NZIC Chemistry IN NEW ZEALAND

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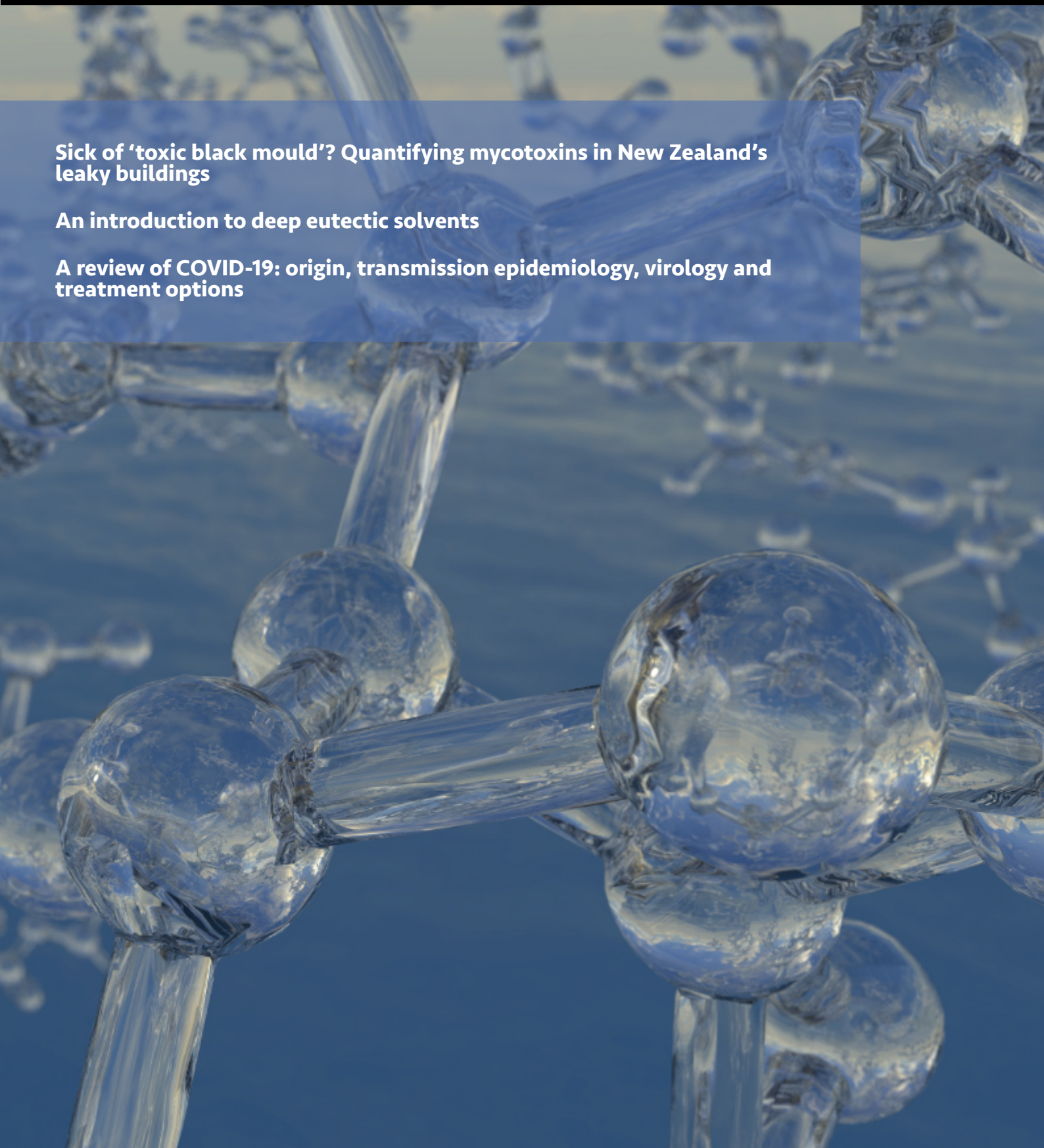

Sick of 'toxic black mould'? Quantifying mycotoxins in New Zealand's leaky buildings

An introduction to deep eutectic solvents

A review of COVID-19: origin, transmission epidemiology, virology and treatment options

Published on behalf of the New Zealand Institute of Chemistry in January, April, July and October.

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NZIC Chemistry IN NEW ZEALAND

Volume 85, No.1, January *2021*

Articles and features

Other Columns

Comment from the President

Kia ora and greetings!

I bid you all a hearty welcome to the beginning of a new year and to the first issue of *Chemistry in New Zealand* for 2021. In writing this first column as the incoming President, it is fitting that we acknowledge the tireless efforts of our past president, Associate Professor Sarah Masters, over the past two years in promoting the discipline of chemistry in her role during 2019 and 2020 and in spite of the COVID-19 challenge presented to us in 2020. I look forward to further working with her this year on the Executive as I take up this role and hope to benefit from her experience and advice. It was also the first time we had a president serving a two-year term, a change advocated by a past NZIC President (Professor Penny Brothers) approximately 3 years ago.

It would be completely remiss of me not to mention how 2020 has been a harrowing year for all of us in regard to the far-reaching impacts of COVID-19 and the associated lockdown in New Zealand (and overseas) on all of our lives, on businesses, research institutes as well as all branches of education and many other aspects of life.

Looking back to that year (and especially in the lockdown), it all seemed somewhat surreal. Many of us working in education had to adapt to a new way of teaching which shunned personal face-to-face contact and instead embraced online learning. In the secondary school sector, I have heard that schools across the country experienced mixed impacts in terms of their decile rating and the variable experience of staff in using any supplied electronic devices to teach students. In universities, we needed to engage entirely with online teaching methods or blended ones when the alert levels subsequently went down. As well as forcing us to learn new skills, it also increased workloads and made us more aware of some of the perils posed to academic integrity when conducting exams "online". For chemistry students pursuing degrees which contained some research element, the lockdown meant a complete denial of access to the all-important laboratory or instruments which are necessary for generating research results. Some of our international PhD students who happened to leave the country prior to our borders being closed were also denied entry back in, effectively putting their degree programmes into limbo. This impact of restricted human movement has taken a big hit on many secondary and tertiary institution budgets with obvious consequences on employment. Although the border closures are an effective and necessary tool in keeping COVID-19 at bathere will always be that inevitable sense of limbo as we wait for a return to some sense of "pre-COVID" normality.

In terms of the New Zealand Institute of Chemistry, COVID-19 restricted many of our activities branch-wise and "globally". We had to Zoom meet for our Council and Executive meetings after the late March/April lockdown. Any social activities within branches, especially during lockdown, had to be cancelled, drastically curtailed or postponed. The most significant effect for us perhaps was the postponement of the Hawaii-hosted Pacifichem conference that would have taken place in December 2020 but is now planned for December 2021 and where the assumption is being made that borders and travel might become a little freer to facilitate travel overseas. If it transpires that

we do have a return to pre-COVID normality for travel, it would be worthwhile for members to consider sending in an abstract to this. As a participating chemical society, we derive some partial financial benefit from the registration fees paid and of course it would be extremely nice to attend a face-to-face conference again!

Of course with Pacifichem scheduled for the end of 2021, we also had to make the decision to push the NZIC National Conference out to 2022 to avoid competing events being on at the same time. This will be hosted by Auckland branch.

What do we have planned for 2021? There was much discussion in 2020 meetings about seeing the *Chemistry in New Zealand* journal go to a more digital format, certainly as we move headlong into an increasingly digital age. Of course there are still some who prefer the hard copy form of the journal. The appointment of Raoul Solomon as our new publishing editor is to go a long way into achieving that digital future for the journal. Already we have seen some interesting design changes that may come our way soon in the journal. I would also like to acknowledge the fantastic efforts of our present Editor, Dr Catherine Nicholson, in preparing our quarterly editions of the journal.

I would also like to acknowledge the fine efforts of Joanna Dowle who, as Sarah mentioned in her last column, resigned from her role as administrator last year. Her help in bringing me up to speed with the timetable of events for NZIC has been invaluable and I wish her well in her new role. I am pleased to announce the appointment of Samantha Eason as our new administrator. Samantha started with NZIC in November and you can read a little about her in this issue. I am also grateful to Hamish McDonald for keeping our accounts in order.

I will close by saying that hopefully we will return to normal activities in 2021 and I will look forward to travelling to your branches over the course of my two year period as President to meet you all.

Noho ora mai

Michael Mucalo

NZIC President

Biographical Note

Michael Mucalo is an Associate Professor at the University of Waikato. He obtained his Bachelors, Masters and PhD degrees in chemistry from the University of Auckland under the supervision of Professor Ralph Cooney (1989-1991) where he carried out spectroscopic and microscopic studies of surface processes/adsorption on colloids and electrodes. In 1991 he was one of the first recipients of the Foundation for Research, Science and Technology (FRST) post-doctoral fellowships which he held in the former Inorganic and Materials Chemistry Division at Department of Scientific and Industrial Research (DSIR) Chemistry at Gracefield, Lower Hutt where he studied ceramic coatings formed from preceramic polymer pyrolysis. During that time he witnessed the birth of the Crown Research Institutes in New Zealand as "DSIR Chemistry" became "Industrial Research Limited". Subsequently he was fortunate to get a Japanese Science and Technology Agency (STA) postdoctoral fellowship that was held in the National Industrial Research Institute of Nagoya where he became interested in calcium phosphate chemistry from a biomaterials viewpoint. He was offered a lectureship in the then Chemistry Department at the University of Waikato in 1995 where he has been since, becoming an Associate Professor in 2015. His research interests have spanned broad areas such as colloid chemistry, biomedical materials, IR spectroelectrochemistry, controlled release drug delivery, and repurposing of waste materials. He has also had extensive collaborations with industry. Michael has been on the NZIC Waikato branch committee since 1997 as treasurer and has also had stints as branch president.

AUCKLAND

The University of Auckland

EVENTS

School of Chemical Sciences Research Innovation Showcase
In November the annual SCS Research Innovation Showcase was held, with a varied range of research from the school on display.

Faculty of Science Poster Competition
Also in November, the Faculty of Science held its Poster Competition: Show & Tell.

Royal Society Te Apārangi Video Competition
Sponsored by Royal Society Te Apārangi and MBIE, 180 Seconds of Fascination is a film competition for early career researchers to showcase their work. Dr *Joel Rindelaub* entered the contest, submitting a video titled, In the air tonight: https://www.youtube.com/watch?v=nO5VKxSFxEI

A still shot from Joel Rindelaub's entry into the 180 Seconds of Fascination video competition

School of Chemical Sciences Seminars

Inaugural lecture
Professor Bob Anderson presented on, A radical chemist's ventures into preclinical cyclic research. Professor Anderson established the Free Radical Research Facility for teaching and research, which has become a regional resource within the Australian Institute of Nuclear Science and Engineering (AINSE). His major research continues in the study of fast radical reactions underlying mechanistic aspects in the development of bioreductive anticancer drugs at the Auckland Cancer Society Research Centre.

Sarah Hillary (Principal Conservator, Auckland Art Gallery Toi o Tāmaki) gave a talk entitled, Beneath the surface: painting conservation research.

NZIC Auckland Branch Seminars
The NZIC Auckland Branch Seminar was delivered by Associate Professor Sarah Masters. Sarah gave the NZIC Presidential Address entitled, Resilience and opportunity in career pathways.

STAFF SUCCESSES

Hercus Fellow
Dr *Michel Nieuwoudt* has been awarded a four-year Sir Charles Hercus Fellowship for a project entitled, Photonic device for real-time measurement of ischaemic tissue margins in surgery. Michel will undertake this project with a team of researchers including *Cather Simpson*, *Hannah Holtkamp* and *Claude Aguergaray* from SCS, together with Professor John Windsor, Dr Michelle Locke, Associate Professor Sanjay Pandanaboyana, Dr Thom Minnee and Dr Marco Bonesi.

Michel intends to combine her expertise in developing photonic tools with the expertise of her surgical collaborators to deliver a photonic device that will enable surgeons to identify and remove mm-regions of any oxygen-starved (ischaemic) tissue during surgery.

Maurice Wilkins Prize for Chemical Science
Geoff **Waterhouse** won the Maurice Wilkins Prize for Chemical Science. This is the top research prize awarded by the New Zealand Institute of Chemistry (NZIC), and the winner is selected on the basis of the excellence and impact of their chemistry.

Dr *Leandro Dias Araujo* has recently been appointed as a lecturer in wine chemistry at Lincoln University in Canterbury. Leandro completed his PhD on Sauvignon blanc aroma and harvesting issues at Auckland under the supervision of Professor *Paul Kilmartin*. Since completing his PhD in 2017, Leandro has worked in the School on further wine projects as a research fellow. The most notable has been the NZ Winegrowers/MBIE-funded Pinot noir programme over the past two and a half years, where he has developed new methods to characterise tannins in New Zealand's leading red wine.

Jakob Gaar and *Margaret Brimble* published a review on Enzymatic and non-enzymatic crosslinks in collagen and elastin and their chemical synthesis (http://xlink.rsc.org/?DOI=D0QO00624F), written in collaboration with Rafea Naffa from the New Zealand Leather & Shoe Research Association (LASRA) in Organic Chemistry Frontiers and featured on the cover.

Dr *Alan Cameron* had his first senior author paper accepted for publication in Angewandte Chemie. The paper was co-authored with *Margaret Brimble* and *Paul Harris*. The paper reports a novel method of preparing allenamide-modified peptides – an unexplored functionality for peptides that provides a versatile chemical tool for effecting chemo-selective inter- or intramolecular bridging

reactions with thiols. This new method is a valuable tool that can be used to constrain peptides to adopt a defined secondary structure – a technique that chemists can use to improve the biological activity of potential new drug targets.

FUNDING

Marsden Grant

Jadranka Travas-Sejdic, *Tilo Söhnel*, and *Alan Cameron* were all successful as PIs in the Marsden Fund. Tilo received his grant for Skyrmion systems: new opportunities for Information technologies. Jadranka and *Eddie Chan* have a project entitled, A new approach to transient organic electronics. *Alan Cameron* received a Fast

The SCS Research Innovation Showcase featured a wide range of engaging presentations

Start grant for his project, A "self-bridging" approach to antimicrobial peptides: disulfide replacement and peptide stapling.

Ivan Leung is an AI on a Marsden grant awarded to Naresh Singhal in the Faculty of Engineering for the project, Exploiting the ancient microbial response to reactive oxygen species to degrade persistent emerging contaminants.

Science in Society Seed Funding
Dr *Joel Rindelaub* was awarded $5,000 seed funding from the Science in society theme for a student-led investigation of airborne plastic pollution in conjunction with Manurewa High School.

MBIE platform grant
A team lead by *Margaret Brimble* was awarded a 5-year $9.2M MBIE platform grant to study new generation peptide antibiotics. The team includes *Paul Harris* and Ghader Bashiri at UoA, together with colleagues throughout New Zealand including at VUW, University of Canterbury, and University of Otago. Their aim is to combine synthetic chemistry, chemoenzymatic synthesis, genomics and synthetic biology to develop and deliver niche lipopeptide antibiotics.

STUDENT SUCCESSES

PhD Completions
Congratulations to the following students on their successful PhD defences:

Martijn Wildervanck defended his thesis at his oral exam on 13 October. Martijn's thesis is entitled, Synthesis, characterisation and DFT calculations of saccharide-BODIPY conjugates. The project and Martijn's stipend were supported by the Marsden Fund, and his work was supervised by *Penny Brothers* and *David Ware*.

Rasangi Sabaragamuwa defended her PhD thesis, Phytochemical profiling and investigation of bioactivities of a New Zealand grown chemotype of Centella asiatica (Gotukola) – a potential neuroprotective herb. Rasangi was supervised by Professor *Conrad Perera* and co-supervised by Associate Professor *Bruno Fedrizzi*. Coincidently, she was also appointed the Dean of the Faculty of Applied Sciences with a staff of over 100 academic and professionals at The University of Sabaragamuwa, Sri Lanka on the same day.

Naasson Mbenza defended his PhD thesis, Studies on the modulation of the enzymatic activity of hypoxia-inducible factor hydroxylases on 13 October. Naasson was supervised by Dr *Ivanhoe Leung* and co-supervised by Professor *Christian Hartinger*. Naasson studied the inhibition and activation of the human oxygen sensing enzyme by gasotrasmitters, organic molecules and metal ions. Naasson co-authored two publications during his PhD, with a couple more in the pipeline. Naasson will be moving to Wellington for his postdoctoral research soon.

PhD Student Prizes
Thuy *Trang Pham* was named on the Dean's List. This is an excellent achievement, with fewer than 30 PhD graduates from all of the University being named to the Dean's List last year. Trang was supervised by *Jon Sperry* on the project, Synthetic applications of the chitin-derived platform 3-acetamido-5-acetylfuran (3A5AF).

The SCS Research Innovation Showcase included student poster presentations and two-minute talks by selected PhD students. The two-minute talks prize winners were Xuan Don (1st), Lewis Green (2nd), and Kapish Gobindlal (3rd). The poster prize winners were Sunandita Ghosh (1st), Fearghal Walsh (2nd), Kapish Gonbindlal (3rd).

Two members of the *Brimble* peptide group were awarded prizes at the School of Biological Sciences Research Showcase last week: Oscar Shepperson was the winner of the student talks and Juliana Tong was runner-up.

Auckland University of Technology

NEW FACES

Cailin Carmichael will be doing a summer studentship under the supervision of Dr *Cassandra Fleming*. Cailin will be working on Development of fluorescent caging groups with improved aqueous solubility.

Olivia Matich was also awarded a summer studentship and will be working with Dr *Jack Chen* on a project entitled, Smart materials – nanoscale containers programmable by light.

EVENTS

Dr *Jack Chen* gave a virtual seminar entitled, Applying concepts from nature for the design of dynamic catalyst systems as part of the MacDiarmid Institute Research Seminar series.

Dr *Cassandra Fleming* gave a seminar at the University of Auckland entitled, Development of light-responsive entities as molecular tools to probe dynamic biological functions.

AUT hosted the NZIC Auckland Branch AGM, an event combined with the presidential lecture by Associate Professor *Sarah Masters* entitled, Resilience and opportunity in career pathways. Thanks, Sarah, for the visit and braving us unruly Aucklanders (elbow bump).

CONGRATULATIONS

Dr *Cassandra Fleming* was awarded a Marden Fast Start for the project, Light-responsive drug delivery systems to probe dynamic biological functions. Cassandra will be developing new light-responsive drug delivery systems to control when and where drugs activate their therapeutic activity. Congrats Cassi!

Professor *Allan Blackman* published an article in Dalton Transactions entitled, Five-coordinate complexes and the value of τ_5. It also made the inside back cover of the issue in which it was published. The illustration is yet another fantastic piece of art from Michael Crawford.

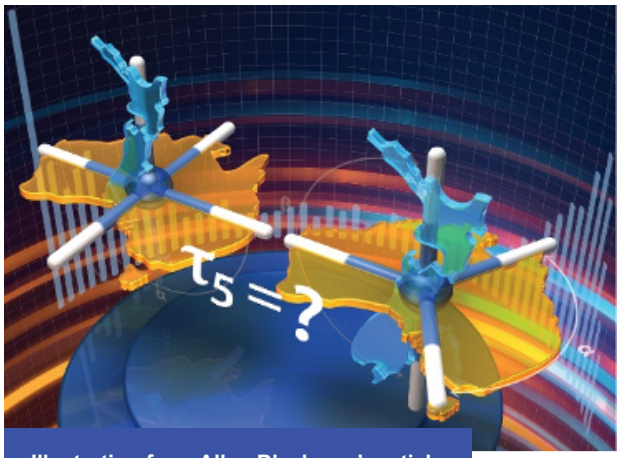


Illustration from Allan Blackman's article

The podcast Elemental is currently getting 300-450 downloads per day, nearly 2 years since the start of the International Year of the Periodic Table, and has totalled 200,000 downloads. It can be found at:
https://www.radionz.co.nz/programmes/elemental

CANTERBURY

Professor Ian Shaw is UCSA's Science Lecturer of the Year 2020
Some of the most inspiring and dedicated staff from the University of Canterbury have received awards in the UC Students' Association 2020 Staff of the Year Awards celebration. Students from all over campus voted on their most enthusiastic and dedicated lecturers, as well as non-academic staff who have also gone above and beyond their role as they support students. The nominator said, "Despite the pandemic, Professor *Ian Shaw* was lovely in every regard and managed to create a wonderful sense of community among his students. He brought enthusiasm to every lecture and empowered students with the hope for the future. He always replies quickly with a detailed answer to students' issues and then relays it to the rest of the class."

PhD successfully defended
Ting Wu successfully defended her PhD thesis on 13 October. Ting's thesis was titled, *Covalent carbon surface modification with iron porphyrin: application to oxygen reduction reaction*, and she was supervised by *Alison Downard* (senior supervisor) and *Chris Fitchett* (co-supervisor). The viva voce exam took place over Zoom and Ting was examined by Professor *Penny Brothers* (ANU). Professor *Richard Webster* (Nanyang Technological University, Singapore) was the external examiner.

In Loving Memory: Russell Wayne Gillard
26 March 1945 – 6 October 2020
Russell Wayne Gillard was born in 1945. He lived in Rex Street, Riccarton, no more than 2 km from Ilam campus but at that time Blenheim Rd had not been built and the Mall was paddocks! He attended Christchurch Technical College (now the Polytech) and started making deliveries after school. Russell's first job after leaving school was delivery work for Farmers and at about the same time he started riding motor cycles.

On 15 May 1961, Russell started in the chemistry department. At that time the mechanical workshop was located in the attic of the old Chemistry Building at Canterbury College (now the Art Centre). The mechanical workshop staff at that stage consisted of Dick Nokes and Russell. When the Engineering School moved to the Ilam campus, the workshop was moved to the other side of the town site near the Worcester St (lecture Theatre D).

The department moved to Ilam in 1966 and the number of technicians increased, as did the amount of equipment and floor space. Heavy equipment was located on level 1, with the apparatus workshop occupied by Russell on the west side of level 5 and the glassblowers and dark room in the area now occupied from 530 to 536.

