CHEMISTRY in Sri Lanka

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Themed Collection

COMBATING THE UNSEEN

Smoking and respiratory virus epidemics

Drug Discovery in Combating Viral Diseases

Student Corner

RT-PCR

Synthetic Organic Chemistry in Sustainable Agriculture

The Tri Annual Publication of the Institute of Chemistry Ceylon

Chemistry in Sri Lanka

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The Tri-Annual Publication of the Institute of Chemistry Ceylon

Founded in 1971, Incorporated by Act of Parliament No. 15 of 1972

Successor to the Chemical Society of Ceylon, founded on 25th January 1941

Vol. 37 No. 2 **May 2020**

Theme for the year -

Shaping Careers of Chemists Through Advancement of Chemical Technology

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Outline of our Institute

The Institute of Chemistry Ceylon is a professional body and a learned society founded in 1971 and incorporated by act of Parliament No. 15 of 1972. It is the successor to the Chemical Society of Ceylon which was founded in 1941. Over 50 years of existence in Sri Lanka makes it the oldest scientific body in the country.

The Institute has been established for the general advancement of the science and practice of Chemistry and for the enhancement of the status of the profession of Chemistry in Sri Lanka. The Institute represents all branches of the profession and its membership is accepted by the government of Sri Lanka (by establishment circular 234 of 9-3-77) for purposes of recruitment and promotion of chemists.

Corporate Membership
Full membership is referred to as corporate membership and consists of two grades: **Fellow (F.I.Chem.C.)** and **Member (M.I.Chem.C.)**

Application for non-corporate membership is entertained for four grades: Associate (former Graduate) (A.I.Chem.C.), Licenciate (L.I.Chem.C.), Technician (Tech.I.Chem.C.) and Affiliate Member.

Revision of Membership Regulation
All Special Degree Chemists can now apply directly to obtain Associate (Graduate) Membership. Three year B. Sc. Graduates (with an acceptable standard of Chemistry) can
(i) directly become Licentiate
(ii) obtain corporate membership in a lesser number of years.

Tech.I.Chem.C.
Those who have passed the DLTC examination or LTCC examination or have obtained equivalent qualification and are engaged in the practice of Chemistry (or chemical sciences) acceptable to the Council are entitled to the designation Tech.I.Chem.C.
Members/Fellows with Membership for Life are entitled to the designation of **Chartered Chemist (C.Chem.)** on establishment of a high level of competence and professionalism in the practice of chemistry and showing their commitment to maintain their expertise.

All corporate members (Members / Fellows) are entitled to vote and become Council/ Committee members whether Chartered Chemists or not.

Membership Applications
Any application for admission to the appropriate class of membership or for transfer should be made on the prescribed form available from the Institute Office.

Current Subscription Rates
Fees should be payed on 1st of July every year and will be in respect of the year commencing from 1st July to 30th June

Fellow	Rs. 2000
Member	Rs. 2000
Associate	Rs. 1500
Licenciate	Rs. 1200
Technician	Rs. 750
Affiliate	Rs. 1200
Membership for Life	Rs. 15000

Entrance Fee

All the grades	Rs. 1000
Processing Fees*	Rs. 500
Processing Fee for Chartered Chemist designation	Rs. 5000
Institutional Members	Rs. 2500

*per application for admission/transfer to any grade

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CHEMISTRY IN SRI LANKA

Chemistry in Sri Lanka is a tri-annual publication of the Institute of Chemistry Ceylon and is published in January, May and September of each year. It is circulated among the members of the Institute of Chemistry and students of the Graduateship/DLT course and libraries. The publication has a wide circulation and more than 750 copies are published. Award winning lectures, abstracts of communications to be presented at the annual sessions, review papers, activities of the institute, membership news are some of the items included in the magazine.

The editor invites from the membership the following items for publication in the next issue of the Chemistry in Sri Lanka which is due to be released in September 2020.

- *Personal news of the members*
- *Brief articles of topical interests*
- *Forthcoming conferences, seminars and workshops*
- *Latest text books and monographs of interest to chemists*

All publications will be subjected to approval of the 'Editorial and Publicity Committee' and the Council of the Institute of Chemistry Ceylon.

Further, prospective career opportunities for chemists, could be advertised in Chemistry in Sri Lanka at a nominal payment. The editor welcomes suggestions from the members for improvement of the publication.

5th Emeritus Professor J.N.O. Fernando Memorial Oration

Memorial Oration

Past, Present and Future challenges to Chemistry Education at the Institute of Chemistry Ceylon

M. D. P. de Costa

Senior Professor, Department of Chemistry, University of Colombo, Sri Lanka

In the 1960's, the government and the university system in Sri Lanka were faced with the challenge of providing university entrance to all Advanced Level qualified students to pursue their tertiary education, owing to the lack of resources and space at that time. It was at this time that a group of determined university lecturers took on the courageous task of commencing the Graduateship in Chemistry (GIC) course for students who, despite successfully getting through their A/Ls, do not gain access to state universities due to the aforementioned reasons. Among them was the late Prof. J N O Fernando who took it on his shoulders to ensure the success of the GIC programme from when it began in 1979 to today. Prof. Fernando was a man of principles who stood strong against unethical practices and was firm and brave when it came to overcoming opposition. He silenced his opponents with logical discussions and outstanding ideas, was firm in his ideas and never gave up. In short, he was a hero of our times.

As much as he was tough when it came to work, he was an extremely kind hearted human being who never hesitated to extend his support, be it towards the students or his colleagues, in their times of need. He headed the establishment of several scholarships and awards in order to provide financial assistance towards students and set up Benevolent Funds and donations to assist the members of the Institute through periods of hardship. This was all possible due to his clear cut, strong policies and great vision.

My close association with Prof. Fernando dates back to 1992 when I became a Council Member of the Institute of Chemistry Ceylon. However, I had known him as a lecturer since my undergraduate years at the University of Colombo in 1980s. Within a short period of time, I was fortunate enough to become a member of the Academic Board in 1991. Thereafter, I held positions such as that of the Assistant Treasurer and the Secretary before being inducted as the President of the Institute in 2011. The reason behind my successful journey in such a short period of time is the remarkable ability of Prof. Fernando to identify the most suitable person for a particular task or position. Among the persons of high caliber that I have encountered in my life, he is one of the best. I owe him immensely for the guidance and the encouragement he provided me in performing my tasks at the Institute, especially in the development of the GIC programme.

When it came to meetings where important decisions had to be taken, he would always listen to what everyone had to say and ultimately, make his own decision which we would also later realize was the correct decision. There have been numerous times where I have had heated arguments and disagreements with him during meetings. However, he never carried any bitterness with him after the meeting. That showcases, how true a professional he was. The knowledge and experience on good governance, financial practices and procedures that I acquired from him assisted me in handling several problems in his absence.

With Prof. J N O Fernando's sudden demise, came the challenging task of achieving the goals set out by him and fulfilling his dream of making the Institute and the College, that he loved so much, leading institutions in the country or perhaps, even in the world. This was not at all an easy task for me. Fortunately, there were many Council members, members and seniors from whom the required support and guidance could be obtained in handling matters related to the Institute and the College after Prof. Fernando's demise. As such, the vacuum created by his demise was so vast that we needed several people to fill it. Time has come for the new generation, as the experts in the field now require their support in the journey ahead. However, the young generation must always remember to seek the advice and experience of their seniors in order to overcome the challenges that lie ahead.

With that I come to the second half of my talk, which is on the "Past, Present and Future Challenges to Chemistry Education at the Institute of Chemistry Ceylon." In 1970's, the Chemical Society of Ceylon, as the Institute was called prior to its inception in 1971, felt the necessity of recruiting trained, middle-level technicians in chemistry at institutions such as research institutions and industries. Accordingly, the Laboratory Technician Training Course (LTTC) was commenced by the Chemical Society as a training programme for technicians and Dr. Senthe Shanmuganathan was appointed as its first coordinator. The syllabus of the course was approved by the Council in 1973 and with the Government Grant that was received by the Institute, the course was commenced with its first batch consisting of 36 students at Aquinas College. The students who registered for the course were mainly from laboratory staff of scientific and technical institutions.

Soon after the founding of the Institute of Chemistry Ceylon in 1971, the Council began considering the possibility of offering a course in Chemistry equivalent to a four year degree in Chemistry. The Graduateship in Chemistry course conducted by the Royal Society of Chemistry (RSC) was studied by a committee appointed for the task. The syllabus for the course, after going through multiple revisions over a period of two years, was finally approved in 1972. However, the Graduateship course did not officially start right after that. As per the practice followed by the RSC at that time, the draft syllabus was made available to anyone wishing to take the Graduateship examination and they could sit for the exam by studying based on the syllabus provided. In December 1978, the first Graduateship examination was held where three out of the seven external candidates emerged successful in Part I. In 1979, the Council decided to start the Graduateship course and with 72 selected students out of 175 applicants, the course was inaugurated on May 16, 1979 at Aquinas College.

The efforts of the Council in gaining recognition for the Institute (IChemC) Membership became successful when it was made official, that, by the Establishment Circular 234 of 09.03.1977, the Sri Lankan Government accepts IChemC Fellowship, Associateship (now referred to as Membership) and Graduateship (now referred to as Associateship) as equivalent to corresponding grades of the Royal Society of Chemistry, UK (FRSC, MRSC, GRSC) for the purpose of recruitment / promotion of Chemists.

The third Graduateship Examination was held in 1980 where the first batch of our own students sat for the exam and 11 out of the 44 candidates, were successful in Part I. Thereafter, the Institute was faced with the humungous challenge of finding a suitable venue to conduct the course, as unfortunately, the Aquinas College rejected the proposal made by the Institute to conduct Part II and a Practical course. However, with the involvement of Prof. Fernando and the help of Government Analyst Mr. G A C Sirimanne, the Institute was able to obtain permission from the Governors of St. Thomas' College, Mt. Lavinia to conduct lectures and the practical course of the GIC programme at the College premises. In order to manage the course, an Education Committee was appointed in 1981 of which Prof. Fernando was the first Secretary.

LTTC course which was revised from time to time after its commencement in 1973 underwent a major revision in 1987 which also resulted in the change of its name to LTCC – Laboratory Technicians Certificate Course. On the other hand, the Graduateship course was shifted to the New Science Laboratory premises of St. Thomas' and revisions were made in Part II of the course with the inclusion of more industrial and applied subjects. First Part II (Theory) and First Part II (Practical) were inaugurated in 1981. This was followed by the commencement of the Second Part I course at Mt. Lavinia in May 1982, with the registration of 70 students selected from a group of 300 applicants.

In 1981, the Council took an important policy decision regarding the management of the surplus of money the Institute was in possession of, by conducting the LTTC and GIC courses. As per this policy, "Educational Funds in general and Graduateship Course money in particular (including interest) must be utilized as decided by the Educational Committee." This vital decision which allowed the Institute to manage its funds wisely originated from the visionary Secretary of the Committee, Prof. J N O Fernando, and was well supported by some key members of the Council at that time.

The Council also took several important decisions with the view of providing benefits to the students and the member of the Institute. The office of the Institute of

Chemistry Ceylon was declared open in April, 1981 at Vidya Mandiraya and the Institute library was opened in Sumanarama Road, Mt. Lavinia with the collection of books received from the UK. In 1982, the Education Committee decided to start a series of monographs which would be of great use to the members and the first of the series, "Textile Fibres", was published in 1986. Further, a Council decision was taken to provide a 25% tuition fee waiver for children of the members studying at the Institute. However, this has now been increased to a full tuition fee waiver.

In 1984, the first batch of graduates consisting of four Graduate Chemists passed out and they were felicitated at a function held in Vidya Mandiraya. In his Presidential Address at the 14th Annual Sessions held in 1985, Prof. J N O Fernando suggested considering the establishment of a Sri Lanka College of Chemistry and also to consider applying to the University Grants Commission (UGC) for approval as a degree awarding Institute. He initiated this task himself, by writing to various institutions which he believed would be able to assist in gaining recognition for the GIC course. As a fruitful outcome to his unwavering efforts, in 1986 the Ministry of Higher Education issued a letter to the Ministry of Industries and Scientific affairs saying that, GIC can be considered as an alternative qualification to a degree with Chemistry as a subject. Further, confirmation was received from the RSC that those who successfully completed Parts I and II of the GIC course are eligible for Associate membership.

Successful completion of the GIC course opens up a myriad of opportunities for IChemC graduates in the industrial sector as well as in the academics. They are employed in diverse research institutions and many universities, both local and foreign, accept GIC graduates to follow their postgraduate degrees. Mr. S J Sarath Kumara, one of our first four Graduate Chemists, was the first to register for a postgraduate degree upon completion of the GIC course. He obtained his M.Phil. from the University of Kelaniya in 1987. Mr. K A Eustace from the second batch of Graduate Chemists (1985) obtained his M.Sc. from the University of London in 1988. In 1988, the Part I course was inaugurated at the University of Peradeniya for the first time. Owing to the high demand for the GIC course, in 1990, the Council decided to offer the Part I Course every year in Colombo. The Council granted approval for the academic dress for Graduate Chemists in 1990 and in the following year, the graduates joined the ceremonial procession for the first time at the Annual Sessions in the newly approved academic dress.

In 1991, on the occasion of the Golden Jubilee of the Institute (the Chemical Society of Ceylon was founded in 1941), a grand seminar was organized on "50 years of Tertiary Chemical Education in Sri Lanka" by the Education Committee and a one rupee commemorative stamp was issued. In his Presidential Address, Prof. E R Jansz accurately identified five challenges that lied ahead of us, most of which we have been able to successfully overcome. In 1993, following the practice adopted by the RSC for its Graduateship Examination, the Institute adopted a new performance criterion which enabled the awarding of classes to our graduates. In the same year, the Institute established its own library for Graduateship students at Mt. Lavinia.

In 1997, the Australian National Chemistry Quiz (ANCQ) commenced in Sri Lanka based on an idea forwarded by Prof. Fernando upon his return from a visit to Australia. ANCQ which is a very popular competition particularly among A/L students in Sri Lanka is conducted by the Institute every year and the candidates who manage to answer all 30 questions correctly are awarded scholarships to follow the GIC course. With major expansion in the course content of LTCC, it was upgraded to a diploma, DLTC – Diploma in Laboratory Technology in Chemistry, in the Silver Jubilee year of the Institute (1998).

In 2000, Prof. R P Gunawardena, the Chief Guest at the 29th Annual Session of the Institute suggested the establishment of a College of Chemical Sciences. Consequently, in 2001, the College of Chemical Sciences (CCS) was established during the Diamond Jubilee Ceremony of the Institute by unveiling a commemorative plaque. The Academic Board of the College was introduced in the same year as a more autonomous statutory Board, replacing the Education Committee that prevailed previously. Prof. J N O Fernando was appointed as the first (Honorary) Dean of the CCS and Mr. N I N S Nadarasa was appointed as the first Registrar. The logo of the College of Chemical Sciences was unveiled by the President, Prof. W. S. Fernando, at the first Convocation held in 2004, where 33 graduate chemists passed out in the Silver Jubilee year of the GIC course. Ms. B C J

Cooray, the daughter of Mr. B A Cooray who followed the first GIC programme in 1979, successfully completed the programme and became a Graduate Chemist.

The foundation stone for the Headquarters Building in Rajagiriya was laid on June 27th, 2002. This land was acquired thanks to the untiring efforts of Prof. Upali Samarajeewa of the University of Peradeniya who left no stone unturned in the process. The total cost of the building project was around 25 million rupees which was just over half of the money we had including the refundable deposits of the current students at that time. After much discussion on the management of the available funds, it was decided, based on an idea forwarded by me, that the construction would take place in one half of the land thereby requiring half of the estimated cost. The project was in very capable hands under the supervision of Mr. Mevan Peiris (President, 2007) and the construction work was completed by early 2005. The ceremonial opening of the building took place on February 25th 2005. This was followed by the winding up of operations at St. Thomas' College by May 2006. The opening of the Instrument Center on Level 3 in 2006 provided better facilities for students carrying out research in fulfillment of Part C optional course which was included in the GIC programme thanks to Prof. H D Gunawardhana who constantly highlighted the importance of research.

Immediately after shifting operations completely to the Rajagiriya premises in 2006, we were faced with the problem of acute shortage of lecture hall space during weekends, despite the availability of a total floor area of 11127 sq. ft. in the newly constructed Headquarters building. This was due to the increased number of students in both GIC and DLTC course which totaled up to around 700. As a solution for this limitation of space, plans for the second half of the building commenced. However, due to the lack of funds it was not possible to start the actual construction work immediately.

In 2007, some students were granted permission to carry out a research project in lieu of a written paper, which was later introduced to the GIC curriculum in 2008. At the Annual General Meeting (AGM) held in June, 2007 it was discussed to appoint a committee to look in to financial and management matters of the Institute. This, I would describe as one of the gloomiest incidents to take place in the history of the Institute which disturbed Prof. Fernando so much that, for the first time, he attended the Annual Dinner that year without his wife. As per the discussion at the AGM, a committee on making recommendations to the Council on reforming and restructuring of financial, management and related matters was appointed by the Council.

2008, the GIC programme received recognition of the Northumbria University, UK. In February and October of the same year, the first and second research sessions of the Institute were held. At the AGM of June, 2008, it was noted that the committee appointed to give recommendations to the Council on financial and management matters had not given any recommendations, and the President thanked Prof. Fernando for his valuable contribution towards the development of the Institute. Hence, things returned to normalcy once again! In 2008, Prof. S P Deraniyagala was recruited as a fulltime Visiting Professor at the Institute and he became the first Head of Faculty. The establishment of the Dr. Sudath Kumarasinghe Memorial Fund also took place in 2008, which proved to be extremely useful in awarding scholarships.

In 2009, I joined as a fulltime visiting professor. In the following year, the groundwork of IYC 2011 – International Year of Chemistry began under the skilled leadership of Mr. N M S Hettigedara, the Chairman of the IYC Steering Committee. In my opinion, IYC 2011 is the highest impact programme that we have conducted in the history of the Institute. The Steering Committee planned several activities to be implemented in contributing to the objectives of the IYC. One such activity was the CHEMEX-1 Exhibition and Trade Fair which was held over a period of four days from 27th to 30th January at the BMICH. The staff and the students of the College played a magnificent role in CHEMEX-1 by actively involving in many activities related to the organization of the event and in the presentation of numerous topics in stalls. The activities of the Institute and the College gained a big boost due to this event where the popularization of the GIC programme, in addition to promoting Chemistry as a subject, was made possible. A stamp and a first day cover were also designed in commemoration of the IYC 2011. The stamp included the portraits of Nobel Laureate Marie Curie and the late Professor M U S Sultanbawa, one of the most distinguished chemists produced by Sri Lanka.

In my year as the President (2011), I strongly put forth the idea that a change should be made in the administrative structure of the CCS so that post of the Dean would be a fulltime one. However, the then Dean, Prof. Fernando, was not willing to become a fulltime academic at the Institute. Hence, the new post of Honorary Rector (part time) was created and Prof. Fernando was appointed as the first Honorary Rector. The amendment to the post of Dean as a fulltime recruitment and the post of the Honorary Rector became effective from July 2011.

In 2012, CCS received accreditation from the RSC. The expansion of the Adamantane House utilizing the remaining half of the land, making available another 8500 sq. ft. of space was also commenced under the supervision of Prof. Gunawadena, who was the Head of this project which had an estimated cost of around 60 million rupees.

By the 2014 Convocation, we had produced a total of 946 DLTC diplomates and 969 GIC graduates, nearing a milestone. Prof. Fernando was elated with this result. His happiness grew several times fold upon the election of one of our alumni, Mr. K.R. Dayananda, as the Vice President of the Institute and Prof. J N O was very happy to include this in his speech at the Convocation.

By the 11th Convocation in 2015, Prof. Fernando was unwell. However, the happiness that filled his heart of having produced a total of more than 1000 diplomates and 1000 graduate chemists made him organize the event in a grand manner. He was unable to be present at the venue to attend to all the arrangements as often as he usually did due to his health condition. I recall, how he visited the place with Mandrupa on the day before the Convocation to make sure everything was in place. All this while, never did he show a sign of sickness. Probably, due to the humungous amount of happiness that occupied his heart at that time.

On 19th February, 2015 at his Convocation Speech as the Rector, he said "We have the pleasure that, from amongst our alumni three Graduate Chemists have functioned as Heads of the Chemistry Departments with one as a Dean of a Science Faculty. None of us who were involved with our education programme ever expected, even a few years ago, that our alumni might occupy such positions of distinctions and importance in our State Universities. We are confident that more of our alumni will follow suit in the years to come." This was his last Convocation Address and in 2015 March, he was no more. What is CCS, the Institute, and the country without him…

Upon the demise of Prof. J N O Fernando, Prof. S.P. Deraniyagala was appointed as the Honorary Rector of the Institute. He was well-supported by the Academic Board and the Council. I believe my little support as the Treasurer was a strength to him. In late 2015, Prof. S Hewage was appointed as the Honorary Rector. In 2016, the Council approval was granted for the Academic Dress for DLTC Diplomates. In July 2018, changes were made in the administrative structure of the Education Programme when the Academic Board decided to start the new B.Sc. Degree Programme. Necessary documents were submitted to the Ministry of Higher Education (MOHE) in order to obtain the degree awarding status. We decided to keep the GIC course also as a professional qualification and to continue the programme without a break. We were successful in the Institutional Review by the MOHE and received the degree awarding status in 2018. In the same year, the degree programme was submitted to the MOHE and we were able to obtain accreditation for the B.Sc. Programme. The DLTC course received approval by the Sri Lanka Medical Council (SLMC) in 2019. The DLTC Director, Mr. E G Somapala worked tirelessly towards that end.

In 2019, we forgot something! That is, the Ruby Jubilee of the Graduateship Programme. Prof. Fernando, had he been here, would have ensured that this occasion was not forgotten. But let's not forget the Golden Jubilee of the Institute and the 80th Anniversary of the Chemical Society of Ceylon, both of which are due in 2021. One of the major challenges that lies ahead of us right now is to get the necessary building space to commence the B. Sc. Programme in 2021. I believe this should be done either by obtaining a suitable place on rent or by completing the proposed project at Malabe. A further challenge we have to overcome in future is to ensure that the B. Sc. Programme is sustainable. I also suggest the commencement of the new BMs Programme by 2023 in celebration of the Golden Jubilee of the DLT course. It is now time for the new generation to take the challenge and move forward. In future, I will be happy to get involved only with teaching as long as the students need me.

In concluding my talk, I would like to specially thank the Council and Mr. Dayananda for giving me the opportunity of delivering the commemoration oration at the 76th Birth Anniversary and 5th Death Anniversary of Prof. J N O Fernando. I would also like to extend my sincere thanks Prof. Ramanee Wijesekera, Prof. Sujatha Hewage, Mr. Sahan,Jayasingha, Mr. N. I. N. S. Nadarasa and the library staff for helping me in the preparation of this talk. I am also thankful to the CCS students for making my teaching at CCS wonderful and to all the senior members of the Institute for giving their fullest support during my career. Last but not the least, I thank you all who are in the audience for listening to my talk.

Guest Editorial

Role of a Chemist in a Pandemic Situation

R.M.G. Rajapakse

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The entire world is severely and adversely affected by the newly emerged Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) infection which was first discovered in the Wuhan Province of China in December 2019. The virus strain causes the disease which now called the coronavirus disease-2019 (COVID-19) that is contagious in humans where human-to-human transmission of SARS-CoV-2 was confirmed on January 20, 2020. The World Health Organization (WHO) has identified it as an ongoing pandemic. The total number of infected persons has now exceeded 3 million (3,083,453) with over 200,000 (212,498) deaths, as of today, 27th April, 2020, and both curves are still increasing exponentially (https://www.worldometers.info/coronavirus/). The respiratory droplets from coughs and sneezes of an infected person carry the virus and the virus is transmitted to another individual who is at a distance where these droplets usually travel in the air. This distance is usually about 1.8 m and hence keeping at least such a distance between people, avoiding crowded gatherings (social distancing), and wearing protective gear would be a way of controlling the transmission of the disease. A recent study published in the New England Journal of Medicine states that SARS-CoV-2 is viable for up to 72 h on plastics, 48 h on stainless steel, 24 h on cardboard, and 4 h on copper and 3 h in the air. However, the main mode of transmission of SARS-CoV-2 is through direct contact of respiratory particles of a patient in air. However, if a person touches a surface that has the virus on it and then touches nose, mouth or eyes, then he or she can pick the virus up and get infected. These surfaces can be disinfected using a suitable disinfectant or by washing with soap. In this scenario, chemist has a huge role to play in recommending most effective disinfectants, suitable usable concentrations, frequency of disinfection application, and their health effects. A simple disinfectant such as a mixture of ethanol (62-71%), hydrogen peroxide (0.5%) or sodium hypochlorite (0.1%) and water can break the delicate envelop that surrounds the virion. The use of soap to disable the virus is effective as it dismantles the lipid outer coating of the virus particles. In order to recommend such a simple yet effective way of destroying the virus, knowledge of the chemical structure and the 3-D arrangement of chemical components of the virus are required. Such a structural elucidation is a task of a chemist in collaboration with a biologist and the recommendation to use soap to disrupt the lipid outer coating is none other than simple chemistry of action of soap.

