A NONCLINICAL SPECTROSCOPIC APPROACH FOR DIAGNOSING COVID-19: A CONCISE PERSPECTIVE

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With the COVID-19 outbreak, many challenges are posed before the scientific world to curb this pandemic. The diagnostic testing, treatment, and vaccine development for this infection caught the scientific community's immediate attention. Currently, despite the global proliferation of COVID-19 vaccination, the specific treatment for this disease is yet unknown. Meanwhile, COVID-19 detection or diagnosis using polymerase chain reaction (PCR)-based methods is expensive and less reliable. Moreover, this technique needs much time to furnish the results. Thus, the elaboration of a highly sensitive and fast method of COVID-19 diagnostics is of great importance. The spectroscopic approach is herein suggested as an efficient detection methodology for COVID-19 diagnosis, particularly Raman spectroscopy, infrared spectroscopy, and mass spectrometry.

Keywords: *COVID-19, coronavirus, polymerase chain reaction, spectroscopy.*

ПЕРСПЕКТИВЫ ДОКЛИНИЧЕСКОГО ПРОСТОГО СПЕКТРОСКОПИЧЕСКОГО ПОДХОДА К ОБНАРУЖЕНИЮ COVID-19

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В связи со вспышкой коронавирусной инфекции (COVID-19) перед научным миром встала задача разработки высокочувствительного и быстрого метода ее диагностики. Диагностическое тестирование, лечение и разработка вакцины от этой инфекции привлекают особое внимание научного сообщества. В настоящее время обнаружение/диагностика COVID-19 на основе полимеразной цепной реакции (ПЦР) является дорогостоящим и недостаточно надежным методом. Кроме того, этот метод требует длительного времени для получения результатов. В качестве эффективной методологии обнаружения COVID-19 предлагаются спектроскопические методы — комбинационного рассеяния света, инфракрасной спектроскопии и масс-спектрометрии.

Ключевые слова: *COVID-19, коронавирус, полимеразная цепная реакция, спектроскопия.*

Introduction. The threat of the highly transmitting and pathogenic coronavirus disease has increased since the outbreak of this pandemic. The disease has been characterized as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1] because the genetic analysis of the causative virus revealed phylogeny matching with SARS-CoV-1. The primary questions of knowing the origin of this dreadful virus and its man transfer still remain unexplored. The current rate of its rapid human-to-human transfer is widely known. As a result, the quarantine and other preventive measures (self or administrative) have received immense atten-

tion. The ideas of physical distancing and the use of sanitizers have spread worldwide [2]. Due to the pandemic, drugs with antiviral and anti-inflammatory properties are used as leading drug compounds to combat this disease. Due to the unavailability of any clinically approved drug against COVID-19, some broad-spectrum antiviral drugs in clinical trials led to the successful recovery of the affected people. This clinical methodology still stands in practice across the globe.

In the human body, the defense/immune system is always responsive to invading microbes, heat, or other toxins in a particular tissue. This responding behavior appears in inflammation, fever, color change, etc. The inflammatory response is mainly expressed by the release of bradykinin, histamine, and prostaglandins by the affected cells, which induces fluid leakage from blood vessels into the respective tissues, thereby causing swelling. Generally, NSAIDs, a class of drugs called nonsteroidal anti-inflammatory drugs, are administered to counter this inflammatory action. These include both selective as well as nonselective inhibitors. The COX2 inhibiting drugs like rofecoxib, celecoxib, and valdecoxib are selective inhibitors, while ibuprofen, diclofenac, aspirin, and naproxen are non-selective ones. In the corona viral infectious state, this countering mechanistic way has raised some concerns associated with the possibility of increased adverse effects [3, 4]. Some evidence indicates the influential role of NSAIDs in treating COVID-19. However, prudent control is needed until further evidence sheds light on this viral strain [5]. Some old antimalarial drugs like chloroquine have shown noteworthy results against COVID-19 [6]. From the literature survey, it is evident that this drug possesses the antiviral potential of broad-spectrum action. In this context, the endosomal pH with the glycosylation of SARS-CoV receptors is highly significant [7, 8]. Hence, as expected, chloroquine represents a potent agent in treating COVID-19 pneumonia. Similarly, several other examples of antivirals are undergoing the same type of evaluation, including arbidol, ribavirin, favipiravir, dexamethasone, etc., which show moderate recovery results against the infection [9–11]. It is also suggested that the combinatory treatment form of such drugs with traditional medicines could enhance the anti-COVID-19 effect [12].