Russell ran the chemistry department liquid air plant which was located in the glassware store. During this time Dick Nokes and Fred Downing were always delighted to see Russell in one piece on a Monday morning. Over the years of motorcycle racing he suffered from broken ribs, a broken collar bone, broken fingers and a broken toe (that was from working in our basement). He managed to never break an arm or leg. When the chemistry department library moved it freed up space on level 1 and the mechanical workshops were combined into one on the east side of level 1. By this stage mechanical workshop numbers had risen to 5 and electronics also had 5.

In 1985 Reg Dalley retired. Russell took over as head of the workshop and gave up motor bicycle racing. Russell retired from UC in December 2008.

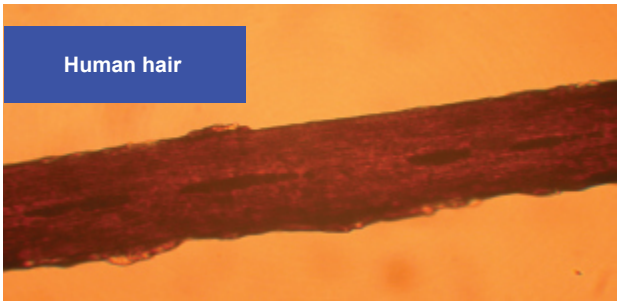
NZIC
Darren Saunders (Food Chemistry Laboratory, ESR Christchurch) gave a presentation on 7 October entitled, *Food forensics: taints to tails, mice to mould*. This was a review of the
food forensics techniques used to investigate safety, quality and aesthetic issues concerning our

food. This presentation touched on the methods used to investigate unwanted foreign matter, apportion legal liability, hunt down the source of taints, determine authenticity and appraise aesthetic issues that affect the food we eat with reference to specific (often gross) examples. The seminar was well attended and generated many questions.


Mouse in frozen vegetables

MANAWATŪ


Mouse tail in toast


Human hair

Leonie Etheridge successfully defended her PhD thesis entitled, *The synthesis and chemistry of [2.2]paracyclophane peptide derivatives*. Leonie was supervised by Associate Professor *Gareth Rowlands* and Professor *Paul Plieger*. She has since started a position as a growth and innovation chemist at Hexion, Tauranga. The *Rowlands* and *Plieger* group would like to wish her the best for her future endeavours.

David Perl successfully defended his PhD thesis entitled, *Hetero-interpenetrated metal-organic frameworks: supramolecular interactions between ligands in metal-organic framework formation*. David was supervised by Professor *Shane Telfer* and Professor *Geoff Jameson*. He has since started a postdoctoral position at SOLEIL, located in Paris. David will be working on new approaches for MOF crystallography.

Fareeda M. Barzak successfully defended her PhD thesis entitled, *Biophysical and biochemical characterisation of DNA-based inhibitors of the cytosine-mutating APOBEC3 enzymes*. Fareeda was supervised by Associate Professor *Vyacheslav Filichev*, Dr *Elena Harjes* and Professor *Geoff Jameson*.

Associate Professor *Catherine Whitby* was invited to give a keynote seminar on *Protein adsorption at the oil-water interface: effect of complexation with polysaccharide*, at the online Australia Japan Colloids Symposium 2020.

On 22 October the APOBEC3 team consisting of Associate Professor *Vyacheslav V. Filichev*, Dr *Elena Harjes* and Professor *Geoff Jameson* presented their recent work on powerful inhibitors of DNA-mutating APOBEC3 enzymes. The work presented is part of a series of webinars APOBEC3 International Translational Working Group. This group was established last year by Professor Reuben S. Harris (University of Minnesota, USA) to translate discoveries made in the APOBEC3 field into clinical reality.

The Massey-Victoria Chemistry Symposium was held on 6 November at Victoria University of Wellington (VUW). *Sidney Woodhouse* of Massey University, along with *Tehreema Nawaz*, *Hellen Nalumaga* and *Matt Brett* from VUW served as organisers. The event featured 8 presentations from students covering a diverse range of research. *Tehreema Nawaz* was awarded the Best Talk Prize for her presentation entitled, *Ferromagnetic* $Ni_{1-x}Fe_x$ *nanofibers produced by electrospinning*. *Bernhard Auer* of the *Telfer* group at Massey spoke on the *Design of novel porous catalyst* and was awarded the best PhD talk. *Suraj Patel* of the *Rowlands* group received the prize for the best 2nd year MSc presentation titled, *Synthesis of a planar chiral foldamer from [2.2]paracyclophane*. *Georgia Richardson* of VUW was awarded the top BSc Hons /1st year MSc / PGDipSci presentation for her talk entitled, *Catalytic nucleophilic alkylation of benzene*.

On 3 November, Associate Professor *Sarah Masters* visited Massey University to present the NZIC presidential talk. Sarah gave an inspirational talk on resilience and opportunity in her career pathway, highlighting the challenges she has had to overcome as well as the battles she has won.

During Sarah's visit, we had the pleasure of celebrating *Barry Scott's* contribution to NZIC as he became an Honorary Fellow of NZIC for his meritorious service to the Manawatū branch.

Georgia Richardson (left) receives her award from Associate Professor Vyacheslav Filichev for the Best Talk in the BSc Hons / 1st year MSc / PGDipSci category

Bernhard Auer (right) receives his award for the best PhD talk from Associate Professor Vyacheslav Filichev

Tehreema Nawaz (left) receives the Best Talk Prize from Associate Professor Vyacheslav Filichev

Suraj Patel (left) receives his award for the best 2nd year MSc talk from Associate Professor Vyacheslav Filichev

Barry has served as a key member of our branch for 21 years, holding several leadership roles including chair of the Manawatū branch, trustee, and trade representative on the NZIC conference organising committee. During his time, he has made a vital contribution by organising industry sponsorship for NZIC awards, events, and sponsorship for the annual student recruitment events run by our branch. On behalf of our branch, we would like to congratulate and thank Barry for his dedication and work for NZIC. A dinner at Little Savannah was attended by students, staff, and branch members joining Sarah and Barry to end the eventful day.

Associate Professor Sarah Masters (left) awards Barry Scott an Honorary Fellowship of the New Zealand Institute of Chemistry

The Massey-Victoria Chemistry Webinar series continued with Austin Evans giving a presentation on 11 September. Austin is a PhD student attending Northwestern University,

Students, staff and Manawatū NZIC members attending dinner at Little Savanah after the NZIC presidential talk

USA, under the supervision of Professor William Dichtel. His talk titled, *Controlled polymerization and emergent properties of 2D covalent organic frameworks* discussed the synthesis of colloidal 2D covalent organic frameworks and their operative mechanisms, investigating a range of emergency properties and first-generation devices based on these materials.

Elizabeth Chernysheva has joined the *Filichev* group as part of a summer scholarship and will be working on a new generation of APOBEC3 inhibitors.

Braydon Nikolaison has joined the *Whitby* group as part of a summer scholarship. He will be investigating the interfacial properties of proteins and polysaccharides.

Liam Mowbray has joined the *Waterland* group as part of a summer research course and will be working with Associate Professor Keren Dittmer (School of Veterinary Sciences) on Raman analysis of the effects of nutritional deficiencies on bone disease in cattle.

OTAGO

University of Otago, Department of Chemistry

PhD student *Ioan Fuller* and *Nigel Perry* attended the East Otago Taiāpure 11th Annual Research Evening at Puketeraki marae. This was a well-attended outreach evening, communicating research projects (mostly marine science) to the community. Ioan outlined his planned work on local kai mātaitai (shellfish) phospholipids.

Courtney Ennis has been selected to sit on the ANSTO Australian Synchrotron User Advisory Committee (UAC). In this role, Courtney will represent users' experience and encourages any users (or potential users) of the synchrotron to contact him with any issues or if they are wondering whether the synchrotron might suit their research needs.

Jaydee Cabral was an invited speaker at the *Bioengineering at Otago* showcase where she gave a talk entitled, *Advanced scaffold fabrication for regenerative tissue applications*.

Jaydee Cabral presenting at the Bioengineering at Otago showcase

At the recent 2020 Blues and Golds awards ceremony, *Sriram Sundaresan*, a final year PhD student in Brookers Bunch, was awarded a University of Otago OUSA Gold for "Outstanding member of the Dunedin community", in recognition of his contributions and service to the Dunedin Hindu and Tamil communities. There has no doubt been a large amount of support needed for many of our communities this year and Sriram's award is very much deserved. Well done, Sriram!

Sandhya Singh, working in Brookers Bunch, has just submitted her PhD thesis, *Spin crossover in iron(II) dinuclear helicates and tetranuclear cages*, so was given a chocolate fish, and the official mallet to ring the ceremonial University bell. Great job, Sandhya!

Sriram Sundaresan with his Gold award for services to the Dunedin Hindu and Tamil communities at the recent OUSA Blues and Golds awards

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Sandhya Singh and other Brookers Bunch members

Sandhya ringing the ceremonial bell for PhD submissions

Sally Brooker has assembled, and is still growing, "team NZ" on green hydrogen as she looks to establish a NZ-Germany Green Hydrogen Alliance with activities ranging from fundamental to applied and industrial with economic benefits to NZ. She recently travelled to visit Robert Holt and his team at Callaghan Innovation to check out a household-sized electrolyser for producing green hydrogen from water.

Anna Garden's group recently travelled to Whakapapa Village for the MacDiarmid Institute's Cluster Hui on Nanoclusters. After a year of virtual meetings it was great for everyone in the group to get a chance to give a talk and meet with researchers around the country. Special mention goes to *Frank Mackenzie*, *Ciaran Ward* and *Sam McIntyre* for their first (and very successful!) conference presentations. Thanks to *Elke Pahl* (Auckland), *Charlie Ruffman* (Otago) and *Alex Smith* (Auckland) for organising such a great meeting!

Sally Brooker with the Hylink household-sized robust electrolyser which produces green hydrogen from water and is powered by sun and wind. It was developed by Robert Holt and his team at Callaghan Innovation and provides instant hot water for showers, taps and radiators as well as fuel for the BBQ (Photo: Robert Holt).

Charlie Ruffman, *Calum Gordon* (now at VUW) and *Anna Garden* published a paper on understanding the mechanism of hydrogen evolution on MoS_2 catalysts in the *Journal of Physical Chemistry C*. Special congrats to Charlie for leading this work and to Calum for his first paper!

From the group of *Keith Gordon*, *Georgina Shillito* and *Joe Mapley's* paper on *Accessing a long-lived ^{3}LC state in a ruthenium(II) phenanthroline complex with appended aromatic groups* was published in *Inorganic Chemistry*. *Kārlis Bērziņš* and *Sara Miller* published several papers, most notably a review article titled, "*Recent advances in low-frequency Raman spectroscopy for pharmaceutical applications* in the *International Journal of Pharmaceutics*. *Chima Robert* published two papers on *Rapid discrimination of intact beef, venison and lamb meat using Raman spectroscopy*" in *Food Chemistry*, and *Diagnostics of skin features through 3D skin mapping based on electro-controlled deposition of conducting polymers onto metal-sebum modified surfaces and their possible applications in skin treatment* in *Analytica Chimica Acta* recently.

KCG group members have been busy presenting and participating in different virtual conferences and workshops. *Sara Miller* gave an invited tutorial talk on *Biophotonics* and *Fatema Ahmmed*, *Samanali Garagoda Arachchige* and *Kārlis Bērziņš* gave talks respectively titled, *Raman and infrared spectroscopic data fusion strategy for quantitative analysis of commercial krill oil*, *Vibrational spectroscopic assessment of consolidated paru dyed harakeke fibres: does consolidation protect from further degradation?* and *Qualitative and quantitative vibrational spectroscopic analysis of macronutrients in breast milk* at the Dodd-Walls Virtual Symposium at the end of the October.

The KCG group welcomed *Peter Remoto*, *Sam Harris*, *Joshua Kirkham* and *Quintin Jane* who joined the group as summer students. Peter will be working with Fatema on the study of micro plastics in fish, Joshua will be working with Sara Miller to develop and test a multi-spectroscopic probe while Sam and Quintin will be working with Joe to study a series of donor-acceptor dyes.

The Garden Group attendees at the 2020 MacDiarmid Institute Cluster Hui on Nanoclusters

Sara Miller has funding for a one year MSc thesis scholarship to work on a project developing a multi-spectroscopic probe for disease diagnosis. If you are interested please contact her (**sara.miller@otago.ac.nz**) or Keith (**keith.gordon@otago.ac.nz**) for more details.

Keith Gordon was part of the team of researchers led by Plant and Food Research who were successful in gaining an endeavour fund research program titled, *Cyber-physical seafood systems: intelligent and optimised green manufacturing for marine co-products*. Keith and Sara are part of the Dodd-Walls Centre CoRE which was recently refunded. Keith is also part of the Riddet Institute and MacDiarmid Institute CoREs which were also successful in the 2020 funding round.

In other news from Plant and Food Research, *John van Klink* had a paper entitled, *Taramea, a treasured Māori perfume of Ngāi Tahu from Aciphylla species of Aotearoa New Zealand: a review of Mātauranga Māori and scientific research* (Aaria Dobson-Waitere, Robin MacIntosh, Matapura F. Ellison, Bruce M. Smallfield and John W. van Klink) accepted by the *Journal of the Royal Society of New Zealand*. This is a collaborative effort between Plant and Food Research, summer student Aaria, Robin at Te Rūnanga o Ngāi Tahu, Christchurch, and Matapura at Kati Huirapa Runaka ki Puketeraki, Karitane. John and Bruce are now working on the extraction and chemical analysis papers that supported commercial production by Ngāi Tahu: **https://meafragrance.co.nz**

WAIKATO

NZIC Analytical Chemistry Competition 2020

This annual event was held on 1 September. Schools in the wider Waikato/Bay of Plenty region were invited to send teams of four students to the university for the day to carry out an analysis. Due to postponement on account of Covid-19 lockdown, fewer schools chose to participate this year but there were still fifteen teams from twelve schools in this year's competition. Running the competition under Level 2 Covid-19 conditions presented some additional challenges for staff and students but all coped very well and the students clearly enjoyed the day.

The task was to analyse a sample of zinc sulfate using a gravimetric procedure for SO_4^{2-} and a volumetric method for Zn^{2+} and to use these values to determine how many water molecules were associated with each zinc sulfate molecule. "Students found the analysis particularly challenging this year, but the winning team produced results which were close to the actual values," said competition judge and key organiser, *Michèle Prinsep*.

The competition allowed enthusiastic Year 13 chemists to spend a day in the university laboratories working on an experiment that would be beyond the resources of their schools. Although competition was intense, the main emphasis was on enjoying the experience of working in a chemistry laboratory at the university and meeting students from other schools. The winning team received $240 and a trophy, with prize money also awarded to all other place-getters thanks to the generosity of the sponsors.

The day involved many of the chemistry department staff in setting up the competition and supervising the labs. Bryant Hall and Student Village provided excellent lunches (sponsored by the Waikato Branch of the NZIC). Hill Laboratories and the Waikato Branch of the NZIC generously donated the prizes.

Results were:

1st Prize: Aquinas College
(Janelle Danbar-Mair, Daniel Nirmalaraj, Elise Oxenham, Put Suthisrisinlpa)

2nd Prize: Tauranga Boys' College
(Lucas Cowley, Alex Hastie, Vijay Nirvaneshwar, Cohen Radich)

3rd Prize: Waihi College
(Trav Anderson, SJ Kleynhans, Madeline Midwinter, Brayden Shaw)

4th Prize: Tauranga Girls' College 1
(Talia Ingham, Olive Pearce, Tania Simpson, Courtney Smith)

5th Prize: Waikato Diocesan School for Girls 1
(Caitlin Davison, Danielle Gordon, Harman Kaur, Aliyah Thotahil)

Sarah Masters gave her presidential address, *Resilience and opportunity in career pathways* to the branch, which was well received and followed by drinks and nibbles.

University of Waikato

Nearly 150 students from 14 schools in the greater Waikato and Bay of Plenty region participated in the annual ChemQuest competition, held at the university. This was a fun-filled evening for students studying NCEA level 2 chemistry and a hard-fought contest. Prizes were awarded as follows:

1st Place: Tauranga Boys' College:
(Colby Butler, Peter Gedye, Grayson Moore)

2nd Place: Hamilton Boys' High School
(Robert Hoskins, Tebijan Kalarathan, Sankalp Lanka)

3rd Place: Waikato Diocesan School for Girls

(Jordana Bremner, Jaime Hayvice, Amelia Le Comte)

4th Place: Hillcrest High School
(Jessica Chan, Janet Gui, Joy Guo)

5th Place: Hamilton Girls' High School
(Alex Matai'a, Sophie Matai'a, Eden Miller)

The quiz was generously sponsored by the Waikato Branch of NZIC (major sponsor), Hill Laboratories and the School of Science, University of Waikato. The question master was *Michèle Prinsep*, ably assisted by numerous other chemistry staff and students.

Aquinas College, first place winners in the NZIC analytical chemistry competition.
From left: Janelle Danbar-Mair, Elise Oxenham, Put Suthisrisinlpa, Daniel Nirmalaraj.

Tauranga Boys' College, first place winners in the ChemQuest competition.
From left: Colby Butler, Peter Gedye, Grayson Moore.

ChemQuest winners, Tauranga Boys' College, with competition sponsors

Geoff Tait who worked with *Michael Mucalo* successfully defended his thesis on alumina gel vaccine adjuvants. Matthew Risi recently completed his Masters degree with *Bill Henderson* on the coordination chemistry of sulfonylthioureas.

Rose Swears completed her Masters project with *Merilyn Manley-Harris* on carbohydrate composition of honeydew honeys and is now working in her dream job at RocketLab.

WELLINGTON

The third triennial Halton lecture in honour of the late Professor Brian Halton was given by Professor *James Crowley* from the University of Otago. Professor Crowley gave a great talk on *Palladium(II) metallosupramolecular cages: self-assembly and molecular recognition*. Professor Halton always admired the work of Professor Crowley, thus this lecture was a perfect way to honour the legacy of Professor Halton.

This was a season of well-deserved prizes highlighting the research excellence of chemistry at Victoria University of Wellington. Congratulations to Professor *Justin Hodgkiss* and Dr *Kai Chen* on winning the NZIC Prize for Industrial and Applied Chemistry. This was awarded for their development of transient grating fluorescence spectrometry which allows for measuring fluorescence spectra on femtosecond timescales. The awards were handed out during Associate Professor *Sarah **Masters***' presidential address.

Professor *Jim **Johnston*** has won TWO KiwiNet Research Commercialisation Awards; the Baldwins Researcher Entrepreneur Award and

Professor Jim Johnston

the BNZ Supreme Award, both for "*a world-*

renowned inorganic and materials chemist focused on commercial outcomes." This is a superb award highlighting Professor Johnston's contribution to applied chemistry throughout his career at VUW.

The annual Massey-Victoria symposium was held at VUW on 6 November. Congratulations to Tehreema Nawaz for winning the Best Overall Talk and Georgia Richardson for winning the Best Talk in BSc category.

Associate Professor Sarah Masters presenting the NZIC Prize to Dr Kai Chen and Professor Justin Hodgkiss (along with his helpers)

Joe Bracegirdle obtained his PhD under the guidance of Associate Professor *Robert* ***Keyzers***. Dr Bracegirdle's thesis focused on the identification of bioactive natural products from NZ and Tongan marine organisms, and resulted in the discovery of new members of four different classes of metabolite. Congratulations, Joe!

Congratulations also to Dr *Nate Davis* for obtaining a Rutherford Discovery Fellowship for his research on *Pushing the limits on renewable energy technology through hybrid organic/inorganic nanomaterials*.

Congratulations to our Marsden Fund grant winners. Dr *Luke Liu* obtained Fast-Start funding for his research on *3D covalent organic frameworks: potential materials to break the porosity record?* Professor *Martyn Coles* and Associate Professor *J. Robin Fulton* have been funded for their research on *Activating substrates for chemical synthesis with reactive aluminium reagents*.

A warm welcome to our new NZIC Administrator!

We are very pleased to have our new NZIC Administrator, Samantha Eason, on board! Samantha joined us in November, replacing Joanna Dowle who has done a fantastic job of keeping our organisation running smoothly during her time with us.

Samantha is based in Christchurch where she lives with her young daughter and fiancé (who is a chemistry academic). She is an arts graduate from the University of Canterbury with a background in history, mass communications and anthropology. Samantha was the Senior Officer of Records, Examinations, and Graduation at UC for almost 10 years during which her main role was organising all aspects of the graduation ceremonies (approximately 70 events during her time in the role). Before that she held roles in the Postgraduate Office, Enrolments and the Library. She also did a 'Big OE' in her early twenties where she was based in Edinburgh and worked in Customer Service for the City of Edinburgh Council. In her spare time she plays the cello and is learning flamenco. Samantha is excited to have joined NZIC and looks forward to learning more about chemistry in New Zealand.

Book review: *Some Forgotten Chemists* by Brian Halton

Part of *Perspectives on the History of Chemistry* series, edited by Seth C. Rasmussen, Springer, Switzerland, 2020

Did you know that the scientist who developed Bakelite had earlier made his fortune by selling his photographic paper technology to Kodak? What about the famous Russian composer, known for the Polovtsian Dances and the opera *Prince Igor*, who was an organic chemist in his day job and at the forefront of chemical reaction discovery? Which chemist was seminally involved in the development of both gas lighting and detecting adulteration of food? These and many more fun facts can be found in this new book, *Some Forgotten Chemists*, by my late colleague, Professor Brian Halton.

Sixteen lesser-known chemists through the ages are featured in this book, which originated as a series of articles in *Chemistry in New Zealand* from 2013 to 2018 under the title *Some Unremembered Chemists*. Each chapter provides fascinating insights into their lives and their manner of working, as well as historical and societal anecdotes. We also find several chemists with links to New Zealand, namely Thomas Easterfield, Joseph Mellor and Philip Robertson. By definition, the great names of chemistry are not specifically featured here, but we see how the "forgotten chemists" that grace the pages were of eminent importance in shaping the scientific environment that enabled more famous discoveries, in training chemists such as Markovnikov and Zaitsev, and in discoveries and developments of their own that greatly benefitted industry, society and science.