Since the virus can remain on various surfaces for hours to days, the surfaces of hospital COVID-19 wards where patients are treated require suitable disinfection mechanisms. An antiviral paint coating on walls and floors of such wards would drastically reduce the risk of virus spreaing out of the wards and would give some protection for the hospital staff who are working in such wards. In controlling the spreading of the virus, silver nanoparticle containing surface paint coating was used in the Wuhan Province hospitals. Silver nanoparticles are known antiviral agents as they are coordinated by S, O, N atoms found in viral enzymes and thereby deactivate the biochemical pathway catalyzed by the enzymes. Ag^{+}

ions present on the surface of silver nanoparticles can also electrostatically attract spike S proteins which are negatively charged in nature. Advancing this further, we developed an antimicrobial paint consisting of hematite and zinc oxide nanoparticles that has five modes of actions for destroying viruses. The paint thus developed has already been applied on the walls and floors of the COVID-19 designated wards of the Homagama Hospital. The attention of the relevant authorities is required to apply this surface coating on walls and floors of buildings that are acting as virus spreading surfaces. However, up until now, no attention has been paid by any responsible authority despite the fact that the paint was supplied free-of-charge. This is possibly due to the fact that there is no responsible chemist in the decision-making authorities such as the Presidential Task Force assigned to deal with COVID-19 in order to understand chemistry in action of this surface coating.

As protective gear is an important strategy for preventing the infection of the virus, we looked into the suitability of masks that are used particularly by the hospital staff and the officers of the forces. The 3M N95 mask is the best option since it is designed to filter at least 95% of the particles of size equal to or greater than 300 nm. The surgical masks used in this country do not satisfy this requirement and the best surgical mask filters up to 40% at 300 nm though some of them currently used has only 10% efficiency. There is a severe dearth in N95 masks throughout the world and in order to address this problem we have designed and developed a novel strategy where we have covered the pores of fabrics using suitable nano-to-micro-size particles in order to prevent particles of size 300 nm penetrating through them. The modified fabrics were observed through improved optical microscope and particle penetration study was performed. Having obtained required properties, the mask materials will be manufactured in the very near future.

Development of antiviral drugs mandatorily requires identification of biochemical pathways of the virus' life cycle. A group of German Biochemists, Virologists, Biophysical Chemists and Chemical Biologists has identified the X-ray crystal structure of the SARS-CoV-2 main protease (M^{pro}, also called $3CL^{pro}$); the enzyme that cuts the polyproteins translated from viral RNA to yield functional viral proteins (https://science.sciencemag.org/content/368/6489/409). The main protease enzyme is a very attractive drug target because this is the enzyme that is responsible for processing the polyproteins that are translated from the viral RNA. The researchers have reported he X-ray crystal structures of the bare SARS-CoV-2 M^{pro} and its complex with an α-ketoamide inhibitor. The pharmacokinetic characterization of the optimized inhibitor has revealed a pronounced lung tropism and suitability of the drug administration by the inhalative route. This is one such methodical approach for antiviral drug design to combat the current pandemic. Computational chemists also have a great role in drug design through molecular docking. Molecular docking is a kind of bioinformatic modelling which involves the interaction of two or more molecules to give the stable adduct. Depending upon binding properties of ligand and target, it predicts the three-dimensional structure of any complex (https://www.omicsonline.org/open-access/molecular-docking-approaches-types-applications-and-basic-challenges-2155-9872-1000356.php?aid=88070). This is the newest approach in drug design and most suitable inhibitor that inhibits enzyme action of the virus could be obtained through such computational chemistry technologies.

The above are some of the key areas where a chemist can help control the COVID-19 pandemic. There are many other areas where a chemist can actively get involved in these projects. There are claims that some ayurvedic preparations are active against SARS-CV-2. A qualified chemist can scientifically analyze the active component present in the formulation and even its right stereochemical structure, structure-property relation, biochemical action, and pharmaco-kinetic studies. What is required is the recognition of the important roles of a chemist in controlling this pandemic and taking appropriate actions to get their services in time before the pandemic goes out-of-control.

Cover Page

The cover image, adapted from gbcghanaonline.com, depicts the Coronavirus centered in an intriguing background of scattered Influenza virus molecules. The Global Pandemic, COVID-19, has brought the entire world to its knees and continues to take lives worldwide. "Combatting the Unseen" is an issue dedicated to the severe and life-threatening varieties of pathogenic diseases and to give an insight into tackling their disastrous effects. The name of the themed collection was proposed quite fittingly by level 4 GIC student, Binelka Siriwardane.

Themed Collection : Combating the Unseen

Themed Collection

Viral Pandemics Through the World History in a Biochemical Viewpoint

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Disease and illness have affected humans since the dawn of life. Some of these diseases might affect a considerable portion of the human population at once while some are limited to a minuscule number. As an example, a sudden episode of a simple food poisoning and HIV/AIDS can be considered. The latter is a widespread problem found throughout the globe at a given time while the food poisoning will be limited to the small group who ingested the food. This differentiation is of enormous importance because the approaches taken to manage the condition will vary with its spread. The words 'Pandemic' and 'Epidemic' are two words used in epidemiology to define the effect of a disease to the human population.

An epidemic is when a disease exceeds the usual prevalence of it in a given population, while being confined to the area defined by geographical borders. In order for it to become a pandemic, it should cross geological and political boundaries and be spread across the world.[1] Throughout history, pandemics have played a key role in shaping the world to the one we live in today. From them, infectious disease pandemics are more significant due to their contagiousness. As now we are facing a global level pandemic caused by a viral disease, looking back and understanding what happened during the past viral pandemics of the world would provide us with a valuable insight.

Before diving into the history, learning some basic concepts of virology will help understand the behavior of those disease patterns and the biochemical basis behind them. Viruses are obligate intracellular pathogens and generally are made of a protein coating and a core containing genetic material. They cannot carry out any biochemical reaction outside a host, and upon infection they hijack the host cell's metabolic processes for replication of their genetic material and the production of more viral particles. Antiviral drugs aimed in treating viral infections are most effective during the replication of viruses as they do not have any other crucial metabolic reactions like bacteria do, which are targeted by antibiotics. The main hardship faced in prescribing antiviral drugs is that symptoms start appearing later during a viral infection and by this time, most of the viral multiplication have taken place. In such an instance, antiviral drugs will have little to no effect in controlling the disease.[2,3]

Having a rough idea about the defense mechanisms in the human body against viruses is also essential in understanding biochemical basis of the therapeutic options used in treating viral infections. Main goal of host defense mechanisms is to slow the replication of the virus and then eradicate it from the system. Interferons (IFN) play an important role in this process. IFNs are glycoproteins which are secreted by cells that are already infected and these IFNs then diffuse to adjacent healthy cells to activate their genes to hinder the viral replication in them. Furthermore, IFNs activate Natural Killer cells which can kill already infected cells and thereby limit the spread of the virus. Two other important proteins involved in this process are antibodies and the complement system. Antibodies have the ability to bind with viral antigens on an infected cell and then make the cell more susceptible to the phagocytic activity of macrophages. Also, antibodies can directly bind with viral particles and inhibit their binding with host cell, and agglutinate viral particles, allowing them to be phagocytosed. Complement proteins can attack some viruses by damaging their protein envelopes. A type of cells involved in the immunity known as T cells can produce cytokines, which give rise to inflammation which in turn inhibit the viral replication, and they can also kill host cells containing the virus following recognition of viral antigens on cell surface.[4]

The Antonine Plague

The first known viral pandemic in history is the 'Antonine Plague', which mainly affected the Roman empire ruled by Marcus Aurelius in 166-180 A.D.

followed by another outbreak in 189 A.D. which also spread to Egypt. It is assumed that 7-10 million people died due to this pandemic and it is considered as a factor that contributed to the downfall of the Roman empire. A detailed account of this pandemic is not found in literary sources. The most accepted and trusted description of it comes from the texts of Galen, a well-known Roman physician and a writer. Galen describes the symptoms of the disease as fever, an exanthem (a rash involving the whole body) and diarrhea in some cases. The exanthem became black due to collection of blood in the blisters and the diarrhea was dark black in some cases due to bleeding in intestines.[5] These features are very much consistent with that of a hemorrhagic smallpox infection. Thus, researchers and historians widely agree to the fact that this pandemic might have been caused due to smallpox.[6]

Smallpox, which is considered to be eradicated now, was caused by variola virus belonging to the Orthopoxvirus genus. It consists of a small genome with 186 kbp and is known to exclusively infect humans with an incubation period of 14 days. It is widely thought that the first spread of smallpox among humans occurred in the southern parts of the African continent.[7] Main treatment method used to control smallpox was vaccination, followed by Edward Jenner's breakthrough findings. These vaccines contained a prototype orthopoxvirus named vaccinia virus. Modern formulations of the smallpox virus vaccine contain either highly attenuated vaccinia virus or non-infections subunits of it like DNA or protein envelopes in contrast to the live vaccines used before. The purpose of the vaccine is to provide prophylaxis, where it acts to increase the immune response of the body through the development of antibodies, T cell response and cytokine profiling that will enable the body to fight against a future smallpox infection. Furthermore, there are antiviral drugs which were suggested to be used in the treatment of smallpox yet have not been tested clinically since these were developed even after the last smallpox outbreak. Cidofovir and Tecovirimat have been tested in animal models and are known to inhibit viral DNA polymerase and prevent its replication, and inhibit protein envelope respectively. Since the eradication, research done on smallpox has been on a minimal level and thus novel treatment methods to control it are not explored in detail.[8]

Smallpox of the New World

The next recorded viral infection outbreak of world history also was due to a smallpox infection epidemic in Japan from 735 – 737 A.D. This did not spread in to neighboring countries to be declared as a pandemic. Smallpox caused a pandemic again in the 16th century and this is commonly known as the smallpox outbreak of the new world. This pandemic started in 1520 with an expedition from Cuba to Mexico with the intention of hoarding the gold and other riches of the Aztec empire. Among the crew of the ship was an African slave who had smallpox. Since the native Aztecs did not have any previous exposure to the disease, there was no immunity against it among them and it is recorded that more than half of the population died due to it. From the coastal area it spread inland and at least half the population living in Mexico succumbed to it and this assured the success of the conquests done by Cubans. From Mexico, the disease spread into the Inca empire in Peru allowing the small band of Spaniards from Cuba to easily claim victory against them. Afterwards, it continued spreading to Caribbean islands, Central America and Chile, being responsible for the deaths of millions of natives. This even spread into the interior of Brazil later through the missions of conversion by Jesuit missionaries. The Spaniards showed a resistance to smallpox due to their childhood infections in the 8th century through the introduction of the disease to their homelands through Moors. This enabled them to easily overcome the plagued natives and conquer their lands for themselves. Smallpox continued to spread through the South American continent until the early 19th century, when vaccination was established against it.[9]

1781-1782 flu pandemic

Influenza marks the next great viral pandemic of the world. The first pandemic of influenza lasted from 1781 to 1782 in the European countries. It is assumed to have started in Russia and then moved towards west across Finland, Germany, Hungary, Vienna to the British Isles. It even spread to the Mediterranean countries, Italy, France, Spain and Portugal by the August of 1781. This pandemic posed a low mortality rate, while only the elderly population and the ones who already suffered from respiratory illnesses were at risk of death. Yet, it is assumed that at least three-fourth of the population of

whole Europe fell ill owing to this influenza pandemic by the first half of 1782. Although the exact identity of the causative virus is unknown, scientists conclude it is different from the viruses which caused the next influenza pandemics of 1889-1890 and 1918-1919 as this illness spread through the continent during the summer and spring whereas other influenza pandemics thrived during winters. Thus, it is a matter of debate among historians whether this pandemic is actually an influenza infection or some other viral infection which caused respiratory symptoms.[10]

Russian flu

Following yellow fever epidemics in Hispaniola, Philadelphia, New Orleans and then a Measles outbreak in Fiji, then came the next viral pandemic of Influenza in the years 1889-1890. This also began in central Asian regions which then belonged to the Russian empire and then gradually spread all over Europe except for Ireland, Northern Great Britain and Sardinia. Furthermore, it spread through ships to United States, Toyo, Hong Kong, Singapore, India, South America, Africa and Egypt. Even though exact figures are unknown, assumption is that at least one third of the world population caught the disease during that period. Causative virus of this 'Russian Flu' pandemic is also uncertain since virology was still a developing field those days.[10]

Spanish flu

Soon after that, one of the most serious pandemics of history occurred with a record of at least 50 million deaths in its wake. Later on, the causative virus for this pandemic was identified as an influenza virus. Thus, before going into specifics of it, having an understanding about the general structure of an influenza virus will be important. Influenza viruses are of 4 types; A, B, C and D, with influenza A viruses being the most common. These viruses are enveloped viruses with an RNA strand as their genetic material. The envelope is derived of host cell lipids and there are three viral proteins in it. The genomic RNA strand is organized into 8 segments. Haemaglutinin (HA) is the viral protein that is needed to attach to a host cell and there are about 18 types of HA in existence. Neuraminidase is needed to cleave the sialic acid molecules on the host cell surface and thereby allow the newly replicated viral particles to be released from a host cell. 11 types of neuraminidase have been identified currently. There are two types of M proteins, M1 which is a matrix protein and M2. M2 acts as a selective channel for protons and this allows the interior of the virus to be acidic and prevent complex formation between the genome and M1 proteins. This helps the viral genome to be transferred into the host cell nucleus. These viral proteins are again needed for the incorporation of the newly replicated genetic material into new virus particles. Immune system identifies these viral proteins as antigens and produces immune reactions against them.

Subtypes of Influenza A viruses are named according to their neuraminidase and haemaglutinin types (eg: H1N1, H5N1 etc.). Two other concepts that are important with regards to influenza viruses are 'Antigenic shift' and 'Antigenic drift'. Antigenic shift occurs due to exchange of genome segments between subtypes of influenza A viruses. This ultimately results in a virus with completely new combination of antigens and thus previously immunized population will also be susceptible for infection. In antigenic drift, slight mutations occur in the antigens and this is known to happen in Influenza viruses A, B and C types. This results in a slightly modified strain of an existing virus, against which a considerable proportion of the population may show immunity.[11]

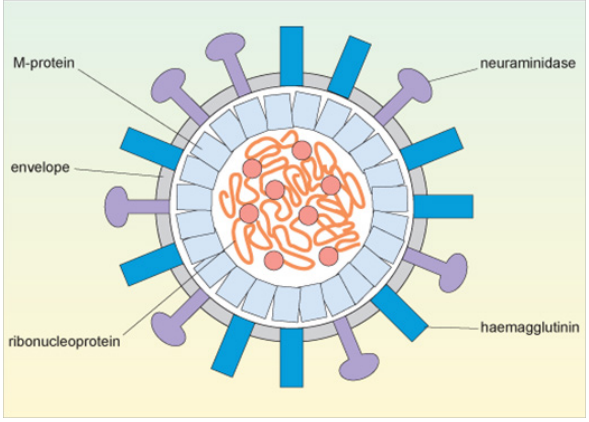

Figure 1: Schematic diagram representing the general structure of an influenza A virus

The 1918 Influenza pandemic is known to be caused by an H1N1 subtype of influenza A virus which is closely related to the influenza virus which caused swine flu recently. It is thought that influenza viruses have been transferred to pigs from its natural reservoir, birds and

pigs, who act as the intermediate host, then acquired the viral infection from humans also. Then these strains underwent genetic segment reassortment (antigenic shift) and formed a new subtype with novel antigens causing the large number of infected cases in humans. This pandemic came in three major waves affecting North America, Europe and Asia. Apart from normal influenza symptoms (which are similar to common cold symptoms), this infection caused pneumonia conditions owing to its high virulence and the ability to replicate throughout the full extent of the respiratory system.[12]

Since antiviral drugs were not yet developed by the time of this pandemic, governments around the world took measures to mitigate the spread of it by encouraging people to wear masks, spraying antiseptics in public places, closure of schools and public institutions and promoting hygienic practices among populations. Some governments even announced quarantine periods and thereby managed to reduce the number of cases.10

Asian flu

Year 1957 marked the emergence of another influenza virus pandemic, which was named 'Asian flu' owing to its origin in China. The causative virus was identified as subtype H2N2 and it subsequently spread throughout the world by September of that year. This pandemic caused more than 1 million deaths among the people who contracted it, and this also had the ability to cause primary influenza virus pneumonia similar to that of the 1918 pandemic. Identification of the virus subtype led to the development of a vaccine against it. Yet the limited amount produced, and low efficacy rendered the vaccine useless in changing the course of the natural progression of the disease.[13]

Hong Kong flu

Another pandemic influenza emerged in 1968. This was first reported in Hong Kong as a large epidemic and thus it was given the name 'Hong Kong Flu'. Virus subtype H3N2 was identified as the culprit. Since only the HA antigen has been changed when compared with Asian Flu virus, spread of the virus was affected by pre-existing N2 immunity among populations. This pandemic was responsible for about 1 million deaths worldwide and the H3N2 virus subtype continues to infect humans as the seasonal influenza while regularly undergoing antigenic drifts.

Swine flu

Again, another strain of influenza virus A, hit the world as a pandemic in 2009, giving rise to the A(H1N1) 'Swine Flu' which first emerged from populations in California and Mexico. Molecular studies done on the genetic material of this virus have shown it has been derived from the combination of several viruses of human, avian and swine origin, that have been circulated in pigs. Gradually the disease spread to 214 countries by April 2010 and it was noticed that mostly the younger population was at risk of infection (60% of patients were 18 years or younger). This observation was explained by the existing immunity in the elderly population who has been exposed to the seasonal flu virus strains. Exact number of infected cases are unknown because mostly the diagnosis was done clinically due to the laboratory diagnostic facilities being limited. However, it is estimated that around 200,000 people died as a consequence of the infection. In most cases the infection was limited to mild flu symptoms while it developed into primary viral pneumonia, respiratory failure and death in some cases. Transmission of the virus was mainly through respiratory droplets of infected people and formites (surfaces with which these respiratory droplets have come into contact). Vaccines were rapidly developed as a treatment method and this included inactivated whole viruses, subunit vaccines and live-attenuated vaccines. Despite the rapid production, manufacturers were unable to cater to the needs of the population all around the world. Thus, vaccination was targeted for groups at high risk including health care workers and pregnant women.14 To understand the effect of antiviral drugs towards this pandemic, first we will go through the principles of antiviral therapy.

Antiviral drugs mainly target different components of the structure of viruses and inhibit them to prevent the replication and infection. Amantadine and rimantadine are two antiviral drugs which are only effective against influenza A viruses. These drugs target the M2 channel protein of the virus and inhibit it, thereby inhibiting transfer of viral genome into the host. For these drugs to be effective, they should be given early in an infection. Another issue with them is the development

of resistance in vruses against them. Viruses which develop resistance against rimantadine becomes resistant against amantadine also and vice versa. Another type of such treatment option is neuraminidase inhibitors. These inhibit the neuraminidase proteins of the virus and thus prevent the release of newly synthesized viral particles from the host cell. Zanamavir and oseltamivir are examples of this category and unlike M2 inhibitors, these can treat influenza B infections as well.[11] In the 2009 A(H1N1) pandemic, oseltamivir was found to be an effective treatment option when given within 36 hours of infection whereas the virus showed resistance against rimantadine and amantadine.

SARS

Prior to the last influenza pandemic of 2009, a small-scale pandemic was caused by a novel coronavirus. This was named as Severe Acute Respiratory Syndrome (SARS) and the causative coronavirus was known as SARS-CoV. Disease outbreak was first noticed in China in November 2002 as an atypical pneumonia. An infected physician from China who stayed at a Hong Kong hotel infected 16 guests from different countries and this led the disease to be spread over the world. As the end result, 8096 cases of SARS were reported from 26 countries with 774 mortalities.

Coronaviruses are large spherical viruses with an envelope formed by a lipid bilayer. In this envelope, viral structural proteins M, E and S are located. Within the envelope lies the nucleocapsid, which is formed by N proteins bound with single stranded RNA genome of the virus. These viruses are responsible for about 30% cases of common cold and upper respiratory tract infections that occur naturally. SARS-CoV is thought to be an unrecognized animal coronavirus which gained virulence against humans with mutations over time. A considerable number of first reported cases were from animal or food handlers in China and bats are thought to be the natural reservoir of this virus. SARS reported to have affected all age groups and both genders similarly. However, it is notable that 22% of infected cases were health care workers. SARS is considered to have low transmissibility and it is spread through respiratory droplets and formites. Common features of the infection were fever, body aches, cough and breathing difficulties with pneumonia, while upper respiratory symptoms were uncommon.

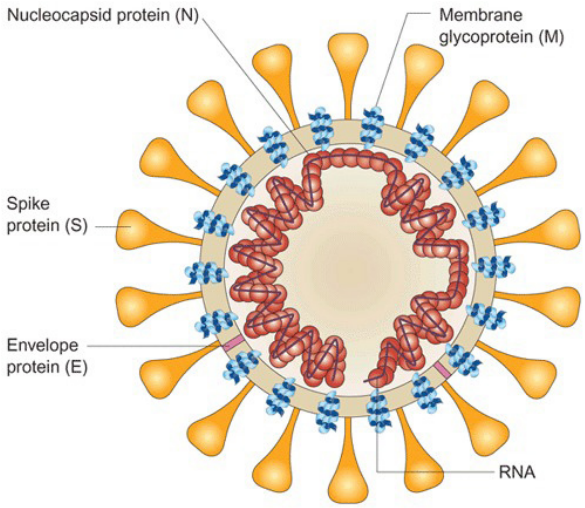

Figure 2: Schematic diagram representing structure of SARS-CoV

Diagnosis of SARS was done by the analysis of upper respiratory tract secretions or plasma component of the blood of suspected cases. Detection of virus under electron microscope after growing them in fetal rhesus monkey kidney cells led to the identification of virus particles consisting of a corona or a halo around it and hence the name, coronavirus. Viral RNA detection was done using Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) and this method is known to be 100% specific for the detection of infection. Immunologic assays such as ELISA and immunofluorescence microscopy was done to detect virus specific antibodies in the plasma. Definitive treatment methods are not available for SARS. Synthetic interferons were shown to be effective in inhibiting the virus and an antiviral named ribavirin was used to treat the infection, which had the ability to inhibit viral replication. Yet, soon it was discontinued from treatment as it showed many side effects among the patients using it. Mainly adjuvant therapy such as inti-inflammatory agents were used in the symptomatic treatment of the disease.[15]

MERS

After a decade of the first coronavirus pandemic, a man in Saudi Arabia developed an atypical pneumonia and died of it. Upon examination of his sputum, another novel coronavirus strain was identified, and this was

named Middle East Respiratory Syndrome Coronavirus (MERS-CoV). This infection gradually spread from the Middle East to other countries via travelers and by April 2016, there were 1,728 confirmed cases in 27 countries with 624 deaths. Similar to the SARS-CoV, MERS-CoV also has a very large RNA genome. Despite the long RNA strand, accumulation of harmful mutations to the virus is kept at check by the proofreading mechanisms available in coronaviruses. Even though usually bats are the natural reservoir of MERS-CoV like coronaviruses, serological studies done on Middle East animals have shown that camels were found to be harboring the virus as well as MERS-CoV specific antibodies. Thus, it is widely accepted that camels act as a natural reservoir for MERS-CoV. The symptoms and clinical features of both SARS and MERS are extremely similar and the diagnostic techniques used were also the same. There is no clinically proven therapy for MERS-CoV; thus, symptomatic treatment and supportive care is given to patients. There are several vaccines which are currently tested on non-human primates against MERS-CoV.[16]

By the time this article is written, a novel coronavirus is affecting the whole world and it is the largest pandemic originated during this millennium. Like all the previous pandemics, this will also gradually diminish after following its natural course. Yet, we can never be certain, but only hope, that may this be the last viral pandemic we will have to experience in our lifetime.

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The Role of Smoking Abeyance During Respiratory Virus Epidemics

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Introduction

Respiratory conditions impose an enormous burden on society. Oodles of people suffering and die because communicable and non-communicable respiratory diseases in every year. Fortunately, most of the respiratory diseases are avoidable by improving the quality of the air. Tobacco smoke, indoor and outdoor air pollution, air containing microbes, toxic particles, fumes and allergens can consider as common sources of unhealthy air. Latest findings show that air pollution exposure suppressed the immune systems regulatory T cells (T lymphocyte, is an essential part of the immune system). T cells are responsible for putting the bracs on the immune system and low function of T cell lead fail to block the inflammatory responses. Tobacco smoke contains more than 7,000 chemical compounds and many of them can harm the immune system and can make the body less successful at fighting against diseases.Tobacco smoking (TS) is associated with both release and inhibition of pro and anti-inflammatory mediators. Since the beginning of the 21st century, we are facing the convergence of several epidemics including tobacco smoking and influenza, SARS-CoV-2 like respiratory viral infections. Therefore, it is very important to understand the interrelationship between TS and respiratory viral infections.

