doi.org/10.36721/PJPS.2022.35.1.REG.157-160.1

Colorimetric chromophoric rapid detection of SARS-CoV-2 through breath analysis

Hamdullah Khadim Sheikh[1]*, Tanzila Arshad[2], Uzma Habib[3], Zainab Sher Mohammad[4], Maaz Uddin Ahmed Siddiqui[1] and Mohtasheemul Hassan[1]

[1]Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi, Karachi, Pakistan
[2]Department of Applied Chemistry, University of Karachi, Karachi, Pakistan
[3]Research Centre for Modelling and Simulation, National University of Scientist and Technology, Islamabad, Pakistan
[4]Health Sciences, Riphah International University, Islamabad, Pakistan

Abstract: Early and rapid detection of SARS-CoV-2 in an infected person is one fundamental part of the strategy against the spread of this virus. As of now, the usual practice is to carry out polymerase chain reaction (PCR) test which provides results in 24-48 hours. Hence, there exists a crucial need for rapid and immediate screening of people suspected to be infected. Presence of volatile organic compounds (VOCs) in the exhaled breath can be one such prospect for detection of virus. In this paper, we have designed chromophoric adducts of VOC's in the exhaled breath that can be formed for colorimetric detection of SARS-CoV-2. We noted the bathochromic shift in λ (nm) of VOC molecules upon chromophore formation for colorimetric detection. If adapted, this research work will result in low cost solution to the requirement of immediate detection of SARS-CoV-2, hence cost and time of testing will be reduced, compared to PCR and antibodies tests. Also VOC's detection in early stage of infection where symptoms are not visible can be advantageous.

Keywords: COVID-19, biosensing techniques, breath tests, volatile organic compounds, DFT calculations.

INTRODUCTION

Detection of SARS-CoV-2 requires painless and immediate method that can help the prevention of the spread of virus. Currently, most common methods include, (i) Detection of viral gene by nucleic acid amplification such as polymerase chain reaction (PCR), gene sequencing (Wang *et al.,* 2020) and CRISPR (Clustered regularly interspaced short palindromic repeats) nucleic acid detection (Ding *et al.,* 2020) (ii) Detection of SARS-CoV-2 related antibodies (iii) Detection of SARS-CoV-2 related antigens. Among these, most effective method is the PCR test, which involves rather unpleasant nasal swab sampling technique while it takes up to 24-48 hours to give out the results. Even though PCR test is highly accurate, but because of the mentioned issues, many researchers are now turning towards the establishment of rapid detection methods (Crozier *et al.,* 2021). One of the reasons of rapid transmission of SARS-CoV-2 is the presence of the virus in exhaled droplets of breath (Sanjuan-Reyes *et al.,* 2021). Keeping in perspective, the above-mentioned drawbacks of molecular and serological techniques, many researchers have turned to analysis of exhaled breath for immediate confirmation of SARS-CoV-2 (Davis *et al.,* 2021). It is now known that SARS-COV-2 results in cellular metabolites resulting into volatile organic compounds (VOCs) present in exhaled breath and cough of infected patient (Grassin-delyle *et al.,* 2021; Ruszkiewicz *et al.,* 2020). VOCs in past have been used for similar purposes (Schivo *et al.,* 2014). Thus, presence of these VOCs can form the basis for noninvasive detection techniques. Cowling et al. (Leung *et al.,* 2020) has suggested the possibility of rapid detection of SARS-CoV-2 from exhaled breath and cough in infected patients, which is now gaining interest of researchers. Studies have shown that (Ma *et al.,* 2020) infected patients exhale millions of SARS-CoV-2 RNA in an hour. This results in exhaled breath having higher positive rate (26.9%) than surface contact (5.4%). It has been proven through dynamic simulation and experimental results that longer resilience of SARS-CoV-2 in the air is one of the reasons for its rapid spread (Goh *et al.,* 2020). One major issue in exhaled breath detection is the need for exhaled breath condensate (EBC) of the collected breath mass which requires several minutes. After the collection, the condensate may have RNA, DNA which can be confirmed by usual methods such as PCR or mass spectroscopy. Need for EBC stems from the fact that the viral load of SARS-CoV-2 in aerosol samples is much lower (Cheng *et al.,* 2020). The presence of VOCs in exhaled breath in the early phase of infection makes the immediate detection even before emergence of symptoms quite possible. SARS-CoV-2 is found to grow microbial flora on the lungs which results in metabolites in exhaled breath (Lamote *et al.,* 2020; Gould *et al.,* 2020). Grassin-Delyle et al. (Grassin-delyle *et al.,* 2021) detected characteristic VOCs in exhaled breath of SARS-CoV-2 infected patients. The four such detected VOCs related to SARS-CoV-2 are 2,4-octadiene, 1-chloroheptane, nonanal