The "forgotten chemists" are ordered in this book by surname, alphabetically. Therefore, reading the book cover to cover may be dissatisfying if the reader is expecting chronological progression. Nonetheless, the foreword specifically states that each chapter is intended to be standalone and I found that dipping in to read a chapter at a time was my preferred *modus operandi*. The inclusion of each chemist's name and lifetime dates in the header of every page is a helpful formatting feature that immediately reminds the reader of the context and historical setting as they go through. Informative illustrations of chemical structures, schemes, portraits and locations complement the text. The chapter lengths make reading one in a single sitting perfectly feasible. In this way we are provided a pleasant and informative interlude that educates us on chemists who contributed many fundamentals of our science.

Joanne E. Harvey
School of Chemical and Physical Sciences
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Sick of 'toxic black mould'? Quantifying mycotoxins in New Zealand's leaky buildings

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Keywords: *mycotoxin, analytical chemistry, leaky building, house dust, black mould*

Health in New Zealand's leaky buildings

The quality of our indoor environment is a significant determinant of our quality of life. As we spend around 90% of our lives indoors, these spaces naturally influence our wellbeing, with warm, dry homes free of noxious agents being conducive to good health.[1] Conversely, poor quality housing has been consistently linked with poor health outcomes, with over thirty years of research evidencing a robust positive correlation between living in cold, damp, mouldy housing and the development of respiratory disorders.[2-5] Such disorders result in 4 million premature deaths annually, yet despite widespread attention, the exact mechanism by which these environments give rise to respiratory illness remains to be elucidated.[3-9]

New Zealand has notoriously poor housing stock with regards to damp and mould. Unsurprisingly, this correlates with a high incidence of respiratory disorders, and in 2015 alone the wider cost of respiratory disease in NZ was estimated to be $7.05 billion.[1,10,11] Some of the worst examples of this housing stock have been exemplified through our 'leaky buildings' (or 'leaky homes') crisis, which developed through the construction of buildings with weathertightness issues from the 1990s through to the mid-2000s. Subsequent moisture ingress into timber framing and wallboard resulted in widespread fungal growth, with many anecdotal reports of respiratory & other illness that occupants believed to be linked to living in their now damp and mouldy homes.[1,12] The impacts of the resulting leaky building crisis range from the substantial economic losses, estimated to be over $11.3 billion to date, through to mental health, with affected homeowners being driven into suicide, marriage breakups, anxiety and depression.[1,12,13] It may seem clear that something in these houses is making people sick, but, as with the thirty-plus years of research into damp, mouldy housing and respiratory illness in general, exactly what agent or agents are responsible for such building-related illness (BRI) remains a mystery.

In studies of housing quality and respiratory health, the strongest correlations to illness are the presence of active leaks and dampness, the presence of visible mould, and the presence of mould odour.[2-5] It is unsurprising then that fungi and the bioactive secondary metabolites they produce – mycotoxins – continue to

be the one of the most often proposed and studied potential causal agents of BRI. In 2002, the landmark Hunn report commissioned by the NZ Government into the leaky homes crisis was published.[14] With concern surrounding the potential health effects of mould, it recommended an investigation into the health risks associated with fungal decay in these buildings.

In a survey of wet rots in NZ's water damaged buildings, the most commonly identified microorganism was the notorious *Stachybotrys chartarum*, which produces some of the most potent mycotoxins yet discovered – the macrocyclic trichothecenes (MCTs). Selected MCTs [satratoxins G & H (SATG & SATH), roridin E (ROE)] and the 'pendant' variant roridin L2 (ROL2) are shown in Fig. 1.[1,15,16] Depending on sampling methods and the source of data, *S. chartarum* in NZ's leaky buildings may be found in anywhere from 49% to 77% of water-damaged building materials, in 20–45% of air samples from indoor locations with 'elevated' spore counts (*c.f.* outdoor levels), and in 13% of tape-lift samples (targeted sampling of buildings with suspected mould contamination, although this is a poor method for detecting *S. chartarum*).[1,17] In light of the reports of illness, the particularly high potential toxicity of this mould and its high prevalence, a second major leaky homes inquiry commissioned by the NZ Government in 2003 specifically recommended an investigation into "the extent of the *Stachybotrys* problem".[18]

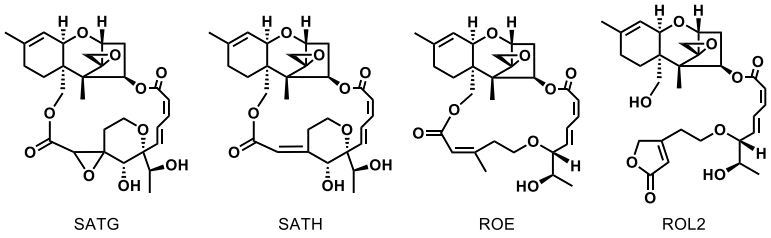


Fig. 1. Key trichothecenes (KTCs) produced by S. chartarum, including the MCTs satratoxins G & H and roridin E, and the non-macrocyclic roridin L2

Toxic black mould

S. chartarum (historically *S. atra*) came to prominence in the late 1980s, where it had been known as a contaminant of animal feed and was subsequently recognised growing in water-damaged buildings.[16,19-21] In agriculture the trichothecenes had earlier been established as the cause of stachybotryotoxicosis, an illness characterised by radiation sickness-like symptoms and sometimes leading to death in the animals and farm workers exposed to *Stachybotrys* contaminated hay.[19] The later notoriety of *S. chartarum* in a building context stems largely from its connection to a cluster of acute idiopathic pulmonary haemorrhage cases in infants in Ohio around 1993.[16,22] Initial investigations suggested that a high level of *S. chartarum* in the water-damaged homes of the infants may have been the cause of illness. However, expert review later described significant methodological shortcomings in the investigation, concluding that no causal link between mould infestation and the observed respiratory symptoms could be established.[16,19,20,22] Despite this critical review, the case lead to a public health hysteria concerning 'toxic black mould', resulting in a significant controversy around mycotoxin-induced BRI that continues to this day.[16,19,22-27]

Although space precludes an in-depth discussion of this controversy, a significant body of research has demonstrated both plausible routes of exposure *via* inhalation and established mechanisms of injury at achievable doses for *S. chartarum* in a BRI context. For example, toxigenic particles from *S. chartarum* can be aerosolised, are found in air samples of infested buildings, are respirable and contain mycotoxins.[28-32] Enzyme-linked immunosorbent assay (ELISA) has been used to detect both *S. chartarum* mycotoxins and antibodies to these toxins in exposed people, suggesting that they do enter the body, and of particular concern are the presence of these toxins on highly respirable sub-micron particles.[33-36] *S. chartarum* fungal fragments – pieces smaller than spores – may reach airborne concentrations over 500 times that of spores, and computer modelling suggests that respiratory deposition of these fragments may be over 200 times greater than that of spores.[29,37] Thus, finding spores on air sampling may indicate exposure to a relatively larger concentration of toxigenic particulates.

While studies suggest that the most potent MCTs are rapidly absorbed, distributed and metabolised, they tend to concentrate in specific cell types such as alveolar macrophages, and as the concentration of toxin at the site of deposition in the lungs will be greater locally than systemically, the environmental exposure required to cause local cellular injury will be lower than that required to achieve acute systemic toxicity.[25,38-40] Inhalation of both *S. chartarum* spores and fungal fragments induces pulmonary arterial remodelling in a murine model, while doses of MCTs based on toxin

concentrations in the air of contaminated buildings were found to cause damage and inflammation in neural cells such as astrocytes, neurons and those of the blood-brain barrier, which may further facilitate the infiltration of toxic substances generally into the central nervous system.[41-43] Intranasal installation of these potent MCTs causes olfactory neuron loss in both mice and monkeys, with repeated smaller doses of the toxins producing cumulative damage equal to or greater than the sum given as a single larger dose.[39,44] As it is established that chronic exposure to sub-acute-effect levels of mycotoxins *via* food can cause population-level increases in illnesses, so this research highlights the potential for harm from low-level chronic exposure to *S. chartarum* trichothecenes in the built environment.[45,46]

Despite this evidence, the effect of *S. chartarum* trichothecenes on respiratory health remains equivocal. Some research finds no significant effect from *S. chartarum* exposure, while trichothecenes are likely not the only component in *S. chartarum*, and fungi in general, that may contribute to such disorders.[47] For example, research indicates that proteinaceous components may also cause or contribute to the observed biological responses, and there may be a multi-factorial aetiology as in many diseases.[48,49] However the notoriety of the trichothecenes and the unanswered questions around their health effects demand more research in order to expose exactly what role they play in the health of our leaky building occupants.

Is toxic black mould making leaky building occupants sick?

In response to the recommendation of the Government inquiry, the relationship between the prevalence of *S. chartarum* mycotoxins in our leaky buildings and the adverse respiratory symptoms reported by occupants is being investigated.[50] To this end, a matched case-control study will be conducted comparing the levels of trichothecenes in house dust swabbed from surfaces and flooring against measures of specific respiratory symptoms in 100 defined leaky homes and 100 control homes. This research hypothesises that with the observed high prevalence of *S. chartarum* in our leaky buildings, low levels of respirable trichothecenes may be present with significant frequency, and in consideration of research demonstrating a plausible route of exposure and mechanism of injury, that low-level chronic exposure to these mycotoxins may be contributing to the prevalence of respiratory disorders reported by leaky building occupants. In order to conduct this study, a comprehensive measurement of the mycotoxins present is required, for which three analytical measures are proposed.

Firstly, the collected dust samples will be subject to a semi-quantitative screen for over 300 microbial secondary metabolites utilising high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) to give information about general microbial toxin exposure.[7] Secondly, the samples will be hydrolysed to convert the majority of trichothecenes produced by *S. chartarum* into the parent diol, verrucarol (VER, Fig. 2) which will be quantified by an ultra-sensitive, isotope-assisted, gas chromatography-mass spectrometry (GC-MS) method, giving a measure of 'total' trichothecene exposure. Thirdly, where trichothecenes are found, quantification of four key trichothecenes (KTCs) – SATH, SATG, ROE and ROL2 (Fig. 1) – will be completed by HPLC-MS/MS. These KTCs represent two of the most potent MCTs (SATH & SATG) and two chromatographically useful proxies for total trichothecene production (ROE & ROL2). While the initial wider microbial toxin screen will be conducted by collaborators in Vienna using established methodology, the research currently proposed here focuses on the development of the ultra-sensitive GC-MS method for quantification of total trichothecene

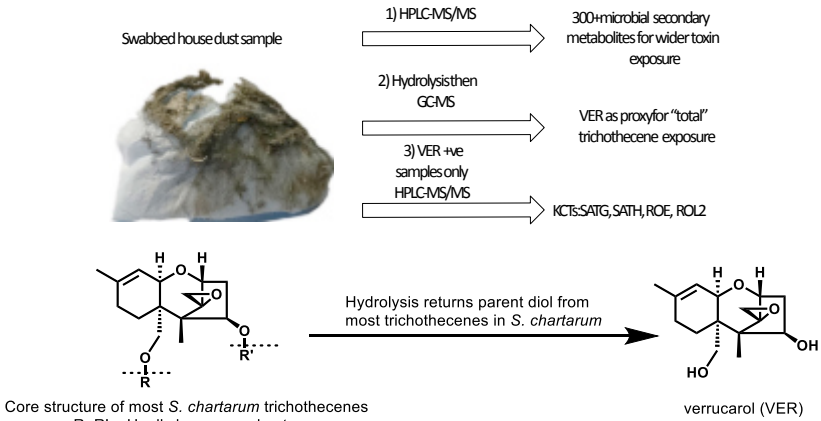


Fig. 2. Proposed analytical approaches to measurement of mycotoxins

exposure and the development of the HPLC-MS/MS method for the quantification of KTCs.[7]

Stachybotrys chartarum & the trichothecenes

S. chartarum has been proposed through chemotaxonomic and genomic analysis to comprise three taxa – *S. chartarum* chemotype (ct.) S, producing the highly potent macrocyclic trichothecenes (Fig. 1), *S. chartarum* ct. A, producing atranones and dolabellanes (Fig. 3), and *S. chlorohalonata*, which also produces atranones and dolabellanes but which was sufficiently different in morphology and genetics to have a unique classification proposed.[20,51-54] A recent genetic analysis of satratoxin and atranone producing *S. chartarum* has suggested that these can be represented by three genotypes; S, which contains the required gene cluster for satratoxin production but not for atranone production, A, which contains the gene cluster for atranone production but not for satratoxin production, and H, which contains the cluster for atranone production in addition to an incomplete set of satratoxin producing genes.[55]

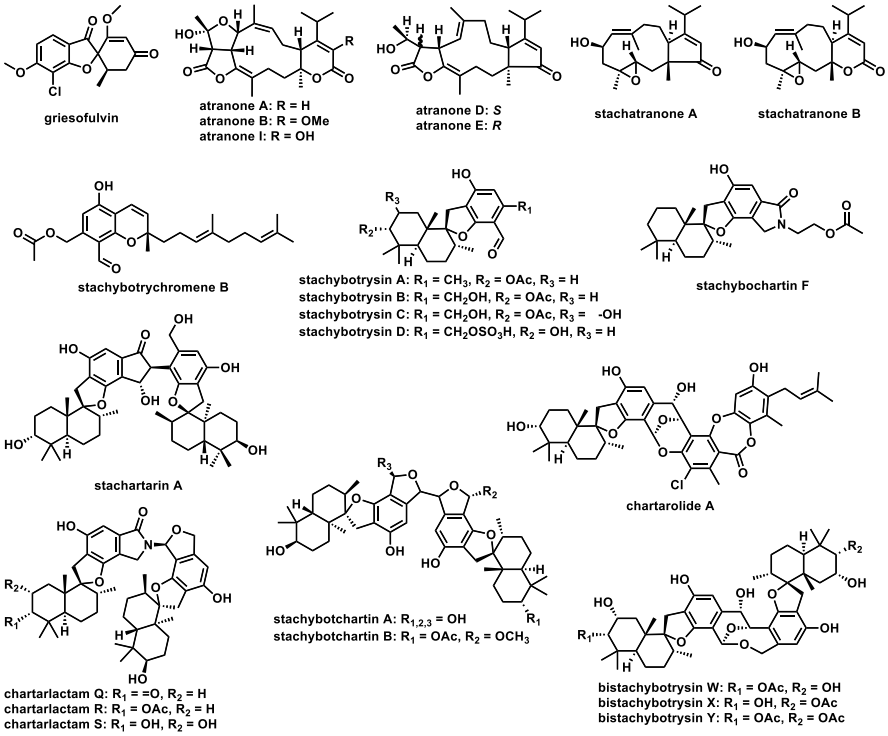


Fig. 3. A selection of non-trichothecene secondary metabolites produced by Stachybotrys

S. echinata (previously *Memnoniella*) grows in the same environments as *S. chartarum* and produces its own mycotoxin profile including simple trichothecenes but in which the metabolite distribution is dominated by griseofulvins (Fig. 3).[9,20,56] The true genetic diversity of *Stachybotrys* remains a matter of debate, and the plethora of compounds it produces is far from extinguished. It may currently comprise perhaps seventy-four species and novel bioactive compounds continue to be reported. These include the stachybotrychromenes and a large number of spirocyclic phenylspirodrimane derivatives; the stachartarins, stachytbotrysins, bistachybotrysins, stachybochartins, chartarolides and chartarlactams (Fig. 3).[56-63]

Over 200 trichothecenes have been identified from a variety of natural sources.[64] They are commonly grouped into four types, A, B, C and D, based on the functionalisation of the core 12,13-epoxytrichothec-9-ene (Fig. 4).[65] Type A trichothecenes are characterised by the presence of either hydroxyl or ester groups at C8, for example in T2, or by lack of substitution as in VER, diacetoxyscirpenol (DAS), trichodermin (TRDM) and trichodermol (TRID) (Fig. 4). Type B have a ketone at C8, for example nivalenol (NIV) and deoxynivalenol (DON), while type C have a C7-8 epoxide such as in crotocin (Fig. 4). Type D trichothecenes contain a macrocyclic ring structure between C4 & C15 as in SATG, SATH and ROE (Fig. 1).

The trichothecenes produced by *S. chartarum* include the type D MCTs; satratoxin F, SATG, SATH, isosatratoxins F, G & H, ROE, and verrucarin B & J (VEB, VEJ), and the 'pendant' type A trichothecenes – non-macrocycles esterified at one or both of C4 & C15; ROL2 (Fig. 1), trichoverrols A & B (TVLA, TVLB) and trichoverrins A & B (TVNA, TVNB) (Fig. 4).[56,66,67] Hydrolysis of the C4 and C15 esters in these trichothecenes returns the parent simple trichothecene diol – VER – the total synthesis of which, as the racemate, was first reported in 1982.[68,69] Other notable trichothecene producers include the agriculturally-relevant plant pathogens *Fusarium* and *Myrothecium*, a toxic mushroom from Japan, *Podostroma cornu-ame*, and the only known plant sources *Baccharis sp.* and *Holarrhena sp.*, although the source in plants may yet be found to be a co-occurring fungus.[64,67,70-72]

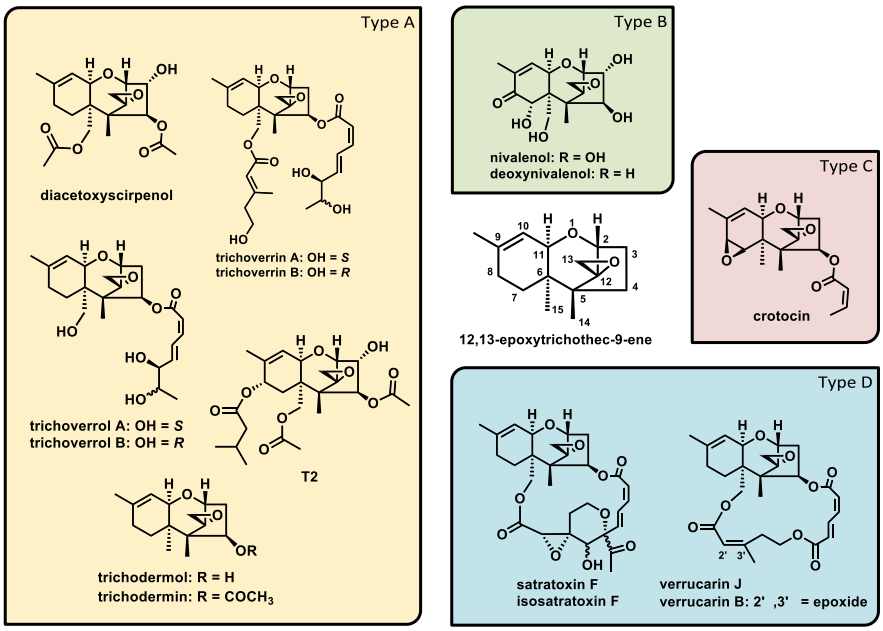
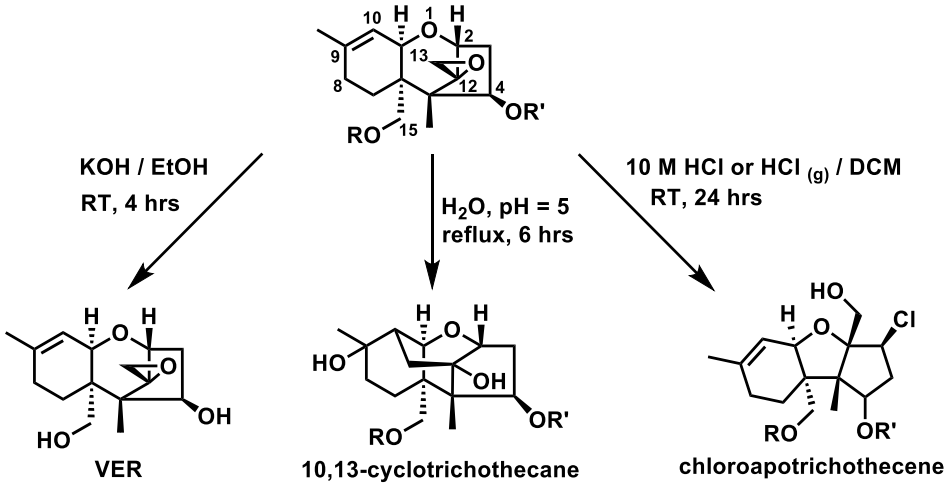


Fig. 4. Representative trichothecenes of each type. The stereochemistries of satratoxin F & its presumed epimer isosatratoxin F remain unresolved.[64]

The chemistry of the MCTs, focusing on the macrocycle, has been the subject of a comprehensive review by Grove (1993).[73] Key reactivities include the base-catalysed hydrolysis of the C4 & C15 esters and the acid catalysed rearrangements of the 12,13-epoxide (Scheme 1). In weakly acidic media, rearrangement gives a 10,13-cyclotrichothecane, presumably *via* protonation of the terminal 12,13-epoxide followed by attack by the 9,10-alkene to form a bridge with concomitant addition of water at the tertiary centre.[74] The propensity for the trichothecene core to undergo this rearrangement is modulated by the functionality of the ring system. For example, substitution of OR at C8 prevents this rearrangement, likely by reducing the nucleophilicity of the 9,10-alkene or by sterically hindering its attack on the epoxide. The presence of a 4,15-macrocycle also prevents such a rearrangement, probably through conformational effects. In strongly acidic media, *e.g.* 10 M HCl or HCl in DCM, an alternative acid-catalysed rearrangement can occur *via* attack of the 1,2-CO bond on the protonated epoxide to give an apotrichothecene.[75] In this scenario, attack from the 9-ene system is suspected to be prevented by its protonation, and the presence of a 4,15-macrocycle does not prevent rearrangement.[73]



Scheme 1. Representative base hydrolysis and acid-catalysed rearrangements of the core trichothecene structure

Matrix effects in mycotoxin analysis

The benchmarks for routine, sensitive and accurate quantification of mycotoxins in the environment are HPLC- & GC-MS based analyses. A major concern in their application is the presence of so called matrix effects (MEs); analyte signal enhancement or suppression caused by other components of the parent material the analyte was extracted from (the 'matrix'), which cause the instrument to over- or under-report the actual analyte concentration. MEs can be divided into two types – effects which affect the slope of a concentration / response curve (rotational effects), caused by other components of the matrix proportionally affecting the ionisation of the analyte, and those which affect the intercept but not the slope of a curve (translational effects) due to a high background / baseline interference (Fig. 5). Three main techniques are used to overcome matrix effects in quantification using MS: matrix-matched calibrations, standard addition, and stable-isotope labelled internal standards.[76]

Matrix-matched calibration uses an external calibration curve prepared in the same or a very similar matrix to the sample. In theory this applies the same MEs to both the calibration and analyte, but it does not correct for inefficiencies in sample extraction or losses during processing. The success of this approach is reliant on how well matched the calibration and sample matrices are, and it ideally uses both a blank matrix and a separate calibration curve for each unique sample matrix, a requirement which can significantly increase the number of analyses conducted when the matrices vary between samples. Standard addition in contrast attempts to correct for MEs by effectively creating the calibration curve within the sample through a series of standard additions of the analyte. In simple terms, by constructing a curve using the data of the known additions and calculating the slope, the background concentration of the analyte can be obtained (Fig. 6). This approach does not require a blank matrix but several standard additions are normally required for each sample, multiplying the number of samples to be analysed. Both of these methods still require considered application, as although they correct for rotational effects they do not necessarily correct for translational effects.[77] Such translational effects must additionally be managed through appropriate sample preparation.