A virus is always acommunicable agent with having both living and nonliving characteristics that is completely dependent on host cell for replication (intracellular parasite). Viruses are metabolically inert, force intracellular parasites, have only either DNA or RNA (but not both like living organisms), which use host living cell machinery to multiply.Moreover, they cannot make energy or proteins without support a host cell. In the case of respiratory viral infections (flu, SARS-CoV-2... etc.), viruses that can affect the breathing passages and cause respiratory illnesses and these viral infections commonly affect the upper or lower respiratory tract.

Viral pathogenesis can be defined as the process by which viruses produce disease in the host cell. There are several complex and dynamic interactions involve between the virus and the susceptible host as the factors that regulate viral transmission, multiplication, distribution and evolution. Considering the spread of a disease in society, it can be distinguished as **endemic** (disease limited to a certain group or region), **epidemic** (widespread in a society), or **pandemic** (spread worldwide).

Coronavirus disease 2019 is an infectious disease caused by severe acute respiratory syndrome coronavirus 2(SARS-CoV-2) which is a RNA virus. The outbreak was first identified in 2019 in Wuhan, the capital of Hubei, China, and has now spread more than 210 countries around the world. SARS-CoV-2 viral pathogens binds to the human angiotensin-converting enzyme-2 (ACE-2) receptor through densely glycosylated spike (S) protein, as the initiation step of the entrance mechanism to human cells.

In biology, **Immunity** is the balanced state of multicellular organisms with sufficient biological protection to fight off infections, diseases or other unwanted biological attacks. Nearly all the cells in the human body have mechanisms to detect viruses and other microbial agents. Pattern recognition receptors (PPRs) are associated with pathogen associated molecular patterns (PAMPs) or viruses, but are usually not found in host cells. Reactive oxygen species induced by TS are

Table 1: Respiratory viral infections

Respiratory virus	Type of virus	Time duration	Global mortality
Spanish flu	H1N1	1918-1919	50 million
Asian flu	H2N2	1957-1958	1-2 million
Hong Kong flu	H3N3	1968-1969	0.7 million
Swine flu	H1N1	2009-2010	0.28 million
SARS-CoV-2	Corona	08.12.2019 – 21.04.2020	>0.17 million

involved in interfering with PPR activity. PPRs important for virus detection include Toll-like receptors (TLRs), retinoic acid-inducible gene I (RIG-I), nucleotide-binding oligomerization domain-like receptors (NLRs) and other cytosolic virus sensors. Activity of the immune system is much critical in limiting virus spread and alerting the respond to the infection. Activation of PRRs in these cells by virus infection triggers production, release of type I, III interferon's(IFNs), and other pro-inflammatory mediators (e.g., cytokines, chemokine's , and antimicrobial peptides) which initiate the host innate and adaptive immune response. Thus, the level of PRR activation throughout the respiratory tract ultimately affects the level of immune cell recruitment and the release of the pro-inflammatory mediator, followed by any immunologic pathology.

Tobacco smoke (TS) isusually a health hazard. It generally has negative health effects, cause more than 7 million deaths per year worldwide (WHO Report on the Global Tobacco Epidemics, 2017).The chemicals that absorbed in to the blood stream by tobacco smoke directly linked to the biochemical changes of immune system.

Changes in the innate immune system along with direct and indirect tobacco smoke exposure lead to a pronounced and chronic inflammation in the respiratory system. This leads to other pathological changes, including re-modeling and destruction of lung tissue. Tobacco smoke exposure can also cause lung infections bacteria and viruses.In human lung, lower respiratory tract stay as sterile. Therefore, the processes of breathing and gas exchange can occur efficiently. When the respiratory system get expose to outside atmosphere, sophisticated system tend to changed, that protects and cleans the lung.

Chemicals in the tobacco smoke enter into the airway lumen by the direct or in direct exposure. Initial contact is with epithelial cells and there exist a numerous types of initial protective mechanisms. These include a mucociliary (self-clearing mechanism of the airways in the RS) transport system and innate defense mechanisms. Signaling from the epithelium triggersadditional innate

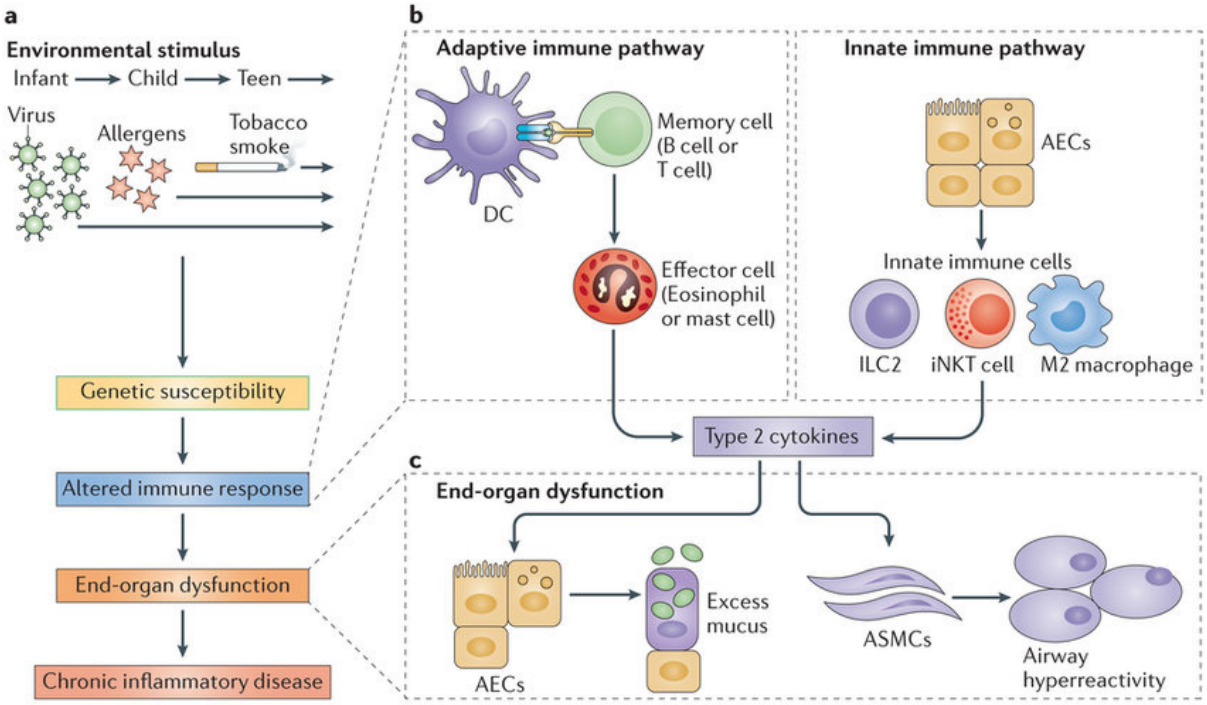

Figure 1: Adaptive and innate immune responses in chronic respiratory disease. a) Environmental stimuli — such as respiratory viruses, allergens and/or tobacco smoke — may act on genetically susceptible individuals to lead to an altered immune response. b) An altered adaptive immune response involves antigen-presenting cells, primarily dendritic cells (DCs), that process and present antigens to memory B cells and T cells that drive the activation of effector immune cells (such as eosinophil and mast cells). Additional T cell subsets that regulate the adaptive immune response include T helper 17 (TH17) cells, TH9 cells and regulatory T cells (not shown). Alternatively, altered innate immune responsesmay include airway epithelial cells (AECs) that activate innate immune cells, such as invariant natural killer T (iNKT) cells, M2 macrophages and innate lymphoid cells (ILCs). c) Effector cells or innate immune cells then produce type 2 cytokines — for example, interleukin 4 (IL 4) and IL 13 — that act on end-organ cells, especially AECs, to produce excess mucus, and on airway smooth muscle cells (ASMCs)

responses. First recruitment of neutrophils and then subsequently monocytes and macrophages. Later still adaptive immunity comes into play with T and B cells playing a role, which might also have an autoimmune component in more advanced and severe (Figure 1).By the down regulation of TRL3, TRL7, RIG-1 type receptors, TS interfere with the recognition of viruses.

In today's global emergency, such as the SARS-CoV-2 outbreak, identification of vulnerable groups is essential. Recent scientific studies show that the SARS-CoV and SARS-CoV-2 share the same receptor, ACE2(angiotensin-converting enzyme 2), and that this receptor is common in the respiratory system of tobacco smokers(Figure 2). According to the latest findings by the research done by University of South Carolina, scientists observed significantly higher ACE2 gene expression in former smoker's lung compared to non-smoker'slung.

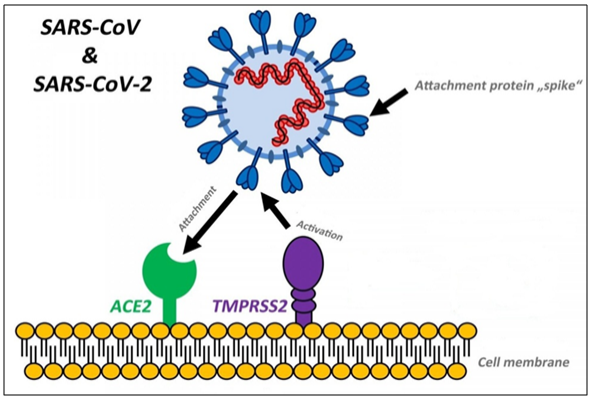

Figure 2: ACE2 receptor, expressed on the cell membrane of lungs, as a binding site of spike protein of SARS-CoV-2 virus.

In addition, they found that the ACE2 gene expressed in specific cell types related to the history of tobacco smoking. Findings of the research explainACE2 actively expressedin current smokers goblet cells and in non-smokers club cells in the bronchial epithelium. Other than that, ACE2 actively expressed in reconstituted type II alveloar cells (in which genes regulating viral reproduction and transmission are highly expressed) of former smokers in alveoli.

Discussion

Tobacco smoke adversely affects the immune system, resulting in immune deficiency, high infection rates (bacterial, viral…etc.) and in many cases of different autoimmune diseases. Surprisingly, immunological pathogenesis of respiratory virus infection reflects a complex interaction that directly influenced by virus and other viral factors, including the response of resident respiratory cells and the recruitment of innate and adaptive immune cells to the lungs. By quitting bad habits like tobacco smoke, people can enhance their immune related defense mechanismsagainst respiratory virus(like SARS-CoV-2 pandemic) and other related infections. In addition, it will help to reduce abnormal expression of ACE-2 receptor(one of the major binding site of SARS-CoV-2 virus to human body) in the respiratory system to reduce riskof the infection of SARS-CoV-2 virus.

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Drug Discovery in Combating Viral Diseases

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Continued efforts toward discovery of novel therapeutic strategies is pivotal in combating viral diseases. Lack of proper therapies for a virus will inevitably cause a localized health crisis to move from an epidemic to a pandemic in a short period of time.

Attacking a host cell

Virus are obligate intracellular pathogenic agents that are vastly different than bacteria parasites or any other pathogens. A virus requires a host cell to multiply. The packaging proteins and the enclosing genomic material are synthesized using the small molecules and the machinery of the host. Viral particles have their genetic material in the form of DNA and RNA enclosed in a protein capsid. In certain viral strains the protein envelop is enclosed in an additional lipid bilayer. Viral particles require hosts for its multiplication. The initial interaction with the host cell is the first key step of the viral infection. Such interactions are largely mediated by viral surface components such as membrane glycoproteins and viral surface proteins. These initial interactions are often less specific and tend to be electrostatic in nature. Once attached, a virus can employ various strategies in entering the host cell include and not limited to; involvement of specific cell surface receptor proteins for specific receptor-mediated entry, activation of intracellular signaling cascades to promote cell entry, direct penetration of the cell membrane, etc.

Once inside the host cell, undergoing intracellular trafficking,some viruses penetrate the cytoplasm or in the case of others migrate to the nucleus to replicate their genetic information. Replication of the genetic material depend on the nature of the genomic material of the virus. Viruses containing DNA often induce a DNA damage response facilitating the integration of the viral genome into the host chromatin. The double stranded or single stranded DNA lesions will allow the integration of the viral DNA molecule. In contrast, RNA containing viruses require different biochemical mechanisms for the replication of the RNA. Viruses containing the plus strand RNA, or the sense strand are able to use the translation machinery on the RNA for the protein synthesis. However, negative and double stranded RNA viruses are packaged with a virus RNA polymerase to synthesize its own RNA prior to translation. It is noteworthy that viruses hold the highest rate of mutations in their nucleic acid sequences. Among all viruses, RNA viruses hold the highest mutation rates followed by single stranded-DNA and double stranded DNA viruses. Viral polymerases are error prone in comparison to cellular polymerases. However, these mutations are not only mere polymerase errors but caused by the ability of the virus to proofread and repair, spontaneous nucleic acid damage and genetic elements in virus that functions to generate mutations.

History of Pandemics

Outbreaks of infections have led to pandemics multiple times through the history. From smallpox in the 16th century to Spanish flu in1918, SARS in 2002 and Ebola outbreak in 2014 to ongoing viral diseases such as HIV/AIDS, MERS, and COVID-19 are few examples of the pandemics. In earlier days the interventions have largely focused on developing vaccinations. Development of a vaccination even during current times may take up to a year. Hence, drug discovery has taken an equal interest during current sudden outbreaks. Historically, drug discovery has been largely dependent on deducing potential molecules that may bind and hinder activity of a protein based on the information on known docking or binging compounds. However, a considerable effort has been made in development of drugs against widespread viral infections. (i) viral hydrolase inhibitors such as (S)-9-(2,3-dihydroxypropyl) adenine (DHPA), (ii) viral replication inhibitors (E)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU) and its derivatives, (iii) viral nucleoside transferase inhibitors 2′,3′-dideoxynucleosides (ddNs), (iv) non-nucleoside transferase inhibitors such as HEPT, TIBO and their derivatives, (v) acyclic nucleoside phosphonates, (vi) bicyclams such as AMD3100, (vii) acyclic nucleoside phosphonates such as (S)-9-(3-hydroxy-2-phosphonylmethoxy-propyl)adenine (HPMPA), etc.

Current Approaches

New drugs have transformed the treatment strategies for viral infections. One of the best examples thus far is the discovery of HIV protease inhibitors in the 1990s. The inhibitor binds to the active site of the protease to inhibit the activity of the enzyme to prevent protein cleavage. As a result, theviral particles produced are immature and are non-infectious. A second drug against HIV, raltegravir acts by inhibits HIV integrase to halt the integration of the viral genome into the host DNA.

During current times the virus particles get isolated from patients and grown in large scale using standard cell culture method. A large component of the experimental work focusses on sequencing the viral genome. However, it is also true that a large number of virus circulating today are either uncharacterized or poorly characterized. The vast diversity of the viruses also arises from their increased mutation rates. Hence, proper characterization can be achieved by using the complete information of the viral genome and the proteome. Secondly, whole genome characterization of novel viruses that lead to epidemic or pandemic situations is beneficial from a drug discovery perspective. Identification of receptors, kinases or polymerases are crucial repurposing drugs that are already being used. Once the proteome of the virus is known, other known viruses can be used to query for shared structures among the receptors or any other important viral enzyme. Simulation software can be used to study docking of available drugs and small molecules for docking to viral proteins. High throughput small molecule screens are often used to finding probable drug targets in parallel to simulations. Small molecule libraries are available through multiple vendors that also include a large fraction of FDA approved drugs. A successful FDA approved drugs will markedly reduce the amount of time for the drug discovery.

Therapeutic strategies in recent outbreak ofnovel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)

Traditional vaccines still remain the preferred and popular choice of therapy. However, during the HINI influenza outbreak in 2009 the vaccine became available after the peak of the infection had passed. There are three strategies that are being suggested. (i) Use of broad-spectrum antivirals such as Interferons, ribavirin, and cyclophilin inhibitors. (ii) Use of the information on the viral genome to design a novel drug that is specific to SARS-CoV-2. Although this may be the ideal strategy, the time that would take to develop a new drug, validation through clinical trial and bring it up to a large-scale production may take a extended time. (iii) Following sequencing of the viral genome, several proteins have become candidates for the potential to be druggable. Current high throughput screening methods are very efficient and reliable and can be performed at a high reproducibility rate. One such candidate peptide is the sequence KRSFIEDLLFNKV of the glycoprotein proteolytic cleavage site on one of the spike proteins of the virus. Therefore, a potential drug candidate is emodin that binds the angiotensin converting enzyme type 2 (ACE2) receptor to inhibit the entry of SARS virus into the host cell. The proteolytic cleavage to activate the spike glycoprotein is predicted to occur thorough Type II transmembrane serine protease (TMPRSS2) where the later ins known to have several variants. In addition, carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1), and various dipeptidyl peptidases have also been proposed as potential candidates.

Various research groups have been successful in preliminary work with some of the potential compounds. Remdesivir, a nucleotide analogue prodrug that inhibits viral RNA polymerases, has shown in vitro activity against SARS-CoV-2.Ivermectin, an FDA-approved antiparasitic drug with broad-range anti-viral activity is shown to inhibit replication of SARS-CoV-2 in-vitro. This drug has shown activity against RNA viruses such as DENV 1-4, West Nile virus and influenza. Patients suffering from advanced pneumonia may be benefited by the regiments used during the SARS outbreak such as protease inhibitors Lopinavir and ritonavir together with the nucleoside analog ribavirin. Results with Favipiravir, also known as T-705 or Avigan, a pyrazine derivative that inhibits viral RNA-dependent RNA polymerase, but is yet to be verified in SARS-CoV-2.

Given this rapid pace in data generation and discovery scientists together with clinicians will use the current evidence to determine the ideal therapeutic interventions to combat SARS-CoV-2

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Virtual Screening for Drug Discovery; Hurdles to Overcome for Better Drug Prediction

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The cost to develop a new drug that would enter the market is found to be $2.6 billion, and only a percentage of less than 12% of new drug candidates that would enter clinical trials would obtain FDA approval as a prescriptible medication.[1] The rational molecular design could potentially change this drastically and save a lot of money and time by eliminating candidates that fail in the process of only selecting candidates with a chance of being ultimately successful. Virtual screening is one such method where computational chemistry simulations are used to screen molecules instead of using conventional biochemical assays. Antiviral drug prediction has recently become a hot topic in science due to the COVID-19 outbreak, where scientists in the whole world are challenged to develop a cure within the shortest period of time in history. In such an endeavor, computational prediction, if correctly executed, could become an ultimate deal-breaker.

However, the question remains as to how far computer-aided drug design (CADD) can bring us in terms of drug discovery. A biological system, in my opinion, is the most complex entity a computational chemist/biologist will ever try to simulate. Any computational model in its core is a type of mathematical expression or correlation to a physical system or phenomena in the real world. A biological system per se would ideally comprise of a countless number of independent/interdependent variables. It is highly unlikely that scientists would be able to address all of them in the near future, even with state-of-the-art computational resources. However, possibly the dawn of commercial level quantum computing would be the next most significant step in technological evolution where pure analytical solutions as such would be a reality. Therefore, computational efforts in this regard are often simplified to overcome the difficulty with the cost of computation. Thus, in order to understand these issues, we should dig into some basics of computational simulations.

Among the many different methods used in Virtual screening, one of the most popular methods is molecular docking. Molecular docking of small molecules to protein binding sites was initiated in the early 1980s, yet continues to be a highly active area of research.[2,3] When

merely the structure of a target protein/enzyme and its active or binding site is located, docking is primarily used as a hit- identification tool. I presume that it is quite reasonable to start addressing certain limitations of the field using this familiar example among many chemists and biologists in Sri Lanka. Even though there may be plenty of other issues pertaining to the field, I would narrow it down to the most compelling three limitations, in my opinion, that one should be aware of when making use of this great toolset. Nevertheless, these can technically be applied to most of the other techniques used in virtual screening in general. The first and foremost is the intrinsic restrictions that are not yet resolved in theoretical chemistry. Second would be the level of accuracy and applicability of the method to the question of interest and its ability to capture the expected experimental outcome. Finally, the limited search space confined to the molecule libraries used, which is more specific to the screening of larger data sets. Thus, in order to understand these issues, we should dig into some basics of computational simulations.

Computational simulations of chemical systems have made its way of becoming its own subdiscipline in chemistry while having a substantial effect on other subdisciplines in chemistry within the past few decades.[4] These simulations have made possible the prior prediction and rational explanation of chemical and physical properties in simple di-atomic systems to extremely complicated biological systems. A chemical system can be modeled using multiple approaches, such as with an analytical method or with a machine-learning method based on empirical data. An analytical approach can either be constructed on simple Newtonian mechanics or rigorous quantum mechanics. Quantum mechanical calculations are proceeded either from an *ab-initio* method or using the electron density as a function of chemical structure with a DFT (Density functional theory) calculation.

Nevertheless, quantum mechanics (QM)-based methods are built on solving Schrödinger's equation while making assumptions. The electronic level nature of QM methods makes them superior in accuracy, thus, they closely resemble the experimental observation. However, solving Schrödinger's equation is quite computationally expensive even for systems with only a limited number of atoms, thus making it unrealistic to be performed on large systems.

In the light of the challenge with the high computational demand, the best remedy is to use classical mechanics to simulate these larger systems, given that the electronic structure properties are sufficiently captured through a forcefield. Even though they are computationally less expensive, the accuracy suffers from not being able to access the systems at the electronic level, unlike QM. This hurdle in using molecular mechanics (MM) is overcome by using a molecular forcefield that helps this simulation to mimic the electronic level properties.[5] Therefore, the accuracy of properties extracted through an MM based simulation vastly relies on the forcefield used. A forcefield cannot be universally applied to any system with the expectation of benchmark performance. This is due to the fact that the forcefield parameters are built upon calculations and experiments performed on dissimilar systems with specific intentions. Simply the forcefield parameters for atoms C, N, and O are not the same when comparing a forcefield made to simulate biological systems and another forcefield made for inorganic polymers. The credibility of such simulations heavily depends on the forcefield parameters used to represent the electronic structure governing effects since MD simulations are not based on quantum mechanics. One such issue that profoundly affects the accuracy is the use of non-polarizable forcefields, which is a common choice in performing MM based simulations due to their low computational cost.

Many virtual screening methods including docking, mostly if not entirely depend on MM that suffer from all aforementioned limitations. In many efforts that use docking for virtual screening, do not involve validating and optimizing forcefield parameters to meet the need of the specific system (enzyme/protein and ligands) we are interested in. Different docking algorithms make use of different scoring functions to grade the small molecules in terms of potency to interact with a defined site on the enzyme. Therefore, the results produced are often subjected to the scoring function that is used, and the order of potency could substantially change between scoring functions. One way to overcome this is again to validate the scoring function with experimental results of similar systems. However, it is well comprehended that validation and finetuning of forcefields and scoring functions are not always feasible or realistic when dealing

with extensive libraries of compounds or experimental access is limited. Thus, one should always be cautious about making statements based on any *in-silico* method used in screening.

Moreover, many virtual screening methods including docking does not take into account the flexibility of the macromolecule in full. However, it should be noted that addressing the side-chain flexibility at least on an explicitly specified binding site is of utmost importance. Similarly, docking is mostly static, in its nature where stability and possible alternative configurations in a time-dependent manner are often neglected. The common practice of overcoming this is accomplished through coupling docking with molecular dynamics simulations. In the account of the first two limitations as mentioned at the beginning, another important fact when using virtual screening is that not every system can be modeled using a method such as docking, especially where molecular interactions alone cannot capture conformational changes that take place after binding.

The most significant advantage of virtual screening is that it allows researchers to screen a massive number of compounds by utilizing molecular libraries, which otherwise would be extremely costly, impractical, or even be rather impossible to be done using *in-vitro* or *in-vivo* methods even with access to sophisticated high throughput automated screening facilities. Nevertheless, it is not rational to assume that the entire chemical search space is screened, although the virtual screening was done with all available commercial and non-commercial databases. It is always essential to be aware of the fact that the search space will always be limited unless a machine learning approach is used.

