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FTIR Spectroscopic Analysis of Normal and Cancerous Human Breast Tissues between 450 Cm^{-1} and 1100 Cm^{-1} using Trend Analysis

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Abstract: The breast pathological tissues are mainly composed of collagen; proline, valine, glycine and phenylamine are the main collagen's amino acids. The change of the cell from normal status to malignant status induces changes in the relative content of the biomolecules. Fourier transform infrared (FTIR) spectroscopy is sensitive to molecular structure. The infrared spectra of human breast tissues were recorded in the frequency range between 400 cm and 1100 cm. In this paper the Trend analysis plot is used for the analysis of FTIR spectrum of human breast tissues. The sensitivity of FTIR spectroscopy for biomolecular changes is used to classify benign and malignant breast tissues. The analysis is carried out for a collection of 43 samples which were histopathologically identified as normal, hyperplasia, fibro adenoma, ductal carcinoma and invasive ductal carcinoma tissues. The difference in the absorbance values for the various cancer grades and also the predictions of absorbance values for critical stages has been analysed using the Trend analysis. Thus it implies that FTIR spectroscopy is useful for the diagnosis of cancerous breast tissues.
Key words: Normal and Cancerous Human Breast Tissues, Trend Analysis, FTIR Spectroscopic Analysis.

Introduction

The breast cancer is the most common malignant tumor found in women in the Western world.Usually, the breast cancer screening involves two steps. The first one is the search for palpable lesions in the annual clinical breast examination. The second one is the X- ray mammography, in which suspicious local density changes could be detected. Whenever the tissue is particularly dense throughout, ultrasound may also be used to locate suspicious regions. If a lesion is found during examination the tissue is submitted to biopsy that could ranges from the needle aspiration of single cells to the surgical removal of the entire suspicious mass by exsisional biopsy**(1)**.

The breast is a large secretory gland composed of 15 to 25 autonomous and empty lobes connected to the nipple. The lobes themselves are divided into smaller units, called lobules, which are connected by ducts. Lobular and duct elements consists of single layers of epithelial and myothelial cells **(2)**. The breast undergoes many changes throughout a women's life, both progressive due to puberty, pregnancy and menopause and cyclical due to menstruation. Hormones regulate these changes. This dynamical activity could induce a lot of opportunities for disease. Usually , breast pathology is extremely diverse, but it could be divided inti two main categories : benign and malignant pathologies. Most benign lesions are part of a spectrum of fibrocystic changes , where as 70% of malignant lesions are invasive duct carcinomas **(3)**.

This paper focuses on the FTIR spectra covering the region between 1100 cm-1 and 2100 cm-1.The study of the spectra enables to identify the spectroscopic differentiation among the various breast carcinoma types namely hyperplasia, fibro adenoma, ductal carcinoma and invasive ductal carcinoma. The

analysis is done using the statistical methods TREND ANALYSIS method.

Materials and Methods:

Human breast tissues were obtained from pathology department at Government General Hospital, Madras Medical College, and Chennai. Each sample was cut into two pieces. The first one was sent to the pathologists for evaluation using their histopathological techniques. The second was frozen in saline water immediately after collecting it and used for IR spectroscopic recording within few hours. For FTIR spectroscopic recording the frozen tissues was taken as thin layer and dried then placed on the BaF_2 window in the spectrophotometer. Totally about 43 samples were collected. Among those 43 samples 10 were diagnosed as infiltrating ductal carcinoma, 10 as ductal carcinoma, 9 as fibro adenoma, 9 as hyperplasia and 5 as normal breast tissues.

Apparatus Description:

The IR spectroscopy is carried out by using FT technique in PERKIN ELMER SPECTRUM ONE FTIR. IR spectroscopy involves the study of interaction of electromagnetic radiation with matter. Due to this interaction, electromagnetic radiation characteristic of the interacting system may be absorbed or emitted. The experimental data consists of the frequency and the intensity of the characteristic radiation absorbed or emitted.

