



The Chemist

Journal of the American Institute of Chemists



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The Chemist

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Editorial

Searching, Researching and Publishing

David Devraj Kumar

Florida Atlantic University

As a researcher and educator, I consider that it is not only important to search and research, but also very important to make one's findings and ideas publicly available so that others can follow up and the quest for understanding the natural world continues. I am delighted that a collection of high-quality reports and articles fills this issue of *The Chemist*. While Calvin Dewitt is making a strong argument for stewardship in "bringing the human use of carbon fully within the bounds of the hospitable climate control system of the biospheric economy and our common home", Vicki Grassian is discussing insights from physical chemistry environmental interfaces in understanding processes taking place on indoor and outdoor environments. C. Saritha and co-authors report on synthesis and characterization of a novel carbazole-based hole transporting material with potential application in the fabrication of solid-state dye-sensitized solar cells. Rajib Choudhury and co-authors assess the efficacy of a donor-acceptor based red light emitting fluorophore in detecting human serum in aqueous samples.

Imelda Rubashvili and co-authors report a HPLC analysis involving sequential extraction for the anthocyanins obtained from agro-industrial waste material – grape skin. Elelu Shehu-Alimi and co-authors discuss the preparation and characterization of absorbents from the Physic Nut plant, a drought resistant perennial plant. John Sarah and co-authors discuss recent advances in the asymmetric Reformatsky reaction. Asha Chacko and co-authors report the oxidation behavior of permanganate functions supported on polymers.

Bryan Nichols, argues how chemical scientists can play a key role by involving themselves more with citizen science and civic science. Micheal Hansen with Hart Hansen recognize the importance of teaching the scientific method in the social sciences and suggests not limiting to the natural sciences.

It has been a privilege editing *The Chemist*, official journal of The American Institute of Chemists. However, it is time to move forward, after resurrecting *The Chemist*, and overseeing the publication of seven volumes (85-91) during 2012-2018 of *The Chemist*, I am stepping down as Editor-in-Chief. In 2018 the journal reached 91 years old and is now indexed in EBSCO. During this period the journal received, processed and published manuscripts from authors across the globe who with confidence chose *The Chemist* as a platform for disseminating their research and scholarly manuscripts and subjecting to the journal's double-blind peer-review process. Since inception in 2012, the Public Understanding of Chemistry Section has published a total of 19 manuscripts from a wide spectrum of authors including a co-author who is a school student.

Though I enjoyed doing this leadership service for seven years pro bono, it is a very time consuming task. Besides evaluating and editing incoming manuscripts, I enjoyed working with members of the review board who helped to maintain the quality of the journal. Thanks to review board members and editorial assistants for dedicated service pro-bono, and Alberto Fernandez for Art & Web

Direction. Also, thanks to Dean Valerie Bristor at the College of Education, Florida Atlantic University for providing a platform to perform all the needed editorial work for *The Chemist*. This is a humbling experience, also a clear example of the support scientists need from people outside of the field in order to move forward. Unfortunately, often out of ignorance, and sometimes out of arrogance, scientists tend to act as if they can make it on their own. Times have changed, and if science needs to move forward in a global society, it needs the support of scientists as well as non-scientists alike.

Human quest for searching and researching this complex natural world will continue on. Part of the quest comes from the chemical sciences which provide a platform for exploring and understanding the complexity of the natural world from the perspective of elements and compounds, their structure, composition and properties, in addition to their physical and chemical changes during reactions. The quest for search and research is built into every one of us and it is a part of being human. "It is the glory of God to conceal a matter; to search out a matter is the glory of kings" (Proverbs 25: 2) (1).

I wish each and every reader of *The Chemist* the best in life!

References

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Carbon, Climate, and Earth Stewardship: Prophetic Teachings of the Biosphere

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Abstract: Human civilization from the agricultural revolution, beginning some 10,000 years ago, and urban development beginning about 5,000 years ago, was supported throughout by a reliable and hospitable climate. Now, however, this hospitability is being compromised, requiring intensified and persistent work and dedication toward its restoration – particularly by institutions, governments, and enterprises and those who scientifically and ethically create, shape, lead, reform, and maintain them. *The present* must be described with exceptional truthfulness and accuracy, with diligence toward full understanding and restoration of Earth’s climate system of self-control. Knowledge of this system must be freely accessible and comprehended by all, uncluttered by false representations and misinformation, informative of relationships of cause and effect, and embracing the entire system, with consequences of human actions expressed and addressed temporally and spatially. Central to this is bringing the human use of carbon fully within the bounds of the hospitable climate control system of the biospheric economy and our common home.

Key Words: Carbon, Atmospheric Chemistry, Climate Change, Climate Economics, Biosphere, Biospheric Economy, Fitness of the Environment, Regulation, “Earth Stewardship and *Laudato Si*” – this last being a key phrase for entry on Google search.

The business of prophecy is not foretelling the future; rather it is
describing the present with exceptional truthfulness and accuracy

– David Ehrenfeld (p. 9) (1)

INTRODUCTION

Scientific discovery in the 20th century brought worldwide attention to the consequences of adverse human actions on the biosphere and its ecosystems at local, regional, and global levels. In response to the challenge these present, a widespread effort was made in America during the decade of the 1970s that brought the U.S. Congress to develop comprehensive legislation to address these assaults on creation, accompanied and followed by similar legislation throughout much of the world. From this, people worldwide are coming to understand the reality that we all live in the biosphere---the vital system that sustains all of our lives and livelihood---and most significantly, that we are adversely

affecting it in many ways, including the destabilization of Earth’s climate control system. Like it or not, this has extended our stewardship, bringing us to realize our new status and responsibility as stewards of the biosphere [2]. This unsettling realization brings challenges to everyone, to our institutions, and to our developing global human civilization. This is the reality of the present.

DESCRIBING THE PRESENT

As we and human society come to understand our new setting and status, we are finding it extremely difficult to describe the present. First, we realize that this requires an immense effort and is challenged by inexperience in describing our global reality – the habitable common home

into which we, unthinkingly, have been born. We have taken this integrated and sustained system *for granted* – simply as a given to us and all life. Never have we thought it would “bite back” – in view of its long history of continued hospitability to us and all of life. An integrative understanding of the intricacies and operating scope of this remarkably integrated life-support system has been so beyond our grasp that many people today don’t really want to know how it works as a wholly integrated system. We would like to have the freedom and the security it gives but without the need for integrated knowledge of its workings. Not that we are lazy about learning and knowing, but because we never expected we would ever have to know this system comprehensively. However, even as we might rather not want to know, we do not like the looks of what we are coming to see. Biospheric changes over only a decade or two contrast strongly with formerly steady and predictable features and futures. We may be reluctant to speak about it, for fear of being called alarmist, but we are coming to find it necessary to describe the present. In this we find ourselves discovering that life is largely atmospheric –with major constituents, carbon, hydrogen, oxygen, and nitrogen, always being reciprocally exchanged with the atmosphere [3]. I am hoping in this realization that religious people are coming to view this in the context of atmospheric providence for which we should respond with gratitude, care, and keeping.

ELEMENTAL & OXIDIZED CARBON

Among atmospheric constituents, carbon dioxide provides the molecular backbone of all living things. Physiologically captured from the air, its carbon is incorporated as the basic structural component of every living organism, and is subsequently sequestered in biomass, detritus, soil, peat, coal, bitumen, oil, and gaseous hydrocarbons. Returning sooner or later to the atmosphere by respiration, oxidation, and combustion principally as carbon dioxide, its atmospheric concentrations have been maintained at 0.28 percent – (280 ppm) over the course of recorded human history – a period of some 10,000 years, rising dramatically above this level only in recent decades and centuries. The trophic importance of this gas can be appreciated by considering that its removal would mean that photosynthesis would end, all green plants would perish, and very soon also every living being. But for some 10,000 years, it has been

right on the mark. Not too much, not too little; always kept at or very near 2.8 hundredths of one percent (280 ppm).