All these bring us to the ultimate question of "Can virtual be really useful in drug discovery, and are there any other methods without these limitations?" The simple answer in my perspective for the first part is "Yes". Virtual screening is extremely useful in this hunt for new drugs. Molecular docking itself has been able to discover new drugs, predict binding modes, understand binding mechanisms and study the effect of mutations in diseases such as cancer, influenza, Zika, Malaria, and HIV etc.[6] However like any other tool used in science it has to be utilized with intuition and understanding of its limitations to yield meaningful results. The answer to the second question is, "Yes, but not entirely". In terms of accuracy, *ab-inito* molecular dynamics simulations (AIMD), does provide a better solution. Specifically, the capability to mimic intermolecular interactions such as π-π stacking and π-cation interactions presents with a high level of confidence, require AIMD or use of polarizable forcefields that are computationally expensive. Many academic and research institutions worldwide, however, have limited computational resources. Thus, a method such as AIMD is not computationally affordable in general. Being able to perform a simulation of the same caliber with less computationally expensive methods such as charge renormalization provides a great opportunity to many researchers.[5] The next solution would be through machine learning (ML). This is nothing new to the field; the theoretical basis of ML is the same as quantitative structure-activity relationships (QSAR) that have been used for virtual screening well before docking has been introduced. The impact of "Big Data" analysis in the modern world, almost in every aspect of human life has become substantial. Global enterprises such as healthcare, education, marketing, business, finance, and economics are heavily dependent on the insights they obtain through ML with big data.[7] Lately, many virtual screening efforts are being directed through ML. Yet again, ML methods are not perfect, but that would be a topic for a separate article. However, in general, the way to go would be through experimental validation of any virtual screening method of interest before implementing a specific project.

In conclusion, virtual screening has served the field of drug discovery for decades and will continue to do so in the future, although methods of virtual screening will change and evolve over time. It is highly doubtful that all these limitations would cease to exist in the near future. There will always be new hurdles to overcome, but researchers will keep on overcoming them as science progresses. After all, is not overcoming hurdles is what science is all about?

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Body and Surface Sanitization | Do's and Don'ts

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Suddenly the world surrendered to an unexpected creature unknown to its overly anticipated future plans. Predictions, anticipations, models, and discussions on the possible nuclear war, environmental disasters, ocean pollution, industry 4.0, or the digitalized world were kept on hold, and everyone was concerned about one thing: Surviving Corona. SARS- CoV-2.

Originated in Wuhan, China, it quickly spread across Asia, Europe, and America creating mayhem in every country. As this article is being written, over three million known infections and 260,000 plus deaths have been reported worldwide.

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-Cov-2) is identified as the virus responsible for the debacle occurring all around in different scales. This is also called as COVID-19 in public health communications. Some countries handle it carefully, and some have become a total disaster zone during its peak depending on the efficiency of the operations management. While all the scientists are working around the clock to beat the virus and to find a cure for COVID-19, still the solution is beyond the horizon with a lot of unanswered questions on the table. Social distancing seems to be the most effective way to mitigate the spreading of the virus, while individual hygienic behavior contributes immensely to hold the virus entering the body.

Generally, transmission of a virus similar to SARS-CoV-2 occurs via respiratory droplets released by coughs and sneezes within a range of six feet, which comes in contact with another person's mouth or nose, seldom through eyes. It mainly enters human cells by binding to the receptor angiotensin-converting enzyme 2 (ACE2). Generally, a person touches his/her face over 12 times an hour unintentionally. The SARS-CoV-2 virus is known to survive on different surfaces with a life span ranging from hours to days. Preliminary research indicates that the virus may remain viable on plastic and steel for up to three days, but does not survive on cardboard for more than one day. People touch these surfaces multiple times during their daily activities. An infected person can easily leave his/her footprint on the surfaces and the rest of the work is in the hands of others in the area. People are never isolated in their daily life, and a mix of healthy, sick and silent carriers roam around in the society undetected. Therefore, the threat of contracting the virus at any given point is extremely high, and owing to the uncertain pattern of deaths and the cure still being unknown, prevention remains to be the best option at this time.

Prevention can be done by isolation, quarantine, or lock-down of suspected spreading areas. All these methods also affect other areas, especially the Economy. In order to maintain daily life and to run daily activities, even at a very minimal level, people's movement and

involvement is a must. Essential services, as well as relief works, need to be continued to run the system at a bare level of normalcy. Although work-from-home is operating in many service-oriented sectors, the necessity of in-person activities is also important to operate the functions smoothly.

Many organizations have taken measures to operate at a minimal level with a high degree of sanitization of the premises and the people involved in the operation. Unfortunately, the people involved may not be tracked well, or they may not reveal the truth of their movements due to fear of being quarantined, shame to be isolated, or other social factors: Sad situation, but this is the reality. Therefore, all the persons involved in such operations and the premises and the equipment need to be sanitized properly and effectively. However, society has a wide spectrum of views and opinions on sanitization.

SARS-CoV-2 is a virus belonging to the ***Coronoviridae*** family which takes a spherical shape, with an image reminiscent of a Solar Corona due to decorations with about 20 nm long petal-shaped surface projections called "peplomers" or "spikes". The virus has a single-strand RNA with a viral genome of 26-32 kilobases in length. The cell wall of the virus is a lipid bilayer and the spikes come out of the bilayer to form the corona structure.

Best is Soap and Water

The best sanitization method recommended by experts and the WHO is using soap and running water. Soap is a bipolar molecule that consists of a polar end of a nonpolar long-chain hydrocarbon tail and a head. The hydrophobic non-polar head and long-chain binds to the organic parts of dirt, oil, lipid, fat, or protein, whereas the hydrophilic polar end which usually consists of a carboxylic or sulphonic acid end with Na+ or K+ ions, dissolves in water and sometimes makes micelles to take dirt/oil out.

The non-Polar head and tail of the soap molecules bind to the lipid bilayer, break it apart, and dissolve in water. This opens up the virus and exposes the genetic material inside, thus destroying the virus. This mechanism generally takes place in a time span of 20 s. Therefore, an effective hand wash needs to be used with a thorough rubbing and cleaning over 20 seconds.

Alcohol? With or Without Soda?

Apart from soap, a quick and instant method for hand sanitization is widely known as a 70% alcohol solution. Generally, it's a 70% Isopropyl/ethanol mixture solution in water with additives such as hydrogen peroxide, glycerin, and other skin-smoothing agents. Moreover, it can be made to a gel form with Carbopol. Whichever the method, it needs over 70% alcohol strength for effective antimicrobial action.

The mechanism of alcohol on the virus is more or less the same as soap. Alcohol is effective in destroying the viruses having both protein and lipid layers. Proteins are denatured by ethanol and lipid layers also get dissolved in the alcohol, disrupting the membrane exposing the genetic material. To have an effective sanitization, a minimum of 65% alcohol strength is recommended. However, most of the alcohol-based sanitizers in the current market do not fulfill the minimum standards, and it's a big question awaiting regulation.

Although hands can be sanitized by either of the above methods, other surfaces are prone to be contaminated by saliva and mucous droplets from uncovered sneezing by patients or carriers. Generally, a sneeze can carry the droplets over 6 feet and a normal saliva droplet can travel about 3 feet while a person is just talking to another. These droplets deposit on the nearest surface, and they can live between 3 hours to many days depending on the surface. These surfaces also need to be cleaned, but with a suitable sanitizing agent that would be effective and safe on that surface as well. The surfaces are not just flat surfaces at the micro-level. They have microscopic grooves, pits, and cavities where Viruses can live for days. If not properly sanitized with an effective sanitizer that would kill the virus, there is a probability of the virus spreading through the surface.

Bleach to Wash out Everything

There are several surface sanitizers in the market with different properties and effectiveness. Also, it depends upon the place and purpose. The most common disinfectant, Bleach, is NaOCl (Liquid Bleach) or $Ca(OCl)_2$ (Bleaching Powder). Depending on the requirement, the solution can be diluted and applied. However, the Bleach solution has a relatively short lifespan and it needs to be prepared frequently.

Industrially, Bleach is prepared by the absorption of gaseous chlorine into NaOH.

Chlorine-based compounds are effective against a wide variety of microorganisms including bacterial spores. They are listed by the World Health Organization as essential medicines in any health system. They are easy to use, widely available, and very cheap. The principal ingredients of a concentrated sodium hypochlorite solution are hypochlorite and sodium hydroxide. Normally sodium hypochlorite is a strong basic solution (pH: 12.5 to 13.5) containing 5 to 12% of available chlorine (AC), but now the pH is adjusted to make it neutral. NaCl formed in eq. 1 is eliminated adequately from the NaOCl product.

This makes metal and other surfaces to be damaged in prolonged use inside buildings and factories. Although the concentration of bleach is less than 1%, the long-term effect is far more damaging.

The Ozone Hole in the Arctic is Closed, But….

Ozone, although it doesn't form naturally, it can be generated mechanically for sanitization purposes. When reacted with water, it forms hydrogen peroxy (HO_2) and hydroxyl (OH) free radicals which have greater oxidizing capacity than Ozone itself and plays an active role in the disinfection process. Ozone is a short-lived unstable molecule, and it needs to be generated on-site.

It is generally believed that the bacteria are destroyed because of protoplasmic oxidation resulting in cell wall disintegration (cell lysis). The effectiveness of disinfection depends on the susceptibility of the target organisms, the contact time, and the concentration of the ozone. Thus, the effectiveness of Ozone disinfection always depends upon many factors. Furthermore, the effectiveness of the Ozonated water depends upon the pH of the solution. Higher pH facilitates ozone decomposition due to increased hydroxyl radical formation; whereas lower pH (less than 7.0) slows down ozone decomposition resulting in higher concentrations of molecular ozone. The rate of ozone decomposition increases significantly (due to •OH formation) when the pH is greater than 8.0. Ozone residuals are difficult to maintain at pH levels greater than 9.0. More basic solutions that have a higher concentration of hydroxyl radicals possess a greater potential of disinfection.

Ozone is a very strong oxidant and virucide. The mechanisms of disinfection using ozone include:

- Direct oxidation/destruction of the cell wall with leakage of cellular constituents outside of the cell.
- Reactions with radical by-products of ozone decomposition.
- Damage to the constituents of the nucleic acids (purines and pyrimidines).
- Breakage of carbon-nitrogen bonds leading to depolymerization.

The generation of Ozone is a very high energy-intensive process. Generally, it's carried out using ambient air with 21% Oxygen or Pure Oxygen of 95% purity. The two main principles of Ozone generation are Corona-Discharge and UV-light. Corona-Discharge, which has no connection with Covid-19, is the primary method with greater sustainability of the unit and higher Ozone production at a low price.

However, in current Ozone Chambers, ambient air is used where purity and the yield of Ozone generated can be lower.

Quaternary Ammonium Compounds (QACs)

Quaternary ammonium compounds are a family of low-level disinfectants primarily derived from benzalkonium. QACs are reacted to provide a variety of chain lengths and molecular structures so that the mix of QACs used in the disinfectant provides a wider range of efficacy than a single chain. QACs are generally used to disinfect countertops, toilets, and other high touch environmental surfaces and floors. They are of low cost and used in many applications. QACs are cationic detergents (surfactants or surface-active agents). They reduce surface tension and form micelles, allowing dispersion in a liquid. The negatively charged anion portion is usually chlorine or bromine and is linked to the nitrogen to form the QAC salt. QACs are further classified based on the nature of the R groups, which can include the number of nitrogen atoms, branching of the carbon chain, and the presence of aromatic groups. These variations can affect the antimicrobial activity of the QAC in terms of dose and action against different groups of microorganisms.

QACs are membrane-active agents that interact with the cytoplasmic membrane of bacteria and the plasma membrane of yeast. Their hydrophobic activity also makes them effective against lipid-containing viruses. QACs also interact with intracellular targets and bind to DNA. They are also effective against non-lipid-containing viruses and spores, depending on the product formulation. At low concentrations (0.5 to 5 mg/liter), they are algistatic, bacteriostatic, tuberculostatic, sporostatic, and fungistatic. At concentrations of 10 to 50 mg/liter, they are microbicidal for these same groups, depending upon the specific organism and formulation. Thus, QACs can be modulated to be more effective against specific targets and safer to humans.

In conclusion, it's extremely vital to recognize the role of a particular disinfectant with the occasion. Considering the chemical and microbiological effects of the disinfectant alone, will not yield expected results as many other factors govern the antimicrobial functions. Every disinfectant has its advantages and disadvantages for a particular situation. Selecting a suitable disinfectant for the application is crucial. Effects of disinfectants on the skin upon prolonged usage need to be carefully analyzed. Applying Chemical Knowledge along with some dosage of common sense is advisable at this point where different opinions galore.

At the end of the day, it's not who's right, but what's best for the society. Once the crisis is over, we need to stand up as one human race who successfully survived a global terror. It doesn't count who contributed more, or less, it ultimately boils down to who survived or not.

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Themed Collection

Fate and Transport of Viruses in Groundwater Environments

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Groundwater contamination

Groundwater is widely used as drinking water supplies around the world, specifically in the developing economies. About 96 percent of all usable freshwater is found as groundwater, which globally provides 25 to 40 percent of the world's drinking water. Aquifers are the source of groundwater that is located subsurfaceis often connected with surface water systems and mostly recharge through rainwater infiltration and percolation and may discharge to surface water sources such as streams and lakes. Contamination of groundwater depends on the risk factors: 1) sensitive aquifers; aquifers in which viruses may travel faster and further than bacteria (e.g. limestone, lateritic or coastal plain sand aquifers, which are high in permeability; 2) shallow unconfined aquifers; 3) aquifers with thin or absent soil cover;4) close to surface water bodies; and 5) high population density areas.

Contamination of groundwater via chemical and pathogenic contaminants is a severe environmental problem that poses a significant threat to human health. Among the pathogenic contaminants such as viruses, bacteria, and protozoa, viruses are readily transported through soils, due to their smaller size compared to bacteria and protozoa. Studies have reported on the fate and transport of viruses in soils and aquifers are necessary to determine the vulnerability of groundwater to pathogenic contamination and to secure safe drinking water sources. However, only a handful of literature reports on the capacity of transport of viruses into groundwater.[1,2] Major processes that govern the subsurface transport of viruses are their rate of

inactivation and their sorption into sediment particles. Inactivation of viruses as well as sorption to soil particles is controlled by the degradation of the viral capsid and by subsurface temperature. Included among the essential hydrogeological factors that can be used to evaluate viral transport are the flux of moisture in the unsaturated zone, the media through which the particles travel, porosity, the length of the flow path, organic matter, dissolved oxygen, presence of other microbes, groundwater chemistry and the time of travel.[2-4]

Sources of viruses in groundwater

It has been a well-known fact that the sewerage and cemeteries are among the chief anthropogenic sources of pollution and contamination of groundwater in urban areas and beyond, in the area of hydrogeology (Figure 1). In the case of cemeteries, 0.4–0.6 liters of leachate is produced per 1 kg of body weight, during the decomposition of a human corpse, which may contain pathogenic bacteria and viruses that may contaminate the groundwater.[5-7] Further, sewerage from hospitals or households or quarantine centers may discharge sewerage and wastewater with viruses (Figure 1). Burial in any means causes soil contamination and then leads to groundwater pollution via the discharge of inorganic nutrients, nitrate, phosphate, ammonia, chlorides etc. and various microorganisms. High biochemical and chemical oxygen demands, ammonia, and organic carbon have been reported as high as several hundreds of mg in L from cemeteries and mass burial sites. In the case of viruses, recent studies indicate that viral may transport in soil with rainfall infiltration and extends specifically to drinking water from an untreated groundwater source.[8] Several scientific publications report virus occurrence rates of about 30 percent of groundwater.[6,7]

In most cases, it is the general thinking that only the enteric viruses are found in groundwater; however, other types of pathogenic viruses have also been reported (Table 1). Severalstudies suggest that certain enveloped virusessuch as SARS, MERS, COVID-19, and avian influenza are capable of retaining infectivity fordays to months in aqueous environments, which implies the danger of untreated wastewater andgroundwater contamination.[9] Given the vulnerability of our groundwater aquifers, and lack of understanding about the behavior of COVID-19 virus, there can be a risk from corpses, septic waste or sanitary waste are having any contact with water sources. Hence, it is advisable to have careful measures in destroying the infected dead bodies, septic, and sanitary waste in proper conditions without provisioning chances in groundwater contamination for any future disease outbreak in any case of viral pandemicity.

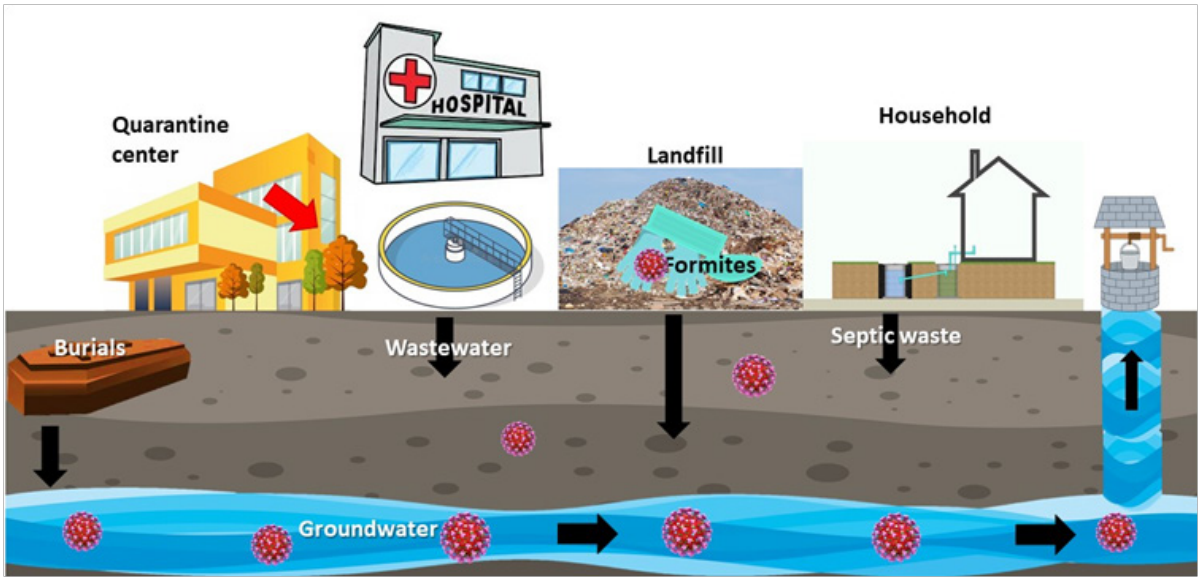

Figure 1: Possible sources of viruses in groundwater

Table 1: Detection of various viruses other than enteric in soil and groundwater environments

Virus common name	*Virus type*	*Associated illness*	*Country*	*Environmental condition*	*Reference*
Lassa virus	H40/1	Acute viral hemorrhagic illness	Germany	Gravel aquifer	[10]
Adenovirus	PRD1	Respiratory disease, pneumonia,gastroenteritis, keratoconjunctivitis	USA	Unconfined aquifer	[11]
	HAdV2		France	Unconfined and confined aquifer	[12]
Enterovirus	Poliovirus	Polio	USA	Unconfined aquifer	
Avian influenza virus	HPAI	Avian influenza	USA	Mississippian limestone	[13]
Hepatitis	HAV	Hepatitis	Korea	Unconfined aquifer	[14]

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Role of Biochemistry in Health Care; Progression from past to present

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'**Biochemistry**' is a relatively young scientific discipline among most of the subject disciplineswe are dealing with today, evidenced by its day by day expansion through new scientific discoveries. Biochemistry can be delineated as a holistic study of life from a chemical perspective and how living organisms arose from lifeless matter, known as biomolecules. In principle, the properties that living beings have, that distinguish them from non-living matter are concerned in Biochemistry. From that preliminary objective, discipline of Biochemistry has branched out to a vast scale, providing many subdivisions, including Animal Biochemistry, Plant Biochemistry and Clinical Biochemistry, hand in hand with other disciplines such as Microbiology, Physiology and Molecular Biology.

Biochemistry related to human health and diseases is known as Clinical Biochemistry. Its practical arm is called clinical chemistry, which mainly deals with the methodology andinterpretation of chemical investigationson body fluids and tissues, leading to assist disease diagnosis and treatments.In early ages of Clinical Biochemistry, main concern was the methodology part rather than interpretation of the test outcomes. However, current trends focus on interpretative aspects and clinical co-relations, emphasizing the professional relationship between clinical chemists who basically performs the tests and practicing clinicians who directly involve in health care management.

Under the era of development of methodologies in clinical Biochemistry, more effort was committed to develop different analytical techniques to measurevarious analytes in alarge number of patient samples, to discover the plausible ways of obtaining biological material and to establish normal ranges (reference values) for each test. More importantly scientists have paid careful attention in quality control of the developed tests. Later on the tests were performed using automated equipment, reducing their laborious nature. As a result of this whole endeavor, tests were developed to measure glucose in blood and urine, non-proteinnitrogen to assess the renal function, amino-acid nitrogen to estimate the nutritional status,plasma and urinary proteins, lipids, enzymes, electrolytes (including calcium,magnesium and phosphorus), and parameters of acid base balance. Moreover, assessinghemoglobin and iron in diagnosis of hematological (blood related) disorders as well as function of the drugs and poisons in the body were actively beingdeveloped.

With thesuccessful progression of the field of clinical chemistry, group of developed test were collectively assigned into '**test profile**'; which represent the function of a specific organ or tissue in the body. Organ and tissue profiles were established mainly for liver, pancreas, bone, muscle,heart and kidney. Most of these profiles are based on the organ specific activity of enzymes. In addition to blood and urine, other body fluids such as cerebrospinal fluid and stools are also used as specimen samples in Clinical Biochemistry. Besides, endocrine (hormonal) function is measured using respective hormones which encompasses assessment of the gonadal function, feto-placentalfunction (during pregnancy), and pregnancy.

Further development of the field was catalyzed through introduction of 'dynamic' functional tests, in which substance such as glucose is first administered to the body and then, its response is monitored in body fluids like blood plasmafor a period of time. To date, with the advancement of technology, such as, radio-immuno assays, florescence based immune assays and enzyme linked immune assays, biochemical investigations related to endocrinologyhas drastically evolved. With the discovery of different bio-markers for prognosis (forecasting on susceptibility) and diagnosis of cancers along with therapeuticdrug monitoring has changed paradigm of the field. However, the measurement of an increasing number of plasma proteins remains within the core of clinical chemistry.

From early ages to-date, gathered and updated knowledge on clinical biochemistry is currently applied inmost of the medical and surgical interventions. The focus was mainly on assessment of water and electrolyte metabolism and hydrogen atom homeostasis (hydrogen balance), leading to diagnosis and treatment of 'novel'

clinical disorders. Diagnosis and monitoring was revolutionized by introduction of glycated hemoglobin (hemoglobin bound with sugar)as a measure of time-causeglycemic (blood glucose level) control and treatment of diabetic coma. One of the critical methodological development in clinical chemistry is 'point of care testing'; in which introduction of a range of portable or small desktop analyzers and dry-reagenttest strips has shown immense contribution on low-volume emergency testing in hospital wards as well as in self-testing by patients.

With the increasing number of patients, almost all the clinical chemistry investigations are now being automated for high volume testing. Therefore issues regarding workflow management and computer system management should be properly maintained with substantial technological support. Under the platform of evidence based practice (clinical practice that relies on scientific evidence for guidance and decision-making) the field of clinical biochemistry represents a predominant position in health care sector, supporting the precisedecision making with respect to the health and diseases, via accurate disease diagnosis, and prognosis, along with monitoring of health status of an individual for a given period of time. In this regard, field of molecular diagnostics (detection and measurement of specific markers of DNA, RNA or proteins, in humans or in the case of infections in microbes) and genetic screening (investigations for systematic early detection or exclusion of a hereditary disease) play a prominent role.

Pediatric clinical biochemistry is one of the latestbranches in clinical biochemistry, which recommendsdifferent reference values from those of adults, with respect to diagnostic/prognostic tests performed for infants and children. It also deals with diagnosis of inbornerrors of metabolism.

In order to provide optimal health care for clients, accurate diagnosis and prognosis of a disease or monitoring of health condition is an essential factor. With respect to Clinical Biochemistry, there is a major role of clinicians to achieve this goal. In one hand, the management of the processof sample analysis, assurance of quality of the process and provision of guidance on the selection of tests and assessment of the significance of the results (particularly with some of the less generally familiar tests) are critical province of a clinician. On the other hand, he or she needs to involve in management of patients according to the decisions made based on the test results.

The field of Biochemistry is an ever evolving field related to patient care, which shed green light on development of health care management. The overview of the chronicle of Clinical Biochemistry with its present picture credibly evidences the brighter future of 'evidence based health care practices' which enables the prevention or early detectionand cure of most of the life threatening diseases.