The interference pattern from a two beam interferometer as the path difference between the two beams is altered, when Fourier transformed, gives rise to the spectrum. The transformation of the interferogram into spectrum is carried out mathematically with a dedicated on – line computer. The Perkin Elmer Spectrum One FTIR consists of Nerst glower as source, an interferometer chamber comprising of KB beam splitter followed by a sample chamber and detector. This instrument cover entire region of 4000 – 450 cm^{-1}. The spectrometer works under purged conditions.

Analysis:

The breast pathological tissues are mainly composed of collagen; proline, valine, glycine and phenylamine are the main collagen's amino acids. It is composed of both an epithelial component and a substantial stroma neoplastic element with collagen deposition. T he spectrum of breast tissues can be considered to be mainly from the overlapping of the epithelial cells and collagen in the connective tissue **(4)**. Proteins, nucleic acids, lipids and carbohydrates are the characteristic functional biomolecules in tissues. The change of the cell from normal status to malignant status induces changes in the relative content of the biomolecules. Fourier transform infrared (FTIR) spectroscopy is sensitive to molecular structure. T hus FTIR measures the changes in the cell status in the biomolecular structure and contents. The infrared spectra of the breast tissue in the frequency region 400 cm^{-1} and 1100 cm^{-1} are focused here.

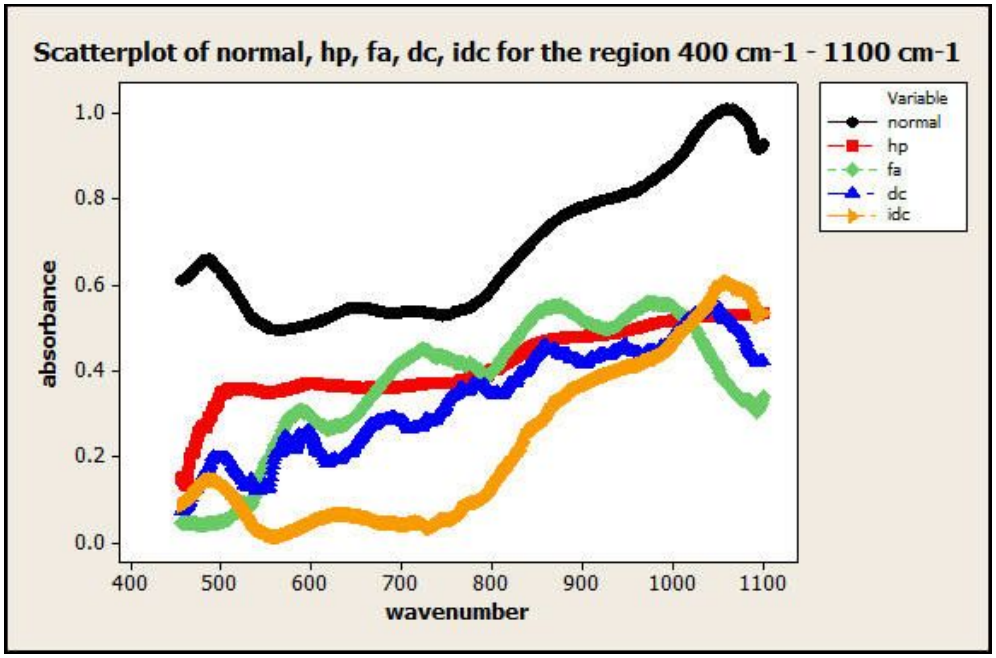

Fig-1: Scatter plot of absorbance Vs wave number for normal, hyperplasia, fibroadenoma, ductal carcinoma, invasive ductal carcinoma.