**Corresponding author:* e-mail: hamdullah.sheikh@uc.pt

(1a) and methylpent-2-enal (1b). Their amount is between 10 to 250 ppb. There has been a lot of research literature published on utilization of VOCs as diagnostic biomarkers for diseases (Broza *et al.,* 2018; Haick *et al.,* 2014). In this research, we are proposing design of a breath analyzer sensor which will show color change upon receiving volatile organic compounds VOCs related to the ketogenesis resulting from SARS-CoV-2 infection. This can be very advantageous since current research on breath analysis requires expensive and time-consuming techniques such as PCR and time of flight high resolution mass spectroscopy (TOF-HRMS).

MATERIALS AND METHODS

Initially, molecular mechanics (MM) based energy minimization of the molecular structures of the VOCs (1a) and (1b) and their imine chromophores (3a-3f) and (4a-4f) respectively, was done through molecular geometry optimization option in Avogadro molecular modeling software. Then quantum mechanical (QM) based geometry optimizations were carried out by using B3LYP/6311G and then Møller-Plesset perturbation theory (MP2)/6-31G model chemistry on Gaussian 09 software (Young, 2001). Ultraviolet-Visible (UV-V) spectrum of all the optimized structures were generated using time dependent density functional theory (TD-DFT) (Foresman and Frisch, 2015) at B3LYP/6311+G(2d,p) as listed in table 1. Only the highest band was noted as $\lambda_{Chromophore}$ (nm). The bathochromic shifts were noted by formula, bathochromic Shift = $\lambda_{Chromophore} - \lambda_{VOC}$. Observed colors of chromophore are given in Part (B) of fig. 1 Chromophore section. All QM Gaussian calculated output files (.log) are given in Mendeley data (http://dx.doi.org/10.17632/5r2ttjs23t.1). Detailed and stepwise methodology is given below:

1) Geometry optimization and frequency analysis of VOC (1-2) and their chromophoric derivatives (3a-3f, 4a-4f)

(1.1) With molecular mechanics (MM) on Avogadro.

(1.2) With B3LYP/6-311G using z-matrix obtained from 1.1

(1.3) With MP2/6-31G using z-matrix obtained from 1.2

2) Energy calculation of excited states of VOC (1-2) and their chromophoric derivatives using TD-DFT method:

(2.1) TD-DFT with B3LYP/6-311+G(2d,p) using z-matrix of from 1.3

RESULTS

VOC (1a) and (1b) gave their λ_{max} at 192.35 nm and 255.5 nm. After chromophore formations, (1a) gave highest bathochromic shift of 219.73 nm after reaction with (2e) to form a chromophore (3f) with $\lambda_{Chromophore}$ (nm) of 412.08 nm. While, VOC (1b) gave a bathochromic shift of 265.59 nm with same (2e) to form a chromophore (4f) with $\lambda_{Chromophore}$ (nm) of 521.09 nm. Thus, both VOC's give high color changes from UV to Visible region upon Brady's reaction (Zhao *et al.,* 2019). table 1 lists the detailed values of shifts while observed colors are given in part (B) of fig. 1.

