Chapter 5

Summary and Conclusion

5.1. Summary

Porphyrin array-based chemical sensing shows promising results for the direct diagnosis of pulmonary tuberculosis in total exhaled human breath. It would be worth evaluating colorimetric porphyrin elements based sensor array in direct diagnosis and prognosis of the active P-TB cases. A new composition of sensor array is required for better accuracy parameters.

It is expected that a unique signature for P-TB patients could be obtained by a proper choice of porphyrin elements that are used to construct the array. If a satisfactory positive predictive value can be obtained even for few porphyrin derivatives, it will open up a new avenue for further studies. Further improvisation can be done by zeroing down on the types of porphyrin compounds to construct future arrays. The interaction properties of the porphyrin can be modulated by variable central metal and peripheral substituent and can be judiciously used to increase the predictive value which is a big advantage for such type of sensor studies.

Exhaled breathe analysis have inimitable advantages that they can be repeated with lesser considerations of time-place-person in contrast to the routine tests avoiding the invasive methods that need ‘universal precautions’ or time spells as in collection of the excretory products and, or repetition like imaging techniques or prohibition of administering several tests in extremes of ages or in critical care survivors. The portability dilemma to outreach
places without constant power supply in the developing countries is solved with real-time point-of-care testing with a hope in applicability in primary care settings.

Unlike methods such as GC-MS that involve component-by-component analysis, colorimetric sensor array can classify complex chemical mixtures without ever explicitly identifying their individual components [218].

Human exhaled breathe provides useful information in health and disease like biometry with hidden data for diseases recognition. Further, our world is moving towards the inexpensive assessing tool and rapid point-of-care diagnostic methods of different diseases. So, for the direct diagnosis of tuberculosis, the breath analysis tools may be worth developing and evaluating as a cost-effective entrant in diagnostic algorithms that may facilitate investigation of individual patients and so enable greater understanding of the factors influencing prognosis for which researchers are working in India and abroad. [45]

In our study, 24 porphyrin derivatives were synthesized indigenously at our laboratory and were coded. The elements were diluted with non-interacting petroleum ether solvent and were spotted onto glass plates from solutions (of a few mM) in thin film spots by dots in construction of the sensor; after spotting the colorimetric sensor plates were dried under vacuum at room temperature for thirty minutes before they were in use in our study. The RGB values of the changes in colour of the sensor element were analyzed and statistical tools were used to evaluate the effectiveness of the sensor in terms of disease detection capability and selectivity.

A single exhaled breath test was done on total 94 participants; 64 consecutive pulmonary tuberculosis cases in two phases of the study presenting at DOTs Clinic at STNM Hospital working under stipulated guidelines framed by RNTCP protocol [183]. Healthy volunteers (n=30) were participants from among the staffs and students of University Colleges.
An outstanding difference of responses in various parameters was observed in many of the coded porphyrins between healthy & P-TB cases. Each of the indigenously synthesized porphyrin based chemicals provided evident changes after exposure to single total exhaled breath in all the participants presenting with pulmonary tuberculosis cases. The different porphyrin derivatives exhibited varying responses.

In the accuracy parameters, high sensitivity (> 80%) was in 8(eight) analytes viz. IB, IIBa, ICh, IAb, ICd, IEc, IAe, ICf in Phase I compared to 3(three) viz. ICh, IEc, IAb in Phase II. In terms of specificity, only ICa emerged as a good sensor element.

In the overall analysis sensor elements could reach up to a maximum sensitivity of 85.42 percent (ICh) and high sensitivity (> 80%) was observed in 3(three) more analytes viz. Analytes ICh (85.42%), IB (82.29%), IAb (81.77%), IEc (81.18%); On the other hand reportable specificity was noted in only in the analyte ICa (78.89%). Time and again good positive predictive values (> 70%) were noted only in ICa in both the phases and in overall data (86.23 %) also. However, few other analytes also showed relatively high (> 70%) positive predictive values. They were ICe (77.06%), I (76.51%), IAb (76.21%), IAc (76.15%), IIb (76.00%), ICd (75.31%), ICg (74.71%), IIAc (74.59%), IAe (73.43%), ICf (72.06%), ICc (71.59%), ICh (70.69%), IEb (70.00%). Conclusive level (>10) of Likelihood ratio positive value (LR+) could not be reached by our sensor elements. Analyte ICa was showing consistent reasonable values viz. 2.39 (phase-1), 2.50 (Phase II), 2.94 (overall) in our series of compounds.

In the binary logistic regression 5(five) analytes viz. ICa, IIb, ICd, ICh, ICe were stronger components showing high statistically significant reaction (p<0.001) in Phase I compared to 2(two) in Phase II; statistically significant reaction (p<0.05) in Phase I was noted in 6(six) viz. I, IAb, ICc, IAe, ICg, IB compared to 7(seven) viz. IAb, I, IIb, IAe, ICd, IB, ID in
Phase II. Overall 12 (twelve) analytes showed as fairly strong components in our sensor array. Of them 4(four) analytes viz. IIBa, ICa, ICd, ICe were showed high statistically significant reaction (p<0.001). However, statistically significant reaction (p<0.05) was also noted in 8(eight) analytes viz. I, IAb, IEc, ICc, IAe, ICg, IB, ICh.

Thus 2(two) components in our sensor array viz. ICa and ICe have consistently shown fairly good response by showing high statistically significant reaction (p<0.001) in phase -1, phase - 2 and in overall analysis. Six more analytes in our series viz. IIBa, ICd, I, IAb, IAe, and IB was also shown be statistically significant (p<0.05) in phase -1, 2 and in overall analysis.