The spectra of normal, hyperplasia, fibroadenoma, ductal carcinoma and invasive ductal carcinoma is shown in figure-1. The figure clearly differentiates benign and malignant breast tissues. The absorbance peak at 538 cm^{-1} is related to the disulphide bridges in cysteine corresponding to the S-S vibrational mode. The absorbance value at this peak is more for benign compared to malignant stages of breast cancer tissues. It was found that the lysosomal cysteine proteases cathepsin B and cathepsin L have been implicated in tumor spread and metastatis which is a underlining factor for tumor associated proteolysis for invasion and metastatis **(5).** In this way the absorbance variation of the FTIR band at 538 cm^{-1} in normal tissue, hyperplasia, fibroadenoma, ductal carcinoma and invasive ductal carcinoma could be well understood when keeping this in mind these changes in cysteine content occurring in tumoral process.

The absorbance peak at 853 cm^{-1} is the next significant FTIR band to be noted in the spectra. It was found that the absorbance is more for benign tissues less for cancerous tissues. The aborbance at this peak is due to the vibrational modes of (C-C) bond and (O-P-O) bond due to the presence of proline, tyrosine and DNA **(6)**. Figure-1 also represents that at the peak near 935 cm^{-1}, the absorbance values of malignant have a higher value compared to benign stages of cancerous tissues. The absorbance here is due to the (C-C) bonding and α – helix bonding due to the presence of proline, valine, protein and glycogen **(7)**. The absorbance peak at 1005 cm^{-1} is due to the symmetric ring vibrational mode due to the presence of phenylalaline. The absorbance is found to be higher for benign tissues compared to cancerous tissues. The peak at 1080 cm^{-1} is related to the (C-C), (C-O), (PO_2),(C-N),(O-P-O) vibrational modes **(8).** It represents the presence of nucleic acids, proteins and carbohydrates.

Comparative analysis of various breast cancer grades using TREND ANALYSIS PLOT method.

The statistical method used here for the variation of the absorbance values with the cancer grade is done by Trend analysis plot. Trend analysis fits a general trend model to the absorbance value. There are linear, quadratic, exponential growth or decay and s- curve models . In this method there are three accuracy measures named MAPE, MAD, MSD. Mean Accurate Percentage Error (MAPE) measures the accuracy of fitted line series values. It measures accuracy as a percentage. Mean Absolute Deviation (MAD) measures the accuracy of fitted line series values. It expresses the accuracy in the same units as the data which helps conceptualize the amount of error. Mean Squared Deviation (MSD) is a more sensitive measure of an unusually large forecast error than MAD. The Trend analysis draws a graph containing the observations, predicted values(the fitted trend equation) and forecast. The predicted value for a cancer grade is obtained by simple calculations using the fitted equation.

- Black symbols – observed (actual) values
- Red symbols – fitted values
- Green symbols – forecasts

The trend analysis procedure also displays along with the graph , three measures to determine the accuracy of the fitted values: MAPE, MAD and MSD. These three measures are used to compare the fits obtained by using different trend models. For all three measures, smaller values generally indicate a better fitting model.