Oceanographer Roger Revelle summarized its significance this way: “Carbon dioxide may be thought of as the most important substance in the biosphere: that part of the Earth’s atmosphere, hydrosphere, and solid crust in which life exists. It has supported the existence and development of life by serving as the source of carbon, the principal element of which all living beings... are made. In past times it was a source of the free oxygen in the air and the ocean that makes animal life possible. By absorbing and backscattering the heat radiated from Earth’s surface, it maintains, together with atmospheric water vapor, a sufficiently high temperature in the air and the sea to allow liquid water, and therefore life, to exist (p. 3) [4].

HOSPITABILITY IN THE BODY & BIOSPHERE

What keeps the biosphere so hospitable – to microbes, plants, animals, human beings and human society? In seeking an answer, it is helpful to ask a similar question for ‘the internal environment’ of the body, as did 19th-century French physiologist, Claude Bernard. His extremely important dictum, “La fixité du milieu intérieur est la condition de la vie libre” (Regulation of the internal environment is the condition for a free life.), provides an extremely important dictum that applies not only to the internal workings of all living creatures, but also provides the foundation for cybernetics and control systems in science, engineering, and society [5]. This statement about freedom is informed by the controls that regulate body temperature, blood sugar levels, alkalinity, pH, and other chemical and physiological processes with some like body temperature regulation, using a kind of *central control* and others using a more *diffuse control* that is broadly present within and throughout the system being controlled. Whether central or diffuse, “La fixité du milieu intérieur” gives freedom to the body, allowing it to live without the need to think a moment-to-moment about operation and control of critical internal processes of sustainable living. In an answer to an exam question one of my university students explained it this way, “For example, there are thousands of little processes going on inside my body right now that I am not conscious of, but which are doing their best to facilitate my continued existence. If I had to consciously think about every process and control them without the help of internal regulation all my time and

energy would be spent doing that and I effectively would be a prisoner to this system of control and change, unable to live a 'free' life!" [6].

LEVEL & PRECISION OF TEMPERATURE REGULATION IN THE BODY

Regulation, be it the temperature of the body or the concentration of a gas in the atmosphere, or pH of the ocean, can be evaluated in terms of both its level and its precision. For human body temperature, the level of regulation is often placed at 37°C (98.6°F). Recent studies, however, put the level at about 36.6°C (97.9°F) and its precision is expressed as the central 95% range of 35.7 to 37.3 °C, or 1.6 Celsius degrees [7]. In contrast to endothermic mammals and birds, body temperature regulation achieved behaviorally by ectothermic animals is less precise, with intraabdominal body temperatures of the Desert Iguana, for example, having a level of 38.5°C (101.3°F) and precision, as the central 95% range, is 33.2 to 41.8 °C or 8.6 Celsius degrees [8].

ATMOSPHERIC CARBON DIOXIDE

Scientists often investigate things simply from their love to know, and this has resulted in important discoveries. It was from such research that physicist John Tyndall came to give a remarkable Friday evening lecture at the Royal Institution in 1859 [9]. He had set up a projector and screen to put a full spectrum of light on display, directing a stream of ignited oxygen and hydrogen at a cylinder of lime, calcium oxide (CaO), to produce a bright beam of the limelight. He passed this light through a prism he had fashioned from rock salt to the screen, thereby showing a spectral array with the colors of the rainbow: red, orange, yellow, green, blue, indigo, and violet. He then took a thermopile he had prepared for measuring temperature and moving it across the screen proceeded to show that light of every color warmed the screen. Next, he moved the thermopile past the red light to an area that was dark on the screen, showing that again the thermopile was heated. Clearly, radiation was still hitting the screen, but it was not visible. Calling this "dark radiation," he speculated that the aqueous and vitreous humor of the eye absorbed this light and might prevent it from passing to the retina. Upon which he brought up a cow's eye he had picked up from the local butcher and pouring its contents into a vial made of rock salt, put these eye fluids into the path of the dark

radiation. Remarkably, the thermopile at the place where dark energy had been hitting the screen, was no longer warm. The dark light - what we now call infrared radiation -- had been stopped in its course by the humor of the eye.

Tyndall then told his audience how, after finding this, he had prepared a long tube through which he could project a beam of light, filling the tube with various gases. He said that he first was disappointed that nothing seemed to stop the dark radiation as had the material from the cow's eye. But gas from the spigot on his lab bench, while passing visible light fully, blocked the dark radiation completely. And carbon dioxide, while transmitting visible light fully, also blocked infrared radiation completely. With this he realized the significance of this for Earth's atmosphere, concluding that carbon dioxide would allow light from the sun to pass through the atmosphere to the earth, but would hinder infrared radiation from leaving back through the atmosphere.

A person born in the very year that Tyndall gave this lecture, physical chemist Svante Arrhenius, brought things further in the late 1800s by calculating the effect that a doubling of atmospheric carbon dioxide would have on Earth's average surface temperature [10]. This prominent Swedish scientist used the measurements made by astronomers Samuel Langley and Frank Very of infrared rays radiated from the moon at various angles to Earth's horizon. For these measurements, they used an instrument Langley had invented, the "bolometer," that could measure temperature differences as little as 1/100,000 of a degree C. Using Langley and Very's measurements of infra-red radiation from the moon at different heights above the earth's horizon, Arrhenius calculated the absorption of infrared radiation through different thicknesses of atmospheric carbon dioxide and water vapor. Then, from his calculations, Arrhenius computed from his knowledge of basic physics that a doubling of atmospheric carbon dioxide would raise the atmospheric temperature at Earth's surface by 5 Celsius degrees. Building upon these discoveries by Tyndall and Arrhenius, other scientists also came to realize the importance of carbon dioxide's role in maintaining a habitable Earth, among these being geologist Thomas C. Chamberlin, a professor and president of the University of Wisconsin and later chair of the Geology Department of the University of Chicago.

Chamberlin's monumental three-volume textbook, *Geology*, written with his colleague and former student Rollin Salisbury in 1909 [3] includes a section on Life material chiefly atmospheric where they write:

"In the building up of the organic compounds, a necessary step is the decomposition of certain inorganic compounds. The chief of these is carbon dioxide of the atmosphere and hydrosphere, the decomposition of which furnishes the carbon needed for the organic compounds. On this account carbon dioxide may be regarded as in some sense the basal material or the fundamental food of the organic kingdom, and hence it plays a radical role in the life history of Earth. Water, and the constituents of water, oxygen, and hydrogen, play a larger part quantitatively, but a less distinctive part. Nitrogen is also an essential element and usually stands next to carbon, oxygen, and hydrogen in quantity. These, it will be noted, are all atmospheric constituents, and the material of life is, therefore, dominantly atmospheric" (p. 638) [3].

Chamberlin and Salisbury developed a focus on carbon dioxide, and even though it is among the least abundant atmospheric gases, described it as the chief inorganic compound. And, on "the climatic effects of organic action" they write:

"The atmosphere blankets the earth and equalizes its temperature...[and] while the solar rays come in rather freely and heat the surface of the earth, the dark rays which the earth radiates back are measurably arrested by the carbon dioxide and vapor of water and serve to keep the air warm" (p. 642) [3].

ATMOSPHERIC CARBON DIOXIDE FOR 10,000 YEARS

During the development of human civilization, society began to change from nomadic to settled lifestyles as the agricultural revolution began some 10,000 years ago and enabled more compact human communities. Many of these communities were further enabled by increased agricultural productivity and security to develop into cities beginning about 5,000 years ago, mostly along streams and rivers that assured reliable sources of water [11]. Accompanying these 10,000 years of agricultural and societal development - and its sustained productivity of food, community security, and regularity of natural water supplies - was a reliable, sustained, and predictable climate that, if not seasonally hospitable, was successfully addressed by fabrication of adaptive clothing and seasonal food storage.

A highly significant indicator of the reliable, sustained, and predictable climate during these ten millennia is the regularity of atmospheric carbon dioxide at or near 280 ppm. With measurements of atmospheric carbon from ice cores, and continuous measurements of atmospheric carbon, begun by chemist Charles David Keeling in 1958 at the Mauna Loa Atmospheric

Observatory in Hawaii, we have learned that carbon dioxide concentration for this 10,000 year period has been maintained at or near the 280 ppm level, all but for its final centuries. Data from the Law Dome, Antarctica ice core show remarkable regularity from 0 to 1600 years A.D., with its 43 records having an average concentration is 279.5 ppm (standard deviation: 2.05 ppm) and range from 276 to 284 ppm [12]. In earlier years, from 2342 to 10,123 years BP, for the seven records from the Vostoc, Antarctica ice core, the median concentration is 262.2 and range from 254.6 and 284.7 ppm. This remarkable regularity over the ten millennia course of development of human civilization indicates that atmospheric carbon dioxide has been controlled at or near 280 ppm level during this span of 10,000 years.