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Guest Articles

Drug Discovery *via* Synthetic Biology Approach

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Higher percentages of human medicines such as anticancer, anti-infective, and cholesterol lowering drugs using in the pharmaceutical market during last three decades were origins of fungal natural products or its derivatives. Even though Food and Drug Administration (FDA) approves natural products originated compounds as human drugs, the major drawbacks on them are producing lower amounts per extracting batch and difficulties in synthesizing which eventually lead rich natural sources away from the pharmaceutical industry. Recently, emerging scientific platform, synthetic biology, which is redirecting the field of drug discovery and opening up huge chemical space of natural product derivatives *via* genetic manipulation, engineering the related biosynthetic gene clusters, and transformation to highly replicating microorganisms. Two Nobel prizes were awarded for this field, first one in 2015 for physiology or medicine, co-recipient was Chinese Professor Tu Youyou for discovery and heterologous bio production of antimalarial drug artemisinin, second in 2018 for chemistry, co-recipient was American Professor Frances H. Arnold, for directed evolution of enzymes, in other terms the biocatalysts, which results can be focused environmental friendly manufacturing of chemical substances. Once Professor Arnold stated that "*microbes are brilliant chemists. If we could have reprogrammed these microbes to make new chemicals, they can serve as chemical factories of the future*". Therefore, the genome mining by bioinformatics analysis, heterologous expression of biosynthetic gene clusters (BGC) and gene deletion, pathway design, and product detection have been intensively studied.

Genome mining is the key tool to discover fungal natural product diversity. Genes responsible for the natural product biosynthetic pathways are usually clustered together on the chromosomes, consequently, they can be rapidly identified using genome sequence of a single microbial strain using in silico methods such as Softberry and Geneious. Furthermore, comparative analysis with known BGCs and prediction of the chemical components producing by relevant BGCs can be done by antiSMASH which strengthens and validates the initial predictions. Subsequently, identification and characterization of biosynthetic gene cluster, and then pathway reconstruct using different techniques such as heterologous reconstitution and target gene inactivation.

Synthetic biology tool which is based on heterologous expression of biosynthetic gene clusters, is promising, fast, and relatively inexpensive strategy for natural product discovery. Heterologous expression (HEx) is a prompt platform which is important to overcome uncultivable conditions and activate transcriptionally silent genes in fungal strains. Biosynthetic pathway of norsequiterpenes (C14), aculenes, were heterologous reconstituted in both *Saccharomyces cerevisiae* and *Aspergillus oryzae*. Biosynthetic pathway of complex fungal (in both *Penicillium sp.* and *Aspergillus sp*.) indole alkaloids, okaramines, were reconstituted in *Saccharomyces cerevisiae*. Highly active promoter (ex: *AmyB*, *gpdA*) and suitable vector selection (ex: pPTRI, pTAex3), cloning of relevant genes to vectors, transformation *via* high quality protoplasts, and using appropriate selection markers are the key steps to success the heterologous expression. Gene deletion can be used to find genes occupy in the biosynthetic pathway, which can be executed by two methods such that double-crossover recombination with selection marker resistant gene and clustered regularly interspaced short palindromic repeats- associated RNA guided DNA endonuclease (CRISPR-Cas9) where ribonucleoprotein (RNP) together with single guided RNA (sgRNA) introduce to fungal protoplasts during transformation, where first method indicated higher random integration, thus second was more successful in homologous recombination. Additionally, silent genes can be activated by using over expression of transcriptional factor and promoter. Consequently, we

can map the biosynthetic pathway using heterologous expression and gene deletion results.

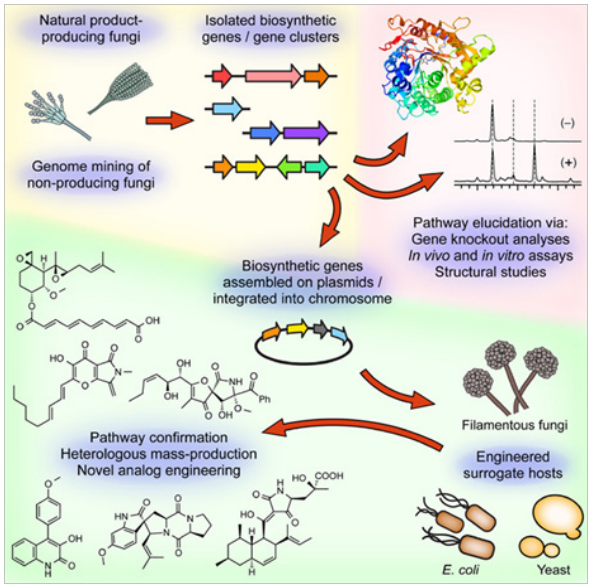

Figure1: Workflow for heterologous expression, Kishimoto, S., Tsunematsu, Y., Sato, M. and Watanabe, K., Elucidation of biosynthetic pathways of natural products. *The Chemical Record*, **2017**, *17*(11)

In addition, *in vitro* experiments are carried out for confirm the relevant enzyme activity. By using computational tools, first predict the enzymes are plasma soluble or membrane bound, furthermore, genes express in *E. coli* or *Saccharomyces cerevisiae* hosts and then, purify soluble proteins using different tags (ex: *His, GST, strep*) and microsomes extract for membrane bound proteins.

With new synthetic biology tools, microbes can now potentially be designed or redesigned rapidly for engineered biosynthesis of natural products to reasonably access the natural products with novel structures which can be potential drugs in the future. To identify the target natural products, the resulting metabolite profiles are evaluated and characterized by advanced powerful metabolomics and detection techniques such as LC-MS, GC-MS, Tandem MS, and NMR. Building upon the knowledge of enzymes and their catalytic power will nurture new biological methods to manipulate nature's chemical tools. In addition, future applications to develop enzyme catalysts will promote green chemistry. Moreover, if some enzyme shows novel activity other than the predicted, then essential to perform protein structure elucidations by traditional x-ray crystallography or by using new technique, cryo-electron microscopy (cryo EM) which can be determined high resolution (less than 2.0°A) macro molecule structures in solution form.

"*Nature has explored only a tiny fraction of the life and life's molecules that are possible. With evolution in our hands, with the ability to set genetic diversity and to tailor the forces of selection, we can now explore paths that Nature has left unexplored* – Frances H. Arnold"

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Electrohydrodynamics in Fabricating Drug Delivery Systems

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Background

Modern therapies clearly demonstrate the need for pharmacokinetic and pharmacodynamic principle-driven administration of drugs. The term 'drug' has been broadened over the years to include bioactive proteins, growth factors, and nucleic acids. This evolution of new therapeutic agents demands for development of novel drug delivery systems (DDS) to realize the actual therapeutic potential of these delicate bioactive agents. One exciting development in this area is the application of electrohydrodynamics (EHD) to fabricate drug-loaded nanofibers and nanoparticles. The former is mostly used in creating an optimal microenvironment for regenerative medicine, and the latter being developed to meet the demand of targeted and intracellular delivery of therapeutics. Especially the electrohydrodynamic atomization technique, commonly known as electrospraying, offers several main advantages over the other techniques such as improvement of dissolution rate of poorly water-soluble drugs, batch-scalability, reproducibility, effective encapsulation in a single step microparticle fabrication. In this process drug release characteristics are tuned by using suitable biodegradable polymer carriers, leading to a sustained release of encapsulated drugs. Moreover, the novel concepts like multi-pharmacy or polypharmacy can be made possible by loading different drugs into multi-layered particles using the electrospray technique.

Electrohydrodynamics (EHD)

EHD also known as electro-fluid-dynamics or electrokinetics, is the study of the dynamics of electrically charged fluids. EHD is actually at the interface of electrodynamics and fluid dynamics. In EHD, when a liquid is infused into a nozzle (needle) and a free-spherical-droplet is formed at the tip of the nozzle. However, when a strong electric field is applied, a charge is induced on the surface of the droplet. When the force generated by the electric field is strong enough to overcome the surface tension of the liquid, the droplet deforms into a Taylor cone, and a charged jet of the liquid is propelled to the collector, which is of opposite charge to the droplet or grounded (Figure 1). If a polymer solution is being fed to the nozzle, the solvent evaporates forming solid products at or before the collector. EHD technique has two subcategories: Electrospraying and electrospinning, they differ only in their final products. Both of these processes transform liquid droplets emerging from a nozzle, under a strong applied electric field, into micro and nano products. In electrospraying, typically a low-viscous conductive liquid is dispersed into fine droplets. These charged droplets stay stable till they reach the Rayleigh limit and then undergo coulombic fission resulting in even smaller droplets. Electrospinning is a process in which a high-viscous solution or a melt can be spun into fibers with smaller diameters. The electrospinning process could generate fibers of various diameters and lengths from almost any soluble polymer.

Electrospun fibers

Electrospun fibers have attracted much attention in the field of tissue engineering because of its ease of fabrication and its resemblance to nanotopographical elements in the extracellular matrix of tissues. There is also an increased interest to incorporate drugs into the fibers to get an enhanced functionality of these scaffolding materials. Drugs can easily be embedded in the fiber through dissolution or dispersion in the polymer solutions. Controlled release of the drug integrated into a tissue engineering scaffold offers temporal spatial gradient to mimic the complex tissue microenvironment for tissue development or regeneration. Many biochemical factors needed for tissue development are protein or nucleic acid in nature, and most importantly, they do not dissolve in common organic solvents. In addition, they may lose their bioactivity if dispersed in the polymer solutions. Co-axial electrospinning would be an ideal alternative for this issue, here the drug is dissolved in an aqueous core

solution and the polymer in an organic shell solution.

Electrospray

Electrospraying on the other hand has generated immense interest as a facile single-step method to generate nano/micro particles. Several other techniques have been established to make nano/micro particles encapsulating therapeutic agents such as spray drying, emulsification-evaporation/extraction, salting-out/emulsification, nanoprecipitation, and ionic gelation. Emulsion-based methods are the most extensively studied in terms of DDS fabrication.

It would be highly beneficial to synthesize nano/micro particle based DDS with the following features: 1) elimination of high shearing forces (stirring or sonication); 2) high encapsulation efficiency; 3) high loading level; 4) uniform drug distribution in the matrix; 5) efficient removal of residual surfactant; and 6) convenient and easily scalable. Electrospraying, especially the coaxial variant (Figure 2) has the capability to address these requirements. In general, electrospraying is very similar to electrospinning except the fact that the jet breaks down into droplets. Drying effects along with residual charges on the particles prevent aggregate formation once they land on the target. Spheres with a diameter of < 10 nm can be generated using this electrospray technique compared to mechanical atomizers, which typically produce particles with micron dimensions. Especially, the absence of continuous high-energy shearing force is beneficial in protecting sensitive therapeutics like proteins or drugs.

One of the crucial factors for drug effectiveness is the water solubility ratio, especially for the oral route. The electrospraying process can be used to improve solubility of poorly water-soluble drugs. Moreover, different release profiles for the drug can be obtained by using different ratios of different polymers or polymer composites. Electrospraying has also been used for targeted delivery of therapeutics. Through this method, drug release can be predictable in a sustained manner and more importantly the drug can be released to provide enough and exclusive accumulation of the drug at the specific unhealthy location. Especially, this system can protect the drug from degradation and loss of bioactivity effectively compared to other conventional dosage forms.

EHD method can in principle provide a uniform dispersion of a particular drug within the polymeric matrix with high loading capacity and minimal drug loss. Use of multiple electrospinning/electrospraying spinnerets will make the process high throughput. Ease of operation and cost-effectiveness of this process are two major benefits. The aforementioned characteristics along with the ability to spray/spin virtually any soluble polymer into micro/nanoparticles and fibers has made electrosprayed particles and electrospun fibers attractive drug delivery vehicles.

Fabrication techniques

Electrospinning

Monoaxial (one needle) electrospun fibers have been prepared to incorporate and release proteins, antibiotics and other therapeutics in a sustained manner. However, in this version distribution and release of drugs from the fibers are poorly controlled. Moreover, therapeutics like growth factors and cytokines embedded in polymer matrices could lose their bioactivity. The co-axial electrospun fibers offer better delivery systems with better drug stability, efficient drug encapsulation, and well controlled release kinetics compared to monoaxial fibers. Changes made in the shell and core material properties by varying the molecular weight, polymer type and addition of porogens enable us to fine-tune the release profile.

Drug is usually incorporated in the core of core-shell (Figure 2) electrospun fibers as opposed to the random distribution of the drug throughout the fiber matrix in monoaxial fibers. In core-shell design, a higher controlled release barrier can be achieved by using higher molecular weight and concentration of the polymer. The polymer-drug interaction is another variable that significantly influences the extent of drug release. This interaction is governed by charge density, hydrophilicity, and degradability of a polymer. Additionally, increase in encapsulated drug concentration leads to a higher diffusive driving force for drug release.

Electrospraying

Unlike electrospinning, in electrospraying the jet breaks into droplets. Due to surface tension effect the fragments of the jet consequently acquire a spherical

shape before reaching the grounded substrate. An increase in voltage, conductivity and surface tension of sprayed solution results in decrease in particle diameter. An increase flow rate, density and viscosity of sprayed solution gives larger particles. The following techniques can be used for loading of drug (s) into the sprayed vehicle.

Adsorption— in this method the drug is adsorbed onto the sprayed particle by exposing them to a drug solution. The major disadvantage associated with this method is that most of the drug is often loosely attached to the particle surface and results in a prominent burst release. In addition to that the period of sustained release also tends to be short for such DDS.

Encapsulation—encapsulation can be achieved by using several methods.

1. collision of droplets of opposite charge: Two adjacent capillaries are used in this process. As droplets emerge from two adjacent capillaries, they attract one another due to columbic forces and subsequently fuse to make a one particle. Therefore, this can be used for drug encapsulation if one charged species is polymer and the oppositely charged species is the drug
2. Electrospraying: The drug-polymer solution/ suspension is electrosprayed and the solvent is evaporated as the jet travels towards the collector. Thus, the drug gets encapsulated in the dried polymer spheres.
3. Electrospraying of a drug dissolved/suspended in a polymer followed by solidification by a chemical or ionic cross-linker: The drug-polymer solution/ suspension is sprayed into a collection bath containing a cross-linker. Here the drug becomes entrapped in the polymeric network.
4. Coaxial electrospraying: This method offers immense potential as the core-shell structure will reduce any burst release and may follow near zero-order release kinetics. Additionally, this procedure is quick to fabricate and offers high encapsulation efficiency and loading capacity.

Summary and the outlook

Evidently, micro/nanomaterials have the ability to mimic the size range of biological molecules and entities, hence they have a great potential in the medicinal and pharmaceutical field. Biocompatible polymer-based nano/microparticles can be used as vehicles for the controlled delivery of various therapeutics such as anticancer, antidiabetic, antihypertensive drugs, hormones, immunomodulatory agents, vitamins, nucleic acids, proteins, and antibodies. Polymers such as PLGA, PCL, PLA, are approved to be used for the aforementioned purpose by the Food and Drug Administration (FDA).

The main purpose of controlling the release of a drug is to improve the effectiveness of a particular therapy, preventing both deficient and overdosing intake. Moreover, controlled-delivery systems have the ability to maintain the level of the therapeutic agent within the desired range, decrease the frequency of dosage, ensuring better stability of the incorporated substances against degradation (e.g. enzymatic), reduce toxicity, and increase patient compliance. The biggest challenges in adapting electrosprayed microparticles for drug delivery at a commercial level are mass production and reliability of dosage. This will require modern but simple and economical engineering to control the size distribution of the particles precisely. Overall, electrohydrodynamic routes are actually in competition with emerging new technologies such as gyration and microfluidic methods. Therefore, rapid investment is needed to take this perfectly viable laboratory scale method to an industrial scale operation.

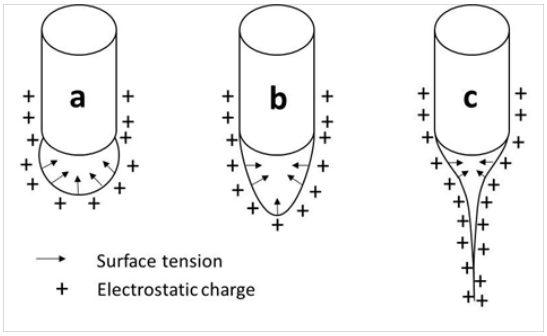

Figure 1: Taylor cone formation at the capillary tip under an applied electric field., (a) primary droplet held by high surface tension, (b) increased electrostatic charge overcomes the surface tension and droplet undergoes deformation (c) at the critical voltage the solution overcomes the surface tension and the electric charge causes the solution to elongate and assume a cone shape, known as Taylor cone.

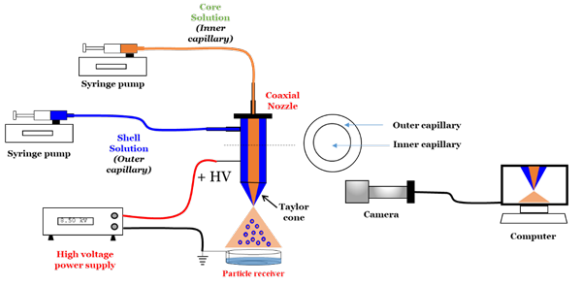

Figure 2: The basic setup for electrospraying consists of several components: syringe pump(s), a metal nozzle connected to a high voltage power source, a grounded substrate as a collector and a monitor

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Guest Articles

Understanding the Relationship Between Protein Structure and Function Using NMR Spectroscopy

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Nuclear magnetic resonance (NMR) spectroscopy uses the magnetic spin properties of certain atomic nuclei within a molecule to enable identification of atoms that are close together in space. NMR is ideal for protein studies, as many structural and motional "spin probes" are uniformly distributed throughout any given protein. These atoms can be nearby either because they are bonded together or because folds of a protein chain bring them together. This information is used to derive a three-dimensional model of a protein in solution phase or a "solution structure". Since protein structure and function are deeply related to one another, several experimental methods have been designed to determine protein structures. An NMR instrument allows the structure of a material to be analyzed by producing atom-atom distances between close atoms of the structure. Important technical improvements of NMR methods for protein structure and dynamic determination have occurred during the past few years.

Many biological processes are in principle driven by protein conformational changes. The polypeptide chain of most proteins fold and fluctuate around an average three-dimensional structure. Understanding the mechanisms of how proteins "morph" their three-dimensional structures into alternative conformations provides a deeper understanding of the protein function. Protein conformational change is a pervasive regulatory mechanism in biology and has recently emerged as a topic of broad appeal to a wide range of biological research areas, including drug design and protein engineering.

The focus of this presentation is understanding how various substrate sequences modulate the affinity and inter-domain dynamics of Pin1, an essential Peptidyl-prolyl isomerase (PPIase). Pin1 is a 163 amino acid polypeptide with an N-terminal binding domain and a C-terminal rotamase (PPIase) domain. A flexible linker of 12 residue connects the two domains. Both domains have binding sites that specifically recognize phospho-Serine/Threonine-Proline motifs. Pin1 catalyzed isomerization supports a major conformational switch between cis-trans isomerization of common pSer/Thr-Pro sequence motif common in cellular response to a variety of signals. However, the binding sites of the two domains are far apart, separated by an inter-domain interface. The diverse number of potential binding sequences for Pin1 made it difficult to interpret its role in pathogenesis in human diseases such as cancer, frontotemporal dementia (FTD) and Alzheimer's disease (AD).

Our research aims to characterize the influence of target peptides on domain interactions and dynamics of full-length Pin1 in solution utilizing high resolution NMR methods. Here, we sought to investigate the molecular mechanism of the Pin1-histone H1 interaction with respect to inter- domain association, binding equilibria, and allosteric activation. NMR titrations were performed for each peptide using both full-length Pin1 as well as the binding domain alone. An analysis of dissociation constants (KD) values reveals complexity in the energetics of interaction between the peptide substrate, the PPIase domain, and the binding domain. 15N relaxation and residual dipolar couplings (RDCs) were used to monitor the degree to which peptide binding induced inter-domain interactions. When combined with molecular simulations, our results suggest a structural basis for how substrate binding can alter the inter-domain dynamics. Finally, we investigated whether our synthetic peptide sequences could alter catalysis (kex) using NMR 1H-1H EXSY (Exchange

Spectroscopy) experiments. Interestingly, no relationship was found between kex and either peptide affinity or inter-domain interaction, suggesting a lack of allosteric control for this series of peptides. Thus, while our results suggest that peptide binding can alter the interaction between the PPIase and binding domains, altering the inter-domain interaction by itself does not appear to modulate catalysis in the PPIase domain.

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Guest Articles

Biophysical Techniques to Probe Nanoparticle-Protein Interactions

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Nanoparticles (NP) have been used in the advancement of medicine and biotechnology, with applications ranging fromclinical diagnosis, drug delivery, tobiosensing. Nanoparticlecomposition canvary significantly from metals to inorganic semiconductors. Inaddition, there are a variety of surface modifications available depending on the desired physiochemical and biological activities. Identifying nanoparticle interfaces that facilitate both specific and non-specific interactions in biological media is a daunting task. Nano-bio assemblies require the use of various analytical approaches to gain fundamental insight on these novel materials. Furthermore, when NPs are introduced into biological media composed of nucleic acids, lipids, and proteins, theywill spontaneouslyinteract forming a biomolecular corona.Due to these phenomena, and given the broad medical applications of NPs, understanding NP-protein interactions isof paramount importance.It ishypothesized that thetranslocation of a protein fromaqueous medium to NP corona can affect the protein's conformation and dynamics, which in turn can interfere with thesurface properties of the NPs. Nanoparticle curvature is prone to induce conformational changesin both the secondary and tertiary structures of many proteins.

Different analytical tools have been employedto characterizebiocoronaformation on NP surfaces. These techniques include surface plasmon resonance (SPR), transmission electron microscopy (TEM),mass spectrometry(MS) based proteomics, chromatography, fluorescence spectroscopy,CDspectroscopy,electrophoresis,dynamic light scattering (DLS), isothermal titration calorimetry (ITC),and nuclear magnetic resonance spectroscopy (NMR). These techniques have been used to identify

the structural and conformational changes of various proteins onto the NP surface.

Under physiological conditions, biological molecules spontaneouslyadsorbonto NP surfaces.This in turn changes the surface properties of the NP by the formation ofa biocorona. Understanding the biophysical characteristics of the NP biocoronacan leadto new applicationsin thebiological and medical fields. The biocorona consists of proteins, carbohydrates, lipids, and nucleic acids that either loosely (soft corona) or tightly (hard corona) associate with the NP surface.Determinationof binding affinities, binding capacities, association and dissociation rates, and stoichiometries of these biomolecules are essentialfor a thoroughunderstanding of the corona's properties. Different analytical techniques have been employed to study NP-protein interactions, specifically UV-Vis, MS, DLS, ITC, and CD. Each techniquehas unique advantages and disadvantages when investigating NP-protein interactions.

In UV-Visible(UV-Vis) spectroscopy, the localized surface plasmon resonance (LSPR) phenomenon is used to characterizethe metallic NPs, such as gold nanoparticles(AuNPs) and silver nanoparticles (AgNPs), and its conjugates. LSPR is only present in plasmonic NPs and cannot be used in non-metallic systems, such as liposomes and SiNPs. The LSPR peak shape and size is caused by a collective oscillation of free electrons of the metallic particle. A shift and broadening of the absorption spectra for the NP-protein complex will depend on the bioconjugate size, aggregation state, and the local dielectric environment. Due to this phenomenon, UV-Vis is widely used to quantify metallicNPs and qualitatively measure conjugate binding. UV-Vis is a non-invasive method, where the integrity of the sample is not compromised,and is aninexpensivetechnique that requires little sample preparation. The major disadvantage of UV-Vis is that the adsorption spectrum ishighly influenced by the solvent, pH, temperature, and high electrolyte concentration. Moreover, very little can be learned about biomolecular structures using UV-Vis spectroscopy alone.

Fluorescence, Raman, and CD spectroscopy can be effectively used to detect NP-protein binding. Theadvantage of employing these techniques isthat they do not require plasmonic NP systems. However, there are significant factorswhich can complicate each of these methods. For example, fluorescence spectroscopy typically requires natural fluorophores, such as tyrosine or tryptophan in proteins.If these are not present, cysteine or aminescan be labeled with fluorescent probes, such as fluorescein, to study their structural and dynamic properties.Even though fluorescence is a highly sensitive technique,its main disadvantage is that the addition offluorescent probes can alter the NP-proteininteraction. Moreover,the inner filter effect (IFE) and light scatteringfrom the proteins or NPs may complicate the interpretation of fluorescence experiments.

Some of the drawbacks faced by fluorescence spectroscopy can be avoided using Raman spectroscopy, a non-destructive, highly specific technique thathas a distinct fingerprint for solid and liquid solutions. Ramanspectroscopy measures the NP bioconjugatein aqueous solutions with great spectral resolution.Surface enhanced Ramanspectroscopy (SERS) has improved the measurements of Raman spectroscopy with higher sensitivity and higher selectivity of chemical groups. SERS has been used todetermine the structural and conformational changes of protein on metal NPsurfaces. Apart from protein conformation data, the morphological changes in AuNPs when interacting with proteins, are also detected through SERS spectra. One drawback is the intense laser heating the NP-protein conjugate, which can alter its structure and conformation, giving rise to misleading results.