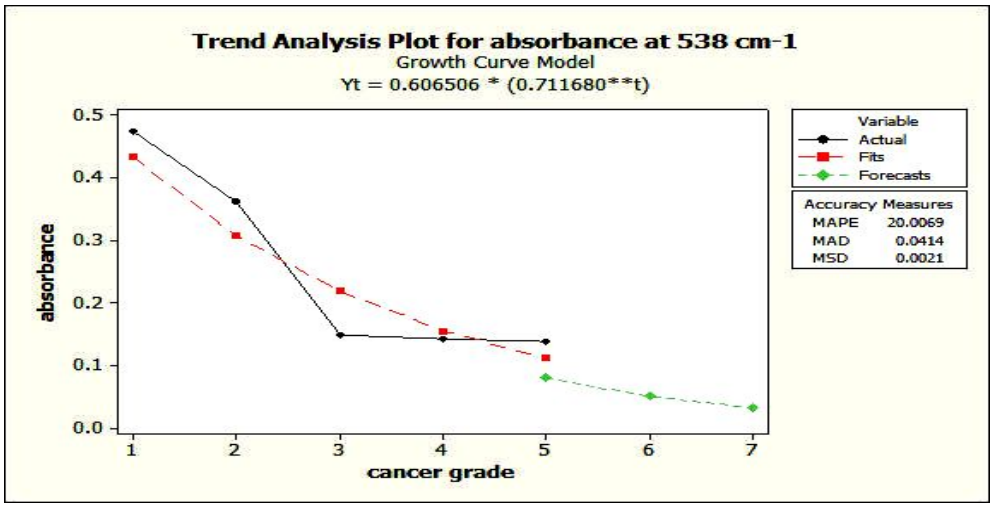

Figure-2(a) :Trend Analysis Plot for absorbance at 538 cm^{-1}

Trend Analysis for absorbance

Data **absorbance**
Length 5

Fitted Trend Equation

Yt = 0.606506 * (0.711680**t)

Accuracy Measures

MAPE	20.0069
MAD	0.0414
MSD	0.0021

Forecasts

Period	Forecast
5	0.0795848
6	0.0507347
7	0.0323430

Cancer grade	mean	Trend	Detrend
1	0.474000	0.431638	0.0423617
2	0.361111	0.307188	0.0539227
3	0.148889	0.218620	-0.0697311
4	0.142000	0.155587	-0.0135875
5	0.138000	0.110729	0.0272715

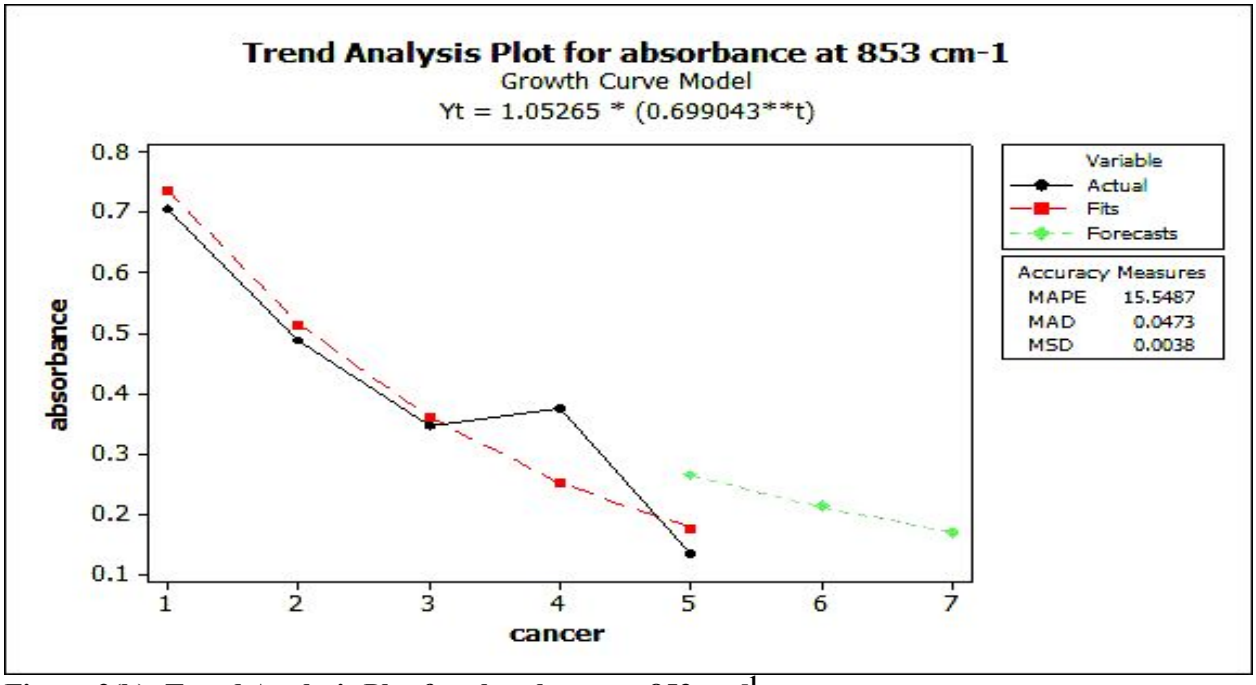

Figure-2(b) :Trend Analysis Plot for absorbance at 853 cm^{-1}

Trend Analysis for absorbance

Data absorbance
Length 5

Fitted Trend Equation

Yt = 1.05265 * (0.699043**t)

