Fabrication of Porphyrin Based Colorimetric Sensor for the Detection of Liver Cirrhosis Biomarker

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ABSTRACT

The early detection of liver cirrhosis biomarkers is crucial for timely medical intervention and improved patient outcomes. In this study, we present the fabrication of a novel colorimetric sensor based on porphyrin derivatives for the specific and sensitive detection of a prominent liver cirrhosis biomarker. The sensor design capitalizes on the unique optical properties of porphyrins, allowing for a rapid and visually detectable response upon biomarker binding. The synthesis and characterization of the porphyrin receptor are detailed, highlighting its structural and spectroscopic properties. The sensor's performance was evaluated using RGB analysis demonstrating exceptional selectivity and sensitivity towards the target biomarker. Importantly, the sensor's response mechanism is elucidated, shedding light on the underlying molecular interactions. The proposed porphyrin-based colorimetric sensor offers a promising avenue for the early diagnosis of liver cirrhosis, paving the way for point-of-care applications and enhancing disease management.

Keywords: Liver cirrhosis, biomarker detection, early diagnosis, colorimetric sensor, porphyrin derivatives, synthesis, characterization, structural properties, spectroscopic properties, RGB analysis, selectivity, sensitivity, disease management.

Introduction

Liver cancer, also known as hepatic cancer, is a malignant tumor that originates in the liver. It is one of the leading causes of cancer-related deaths worldwide. The incidence of liver cancer has been on the rise in recent years, and it is estimated that by 2030, it will become the second leading cause of cancer-related deaths worldwide.[1]

When liver cells are damaged or destroyed due to various factors, such as chronic hepatitis B or C infections, excessive alcohol consumption, or exposure to toxins, the liver tries to repair itself by regenerating new cells. However, this process can sometimes go awry and result in the formation of cancerous cells.[2]

A variety of tests may be carried out to corroborate the diagnosis of liver cancer if it is suspected. These include imaging tests like CT or MRI images, blood tests to check for tumor markers and liver function, and liver biopsies to look for cancer cells in tissue samples. But these examinations are frequently costly, time-consuming, and invasive.

Moreover, the Live Cancer Survival Rate mainly depends on the disease's stage as shown in the figure 1.[3] Therefore, for successful treatment and a high patient survival rate, early detection of liver cancer is the holy grail in liver cancer study.

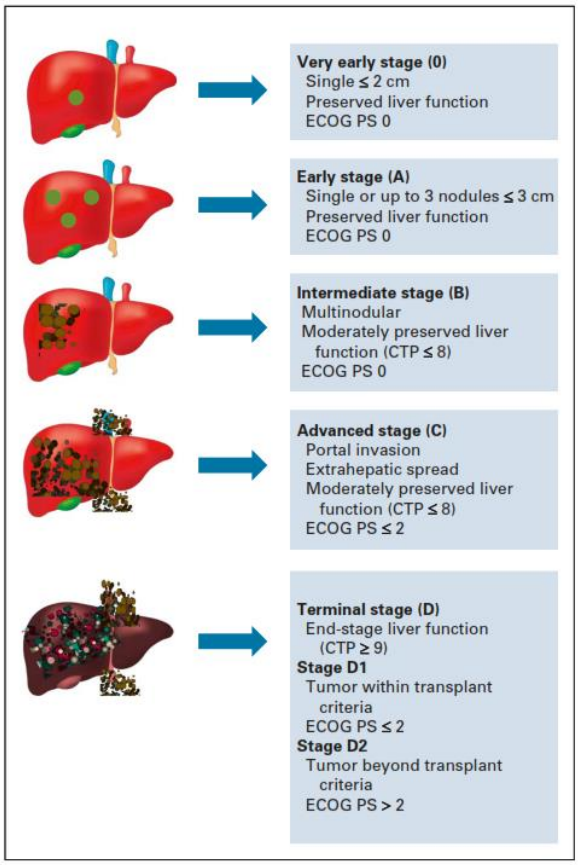

Figure.1.. INASL-modified BCLC staging of HCC. BCLC, Barcelona Clinic Liver Cancer; CTP, Child-Turcotte-Pugh; ECOG, Eastern Cooperative Oncology Group; HCC, Hepatocellular Carcinoma;INASL, Indian National Association for Study of Liver; PS, performance[4]

It is found that many patients with liver disease like hepatocellular carcinoma, liver fibrosis, and cirrhosis etc. exhaled mixture of specific VOCs which would have normally not present if the liver would have functioning well.