This remarkable regularity is the consequence of carbon dioxide as a principal temperature "control knob," identified as such by chemist Andrew Lacis, and colleagues in their 2010 paper, "Atmospheric CO₂: Principal Control Knob Governing Earth's Temperature" [13]. Their paper was summarized by an editor of the journal *Science*, where it was published, as follows:

"The physical effect of atmospheric carbon dioxide on Earth's energy budget - that is, its 'greenhouse effect' - has been understood for more than 100 years, but its role in climate warming is still not universally accepted. Lacis et al. (p. 356) conducted a set of idealized climate model experiments in which various greenhouse gases were added to or subtracted from the atmosphere in order to illustrate their roles in controlling the temperature of the air. The findings clearly show that carbon dioxide exerts the most control on Earth's climate and that its abundance determines how much water vapor the atmosphere contains, even though the radiative effect of the water vapor is greater than that of carbon dioxide itself." (p. 471) [14].

For ten millennia, this regularity has given what physiological body temperature has given each of us: the freedom not to have to think about it. The statement by my student about physiological regulation also applies to the external environment. "La fixité du milieu extérieur est la condition de la vie libre" applied to the biosphere can be rendered:

Regulation of Earth's temperature is a necessary condition of a free life. This is a dictum we now can rightfully use to accompany the one given for our "milieu interieur" by Claude Bernard a century ago.

GREAT REGULARITIES OF EARTH'S TEMPERATURE & OCEAN NEUTRALITY

As carbon is key to the regulation of Earth's temperature, it is also key to the regulation of the chemical neutrality of the ocean. "The most striking of all the ocean's qualities is its constancy," wrote Henderson in 1913 (p.164) [15]. This chemist's discovery of this constancy, including the extraordinary property of carbonic acid in preserving chemical neutrality - familiar to every chemist as expressed in the Henderson-Hasselbalch Equation - brought him to understand the regulation of the pH of the blood, but also of the ocean and other water bodies of the Earth. In wonder, he declared for carbonic acid: "no other known substance shares this power" (p. 153) [15]. "Moreover, the chemist has discovered no means of rivaling the efficiency and delicacy of adjustment of the process... Almost wholly, through this mechanism, the oceans are always nearly neutral" (p. 153) [15]. Here there is no *central control* in contrast with vertebrate body temperature regulation, but *the diffuse control* that is broadly embedded within the system being controlled.

OVERWHELMED REGULARITIES

The data of chemist David Keeling from Mauna Loa and Antarctic ice cores show, however, that these remarkable regularities no longer exist. Atmospheric carbon dioxide is rising exponentially, increasing by 40 percent, from about 278 ppm in 1750 A.D. to 390.5 in 2011. And, widespread acidification of the ocean is underway, as the remarkable buffering system described by Henderson is being overwhelmed beyond buffering capacity, by absorbing much greater amounts of carbon dioxide as it is driven by much higher concentrations in the atmosphere. Ocean pH has decreased from 8.13 to 8.05 (nearly 0.1 pH unit, or about 28%) and is predicted to decrease further, by 0.4 pH units, by 2100 [16].

When atmospheric CO₂ concentration reached 400 ppm in 2013, physical chemist Charles Miller wrote, "Current CO₂ values are more than 100 ppm higher than at any time in the last one million years...This new record represents an increase of 85 ppm in the 55 years since chemist David Keeling began making measurements at Mauna Loa. Even more disturbing than the magnitude of this change is the fact that the rate of CO₂ accumulation in the atmosphere has also been increasing over the last few

decades, meaning that future increases will happen faster." [17]. And so too for ocean acidification, as this is driven by increasing atmospheric CO₂ levels.

On October 8, 2018 the Intergovernmental Panel on Climate Change (IPCC) announced publication of their Special Report on *Global Warming of 1.5°* [18], at the invitation of the Paris Climate Accord of 2015, in which they reported that human activities, particularly increased atmospheric carbon dioxide, has resulted in about a 1 Celsius degree of global warming above pre-industrial levels (*likely* range: 0.8 to 1.2 C deg) with expected increase to 1.5 Celsius degrees between 2030 and 2052 (*high confidence*) at current rates of increase. They also reported that the ocean has absorbed about 30% of the anthropogenic carbon dioxide, resulting in ocean acidification and changes to carbonate chemistry that are unprecedented in at least 65 million years (*high confidence*), with expected impacts on a wide range of marine organisms, ecosystems, and on aquaculture and fisheries (*high confidence*).

Ocean ecosystems are changing on a large scale, with critical thresholds expected to be reached at 1.5 C degrees and above (*high confidence*). And moving to a 1.5 C degree increase will bring ecosystems such as kelp forests and coral reefs high rates of mortality and loss (*very high confidence*). As an example, multiple lines of evidence indicate that most warmer water coral reefs (70-90%) will largely disappear when global warming exceeds 1.5 C degrees (*very high confidence*). Moreover, this loss of 90% of coral reefs will remove resources and increase poverty across tropical countries worldwide. And protection of shorelines by coral reefs is at risk and will be amplified by sea level rise.

Importantly, the IPCC's Figure SPM.2 illustrates graphically the impacts and risks of increasing global warming for people, economies, and ecosystems for what they call Five Reasons for Concern (RFCs) [18]. Because the remarkable regularity within Earth's climate system has thus been overwhelmed, it is vitally necessary that a clear and decisive restorative response is necessary. Earth's climate system and ocean buffering system need restoration to the self-control that has been sustaining Earth's life and human civilization for ten millennia and more.

RESPONSIBILITY FOR RESTORING & MAINTAINING THE CAPACITY FOR REGULATION

When the system that maintains biospheric temperature control is overwhelmed, by whatever its drivers, our task is much like the physician's: we must restore the conditions of health so that the thermoregulatory system can do the work it normally does, including its sustaining vibrant life. Body temperature regulatory systems teach this well: if they are pushed off track by hypothermia or hyperthermia, the physiology of the body does not accommodate to a new body temperature above or below the 36.6°C (98°F) level. Instead, its capacity to self-regulate must be restored. Work that puts the regulatory process back into full self-control is required, thereby to give the freedom and security to live a fruitful and abundant life.

SHOULD WE LIVE OUR LIVES WITH A CONTINUOUS FEVER?

We know from experience that if our body temperature is 40°C (104°F), meaning that we have a fever, it is good to bring it down to a 39°C (102.2°F) fever. But we also know that it is not good for our bodily economy to operate at this, or any, feverish temperature continually. We simply must restore the conditions that allow our body thermoregulation to operate with full health, meaning that it again operates at its normal level and precision. So too, as it may be a very worthy goal today to bring atmospheric carbon dioxide to 350 ppm, we also know that we cannot simply make this still feverish level the new norm!

The aim of restoring health – be that of our body or biosphere – is to get things back under self-control. As the goal of the physician and nurse is to restore body temperature to normal, not having it work with a marginal fever, so too with Earth's climate system. From physiology and medicine, we learn this: It is always the greater part of the physician's task – including the Earth physician – to restore the self-regulatory processes of the system to normality; disabling the drivers that degrade its self-control.

There often will be frustration, even with colleagues, as we work to restore integrity and normality to the 'external environment' in which all living things live. Among such frustration is the experience of fellow scientist Louis Sytsma, with whom I taught at Au Sable Institute. Upon visiting a waste chemical injection well in Kalkaska County, Michigan he found another chemist in

charge doing calculations on what could be injected into the deep ground beneath such that the pure waters below could be raised up to the limits that had been set for deep groundwater pollution. The limit had become the goal, even as maintaining the relative purity of deep underground formations from pollution was being sacrificed. The position of my colleague was that his profession of chemistry should call him to find and have implemented methods to maintain groundwater quality and develop ways to use the materials that were to be injected as a resource, not simply discard them without regard to consequences.