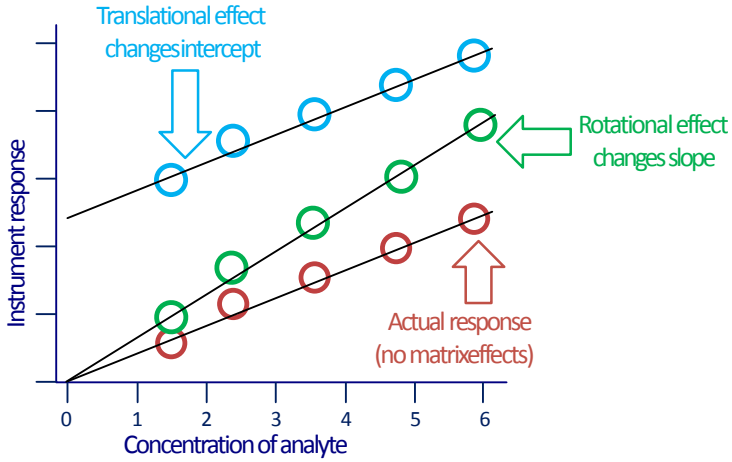


Fig. 5. Rotational & translational matrix effects

Adding a known amount of internal standard (ISTD) to samples before processing can overcome MEs if the ISTD has the same response to processing and instrumental analysis as the analyte. The ideal standard in this approach therefore has exactly the same chemical properties as the analyte. When MS is used as a detection method, this is possible by using a stable isotope (SI) labelled analyte as the ISTD – chemically identical, yet distinguishable upon MS detection due to its mass difference. This approach is known as stable isotope dilution analysis (SIDA, Fig. 7). As the amount of SI labelled ISTD (SI-ISTD) is known prior to analyte extraction, the ratio of SI-ISTD to analyte found *via* MS gives the analyte concentration with compensation for both MEs and processing losses.[76,78] SIDA represents the gold standard in the analysis of mycotoxins in the environment, generally giving excellent quantification of analyte recovery with a very low limit of quantification (LOQ) and limit of detection (LOD).[78]

The main challenges facing SIDA include the stability of labelling and isotope effects (IEs). For example, deuterium placed at labile sites can participate in $^{1}H/^{2}H$ exchange resulting in loss of label, while the mass difference of isotopologues can result in slightly different retention times in GC or HPLC, or changes in fragmentation patterns.[79] This effect is most notable with ^{2}H as opposed to with ^{13}C or ^{15}N labelling as the relative change in mass to the nucleus is significantly greater. IEs can be minimised by only introducing one or two labels, however sufficient difference in mass between analyte and SI-ISTD, ideally ≥ 3 Da, is desired to avoid spectral overlap in the mass spectrum resulting from

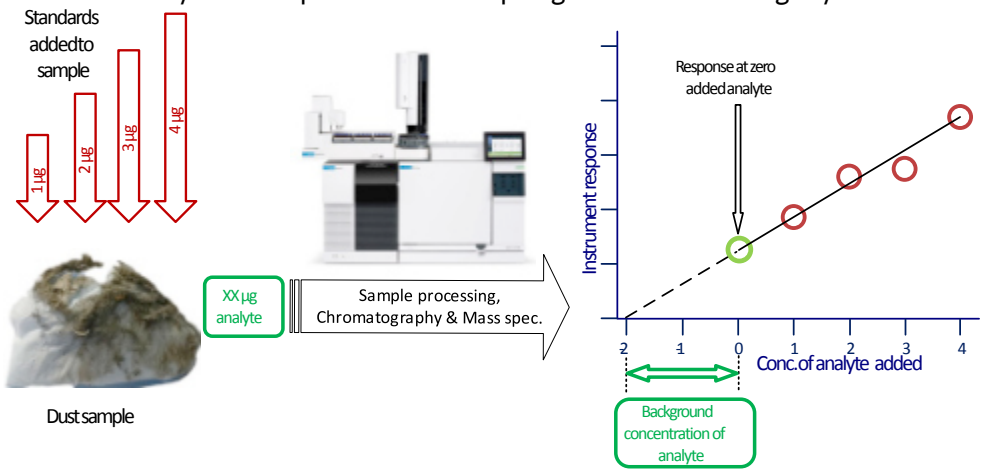


Fig. 6. The standard addition method workflow

naturally occurring isotopologues of either the SI-ISTD or analyte inflating the signal of one another.[78-80]

Isotope assisted trichothecene quantification

The SIDA analysis of trichothecenes in the environment is dominated by measuring simple agriculturally relevant trichothecenes such as DON and NIV in food and biological fluid due to their established health risks. In this context, the use of SIDA is increasing in order to meet food production regulations and more accurately track mycotoxin exposure in populations. Due to this demand, SI-labelled standards of agriculturally relevant trichothecenes are becoming more widely available and utilised, and this has enabled the development of highly sensitive analyses in previously challenging matrices.

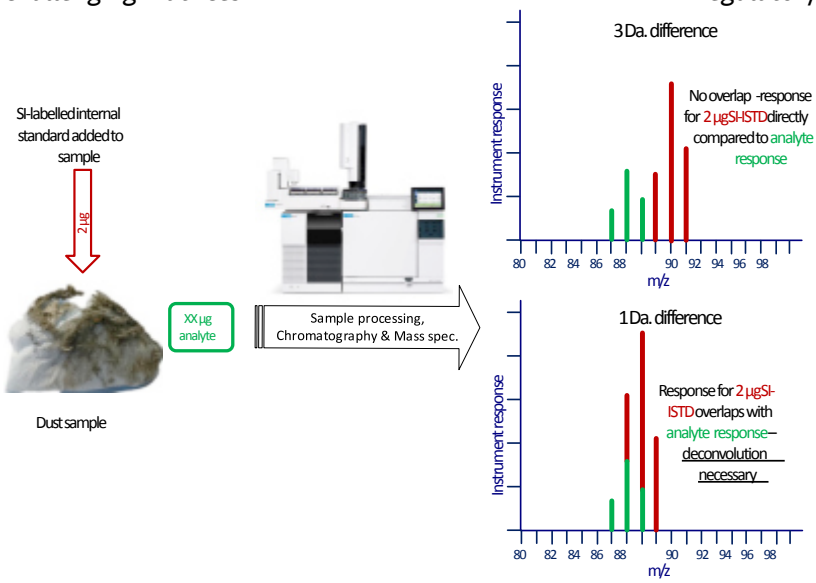


Fig. 7. Simplified SIDA workflow. An insufficient mass difference between standard and analyte can cause isotope overlap, requiring mathematical correction.

Commercially labelled standards are typically fully ^{13}C labelled, although as noted this could lead to undesirable IE, while laboratory-prepared standards are often labelled through facile synthetic steps at just a few sites, *e.g.* a single deuteration or the introduction of two ^{13}C through acetylation with an SI-labelled reagent (Fig. 8). For example, in a report of a SIDA method for the detection of type-B trichothecenes, Bretz *et al.* prepared deuterated DON (15-[$^{2}H_1$]-DON) by semi-synthesis from its natural precursor 3-acetyl-DON (3-Ac-DON).[81] With the SI-ISTD, they were able to mitigate MEs in the HPLC-MS/MS analysis of DON in food samples. Labelling was carried out by oxidising the C15 primary alcohol of 3-Ac-DON to an aldehyde and reducing this with $NaB^{2}H_4$ to return the $^{2}H_1$ labelled C15 carbinol. Deacetylation then gave 15-[$^{2}H_1$]-DON. An analysis of fragmentation patterns *via* MS gave the authors confidence that the chemically feasible mono-labelling at the C15 primary alcohol would be sufficient for use as an SI-ISTD. With only a single mass unit difference, some spectral overlap was found in the mass spectrum, however, these effects were adequately managed through mathematical correction.

The same authors had also previously prepared [$^{2}H_3$]-3-Ac-DON through mono-[$^{2}H_3$]-acetylation of DON.[82] McMaster recently also used SIDA with a singly deuterated [$^{2}H_1$]-DON in a GC-MS method to quantify DON in 196 sorghum samples, after a traditional non-SI assisted method produced inconsistent recoveries.[83] The SIDA method found more samples exceeding the FDA regulatory limits than the traditional method, including thirteen that would have otherwise passed the 1.0 µg g^{-1} advisory limit under the non-SI assisted method, and six that would have otherwise passed the 5.0 µg g^{-1} advisory limit. Spectral overlap resulting from the small mass difference of the analyte and standard was observed but was again mitigated through mathematical correction.

In another example of facile labelling, Asam *et al.* reported on the use of ^{13}C labelled ISTDs in the HPLC-MS/MS analysis of type-B trichothecenes in food.[84] The authors stated that the accuracy of MS analysis for these compounds had been restricted by the lack of an SI-ISTD, and noted that research using a chemically related surrogate would have suffered from MEs and variation in extraction efficiencies. Three ^{13}C labelled trichothecene standards, [$^{13}C_2$]-3-Ac-DON, [$^{13}C_2$]-15-Ac-DON, and [$^{13}C_2$]-4-Ac-NIV, were prepared by $^{13}C_2$-acetylation of the commercially available deacetylated precursors. The standards were found to be isotopically stable at pH 3 & 7 over 2 days. This was an advantage over the previously synthesised ^{2}H-labelled [$^{2}H_3$]-3-Ac-DON by Bretz *et al.* where the location of the ^{2}H label in the acetyl group could undergo $^{1}H/^{2}H$ exchange during sample clean-

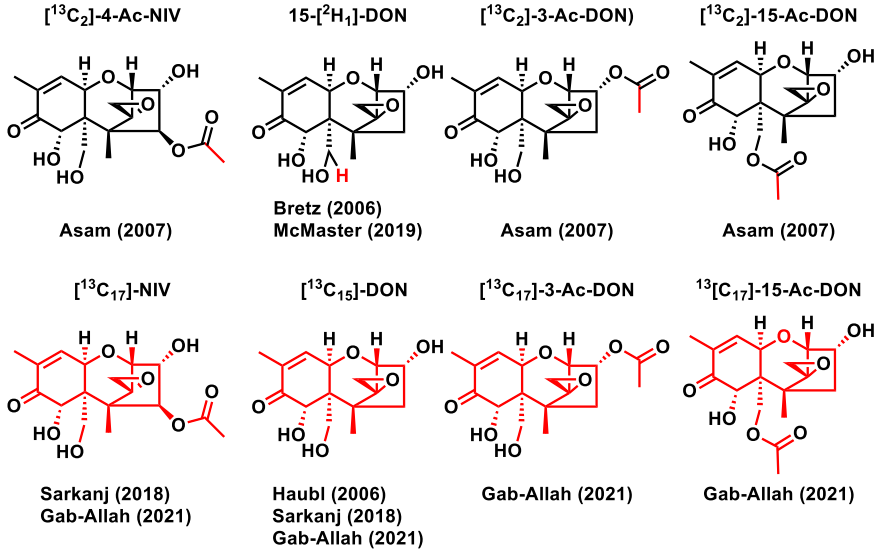


Fig. 8. Isotopically labelled standards used for trichothecene analysis in food via SIDA. Red areas indicate label locations, with the bottom row representing fully ^{13}C labelled standards.

up.[82] A commercially available, fully ^{13}C labelled [$^{13}C_{15}$]-DON was used as a standard for DON. In this case, a significant IE was found where the calibration slope between SI-ISTD and unlabelled analyte was much greater than unity (1.7 *versus* 1). Whereas the mass difference (15 Da) was too high to give isotope overlap, the ratio of [^{13}C]-formaldehyde to water fragments in the SI-ISTD was different to the ratio of formaldehyde to water fragments in the analyte. The higher abundance of [^{13}C]-formaldehyde fragments had thus resulted in a higher slope. Overall the standards allowed excellent sensitivity with low LOD & LOQ and good precision.

Despite the potential for IE with fully labelled standards they have proved quite useful. Häubl *et al.* illustrated the power of using SI-ISTDs in circumventing MEs when in 2006 they used the commercially available [$^{13}C_{15}$]-DON to analyse maize and wheat for DON by HPLC-MS/MS without sample clean-up.[85] Simple acetonitrile / water extracts with no ISTD gave apparent analyte recovery of 29±6% in wheat and 37±5% in maize, while use of the SI-ISTD gave recoveries of 95±3% and 99±3% respectively, illustrating the potential for SIDA to provide reliable and accurate quantification with even minimal sample clean-up.

More recently, in a study which demonstrates the state-of-the-art, Šarkanj and co-workers in 2018 developed a robust and ultra-sensitive UHPLC- (Ultra-HPLC-) MS/MS method for the detection of multiple mycotoxin biomarkers in urine using SIDA and a range of 12 SI-labelled mycotoxin ISTDs.[86] Urine is particularly susceptible to MEs, and as SIDA is considered an ideal way to quantify the recovery of analytes from varied matrices, it was hoped that this method would be applicable to the wide range of urines and mycotoxins expected in epidemiological studies. Compared with the same samples previously analysed in a 'dilute and shoot' (minimal sample clean-up) method using un-labelled standards, Šarkanj's method found more positive samples (above LOD) and a greatly increased number of quantifiable samples (above LOQ) for all comparable analytes, including the type-B trichothecenes NIV, DON and de-epoxy-DON (Fig. 9). It was proposed that the SIDA method would allow a realistic assessment of mycotoxin exposure in large scale epidemiological studies.

Finally, Gab-Allah *et al.* used SIDA in the development of an ultra-performance liquid chromatography- (UPLC-) MS/MS reference method for the analysis of type-B trichothecenes in cereal grains using fully ^{13}C labelled [$^{13}C_{15}$]-DON, [$^{13}C_{15}$]-NIV, [$^{13}C_{17}$]-3-Ac-DON and [$^{13}C_{17}$]-15-Ac-DON.[88] Excellent accuracy, high reliability and low LODs & LOQs were achieved, with the authors noting the LODs and LOQs were the lowest amongst a selection of recent SIDA LC-MS trichothecene analysis reports.[87] These studies are but a few that have leveraged the power of SIDA to overcome the challenges associated with the highly sensitive detection of mycotoxins in complex matrices.

Quantifying trichothecenes in indoor matrices

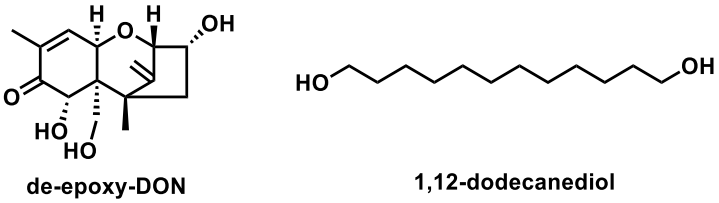
The analysis of trichothecenes in buildings typically focuses on house dust, as particulate inhalation represents the expected route of exposure. House dust has been found to be particularly prone to MEs and so analyses need to be carefully approached in this regard. Unfortunately, the application of SIDA to the quantification of building related trichothecenes (*e.g.* VER, SATG, SATH & ROE) is decidedly lacking due to an absence of suitable SI-labelled standards.[78] It is likely that this absence of standards reflects low demand due to the lack of regulation (*c.f.* agriculture), which in turn is rooted in a lack of knowledge about the risks from trichothecenes in these environments, as well as the tech-

nical challenges associated with preparing SI labelled standards. The mitigation of MEs in building-related matrices has thus typically used matrix-matched calibrations and internal standards which differ from the analyte. Compared with the results seen using SIDA in an agricultural context, this can severely limit the quality of analysis.

Such situations are illustrated across the literature. A 2009 paper by Vishwanath *et al.* describes the simultaneous detection of 186 microbial secondary metabolites including trichothecenes in indoor matrices by HPLC-MS/MS.[88] For a dust matrix, analyte signal was supressed by more than 50% in a third of the analytes, and apparent recoveries of below 50% for half of the analytes was observed. It was proposed that this was due to incomplete extraction and the severe MEs presented by house dust. This was noted to be an extremely challenging matrix, and a significant difference in MEs between dust samples was found. It was proposed that matrix-matched calibrations would likely be unsuitable for dusts due to the severe MEs, and that SI-ISTDs would be required to overcome these.

In 2012, Pietzsch *et al.* reported a follow-up analysis of schools in Europe again for 186 microbial secondary metabolites using HPLC- and GC-MS/MS.[89] Approximately 10 samples each of settled dust and swabs of mouldy surfaces were collected preferentially from locations where dampness or moisture damage had been noted. HPLC-MS/MS was used to screen for the metabolites using external calibrations prepared with a multi-analyte standard. No attempt was made to assess or correct for extraction losses or MEs as in previous research[89] they had found matrix-matched calibrations unsuitable for correcting these effects in extremely heterogeneous matrices such as dust. A GC-MS/MS method was used specifically for the detection of trichothecenes, where hydrolysis of samples prior to analysis generated VER and TRID as proxies for total trichothecene exposure. An ISTD of 1,12-dodecanediol (Fig. 9) was added to the samples prior to hydrolysis. Trichothecenes, as measured by the detection of VER, were found in only 4 of 675 samples. The use of unsuitable ISTDs and the presence of MEs make it difficult to draw any conclusions with regards to the presence of trichothecenes, again underlining the desirability of SI-ISTDs.

These researchers would go on to conduct one of the most comprehensive studies of its type, when Kirjavainen *et al.* surveyed dust samples using HPLC-MS/MS for the presence of 333 microbial secondary metabolites in 93 homes of 1-year old children in Finland.[7] Microbial secondary metabolites were ubiquitous, being found in all houses. A moderate but significant negative correlation was found between overall metabolite abundance and the prevalence of asthma symptoms at 6 years of age. External calibration curves derived from serial dilutions of a multi-analyte standard were used with no matrix-matching, and no S. chartarum trichothecenes were found. Authors from this group had in previous research[88,89] stated the particular difficulty that dusts present in terms of MEs, both within the individual sample and due to the great variability that is expected between samples. It has been argued that such multi-analyte approaches, while valuable for qualitative screens, can only be considered semi-quantitative due to the inability to apply MEs mitigation such as matrix-matching to the large range of analytes.[76]



de-epoxy-DON 1,12-dodecanediol

Fig. 9. Standards used in building-relevant trichothecene analysis

The difficulties presented by MEs in such heterogeneous indoor environmental samples were further highlighted by Saito et al. in a 2016 analysis of MCTs in the floor dust of water damaged buildings using GC-MS/MS.[90] By subjecting dust samples to hydrolysis conditions, trichothecenes were converted to VER which was used as a proxy for total MCT content. Previously established GC-MS/MS methods had used an ISTD of 1,12-dodecanediol, and this was examined for its ability to correct for MEs alongside a matrix-matched calibration method. With no ISTD adjustment, a 280% enhancement of the analyte signal was observed. However, the ISTD was found to suffer severe MEs compared with the VER analyte, with the consequence that, in 21 dust samples analysed with ISTD correction applied, VER was not found. In contrast, with a matrix-matched calibration curve and no ISTD adjustment, VER was detectable in 38% of samples, and a 94% recovery was observed in spiking experiments. The authors proposed that the use of this particular standard

for VER analysis could therefore result in a significant over- or under-estimation of trichothecene content in dust. Attempts at preparing stable isotope labelled VER, considered to be the ideal ISTD, were fruitless, and it was suggested that matrix-matched calibrations may provide a useful alternative to overcome MEs when a suitable ISTD is not available.

The ability of matrix-matching to correct for MEs in these contexts was examined specifically by Jaderson & Park who investigated MEs in the measurement of microbial secondary metabolites, including VER, in dust using UPLC-MS/MS.[91] They compared the use of the trichothecene de-epoxy-DON as an ISTD against the use of matrix-matched calibration curves. By comparing the results of standards prepared in neat solvent, standards prepared in a solvent extract of the dusts (matrix-matched), and standards prepared by spiking dusts with a known amount of standard prior to extraction, they were able to gauge MEs and extraction efficiency. Signal suppression due to MEs was found in all 31 microbial secondary metabolites studied. MEs varied significantly by the building from which the dust was collected, the analyte of interest, and the concentration of the spike. It was concluded that MEs could result in a significant underestimation of microbial secondary metabolites in dust samples. Matrix-matched calibrations were found to provide acceptable compensation for MEs and extraction loss, and it was suggested they could provide reasonable results when it was practical to prepare them. However, the increased number of samples required to generate a calibration curve for each matrix and analyte, and the availability of a blank matrix for each sample, were both noted as obstacles to the wider application of the technique. De-epoxy-DON was not found to be a suitable universal ISTD, and where an SI-ISTD for each analyte would be ideal, the impracticalities of achieving this meant that investigations into other correction methods such as standard addition were advised.