T Sukaram et. al studied the VOCs profile in exhaled breath of 97 HCC patients and 111 controls using gas chromatography–mass spectrometry and Support Vector Machine algorithm it was found that the combination of acetone, 1,4-pentadiene, methylene chloride, benzene, phenol and allyl methyl sulfide provided the highest accuracy of 79.6%, with 76.5% sensitivity and 82.7% specificity. [5]

Though GC-MS techniques used in the above studies are more or less accurate, but these techniques are highly sophisticated and costly and required high-end facilities.[6]

Therefore, the alternative methods which would be easy and cost effective and could be performed in a self-testing manner is the need of the hour. Like the testing should as easy as the pregnancy test kits.

Porphyrins, a class of tetra pyrrole macrocycles, have garnered considerable interest for their unique structural and electronic properties. Their planar, cyclic framework comprises four pyrrole rings linked by methine bridges, encircling a central metal atom, typically iron, cobalt, or zinc[7]. Porphyrins exhibit distinct optical and electronic characteristics, making them ideal candidates for transducing molecular interactions into measurable signals. Moreover, their versatile coordination chemistry enables the customization of sensor properties by modifying ligand structures and metal centers.[8]

Colorimetric sensing, which relies on visual color changes as an indication of analyte presence, has gained popularity due to its simplicity, low cost, and potential for on-site detection.[9] Porphyrins, when appropriately designed and tailored, can undergo significant alterations in their optical properties, such as absorbance or fluorescence, upon interaction with specific VOCs. These changes arise from the modulation of electronic transitions within the porphyrin ring, triggered by interactions ranging from hydrogen bonding and host-guest interactions to coordination chemistry.[10]

This paper explores the burgeoning field of porphyrin-based colorimetric sensors for VOC detection. By harnessing the distinctive structural and optical properties of porphyrins, researchers are pioneering innovative sensor designs that exhibit high sensitivity, selectivity, and real-time response to various VOCs. The integration of porphyrins with appropriate transduction mechanisms, such as color changes observable to the naked eye or through portable devices, presents a promising avenue for the development of practical and efficient VOC detection systems.

These colorimetric sensor can also be designed to fit in specific VOCs in liver cancer patients and hence give signal electronically or visually by changing its color. What we desire is to detect these by visually.

As per study by T Sukaram et. al. people with liver cirrhosis/cancer are not able to

digest limonene properly hence their breath contains appreciable amount of D-limonene which is present as most of the food additives and citrus fruits.[11] So presence of D-limonene in the liver cancer patients can be taken as a gold standard of early liver cirrhosis or cancer. Also as shown in the Figure .4. the asterisk carbon is a chiral carbon. Therefore, we expect that the porphyrin based colorimetric sensor can specifically and sensitively interact with chiral limonene to produce the signal as color change.[5]

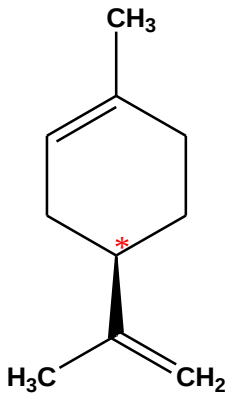

Figure.4. D-Limonene

1.2 OBJECTIVES

Porphyrin Selection and Modification:

Identify suitable porphyrin compounds with the potential to interact with the targeted liver cirrhosis biomarker. Modify the porphyrin structures to optimize their sensitivity and selectivity for the biomarker of interest.