Neither fluorescence norRaman spectroscopy can detect secondary or tertiary structuralchanges in proteins; however,CD is used extensively to determine the secondary structure of proteins and how these structures change upon binding to NP surfaces. To exhibit a CD signal, a molecule must be chiral.However, NP surfaces are not typically chiral and will not generally interfere with signal and interpretation of data.As a drawback, UV-CD provides only a rough estimation of conformational changes, since the unbound (native) protein is typically left in the cuvette when the NP-bound protein is measured, and the unbound protein often dominates the observed signal. Separating the NP-bound protein by centrifugation, or performing a difference measurement are viable alternatives, althoughthe signal originating solely from NP-bound proteins is often very weak.

Other, non-spectroscopic approaches are also useful, and include dynamic light scattering, chromatography, mass spectrometry, and isothermal titration calorimetry. Dynamic light scattering (DLS) is a popular technique used to determine the hydrodynamic size of NPs in suspension.DLS measures the scattering intensity fluctuations caused by the Brownian motion of NPs in solution and uses the Stokes-Einstein equation to relate the diffusion coefficient to the NP size. The measured hydrodynamic diameter reflects the dimensions of the NP as well as thecorona layer bound to the NP surface in solution. The hydrodynamic radius measured by DLS can also be used to determine the binding ratio of protein to NP. Although DLS is a non-perturbative, fast, and accurate method,it requires adust free and dilute sample having a monodisperse population. Furthermore, this method suffers from low sensitivity toward small particles and possible interferences from light-absorbing species.

Chromatographic methods,like size exclusion chromatography (SEC) or gel filtration chromatography,are frequently used to separatecomplex mixtures of biological compoundsbased on their size. Gel filtration chromatography has been used to detect proteins bound to NPs and determine the exchange rate by comparing the bound versus free protein elution profiles.There are inherent drawbacks owing to the sensitivity of the technique, including lower precision, accuracy, and longer acquisition times. Electrophoresis is another useful technique to separate complex NP-protein mixtures thatprovidesqualitative and quantitative analysis. Polyacrylamide gel electrophoresis (PAGE) is one of the most widely used methods to separate NP-protein complexes. Macromolecules are differentiated according to their electrophoretic mobility, which is a function of the molecule'slength, conformation, and charge. For proteins, sodium dodecyl sulfate (SDS) is used to denature proteins and give them a uniform charge/size ratio.Even though SDS-PAGE is an effective tool to identify the composition of the protein corona, it suffers from poor protein separation if the protein mixtureis too complex, resulting in comigration of several proteinswith similar size.

Mass spectrometry (MS) is a high throughput, sensitive analytical technique used to monitor larger proteins (up to ~100 kDa) interacting with NPs. The two main ionization methods used to investigate biomolecules are matrix-assisted laser desorption ionization (MALDI) and electrospray ionization (ESI). Protein samples are often digested using proteolytic enzymes, into smaller fragments that are more suitable for the mass range of instruments. MS can provide both qualitative and quantitative information regarding the protein mixtures present on NP surfaces andcan be used in parallel with chromatographic and gel-based methods to identify the composition of the protein corona.Primarily, the use of protein fragments enables one to investigate structure on the NP surface. Even though MS is a destructive method, it provides qualitative and quantitative values that reflect the protein abundance in the protein corona, and it often uses comparatively small amounts of sample.

Isothermal titration calorimetry (ITC) can be used to directly measure the enthalpy change when proteins interact with NPs. In general, to measure the enthalpy change, the protein of interest is gradually added to a solution containing NPs and the evolved heat of binding is measured. These heats are calculated using the power required to maintain isothermal conditions.Fitting a thermodynamic model to the heats produces parameters like stoichiometry and the enthalpy of binding. Several drawbacks exist when using ITC to study protein-NP binding. For one, the method requires high sample concentrations (0.1-1 mM) and volumes (~1 mL). Secondly, adsorption may not produce a measurable heat, even when NPs are quite concentrated. Finally, if multiple steps occur (e.g. binding and unfolding), ITC thermograms may be challenging/difficult to interpret. Despite these challenges, ITC remains a useful tool for understanding the strength of biomolecule-NP interactions.

In summary, each of the analytical methods mentioned abovehas their own advantages and disadvantages that coincides with the physical properties being measured.

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Guest Articles

Role of Synthetic Organic Chemistry in Sustainable Agriculture

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Introduction

Undernourishment and malnutrition are major human health concerns in the developing world and these issues are becoming major threats with the increasing population.[1] According to the FOA, in 2050 the world's population is expected to be increased by 2 billion.Providing nutritious foods in adequate amount for the increasing world population is a challenging task.[2] Sustainable development in agriculture is critically important to improve theglobal food security and food nutrition. To achieve this goal world's agriculture has to increase the quantity and diversity of foods.

Synthetic organic chemistry has playing a major role in crop protection and food protection. The control of pathogens, insect pests and weeds has been the major contributor to the agricultural yield. New agro-chemicals has got escalating demand. Safe and efficient new organic molecules with new mode of actions will

claim a place in agriculture for many years to come.On this note, synthetic organic chemistry so far has made a huge impact on discovering and manufacturing of new organic molecules for crop protectionas well as other critical issues in agriculture. The major aspects we cover from this article are the approaches of synthetic organic chemistry on some of the major concerns in sustainable agriculture such as climate change, minimizing post-harvest losses, reduction of fertilizer consumption and crop protection.

(i) Climate Change

Climatic changes due to fluctuations in temperatures and rainfall patterns are playing an essential role in global agricultural productivity.[3] Plants respondto theseclimatic changes by altering metabolism pathways.[4] It has been recognized that most of these metabolic pathways mediated by plant based small peptides.[5] The essential role of these small peptides (<100 amino acids) in plant growth and development can be used to mitigate the plant stress during climatic changes.Naturally these peptides are produced in plant cells. Some of these essential plant peptide sequences have already been recognized and summarize in Table 1.

Table 1

Peptide	Function
CLE 25[6]	Resistance to dehydrative stress
Calreticulin[7]	Drought and high salt tolerance, better root growth
Ph-AMP[8]	Broad spectrum antifungal activity
BPCH7[9]	Effective gene transfer in cells

These peptides can be synthesized using solid phase peptide synthesis (SPPS)10 and use as plant external plant stimuli to relive the plant stress during extreme conditions.

(ii) Water scarcity

Global agriculture accounts for about 70% of global water withdrawals, and also constantly competing with domestic, industrial and environmental uses even with limited availability.[11] Scarcity of water for agriculture is one of the future challenges to overcome to increase the crop production for the increasing population. Sustainable use and water management arecritically important to conserve water for agriculture and other uses. Innovations are required to manage sustainable irrigation practices for the crop production.

The three dimensional polymeric network materials which are capable of absorbing large amounts of water are termed as hydrogels.[12] These hydrogels can be able to retain water and nutrients as well as release them accordingly when the root zone of plants start to dry up. Harnessing the high swelling and slow water retention of hydrogels opens the novel avenues in agricultural applications.[13] Most of the developments in the area of hydrogel are based on natural polymers. However polypeptides,[14] polysaccharides[15] and synthetic polyvinyl and polyester can also be used as alternatives. These polymers can be synthesized and optimized using Synthetic organic techniques.[16]

(iii) Minimizing post-harvest losses

One-third of the global food production (worth about US $1 trillion), is lost during postharvest operations every year.[17] The reduction of postharvest losses can give higher returns compared to increasing crop production to meet escalating global food demand. Novel strategies to reduce postharvest losses can be achieved by using innovative multidisciplinary approaches involving synthetic organic chemistry.

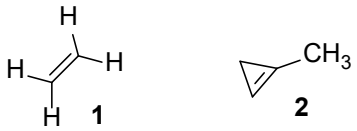
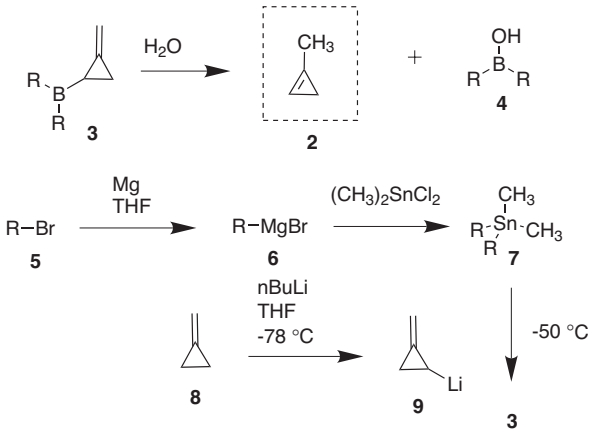


Figure 1: Structure of plant hormone ethylene,1 and ethylene inhibitor 1-methyl cyclopropene,2

Plant hormone ethylene can play a significant role in ripping fruits and yellowing leaves.[18] Uncontrolled ethylene exposure can induce significant changes in quality parameters of perishable plant products during the post-harvest storage. 1-methyl cyclopropene (1-MCP) is one of the competitive inhibitors in ethylene receptors of the plant and this compound can prevent ethylene binding even at minimum concentrations.[19]

Gases nature of 1-MCP at ambient temperature renders its usage in open field applications. Therefore stable complexes which can release 1-MCP gradually at ambient temperature could be much effective. These stable 1-MCP complexes can be synthesized using

synthetic organic techniques. Scheme 1 depicted the synthesis of stable Boronated1-MCP complex[20] which releases the active component upon contact with water.



Scheme 1: Synthesis of 1-MCP boron complexes

(iv) Reduction of fertilizer consumption

Synthetic inorganic fertilizers are playing a key role in enhancing crop production.[21] However, overuse of these inorganic fertilizers in many parts of the world has contributed to soil, water and air pollution. The demand for inorganic fertilizers in the future is predicted to be increasedfurther with the world's population growth. New technologies employing to increase nutrient use efficiency (NUE)[22] and thereby reduction of fertilizer usage can be used as effective mitigation alternatives to control the environmental impacts of the inorganic fertilizers.

Chemically decomposing organic compounds such as isobutylidene-diurea (10)[23] andcrotonylidene-diurea (11)[24] can be used as controlled release N-fertilizers. These compounds can be prepared by using excess urea aldehyde condensation reaction.

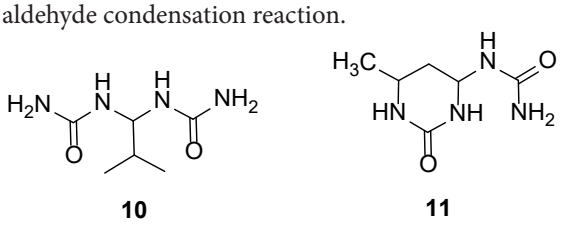


Figure 2: Chemically decomposing urea compounds

Inhibition of the conversion of ammonium-N to nitrate-N by soil bacteria can be a useful process to prevent leaching and denitrification.[25] Several small organic molecules can be used in this regard as inhibitors as shown in figure 3. Substituted schiff base type compounds have also been synthesized as potential inhibitors.[26]

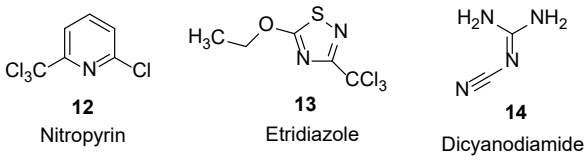


Figure 3: Key Inhibitors of nitrification reaction

(v) Modern Crop protection chemicals

Crop protection by using chemical strategies have been in practice for many decades. However the industry is constantlydemanding new moleculesdue to changing the agricultural environment and the cropresistance.[27] The three main groups of agrochemicals are herbicides, insecticides and fungicides.Among these,half of the crop protection chemicals are in fact herbicides. However, it has been noted that the resistance is appearing even for the most used herbicide Glyphosate.[28]

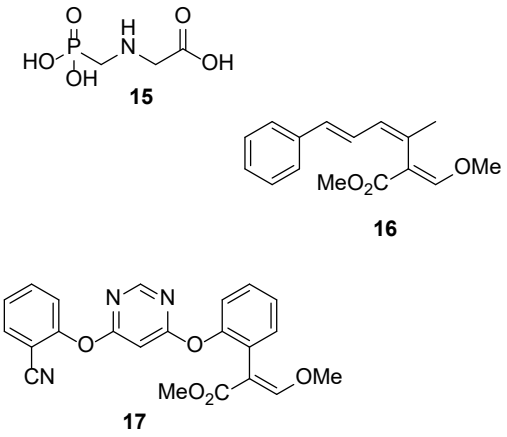


Figure 3: Structure of Glyphosate (15), natural product stobilurinA (16) and Azoxystrobin (17)

The resistance breaking nontoxic molecules with broad weed spectrum activity are constantly in need for the industry. Natural product stobilurin-A derived fungicide, azoxystrobin is a good example of broad spectrum fungicide which is effective against over 400 crop disease systems.[29] The invention of new agrochemicalsis exclusively relying on biological efficacy. However, there is a well establishing way of developing safe, effective and economical agrochemical leads. This involves screening of the biological target, use of bioactive natural products and their rationally designed analogs and mechanism based design.

Nature has been a frequent resource for many bioactive molecules. Even though most of the natural products are lacking the properties required for successful crop protection, they have provided better leads for crop protection chemicals. The structurally optimized target molecules then can be synthesized using synthetic organic chemistry.[30] Synthetic pyrethroid insecticides were perhaps the most enduring example in this regard which were derived from pyrethroids natural products (figure 4).[31]

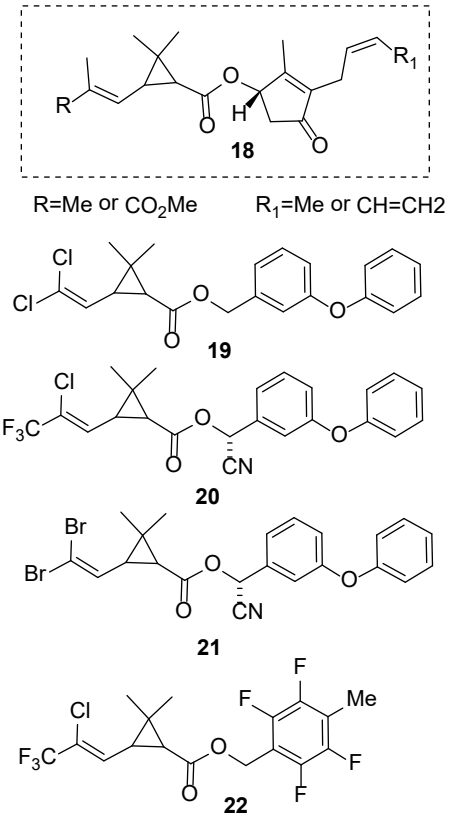


Figure 4: Natural and synthetic Pyrethroids

Summary

Constant supply of food forthe growing world is critically important. With its unique ability of bridging the structure and function, organic synthesis became an enabling science for many applied disciplines. In concert with other key science segments, organic synthesis is poised to address the key issues in global agriculture and food safety. Continues multidisciplinary research on agriculture organic interfacewillimmenselycontribute to economic developmentvia producing new compounds for safe, efficient agricultural practices. These new molecular architectures will facilitate the novel approaches to mitigate some of the key challenges in agriculture in order to increase the crop production especially in an agriculture dependent tropical country like ours.

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Guest Articles

Chemical Nature of Pesticides

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The phrase, "The Granary of the East" was used to describe the success of agriculture-based economy of our ancient nation. After European invasion, we lost our self-sustaining golden era, and gradually we started begging from developed countries and now we depend on their monetary loans and products. Pesticides are one of the major hazardous products, which we import without proper specifications, guidelines and monitoring. Today, it seems that these pesticides are making us sick or killing silently, particularly those who live in cultivating areas of Sri Lanka.

The substances or mixtures that use for controlling, preventing, destroying, repelling or attacking any biological organism are known as "**pesticides**". First historical evidence of pesticides came from Sumerian civilization, 2000 BCE. According to the archeologists, they have used sulfur to control mites and insects. Presently, about 2 million tons of pesticides are utilized globally, out of which 47.5% are herbicides, 29.5% are insecticides, 17.5% are fungicides and 5.5% are other pesticides.

Pesticides can be classified into two main groups based on;

- **Targeted use** - *e.g.* Algaecide (foralgae), Acaricide (for mites), Avicide (for birds), Bactericide (for bacteria), Fungicide (for fungi), Herbicide (for weeds), Insecticide (for insects), Biopesticide (derived from natural materials, *e.g.* baking Soda), *etc.*
- **Chemical nature** - This article intends to shine some light in this regard.

Pesticides based on chemical nature

Pesticides are classified depending on their chemical compositions, and are broadly classified as either organic or inorganic pesticides.

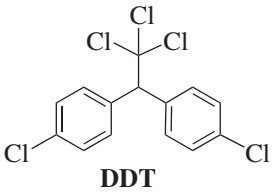
Inorganic pesticides are simple compounds

which have a crystalline, salt-like appearance; these are environmentally stable and usually soluble in water. These are the earliest chemical pesticides, *e.g.* sulphur, aluminium phosphide, lime, arsenic, copper and mercury salts. They are generally toxic and can remain in the environment for a long period of time.

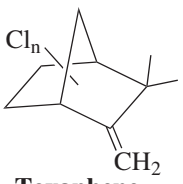
Organic pesticides are water insoluble, but readily soluble in fatty acids. Most of the modern pesticides are organic chemicals, which often contain oxygen, phosphorus, or sulphur in their molecules. They are further classified as either organochlorines, organophosphates or carbamates *etc*.

1. **Organochlorines (OCs)**

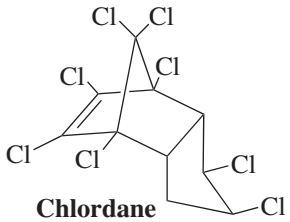
This broad-spectrum pesticide group contains chlorinated organic compounds. Nowadays, most pesticides belong to this group are banned or restricted in many countries as it is labeled as **persistent organic pollutants** (POPs). Organochlorines are mostly used as insecticides and its usage ranging from pellet application in field crops to sprays for seed coating and grain storage. These are strong neurotoxins; though it can be harmful to mammalians as well as insects. Dichlorodiphenyltrichloroethane (DDT) is one of the infamous banned insecticides that successfully used to control malaria. Endrin, Methoxychlor, Chlordane, Endosulfan, Chloropropylate, Aldrin, Toxaphene (Camphechlor) are some of the popular examples among OCs.



DDT



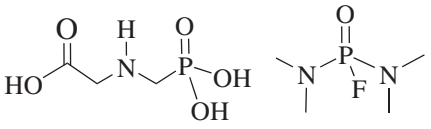
Toxaphene



Chlordane

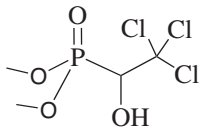
2. **Organophosphates (OPs)**

Esters of phosphoric acid and its derivatives are included in this group. These chemicals consist of phosphoric (P=O) or thiophosphoric (P=S) bond, and a leaving group, which can be replaced by the oxygen of serine in the acetylcholine esterase (enzyme that responsible for the metabolism of the neurotransmitters) active site. Toxicity of the pesticide depends on the leaving group. Today in Sri Lanka, **glyphosate** has become the main controversial OP pesticide that contains arsenic. However, OPs degrade rapidly by hydrolysis on exposure to moisture, air and soil; though small amounts can be detected in food and drinking water, *e.g.* Methyl Parathion, Dimefox, Malathion, Fenthion, Trichlorfon, glyphosate *etc*.



Glyphosate

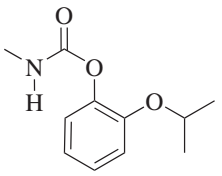
Dimefox



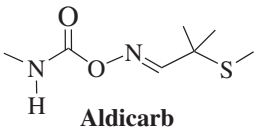
Trichlorfon

3. **Carbamates**

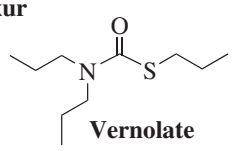
Carbamates are derivatives of carbamic acid, which has a similar structure to phosphoric acid. Carbamylating of the active site of acetycholine esterase is the main inhibition mechanism used by this pesticide group. Though carbamates are biodegradable, it is not as fast as OPs. Prupoxur, Carbofuran, Aldicarb, Vernolate, Thiourea are few examples of carbamates.



Propoxur



Aldicarb

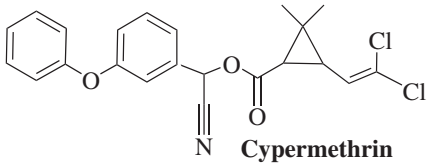


Vernolate

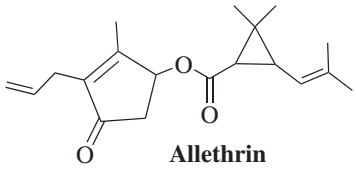
4. **Pyrethroids**

Chrysanthemum coccineum and *C. cinerariaefolium* flowers are used to isolate pyrethroids, as it exhibits natural insecticidal properties. Pyrethroids affect the sodium channels and lead to paralysis of the organisms. These types of pesticides have high biodegradable capacity, *e.g.* Allethrin, Tetramethrin, Cypermethrin,

Furethrin, Tetramethrin



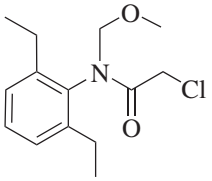
Cypermethrin



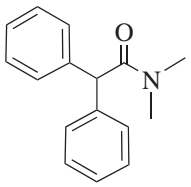
Allethrin

5. **Phenyl amides**

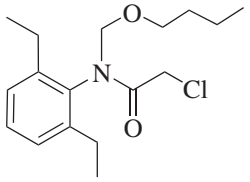
These are well-known fungicides, which added to the soil to enhance plant growth and yield. Barban, Alachlor, Diphenamid, and Butachlor are some examples for the pesticides which belong to this category. These fungicides have an impact on mitosis and cell division in target fungi.



Alachlor



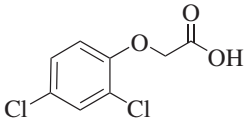
Diphenamide



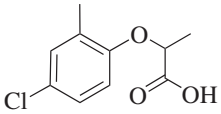
Butachlor

6. **Phenoxyalkonates**

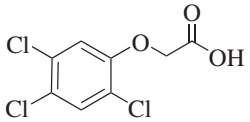
This herbicidal group is mainly used to control weeds in agriculture. Nearly almost all of the compounds of this group are degraded by microorganisms. Mecoprop, Erbinox, 2,4-D (2,4-dichlorophenoxyacetic acid), 2,4,5-T (2,4,5-trichlorophenoxyacetic acid), Sesone, and Dichloroprop are some of the members of this category.



2,4-D



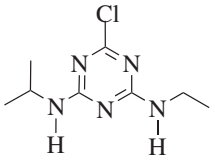
Mecoprop



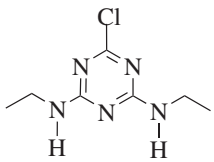
2,4,5-T

7. **Trazines**

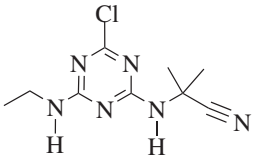
Atrazine, Simazine, Ametryn, Atratone, Chlorazine, Cyanazine can be included under this wide range of herbicide category. These chemicals are also used as insect chemo-sterilants.



Atrazine



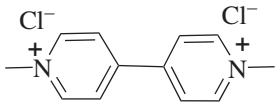
Simazine



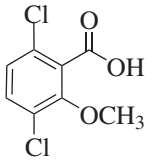
Cyanazine

8. **Others**

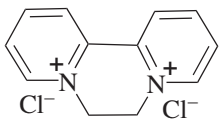
Benzoic acid and dipridyl derivatives which include paraquat and diquat are used as herbicides. Captan, Folpet and Captafol are fungicides which consist of the phthalimide moiety. Heavy metal elements such as iron, lead, arsenic, mercury, zinc, tin, *etc*. and both inorganic and organometallic compounds (*e.g.* Methyl mercuric chloride, sodium arsenate, calcium arsenate, zinc phosphide) are used as pesticides.



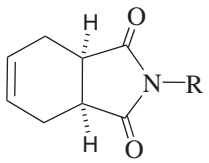
Paraquat



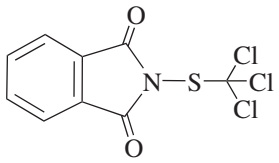
Dicamba



Diquat



Captan R = -$SCCl_3$



Folpet

Captafol R = -$SCCl_2CHCl_2$

Threats and concerns

Pesticides are well known carcinogenic agents. These toxins can enter the human body *via* ingestion (swallowed or eaten), inhalation (as a mist, dust, fumes

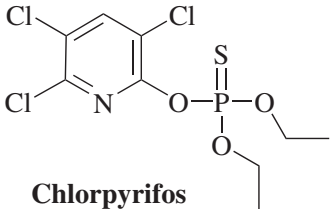
or smokes), or dermal contact (absorbed through skin or eyes). The toxic effect varies with the quantity, quality and potency of the pesticide, time of exposure, type of the injury and health state of the victim.