In other instances the use of matrix-matching has been reported as inadequate in accounting for MEs. For example, Došsen et al. developed a semi-quantitative UHPLC- (Ultra-HPLC-) QqQ (triple quadrupole MS) method for the detection of S. chartarum secondary metabolites in dust and wall swabs from a water damaged building.[92] UHPLC-qQTOF (quadrupole-quadrupole-time-of-flight MS) analysis of S. chartarum culture extract as a preliminary screen identified several novel potential biomarkers as well as satratoxins and atranones through dereplication against mass-spectral databases of known S. chartarum compounds. These biomarkers were then quantified using UHPLC-QqQ and calibrations based on similar compounds for which reference materials were available. MEs were assessed for the Kimwipes™ used to collect the dust but not for the dust itself, and ion enhancement and suppression were observed even in this presumably less-complex matrix. Thus it was concluded that matrix-matched calibrations would not be adequate to account for the observed MEs and that SI-ISTDs would be preferable.

Next steps

The lack of an ideal analytical tool for the measurement of S. chartarum mycotoxins in indoor environments compels us to develop GC-MS and HPLC-MS/MS techniques using SIDA and standard addition quantification methods respectively. To this end we are preparing an SI labelled VER standard via semi-synthesis from the closely related DAS and pure examples of KTCs by extraction from natural sources. In ongoing work, strains of S. chartarum from the Manaaki Whenua International Collection of Microorganisims from Plants have been cultivated on potato-dextrose agar before inoculation onto Uncle Ben's par-boiled rice, a medium which is known to facilitate the production of MCTs.[93] After 4–6 weeks incubation at room temperature, extraction with 1:1 chloroform:methanol has yielded a crude extract suitable for purification using established techniques.[93] In this way, the KTCs SATG, SATH and ROE have been prepared successfully, while we hope to soon isolate ROL2.[66,94,95]

With regards to the GC-MS method, synthesis of the proposed SI-VER has commenced with the production of a mono-labelled variant in which we hope to shortly introduce further isotopic labelling. These approaches to trichothecene quantification, along with the high mould contamination rates in our leaky buildings, a large sample size, well-matched controls and objective measures of illness, should provide us with a particularly powerful method with which to investigate the relationship between the prevalence of these potent mycotoxins and the health of NZ's leaky building occupants. Overall, this study will further inform the wider controversy surrounding the role of S. chartarum tri-

chothecenes in the development of respiratory disorders, while the SIDA technique will facilitate future investigation into the presence of these building-relevant trichothecenes in a variety of contexts.

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An introduction to deep eutectic solvents

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Introduction

Deep eutectic solvents (DESs) have evolved from ionic liquids (ILs) as a solvent system with a greener basis, easier synthesis and lower cost. As the field has evolved, different types of DESs have been identified, key among these being Lewis acidic DESs and H-bonding DESs. The field is still rapidly developing and new types/classifications of DESs are emerging. However, DESs have a more complex composition than ILs and are not well understood at a fundamental molecular level. Compared to ILs, the ionic interactions within DESs are reduced and H-bonding is recognised as taking a more important role. Understanding H-bonding in DESs is key to determining the physico-chemical and solvation properties of DESs. Like ILs, DESs can be used in a particularly wide range of applications. As solvents, the dominant areas of application for DESs involve dissolution of a reactive or key species; synthesis, bio-transformations, electrochemistry and extraction /separation processes. The following sections will first introduce DESs and briefly describe the different types of DESs. The second section will probe H-bonding within DESs and the last section will focus on some of the wide range of applications that employ DESs.

Deep eutectic solvents

The term deep eutectic solvent is associated with the formation of a liquid from the combination of a salt (often an IL) with a neutral molecule in a specific molar ratio. The two individual components melt at higher temperatures, but the mixture forms a eutectic, a minimum in the melting point vs composition diagram. Typical notation defines the salt or IL ion pair, cation (C^+) and anion (A^-), in a given molar ratio, x, with the neutral molecule (N): [C][A]:xN.[1]

Traditional salts, such as sodium chloride, exhibit high melting points (≈ 801C for NaCl). In contrast, ILs have melting points (by convention) below 100ºC.[2] ILs are composed of ions that are larger with a reduced charge density leading to reduced Coulomb interactions and a reduced enthalpy of association. The ions are typically asymmetric, reducing packing efficiency, increasing the number of conformers/arrangements possible and thus increasing the entropy.[3] The combined effect of decreased enthalpy and increased entropy lowers or even eliminates the melting point. Many ILs exhibit a glass transition rather than a true melting point.

Larger organic cations are common, including alkylimidazolium, pyridinium, pyrrolidinium, ammonium and phosphonium ions. Inorganic anions are common, including halides, fluoroborates, trifluormethanesulphonate (and related anions), cyanine-containing species such as dicyanamide, alkyl-sulphate and alkylphosphate anions.[2] The move away from inorganic monoatomic ions introduces additional intermolecular interactions, most notably hydrogen bonding, dispersive and pi-interactions.[4]

Eutectic mixtures have long been used in the molten salt field to create lower melting point systems. The earliest recognised IL-based DES is a chloroaluminate comprised of 1-ethyl-3-methyl-imidazolium chloride, $[C_2C_1im]Cl$, and $AlCl_3$.[5] This is a Lewis acidic DES due to the inherent Lewis acidity of $AlCl_3$. $AlCl_3$ is able to combine with Cl^- from the IL forming a new covalently associated anionic species $[AlCl_4]^-$ and $[C_2C_1im][AlCl_4]$ is formed.

Lewis acidic DESs have now been extended across much of the periodic table.[6] The DES is formed by

adding solid MCl_n (M is a metal such as Sn, Fe, Co, Ni, Zn, Cr, Cu, Au, Al, Ga, In) to a traditional IL such as [C]X (C=cation, X=halide Cl, Br, I). MCl_n will react with Cl^- in the IL; the exact nature of the anion (or anions) formed varies with the amount of MCl_n added. If a 1:1 molar ratio of MCl_n:Cl is present then $[MCl_{n+1}]^-$ forms.[6a] If more MCl_n is added (than Cl^- present), then there is a "shortage" of Cl^- and dimers $[M_2Cl_{2n+1}]^-$, trimers $[M_3Cl_{3n+1}]^-$ and larger clusters can form (Fig. 1). If less MCl_n is added then there is an excess of Cl^-. Anion formation is not limited to a single species since multiple anionic species can exist at the same time. The liquid is now a structurally complex and disordered mixture of multiple molecular ions, where complex equilibria can be established between multiple anionic species. A key characteristic of DESs (in general) is the existence of a complicated set of equilibria governing anionic speciation, dependent on the identity and mole fraction of the complexing agent.[6b]

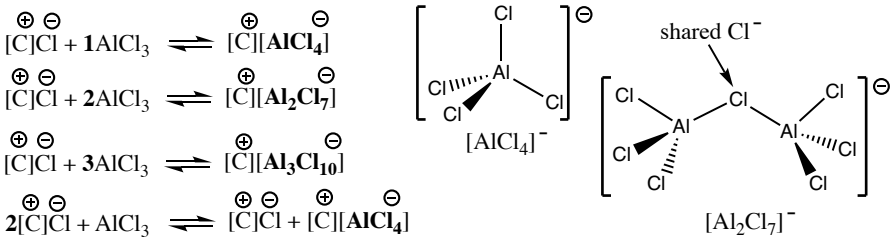

Fig. 1. Examples of species formed from different molar ratios of IL components with neutral $AlCl_3$

Early in the development of DESs, another type of DES was formed via the combination of an ammonium salt with a neutral hydrogen bond (H-bond) donor. A prototypical example is formed from [Ch]Cl (Ch=choline: $N(CH_3)_3(CH_2CH_2OH)$, 2-hydroxyethyl)trimethylammonium) and urea (U: $(NH_2)_2C=O$ carbonyl diamide) in a ratio of 1:2, [ChCl]:2U.[7] This class of DES is identified as H-bond DES. Rather than forming a new covalent complex, H-bond DESs form new species through H-bonding. The newly associated species reduce ionic interactions and thus the melting point. Typical neutral organic species include amides, carboxylic acids or polyols.[1,8] Ethylene glycol is another commonly used H-bond donor.

For [Ch]Cl:2U it was originally proposed that the anionic species $[Cl•2U]^-$ formed.[1] However, more recent investigation has shown that the species formed are more likely to be [Ch•U][Cl•U].[9] Moreover, addition of neutral organic species (e.g. urea, ethylene glycol) to $ZnCl_2$ has been shown to lead to a wider range of complexed species, for example cations $[ZnCl•urea]^+$ and anions $[ZnCl_3]^-$.[10] Cation complex formation in DESs is an area that has not yet been explored.

Independently of the precise species formed, the H-bonding network within the DES will make a significant impact on the entropy of the system, contributing to a melting point reduction.[9] H-bond donors include the -OH and -CH of choline and -NH of urea, H-bond acceptors include Cl and the C=O of urea. The wide range of different types of H-bonding interaction contrast with traditional solvents such as water.[9]

Further development has seen DESs comprised of coordination complexes with water as a neutral coordinating ligand, for example [Ch]Cl:$CrCl_3•6H_2O$.[11] The range of non-hydrated metal salts with a sufficiently low melting point is limited, thus hydrated metal salts offer a cheaper alternative; $MCl_x•nH_2O$, (M=Ca, La, Cr, Co, Cu, Ni, Fe) all form eutectics.[6a,12] $CrCl_3•6H_2O$ with [Ch]Cl has been characterised in depth.[13] Other metal salts are also possible such as $LiNO_3•4H_2O$ and $Zn(NO_3)_2•4H_2O$.[13]

Most recently, NAtural DESs (NADES) have emerged.[14] NADES employ natural compounds for either the anion or the H-bond donor or both. The natural compounds employed include organic acids and bases, amino acids, sugars and polyalcohols. For example, [Ch]Cl:5lactic-acid is a NADES. NADES are also typically water saturated adding a further level of complexity. In this context, the liquid formed is unlikely to be a DES but the name has remained. NADES are regarded as more environmentally friendly DESs.

DES-like systems employing neutral chelating ligands have also been developed, for example Li[NTf_2]:G1 where G1 is monoglyme CH_3-OCH_2CH_2O-CH_3 and NTf_2 is bis(trifluoromethylsulfonyl)imide).[15] There is a drive to develop liquid-based pharmaceuticals. These can be formed by adding a (solid) pharmaceutical to an IL, effectively forming a DES.[16] Moreover, the IL component can itself be formed from pharmaceutical anions (or cations). Thus, a drug molecule can be added directly (dissolved) in a NADES, or it can form a component of the DES.[17]

Aprotic-ILs have fully alkylated quaternary centres. However, protic-ILs are formed through the reaction of a Brønsted acid (XH) and base (Y) in a 1:1 ratio as shown in Eq. 1.

$$XH + Y \rightleftharpoons [X]^- + [HY]^+ \qquad \text{(Eq. 1)}$$

A distinguishing feature of protic-ILs is the introduction of a more acidic (N-H) proton donor site on the cation (Fig. 2). As protic-ILs are derived from a simple acid-base reaction, the ions are in equilibrium with the neutral parent acid and base, with the position of equilibrium depending on the acid and base combination. Protic-ILs formed with strong acids, such as H_2SO_4, are almost exclusively ionic, whereas those formed from weaker acids, such as carboxylic acids, have a larger percentage of neutral species and are a form of DES.[18] As the neutral species are hydrogen bond donors and acceptors these will fall into the class of H-bond DESs.[19]

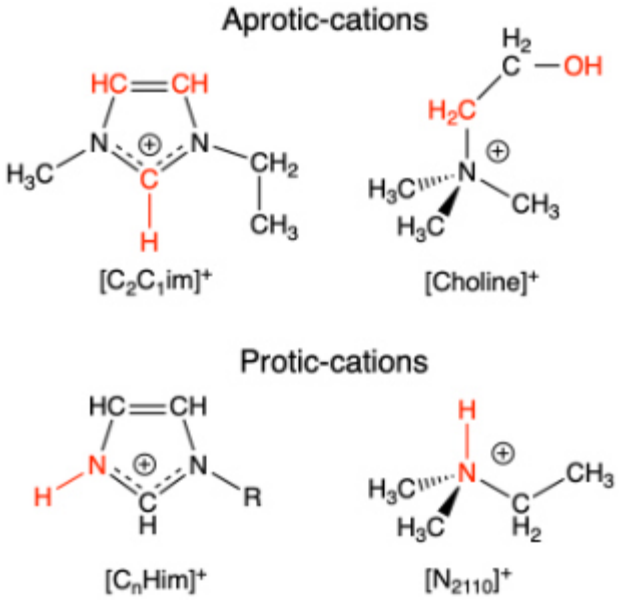

Fig. 2. Illustrative examples of aprotic and protic IL and DES cations. Red indicates the most active H-bond donor sites. Protic H-bond donor X-H are significantly more H-bonding than aprotic X-H sites.

Linked to the protic-IL based DESs are Brønsted acid DESs. The DES is formed when excess acid is added, for example, $[(HSO_4)(H_2SO_4)_x]^-$,[20] or $[(CH_3COO)(CH_3COOH)_x]^-$.[21] Analogous to Lewis acidic DESs, which form larger covalently bound anions, Brønsted acids form larger H-bonded clusters, generally of the form $[A(HA)_x]^-$ (where A^- is typically the IL anion, and conjugate base of added acid HA). Complex equilibria, dependent on the identity and ratio of the components, govern anionic speciation.

Less common but also possible, is a Brønsted acid added to an aprotic-IL. An example is HCl previously added to $[C_2C_1im]Cl$ resulting in the formation of complexed anions $[Cl(HCl)_x]^-$.[22] A protic-IL DESs can be readily tuned through the addition of excess base.[23] However, the speciation of such systems is even less well established than for the excess acid analogues. The addition of excess base to a protic-IL formed from a poly-protic acid may lead to deprotonation of the protic anion.

Water is often present as a contaminant post synthesis, or as a result of the hygroscopic nature of both metal-halides and the IL component of a DES. In addition, DESs are often exposed to moisture in the air or aqueous solutions (during extraction processes or within the body). Water can also be added to reduce the viscosity of the DES or as an antisolvent in extraction processes. Thus, water can play an important role that is often not fully acknowledged. IL-based aqueous biphasic systems (ABS) for the extraction of metals has emerged recently.[24] ABS consist of two water-rich but immiscible phases, one salt-rich and the other IL-rich. ABS based around DES have also evolved.[25]

The level of moisture within a DES can strongly affect the DES properties requiring pre-saturation and allowing water to reach equilibrium within the DES.[26] While a more complex mixture of species forms when water is added into the DESs, air/moisture sensitivity is reduced, which is a key advantage over ILs. Understanding the influence of water is essential for controlling the performance of DESs.[27] Water-free DESs are required for some applications, for example to avoid a restricted electrochemical window. In other DESs, the presence of water can make the liquid cheaper and/or positively modify the DES properties, for example by reducing the viscosity.[14b]

Formally a DES needs to exhibit a eutectic point, where the melting point is lowered more than can be predicted through ideal mixing. However, the term has become more loosely applied to systems with an IL and neutral component.[28] The combination of an IL and traditional molecular solvent in a liquid mixture is not a true DES. Many ILs already have low melting points and further depression can be difficult. Never-

theless, there are many low melting point salts with large ions, or high melting point ILs with small ions which can form a DES.

DESs span a very wide range of chemical species and complexity. Most of the focus to date has been on the potential applications of DESs and/or reporting properties of novel [C][A]:xN combinations. Speciation within DESs is very complex, but also very important. Understanding speciation profiles will be critical to controlling electrochemical activity, solvation ability and physical properties of DESs. Many questions remain open regarding the intermolecular interactions within DES systems, including questions relating to the role of H-bonding and the nature of the complexed ions.

Hydrogen bonding

H-bonding plays a critical role in the formation and properties of many DESs. More generally, H-bonding has been recognised as playing an important role in influencing the secondary structure and properties of conventional ILs. All ILs exhibit Coulombic interactions between the +1 cations and -1 anions, thus IL are differentiated by their secondary interactions, such as H-bonding. In a DES, the ions are further separated by neutral molecules and experience weaker Coulomb interactions, leading to a more dominant contribution from H-bonding.

The H-bonding in ILs and DESs differs substantially from traditional notions of H-bonding. We are used to the traditional form of H-bonding as shown on the left hand side of the equilibrium represented in Fig. 3, while doubly ionic H-bonding explores the right hand side of the equilibrium. One way of conceptualising the H-bond is as a stage in the proton transfer reaction before the transition state is reached. The position of the proton along the proton transfer reaction coordinate will determine whether a weak or strong H-bond is formed. For the neutral H-bond, a weak H-bond corresponds to an early stage in the proton transfer, while a strong H-bond represents a more advanced stage.[29]

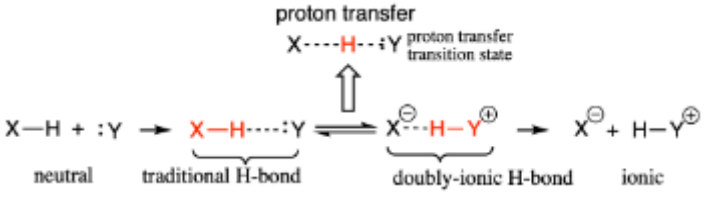

Fig. 3. Formation of the doubly ionic H-bond

For a doubly ionic H-bond the proton transfer has occurred and the H-Y$^+$ cation forms a H-bond with the anion. A strong doubly ionic H-bond corresponds to a late stage in the proton transfer. Once H-bonding has been established, an equilibrium exists between the neutral (X-H:Y) and (doubly) ionic forms (X:$^-$ H-Y$^+$). For aprotic-ILs the equilibrium lies exclusively on the ionic side, for protic-ILs (DESs) the position of the equilibrium leaves some neutral species present. Doubly ionic H-bonds represent a wide area of H-bonding which has yet to be fully recognised, characterised or explored.[19]

There are many similarities but also key differences between traditional and doubly ionic H-bonds. Traditional H-bonding is typically evaluated between neutral molecules (H-bond), or between a neutral molecule and an anion (ionic H-bond). In an IL/DES the H-bond exists between two ions thus forming the "doubly ionic" H-bond.[19] Traditional H-bonding is typically evaluated for a single strong almost linear H-bond, however in IL/DESs there are multiple H-bonds occurring at the same time leading to bifurcated and chelating H-bonds that are not linear.[19] H-bonding in traditional solvents usually involves a single type of H-bond. For example, in water (to a first approximation) there is a single type of H-bond; OH-O. However, in ILs, and even more so in DESs, there are a multitude of different types of H-bond. A DES contains neutral as well as ionic species and thus there is potential for neutral, ionic and doubly ionic H-bond formation to occur concomitantly. Thus, examination of H-bonding within a DES provides the opportunity to compare and contrast these different types of H-bonding within a single system.

The characteristics of doubly ionic H-bonds have led to problems in recognising and evaluating H-bonds in IL/DESs. The ionic nature of the ion-pair dominates interactions, and it is no longer possible to evaluate the H-bond using the traditional method of computing an association energy. To evaluate the doubly ionic H-bond, more localised evaluation techniques are required.[19] The multiple H-bonding interactions occurring within IL/DES mean that there is no single strong H-bond to evaluate. Thus, the traditional method of using acceptor Y:H distance and deviation from linearity in the H-bond angle to evaluate H-bond strength is no longer appropriate. In contrast, *ab initio* computed parameters such as

the electron density at the bond critical point (ρ_{BCP}) obtained from quantum theory of atoms in molecules (QTAIM),[30] qualitative molecular orbital theory and natural bond orbital (NBO)[31] analysis using $E^{(2)}$ provide local and relevant measures of doubly ionic H-bonding.

ρ_{BCP} is a diagnostic parameter for identifying the presence of a covalent bond. The bond critical point occurs where density decay along the line between two bonded atoms is at a minimum (the density is a maximum in directions perpendicular to the bond). As shown in Eq. 2, $E^{(2)}$ is proportional to the amount of electron density (q_i) donated from a filled donor lone-pair orbital (with energy ε_i on Y:) into an empty $\sigma*$ orbital (with energy ε_j on X-H $\sigma*$) computed using $F(i,j)^2$ (where F(i,j) is the off-diagonal or coupling NBO Fock matrix element), moderated by the energy difference between these two fragment orbitals ($\Delta\varepsilon=\varepsilon_i-\varepsilon_i$). Both QTAIM and NBO methods are established and robust mechanisms for understanding and interpreting traditional H-bonds.[32]

(Eq. 2)

$$E^{(2)} = \frac{q_i F(i,j)^2}{\varepsilon_i - \varepsilon_j}$$

Using ρ_{BCP}, MOs and $E^{(2)}$ it has been found that doubly ionic H-bonds cover a full range from weak to very strong H-bonds.[19] The multiple H-bonding types present in IL/DESs systems mean that there is a dynamic equilibrium and active network of H-bonds forming and breaking over time. At one instant there could be a smaller number of stronger H-bonds and a moment later (in the liquid) a larger number of weaker H-bonds, all active within a small energy window.[9,33] Thus, there is a range of iso-energetic states contributing significantly to the entropy of the liquid state. The dynamic nature of the H-bonding also necessitates that a range of motifs and conformers be examined for any system.[34]

The first in-depth examination, employing quantum chemical methods, of intermolecular interactions within an example DES was applied to the archetype system [Ch]Cl:2U.[9] Here it was found, as expected, that the relative ordering of the association energies, E_a, of the pairs of components reflected the dominant electrostatic contribution (choline-chloride >> urea-chloride > choline-urea > urea-urea). Thus, insight into H-bonding using this parameter was minimal. Each of Ch^+, Cl^- and U can interact, the pairs [Ch]Cl, $[Cl•U]^-$ and $[Ch•U]^+$ (Fig. 4a) interact via H-bonding in different ways, through different types of H-bond, leading to a large number of low energy configurations.