Synthesis and Characterization:

Synthesize the modified porphyrin compounds and thoroughly characterize their chemical structures using spectroscopic and analytical techniques. Confirm the successful modification and structural integrity of the porphyrins.

Interaction Studies:

Investigate the interactions between the modified porphyrins and the specific liver cirrhosis biomarker. Assess the RGB change porphyrin-biomarker complex formation.

Optimization and Data Analysis: Optimize the conditions that induce colorimetric changes in the porphyrin upon binding with the biomarker. The optimization of the device to achieve the most noticeable and reproducible color changes and data would be analyzed.

Sensitivity and Selectivity Enhancement:

Enhance the sensor's sensitivity and selectivity by fine-tuning the porphyrin structure and experimental parameters. Minimize potential interferences from other compounds commonly found in biological samples.

SYNTHESIS, PHYSICAL METHODS AND CHARACTERIZATION

2.1 Introduction to Synthesis

Synthesis of heterocycle appended porphyrin is of major interest as heterocycles are excellent template for generation of robust supramolecular assemblies and molecular wires. Additionally, heterocycles appended porphyrins become a good sensor element as the heterocycle can interact with a variety of analytes which in turn will lead to a detectable change in the absorption and emission properties of the porphyrin. In our synthesis Alder and Longo method gave the product in moderate yield however the simplified purification process is an added advantage. Other methods failed to give the product within the experimental conditions used.

2.2 Chemical, Instruments and Physical Methods

2.2.1 Chemicals :

Pyrrole, Benzaldehyde ,copper acetate, manganese acetate, nickel chloride, sodium acetate, lead acetate.

2.2.2 Solvents :

Propionic acid, acetic anhydride, chloroform, Methanol

2.2.3. Adsorbent Used

Silica gel (60-120 mesh, Merck).

2.2.4Instruments

• RB-50ml • Magnetic stirrer • Heating plate • Oil bath • Micropipette • Beaker 5 • UV-Vis spectrophotometer

2.2.5 Physical Methods

The UV-visible spectroscopy was recorded with Shimadzu Perkin Elmer Lamda 265 spectrophotometer. The data was processed using UV-probe software manager. In all the experiments, the scan speed was kept at 400 nm per minute with a slit width of 0.1 nm.

2.3 SYNTHESIS OF TETRAPHENYLPORPHYRIN (H_2TPP) AND MONITORING BY UV VISIBLE SPECTRA: 250ml of propanoic acid and 1 ml of propanoic anhydride was added in 50 ml round bottle flask and heated till it cross 100^0 C.Then 0.74 ml(740micro liter) Benzaldehyde and 0.505(50micro liter) Pyrrole was added in solution and refluxed for 1 hrs.After each 45 min UV-Visible spectra is taken. With the help of glass rod we took the sample in the cuvette and it was mixed with the chloroform and the structure of pure final product was studied by UV-Vis spectroscopy. And then filtration was done with the help of vacuum pump and purple precipitate was found. The purple precipitated filter paper was washed with heated water. After that with another dropper methanol was poured dropwise until the residue coming out was colorless. As long as the liquid coming out is black in color, it is impure, again dropwise methanol was added then colorless liquid came. A purple precipitate came out in the filter paper. Put it in the veil and measure the weight. The weight of the precipitate was 136 mg.

2.4.1COPPER TETRAPHENYLPORPHYRIN(Cu-TPP):- In a 50 ml round bottom flask 0.314 mg TPP and 50ml DMF and 5.4 mg copper chloride was taken and it was refluxed for 2hrs.. . With the help of glass rod we took the sample in the cuvette and it was mixed with the chloroform and then the structure of pure final product was studied by UV-Vis spectroscopy. After that, column chromatography was being done where at first silica gel with chloroform was poured in the burette and then the refluxed product was poured. All the organic solvents came out from the burette. These organic solvents are then poured in the conical flask by filter paper. The spectra of the

2.4.2 IRONTETRAPHENYLPORPHYRIN(Fe-TPP): In a 50 ml round bottom flask 15.3 mg TPP and 15ml DMF was taken and it was refluxed. After that we

added iron (II) chloride in that round bottom flask and boiled for one and half hours. With the help of glass rod we took the sample in the cuvette and it was mixed with the chloroform and then the structure of pure final product was studied by UV-Vis spectroscopy. After that, column chromatography was being done where at first silica gel with chloroform was poured in the burette and then the refluxed product was poured. All the organic solvents came out from the burette. This organic solvent was then poured in the conical flask by filter paper.