With the coronavirus pandemic, many concerns regarding the detection of this disease have emerged. RT-PCR serves as an emergency diagnostic tool for COVID-19. Because of the scarce access to equipment, reagents, and target, the test is less reliable. Based on the requirement of accuracy, patient care, and medicine, such diagnostics must be less time-consuming and more reliable. Therefore, the test sensitivity, cost, and time taken by routine techniques made the scientific community search for more sensitive and high economic ways of the virus detection [13–15]. Mass spectrometry, Raman spectroscopy, infrared spectroscopy, etc., can solve these issues [16, 17].

Similarly, biosensing detection based on a field-effect transistor (FET) has been suggested as an alternative diagnostic method using the immunological concept [18]. Some coupled techniques like ultra-high-pressure liquid chromatography combined with high-resolution mass spectrometry (UHPLC–HRMS) have also been suggested in this context [19]. Therefore, on-time detection and clinically satisfying specificity and sensitivity are the most crucial forefront of COVID-19 detection analysis. Spectroscopic analysis can thus be a promising detection method using potential chemical biomarkers [20, 21] to furnish the stage of infection. Such techniques prove helpful in recognizing coronaviruses at the molecular level. So, using spectrophotometers, one can also reveal the biomolecular structure involved in the viral invasion. Spectroscopy is prominently involved in the structural analysis of unknown or known compounds [22–24]. With our constant contribution to bioconjugated molecular research [25–29] and the recent COVID-19 related reports from our laboratory [30–32], herein a literature review of the nonclinical spectroscopic methods of COVID-19 testing is presented. The beneficial aspects of this approach over the PCR method are discussed in this work.

The general light sensitivity approach for COVID-19 detection. The expression of proteins or enzymes associated with COVID-19 severity or COVID-19 associated death can serve as infection markers. These include D-dimer for blood coagulation (Fig. 1), lactate dehydrogenase indicative of cell damage (Fig. 2), and C-reactive protein showing the inflammatory response (Fig. 3). An accumulation of evidence relating to COVID-19 with SARS suggests that protein biomarkers can define these investigations [33]. From the identity of their absorption and emission bands, it can be possible to find the extent of infection. The consistency of different vibrational and rotational modes in bioaerosols (like coronavirus) is detectable in the presence of a light-sensitive material [34]. Even smart phone-assisted electrochemical signaling can help in the detection [35]. Optical theranostics serve to identify the pulmonary severity of COVID-19 quickly [36]. Noncontact nanomaterial-based optical methods are significant in both detection and surface disinfection [37]. Therefore, the electromagnetic radiation (EMR) based interactive approach is important in this context for both qualitative and quantitative analysis [37, 38].

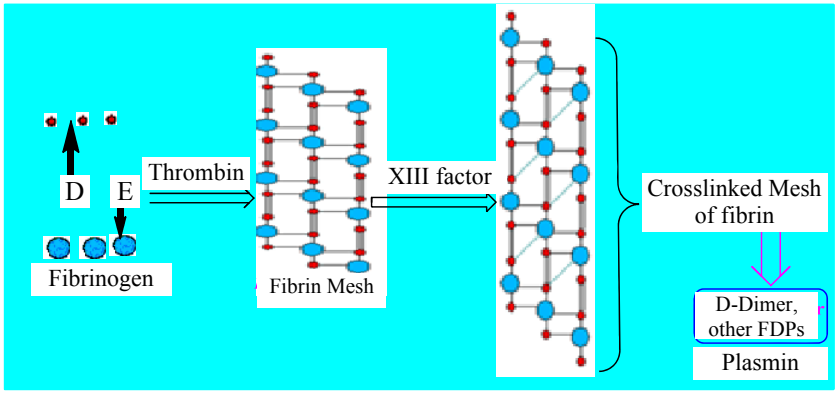

Fig. 1. Diagram showing the role of the D-dimer in blood coagulation.