DISCUSSION

Major problems with current SARS-CoV-2 detection are the cost, unpleasant experience and time consumption. In this research, we proposed rapid colorimetric detection of volatile organic compounds VOCs exhaled out in breath. Through QM calculation we designed reactions of VOCs with substrate (2a-2f) that will form chromophoric adducts resulting into visual confirmation that VOCs due to SARS-CoV-2 infection were present in the exhaled breath (Reaction of 1a and 1b in part (A) of fig. 1). We selected two -CHO containing VOCs (1a) and (1b) in part (A) of fig. 1 and used QM approach to design multiple imine C=N chromophoric adducts derivatives [(3a-3f) and (4a-4f), given in part (B) of fig. 1. Some of these adducts gave strong bathochromic shift and absorb in the visible region as in case of reaction with (2f), thus confirming the SARS-CoV-2 related VOC's in the breath of the person. Substrate (2f) can be fit inside a vessel in which VOCs in the exhaled breath will react with the designed molecules and show color upon presence of VOCs.

VOCs (1a) and (1b) and 2,4-dinitrophenylhydrazine (2f) can form conjugated chromophores (3f) and (4f) with higher energy highest occupied molecular orbital (HOMO) and lowest energy unoccupied molecular orbital (LUMO), hence the electronic transition energy ΔE reduces. This increases the wavelength λ (nm) of absorption, thus bathochromic shift results in absorption in visible region as shown in table 1. VOCs (1a) and (1b) contains C=O functional groups which can be converted into imine C=N bonds upon reaction with $-NH_2$. 2,4-dinitrophenylhydrazine (2f) is known to form colored imine chromophores in Brady's reaction (Zhao *et al.,* 2019). The C=N bond in (3f) and (4f) is in conjugation with other nonbonding electrons on the second $-NH_2$ group resulting into longer wavelength absorption. Brady's reaction is well documented as Schiff base formation reaction that forms imine derivatives with sharp melting points. This rapid reaction starts with protonation (H^+) of C=O makes its C atom more Lewis acidic and more vulnerable towards acyl addition by Lewis basic $-NH_2$ of (2f). The protonated C=O bond then undergoes acyl addition with $-NH_2$ to form amino alcohol. Acid catalyzed elimination of H_2O and deprotonation of N results in C=N bond formation in conjugation with aryl ring. This takes the colorless VOCs -CHO into the visible region by chromophoric formation. In order to give the colorimetric change right away the reaction should be rapid. Another advantage is detecting the infection in

Table 1: Bathochromic shifts of designed C=N chromophores.

Nonanal (1a) $\lambda_{VOC(1a)}$ = 192.35 nm			2-methyl-2-butenal (1b) $\lambda_{VOC(1b)}$ = 255.5 nm		
S. No.	$\lambda_{Chromophore}$ (nm)	Bathochromic Shift (nm) = $\lambda_{Chromophore} - \lambda_{VOC(1a)}$	S. No.	$\lambda_{Chromophore}$ (nm)	Bathochromic Shift (nm) = $\lambda_{Chromophore} - \lambda_{VOC(1b)}$
3a	207.14	14.79	4a	263.4	7.9
3b	220.68	28.33	4b	286.4	30.9
3c	288.33	95.98	4c	343.49	87.99
3d	339.53	147.18	4d	371.29	115.79
3e	296.73	104.38	4e	386.64	131.14
3f	412.08	219.73	4f	521.09	265.59