2.4.3 ZINC TETRAPHENYLPORPHYRIN (Zn-TPP): In a 50 ml round bottom flask 30 mg TPP and 5ml chloroform was taken and it was refluxed. After 1 hour 30 minutes when the temperature rose to 120°C, a mixture of 14 mg Zinc acetate with 10 ml methanol,measured in a measuring cylinder, was poured in that round bottom flask. With the help of glass rod we took the sample in the cuvette and it was mixed with the chloroform and thenthe structure of pure final product was studied by UV-Vis spectroscopy. After that, column chromatography was being done where at first silica gel with chloroform was poured in the burette and then the refluxed product was poured. All the organic solvents came out from the burette. This organic solvent was then poured in the conical flask by filter paper also water was added dropwise. The spectra of the residue was being checked and then it was kept for drying

2.4.4 MANGANESE TETRAPHENYLPORPHYRIN(Mn-TPP): In a 50 ml round bottom flask 30 mg TPP and 5ml chloroform was taken and it was refluxed. After 1 hour 30 minutes when the temperature rose to 120°C, a mixture of 13 mg Manganese acetate with 10 ml methanol,measured in a measuring cylinder, was poured in that round bottom flask. With the help of glass rod we took the sample in the cuvette and it was mixed with the chloroform and then the structure of pure final product was studied by UV-Vis spectroscopy.After that, column chromatography was being done where at first silica gel with chloroform was poured in the burette and then the refluxed product was poured. All the organic solvents came out from the burette. This organic solvent was then poured in the conical flask by filter paper also water was added dropwise. The spectra of the residue were being checked and then it was kept for drying.

3.1 Sensor Plate Construction

- The synthesized compounds was dissolved in respective solvents.
- The glass slides was thoroughly washed and dried.
- Using the Micropipette tips the thin flim was formed in the glass slied as shown in the figure There were six thin films where made on single glass slide and the slides were numbered form 1 to 6