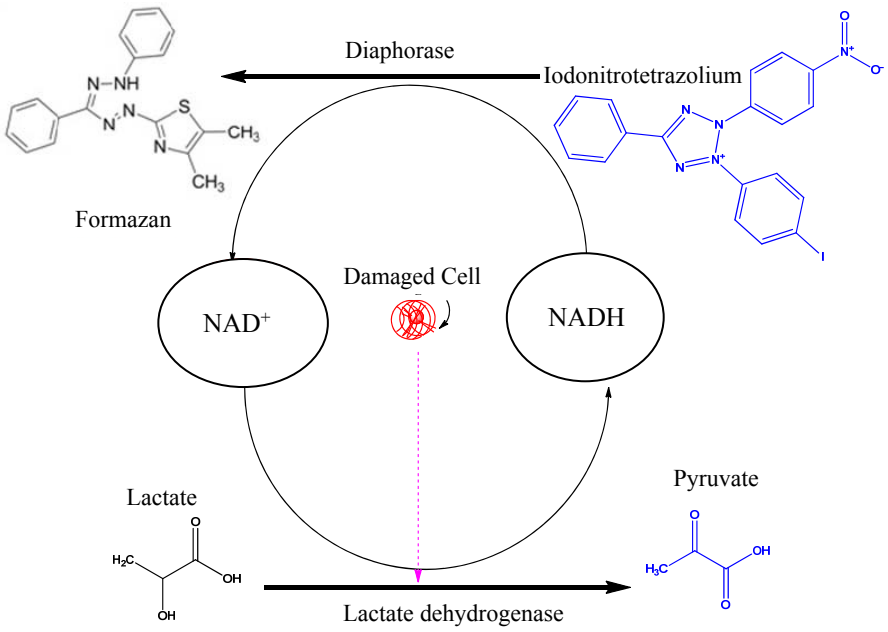

Fig. 2. Representation of lactate dehydrogenase (LDH) action induced by cell damage and the detection of the LDH response by the enzymatic reaction resulting in change in the formazan color of tetrazolium salt to a red color by diaphorase.

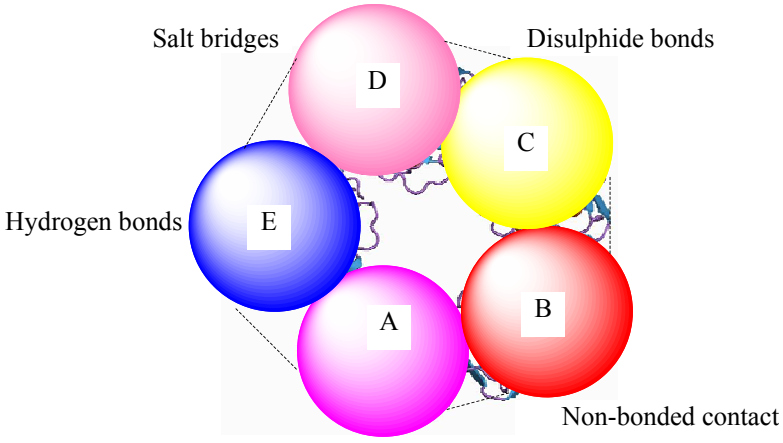

Fig. 3. The 3D structural model of C-protein displaying five subunits with the intra-bonding parameters.

The Raman spectroscopic approach. Raman spectroscopy is a reliable tool for bacterial identification and hence can similarly be used in COVID-19 detection [39]. Low-frequency Raman (LFR) spectroscopy is a robust theoretical technique eventually valuable for developing a diagnostic tool for such type of analysis. LFR Nanostructure mapping distinguishes the nanostructure details, thermal behavior or other fragmentation views, solution parameters, etc. [40].