Accuracy Measures

MAPE	15.5487
MAD	0.0473
MSD	0.0038

Forecasts

Period	Forecast
5	0.2638500
6	0.211209
7	0.169070

Cancer grade	mean	Trend	Detrend
1	0.704000	0.735850	-0.031850
2	0.488889	0.514391	-0.025502
3	0.346667	0.359582	-0.012915
4	0.376000	0.251363	0.124637
5	0.134000	0.175714	-0.041714

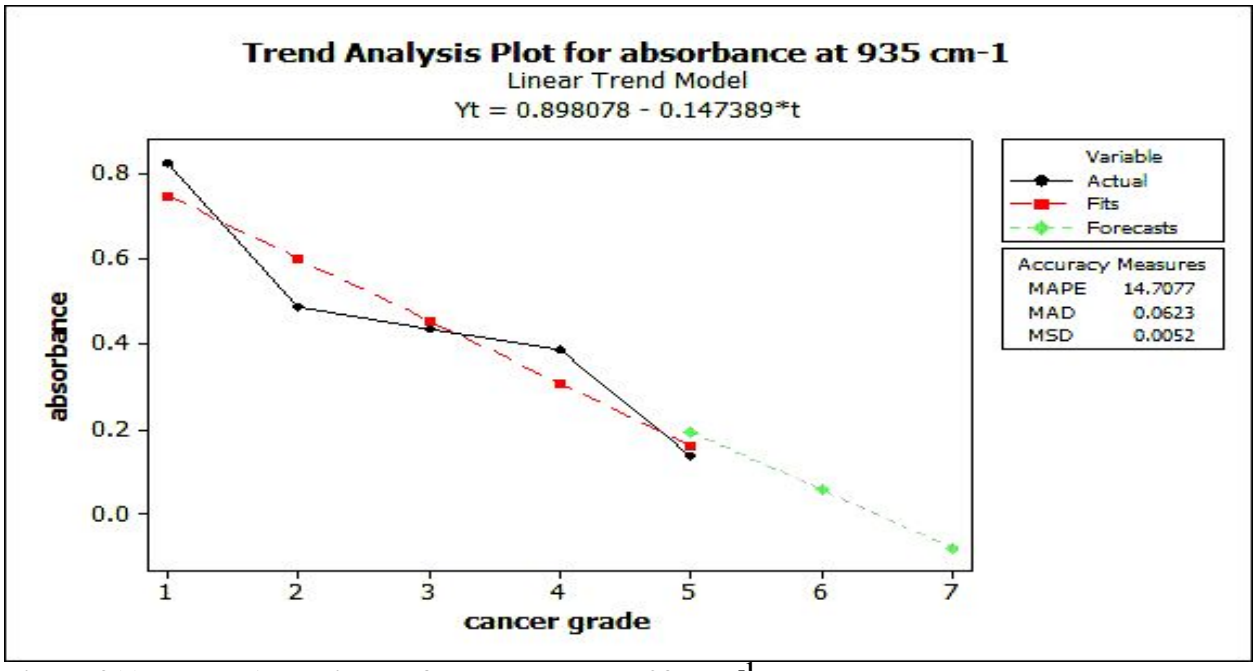

Figure-2(c) :Trend Analysis Plot for absorbance at 935 cm^{-1}

Trend Analysis for absorbance

Data absorbance
Length 5

Fitted Trend Equation

Yt = 0.898078 - 0.147389*t

Accuracy Measures

MAPE	14.7077
MAD	0.0623
MSD	0.0052

Forecasts

Period	Forecast
5	0.194333
6	0.058011
7	-0.078311

Cancer grade	mean	Trend	Detrend
1	0.826000	0.750689	0.075311
2	0.488889	0.603300	-0.114411
3	0.436667	0.455911	-0.019244
4	0.389000	0.308522	0.080478
5	0.139000	0.161133	-0.022133