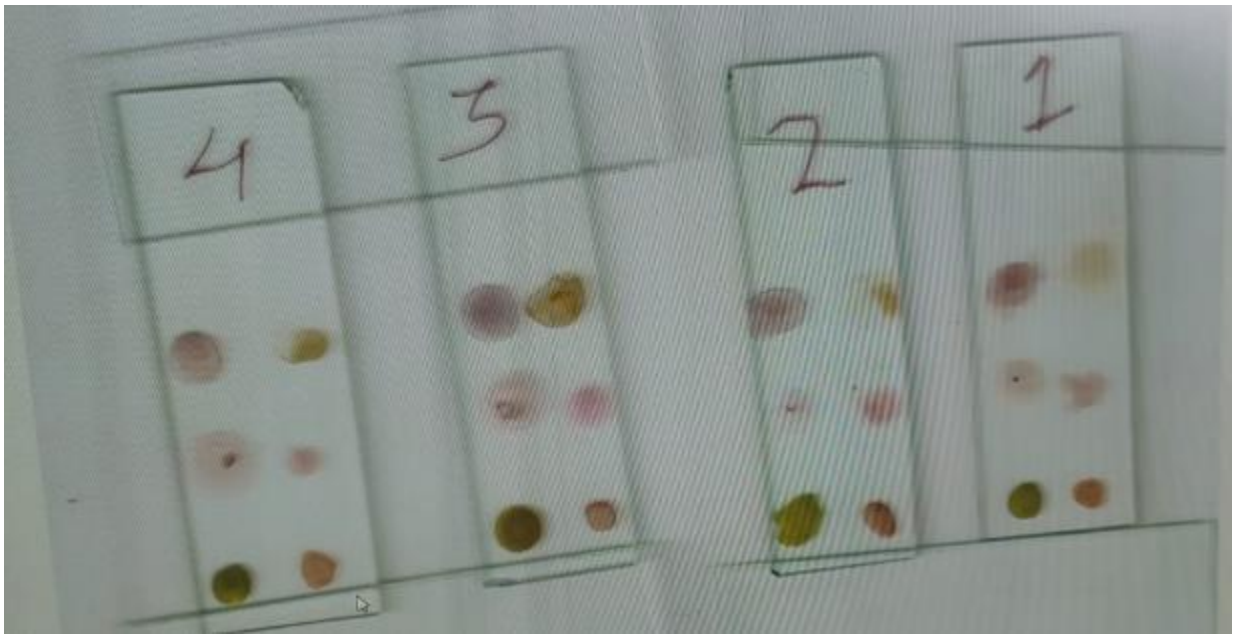

Fig.13. Fabricated Thin Film Sensors

- Following were the pattern in which the sensor elements were placed in the Sensor Plate

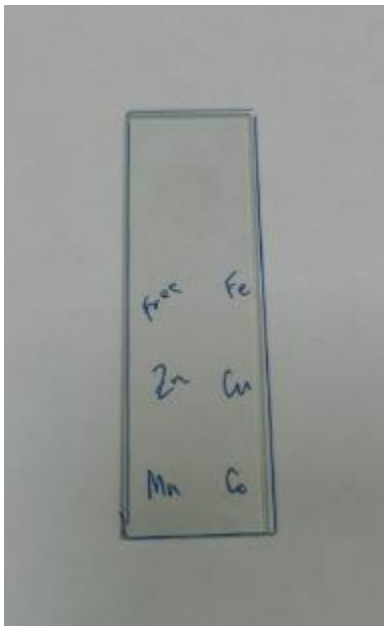

Fig.14. Allocation of sensor elements on sensor plate

Where Free = Free Based TPP, Fe = Iron TPP, Zn = Zinc TPP, Cu = Copper TPP, Mn = Manganese TPP, Co = Cobalt TPP

- The slides were vacuum dried in vacuum chamber and ready for analysis **Testing of Device with Cirrhosis Biomarker**

- The two sets of experiment were designed in which the source of Limonene were Lemon and Sweet Lime was used.

- The reason for choosing Lemon and sweet lime is they are the good source of D-Lemonene and could be standardized for testing Liver Cirrhosis Patients due to common consumption and easy availability.
- Before the exposure, the Sensor plated where vacuum dried for 30 mins.
- The sensor plates were then scanned using HP LaserJet M1005 MFP scanner at highest resolution. Immediately after scanning the sensor plates were exposed to limonene vapor at room temperature.
- Exposure time was maintained to be 5 mins.
- The Glass device used for exposure was of 100 ml capacity. **The data obtained were put for RGB analysis.**

4.1 Result Analysis

4.1.1 Data Acquisition

The scanned sensor plates were feed into the computer and RGB values were noted for Before Expousre and After Exosure to Limonene.
It was kept in mind that while getting RGB values same spot was taken from sample before and after exposure.

4.1.2 Data Process

Follwing is the methodology used to calculate RGB diffrence and representation

Before Exposure		After Exposure		RGB Diffrence
R_1 G_1 B_1	-	R_2 G_2 B_2	=	$(R_1 - R_2)$ $(G_1 - G_1)$ $(B_1 - B_2)$

Where R, G , B are Red , Green and Blue values of the elements of Colorimetric Sensor strip .
All the data where put in the tabular form and the RGB diffrence was obtained as shown below

Source: *Sweet Lime* **Slide No:** 1

Before Exposure		After Exposure		Difference	
191,166,176	261,195,189	71,99,265	215,216,233	50,67,89	120,21,44
206,105,165	206,171,215	265,190,195	165,225,125	59,85,30	41,54,90
164,195,75	191,193,119	122,135,198	155,43,162	42,60,123	36,150,43