A small subset of the low energy [Ch]Cl ion-pair conformers, those exhibiting very different H-bonding motifs, are shown in Fig. 4b.[9] The positive charge of the choline cation facilitates the formation of C-HX doubly ionic H-bond interactions that would be too weak to occur between two neutral species. A tripodal motif was found to be a recurring feature of pairwise interactions with choline. The concurrent formation of three C-HCl H-bonds in the tripodal arrangement leads to a stronger overall H-bonding interaction than for a single (more traditional) O-HCl H-bond.[9] Within the pairs, the doubly ionic C-HCl H-bonds are found to be competitive (in terms of strength) with the urea N-HCl H-bonds, despite N-H groups typically being considered as the stronger H-bond donor.

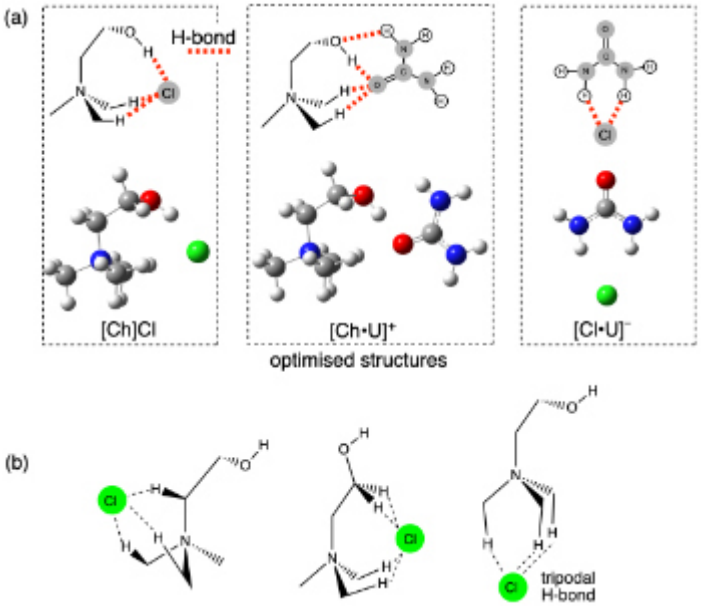

Fig. 4. (a) Lowest energy pair conformers for [Ch]Cl, $[Ch•U]^+$ and $[Cl•U]^-$ where U = urea, Ch = choline. (b) A selection of low energy [Ch]Cl ion pairs exhibiting doubly ionic H-bonds[9]

Somewhat unexpected was the strength of the O-HO=C choline-urea H-bond. Urea had been expected to interact with the chloride anion forming a more diffuse anion complex $[Cl•2U]^-$, however the Cl-urea H-bond was the weakest examined for this system (Fig. 5). It was shown that $[Ch][Cl•2U]^-$ is less viable than [Ch•U][Cl•U] ion pair formation.[9]

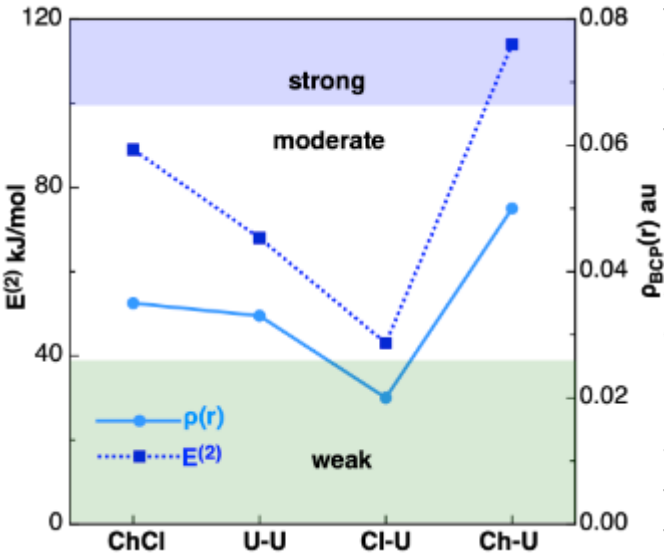

Fig. 5. Comparison of H-bond strength parameters, QTAIM r_{BCP} and NBO $E^{(2)}$ for different H-bonds within [Ch]Cl:2U

A large number of different H-bond types can be identified in [Ch]Cl:2U, a "soup" of possible intermolecular interactions: O-HO=C, N-HO=C, O-HCl, N-HCl, O-HNH, C-HCl, C-HO=C, N-HOH and N-HNH.[9] This contrasts with the more homogeneous H-bonding found in most traditional solvents. The large number of different H-bond types identified within the DES will also increase the disorder within the system, favouring the formation of a liquid. The [Ch]Cl:2U DES has been found to solubilise a wide range of materials, including salts and polar organics.[1,35] We rationalise that the different H-bond donor and acceptor groups play an important role in enabling the DES to "adapt" to the solute in terms of H-bonding.

H-bond DES applications

Interest in DESs has grown significantly in the last few years, with a continuously diversifying range of applications being explored.[1,8,36] As such, there has been a notable increase in the number of publications relating to DES since they were introduced in the early 2000s (Fig. 6). Each class of DES (Lewis acidic, H-bonding, NADES) has a set of highly desirable properties, most often related to a highly favourable solvation ability. The ability to tune the bulk phase properties simply by changing the mole fraction of the neutral molecule is a very attractive feature, not yet fully exploited.

DESs exhibit many of the favourable properties found for ILs; low vapour pressure**,** large liquidous range, high thermal stability, good ionic conductivity and high electrochemical stability. Key for DESs is a favourable solvation behaviour and thus the ability to act as solvents for extractions, catalysis, organic synthesis and electrochemistry.[37] Like ILs, DESs are also "designer" solvents, in that the constituent ions or neutral molecule can be altered to control physical properties. Moreover, like ILs, DESs are "task-specific" in that tailored chemical functionalisation can achieve particular goals. DESs have the potential to be tailored or designed to offer a range of novel functional properties. However, predictive capabilities depend on a well-developed understanding of the fundamental links between molecular interactions and bulk phase properties. At present, our fundamental understanding of DES is very limited.

Despite the demonstrated potential of ILs in new and established areas, the cost of many ILs is prohibitive. A high viscosity has been an ongoing issue, on the macro scale limiting stirring and pumping and on the molecular scale limiting applications where diffusion of ions or mass transport of reactants is important. Many of the best studied ILs (first generation) are toxic (to fish) with poor biodegradation characteristics.[14a] H-bond DESs are easy to synthesise from cheap, abundant or bulk commodity chemicals and are tolerant to impurities. Many H-bond DESs have low cytotoxic effects and are biodegradable. Thus, DESs have the potential to replace hazardous complexing agents such as cyanide, to

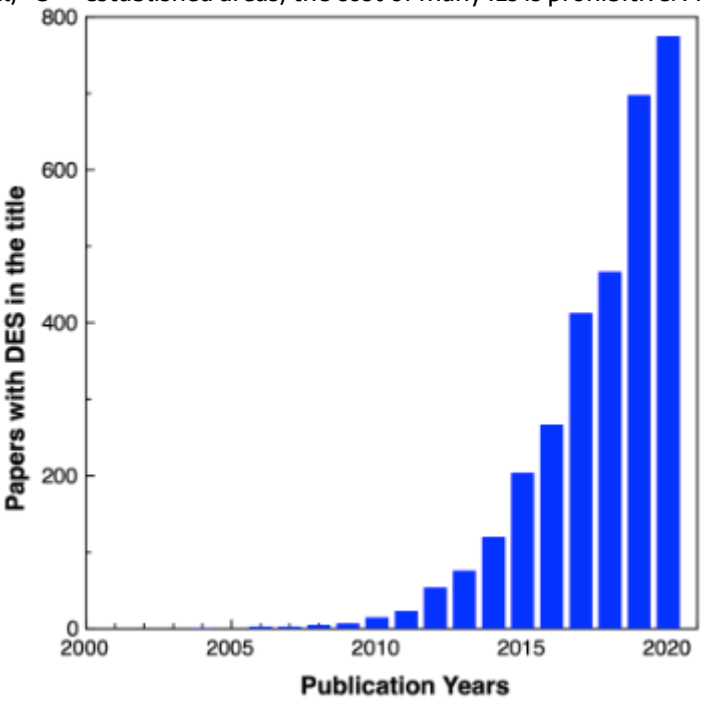

Fig. 6. Papers with "deep eutectic solvent" in the title from 2000-2020 (sourced from Web of Science)

suppress corrosion and to replace processes that employ concentrated acid/alkali solutions. DESs have a low volatility and a low flammability, offering improved cyclability and reduced operational temperatures. DESs with good air and moisture stability can be selected. Overall, DES processes are environmentally more sustainable. Thus, DESs are seen as more favourable than ILs for many applications.

H-bond DESs offer considerable advantages over traditional organic and aqueous electrolytes and are suitable candidates for large-scale industrial applications.[37] DESs offer high metal ion solubility leading to a wide range of applications. For example, H-bond DESs are of significant interest for metal dissolution, electrowinning, electropolishing, deposition, electroplating and electrorefining processes.[1] DESs form good electrolytes for batteries and supercapacitors. DESs offer novel capabilities simply not obtainable with traditional electrolytes. For example, deposition of new alloys and semi-conductors with controllable, novel compositions and/or surface deposition morphologies.[1,36]

DESs exhibit a much wider electrochemical window than aqueous solutions giving access to electrochemical reactions not possible in aqueous systems. For example, new coating methods for wear and corrosion resistant metals such as Ti, Al and W are possible. Clean production of elements that currently cannot be electrodeposited from aqueous solution (Al, Ge, Si, Ta) are possible.[6a] Aqueous phase electodeposition has significant drawbacks including a narrow potential window, H_2 production leading to hydrogen embrittlement and O_2 production leading to passivation of substrates and electrodes (due to surface formation of insoluble oxides and/or hydroxides). DESs are not hampered by the O_2 and H_2 oxidation/reduction limitations of aqueous solutions, even when a small amount of water is present.

The wide and changing electrochemical window for metals in DESs (i.e. different from those in aqueous solution) allow for electrochemically driven separation of complex metal mixtures. New sequential electrodeposition processes for metals are possible.[11] The dissolution and deposition advantages are particularly important in the recovery of metals from complex media such as waste electronic and electrical equipment (WEEE) and rare earth magnets.[38]

DESs deliver increased solubility of recalcitrant materials, such as metal oxides,[39] offering the potential for enhanced and novel processing routes. DESs also offer options in the synthesis and shape control of novel nanostructures including nanowires, nanocrystalline films and nanometre sized crystallites.[36] DESs are used in a wide range of extraction processes.[40] There is the potential to employ liquid/liquid extraction to recover, separate or concentrate metal ions from aqueous sources.[38] DESs are also effective in extraction processes for natural products including amino acids, carbohydrates, phenols, alkaloids and organic acids.[14a,b] NADES applications have related strongly to biological uses such as extraction of (targeted) bioactive compounds from natural sources, media for enzymatic or chemical reactions, preservatives, and solvents for macromolecules such as polysaccharides (cellulose) and lignins.[14c]

Conclusion

DESs have evolved over the last 20 years, with many new forms emerging. H-bonding plays a critical role in many DESs, and when compared to traditional solvents, DESs exhibit some unusual and novel H-bonding interactions. DESs are finding use in an increasingly wide range of applications. However, DESs are still not well understood at the fundamental and molecular level and there is a strong need to develop underpinning knowledge. Many questions remain open, especially related to speciation, solvation and H-bonding.

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A review of COVID-19: origin, transmission epidemiology, virology and treatment options

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Abstract

The world is currently grappling with a pandemic caused by the outbreak of novel SARS-CoV-2 virus causing COVID-19. It causes acute symptoms of respiratory distress and it has affected over 200 countries, killing hundreds of thousands of people and affecting many more with long-term after effects. This number is still increasing day by day as countries attempt to contain the spread of the virus. The structure of the virus is largely known due to its close similarity with SARS-CoV which was responsible for the pandemic of 2002-2003. The virology of the virus has been widely reported but there lacks a proper summary explaining how it actually interacts with the host and infects the lung cells, along with the therapeutic options that are currently available. This review is an effort to give an overview of the current epidemiology of the disease, the origin and possible mode of action of the virus, and the available suggested clinical trial options that can used for treatment to date.

Introduction

COVID-19 is a local pneumonia outbreak that initiated in China,[1] caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The first case was reported on 31 December 2019 in Wuhan. It has quickly spread and affected over 213 countries, and the WHO has declared it as a global health emergency.[2] As of December 2020, the number of confirmed cases globally is more than 14 million with 612,000 deaths.[3] This number sadly continues to rise.

Epidemics are not new and even in the last two decades several have occurred. SARS virus was observed in the early 2000s with nearly 8,000 people diagnosed with the disease. 774 people died as a result of that epidemic. It was alleged to be of zoonotic origin, with bats being the primary host and palm civets being carriers.[4] A swine flu epidemic of virus H1N1 influenza in 2009 affected 126,168 people[5] and caused over 3,205 deaths worldwide.[6] With the alarming mortality rate of 60%, a virus known as Middle East Respiratory Syndrome coronavirus (MERS-CoV) was removed from a septum of an individual suffering from the disease in Saudi Arabia in 2012.[7] The reported confirmed cases globally from MERS-CoV totalled 1,321 with 466 deaths.[8] The origin was again proposed to be bats with dromedary camels as the reservoir[9] and an intermediate host was proposed to be the masked palm civet.[10]

Origin of COVID-19

The origin of COVID-19 virus is still the subject of debate, with several theories existing. It is believed to have an animal origin,[11] most likely in bats, as there is found to be a 96-96.2% genetic similarity between the bat coronavirus RaTG13 and COVID-19.[12, 13] It also shows 79.5% identity to SARS-CoV.[14] The likelihood of involvement of an intermediate host is also present[15,16] as the spillover of coronavirus to an intermediate host is not new, having occurred previously for other coroaviruses.[17] The intermediate host might also undergo homologous combination which can then infect humans[18] and the severity of that infection is higher. The expected and most probable intermediate animal that is involved in the process is the pangolin which shows a similarity of 99% to that of SARS-CoV-2 in a report that was released on February 7, 2020.[19] It is also

plausible that it may have originated from bats which went through the process of evolution and natural selection before the jump to zoonotic transfer occurred. Despite the remarkable similarity between bat coronavirus and SARS-CoV-2, the spike protein of the two differs in the protein known as variable receptor-binding domain (RBD). This RBD attaches to the cell surface enzyme of the host such as the angiotensin-converting enzyme-2 (ACE-2) receptor that is present in the outer surface of the heart, lungs, kidneys and gastrointestinal tract. It is suggested that its attachment to the host human ACE2 is rather weak.[20] However, there is also some research suggesting that the RBD in the spike protein strongly interacts with ACE2 in both humans and bat and SARS-CoV. RBD-based vaccines could potentially be used to prevent the COVID-19 virus.[21] Targeting the spike RBD protein to develop a possible vaccine for COVID-19 has been recently discussed in the literature as it is considered a safer option that has less chance of inducing Th2 type immunopathology.[22] SARS-CoV and SARS-CoV-2 both show similarity in binding, genes and mode of action and therefore are suggested to be evolved from the same host i.e. bats.

Some pangolin coronaviruses also display a strong resemblance to COVID-19 in the RBD which indicates that the spike protein is optimised for binding to human-like ACE2. Spike proteins of the virus have undergone some changes to increase its efficiency for effectively binding to ACE2 which is caused by natural selection. The occurrence of RBD in this intermediate pangolin animal is strikingly similar to the COVID-19 virus which indicates that this was present in the virus that jumped to humans.[20]

Undergoing homologous mutation in the origin animal prior to its infection in the intermediate animal is also a possibility. The RBD binding tendency appears to have increased for the receptor ACE2 of humans due to mutation.[23] The rate at which the virus is transmitting and spreading is alarming and it is presumed, although not confirmed, that the virus itself is mutating to improve its binding affinity to the host receptor ACE2. Any type of adaptation that the virus uses will increase its chances of being more deadly and boost its virulence.[24]

Structure of SARS-CoV-2

Scientists are trying to discover possible drugs to combat COVID-19[25] and to this end identification of the structure is an important step to enable a suitable approach for the synthetic process. COVID-19 is a member of the *Betacoronaviruses* subgenus *sarbecovirus*, *Orthocoronavirinae* subfamily. So far there are seven coronaviruses identified that can infect humans,[26] including the newly emerged SARS-CoV-2, SARS-CoV, MERS-CoV, HKU1, NL63, OC43 and 229E. Of these, SARS-CoV and MERS-CoV cause acute and potentially deadly respiratory tract infections,[7] while the rest are associated with mild respiratory and common cold-like symptoms. Of the four genera of coronavirus, two, namely α- and β-CoV, are known to infect mammals, whereas γ- and δ-CoV can infect birds.[14]

The "corona" is so named because of its appearance which is a crown-like halo of viral spike proteins that are present on the surface of the virus as shown by electron microscopy.[27] The COVID-19 virus is an enveloped, single-stranded, non-segmented, positive-sense RNA virus, simply called +ssRNA, with a genome size of 29.9kb - 30kb.[28,14] There are two categories of proteins that are present in the virus, both originating from RNA translation. These are the structural protein that is involved in the formation of the capsule of the virus and non-structural proteins (NSP) that are encoded by the virus.

Structural proteins include the spike (S), matrix (M), envelope (E) and nucleocapsid (N) proteins. and non-structural proteins such as RNA-dependent RNA polymerase (RdRp) (nsp12) and hemaglutein estemase dimer.[29, 30] The genome of coronaviruses indicates the presence of an open reading frame (ORF) that has the ability to translate in variable numbers. 66% of RNA of the virus is located in the first ORF region which, with the help of ribosomes, translates into two types of polyproteins, pp1a and pp1ab, encoding 16 NSP. The remaining 33% of the virus genome encodes the synthesis of gene products that in this case are the four essential structural proteins (S, E, M, N), and also several accessory proteins that affect the human natural immunity.[14]

The spike proteins of COVID-19 were tested for binding affinity against immunoglobulin heavy chain-binding protein also known as GRP78. The result showed that the protein can in fact bind to COVID-19. This sequence and structural similarity suggest four regions of spike proteins to be the most probable site for binding that

are involved in binding to GRP78 with the probable recognition of the spike being the cell-surface GRP78 upon cell stress. The importance of the spike protein is that the entry of the virus into the cell after attachment to the host cell is achieved *via* these spike proteins. The receptors of host cells like GRP78 are the targets for these proteins. This led to the idea that by decreasing the interaction between these host cell receptors and that of spike proteins it is most likely to decrease the rate of infection caused by the virus. Thus, the developed vaccine should be an inhibitor that uses the same approach to target spike proteins.[31]

Despite sharing genetic similarity, COVID-19 differs from SARS-CoV in terms of the infectious period, transmissibility, clinical severity and extent of community spread.[32] The protein size, receptor binding region, mortality rate, R_o value and survival in the environment are all different.[33] However, it suggests that both of these viruses indeed use a common ACE2 receptor for binding to the host cell membrane to pass on a disease to humans.[14] Despite the amino acid mutations at some RBD,[34] it is proposed that ACE2 acts as a dimer which forms a complex with the membrane protein and acts as a chaperone. The two trimeric spike proteins of the virus bind to this ACE2 dimer[35] and interact with the lung epithelial cells. This then triggers the inflammasome NLRP3 activation and unfolded protein response which cause an inflammatory response, the classic symptom that is associated with the disease.[36]
Another study also suggests the involvement of spike protein used for host cell attachment. The spike glycoproteins in both of its forms, i.e. ligand free and bound conformation, is involved in the process. The spike glycoproteins use newly identified N and O sites for the process of glycosylation and this is what enables the virus to shield itself from the immune system of the host. The S1 domain of the SARS-CoV-2 spike glycoprotein is believed to interact with CD26 cell surface glycoprotein, a key immunoregulatory factor for hijacking and responsible for the severity of the disease.[37]

Types of SARS-CoV-2

Genetic mutation gives diversity to a population and it is something that is observed in living organisms[38] as well as viruses. These mutations can increase the binding affinity to the host cell receptor, something that was observed in A/H5N1 influenza viruses.[39] Such mutations are observed with the COVID-19 virus. Point mutations are observed in NSP2, NSP3, spike protein and in RBD. It is believed that the mutations in NSP2 and NSP3 play a part in the infection that the virus is capable of and it also makes it distinguishable from the mechanism that is followed by SARS-CoV-2.

SARS-CoV-2 is classified into S and L types. The strains in L type are derived from S type, and as a result are evolutionarily more aggressive, spread more quickly and are very contagious. Approximately 30% is S type, for which the cases show less severe symptoms and less aggressive action, and 70% is L type, for which cases are more intense and severe. However, there are also research articles that indicate the L type can be asymptomatic.[40] The S type has potentially originated from an animal, most likely a bat, so there is a form of zoonotic connection, while the L type is a recombination of S type.[14, 41]

Mechanism of entry to host cell and lung cell infection
As previously mentioned, coronaviruses have glycoproteins on the surface of the envelope which initiate entry of the virus by identifying the cell receptors sites of the host membrane. This is usually followed by conformational changes to its structure to facilitate fusion to the cell membrane of the host, pushing its genome into the cytoplasm of the host. It is well known that the mRNA sequence is used as a template to assemble amino acids which are then used for protein synthesis.
The protein used by SAR-CoV-2 is the spike S1 protein.[42] The COVID-19 virus operates in the same way where the 20nm complex crown-like structure on the virus, known as spike glycoprotein of SAR-COV-2, has a RBD (through S1 subunit of RBD) which recognises and binds to humans receptor ACE2 (through S2 subunit of RBD) by membrane fusion.[21] The S2 subunit containing a fusion peptide, a transmembrane domain and a cytoplasmic domain is highly conserved. Thus, it could be a target for antiviral (anti-S2) compounds.[11] The RBD receptor-binding motif (RBM, particularly Gln493) has a particularly favorable interaction and directly contacts ACE2.[43] These target the antibodies of the host and form corona spikes enclosed in the viral envelope in the host cell. These S proteins are important as they are crucial components to understand the capacity of the virus to infect other cells, as well as rate of transmission.[44] ACE2 cleaves the angiotensin (Ang) I to produce Ang-(1-9) in addition to providing a binding site for S spike proteins. It is in a metastable state and under-

goes a dramatic structural rearrangement to fuse the viral membrane with the host cell membrane. The S1 subunit of RBD undergoes structural rearrangement to facilitate the fusion process. This fusion is rather strong and it has 10-20 times larger binding affinity than SAR-CoV.[45]

COVID-19 pathogenesis is believed to depend on various factors that include the interaction of the virus with the host cell receptor, the way it identifies and attaches to the receptor, the recognition that is followed by RBD, the receptors of the host it is targeting, protease cleaving, the process it uses to fuse its membrane to the host cell membrane, host cellular transmembrane serine protease (TMPRSS) and its ability to strongly bind to the host cell receptors. In humans, the receptor ACE2 is expressed in the lung AT2 cells, oesophagus epithelial cells and in colon and ileum cells. This is one of the reasons why, when the virus infects an individual, the symptoms are usually either respiratory or gastrointestinal such as sore throat, difficulty in breathing and diarrhea.