Virus detection by combined Raman-UV-Visible spectroscopy represents a fascinating approach in this context. Figure 4 shows the Raman setup followed by Manato et al. [41], with the fixed Raman excitation at 527 nm under a single-mode diode laser (Evolution Nd: YLF, diode-pumped, Q-switched) of 1 kHz repetition rate with a 5 μs pulse duration. The study involved an expanded laser beam by incorporating a two-lens telescope (L1 and L2) followed by an intervening 100× microscope objective (NA = 1.25) deliverable to the sample unit. A dichroic mirror removed the Rayleigh scattering and senses the objective lens. The spot size of the beam at the sample was approximately one μm with a power of 10 mW. After passing through a notch filter (to further remove the Rayleigh scattering), the backscattered Raman signal was collected and guided to a spectrograph (Andor Shamrock spectrometer) and a deep cooling CCD camera (Newton, DU9-20P, Andor CCD camera) through an optical fiber. In addition to these components, a grating with 1200 g/mm and 500 nm acted as a wavelength dispersal unit for the Raman signal. Meanwhile, a UV-Visible spectrometer was also used to record the observations. Hence the study concludes with the identification and quantification of coronaviruses under this setup. The procedure shall involve the immobilization of the viral spike antibody on a salinized glass. The sampler substance could be then added to the substrates, followed by the virus capture immobilized via the antibody interaction. In similar approaches, the identification of virions based on modular atomic force microscopy (AFM) in association with Coherent Anti-Stokes Raman Scattering (FASTER CARS) presents remarkably high sensitivity in this regard [42, 43]. Thus, the diagnosis and detoxification can be achieved by these spectroscopic approaches [44, 45].

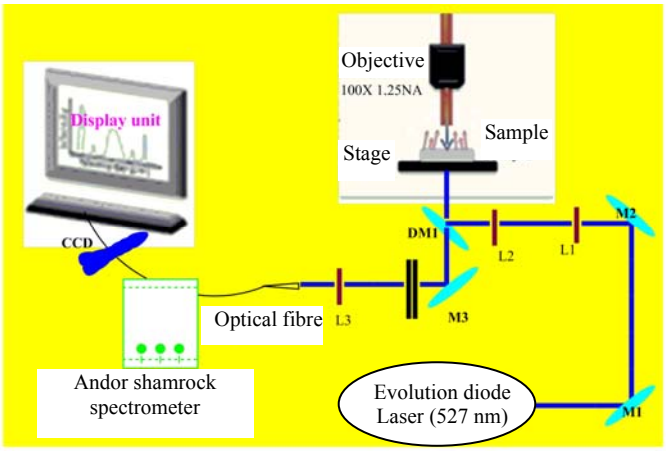

Fig. 4. The representation of the Raman spectrometer set-up for sample analysis.

The Fourier-transform infrared spectroscopic approach. Infrared spectroscopy can easily verify the functional groups present within the structural composition of a virus and the respective pathogenicity (Fig. 5). Figures 1–3 are the possible compounds that determine the level of infection of coronavirus. More straightforwardly, attenuated total reflection Fourier-transform infrared spectroscopy (ATR-FTIR) is one of the famous techniques working in the mid-IR region [46]. ATR-FTIR analysis is said to be highly economical because it is less time-consuming and no reagent is required. Generally, significant vibrational modes of biological samples fall within the 1800–900 cm^{-1} region, and the region is called “biofingerprint” (Fig. 6). Since this region is a source of sufficient information about the biochemical bonds [47], the nucleic acids specific to viruses, nucleic acid variation, and other biological insights are detected using ATR-FTIR. As mentioned earlier, the spectroscopic approach can also reveal the complex dynamics during the infection. A similar approach was experimented by McIntyre et al. [48] in finding the diversity of the viral epitranscriptome (biochemical modification of RNA within a cell). Santos et al. [49] recently reported FTIR as the potential spectroscopic tool for detecting changes that occur in biological samples due to the virus invasion. Therefore, FTIR-based detection is preferable over the available PCR technique due to it being nondestructive, user friendly, highly sensitive, and less time-consuming.