In spite of the results being not close to clinically acceptable accuracy paarmeters, it is noteworthy that many of the porphyrins utilized in this study have the potential of discriminating healthy and diseased person. The commonality in the Phase I and Phase II study amongst the sensor element exhibiting good response validates the possibility of using porphyrin derivatives as a viable disease marker. As elaborated earlier, the most important findings of the study and in a way path-breaking has been that variations in base porphyrin, the central metal and substituents at the periphery significantly modulate the responsiveness of these porphyrins. Especially the impact of a particular electron withdrawing group was very large on the overall responsiveness of the porphyrin.

Another interesting aspect of the study has been the identification of porphyrin that exhibits good colour change with healthy persons and negligible colour changes with patients suffering from pulmonary tuberculosis.

With so many porphyrin derivatives that can be synthesized and the metal ions that can be incorporated in core supplemented by the wide variety of electron withdrawing groups that can be substituted at the periphery, a huge bank of possible sensor elements is at hand that needs to be explored in detail to achieve a much better result finally leading to clinically acceptable response.
5.2. Limitations and Scope for Further Studies

To the best of our knowledge, we are first to conduct and report colorimetric sensor array studies using porphyrin based elements pertaining to exhaled breath study of pulmonary tuberculosis cases. This colorimetric sensing can be visualized as a futuristic model of non-invasive diagnosis to fix the chemicals of breath. Our encouraging results augment reliability to porphyrin based sensors to find novel and reliable non-invasive clinical diagnosis with a far reaching impact to establish new range of self-administered instrument.

Generally, the colour of the sensor elements changed noticeably even with naked eye after exposure with single total exhaled breath. The different analytes interacted differently thus exhibiting varying colour change and is a good indicator for possibility of identifying a unique signature pattern. Strengths of our study were that we synthesized all compounds with ultra-high purity. Secondly, diagnostic accuracy was assessed including binomial regression. Thirdly, we used higher cut-offs of more than 10 RGB difference for interpretation.

Breath testing provide us to reflect beyond ‘glass box’ of available invasive and non-invasive procedures like patients in critical care units or cost-effective screening in kidney or liver ailments or in cancer with uninterrupted analysis even under stress for improved understanding of physio-chemical processes. Apart from being noninvasive character breath analysis can be repeated without sanction to time, place and person as real-time personalized medicine at point-of-care, and it increases the potential for even in the remote domiciliary settings of the developing countries. On the downside, there are multitude of possible confounders, from host diurnal and seasonal variations to even dietary patterns and environment. Lack of standardization is a major problem; sampling and timing in relation to the respiratory cycle with control of flow, pressure, and ambient conditions are key issues.
5.2.1. Limitations of Our Study

Our study was limited by the lack of expected sensitivity and specificity, predictive value and likelihood ratios possibly due to small sample size. Secondly, we worked in the empirical methods in the resource poor settings. Thirdly, the study was conducted in an urban tertiary care hospital chest clinic. Fourthly, due to administrative reasons we included all the P-TB cases irrespective of the status of diagnosis or therapy.

We were not able to conclusively establish the unique signature pattern that was clinically acceptable even though many of the sensor elements were able to discriminate the healthy and diseased states.

The participants under study, pulmonary tuberculosis patients (case) and health volunteers (control), were grossly heterogeneous in terms of socio-demographic parameters with the state of morbidity. In the resource poor settings of this non-funded research project in a sparsely populated north-eastern state of India, we had to omit restrictions on age, gender, ethnicity, occupation, tobacco use, dietary patterns, co-morbidities and medications were set aside for participation requisites of inclusion or exclusion.

It would be ideal to have a stricter control on the sample set of participants which would naturally lead to results with higher precision.

Further, the sample size was small due to constraint in the number of patients available at the hospital and being voluntary in nature. A larger sample size would have allowed us to achieve a much better result in statistical analysis.
5.2.2. Scope for Further Studies

Sputum microscopy consumes two days, sputum culture up to three months though they remain the basis of laboratory diagnosis. So there is an urgent necessity for novel precisioned diagnostic tools to halt the pandemic of tuberculosis that will be cost-effective, noninvasive, and suitable for use in primary care settings. The present study established the efficacy of porphyrin derivatives in discriminating healthy and persons suffering from pulmonary tuberculosis. The findings of this study can be extended to investigate other classes of porphyrin and its derivatives for identifying sensor elements that can produce the best possible response. It will be followed by construction of sensor arrays of such elements to achieve clinically acceptable results. Further studies are required to determine the accuracy parameters when compared to standardized microbiological and clinical indicators of tuberculosis disease to obviate the potential bias in a large multicenter trial even extending to comparison with the "gold standard" of sputum culture. The use of the colorimetric sensor arrays can be extended to other lungs diseases and diseases like diabetes which produces significant amount of ketone bodies in breath. In principle there is no limit for the investigation of types of disease as analysis is not based on specific analytes but on overall composition of breath biomarkers in the total exhaled breath. This screening test need to be highly selective as well as simple, rapid, inexpensive and non-invasive viable method of diagnosis for tuberculosis. The quality of interaction of porphyrin with VOCs can be controlled by suitable changes in porphyrin macrocycle like peripheral substituent and central metal. This might lead to fine tuning of senor array by identifying responsive porphyrin derivatives with better predictive value that has to be further standardized to achieve unique colour ‘chemical signature’ pattern. To obviate all the potential biases large multicenter trial are needed to compare P-TB diagnosis with the “gold standard” of sputum culture.