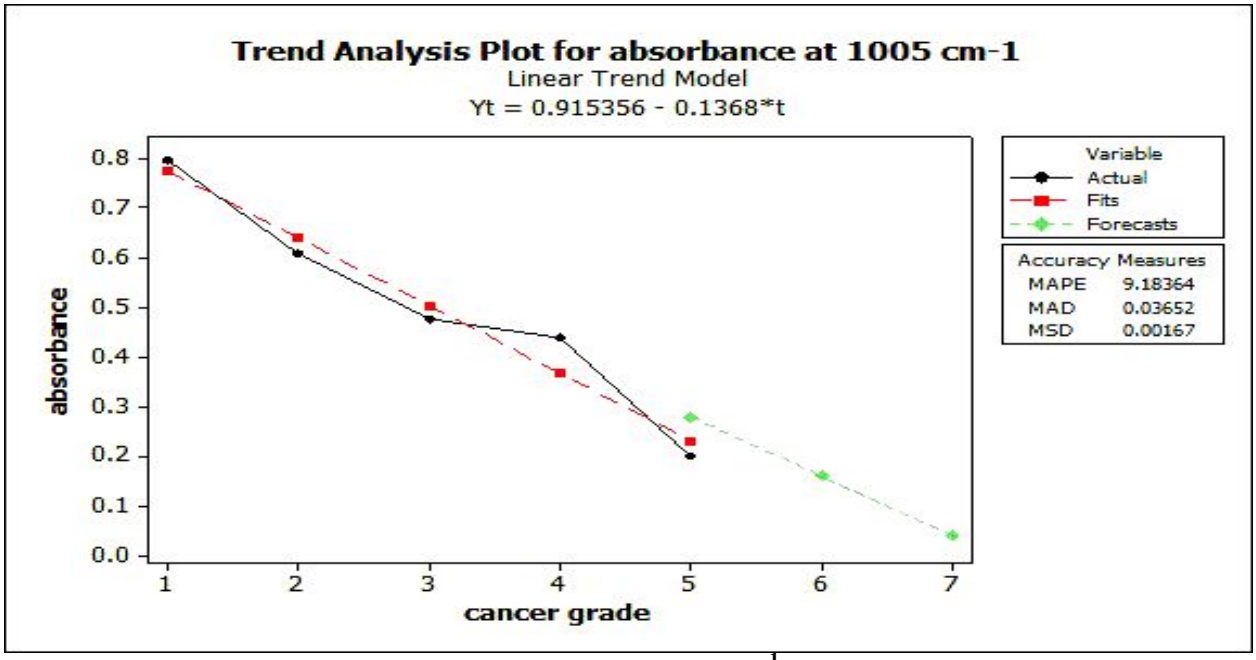

Figure-2(d) :Trend Analysis Plot for absorbance at 1005 cm^{-1}

Trend Analysis for absorbance

Data **absorbance**
Length 5

Fitted Trend Equation
Yt = 0.915356 - 0.1368*t

Accuracy Measures

MAPE	9.18364
MAD	0.03652
MSD	0.00167

Forecasts

Period	Forecast
5	0.279889
6	0.159267
7	0.038644

Cancer grade	mean	Trend	Detrend
1	0.798000	0.778556	0.0194444
2	0.610000	0.641756	-0.0317556
3	0.477778	0.504956	-0.0271778
4	0.440000	0.504956	0.0718444
5	0.199000	0.231356	-0.0323556