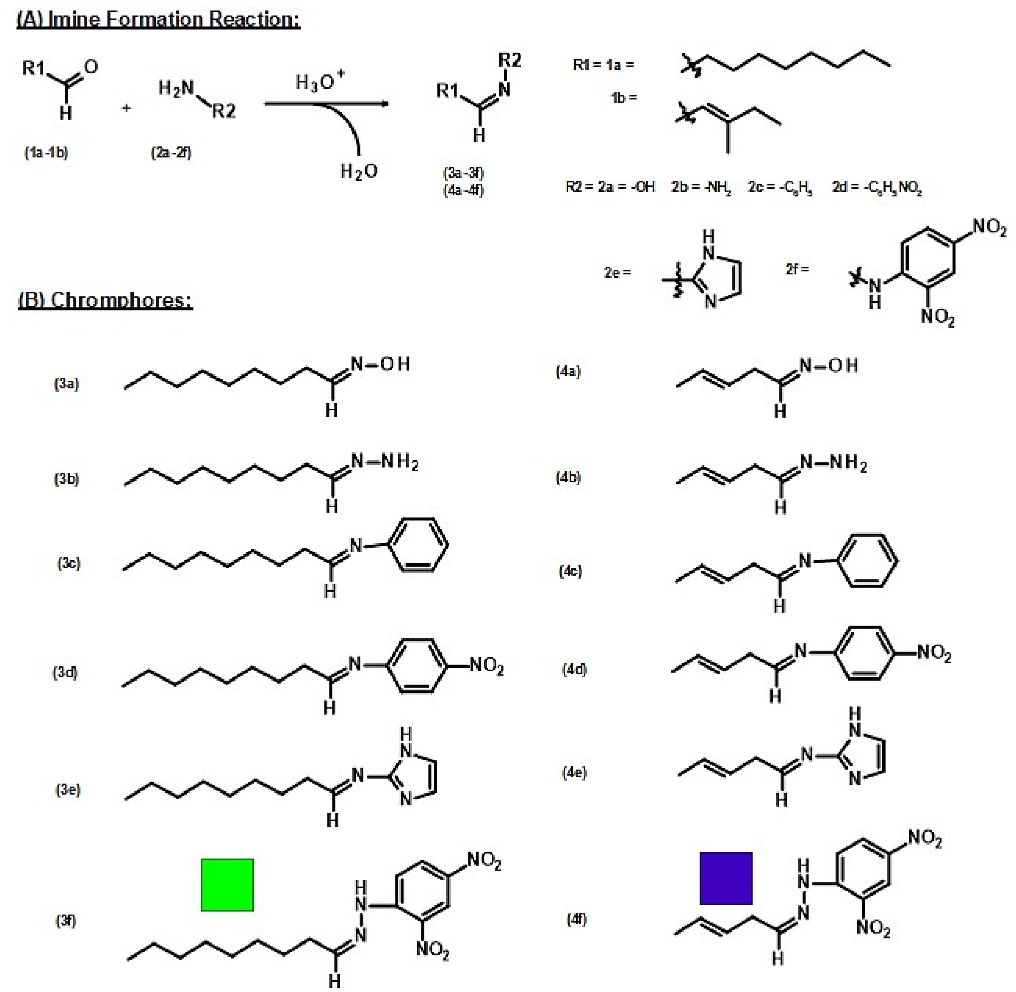

Fig. 1: (A) Scheme for conversion of VOCs (1a) and (1b) functional group –CHO into chromophores (B) Chromophores (3a-3f) and (4a-4f) respectively.

early stages as VOCs are found in patients which are just infected yet not showing any visible symptoms.

CONCLUSION

In this work, we discussed the prospect of use of volatile organic compounds (VOCs) in exhaled breath of SARS-CoV-2 infected person. We reviewed the current work being published on this matter. We discussed the importance of immediate detection of the virus compared to current standard time consuming tests. Through MP2 and TD-DFT calculations, we designed C=N based chromophoric derivatives of two VOCs, nonanal and 2-methyl-2-pentenal. As a result of formation of these compounds, colorless VOCs form colored chromophores as confirmed by high bathochromic shifts up to 219.73 nm and 265.59 nm in UV spectra of designed C=N chromophores. This colorimetric confirmation through

Brady's imine formation reaction can result in a cost-effective device that can confirm the SARS-CoV-2 rapidly. As VOC's are found in patients in early stages of infection without any visible symptoms, detection can be quite beneficial. Our future plan is to create such a VOC analyzer device and make it undergo clinical testing as well.

REFERENCES

Broza YY, Vishinkin R, Barash O, Nakhleh MK and Haick H (2018). Synergy between nanomaterials and volatile organic compounds for non-invasive medical evaluation. *Chem. Soc. Rev.,* **47**(13): 4781-4859.

Cheng VC, Wong SC, Chan VW, So SY, Chen JH, Yip CC, Chan KH, Chu H, Chung TW, Sridhar S and To KK (2020). Air and environmental sampling for SARS-CoV-2 around hospitalized patients with Coronavirus disease 2019 (COVID-19). *Infect. Control Hosp. Epidemiol.*, **41**(11): 1258-1265.