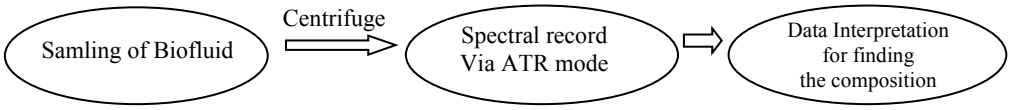

Fig. 5. Flowchart for recordin g the FTIR spectrum of a biological sample.

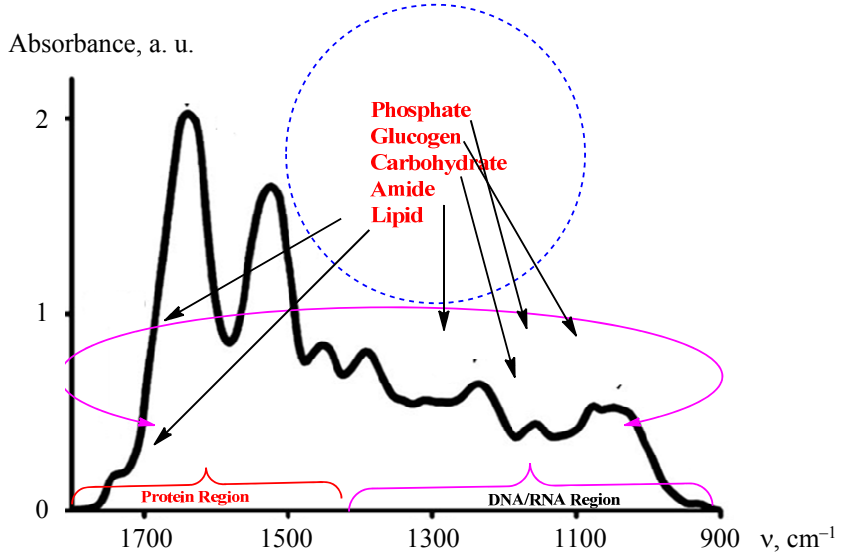

Fig. 6. Significant biofingerprint regions related to the viral infection
and the infrared spectral region.

The mass spectrometry approach. Mass spectrometry is used to determine the elemental percentage of a sample and can be used to quantify the mass of the chemical species. In dealing with the detection of the coronavirus infection, mass spectral data could make it easy to guess the infection stage by knowing the viral invasion implications regarding the expression of specific proteins and even the DNA/RNA content. For instance, the fragmentation pattern of spike protein and replicase protein, if observed, can help in the detection of the infection. Testing incapabilities can be eradicated by allowing the simplified assays to be followed and not reagent-dependent. One such analytical tool with the potential to minimize treagent use is matrix-assisted laser desorption/ionization combined with mass spectrometry (MALDI-MS). Recently, Nachtigall et al. reported the development of a MALDI-MS method for the diagnosis of the SARS-CoV-2 infection [50]. Similarly, SoRelle et al. [51] suggested MALDI-MS as an efficient indirect detector for current and future interest in relevance to an infectious disease.