ACE2 and transmembrane protease serine 2 (TMPRSS2) are co-localised in the same host cells. TMPRSS2 causes hydrolytic effects accountable for the S-protein attachment and entry into target cells. The mechanism the virus uses to cause gastrointestinal symptoms is, however, largely unknown and further investigation is required. The drugs that need clinical attention are the ones that use ACE2 fusion proteins and TMPRSS2 inhibitors. One other less-identified symptom is damage to the liver that is also observed in patients that are infected with SAR-CoV-2A recent study also suggested that an increased ACE2 expression was observed in epithelial cells of the bile duct (59.7% of cells) rather than liver cells (2.6% of cells), which indicates that the virus may be causing direct damage to intrahepatic bile ducts.[34]

The coronavirus infects the lung cells and produces a massive cytokine storm as well as the presenting symptoms. The virus S-glycoprotein of the spike proteins is used for attachment to the host cell, i.e. type II pneumocytes in the case of lungs.[14] The receptor ACE2 on the surface of the human cell is known to be targeted as it is highly expressed in the lungs. The S protein then needs to be cleaved by TMPRSS2, termed S protein priming.[46] Thus TMPRSS2 and ACE2 are believed to work in harmony for S protein activation to facilitate the process of fusion and cell entry of the virus into the cell via the S protein. ACE2/TMPRSS2 are co-expressed cells within type II pneumocytes, absorptive enterocytes, and nasal goblet secretory cells.[47] The role of autophagy and its involvement in the said process is debated but it is suggested that the novel coronavirus uses clathrin-dependent and independent endocytic pathways to enter the host cell. After entry, the virus is enclosed in a vesicle following early and then late endocytosis. The endosome matures into lysosome which finally encodes and is released from the viral envelope to give +ssRNA. This nucleic acid is released into the cytoplasm and can now bind to the ribosome.[46, 48]

The endocytosis cellular pathways were therefore suggested as a drug development target for COVID-19.[49] This receptor mediated endocytosis pathway is recommended but it is also suggested that the virus can enter into the host cell by direct membrane fusion to the cell membrane receptor of the host cell. Following either of the pathways, the virus +ssRNA after fusion undergoes transcription and then translation by the host cell which then synthesises the two polyproteins pp1a and pp1ab in the ribosome leading to viral protein production. These polyunits act as structural units to synthesise the structural and non-structural proteins of the virus. Production of these structural proteins leads to completion of assembly and release of viral particles.[50] These proteins are then cleaved by viral proteases to form replication-transcription protein complexes (RTCs) by a viral protease 3-chymotrypsin-like protease (3CLpro) producing viral replication machinery.

The envelope of the virus also has a crucial role in virus pathogenicity as it promotes viral assembly and release.[11] The viral RNA is protected from detection by the host immune response which is usually accomplished by opposing interferon signaling protein and with the replication of RNA carried out in double membrane vesicles in replication-transcription protein complexes, which includes the RNA-dependent RNA polymerase. The produced viral mRNA is then translated and the virions are assembled in the endoplasmic reticulum and golgi bodies.[27] The packaged golgi vesicle assemblies[51] leave the infected cell by a process of exocytosis (Fig. 1).[52] The released viruses cause infection of surrounding cells and thus cause the appearance of symptoms.[53]

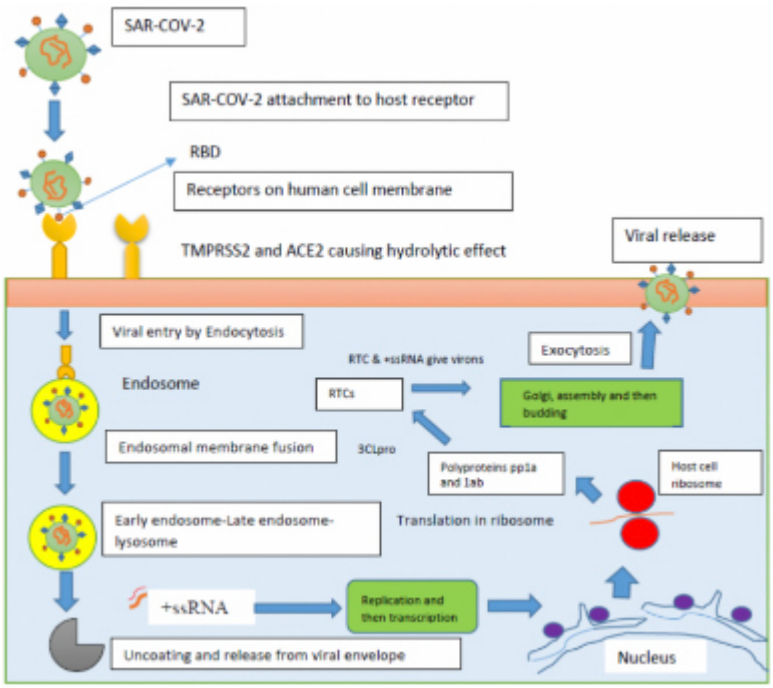

Fig.1. Virology of COVID-19 and entry into the human host cell

It is reported that SARS-CoV-2 leads to downregulation of the ACE2 receptor, but not ACE, through binding of the spike protein with ACE2. This leads to viral entry and replication, as well as severe lung injury.[54] This attachment of the S protein to the receptor most likely contributes to pathogenesis as it not only down regulates ACE2 but also elevates the formation of angiotensin II which increases the flow of small molecules in and out of the ACE expressed cells such as those in the lungs.

The virus damages the cells around it and these damaged cells of the alveoli release inflammatory molecules with an associated molecular pattern. These are usually cytokine molecules such as interleukin-1β (IL-1β), induced protein 10 (IP10) and monocyte chemoattractant protein-1 (MCP-1).[55] These cytokines alert alveolar macrophages in the vicinity to form potent inflammatory mediators as it encourages the production of cytokines and chemokines. This is known as a cytokine storm that includes interleukin-2, interleukin-4, interleukin-7, interleukin-8, interleukin-10, interferon-γ, tumor necrosis factor-α and macrophage inflammatory protein-1-α, suggesting a broad type 1 and type 2 helper T-cell response.[27,46] Targeting these inflammatory cytokines, for instance interleukin-6 (IL-6), is also a considered treatment option.[55]

Alveolar macrophages also blast out inflammatory molecules and these cytokines can increase the activity of procoagulants and decrease the activity of anticoagulants which will lead to clot formation and eventually to pulmonary emboli.[56] Disturbed coagulation function is found in some patients.[57] GM-CSF and IL-6 are the major cytokines that increase inflammatory response and lead to elevated alveolar-capillary blood-gas exchange dysfunction, especially impaired oxygen diffusion, and eventually lead to pulmonary fibrosis. Tocilizumab interferes with IL-6 and has been recommended as a therapeutic compound against SAR-CoV-2.[58] Some of these inflammatory cytokines, such as interleukin and TNFalpha, move out into the pulmonary capillary area. As a result, fluid starts leaking out in between the pulmonary capillaries and into the alveoli and cause alveoli oedema. In order for gases (oxygen from alveoli into the blood) to move they need a very thin respiratory membrane. The oedema causes the membrane to become thick as less oxygen is moving into the blood. As a result, there is less partial pressure of arterial oxygen into the blood, causing hypoxemia. This is a potential effect that can result in the inability of lungs to oxygenate the blood and causing heavy breathing or difficulty in breathing.[59] Patients with SAR-CoV-2 demonstrate a decreased ratio of arterial oxygen partial pressure to fractional inspired oxygen (PaO_2:FiO_2 ratio) with associated low oxygen (hypoxia) and rapid breathing (tachypnea).[60]

Abnormally high CO_2 levels in the blood (hypercapnia or hypercarbia) is found to be common in COVID-19-related acute respiratory distress syndrome (ARDS) patients with low tidal volume ventilation.[61] This hypercapnia is due to the fact that over time, as the lungs start struggling and patients are not able to breathe as rapidly, the muscles get weak and the CO_2 will not be released from the blood into the alveoli, subsequently escaping into the exhaled air. This can lead to conversion to carbonic acid that can break down generating protons. The blood becomes acidic and potentially results in respiratory acidosis.[62] Another report also suggests that in patients with COVID-19, the arterial blood gas analysis showed severe metabolic acidosis

with excess lactic acid (hyperlactacidaemia) and low oxygen (hypoxaemia) in the blood.[63] This leads to shortness of breath as not enough oxygen is delivered to the tissues. Blood gases of patients with severe illness also showed a stark decline in arterial oxygen due to an increase in alveolar-arterial oxygen difference, thus hypoxia and hypocapnia are more commonly seen in acute cases. Post-mortem examination of patients from SARS-CoV-2 demonstrated that the mortality rate of these patients is due to damage to both sides of alveoli along with pulmonary oedema, an increase in concentration of pro-inflammatory molecules and signs of initial signs of ARDS. Over 70% of patients with COVID-19 showed increased lactate dehydrogenase levels which may also be due to hypoxia.[60] The disease presents differently in different patients, with each phenotype approached and treated individually.[64] The damaged alveoli cells produce inflammatory molecules which have the ability to activate particular receptors in the central nervous system (CNS). Our CNS generates the cough reflex that results in a non-productive[2] or productive cough.[65] Researchers also confirmed the presence of SARS-CoV-2 in cerebrospinal fluid by genome sequencing, adding support to the theory that this new pneumonia virus can also cause nervous system damage.[51]

Epidemiology

The distribution of the disease in over 200 countries severely stretched the available health facilities of even some of the most developed countries. One such example is China where, despite the prompt and large scale control measures initiated by the Chinese government, the COVID-19 epidemic spread drastically over the course of just 30 days, expanding from Hubei to the rest of mainland China.[66] The possibility of asymptomatic transmission of the disease is also present with COVID-19, which is a recipe for a bad outbreak,[67] but the proportion of such cases is low. The Chinese Center for Disease Control and Prevention summarised 72,314 cases in which 1% (889 cases) were asymptomatic, 62% were confirmed cases and the majority of cases (81%; 36,160 cases) showed mild symptoms. 14% were reported as severe and 5% as critical. The overall fatality rate was reported to be 2.3%.[68] The most affected patients were over 50 years of age and had an underlying health issue. Younger children were rarely reported to have the disease and were mostly asymptomatic but may assist in transmission.[11, 69] One case study suggests that more than 90% of children had asymptomatic, mild, or moderate cases.[70]

As at December 2020, vaccines are just becoming available and therefore for many the best preventative is self-care, quarantine and minimising social interactions *via* so-called "social distancing".[71] From the percentile estimation of the incubation period the suggested length of quarantine should be at least 14 days.[72] However, there are reports of this being longer than recommended based on community size.[73]

The global pandemic has affected all countries, some more severely than others. For instance, Italy reported its first COVID-19 case on 18 February 2020, soon after the initial reports in China.[74] The health care system was overwhelmed and the system capacity to hold patients in care was far less than demand. There were 105,792 reported cases of affected people.[75] The severity of the disease and the symptoms presented was so intense that ICU admissions represented 12% of the total positive cases and 16% of all hospitalised patients. This rate is higher than that reported from China, where only 5% of patients who tested positive for COVID-19 required ICU admission.[76] This is most probably due to large social movement that was initially neglected in Italy and only later forcibly imposed on 9 March 2020.[77] The deaths reported in Italy were as high as 839 in just one day with 105,792 confirmed cases on 1 April 2020.[78,79] The majority of the population in Italy is of advanced age[80] which is a major risk factor and hence the fatality ratio in these countries is observed to be higher due to the older age ratio. This may also explain the higher mortality rate.[81]

In 2017, 76.9 million people visited the USA from other countries for different purposes and this number increased even further in 2019.[82] The virus appears to have been circulating in the USA from mid-February 2020, long before the first case was reported there. Travellers were going back and forth from other countries, as well as domestically over land and by air which may have increased the chances of spreading the infection as a result of primary or secondary infection.[83] In the USA, the first patient officially identified to have COVID-19 was reported on 20 January 2020 in a 35-year-old male[84] and the first death that was officially announced was on 29 Feb 2020.[85] The total number of cases as at 4 December 2020 was 13,563,731 with the number of deaths reported to be 268,482.[86]

There are other countries which approached the problem more efficiently, an example being Japan.[87] They used the method of contact tracing in which a patient who tested positive for the disease was isolated immediately and the individuals they were in contact with were tracked down using the patient's cellphone Bluetooth data and signaling. These individuals were tested and isolated if required.[88] This helped identify the potential cases more promptly with great success. New Zealand has mainly used the approach of lockdown to contain the virus, with great success.

There are some countries that are more severely affected by this epidemic but whose testing capacity is not sufficient to be able to determine the actual mortality rate and diagnosed cases. These include low income countries like India[89] and Pakistan.[90]

Transmission

The spread of this contagious disease is from person to person by direct or indirect contact with an affected individual and hence characterised as direct or indirect transmission.[91] The person can also be an asymptomatic carrier of the virus where they themselves are not infected and show no signs of infection that is usually experienced with COVID-19 and yet are still a carrier.[40] Droplet spray in short ranges that may contaminate objects around it and a possibility of it being an aerosol is also another mechanism by which it can be spread[92] but no conclusive evidence is present for it being airborne.[93] SARS-CoV-2 can survive in urine and faeces of a patient for one to two days so there is the possibility it being transferred via body fluid. The surfaces that might be in contact with these can also become a probable source of contamination and lead to infection.[74, 94] Salivary gland transmission is also reported.[34] Reports also suggest it is spread via fomites,[95] a form of indirect transmission, where the surfaces it survives on vary such as aluminium (8 days), cardboard (24 hours), plastic and stainless steel (2-3 days),[96] glass and metal (≤5 days) and wood (4 days).[91] In fomite form, COVID-19 can be present in the environment for up to 3 days.[97]

The rate of spread via transmission varies, as it is reported that the infection is transmitted more slowly and then elevates gradually before any symptoms starts appearing.[98] According to WHO, asymptomatic patients are not drivers of disease transmission. However, there are some contradictory reports and the consideration of it being rare or non-transmittable via asymptomatic patients may be underestimated.[99] Transmission is described as pre-symptomatic, symptomatic and asymptomatic based on the symptoms that appear in an individual.[100]

There are some reports suggesting that it is not possible for COVID-19 to spread from mother to foetus.[101] Mothers who were tested positive for COVID-19 gave birth to newborns that did not test positive for COVID-19,[102] although there is research proving that pregnant women are themselves at greater risk of associated respiratory problems with the disease.[103] However, it should be noted that the data is limited for pregnant women with COVID-19 and it is not yet proven that there is no intrauterine infection and transmission from mother to infant.[104]

Symptoms

Common symptoms of COVID-19 include cough, fever, myalgia or fatigue[105] while less common ones include headache, diarrhoea, coughing of blood (haemoptysis), and pneumonia-like features including respiratory distress, acute cardiac injury,[106] shortness of breath (dyspnea), abdominal pain/diarrhoea, pharyngeal discomfort and chest pain. COVID-19, in most cases, presents with mild symptoms but can develop into severe illness. This severity is often in the form of acute pneumonia, accumulation of excessive fluid in the lungs causing pulmonary oedema, inflammation in the lungs with a common complaint of shortness of breath or rapid breathing leading to ARDS, multiple organ failure, or even death. Death is predominantly as a result of alveoli damage leading to respiratory failure. Serious cases report chest pain which arises because of inflammation of pleura and dyspnea leading to alveoli damage. Segmental pulmonary embolism has also been reported in one patient.[107]

The proteins present on coronavirus affect the human gastrointestinal system, heart, kidney, liver and CNS leading to several different organs being damaged.[29] A study showed that the lungs of these infected individuals exhibit oedema, proteinaceous exudate, focal reactive hyperplasia of pneumocytes with patchy inflammatory cellular infiltration and multinucleated

giant cells. These changes can be used for the initial identification and lung pathology of COVID-19 pneumonia.[108]

Airway bleeding (hemoptysis) along with lung lesion has also been reported in one patient during the first ten days of infection. The atypical pneumonia was considered due to second- or third-generation transmission of the viral infection. A CT scan also showed focal ground-glass opacities (GGO) with non-central distribution.[109] However, hemoptysis is described as an infrequent COVID-19 clinical symptom (0–5%) by retrospective analysis.[107] In a study of 140 patients with COVID-19 from China, two patients presented with hemoptysis. Thus it might be a possible clinical presentation for COVID-19 although it is not considered common.[110]

After the usual incubation period of five days, symptomatic patients experience common cold-like symptoms. The incubation period before symptoms appear can be longer than five days and can be as long as 10 days. Following incubation, the most common symptom to appear is a high fever and either a dry or productive cough. The mucus membrane of the voice box, trachea, bronchial tubes and finally the spongy alveoli sacs are affected once the disease progresses.[111]

High temperature is an onset symptom reported in 78.2% of patients which is also an indication that the immune system is highly activated.[112] Traction bronchiectasis is also observed in a certain proportion of patients. Lesions reported in CT are either peripherally distributed or have bilateral involvement. They are predominantly present in the lower lung although they can be multifocal. Architectural distortion, traction bronchiectasis, is another symptom that is present in COVID-19 patients.[113] A further report suggested similar findings that high temperature followed by cough and GGOs present in the bilateral lung is the most common pattern found in CT of COVID-19 patients.[114] The pathology is rarely reported due to the inaccessibility of biopsies and autopsy results. One report suggested that histological examination results show bilateral diffuse alveolar damage with cellular fibromyxoid exudates,[13] bronchial wall thickening in severe cases and lymph node enlargement, pericardial effusion and pleural effusion. These severities were mostly age-related and critical cases were reported in patients of older age that had more underlying diseases with weakened immune systems.[112]

The CT scans showed that the asymptomatic patients displayed increased GGOs when compared with CT scans of symptomatic patients.[115] This is contrary to the common misconception that asymptomatic patients are apparently not affected by the disease. Another report suggested similar findings in that 20.8% of patients with COVID-19 develop mild typical symptoms like cough and fever. However, in these patients, 50% of them had GGOs in the CT scan and 20.8% had shadowing in their lungs.[116] The ratio of asymptomatic cases can vary depending upon the type and number of population selected for the study. One report suggests this ratio to be 41.6% for 565 individuals tested.[117]

Symptoms associated with the nervous system are grouped according to which part of the nervous system is affected. The first category is the CNS, displaying symptoms including headache, dizziness, impaired consciousness, ataxia, acute cerebrovascular disease, and epilepsy. The second category is the peripheral nervous system (PNS), displaying symptoms including hypogeusia, hyposmia, hypopsia, neuralgia and skeletal muscle injury.[118]

Risk factors

The mortality rate and overall rate at which COVID-19 spread worldwide has caused a "pandemic of the decade". This was very alarming for older patients as increased morbidity and mortality is more common in people over the age of 60 and patients who are already suffering from diabetes, hypertension or obesity. According to the USA national diabetic statistics report, 34.2 million people have diabetes and 88 million have prediabetes in the USA in 2020.[119] So far there are several reports suggesting that diabetes is a risk. A case study of 1099 patients who tested positive for the disease indicated that 173 were associated with some form of comorbidities such as hypertension (23.7%), diabetes mellitus (16.2%), coronary heart disease (5.8%) and cerebrovascular disease (2.3%). Another case study included 140 patients and suggested that 30% these patients infected with the virus had hypertension and 12% had diabetes.[120]

Age

The incubation period of coronavirus is from 6 to 41 days with a median of 14 days. However, this period is related to the age and immunity of the patient. Typically older people that are over 70 display a shorter incubation period that for those who are less than 70 years old.[121]

Comorbidity

A retrospective study of 191 patients showed that 48% of these patients had a comorbidity, with hypertension being the most common (30% of patients), followed by diabetes (19%) and coronary heart disease (8%). Along with older age, a sequential organ failure assessment score was also used which gives the rate of organ function or organ failure. When the patients were in an intensive care unit, the score was recorded as very high and D-dimer elevations and disseminated coagulopathy was also found to be enhanced in COVID-19 patients. This can be considered as a risk factor for death of adult patients with COVID-19.[122, 123]

Cardiovascular disease

Patients that have a history of heart disease or have an underlying metabolic disorder are at a greater risk of showing severe symptoms if infected with COVID-19.[124] The virus itself can intensify heart damage further putting these patients at greater risk.[125] The exact percentage of the people who suffer from cardiovascular disease (CVD) who also had COVID-19 may vary from one report to another but it has been suggested that nearly 36% of patients who tested positive for COVID-19 had associated cardiovascular complications. One study reported this percentage to be 5% - 30% in hypertensive patients with 2.5% - 15% having CVD.[126] Some patients showed an increased troponin t level which indicates some form of myocardial injury occurred in these infected individuals.[122] Another report suggested this injury is present in more than a quarter of patients, i.e. more than 7% of the patients suffered from myocardial injury from the 22% of the patients that were severely ill.[127]

ACE2 adheres to the cell membrane of heart tissue and is therefore involved in heart function, and hypertension. The virus, using its spike proteins, binds to the receptor ACE which are overly expressed in the heart. COVID-19 affects the respiratory system and causes alveoli damage, however patients with CVD display severe symptoms due to elevated secretion of ACE2 in contrast to a normal individual. For the same reason it is recommended that patients who are on antihypertensive medicine using ACE inhibitors and also suffering from COVID-19 should use alternative therapeutic medicine other than ACE inhibitors.[128] A numerical model predicts that in addition to the age of an individual and their gender, the increased formation of heart proteins like NT-proBNP and cardiac troponin **(**cTnI) can be used as biological markers for assessment of muscle injury of the heart due to COVID-19. An elevated level of higher sensitivity C reactive protein (hs-CRP) and increased creatinine levels were also found to be significantly related to the severity of the disease.[129]

Diabetes mellitus

Most of the patients that suffer from diabetes mellitus (DM) are between 41-60 years which puts them more at risk of developing the disease with severe symptoms.[130] The innate immunity of these patients is also comprised allowing the pathogen to infect the host more easily. In diabetes mellitus the cytokine response, particularly (IL)-1, IL-6 and tumor-necrosis factor (TNF)-α, is increased. This response is further elevated when a person is suffering from COVID-19. The basic purpose of ACE2 is to break the angiotensin-II down to angiotensin, angiotensin I and smaller peptides. ACE expression is decreased in patients with DM, perhaps because of glycosylation, and this may be one of the reasons that an elevated predisposition is present in lung injury and ARDS with SARS-CoV-2.