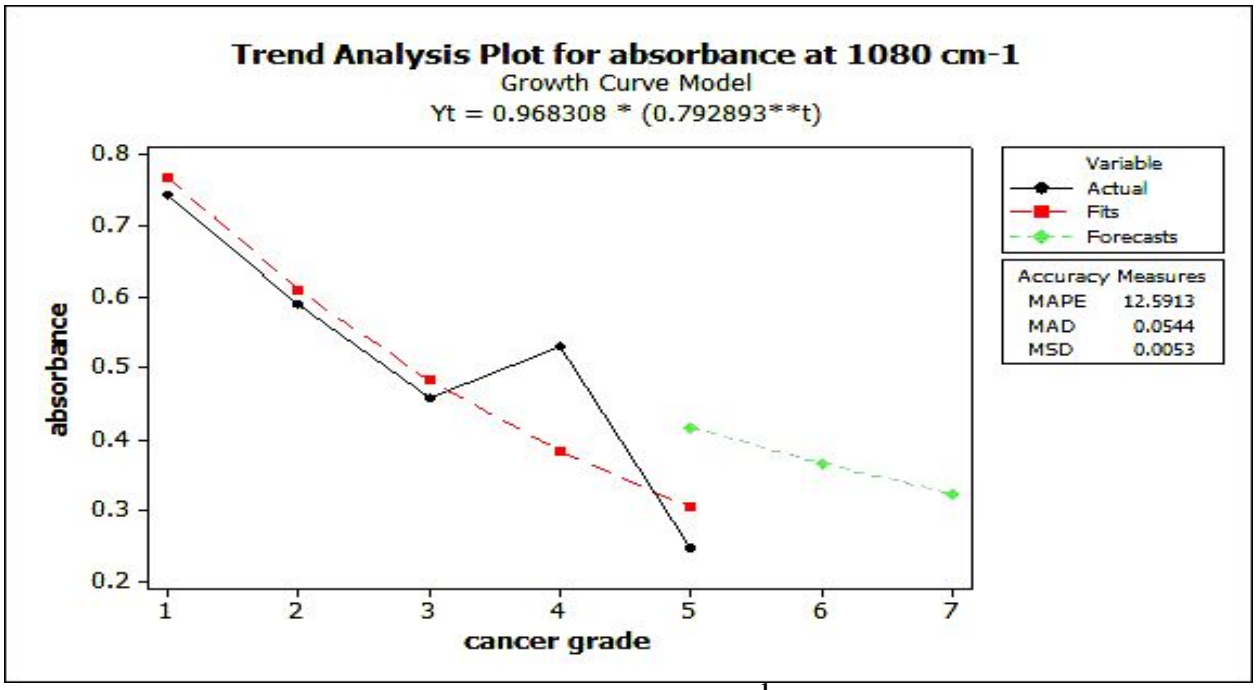

Figure-2(e) :Trend Analysis Plot for absorbance at 1080 cm^{-1}

Trend Analysis for absorbance

Data **absorbance**
Length 5

Fitted Trend Equation
Yt = 0.968308 * (0.792893**t)

Accuracy Measures

MAPE	12.5913
MAD	0.0544
MSD	0.0053

Period	Forecast
5	0.415735
6	0.366107
7	0.322403

Cancer grade	mean	Trend	Detrend
1	0.744000	0.767765	-0.023765
2	0.590000	0.608756	-0.018756
3	0.457778	0.482679	-0.024901
4	0.530000	0.382713	0.147287
5	0.246000	0.303450	-0.0574

Conclusion

In this study it has been analyzed that the Infra Red spectra differentiated the normal and tumoral breast tissues including hyperplasia, fibro adenoma, ductal carcinoma and invasive ductal carcinoma. The collected samples were histopathologically classified into five groups. Through qualitative analysis comparative study has been done using the Trend analysis plot. The forecasts clearly establish the carcinogenesis process when absorbance values reach an extreme smaller value. The changes in the absorbance values for the different cancer grades were because of the changes in the biomolecular changes in the tissues. We were able to establish the biochemical basis for each spectrum by relating the observed peaks to specific biomolecules that have a significant role in the carcinogenesis process. Thus FTIR has a great role in the qualitative analysis of cancerous breast tissues.

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