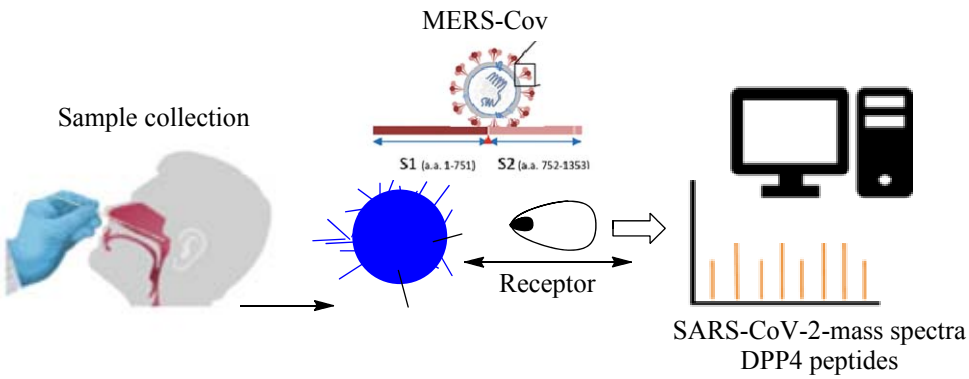

Fig. 7. Representation of the mass spectrometer for the identification of receptors
in diagnosing COVID-19.

Conclusions. This paper aims to introduce spectroscopic diagnostic as a more reliable methodology than PCR for COVID testing. By employing familiar techniques like mass, infrared, and Raman spectroscopy, it becomes easy to understand the stage of the coronavirus infection and to render more accurate results in less time. Also, for monitoring the virus mutation and investigating the respective drug development, the spectroscopic detection represents an efficient tool. Therefore, spectroscopic identification of COVID-19 is an efficient and less expensive method. It is worth noting here a quotation displayed on the WHO website (12th January 2021) that reads, "With a fast-moving pandemic, no one is safe unless everyone is safe." In the several approaches brought to light through this survey, it is demonstrated that viral strains keep changing their functionality concerning the environment. Hence, scientists should be ready to encounter any pandemic like COVID-19 in the future. Very recently, a second type of coronavirus strain has renewed COVID-19 pandemic-like fear. Several countries have restarted lockdowns to protect people from this novel strain (possessing 70% more transmissivity than the previous form). Therefore, reaching the vaccination stage and bringing COVID-19 under control does not mean that we are safe. Nonclinical spectroscopic diagnostic is hence a more reliable viral analysis tool than RT-PCR.