As my chemist colleague used his knowledge of chemistry to gain insight into the responsibilities of chemists, so did Walter Cannon for physiologists and medical doctors. Cannon, who coined the word *homeostasis* to describe the consequences of self-regulation, had written *The Wisdom of the Body*, a book on this subject [19]. His publisher, W. W. Norton, encouraged him to make applications from his research to society as part of his book, but Cannon refused. Only after repeated urging he reluctantly did so, but only as an epilogue in a later edition. Nearly a decade later, however, when giving his address as the outgoing president of the American Association for the Advancement of Science in 1940, he put things together, speaking on "The body physiologic and the body politic."

Physiological homeostasis would suggest, further, that stability is more important than economy... Extra blood volume, lung capacity, blood pressure, and cardiac power --much more than ordinarily required --all indicate generous preparations for meeting unusual demands, which might create disorder if they were not met. In personal and governmental practice, also, the principle of preferring security to the economy has been to some degree recognized. Life insurance and accident insurance may be paid year after year with no advantage except a sense of being protected. Fire departments are maintained, and armies and navies are kept in fighting trim at great expense, again with special regard for safety rather than economy (pp. 7-8) [20].

We can profitably do as medical doctor Walter Cannon did, to apply our understanding of the regulatory and control systems of the human body, or of chemical regulation in the body and oceans, as Lawrence J. Henderson did in chemistry [15] to the body politic. More than that, however, we can apply this understanding to the operations of Earth and its atmosphere, wisely employing limits we might set, but always aiming to go beyond setting limits to achieve normal levels of self-regulation.

CONTROLLING OURSELVES

Unlike the assessment of *Man's Role in Changing the Face of the Earth* in 1956 which described increased human influence upon earth's systems, we now know six decades later that we human beings have not only achieved domination, but also are seemingly unable to control our domination, even as we find ourselves destabilizing Earth's climate regulation [21]. The greatest unsettling truth at present is that through our actions, we have so transformed the biogeographic, trophic, and climate systems that sustain us and all life that we have propelled ourselves into a new role: like it or not, our responsibility has been extended to become responsible stewards of the biosphere. Our work is not to further press and overwhelm our climate system's operations, not to presume we can take over its control and put it into our own hands. Instead, it is to discover the systems that control Earth's climate system and to bring ourselves in accord with that control.

Beyond this, we must be persistently diligent in restoring the conditions that once again allow earth's controls to operate in service to life on earth – as it has been providing for past centuries and millennia. For this, we need fully to understand the biosphere's operations as a controlled system. This return of the economy of the biosphere to its self-control requires a more comprehensive and integrative understanding of the biosphere that we have put together –integratively, with full knowledge of how its level and precision are being compromised by human drivers. In this, we need vitally to enculturate this understanding into our practices and institutions to restore and sustain a habitable earth. Simply put, we must come to rule ourselves with truth and grace.

INSTITUTIONS & THEIR ROLE

Global changes that contribute to unsustainability at all levels – including climate, weather events, degraded ecosystem services, and regional public health issues – require understanding not only of the biosphere but also of *institutions* [22]. For it is institutions that produce and can correct the various social drivers both of sustainability and unsustainability. Institutions, according to institutional economists, are the sets of rules, conventions, arrangements, and frameworks that form and shape human actions in the biosphere. They shape human relationships within the world, either in support of the

way the biosphere works, contrary to it or indifferent toward it. They are the social constructs that frame human action in the world, whether that be at the level of the family, profession, church, club, community, business, economy, organizations, or government and are determined by beliefs about society and the wider world, beliefs that institutional economist and Nobel laureate Douglass C. North describes as 'internal representations' of the world (p. 49) [23]. Thus, institutions are external manifestations of our beliefs – manifestations of our internal representations of the world.

While our society becomes aware of the consequences of destabilizing the regularity of atmospheric carbon dioxide and its effects on our biosphere's climate system, including highly consequential global warming and ocean acidification, we find that our out-of-control system for atmospheric carbon dioxide regularity has not yet been sufficiently encompassed within our institutions – a conclusion that is self-evident from describing the present with exceptional truth and accuracy. A belief that all people hold in common is that our society must not degrade or destroy the habitability of our biosphere – our common home. This basic belief is deeply embedded in us and vital to each of us and to our civilization. When institutions are not adequately matched to what is necessary to sustain the biosphere, like the climate control system that has held Earth's thermostat very near 280 ppm for 10 millennia, they require reform that matches the needs for restoration of normality. Specifically institutional reform, innovation, and creation is needed immediately that (1) recognizes that the term *fossil fuels* as a very serious misnomer for what in fact is sequestered *fossil carbon with a key storage function within Earth's climate control system*, (2) recognizes that release of carbon dioxide from fossil carbon turns up the control knob of Earth's temperature control system, (3) works to put the human carbon economy back into its proper place within the larger biospheric carbon economy, and (4) recognizes that negative feedback has to be built into our economy that restores atmospheric carbon dioxide the normality it has had for 10,000 years.

CARBON, CLIMATE, & STEWARDSHIP

The distinguished historian of chemistry, Colin Russell, Fellow of the Royal Society of Chemistry, gave a series of lectures at Cambridge University that examined the prospects for restoring environmental integrity on earth. In 1994 these lectures on his comprehensive historical

inquiry were published, on the present condition and prospects for the future of our planet, with special attention to the history of chemistry and the chemical industry [24]. Importantly, he concluded that science and technology are not the problems. Instead, he located the problem in what he called *the motivating springs of active degradation: human arrogance, ignorance, greed, and aggression*. Human beings know what environmental integrity means and yet they degrade the Earth. This is the human predicament.

Importantly, Russell evaluates the prospects for addressing this predicament through the postmodern organismic view of Earth but finds this countering the very science needed to restore environmental integrity. He evaluates the conventional reductionist view but finds this inadequate for addressing this human predicament and the breadth of its impact. In seeking a third way, he comes to an ancient response to this predicament, summarized by the keyword, *stewardship*. Russell's proposition is this: human beings have an instrumental stewardship function in Earth's recovery, whatever theological views they may or may not hold. At the *conceptual level*, stewardship makes the great effort to understand the complex systems of the planet and biosphere, says Russell. It recognizes that in times of environmental degradation the need for public understanding of science is greater than ever. At the *perceptual level*, stewardship leads people to value the Earth highly as a treasure held in trust. It develops empathy with nature and sympathy for those who work for environmental integrity. And at the *relational level*, stewardship elicits practical strategies for relating people to the Earth as responsible members who are obedient to the dictates of conscience [25].

It is in this context of Russell and the keyword *stewardship* that the announcement on October 8, 2018 of the Nobel Prize in Economic Sciences awarded to William D. Nordhaus "for integrating climate change into long-run economic analysis" is particularly important. His conceptual development of the social cost of carbon (SCC) has already delivered more than \$1 trillion dollars of benefits by actions of various U.S. agencies, with the SCC defined as the monetized value of the net impacts from global climate change that result from an additional ton of CO₂." [26] when motivated by the ethical motivations of responsible stewardship – as these were embedded in U.S. federal requirements that were in place that incorporated the SCC (p. 5) [27]. But it fails when the motivating springs – arrogance, ignorance, greed, and aggression – overwhelm responsible stewardship. Thus, internationally there will be free-riders who contribute little or nothing to

reducing carbon dioxide but reap the benefits of the efforts of other parties. And, the benefits often will be "diffuse in space and time" (p. 29) [27], and escape most everyone's attention and concerns. In response to the problem of such free-riding and diffuse benefits, Nordhaus proposes Climate Clubs [39] which, properly designed, could harness Russell's motivating springs to drive desired outcomes. Such design would have the potential of embedding the SCC into the interacting climate and economic system to bring this system fully into accord with its operations in natural systems, with a more diffuse control. Use of Global Carbon Budgets will be helpful in this design [28]. Colin Russell's analysis, in either case, still requires an over-riding stewardship ethic.