The increased expression of ACE is also harmful in COVID-19. The virus utilises the ACE2 cell surface receptor for attachment and entry to the host pneumocytes. The drugs that are designed to control DM are mostly ACE blockers or angiotensinogen receptor blockers (ARBs). Renin-angiotensin system (RAS) activation is increased in patients that are suffering from DM. The ACE inhibitors (ACEIs) and ARBs increase the expression of ACE2 receptor present in the heart in these patients which allows the virus to enter into the heart tissues and can cause heart arrhythmia. The use of drugs such as ACE2 restoring drug will ease the path of COVID-19 pneumocytes and subsequently result in acute and deadly disease. Pioglitazone[131] and liraglu-

tide[132] are diabetes drugs that are involved with ACE2 in increasing the response to a stimulus in animal studies. However, no conclusive study has been conducted to investigate this further.

One other report suggests that severe and critically ill patients with COVID-19 have increased and widespread presence of low blood potassium (hypokalemia) as a result of renal potassium wasting. The decrease in production of ACE2 after viral invasion causes a drop in effectiveness of angiotensin-II, elevated aldosterone secretion and subsequently an elevated loss of potassium in urine. An initial normalisation of serum potassium is recommended as a marker for good diagnosis of COVID-19 patients.[133] Laboratory trends such as an elevated ferritin level and reduced platelet counts, or erythrocyte sedimentation rate and the HScore is also observed in COVID-19 patients.[134] As previously mentioned, the virus attaches to its host cell using the ACE receptor which is overexpressed in patients with diabetes who are treated with ACE and ARBs inhibitors. Hypertension is also treated with the same blockers and results in upregulation of ACE2. This increased ACE2 expression increases the risk of infection by COVID-19. Patients with cardiac diseases, hypertension or diabetes and that are on ACE2 stimulating drug thus have an increased risk of developing severe and fatal COVID-19.[135] The available data about the number of patients that have diabetes and COVID-19 is currently limited. One study performed on 140 patients in China indicated that diabetes was not considered a risk factor. On the other hand a report that included 150 patients showed that the presence of comorbidities is most likely a good predictor of fatality rate with diabetes included as one of the factors.[136] What is clear is that older age and the presence of diabetes, hypertension and/or obesity considerably elevates the risk for hospitalisation and subsequent death in COVID-19 patients.[137]

Hypertension

The predominant comorbidity accompanying patients with SARS-COV-2 is hypertension and diabetes. This subsequently leads to CVD and respiratory system disease.[138] There is enough research to suggest that tehe renin-angiotensin system (RAS) has been weakened in these patients but it is unclear if using RAS system inhibitors such as ACE or ARBs will have a positive or negative impact on individual health. One study showed that patients suffering from COVID-19 and provided with ACEI or ARB treatment displayed less severe symptoms of disease and a decreased level of IL-6 in the peripheral blood. Moreover, ACEI or ARB treatment also increased CD3 and CD8 T cell counts in peripheral blood and decreased the peak viral load compared to other antihypertensive drugs. Therefore, using ACEIs or ARBs can significantly improve the clinical results of patients with COVID-19 and hypertension.[139]

The coronavirus also has an impact on the physiological behaviour of people as it creates anxiety and depression among individuals. From 31 January to 2 February 2020 an online survey was conducted to evaluate the physiological impact of COVID-19 on individuals and it was reported that, of the 1210 respondents from 194 countries, more than half reported anxiety, depression and stress[140] which further increases the risk of being infected with the virus. Various important studies have been conducted since then with similar findings.

Diagnosis

Chest CT scan

Individuals with SARS-CoV-2 are recommended to have a chest CT scan because GGOs are diffused and the lungs are compressed with fluid in them. When the GGOs are surrounded by either complete or partial rings of consolidation then this is called a halo sign which is found in these patients. This can act as a radiological marker for the identification of the disease in these patients.[141]

Biomarkers

Considerably elevated levels of cytokines and chemokines in blood were noted for patients with COVID-19 infection.[121] A decrease of lymphocytes acts as an indicator in severe cases showing the consumption of immune cells and hence can be used as an indication for determining the severity of the disease.[112] Blood analysis demonstrated high inflammatory markers and lymphopenia (white blood cell count 23080/mm^3, neutrophils 91.4%, lymphocytes 1.4%, C-reactive protein 52.7 mg/L) and increased hs-TnI (4332 ng/L) in these patients.[63]

Treatment options

Passive transfer of antibodies

The passive transfer of antibodies is a suggested treatment option that can be prophylactically used. The antibodies introduced to the affected person will help them recover.[142] This was the approach used for the 1918

flu epidemic, as well as mumps, measles and polio and the SARS epidemic of 2003.[143] The antibodies that are extracted from patients who were infected and subsequently recovered from COVID-19 can be used as a treatment option known as human convalescent serum treatment. The blood from the recovered patient is used to screen for antibodies. A serum is then made from these antibodies and introduced into the patient in a prophylactic manner for the purpose of preventing infection in critical cases such as patients with comorbidities. Treatment with convalescent serum is effective in decreasing symptoms and death rate. The effectiveness of the method is debated but can be considered as a treatment option for COVID-19.[144]

Chloroquine phosphate

Chloroquine phosphate is an antimalarial drug on clinical trial that seems to be safe and effective against COVID-19 associated pneumonia.[102, 145] It is also reported that COVID-19 patients have a subgroup that suffer from cytokine storm syndrome. With the elevation in mortality rate from 3.7 % the need to use a safe approach for treatment is necessary. The increased ferritin level in both recovered and deceased patients suggested that the higher fatality rate is due to hyper inflammation that is associated with the disease. Critically ill patients diagnosed with COVID-19 could be tested for increased inflammation by testing for elevated levels of ferritin, declining platelet counts, or erythrocyte sedimentation rate and the HScore11 to classify the subcategory of infected individuals for whom immunosuppression could increase the rate of fatality. Treatment options include steroids, intravenous immunoglobulin, selective cytokine blockade such as anakinra or tocilizumab and JAK inhibitors.[134]

Secondary haemophagocytic lymphohistiocytosis (sHLH) is an increased inflammation within a number of organs that malfunction and eventually lead to death, and is usually triggered by a viral infection. By comparing the cytokine profile to that of sHLH they are found to have similarities in patients with COVID-19. They are most commonly identified by increased interleukin (IL)-2, IL-7, granulocytecolony stimulating factor, interferon-γ inducible protein 10, monocyte chemoattractant protein 1, macrophage inflammatory protein 1-α, and tumour necrosis factor-α. The use of IL-1 blockade (anakinra) in sepsis showed improved chances of survival for COVID-19 patients with hyper inflammation.[134]

Chloroquine and hydroxychloroquine

A study conducted in China of patients suffering from COVID-19 appeared to prove the effectiveness of chloroquine and hydroxychloroquine use for treatment. A daily 600 mg dose of hydroxychloroquine was given to patients. A nasopharyngeal swab was taken daily which is commonly used as a diagnosis method for COVID-19, collecting the sample from the surface of the respiratory mucosa.[146] Azithromycin was used alongside as a treatment option. Untreated patients acted as a control. The 20 patients used for the study displayed a considerable decrease in transmission of the virus after day 6 of treatment in contrast to the control group, and a significantly lower average carrying duration was observed than for those patients who were not treated. Thus addition of azithromycin to hydroxychloroquine was considerably more effective for decreasing the activity of the virus. The study also confirmed that the use of hydroxychloroquine is related to viral load reduction/disappearance in these patients and its effect is improved when used in combination with azithromycin.[147] Chloroquine and hydroxychloroquine are 4-aminoquinolines which act as a weak base to increase the pH of the endosome in host organelles. This inhibits the fusion of autophagosome and lysosome and thus prevents the replication process.[148]

There is much controversy revolving around this use, with some reports suggesting it as an effective approach and consider it praiseworthy in giving fast clinical results and should be used as an experimental drug[148] while others consider it less effective[149] and even dangerous with adverse side effects such as cutaneous adverse reactions, heart failure[150] and bone marrow dysfunction.[151] Most of the case studies reporting its effectiveness are either non-randomised, falsely reported or considered only a very small number of patients and the hypothesis for its effectiveness does not appear to be sound.[152] One study suggested that 70% of patients who were treated with hydroxychloroquine quickly recovered from COVID-19 but this study opted out cases in which patients were admitted to the ICU post-treatment with the drug or when patients died after dosage.[153] These drugs may well do more harm than good and a large, carefully controlled clinical trial with randomised testing would be required to test

effectiveness before recommendation as a clinically effective drug could be made.[154]

Remdesivir

There is a single case study where a clinical trial of remdesivir drug is used on a COVID-19 patient in the USA and the results appear to be promising.[114] RNA dependent RNA polymerase or RdRp is an enzyme that is essential in the life cycle of RNA viruses, including COVID-19. The COVID-19 RdRp model was built and molecular docking was performed for direct-acting antiviral drugs against the disease. The results were good enough to consider their possible use against COVID-19. This report also recommended that using direct-acting antiviral drugs such as sofosbuvir, ribavirin, and remdisivir against COVID-19 proved to be effective. The use of guanosine triphosphate derivatives was also recommended.[30] In a separate trial remdesivir was claimed to be effective against coronavirus when tested on animals. Six rhesus monkeys were first infected with respiratory disease and then given the drug intravenously 12 hours post infection. The amount of virus was decreased and, in 5 out of 6 monkeys, breathing was considerably improved. A drug trial in Chicago on 113 patients suffering from COVID-19 showed they recovered in less than a week.[155]

There are some reports suggesting the use of RdRp inhibitors such as arbidol and favipiravir for the treatment of COVID-19.[156,157] Favipiravir is an RdRp inhibitor which has the ability to block the replication of RNA viruses.[158, 159] It is converted into an activated favipiravir-RTP which is identified as a substrate by viral RNA polymerase, thus selectively inhibiting RNA polymerase activity. Following the same mechanism, an RNA virus of COVID-19 can therefore be controlled using favipiravir.[156]

Table 1 summarises other drug options that have been considered.

Table 1. Drugs recommended in the literature for COVID-19 treatment

Drug	Description
Barcitinib	A reversible[160] Janus kinase (JAK) 1 and 2 inhibitor[161] as well as NAK inhibitor, with high affinity for AAK1,[162] and Cyclin G associated kinase. Prevents endocytosis and decrease viral assembly.[163] It also reduces cytokinase release[164] and serum levels of IL-6, IL-1β, and TNF-α, recovery of circulating T and B cell frequencies, and increased antibody production against the SARS-CoV-2 spike protein[165]. This drug also significantly improves SpO2, PaO2/FiO2, CRP, and MEWS.[166]
Lopinavir–Ritonavir	The effectiveness against COVID-19 is rather controversial[167] with some studies suggesting it less or not effective[168] against coronavirus when used alone.[48] Some studies suggest a decrease in viral load and coronavirus titers after administration of lopinavir–ritonavir, but its clinical efficiency is still in doubt with further investigation needed to be classify it as a safe drug option.[169] Utilising a triple therapy by administrating interferon beta-1b, lopinavir–ritonavir and ribavirin is found to be superior in treatment when compared to using it alone.[170]
Favipiravir	Favipiravir is a small purine analogue and converted into its active ribofuranosyl 5'-triphosphate metabolite in the cell.[171] It is an oral broad-spectrum inhibitor of viral RNA-dependent RNA polymerase and an effective antiviral agent.[172] When administered in a controlled experiment, a significant improvement in chest CT clearance, virus clearance and fever reduction was observed.[159] It also improves the latency to relief for pyrexia and cough[173] and controls inflammatory mediators.[174] The side effect associated is the increased uric acid in serum.[173] A case study suggested that the favipiravir and nafamostat combination inhibits hypercoagulopathy and decreased mortality against COVID-19 patients.[175] It has also been suggested to use in combination with umifenovir.[176]
Dexamethasone	Dexamethasone is a synthetic corticosteroid. Patients treated with dexamethasone showed a decrease in mortality.[177] Timing of dexamethasone is important with administration only for patients with hypoxaemia that usually presents 7 days after the onset of symptoms.[178] It limits the release of cytokines and their side effects and also inhibits B cells to synthesise antibodies causing elevated plasma viral load. It also blocks macrophages from clearing infections. Dexamethasone is considered effective for short term critical cases[179] which require respiratory support.[180]
REGN-COV2	REGN-COV2 is a combination of antibodies namely REGN10987 and REGN10933 recommended for treating COVID-19. It works by attacking the non-overlapping epitopes on the spike protein of coronavirus. Tests on animal models suggest that REGN-COV-2 can significantly decrease viral load in the lower and upper airways as well as decrease the virus induced pathological sequalae when administered prophylactically or therapeutically.[181]
Tocilizumab	IL-6 level is found to be higher in patients with chronic COVID-19. Tocilizumab is an IL-6 antagonist that is found to be effective against COVID-19.[182] A study suggests that treating severe COVID-19 patients with tocilizumab results in a significant decrease in inflammatory markers, reduced ventilatory support requirement[183] and improved lung function.[184] These studies recommend its prompt use in the severe stage for critically ill patients.[185] There are some studies doubting its effectiveness against the coronavirus[88, 186] with no improvement seen in moderately ill patients.[187] Post treatment of tocilizumab results in late onset infection.[188]
Corticosteroids	Just like dexamethasone the time at which the dose of corticosteroid is administered is important. In severe cases of COVID-19 the clearance of virus is relatively slow and mortality rate is increased when patients are treated at the late stage of pneumonia.[189]

Vaccination

In April 2020 there were 115 vaccine candidates for COVID-19, with 78 recognised as active vaccines. Of these active vaccines, 73 were at preliminary testing stage. As of December 2020 there are several vaccine candidates that are currently in advanced stages including mRNA-1273 from Moderna, Ad5-nCoV from CanSino Biologicals and INO-4800 from Inovio.[190] COVID-19 vaccines are usually divided into the following basic categories: viral-based vectors, RNA and DNA-based vaccines (also collectively known as nucleic acid based vaccines), protein-based (or subunit) vaccines and finally viruses themselves that include activated and inactivated viruses and virus-like particles that use the non–SARS-CoV-2 virus carrier to stimulate the immune system of the host.[191]

Viral-based vectors

A replication-incompetent recombinant adenovirus, Ad5-S-nb2 carrying a codon-optimised gene encoding spike protein was recently synthesised which can express SARS-CoV-2 S protein in infected cells. An intramuscular injection and intranasal non-injection route was used to test on animals to measure the effectiveness of the designed vaccine. It was concluded that the given vaccine brings out the immune response that is specific to S protein antibodies. After a period of 30 days a single dose of vaccination protected the animals against coronavirus infection making it a candidate for further trial tests. The vaccine works by expressing the S protein of the virus in infected cells.[3]

AZD1222 (the "Oxford" vaccine) formally known as ChAdOx1 nCoV-19 is a newly synthesised version of adenovirus vaccine vector that works on the principle that when the virus is administered to patients, it produces spike proteins. The dosage causes immune priming to identify and attack the COVID-19 virus if a vaccinated individual is infected with it in the future.[192] The vaccine, developed by AstraZeneca, is in phase III trials and claims to be highly efficient against COVID-19. Administration of the primer dose followed by the booster dose resulted in an average efficacy of 70%. The virus can be kept at 2-8C and is therefore manageable from a storage perspective.[193]

The participants were all healthy adults between the ages of 18-55 years with no confirmed cases of COVID-19. A booster dose was administered 28 days after the first dose. The report suggested that the spike-specific T-cell responses peaked on day 14 and anti-spike IgG responses elevated by day 28 with no serious side effects observed in participants.[194] The vaccine trial was temporarily paused due to an unexplained illness in a study group but experts believe that it is common practice to halt trials to assess the safety of a vaccine.[195] The Data and Safety Monitoring Board recommended that the trial restart.[196,220]

The Russian vaccine "Sputnik V" was approved and claimed to combat COVID-19. It is based on two human adenovirus vectors and the spike S protein to generate an immune response. Two trials were conducted and in both of these the vaccine was intramuscularly administered. In one trial participants were administered the vaccine that used a recombinant adenovirus vector based on the human adenovirus type 26 containing the SARS-CoV-2 S protein gene, while the other group was administered with a similar vaccine but using human adenovirus type 5 vector. One group received both types of vectors. The results were promising and helped to develop immunity.[197] However, there are some serious concerns among scientists as it is being approved without large scale testing[198] and the data for the Phase II trial is not sufficient to approve it for Phase III trials.[197]

Nucleic acid-based vaccines

A nucleic acidbased synthetic vaccine is another approach that is being well received by the medical community with fairly good results on the clinical stage. These vaccines target the S protein of the virus. INO-4800 induced robust expression of the S protein in vitro and generated antibody and T cell responses following a single immunisation in mice and guinea pigs. The preliminary dataset identifies INO-4800 as a potential COVID-19 vaccine candidate, supporting further study for mobilisation against this emerging disease threat.[199]

BNT162b1 is another mRNA which is delivered via a lipid nanoparticle. The nucleosides that are modified are often required for enhancing immunity. These modified nucleosides in the mRNA encode RBD of the SARS-CoV-2 S protein. When participants were treated with two doses of the vaccine, the T-cell and antibody

response was increased. The RBD-binding IgG concentration was also higher in the serum when compared with COVID-19 patients. Participants were given a dose of vaccine and then a booster dose on day 22 with a different dose level. A single dose was also given to 12 patients on day 1 only. Patients experienced common expected adverse reactions (reactogenicities) which were tolerable and manageable with simple treatment.

An increased $CD8^+$ and $CD4^+$ T cells response was observed in all patients and can be used as a biomarker.[200] IFNγ cytokine response was also produced after vaccination which suggested that it has the potential to protect against COVID-19 through multiple beneficial mechanisms.[201]

Moderna's clinical candidate mRNZ-1273 is an mRNA vaccine also delivered via lipid nanoparticles. Following injection into the patient, their cells replicate the S protein the coronavirus uses. The immune system then makes antibodies and other immune cells to neutralise it. No live viruses are needed at any stage and the vaccine was designed on computer using genetic data. Nanotechnology has proved very useful to improve the stability and delivery of mRNA based vaccines.[202] mRNZ-1273 is suggested to be a lead vaccine in treating COVID-19.

Protein-based / subunit vaccines

Protein vaccines are often less immunogenic and require a large dosage to enhance antigen-specific immunity. A pre-clinical vaccine by scientists at the University of Queensland used a molecular clamp which is a novel technique to synthesise a subunit vaccine for COVID-19. The thermally stable vaccine has the potential to successfully activate the immune response.[203]

The trial vaccine Novavax is a protein-based vaccine planning to be tested in South Africa.[204] Previously only a very small number of protein vaccines have been approved for use as they require an adjuvant to enhance the immune response.[205]

Inactivated whole vaccine

The inactivated whole vaccine is an interesting approach for development which has been utilised by researchers for COVID-19. The immune system of the host identifies the multiple proteins that are located on the surface of protein.[206] The whole inactivated viruses require an adjuvant to produce immunogenicity due to non-replicative nature of the viruses.[207] Several whole inactivated virus vaccines are in clinical trials for treatment of COVID-19.[208] One Phase I trial constituted 96 participants who were administered with a whole inactivated virus vaccine. By the end of the 28 day course, it was reported that mild side effects were observed in patients with immunogenicity being observed in treated participants.[209]

COVID 19-and New Zealand

New Zealand has used several successful strategies to control COVID-19 including implementation of advice from disease plan modelling and up-to-date science advice. The basis of the planning was a mitigation model with a suppression strategy to "flatten the curve" with a view to eliminating the virus in New Zealand. A prolonged lockdown, digital tracing[210] and quarantine is the strategy until the vaccine becomes available.[211]

Pursuing elimination and achieving zero community cases of COVID-19 is possible and has been achieved in New Zealand although there has been an economic cost.[212,213] The active cases in New Zealand as at December 2020 were imported cases held at the border in managed isolation and quarantine.[214] The New Zealand government has signed an agreement with Pfizer to import 1.5 million vaccine doses which will be sufficient to vaccinate 750,000 people.[215]

Summary

As of December 2020, a majority of countries worldwide are reporting new cases of COVID-19 daily despite extensive testing and isolation which has generally been used to control the spread of the virus. Even an advanced country with extensive medical facilities will be vulnerable to COVID-19 unless enforced physical distancing with strict quarantine is imposed along with the implementation of vaccination. Ultimately COVID-19 has brought global social and economic impact and shown that everyone is vulnerable to a rapidly evolving disease. In the future, development of good hygiene habits and lifestyle considerations, as well as considerations around international preparedness for a pandemic will be at the forefront of global discussions.

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