REFERENCES

1. M. A. Shereen, S. Khan, A. Kazmi, N. Bashir, R. Siddique, *J. Adv. Res.*, **24**, 91–98 (2020).
2. Y. Zhu, C. Wang, L. Dong, M. Xiao, *Brain. Behav. Immunol.*, **87**, 142–143 (2020).
3. B. Russell, C. Moss, A. Rigg, M. Van Hemelrijck, *Ecancer Med. Sci.*, **14**, 1023 (2020).
4. P. Little, *BMJ*, **368**, 1185 (2020).
5. B. Russell, C. Moss, G. George, A. Santaolalla, A. Cope, S. Papa, M. Van Hemelrijck, *Ecancer Med. Sci.*, **14**, 1022 (2020).
6. J. Gao, Z. Tian, X. Yang, *Biosci. Trends*, **14**, 72–73 (2020).
7. A. Savarino, J. R. Boelaert, A. Cassone, G. Majori, R. Cauda, *Lancet Infect. Dis.*, **3**, 722–727 (2003).
8. Y. Yan, Z. Zou, Y. Sun, X. Li, K. F. Xu, Y. Wei, N. Jin, C. Jiang, *Cell Res.*, **23**, 300–302 (2013).
9. A. Sternberg, D. L. McKee, C. Naujokat, *Curr. Top. Med. Chem.*, **20**, 1423–1433 (2020).
10. L. L. Ferreira, A. D. Andricopulo, *Curr. Top. Med. Chem.*, **20**, 1577–1580 (2020).
11. W. Liu, H. L. Zhu, Y. Duan, *Curr. Top. Med. Chem.*, **20**, 603–605 (2020).
12. C. M. Chu, V. C. C. Cheng, I. F. N. Hung, M. M. L. Wong, K. H. Chan, K. S. Chan, R. Y. T. Kao, L. L. M. Poon, C. L. P. Wong, Y. Guan, J. S. M. Peiris, K. Y. Yuen, *Thorax*, **59**, 252–256 (2004).
13. R. S. Khan, I. U. Rehman, *Expert Rev. Mol. Diagn*., **20**, 647–649 (2020).
14. K. Wu, R. Saha, D. Su, V. D. Krishna, J. Liu, M. C. Cheeran, J. P. Wang, *arXiv preprint arXiv:2007.04809* 2020, *arXiv preprint arXiv:2007.04809*.
15. S. Mahapatra, P. Chandra, *Biosens. Bioelectron.*, **165**, 112361 (2020).
16. C. Jenkins, B. Orsburn, *BioRxiv* (2020), https://doi.org/10.1101/2020.03.08.980383.
17. L. F. D. C. de Silva, M. S. N. de Carvalho, *Photodiagn. Photodyn. Ther*., **30**, 101765 (2020).
18. G. Seo, G. Lee, M. J. Kim, S. H. Baek, M. Choi, K. B. Ku, C. S. Lee, S. Jun, D. Park, H. G. Kim, S. J. Kim, J. O. Lee, B. T. Kim, E. C. Park, S. J. Kim, *ACS Nano*, **14**, 5135–5142 (2020).
19. I. Mahmud, T. J. Garrett, *J. Am. Soc. Mass Spectrom.*, **31**, 2013–2024 (2020).
20. C. Sheridan, *Nat. Biotechnol.*, **38**, 382–384 (2020).
21. B. A. Taha, Y. Al Mashhadany, M. H. Hafiz Mokhtar, M. S. Dzulkefly Bin Zan, N. Arsad, *Sensors*, **20**, 6764 (2020).
22. J. M. Mir, N. Jain, P. S. Jaget, R. C. Maurya, *Photodiagn. Photodyn. Ther.*, **19**, 363–374 (2017).
23. J. M. Mir, N. Jain, P. S. Jaget, W. Khan, P. K. Vishwakarma, D. K. Rajak, B. A. Malik, R. C. Maurya, *J. King Saud Univ. – Sci.*, **31**, 89–100 (2019).
24. J.M. Mir, R.C. Maurya, *J. Chin. Adv. Mater. Soc.*, **6**, 434–458 (2018).
25. J. M. Mir, B. A. Malik, R. C. Maurya, *Rev. Inorg. Chem.*, **39**, 91–112 (2019).
26. J. M. Mir, R. C. Maurya, *Rev. Inorg. Chem*., **38**, 193–220 (2018).
27. R. C. Maurya, J. M. Mir, In: *Advances in Metallodrugs: Preparation and Applications in Medicinal Chemistry*, Wiley, New Jersey, 157–201 (2020).
28. J. M. Mir, R. C. Maurya, *Annal. Ophthalmol. Visual Sci*., **1003**, 1–4 (2018).
29. R. C. Maurya, J. M. Mir, *Int. J. Sci. Eng. Res.*, **5**, 305–320 (2014).
30. J. M. Mir, S. A. Majid, A. H. Shalla, *Rev. Inorg. Chem*., 3493 (**2021**), doi.org/10.1515/revic-2020-0020.
31. J. M. Mir, R. C. Maurya, *New J. Chem*., **45**, 1774–1784 (2021).
32. J. M. Mir, R. C. Maurya, *J. Biomol. Struct. Dyn.* (**2020**), doi.org/10.1080/07391102.2020.1852969.