RESOURCEFUL EARTH VS. EARTH AS TEACHER

A consultation was held at Windsor Castle on September 15-17, 2000 by the John Ray Initiative to explore the value and robustness of *stewardship* as a theological, philosophical, scientific, and pragmatic concept. Its intention was to investigate the biblical and traditional roots of stewardship, together with any implications from scientific perspectives, and to inquire whether these provide an adequate description for general use in the secular as well as religious context. The consultation was under the leadership of Sir John T. Houghton, co-chair of the first Scientific Assessments for the IPCC, and geneticist R. J. "Sam" Berry of University College London; and I was one of four speakers in four half-day sessions attended by 24 key thinkers. The arrangement was that one of us speakers would be asked after the first four lectures to give a second presentation, informed by the first two days, as a conclusion who turned out to be me and I wrote:

A worldview that perceives human life and economy within the wide embrace of Creation's economy is a necessary component of every successful culture. What UCLA geographer and biologist Jared Diamond describes for the collapse of Easter Islanders can become a metaphor for our earth. (p. 148) [29]. "When the Easter Islanders got into difficulties, there was nowhere to which they could flee, nor to which they could turn for help..." (p. 119) [30].

Successful cultures and civilizations must shape and reshape human behavior in the direction of maintaining individual, community, and environmental sustainability... They had to understand their world and its workings by direct experience and accumulated knowledge (scientia), had to gain from their experience and culture an understanding of what constituted right

living in the world (ethics), and had to put an interactive and coherent understanding of the world, and how rightly to live, into practice (praxis). Their behavior had to flow from the interactive and coherent engagement of scientia, ethics, and praxis... This is the essence of stewardship. *Stewardship dynamically shapes and reshapes human behavior in the direction of maintaining individual, community, and biospheric sustainability in accord with the way the biosphere works.* (p. 150) [29].

WINDSOR CASTLE & THE LONG WALK

Following the concluding session of the Windsor consultation on stewardship, John Houghton and I strolled along the Long Walk out from Windsor Castle, talking about what we should do next in our life's journey. We concluded that it was vital to bring together leading climate scientists and evangelical leaders at a forum to discuss global warming and our common home. About this a pioneering atmospheric physicist and author of a highly-regarded book, *Global Warming: The Complete Briefing*, a book that in its 5 editions had a chapter on Why We Should be Concerned, NRC meteorologist and climatologist, John S. Perry, in an editorial review in the *Bulletin of the American Meteorological Society*, wrote:

John Houghton has drawn on the exhaustive efforts of the Intergovernmental Panel on Climate Change to produce a notably compact, impeccably concrete and authoritative, meticulously balanced, and lucidly presented guide to the complex yet vital issue of global warming. Houghton holds that we humans are thus on earth for a purpose – to serve as its stewards, not just on behalf of the future carriers of selfish genes but on behalf of God. Many avowed agnostics such as myself, will find this forthright declaration of religious belief and divine purpose a bit startling in an otherwise rigorously scientific volume. However, in a line of argument that I have no difficulty at all in supporting, Houghton demonstrates that the domains of science are simply complementary ways of looking at the truth. The former deals with how the world works and the latter with why [31].

The Forum on Global Warming and Climate Change was held at St. Anne's College, Oxford University, July 14-17, 2002. Its some 70 leading climate scientists, policy-makers and Christian leaders from across 6 continents produced the Oxford Declaration on Climate Change [32]. It declared that activities, especially the burning of coal, oil and natural gas, are rapidly increasing the concentrations of greenhouse gases, especially carbon dioxide, in the global atmosphere... And it concluded that the Christian community has a special obligation to provide moral

leadership and an example of caring service to people and all God's creation and proclaimed that human-induced climate change is a moral, ethical and religious issue and a matter of urgent and profound concern. It referenced the biblical teaching on loving your neighbor, with the new implications of this in the face of present and projected climate change (Matthew 22:37-39), on reconciliation of all things (Colossians 1:20) and on the human calling to the ministry of reconciliation (2 Corinthians 5:18-19) [33]. Finally, the Oxford forum participants called upon leaders in churches, business and government to join them in recognizing human-induced climate change as a moral and religious issue and to take necessary action to maintain the climate system as a remarkable provision in creation for sustaining all life on Earth.

OUR COMMON HOME REQUIRES A COMPELLING UNDIVIDEDNESS

Civilization, from the agricultural revolution beginning some 10,000 years ago and urban development beginning about 5,000 years ago, has had a reliable and hospitable climate. As for the past ten millennia, it can be in the future. At present, however, Earth's climate regulation system is being seriously compromised. It is this that brought Jesuit chemist and pope, Jorge Mario Bergoglio, to write, "Now, faced as we are with global environmental deterioration, I would like to enter into dialogue with all people about our common home" [34]. He had taken on the name Pope Francis, whose namesake from Assisi is his guide and inspiration and with this invitation introduced his comprehensive treatise, *Laudato Si' – On Care for Our Common Home* – a scientifically and ethically grounded treatise on climate change, biodiversity loss, ocean degradation, atmospheric pollution, and social degradation [34].

As I was finishing my review of his treatise – for *The Quarterly Review of Biology* in 2015 [35] at the invitation of its editor-in-chief, Daniel Dykhuizen – I found myself being drawn to a compelling conclusion: *Laudato Si'* is so clear-sighted in its prophetic description of the present, so integrative of science and religion and of human and natural ecology, and so vital toward caring for Earth as Our Common Home, that it clearly earns the status of *required reading* for everyone, even as it embraces everyone and everything. For chemistry, physics, biology, ecology, and all of the natural sciences, it is particularly significant as it compels *undivided perseverance* on Care for Our Common Home. So too for science and religion as I have described in "Earth Stewardship and *Laudato Si'*: Care for

Our Common Home Compels Undividedness of Science and Religion,” a blog article for the International Society for Science and Religion [36].

In this treatise, he is aiming not for mere dialogue but the dialogue that results in appropriate action that is at once swift and deliberative. Human civilization, and indeed life on Earth, has come to global crisis – specifically in the sense of *crisis* – the Greek rendition of *crisis*, meaning: “The point in the progress of a disease when an important development or change takes place which is decisive of recovery or death.” *Krisis* is “the turning-point of a disease for better or worse... a state of affairs in which a decisive change for better or worse is imminent” [37]. It is with this meaning that he writes “today’s problems call for a vision capable of taking into account every aspect of the global crisis” in an *integral ecology* (p. 93) [34].

All of which makes Pope Francis’ *Laudato Si’ – On Care for Our Common Home* required reading as a remarkably comprehensive treatise on understanding and caring for the earth system as our common home. And its title invites every person on earth to “gratefully admire the beneficent arrangement which permits the Earth to be clothed with verdure and abundant life,” as astronomer Frank Washington Very presaged this in 1900 (p. 130) [38].

We have come to realize, with Francis, that “Given the scale of change, it is no longer possible to find a specific, discrete answer for each part of the problem. It is essential to seek comprehensive solutions which consider the interactions within natural systems themselves and with social systems. We are faced not with two separate crises, one environmental and the other social, but rather with one complex crisis which is both social and environmental. Strategies for a solution demand an integrated approach to combating poverty, restoring dignity to the excluded, and at the same time protecting nature.” (p. 104) [34]. My “Earth Stewardship and *Laudato Si’* ” [35] develops these topics and themes in a more detailed and more-comprehensive 14-page paper that is available as a free download at [https://doi.org/10.1086/688096_and Pope Francis’s *Laudato Si’ – On Care for Our Common Home*](https://doi.org/10.1086/688096_and_Pope_Francis's_Laudato_Si'_-On_Care_for_Our_Common_Home) is available as a free download at www.papalencyclicals.net/.