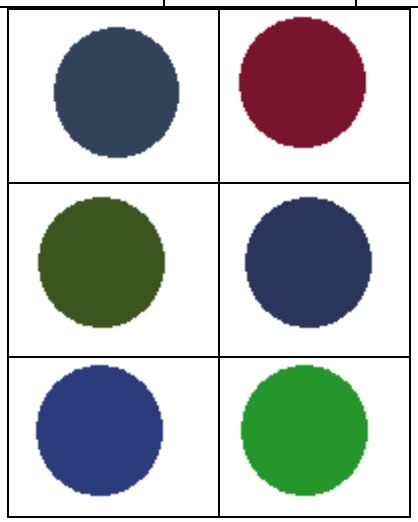

Fig.19. Color Difference for Sweet Lime Slide 1

Source: Sweet Lime **Slide No:** 2

Before Exposure		After Exposure		Difference	
172,146,163	165,163,151	192,180,188	295,195,204	20,34,25	130,32,53
185,145,160	132,189,208	207,235,215	204,152,108	22,90,55	72,37,100
175,160,167	145,136,127	144,125,46	186,180,194	31,256,121	41,120,67

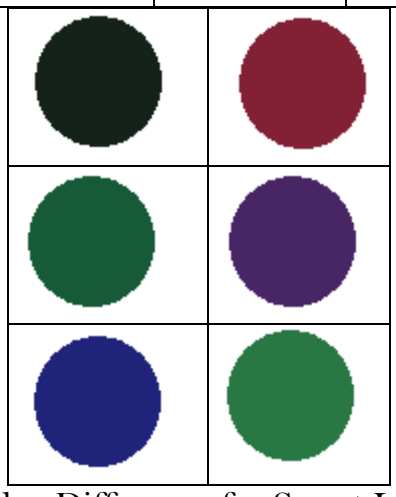

Fig. 20. Color Difference for Sweet Lime Slide 2

Source: Lemon **Slide No:** 3

Before Exposure		After Exposure		Difference	
159,144,166	168,155,123	197,192,201	204,193,175	38,48,35	85,38,52
163,136,194	203,190,172	196,190,153	133,144,292	33,54,41	70,46,120
174,157,74	184,159,51	138,77,111	145,239,115	36,80,37	39,80,64

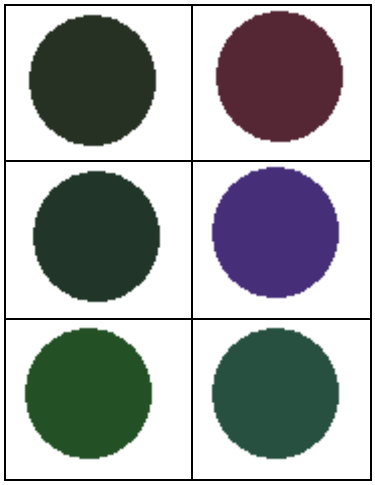

Fig.21. Color Difference for lemon Slide 3

Source: Lemon **Slide No:** 4

Before Exposure		After Exposure		Difference	
210,195,250	242,123,194	174,164,189	172,176,148	36,31,61	70,53,46
258,154,286	269,191,263	206,196,209	213,253,143	52,42,77	56,62,120
173,58,52	199,194,189	205,148,92	160,109,129	32,90,41	39,85,60

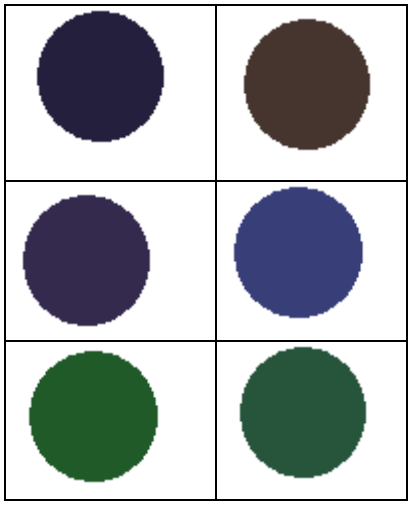

Fig.22. Color Difference for lemon Slide 4

Source: Water **Slide No:** 5

Before Exposure		After Exposure		Difference	
197,182,197	212,205,201	190,180,193	206,199,192	7,2,4	6,6,9
208,202,218	218,194,214	201,195,211	217,196,213	7,7,7	1,2,1
166,151,82	199,172,153	190,186,85	183,154,137	24,-35,-3	16,18,16