Short-term effects of pesticides are headache, skin-irritation, eye-irritation, muscle pain, fever, and weakness. It also adversely effects on nerve system (*e.g.* Alzheimer's disease, Parkinson's disease) and cardiovascular system, decreasing immunity, and cause infertility and severe birth defects. Pesticides are also known to cause gene mutation, chromosomal alteration and DNA damage. Unfortunately, some Sri Lankan farmers use pesticides to suicide.

Chronic kidney disease of unknown origin (CKDu) in rural areas of our country (especially in Polonnaruwa and Anuradhapura) is one of the tragic examples which imply the hazardous nature of pesticides. According to the latest researches, arsenic is the possible etiological factor for this emerging epidemic of CKDu. Phosphate agrochemicals are the main source of arsenic in Sri Lanka; and long-term application of this contaminated agrochemicals lead to the accumulation of arsenic in the soil.

Today, pesticide manufacturing companies use latest gene technology to produce "pesticide resistant crops". Roundup Ready crops are type of genetically modified crops invented by the Monsanto Company. However, researchers have revealed that these GM Crops are the main reason for many diseases including various types of cancers, infertility, birth defects and autism.

Chloropyrifos is an OP which has become the latest controversial pesticide as, it traces back to nerve agents developed in a Nazi laboratory.



Chlorpyrifos

Pesticides adversely effect on the complete eco-systems. There are many micro-organisms, invertebrates and vertebrates that support to improve the soil-structure and soil fertility. Most of pesticides that farmers are using today belong to the broad-spectrum category. Hence, these friendly creatures also get eliminated with the pests due to the chemical activities. Thus, soil has become a barren land. According to the Darwinian Theory, well adopted organisms will survive from the struggle of living. Mainly organochlorines act as an air pollutant; chlorinated compounds create free radicals and ultimately cause the depletion of the ozone layer. Eutrophication of water bodies is one of the serious water polluting method. Downstream drift of the pesticides is another way of polluting water. This polluted water runoff *via* drinking water sources and finally every water drop that we consume could become poisonous.

How to control the effects of pesticides

It is important to rinse vegetables and fruits with saltwater or vinegar or turmeric water or baking soda, followed by clean water, before cooking or consuming. The best possible way to reduce the intake of pesticides is to grow your own food in your garden. If you do not have enough garden space, you can use a hydroponic system.

Unlike most Sri Lankans, Europeans buy organically grown food for their daily usage to keep them healthy. Usage of many pesticides is banned in European countries, though they are selling low quality pesticides to third world countries. It should be mentioned that Sri Lanka has banned some of the toxic/harmful pesticides such as 2,4,5 T, arsenic, chlordane, DDT, dibromoethane, ethyl parathion, fluoroacetamide, mercury, thallium sulphate, methyl parathion, captafol, endrin, aldicarb etc.

Nanotechnology is the latest revolutionary trend in the modern world. Scientists have revealed that action in pesticides can be controlled with the help of nanotechnology. Engineered nanoparticle in the nano pesticides can be formulated to control the release of its active ingredients. This may reduce the environmental contamination through the reduction of pesticide application rates. Inventors of these nano-pesticides believe that development of this latest method will help to minimize the misuse. The other ways of avoiding exposure to toxic pesticide are to develop pest-resistant crop varieties, use of traditional agricultural methods such as crop rotation with locally produced fertilizers, and use of effective biopesticides.

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Guest Articles

Coke Reactivity and Its Applications in Blast Furnace Iron Making

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Greenhouse gas emission is the biggest challenge for the blast furnace iron making process. Even though intensive research has been undertaken in developing new iron making technologies, the blast furnace process still dominates in the iron making.

The iron making blast furnace is a smelting reaction chamber. It is a counter-current reactor where raw materials are added to the top of the furnace and gases are injected into the bottom. The raw materials consist of iron-bearing materials (oxides), fluxes and coke.[1] A low carbon operation in the blast furnace is a possible solution to reduce greenhouse gas emission. Low carbon usage means optimising the metallurgical coke usage in the blast furnace. Coke is the fuel and the primary source of the reductant (CO) of iron oxide in the blast furnace. It also gives the structural support to the furnace to ensure high permeability for high productivity.[1] One way to reduce coke usage is to inject supplementary fuels such as coal, natural gas and oil. These supplementary fuels can provide the heat and reductant for the iron making process but unable to provide the mechanical support to the burden and hence cannot be replaced entirely. Understanding coke reactivity and effect of coke mineralogy lead to optimise the coke usage to achieve high production rates required for profitable iron making in a blast furnace.

Coke characterisation

Coke is a complex material composed of organic components, inorganic minerals and pores. Further, it often displays significant heterogeneity in any metric(s) used to characterise its mineralogy, phase desperation, morphology and porosity.[2]

The mineral matter in the coke is the inorganic component. Coke mineralogy is an essential factor that in part determines coke reactivity. The mineral content of coke is typically 8-12 % by mass. Generally, the chemical composition of inorganic matter contains elements such as O, Si, Al, Fe, Ca, S, Mg, K, Ti and Na and less commonly P, Mn, C, H, N, Ba, Sr, F and Cl. Alkali metals, alkaline earth metals and transition metals such as iron and their oxides have been observed to have the ability to increase coke reactivity with CO_2.[2]

Key reactions in the blast furnace

A schematic of a blast furnace showing temperature profiles and key reactions is given in Figure 1. From this figure three distinct zones can be identified: the direct reduction, thermal reserve and pre-heat zones.

The oxygen from the hot blast, injected at the bottom, tuyere level, reacts with the coke carbon to produce CO_2 (Reaction 1). CO_2 is thermodynamically unstable in the presence of carbon at above ~900 °C and produces CO gas (Reaction 2). Reaction 2 is an endothermic reaction generally known as the Boudouard reaction, gasification reaction or solution loss reaction.[1]

$$C_{(s)}+O_{2(g)} \rightarrow CO_{2(g)} + \text{heat} \quad \Delta G^0 = -394100-0.84T \text{ J mol}^{-1} \quad [1]$$

$$CO_{2(g)}+C_{(s)} \rightarrow 2CO_{(g)} \quad \Delta G^0 = 165300-170.76T \text{ J mol}^{-1} \quad [2]$$

The Reaction 1 is highly exothermic and is likely to dominate at low temperatures and high oxygen partial pressures, while the Reaction 2 dominates at high temperatures and low oxygen partial pressures.[1]

In the indirect reduction zone (Figure 1) iron oxide reacts with CO(g) through exothermic reactions (Reaction 3 – 5).[4]

$$FeO_{(s)}+CO_{(g)} \rightarrow Fe_{(s)}+CO_{2(g)}$$
$$\Delta G^0 = -20100+22.14T \text{ J mol}^{-1} \quad [3]$$

$$3Fe_2O_{3(s)}+CO_{(g)} \rightarrow 2Fe_3O_{4(s)}+CO_{2(g)}$$
$$\Delta G\text{^}0 = -30260+51.54T \text{ J mol}^{-1} \quad [4]$$

$$Fe_3O_{4(s)}+CO_{(g)} \rightarrow 3FeO_{(s)}+CO_{2(g)}$$
$$\Delta G\text{^}0 = 32560+10.14T \text{ J mol}^{-1} \quad [5]$$

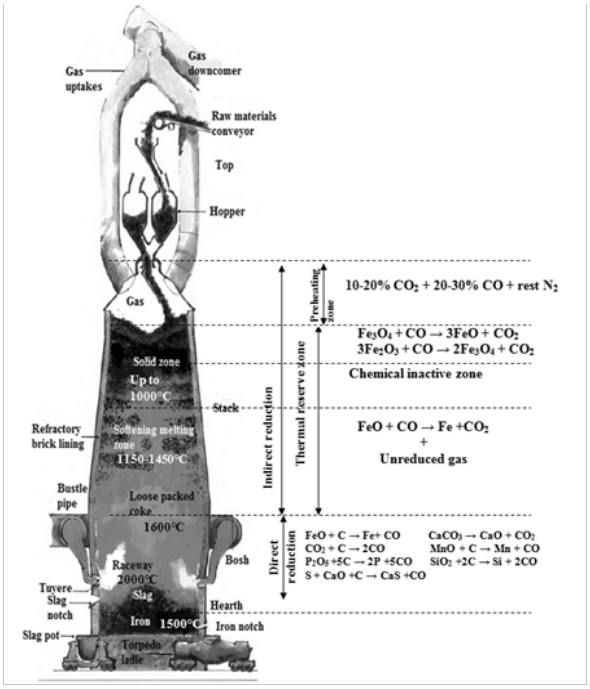

Figure 1: A schematic diagram of a blast furnace and reactions at each region[1,3]

In the direct reduction zone (Figure 1), the lower part of the furnace, at temperatures higher than 1000 °C, iron oxide reacts with carbon through endothermic reactions (Reaction 6-7).[4]

$$FeO_{(s)}+C_{(s)} \rightarrow Fe_{(s)}+CO_{(g)}$$
$$\Delta G^0 = 118300-131.11T \text{ J mol}^{-1} \quad [6]$$

$$FeO_{(l)}+C_{(s)} \rightarrow Fe_{(l)}+CO_{(g)}$$
$$\Delta G^0 = 132107-138.72T \text{ J mol}^{-1} \quad [7]$$

The overall reaction inside the blast furnace can be summarised as the Reaction 8.

$$Fe_2O_{3(s)}+3CO_{(g)} \rightarrow 2Fe_{(s)}+3CO_{2(g)} \quad [8]$$

Mechanism of coke – CO_2 gasification

The reaction mechanism of a coke – CO_2 reaction is based on the ability of coke carbon to dissociate an oxygen atom from a carbon dioxide molecule and retain it on a specific site by chemical bonding. These sites are known as active carbon sites. Unit areas of coke surfaces may not have an equal susceptibility to the reaction even under standardised gasification conditions, and the reaction occurs principally at active sites. Active carbon

sites are formed by surface irregularities such as carbon edges, dislocations, inorganic impurities and oxygen and/ or hydrogen functional groups, which have the ability to chemisorb a gas phase through electron transfer.[5]

The most widely accepted mechanism for a coke – CO_2 reaction [5]:

$$C_f + CO_{2(g)} \underset{j_2}{\overset{j_1}{\rightleftharpoons}} C(O) + CO_{(g)} \quad [9]$$

$$C(O) \xrightarrow{j_3} CO(g) + C_f \quad [10]$$

where;

C_f = active carbon site

C(O) = chemisorbed oxygen on carbon (intermediate)

j_1, j_2 and j_3 = rate constants

In the first step (Reaction 9), CO_2 dissociates into CO forming an oxidised surface complex C(O). This step is known as an oxygen exchange reaction which has been proven by isotopic tracer methods. The surface oxygen complex has a broad range of chemical functionality (e.g. lactone, carboxyl, quinone, ketene etc.). In the second step (Reaction 10), the surface complex produces another CO molecule leaving a free active C site for further reaction. The second step is considered as the rate controlling step. Both the forward and backward reactions of the reaction given in Reaction 9 are very rapid, and therefore there is an equilibrium between CO and CO_2 in the system.

Kinetics of coke gasification

Gasification of a porous particle involves several steps: transport of the reactant gas to the particle surface, diffusion of the reactant inside the particle through the pores to the reaction sites, reaction between gas and solid and elimination of the products (Reaction 9,10).[5]

Coke kinetics has been studied using the Arrhenius relationship:

$$\mathbf{Rate = k_0 e^{\frac{-E_a}{RT}}}$$

where; k_0 = rate constant

E_a = Activation energy

R = Gas constant

T = Absolute temperature

This lead to identify three 'zones' depending on the step that limits the reaction rate, namely, chemical kinetics (zone I), pore diffusion (zone II) and gas phase mass transfer (zone III) based on the operating conditions (Figure 2).

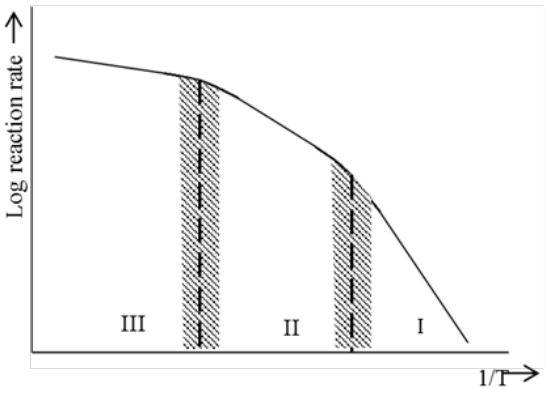

Figure 2: Schematic representation of three zones of gas-carbon reaction (after [5]). The hatched area indicating a transition between zones

Zone I is the low temperature chemical reaction controlled zone, where the rate is controlled by the chemical reaction(s) given in Reaction 9 and 10. Zone II, the intermediate temperature zone, is a mixed control region, where the rate is controlled by both steps pore diffusion and chemical reaction. In zone III, the high temperature zone, the rate is controlled by the gas phase mass transfer.

Novel approach for coke reactivity studies – coke analogue

When exposed to high temperatures and reactive atmospheres, the inherent complexity and heterogeneous compositional and structural features of coke make it difficult to isolate the effects of specific components on coke behavior and reaction kinetics. This has limited the progress in coke studies in assessing the impact of minerals on reactivity and reaction kinetics. A coke analogue has been developed by the Pyro-metallurgy research group in University of Wollongong, Australia using laboratory grade materials (graphite, Bakelite, Novolac and minerals) for use in examining coke reactivity to address these complexity and heterogeneity issues of industrial cokes].[6] Use of the coke analogue offers control in the selection and combination of minerals, mineral particle size and dispersion, and

analogue porosity.

For the coke analogue to represent metallurgical coke, there should be similarities in the key characteristic properties such as porosity, carbon bonding, and general reactivity with CO_2 gas and so on of both materials. Studies using the coke analogue with added mineral matter, of a composition chosen to mimic industrial coke, showed similar dissolution behavior in liquid iron to that of industrial coke. Use of the coke analogue allows control of the porosity, mineralogy, mineral particle size and distribution and the general reactivity with CO_2. This similarity and the control of those properties make the coke analogue a useful laboratory tool in coke studies.[3]

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Student Corner

Student Corner

RT PCR

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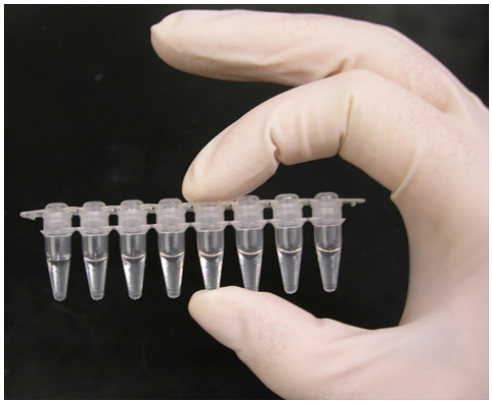
The whole world is engulfed with uncertainty, emptiness, lack of hope and pretty much one-third of the world is under some sort of lockdown due to the Corona Virus threat spreading across the world. While there are many preventive methods such as social distancing, hygienic practices, washing hands, using sanitizers, etc, a viable method of identifying the patients still at the top of the list. Identifying and isolation cut down the community spread of the disease significantly, so highly populated regions can be controlled and prevent the spreading of the virus. Unlike other diseases, COVID-19 seems to hide inside a person for quite a long time before showing symptoms, where he/she can spread across the community unknowingly.

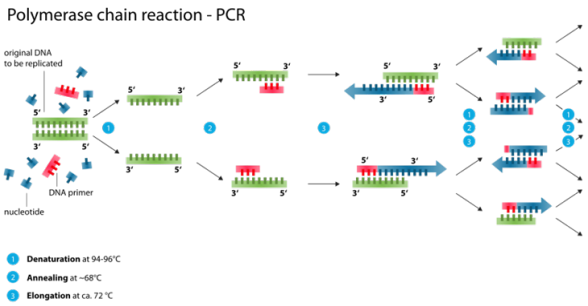
It all comes down to the availability of facilities to handle the pandemic situation. Developed countries have their own strategies in handling the situation, but one thing is common for all. Testing the suspected patients and infected using a PCR Test. Test, Test, Test!

PCR, as it stands for Polymerase Chain Reaction, which is self-explanatory among the chemical community. The goal of general PCR or Polymerase Chain Reaction is to amplify a target region of DNA using the DNA polymerase enzyme for analysis and further studies. In living organisms, DNA is replicated to increase the number of copies. Since the replication process takes place under biological conditions, many enzymes are involved. However, in PCR this replication is done in a test tube using DNA polymerase as the only enzyme and other ingredients. The majority of PCR methods depend on thermal cycling, which involves exposure of reactants to repeated cycles of heating and cooling to initiate a specific reaction at each temperature. The reaction mixture contains a DNA template, two primers, dNTPs (deoxy-nucleotide triphosphate), $MgCl_2$, DNA polymerase, and sterile water or buffer. In all PCR reactions the DNA polymerase that has been used is a heat-stabIe enzyme. In a general PCR cycle, there are three main steps 1. Denaturing 2. annealing of primers and 3. Extension.

Denaturation of DNA

The structure of DNA is a double-stranded helical form; therefore, the two strands have to be separated for amplification. This is done by heating the reaction mixture that contains the template DNA (original DNA) to about 98 ^{0}C. At this temperature, the two strands will be separated and this is called the melting of DNA strands. In traditional PCR there is an initial denaturation for about one minute and at each cycle, denaturation is done for about 30s.





Primer Annealing

All DNA polymerases can only add dNTPs (deoxynucleotide triphosphate) to an existing DNA strand. Therefore, in order for the DNA polymerase to act it needs a starting point called a primer, which is a short sequence of deoxy-nucleotides (about 20 bases in length). Since DNA is double-stranded each PCR mixture needs two deoxy-primers. The primers bind to the template DNA by complementary base pairing. Primer annealing takes place at a lower temperature than melting, usually around 68 ^{0}C for about 30s.

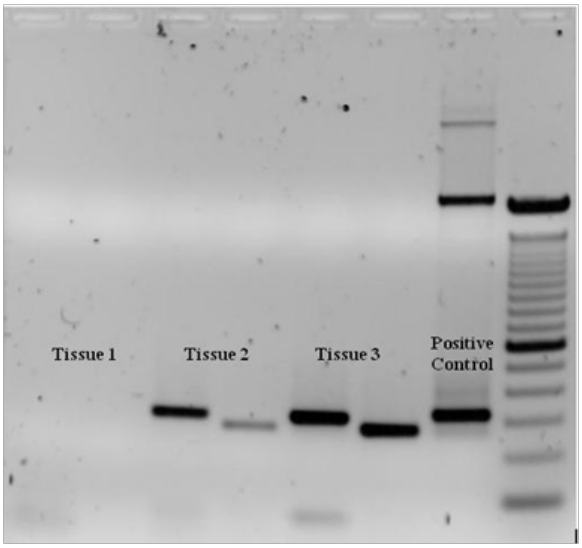
Extension/Elongation

In this step, dNTPs are added to the primer by DNA polymerase. The temperature at this step depends on the thermostable DNA polymerase used and the commonly used temperature is around 72 ^{0}C. In this step, DNA polymerase synthesizes a new DNA strand, which is complementary to the DNA template strand by adding free dNTPs from the reaction mixture. The precise time needed for elongation depends both on the DNA polymerase and length of the amplifying region of the target DNA. At their optimal temperature, most DNA polymerases can add thousands of bases per minute. At this step, the amount of DNA is doubled. With each successive cycle, the original DNA strand and all the newly generated strands become template strands for the next round. In most cases, about 35-40 cycles (denaturation, primer annealing and, extension) are carried out to obtain high enough concentration of DNA for further studies.

After this cycling routine, there is the final elongation step, where the reaction mixture is allowed to undergo the last extension step for about 5-15 min at the same extension temperature used in order to make sure that any remaining single-stranded DNA is fully elongated.

Finally, there is a cooling step (cools to around 4 ^{0}C) where the samples can be stored until they are used for further analysis.

The purity of the PCR products is checked using DNA agarose gel electrophoresis using ethidium bromide as the staining agent. A DNA ladder (a mixture of DNA fragments with different sizes) is used to determine the size of the PCR products. Picture of an agarose gel containing ethidium bromide- stained PCR products is shown below.



Ethidium bromide-stained PCR products after gel electrophoresis. Two sets of primers were used to amplify a target sequence from three different tissue samples. No amplification is present in sample #1; DNA bands in sample #2 and #3 indicate successful amplification of the target sequence. The gel also shows a positive control, and a DNA ladder containing DNA fragments of defined length for sizing the bands in the experimental PCRs.

Applications of the technique include DNA cloning for sequencing, gene cloning and manipulation, gene mutagenesis; construction of DNA-based phylogenies, or functional analysis of genes; diagnosis and monitoring of hereditary diseases; amplification of ancient DNA; analysis of genetic fingerprints for DNA profiling (for example, in forensic science and parentage testing); and detection of pathogens in nucleic acid tests for the diagnosis of infectious diseases.

There are several advantages of doing PCR. It is fairly simple, easy to use, and understand, produce rapid results and the technique is very sensitive for producing billions of copies of DNA fragments.

This technique can be used in high sensitivity even when only RNA is present in the sample. However, in such cases, RNA code has to be first converted to its complementary DNA (cDNA) molecule and this process is called reverse transcriptase reaction. This is followed by a normal PCR procedure where billions of copies of cDNA can be obtained for further analysis. This process is called Reverse transcriptase PCR or RT-PCR. There are two ways for quantification of cDNA present, which are endpoint analysis or real-time analysis. Endpoint analysis could take an average of 6-8 hours, whereas real-time RT-PCR could only take about three hours. Also in real-time RT-PCR, a specific fluorescence marker is used to observe the development of the desired cDNA. Therefore, real time RT-PCR technique is highly sensitive, specific, and fast. Compared to other available methods, the real-time RT-PCR has a lower potential for contamination or errors as the entire process can be done within a closed tube.

Thus, PCR tests can be done to identify possible infected patients for COVID-19, however, there's only a 70% success rate in PCR tests in the identification of Positive results.

The accuracy of a medical test is determined by measuring two things: sensitivity and specificity.

- A sensitive test will correctly identify people with the disease. Sensitivity measures correct positive results.

  If a test is 90% sensitive, it will correctly identify 90% of infected people – called a true positive. However, 10% of people who are infected and tested would get a false negative result – they have the virus, but the test indicates they don't.

- A specific test will accurately identify people without the disease. Specificity measures the correct negatives.

  If a test is 90% specific, it will correctly identify 90% of people who are not infected – registering a true negative. However, 10% of people who are not infected will test positive for the virus and receive a false positive.

Most experts believe that problems with sample collection are the main culprit behind inaccurate testing. False negative results are likely occurring because health care providers aren't collecting samples with enough of the virus for the tests to detect.

This can happen because the person who collects the sample doesn't insert a swab deep enough in the nose or doesn't collect enough of the sample. False negatives could occur if a person is tested too early or too late during their infection and there isn't a lot of virus in their cells. And finally, errors can happen if a sample sits too long before being tested, which allows the viral RNA to break down.

However, there are many fully automated PCR's equipped with cutting edge technology, where everything except sample collection can be completely automated. The above script is just a general description of most common practices in the PCR technique and individual requirements and specific testing procedures may vary.

Nonetheless, it's important to conduct PCR tests in this type of outbreak and a pandemic situation to control community spreading. Lack of trained personnel and price of Testing materials, chemicals are always challenging to an upper-middle-income country like us, but it's vital to invest in these not only for the current situation but for future possibilities as well.

Crystal Field Theory

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Crystal Field Theory (CFT) supersedes valence bond theory as it provides a simple method to explain the **electronic spectra** and **magnetism** of transition metal complexes. CFT is based on the influence of incoming ligands on the **five** *d*-orbitals in the metal centre; which depends on the number of such ligands and the **geometry** of the compound. According to the shapes and orientation of *d*-orbitals, they are divided into two groups.

t_{2g} orbitals - d_{xy}, d_{yz} and d_{xz} orbitals with lobes (*i.e.* electron density) located between the x, y and z axes.

e_g orbitals - $d(_x^2\text{-}_y^2)$ and d_z^2 orbitals with lobes pointing along the axes.

CFT is based on four main **assumptions**:

a. Ligands are considered as point charges.

b. The bonding between the metal and the ligand is entirely electrostatic.

c. There is no interaction between the metal orbitals and ligand orbitals.

d. In the free metal atom, the *d*-orbitals have the same energy, *i.e.*, they are degenerate.

