer patients show peaks at 355,420, 447, 468, 522 and 655 nm and a hump at 406 nm. It is noted that the primary peak of normal subjects is centered at 355 nm where as in the case of MDSCC cervical cancer patients the same peak is red shifted to 10 nm with respect to normal subjects. The red shift is more for MDSCC, whereas other conditions of cervical cancer patients showed minimal red shift of the order of 3-4 nm with respect to normal subjects. The peaks may be due to the presence of NADH / NADPH, FAD, lipids such as lipofuscin ceroid, vitamins and bilirubin etc. It is also observed from the Figure 8.3 (b), that the contribution of NADH and vitamins is more of the order of PDSCC > MDSCC > normal >
WDSCC. Further, it is also observed that the peak at 522 nm in the normal subjects minus WDSCC clearly indicates that, the WDSCC patients are having some higher concentrations of flavins with respect to the other histopathological classification.

Further to understand the spectral difference between the acetone extracted blood formed elements of normal subjects and different histopathologically classified cervical cancer patients such as MDSCC, WDSCC and PDSCC, the difference spectra were computed by subtracting then from the normal subjects. From the Figure 8.3(c) it is found that, the difference spectrum between normal subjects and MDSCC cervical cancer patients has three positive peaks at 335, 417 and 462 nm with a positive hump at 548 nm and two negative peaks centered at 366 and 448 nm.

The difference spectrum between normal subjects and WDSCC patients, it is found that there is a higher difference with the fluorescence intensity both in the positive as well as negative side of the spectrum with respect to the other two categories spectra. It is also observed that the intensity of the positive peaks at 335, 417 and 462 nm, intensity is more when compare to the MDSCC. Further, it is seen that the sharp negative peak at 400 nm is completely absent in other histopathological conditions. However the exact reason for the same has to be identified. The difference spectrum between normal subjects and PDSCC cervical cancer patients shows moderate intensity difference in positive side and high difference at the negative side of the spectrum with respect to the other two categories.
Figure 8.3  SL Spectra of (a) Averaged (b) Normalized spectra of acetone extracted blood formed elements of normal, Histopathologically classified cervical cancer patients and (c) Difference spectra
8.4 STEPWISE DISCRIMINANT ANALYSIS OF SLS CHARACTERISTIC OF ACETONE EXTRACTED BLOOD FORMED ELEMENTS OF NORMAL SUBJECTS AND CERVICAL CANCER PATIENTS

From SLS spectra of acetone extracted blood formed elements of normal subjects and cervical cancer patients, 66 ratio variables were calculated using luminescence intensities at wavelengths 301, 335, 362, 370, 388, 418, 440, 451, 485, 524, 551 and 590 nm of the acetone extracted blood formed elements of normal and cervical cancerous subjects. The linear discriminant analysis performed across the normal and cervical cancerous groups, using these 66 ratio variables as input, resulted in the following expression for a canonical discriminant function (8.1).

\[
DF_{21} = 21.789 \times I_{335/362} - 13.718 \times I_{335/418} + 7.172 \times I_{362/41} - 4.658 \times I_{388/418} + 3.725 \times I_{451/485} + 0.265 \times I_{485/590} - 14.800 \quad (8.1)
\]

Out of the 66 input variables, only six ratio variables viz., \(I_{335/362}\), \(I_{335/418}\), \(I_{362/41}\), \(I_{388/418}\), \(I_{451/485}\) and \(I_{485/590}\) resulted in the significant in discriminating the acetone extracted blood formed elements of cervical cancerous subjects from normal subjects. Figure 8.4 shows the scatter plot of the discriminant score \(DF_{21}\) of normal and cervical cancerous subjects. It is found that \(DF_{21}\) classified 27 normal subjects and 63 cervical cancerous subjects correctly, resulting in a specificity and sensitivity of 90\% and 92.6\% respectively. Five normal subjects were misclassified as cervical cancer and 3 cervical cancers were misclassified as normal subjects. Hence 91.8\% of original groups were classified correctly.
Figure 8.4 Scatter plot showing the distribution of the discriminate score of the acetone extracted blood formed elements of normal and cervical cancer for SLS

8.5 DISCUSSION

The synchronous luminescence spectra of formed elements of normal and abnormal cervical cancer patients were subjected to statistical analysis. From the Figure 8.4 it is observed that, synchronous luminescence spectroscopy provides 90% sensitivity and 92.6% specificity. On comparison with synchronous luminescence spectroscopy of blood plasma, formed elements proved better statistical significance.