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the Vatican Pontifical Council for Justice and Peace, the U.S. Catholic Conference of Bishops, the Center for Catholic Studies at the University of St. Thomas, and St. Paul Seminary, St. Paul, Minnesota, June 3-5, 2015; (2) a paper presented at the Consultation on Faith and Science at the invitation of The Committee on Doctrine of the United States Conference of Catholic Bishops and The Catholic University of America (CUA) responding to Pope Francis’ letter *Laudato Si’ – On Care for Our Common Home*, CUA, Washington, D.C., October 26, 2015; and (3) an invited review article on this encyclical letter for *The Quarterly Review of Biology*, published in 2016. I’m indebted also to my distinguished scientist-colleagues and collaborators, Sir John T. Houghton and R. J. “Sam” Berry and to Sam’s introducing me to The Research Scientist’s Psalm, (111:2) about which he has written and with which he has closed even his last written words. I am also grateful to The University of Michigan-Dearborn for support of my course in Regulatory Physiology and the University of Wisconsin-Madison for support of my course on Modeling and Analysis of Environmental Systems, from which this paper benefits. The author also is grateful to the journal editor and two anonymous reviewers whose comments were very helpful.

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Physical Chemistry of Environmental Interfaces: Aerosols, Nanomaterials and Indoor Surfaces

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Abstract: The 2018 Chemical Pioneer Award symposium provided an opportunity to give an overview of the physical chemistry of environmental interfaces. These interfaces include atmospheric aerosols, nanomaterials in the environment and indoor surfaces. As discussed below, detailed physical chemistry studies on these complex surfaces are challenging yet can provide important insights into processes occurring on interfaces in various indoor and outdoor environments.

Key Words: Environmental interface, Aerosol, Nanomaterial, Indoor surface.

OVERVIEW OF AWARD ADDRESS

This article provides a brief summary of the Chemical Pioneer Award address entitled “Physical Chemistry of Environmental Interfaces: Aerosols, Nanomaterials and Indoor Surface”, presented on May 10, 2018 at the annual meeting of the American Institute of Chemists held in Philadelphia at the Science History Institute. The focus of this address was on environmental interfaces. Environmental interfaces are defined here as any surface in equilibrium with its surrounding environment. From this broad definition, there is a myriad of different types of environmental interfaces that include atmospheric aerosols, nanomaterials, and indoor surfaces.

The physical chemistry of environmental interfaces puts an emphasis on molecular and nanoscale level understanding of the detailed interactions that occur in these inherently complex systems. Examples of the complexity of these interfaces and how a deeper understanding can be obtained through a physical chemistry, molecular-based studies approach were highlighted in the award address.

ENVIRONMENTAL INTERFACES: ATMOSPHERIC AEROSOL SURFACES

Atmospheric aerosols which are defined as solid or liquid particles suspended in air with diameters between about 0.002 μm (2 nm) to about 100 μm . Aerosols are an important component of the Earth’s atmosphere and come from a variety of different sources. This is a large size range (5 orders of magnitude) which reflects the large difference in sources and formation mechanisms. There is also a large range of very different chemical compositions across this size range.

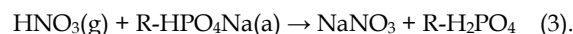
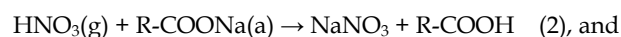
Two main primary sources of atmospheric aerosols include desert regions and oceans, which give rise to mineral dust and sea spray aerosols, respectively. Once lofted into the air, the surface of these two aerosols can interact with atmospheric gases, including water vapor and trace gases. Focusing on sea spray aerosol, recent studies have shown the complexity of SSA which on an individual aerosol particle level contains a mixture of salts, organic compounds and biological components [1-3]. The relative amounts of each of these depend on particle size and formation mechanism, whether formed through jet or film drops [4]. Recent studies have focused on understanding the physicochemical properties of this important component of the Earth’s atmosphere.

Figure 1 depicts some recent developments from single particle analysis in determining composition from electron microscopy [2], Raman microspectroscopy [3] and reactivity studies [5]. For reactivity studies, the reaction of nitric acid with sea spray aerosol was investigated. The current paradigm for the formation of particulate nitrate in the marine boundary layer involved the well-known reaction of the nitric acid gas with sodium chloride in sea spray aerosol, as shown in reaction (1):



However, little was known about how other components within sea spray aerosol reacted with this trace gas in the atmosphere. For these reactivity studies,

both authentic and model systems for sea spray aerosol were exposed to the nitric acid vapor. Two new reaction pathways were found and involved, carboxylate and phosphate groups present within lipopolysaccharides (LPS) in the aerosol. These reactions can be written as follows:



Kinetics for these reactions differ with the rate of reaction for LPS [reactions (2) and (3) being three to four times slower than that of NaCl (reaction (1))].

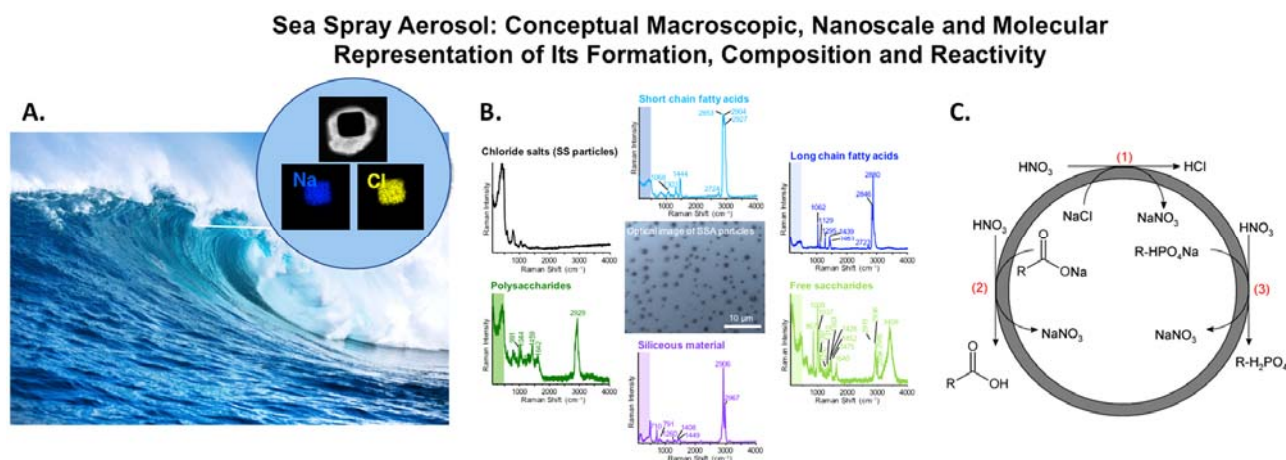


Fig 1. Sea spray aerosol, produced from wave action and bubble bursting at the ocean-atmosphere interface, is composed of salts, organic compounds and biological components. Understanding the physical chemistry of sea spray aerosol requires a single particle analysis approach using different microscopic and spectroscopic probes to unravel the nanoscale and molecular representation of the composition and reactivity. A. Electron micrograph identifying with energy dispersive electron spectroscopy the inorganic core of a sea spray aerosol particle with an organic outer coating; B. Raman spectroscopy identifying organic and biological components of sea spray aerosol and; C. Reactivity studies of sea spray aerosol identify new reaction pathways with nitric acid and sea spray aerosol due to the various functional groups within different components of sea spray aerosol.

Environmental interfaces: Nanomaterial surfaces and their interaction with atmospheric gases

Nanomaterials have high surface to volume ratios. This means that the energetics and properties are controlled to a large extent by the surface. Given that the surface plays such an important role in the properties of

nanomaterials, it is important to understand how common nanomaterials, e.g. metal oxide nanoparticles, interact with atmospheric gases.

In a recent tutorial review, Mudunkotuwa and Grassian provided insights into how nanomaterials transform in the environment [6]. In particular, several examples of nanomaterial transformations that can occur

in atmospheric and aqueous phase environments was laid out which show a wide range of transformation are indeed possible. In atmospheric environments metal nanoparticles can be easily oxidized to form metal oxide nanoparticles. In the case of Cu nanoparticles, these nanoparticles change over time [7]. Initially, the nanoparticle surface region is oxidized on top of a metal

core. This core shell morphology changes over time as the metal core shrinks and the oxidized layer grows with time due to oxidation with atmospheric oxidants including molecule oxygen. Additionally, it has been found the oxidized layer is composed of two phases, Cu₂O and CuO, with the most oxidized layer CuO at the outer surface of the particle.