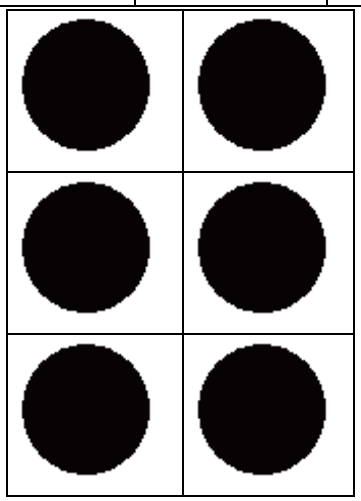

Fig.23. Color Difference for lemon Slide 5

Source: Water **Slide No:** 6

Before Exposure		After Exposure		Difference	
196,180,205	197,17,131	187,171,185	190,171,143	3,1,13	4,4,16
208,197,202	206,185,204	196,186,198	199,183,198	-3,-8,-1	8,9,3
174,157,89	195,176,158	158,142,68	181,163,145	1,-3,22	5,2,8

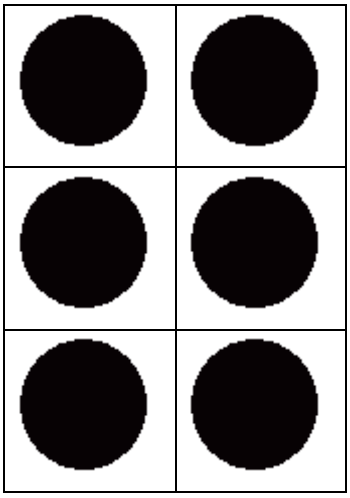

Fig.24. Color Difference for water vapour Slide 6

4.2 Data Analysis

It was found that there is considerable RGB diffrence between before and After exposure to Limonene from Sweet Lime and Lemon.

Free base TPP, Cu-TPP and Mn-TPP has shown less RGB difference than Fe-TPP, Zn-TPP and Co-TPP. Among them Fe –TPP and Co-TPP has shown considerable color change on exposure. This is probably due to the prsence of reactive vacant sites in these metal TPPs due to which they are interacting with the Biomarker and hence changing the coclor considerably. Similar is the case for activity of Haemoglobin and Vitamin B_{12} which contains Fe and Co at their porphryin cores and hence coordinates with incming species effectively showing the properties.

The other important result form the study is that when the senor was exposed to water vaopur , there is no chamge in color of the sensor which means the device can diffrentiate between the Health and Cirrohosis Patienst. Also even patients exhales water along with the Bimarker and hence water might not interfree in the Diagnosis of patients.

5.1 Conclusion

So we conclude that the study is highly succesful is sensing Biomarker fo Liver CIrrohosis at very low concentrations. The resulting pattern coming out of the sensor when exposed to the Biomarekr encorages the resercher to furtehr proceed with the research. The hardest part of the work was synthesising sensor elements at ultra high purity.

5.2. Future Aspects

The work can now be extended to field survey, where cirrhisis patient may be asked to breath in the chamber containg the sensor elemnet. If the color change is nakedly visible by eye its well and good , otherwise an app can be developed which can process the iamges and can give the results in seconds.

In either of the case, the study has high probability of success which can result us in fast, cost effective, non-invasive diagnisisi device for early detection and monitering of liver cirrohsis with high sensitivity and specificirty. The product is high;y feasible to be commercialised. The stduent and guide explores for funding possibility of the research with State, National , Private and Inetrnational funding agencies.

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