Crozier A, Rajan S, Buchan I and McKee M (2021). Put to the test: Use of rapid testing technologies for Covid-19. *BMJ*., 372(8278): 208.

Davis CE, Schivo M and Kenyon NJ (2021).A Breath of Fresh Air - The potential for Covid-19 breath diagnostics. *EBio. Medicine*, **63**: 103183.

Ding X, Yin K, Li Z, Lalla RV, Ballesteros E, Sfeir MM and Liu C (2020). Ultrasensitive and visual detection of SARS-CoV-2 using all in-one dual CRISPR-Cas12a assay. *Nat. Commun.*, **11**: 4711.

Foresman JB and Frisch A (2015). Modelling excited states. *In*: Exploring chemistry with electronic structure methods, 2nd ed. Gaussian Inc., Wallingford, pp.331-333.

Goh GK, Dunker AK, Foster JA and Uversky VN (2020). Shell disorder analysis predicts greater resilience of the SARS-CoV-2 (COVID-19) outside the body and in body fluids. *Microb. Pathog.*, **144**: 104177.

Gould O, Ratcliffe N, Krol E and de Lacy Costello B (2020). Breath analysis for detection of viral infection, the current position of the field. *J. Breath Res.,* **14**(4): 041001.

Grassin-Delyle S, Roquencourt C, Moine P, Saffroy G, Carn S, Heming N, Fleuriet J, Salvator H, Naline E and Couderc LJ (2021). Metabolomics of exhaled breath in Critically Ill COVID-19 patients: A pilot study. *EBio. Medicine,* **63**: 103154.

Haick H, Broza YY, Mochalski P, Ruzsanyi V and Amann A (2014). Assessment, origin and implementation of breath volatile cancer markers. *Chem. Soc. Rev.,* **43**(5): 1423-1449.

Lamote K, Janssens E, Schillebeeckx E, Lapperre TS, De Winter BY and Van Meerbeeck J (2020). The Scent of COVID-19: Viral (Semi-) Volatiles as fast diagnostic biomarkers? *J. Breath Res.,* **14**(4): 042001.

Leung NH, Chu DK, Shiu EY, Chan KH, McDevitt JJ, Hau BJ, Yen HL, Li Y, Ip DK and Peiris JM (2020). Respiratory virus shedding in exhaled breath and efficacy of face masks. *Nat. Med.*, **26**: 676-680.

Ma J, Qi X, Chen H, Li X, Zhang Z, Wang H, Sun L, Zhang L, Guo J, Morawska L and Grinshpun SA (2020). Corona virus disease 2019 patients in earlier stages exhaled millions of severe acute respiratory syndrome corona virus 2 per hour. *Clin. Infect. Dis.*, **72**(10): e652-e654.

Ruszkiewicz DM, Sanders D, O'Brien R, Hempel F, Reed MJ, Riepe AC, Bailie K, Brodrick E, Darnley K, Ellerkmann R and Mueller O (2020). Diagnosis of COVID-19 by analysis of breath with gas chromatography-ion mobility spectrometry - A feasibility study. *E-Clinical. Medicine,* **29**: 100609.

San Juan-Reyes S, Gomez-Olivan LM and Islas-Flores H (2021). COVID-19 in the environment. *Chemosphere*, **263**: 1.

Schivo M, Aksenov AA, Linderholm AL, McCartney MM, Simmons J, Harper RW and Davis CE (2014). Volatile emanations from *in vitro* airway cells infected with human rhinovirus. *J. Breath Res.*, **8**(3): 037110.

Wang M, Fu A, Hu B, Tong Y, Liu R, Liu Z, Gu J, Xiang B, Liu J, Jiang W and Shen G (2020). Nanopore targeted sequencing for the accurate and comprehensive detection of SARS-CoV-2 and other respiratory viruses. *Small*, **16**(32): 2002169.

Young DC (2001). Computational Chemistry: A Practical Guide for Applying Techniques to Real-World Problems, John Wiley & Sons, Inc, pp.8-11.

Zhao S, Lei J, Hou C, Huang J, Hou J, Luo X, Huo D (2019). An optoelectronic detector for aldehydes discrimination applications based on CD-like colorimetric chip. *Microchem. J,* **149**: 103979.