Nanomaterials in the Environment: Nanoscale and Molecular Representation of Transformations in Contact with Atmospheric Gases

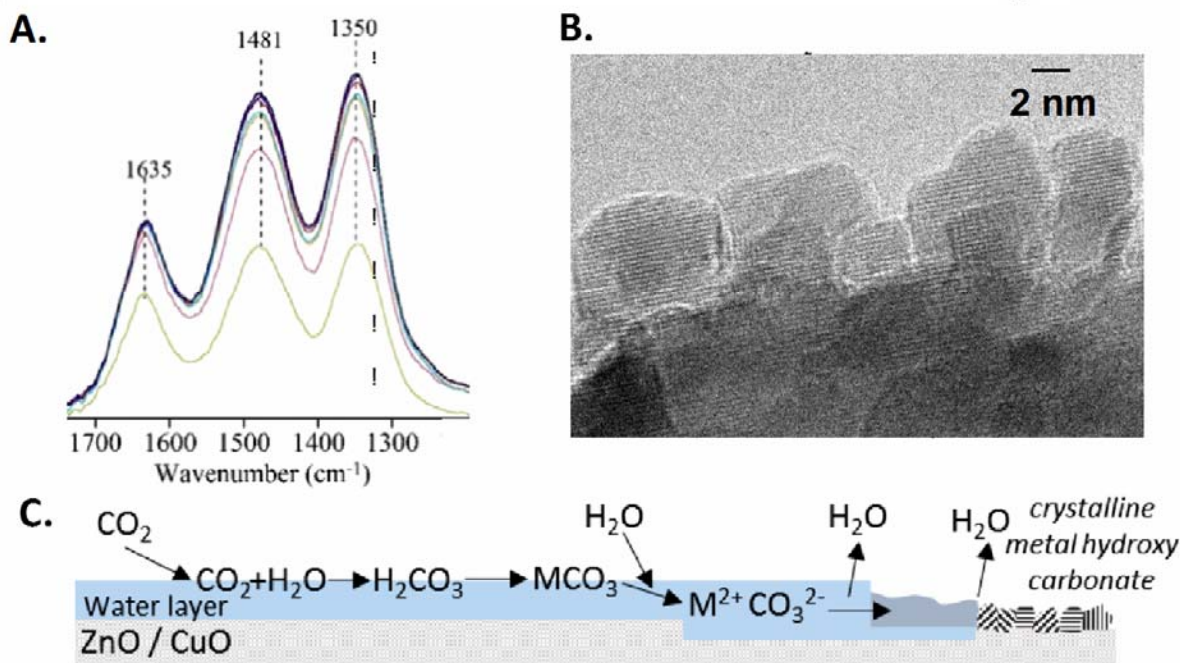


Fig 2. 20 nm CuO nanoparticles, as does ZnO nanoparticles, form metal hydroxy carbonates upon exposure to environmentally relevant concentrations of carbon dioxide and water vapor. **A.** The infrared spectra of CuO surfaces change while being exposed to CO₂ and H₂O. **B.** Analysis of these surfaces with transmission electron microscopy show the formation of crystallites which for CuO is identified as Cu₂(OH)₂CO₃ Cu₃(OH)₂(CO₃)₂. **C.** A pictorial schematic of this chemistry is shown for both CuO and ZnO. Most interesting, this chemistry with atmospheric gases causes the particles to be more soluble leading to higher concentrations of Zn²⁺ and Cu²⁺ when these nanoparticles are dispersed in water. Reprinted with permission from Gankanda et al. 2016 (Ref. 8). Copyright 2016 American Chemical Society.

For metal oxide nanoparticles, the surface of these particles can undergo surface chemistry with trace atmospheric gases that change the properties of the nanoparticles. An example of this comes from Gankanda et al. [8], where reactions of 20 nm ZnO and CuO nanoparticle surfaces with carbon dioxide and water vapor. When adsorbed simultaneously, the nanoparticles were found to be more soluble in aqueous systems.

Figure 2A shows the results from spectroscopy of CuO nanoparticle surfaces in the presence of carbon dioxide and water vapor at environmentally relevant concentrations. These spectra were recorded as a function of time. It can be clearly seen there is adsorbed water (1635 cm⁻¹ band due to the water bending mode) and carbonate ions (1481 and 1350 cm⁻¹, due to the splitting of the asymmetric stretch), which are water solvated [9], on the

surface of these nanoparticles. The surface reaction of CuO with these atmospheric gases is irreversible and transmission electron microscopy reveals the presence of small crystallites identified as copper hydroxy carbonates which form on the particle surface (Figure 2B). Similar reactions occur for ZnO nanoparticle surfaces when exposed to CO₂ and H₂O. The formation of these metal hydroxy carbonates occurs only when nanoparticles are exposed simultaneously to CO₂ and H₂O as shown in the mechanism depicted in Figure 2C.

Environmental interfaces: Indoor surfaces and their interactions with organic compounds

It is increasingly clear that surfaces impact indoor air chemistry and indoor air quality. New molecular-based

research in this area is necessary in order to fully understand processes occurring on surfaces on a molecular and nanoscale level. Many of the challenges and outstanding questions regarding the chemistry of indoor surfaces have been enumerated in several excellent review articles (see e.g. Ref. 10). Moving forward, this area will require new approaches and new methods. We have recently begun a two prong approach to better understanding indoor surface chemistry which involves both fundamental studies of the adsorption and reactivity of indoor surfaces with organic compounds and oxidants and the placement of indoor surfaces in different local indoor environments and analyzing these surfaces with advanced microspectroscopic imaging techniques.

Indoor Surfaces: Conceptual Nanoscale and Molecular Representation of the Interaction of Volatile and Semi-Volatile Organic Compounds with Glass

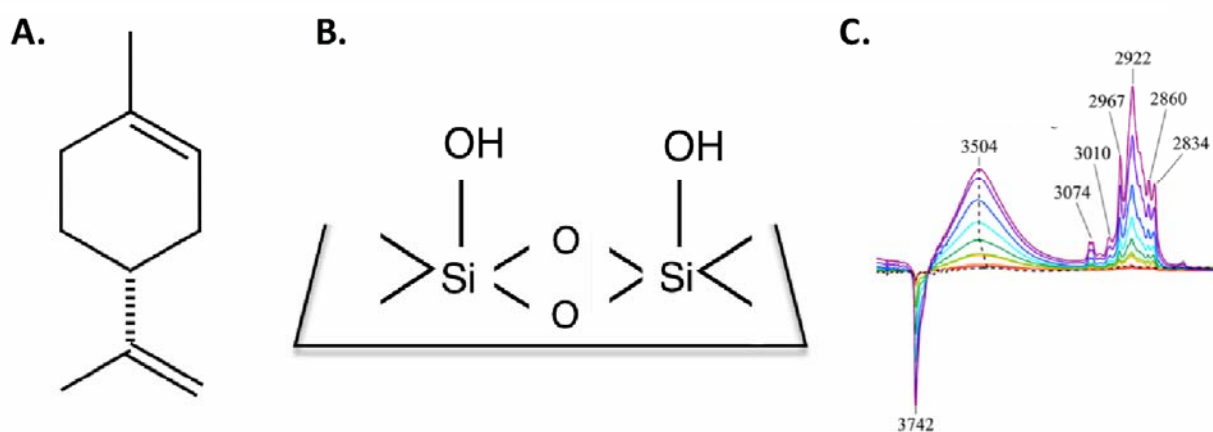


Fig 3. Infrared spectroscopy is being used to better understand the kinetics and thermodynamics of the adsorption of organic compounds on indoor surfaces. (A) Limonene and (B) SiO₂, glass - a common surface found in indoor environments can interact as shown in (C) the infrared spectra as a function of increasing limonene pressure.