33. A. D. Whetton, G. W. Preston, S. Abubeker, N. Geifman, *J. Proteome Res.*, **19**, No. 11, 4219–4232 (2020).
34. R. Singh, P. Su, L. Kimerling, A. Agarwal, B. W. Anthony, *Appl. Phys. Lett*., **113**, No. 23, 231107 (2018), doi: arXiv:1806.06910v2.
35. P. Chandra, *Sensors Int.*, **1**, 100019 (2020), doi.org/10.1016/j.sintl.2020.100019.
36. M. S. Nogueira, *Photodiagn. Photodyn. Ther.*, **31**, 101892 (2020).
37. N. Rabiee, M. Bagherzadeh, A. Ghasemi, H. Zare, S. Ahmadi, Y. Fatahi, R. Dinarvand, M. Rabiee, S. Ramakrishna, M. R. Shokouhimehr, R. S. Varma, *Int. J. Mol. Sci*., **21**, 5126 (2020), doi.org/10.3390/ijms21145126.
38. M. S. Nogueira, *Photodiagn. Photodyn. Ther.*, **31**, 101823 (2020).
39. S. Pahlow, S. Meisel, D. Cialla-May, K. Weber, P. Rösch, J. Popp, *Adv. Drug Deliv. Rev.*, **89**, 105–120 (2015).
40. L. Jacobi, V. H. Damle, B. Rajeswaran, Y. R. Tischler, *Roy. Soc. Open Sci*., **7**, 1–28 (2020).
41. S. L. Manoto, A. El-Hussein, R. Malabi, L. Thobakgale, S. Ombinda-Lemboumba, Y. A. Attia, M. A. Kasem, P. Mthunzi-Kufa, *Saudi J. Biol. Sci.*, **28**, 78–89 (2021).
42. V. Deckert, T. Deckert-Gaudig, D. Cialla, J. Popp, R. Zell, A. V. Sokolov, Z. Yi, M. O. Scully, *Med. Phys*. (**2020**), doi: arXiv:2003.07951.
43. A. M. Elsharif, *Int. J. Res. App. Sci. Eng. Technol.*, **8**, 715–719 (2020).
44. M. H. Jazayeri, H. Amani, A. A. Pourfatollah, H. Pazoki-Toroudi, B. Sedighimoghaddam, *Sens. Bio-Sens. Res.*, **9**, 17–22 (2016).
45. V. X. T. Zhao, T. I. Wong, X. T. Zheng, Y. N. Tan, X. Zhou, *Mater. Sci. Energy Technol.*, **3**, 237–249 (2020).
46. F. L. Martin, J. G. Kelly, V. Llabjani, P. L. Martin-Hirsch, I. I. Patel, J. Trevisan, N. J. Fullwood, M. J. Walsh, *Nat. Protoc.*, **5**, 1748–1760 (2010).
47. J. G. Kelly, J. Trevisan, A. D. Scott, P. L. Carmichael, H. M. Pollock, P. L. Martin-Hirsch, F. L. Martin, *J. Proteome Res.*, **10**, 1437–1448 (2011).
48. W. McIntyre, R. Netzband, G. Bonenfant, J. M. Biegel, C. Miller, G. Fuchs, E. Henderson, M. Arra, M. Canki, D. Fabris, C.T. Pager, *Nucleic Acids Res.*, **46**, 5776–5791 (2018).
49. M. C. Santos, C. L. Morais, K. M. Lima, *Biomed. Spectrosc. Imag.*, **9**, 103–118 (2020).
50. F. M. Nachtigall, A. Pereira, O. S. Trofymchuk, L. S. Santos, *Nat. Biotechnol.*, **38**, 1168–1173 (2020).
51. J. A. SoRelle, K. Patel, L. Filkins, J. Y. Park, *Clin. Chem.*, **66**, 1367–1368 (2020).
52. V. S. Raj, M. M. Lamers, S. L. Smits, J. A. Demmers, H. Mou, B. J. Bosch, B. L. Haagmans, In: *Coronaviruses*, Humana Press, New York, 165–182 (2015).
53. A. M. Zaki, S. Van Boheemen, T. M. Bestebroer, A. D. Osterhaus, R. A. Fouchier, *N. Engl. J. Med.*, **367**, 1814–1820 (2012).
54. W. Li, M. J. Moore, N. Vasilieva, J. Sui, S. K. Wong, M. A. Berne, M. Somasundaran, J. L. Sullivan, K. Luzuriaga, T. C. Greenough, H. Choe, *Nature*, **426**, 450–454 (2003).
55. D. Gouveia, G. Miotello, F. Gallais, J. C. Gaillard, S. Debroas, L. Bellanger, J. P. Lavigne, A. Sotto, L. Greng, O. P. J. Armengaud, *J. Proteome Res.*, **19**, 4407–4416 (2020).