CFT for Octahedral Complexes

As $\mathbf{e_g}$ orbitals have lobes directed towards the ligands, they are strongly interacting with ligands than the $\mathbf{t_{2g}}$ orbitals. Thus, $\mathbf{e_g}$ orbitals have higher energy with respect to the $\mathbf{t_{2g}}$ orbitals. As a result, the *d*-orbitals are no longer degenerate; the energy of the two $\mathbf{e_g}$ orbitals is raised, while the energy of the three $\mathbf{t_{2g}}$ orbitals is lowered by the same amount as shown in Figure 1. The difference in energy between the $\mathbf{t_{2g}}$ and $\mathbf{e_g}$ levels is denoted by the symbol Δ_o. The term Δ(delta) represents the **crystal field splitting**, and the subscript '**o**' refers to the **octahedral**.

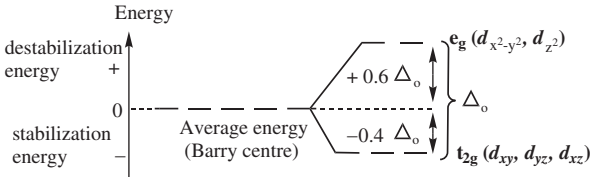

Figure 1: Energy diagram for an octahedral compound

'Δ' depends on the electrostatic field generated by the ligands; thus, some create a **stronger** field than the others. When ligands are arranged in the increasing order of their electrostatic field strength, it is known as the **spectrochemical series**.

Weak field ligands ⟵

$I^- < Br^- < SCN^- < Cl^- < NO_3^- < F^- < OH^- <$ EtOH < Oxalate < H_2O < $NCS^- <$ $EDTA^{4-} <$ NH_3<py<en<bipy<phen (1,10-phenanthroline) < $NO_2^- <$ $PR_3 <$ $CN^- <$ CO

Strong field ligands ⟶

The factors which effect on the crystal field splitting are as follows.

Nature of the ligand - Energy gap Δ varies with the type of ligands as shown below.

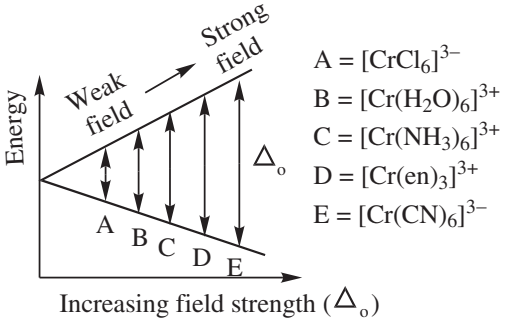

Figure 2: Effect of ligand fields on Δ_o

The charge on the metal - Δ increases with increasing of the charge on the metal ion; as the central ion with a higher charge can polarize the ligand to a great extent, thereby it increases the electrostatic field.

Position of metal centre within the Group - Δ_o increases as the row number increases within the Group; $\Delta_o(3d) < \Delta_o(4d) < \Delta_o(5d)$.

Geometry of the complex - For example, the splitting of $\mathbf{t_{2g}}$ and $\mathbf{e_g}$ levels in octahedral and tetrahedral complexes is quite opposite (see Figures 1 and 4).

Crystal Field Stabilization Energy

The overall energy of a set of electrons in $\mathbf{t_{2g}}$ and $\mathbf{e_g}$ levels is known as crystal field stabilization energy (CFSE). **CFSE = [0.6m-0.4n]Δ_o** for $\mathbf{t_{2g}^n.e_g^m}$ configuration,*i.e.*, for $\mathbf{t_{2g}^1.e_g^0}$, CFSE = $-0.4\Delta_o$; for $\mathbf{t_{2g}^2.e_g^0}$, CFSE = $-0.8\Delta_o$ and for $t_{2g}^3.e_g^0$, CFSE = $-1.2\Delta_o$. For example, Ti^{3+} ion ($\mathbf{t_{2g}^1.e_g^0}$) in $[Ti(H_2O)_6]^{3+}$ is stabilized by an energy amounting to $0.4\Delta_o$. All possible $\mathbf{t_{2g}^n.e_g^m}$ configurations for weak and strong fields are given in Table 1.

For d^4-complexes, two electron distributions ($\mathbf{t_{2g}^4.e_g^0}$ and $\mathbf{t_{2g}^3.e_g^1}$) are possible (see Figure 3). The electron distribution is determined by the **pairing energy** (PE) (*i.e.* the energy required to pair two electrons in an orbital) and the strength of the crystal field (Δ_o).

(a) The $\mathbf{t_{2g}^3.e_g^1}$ configuration is favored when PE>Δ_o. These complexes are known as 'high-spin' or 'weak-field' complexes.

(b) The $\mathbf{t_{2g}^4.e_g^0}$ configuration is favored when $\Delta_o^* >$ PE. These complexes are known as 'low- spin' or 'strong-field' complexes.

Note that $\Delta_o < PE < \Delta_o^*$

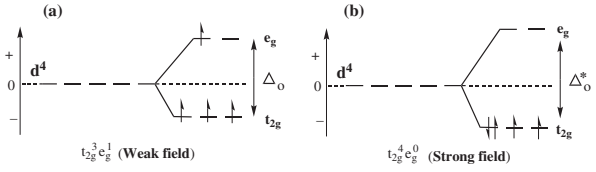

Figure 3: Energy level diagrams for a d^4 configuration for weak field and strong field ligands

Total Stabilization Energy (**TSE**) = CFSE + m x Pairing Energy (**PE**); m = number of orbitals with paired electrons. For $\mathbf{t_{2g}^5.e_g^0}$ configuration,

$$TSE = CFSE + 2\ PE = -2\Delta_o + 2PE$$

Similarly, for the configurations $\mathbf{t_{2g}^6.e_g^0}$ and $\mathbf{t_{2g}^6.e_g^1}$, total stabilization energies are $-2.4\Delta_o$+3PE and $-1.8\Delta_o$+3PE, respectively.

CFT for Tetrahedral Complexes

In tetrahedral complexes, $\mathbf{t_{2g}}$ orbitals situated between x, y and z axes, and strongly interact with incoming ligands than $\mathbf{e_g}$ orbitals. Therefore, $\mathbf{t_{2g}}$ orbitals become less stable due to their closeness to the ligands, while $\mathbf{e_g}$ orbitals become more stable (see Figure 4).

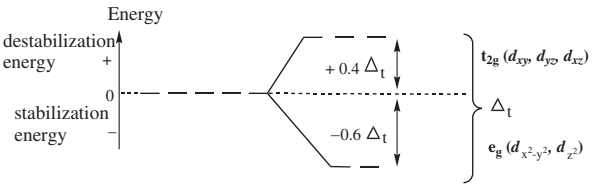

Figure 4: Energy level diagram for a set of five *d*-orbitals in the prescence of a tetrahedral crystal field

Note that $\Delta_t \approx 0.5\Delta_o$ where '**t**' refers to **tetrahedral**.

Tetrahedral complexes are favored when;

- Ligands are large and bulky
- Ligands are weak field where Δ_t is quite small
- Electronic configuration of the metal centre is d^0, d^5 or d^{10}.

For both octahedral and tetrahedral complexes, CFSE is zero for d^0, d^5 and d^{10} configurations. But for all other configurations, the octahedral CFSE is greater than that of the tetrahedral CFSE; thus octahedral complexes are more stable than tetrahedral complexes.

CFT for square-planar complexes

Generally, d^8 configurations show square-planar geometry. It can be achieved by removing two axial ligands of an octahedral complex. The energy of the orbitals in the xy plane is increased, which makes d_z^2 orbital more stable than $d(^2_x - ^2_y)$. Likewise, d_{xy} becomes less stable than d_{zx} and d_{yz} orbitals as shown in Figure 5. The magnitude of Δ_{sp} is roughly 1.3 times higher than Δ_o.

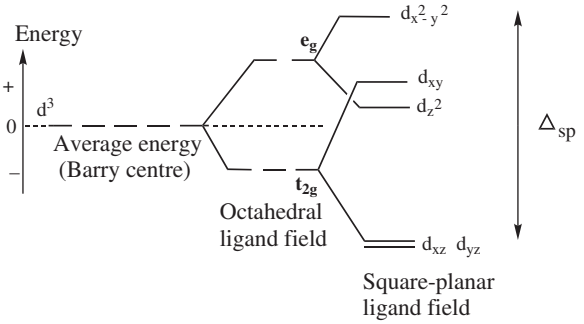

Figure 5: Crystal Field Splitting in a Square-planar complex

Magnetic properties

Total magnetic moment (μ) of an unpaired electron of a paramagnetic complex arises due to its spin about the own axis and its orbital angular momentum. For first-row transition metals, μ is equal to 'spin only' magnetic moment (μ_s), as its orbital angular momentum is negligible. The relationship between μ_s and the number of unpaired electrons 'n' is, $\boldsymbol{\mu_s = \{n(n+2)\}^{1/2}}$. The μ values are given in the Table 1 in Bohr Magneton (BM).

Table 1: Data for an octahedral complex; X = *d*-electron distribution, Y = number of unpaired electrons n with the μ_s value in brackets

d^n	Strong Field		Weak Field	
	X	Y	X	Y
d^1	$t_{2g}^1.e_g^0$	1(1.73)	$t_{2g}^1.e_g^0$	1(1.73)
d^2	$t_{2g}^2.e_g^0$	2(2.83)	$t_{2g}^2.e_g^0$	2(2.83)
d^3	$t_{2g}^3.e_g^0$	3(3.87)	$t_{2g}^3.e_g^0$	3(3.87)
d^4	$t_{2g}^4.e_g^0$	2(2.83)	$t_{2g}^3.e_g^1$	4(4.90)
d^5	$t_{2g}^5.e_g^0$	1(1.73)	$t_{2g}^3.e_g^2$	5(5.92)
d^6	$t_{2g}^6.e_g^0$	0(0.00)	$t_{2g}^4.e_g^2$	4(4.90)
d^7	$t_{2g}^6.e_g^1$	1(1.73)	$t_{2g}^5.e_g^2$	3(3.87)
d^8	$t_{2g}^6.e_g^2$	2(2.83)	$t_{2g}^6.e_g^2$	2(2.83)
d^9	$t_{2g}^6.e_g^3$	1(1.73)	$t_{2g}^6.e_g^3$	1(1.73)
d^{10}	$t_{2g}^6.e_g^4$	0(0.00)	$t_{2g}^6.e_g^4$	0(0.00)

Color of transition metal complexes

Most transition metal complexes are colored, as they transmit the complementary color of the absorbed light. Excitation of electrons between $\mathbf{t_{2g}}$ and $\mathbf{e_g}$ levels (or *d-d* transitions) occurs in the visible region. By analyzing the absorption spectra of these complexes, the size of Δ can be calculated. The possible electron excitations and their intensities are determined by the Laporte and Spin selection rules.

Problems

1.

(a) What are the numbers of $\mathbf{t_{2g}}$ and $\mathbf{e_g}$ electrons of Cr in $[CrBr_6]^{4-}$? Br^- is a weak field ligand.

(b) Calculate the CFSE and TSE in kJ mol^{-1} if Δ_0= 160 kJ mol^{-1}.

(c) Calculate the μ_s of $[CrBr_6]^{4-}$.

2.

(a) What is the *d*-electron configuration of Fe in $[FeBr_4]^{2-}$?

(b) Calculate the CFSE and TSE in kJ mol^{-1} for this anion if Δ_0 = 180 kJ mol^{-1} and PE = 120 kJ mol^{-1}.

(c) Calculate the μ_s of $[FeBr_4]^{2-}$

Substitution Reactions

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A good knowledge in coordination and organometallic chemistry is very useful to a student, who wish to understand chemical transformations taking place around in nature and industry. It also helps to design synthetic routes to target metal complexes. We know that nature very cleverly uses metal complexes to carry out reactions catalytically with a precise selectivity. In enzymes, metal centres such as Zn, Cu, Mo, Fe *etc*. are dressed in organic ligands to perform specific reactions. This article is predominantly focused on the following reaction types; namely, association, dissociation and ligand substitution.

Association reactions

Here, a **coordinatively unsaturated** complex takes up a suitable ligand(s) to form a **coordinatively saturated** (18e) complex, with at least one-unit higher coordination number (C.N.) than the starting complex. Some of these reactions are reversible. For example, (i) $[RuCl_2(PPh_3)_3]$ can form an adduct with CO or PPh_3; (ii) BH_4^- is formed by associating a hydride ion with BH_3.

$$[RuCl_2(PPh_3)_3)] + CO \rightarrow [RuCl_2(CO)(PPh_3)_3]$$

$$[RuCl_2(PPh_3)_3] + PPh_3 \rightleftharpoons [RuCl_2(PPh_3)_4]$$

$$BH_3 + H^- \rightarrow BH_4^-$$

Dissociationreactions

This is the reverse process of an association reaction. Particularly, **coordinatively saturated** compounds undergo this type of reaction to form **reactive, coordinatively unsaturated** compounds. Some of these reactions are reversible. For example, zerovalent Pd(0) complex, $[Pd(PPh_3)_4]$ reversibly dissociate one PPh_3 ligand resulting coordinatively unsaturated complex, $[Pd(PPh_3)_3]$ which can act as a catalyst.

$$[Pd(PPh_3)_4] \rightleftharpoons [Pd(PPh_3)_3] + PPh_3$$

$$[Ni(CO)_4] \rightleftharpoons [Ni(CO)_3] + CO$$

Ligand substitution reactions

At the end of this type of reactions, the oxidation number (O.N.) and the coordination number of the metal centre remain **unchanged**. Thus, valence electron count (VEC) also remains the same. *e.g.*, formation of $[Ni(CO)_3(PPh_3)]$ by reacting one equivalent of PPh_3 with $[Ni(CO)_4]$

$$[Ni(CO)_4] + PPh_3 \rightarrow [Ni(CO)_3(PPh_3)] + CO$$

Substitution of a coordinated ligand by an incoming ligand can take place *via* three pathways;(i) **Associative(A)**,(ii) **Dissociative (D)**, and (iii) **Interchange (I)** pathways.

Associative mechanism (A)

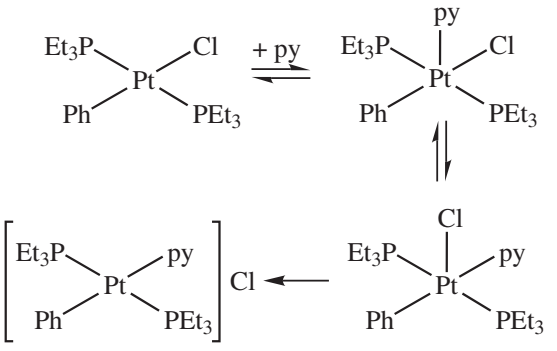
Coordinatively unsaturated complexes, particularly 16e square-planar complexes containing metal centers such as Pt(II), Pd(II), Ni(II), Rh(I), Ir(I) and Au(III) undergo substitution reactions *via* a five coordinate, 18e-intermediate which can have either square pyramidal or trigonal bipyramidal geometry. *e.g.*, pyridine (py) substitutes the chloride of *trans*-$[PtCl(Ph)(PEt_3)_2]$, to give a salt of the type trans-$[Pt(Ph)(py)(PEt_3)_2]Cl$.

trans-$[PtCl(Ph)(PEt_3)_2]$+py

↓

trans-$[Pt(Ph)(py)(PEt_3)_2]Cl$

This reaction proceeds *via* an **associative** pathway.



Dissociative mechanism (D)

Coordinatively saturated complexes undergo this type of substitution reactions; first dissociating a ligand to create a vacant site, and then vacant site is occupied by the incoming ligand. In some cases, solvent molecules with donor atoms can temporarily occupy the vacant site.

Interchange mechanism (I)

An interchange mechanism takes place in a **single step**, where the leaving and entering groups exchange *via* an **activated complex**. This activated complex is not a true intermediate. The rate of the interchange process is expected to depend on the nature of the entering group and its concentration.

Mono-, di- and tri-substitution reactions

In a mono substitution reaction, one labile ligand (*e.g.* MeCN) is replaced by another ligand (*e.g.* L = PPh_3).

$$[IrCl(NCMe)L_2] + L \rightarrow [IrClL_3] + MeCN$$

$$[PtCl_2(NCMe)L] + L \rightarrow [PtCl_2L_2] + MeCN$$

In a di-substitution reaction, two ligands are replaced by another ligand/s. This can beviewed as two mono substitution reactions, taking place one after the other.

$$\textit{trans}\text{-}[PtCl_2(NCMe)_2] + 2\ PPh_3 \rightarrow \textit{trans}\text{-}[PtCl_2(PPh_3)_2] + 2\ MeCN$$

$$[W(CO)_4(pip)_2] \xrightarrow[-2pip]{+\ bipy} [W(CO)_4(bipy)]$$

bipy = 2,2'-bipyridine; pip = piperidine

$$[Fe(CO)_5] + nbd \longrightarrow [Fe(CO)_3(nbd)] + 2\ CO$$

nbd = norbornadiene

In a tri-substitution reaction,three ligandsare replaced by another ligand/s.

$$[W(CO)_6] \xrightarrow[-3CO]{+\ 3MeCN} [W(CO)_3(NCMe)_3]$$

$$[W(CO)_6] \xrightarrow[-3CO]{+\ cht} [W(CO)_3(\eta^6\text{-cht})]$$

(cht = 1,3,5-cycloheptatriene)

Trans effect

The *trans*-effect can be observed in ligand substitution reactions of square-planar complexes, which consider the influence of a ligand on the ligand trans to it. A strong ligand may replace a labile *trans*-ligand by an incoming ligand. The order of the *trans*-effect of some common ligands is given below.

H_2O, OH^-, NH_3, pyridine < Cl^-< Br^-< SCN- < NO_2- < $C_6H_5^-$< PR_3 < CH_2=CH_2 < CN^-, CO

For example, reaction of one equivalent of pyridine with *cis*-$[PtCl_2(Br)(py)]^-$ gives *cis*-$[PtClBr(py)_2]$; and reaction of one equiv. of CO with *trans*-$[PtCl_2(Br)(py)]^-$ gives trans-$[PtCl_2(Br)(CO)]^-$.

$$\textit{cis}\text{-}[PtCl_2(Br)(py)]^- \xrightarrow[-Cl]{+\ py} \textit{cis}\text{-}[PtCl(Br)(py)_2]$$

$$\textit{trans}\text{-}[PtCl_2(Br)(py)]^- \xrightarrow[-py]{+\ CO} \textit{trans}\text{-}[PtCl_2(Br)(CO)]^-$$

Problems

1. Identify the product(s)of the following reactions.
   i. $BF_3 + Et_2O$
   ii. $PtCl_2$ + 2 NaCl
   iii. $PtCl_2$ + 2 MeCN
   iv. $[IrCl(CO)(PPh_3)_2]$ + NaCN
   v. *cis*-$[PtCl_2(PPh_3)_2]$ + 2MeMgI
   vi. $[Mo(CO)_6]$ + 2 piperidine
   vii. $[PdCl_2(nbd)]$ +2 PPh_3
   viii. $[PdCl_2(NCPh)_2]$ + bipy
2. How would you prepare *trans*-$[PtBr_2(py)(CO)]$ from $[PtBr_4]^{2-}$ if the trans effect order is CO > Br^->py?
3. How would you prepare *cis*-$[NiCl_2(CO)(NH_3)]$ from $[NiCl_4]^{2-}$ if the transeffect order is CO > Cl^-> NH_3.
4. $[Pt(NH_3)_4]^{2+}$ undergoes an associative substitution reaction with Cl^- to give an intermediate (**X**) which rapidly loses a ligand (**Y**) to form a square planar complex cation (**Z**). Identify (**X**), (**Y**) and (**Z**).

Oxidative Addition Reactions

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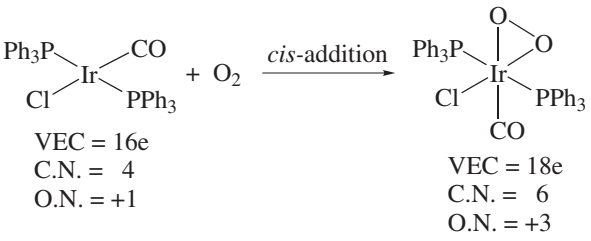
Coordinatively unsaturated metal centres (M^{n+}) (valence electron count, VEC<18) may undergo oxidative addition reactions to form saturated metal complexes by cleaving the X-Y bond of a molecule (*e.g.* H_2, O_2, Br_2, I_2, Cl_2, organic halides (RX), HX, RCO_2H, alkenes and alkynes with electron withdrawing groups).

$$M^{n+} + X\text{-}Y \longrightarrow \underset{\displaystyle Y}{\overset{\displaystyle |}{X\text{-}M^{(n+2)+}}}$$

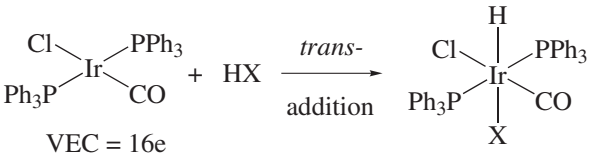
During this process, VEC, oxidation number (O.N.) and coordination number (C.N.) may be increased by either one or two units. Generally, the oxidative addition of non-polar molecules such as H_2, O_2, halogens, alkenes and alkynes is *cis*.



VEC = 16e
C.N. = 4
O.N. = +1

VEC = 18e
C.N. = 6
O.N. = +3

But oxidative addition of polar molecules such as HX, MeI and $ArSO_2Cl$ to Vaska's complex *trans*-[IrCl(CO)$(PPh_3)_2$] is *trans*.



VEC = 16e
C.N. = 4
O.N. = +1

VEC = 18e
C.N. = 6
O.N. = +3

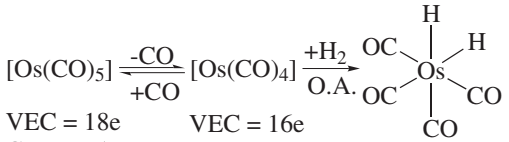
Let us consider the factors which influence the oxidative addition reactions.

- Oxidative addition is facile if the metal centre is coordinatively unsaturated.
- Oxidative addition is facile if the metal centre M^{n+} has an accessible oxidation state $M^{(n+1)+}$ or $M^{(n+2)+}$, *e.g.*

$$Pd(0) \rightarrow Pd(II) \text{ and } Co(I) \rightarrow Co(III)$$

- The nature of other coordinated ligands also influences oxidative addition, for example, electron withdrawing ligands (e.g. C≡O) deactivate the metal centre whilst electron donors (*e.g.* PMe_3) increase the basicity of the metal centre, thus, promotes oxidative addition. Similarly, anionic complexes with more basic metal centres are more activated towards oxidative addition than neutral complexes.

Some coordinatively saturated compounds can also undergo oxidative addition reactions by prior dissociation of a ligand. *e.g.* formation of *cis*-[$OsH_2(CO)_4$] by oxidative addition of H_2 to [$Os(CO)_5$] *via* the intermediate[$Os(CO)_4$].

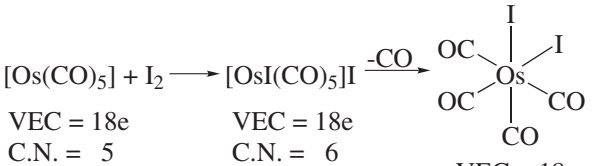


VEC = 18e
C.N. = 5
O.N. = 0

VEC = 16e
C.N. = 4
O.N. = 0

VEC = 18e
C.N. = 6
O.N. = +2

In some cases, a coordinatively saturated metal centre can cleave a X-Y bond *via* a labile, salt-like intermediate as shown below.



VEC = 18e
C.N. = 5
O.N. = 0

VEC = 18e
C.N. = 6
O.N. = +2

VEC = 18e
C.N. = 6
O.N. = +2

1e-Oxidative addition reactions are also known, where two metal centres ($2M^{n+}$) are involved with one molecule (X–Y).

$$2M^{n+} + X\text{–}Y \rightleftharpoons X\text{–}M^{(n+1)+} + Y\text{–}M^{(n+1)+}$$

This type of reaction particularly occurs with 17e-complexes. *e.g.*

$$2[Co(CN)_5]^{3-} + MeI \rightarrow [MeCo(CN)_5]^{3-} + [ICo(CN)_5]^{3-}$$

Problems

1. Draw the structure of the product formed due to oxidative addition reaction of H_2 to *trans*-[IrCl(CO)$(PPh_3)_2$].
2. What structural changes would you expect for a 2e-oxidative addition process of a d^8 metal centre? Explain giving an example
3. The d^{10}-complex $[Pd(PPh_3)_4]$ undergoes 2e-oxidative addition reaction with Br_2 to give a four coordinate neutral complex **A**. Draw the possible structures of **A**.
4. $[(\eta^5\text{-Cp})Ir(PMe_3)_2]$ (VEC=18e) undergoes oxidative addition with MeI to give a saturated salt **B**. Draw the structure of **B**.

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