Focusing on glass surfaces, a ubiquitous indoor surface, we are investigating the physical chemistry, including kinetics and thermodynamics of surface adsorption and desorption, of relevant organic compounds. These studies have begun with d-limonene, a common cyclic terpene found in indoor air. Sources of d-limonene include cleaning products and as an odorant in a variety of consumer products [11]. Figure 3 shows the molecular representation of limonene (A) and surface hydroxyl groups on glass surfaces (B) along with the FTIR spectra of limonene adsorption on silica as a function of increasing gas phase concentration. What can be clearly

seen in the spectral region shown (OH and CH stretching regions) is that the absorption band intensities increase as more limonene adsorbs on the surface and that the OH groups are involved in the interaction. This can be observed as a decrease in the intensity of the isolated Si-OH groups seen at 3742 cm⁻¹ as they hydrogen bond to adsorbed limonene resulting in a broad OH absorption band present at 3504 cm⁻¹. Other absorption bands in the spectra are associated with adsorbed limonene (gas-phase absorptions are subtracted from the spectra).

The nature of this hydrogen-bonding interaction is unclear and the impact of relative humidity (i.e., water

vapor) on the adsorption of limonene and other indoor air relevant organic compounds need to be better understood. Furthermore, there needs to be a better understanding of the adsorption/desorption kinetics as well as surface reactions with oxidants found in indoor environments. Studies that address these issues will provide additional insights into indoor air chemistry, these are all currently underway.

FUTURE OUTLOOK

Environmental interfaces are complex by their very nature. This complexity arises from the fact that they are inherently heterogeneous and because their surface composition is linked to the environmental conditions that they are in equilibrium with. Fundamental physical chemistry studies provide insights into the detailed molecular processes that underlie the macroscopic phenomena observed on indoor and outdoor environmental interfaces. Importantly, there are enormous benefits to society in delineating the fundamental physical chemistry of these environmental interfaces so as to understand and predict how this chemistry impacts human health as well as the Earth's climate in the case of atmospheric aerosols.

ACKNOWLEDGEMENTS

This article gives an overview of the different types of environmental interfaces that have been investigated in the Grassian research group, an area of active inquiry for over two decades. Therefore, the research highlighted here builds on the research conducted by many excellent current and past students and postdocs, a list of all of the individuals involved can be found on the Grassian Research Group website: <http://grassiangroup.ucsd.edu>.

These distinct areas discussed are funded through different programs. The research that focused on atmospheric aerosols, and sea spray aerosol in particular, is part of research within the Center for Aerosol Impacts on Chemistry of the Environment funded by the National Science Foundation (1305427). The research focused on nanomaterials in the environment is currently funded through the National Science Foundation (1606607). The research focused on indoor surfaces is supported by the Alfred P. Sloan Foundation (G-2017-9692).

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Synthesis and Characterization of a Novel Carbazole Based Hole Transporting Material

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Abstract: Carbazole based derivatives are receiving much attention because of their interesting photochemical properties. These compounds constitute a well-known class of hole transporting material. The synthesis of a novel carbazole-based hole transporting material, tris(4-(3,6-diethoxy-9-H-Carbazol-9-yl)phenyl)amine, is carried out using Ullmann Coupling. During this, the intermediate compounds were synthesized by multi-step organic reactions. The synthesized compound is characterized using UV-Visible, FT-IR, NMR, and Mass spectroscopic techniques. These compounds can be used as hole transporting materials for the fabrication of solid-state dye-sensitized solar cells (DSSCs).

Key Words: Carbazole, hole transporting material, Ullmann Coupling, DSSCs.

INTRODUCTION

With the recent increase in awareness of environmental and energy issues, renewable energy sources are given more and more attention. Solar energy provides clean abundant energy and is, therefore, an excellent candidate for a future environmentally friendly energy source. The main aim of solar cell research is to increase the solar energy conversion efficiency at low cost to provide a cost-effective sustainable energy source. Photovoltaic cells are devices that convert the incident photon energy of the solar radiation into electrical energy. Looking at photovoltaics, a dye-sensitized solar cell is considered to be a promising candidate; they have a long shelf life and are low-cost renewable energy sources [1].

Organic materials can be chemically tuned to adjust to physical properties such as band gap and conduction energies, charge transport, solubility, and morphological properties. Small quantities are needed for device preparation. Organic photovoltaic solar cells, therefore, have the potential for development in the search for low-cost modules for the production of domestic electricity [2]. Basic work in our laboratory includes the synthesis of hole transporting materials for the fabrication of solid-state dye-sensitized solar cells [3].

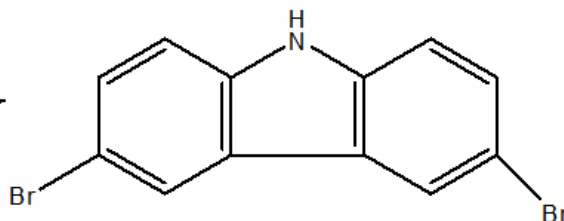
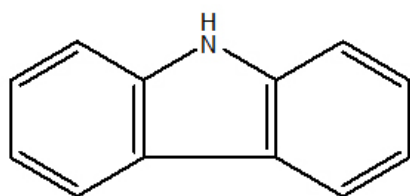
Carbazole based derivatives have attracted much attention because of their interesting photochemical properties [4]. Another fascinating advantage is the versatility of the carbazole reactive sites that can be substituted with a wide variety of functional groups, allowing fine-tuning of its optical and electrical properties [5]. The carbazole derivatives generally possess good thermal stability and hole transport properties [6]. The combination of carbazole derivatives and triphenylamine derivatives is expected to offer improved thermal and morphological stabilities as well as their good hole transport properties [7].

Molecules contain a π -rich heterocyclic or aromatic ring system functionalized with one or more electron donating substituents exhibits good hole transporting properties. The most commonly encountered substituents are amino and alkoxy groups, which contain single bonded heteroatoms possessing sharable lone pairs. These molecules are easily oxidized to resonance stabilized radical cations, which are the actual positive charge bearing species. The most widely used hole transport molecule are aromatic amines, such as anilines, diphenylamines, triphenylamines, carbazoles, and their derivatives. Carbazole based compounds have interesting photochemical properties. Recent interest in the carbazole derivatives has been caused by its good charge transport

function, which can be exploited in the molecular design of new types of HTMS in DSCs [8-9].

The present work focuses on the synthesis of hole transporting materials based on carbazole with triphenylamine derivatives. Carbazole based material constitute a well-known class of hole conducting material. The charge carrier mobility and photoconductive properties of these materials have been studied by various groups. Hole transporting materials based on the carbazole moiety have been the subject of an increasing number of investigations over the last decade. This could be explained by the very interesting features such as low cost of the starting material, good chemical and environmental stability provided by the fully aromatic unit, easy substitution of the nitrogen atom with a wide range of functional groups permitting better solubility and fine tuning of the electronic and optical properties [10-13]. Triphenylamine cored star-burst materials are extensively investigated for their easy modification, superior hole transporting ability and propeller molecular conformation [14].

Triphenylamines were also used as the core to construct an optoelectronic functional star-burst material [15]. Triarylamine based compounds find increasing importance as hole transporting materials in various electro-optical applications like photovoltaic, light emitting devices and photorefractive systems. Triarylamine moiety fulfills the requirement of easy and reversible oxidation and therefore constitutes the building block of many of the hole transporting compounds [16]. Various derivatives of triarylamine have been successfully applied in solid-state dye-sensitized TiO₂ solar cells [17].



Scheme 1

EXPERIMENTAL

Materials

The reagents Carbazole, Triphenylamine, Dimethylformamide, NBS, Potassium carbonate, Copper powder, Copper (I)chloride, Methanol, 1,2-Dichlorobenzene, Chloroform, and Ethylacetate, 18-crown-6 were purchased from Merck. The solvents were distilled before use according to availing procedures in literature. Spectroscopic grade solvents (Merck) were used for UV-Visible spectroscopic measurements.

Infrared (IR) spectra were recorded on a Shimadzu FT-IR 8400 S spectrometer as Potassium bromide (KBr) disc. Ultraviolet-Visible (UV-Vis) spectra of a dilute solution in spectroscopic grade chloroform were recorded on a UV-Vis Shimadzu 1700 using 1.0 cm length quartz tube. ¹H and ¹³C nuclear magnetic resonance (NMR) spectra were recorded on an NMR-JEOL GSX-400 spectrometer with tetramethylsilane as the internal reference using CDCl₃ as the solvent in all cases. Mass spectra were recorded on JEOL GCMATE II GC-MS.

Methods

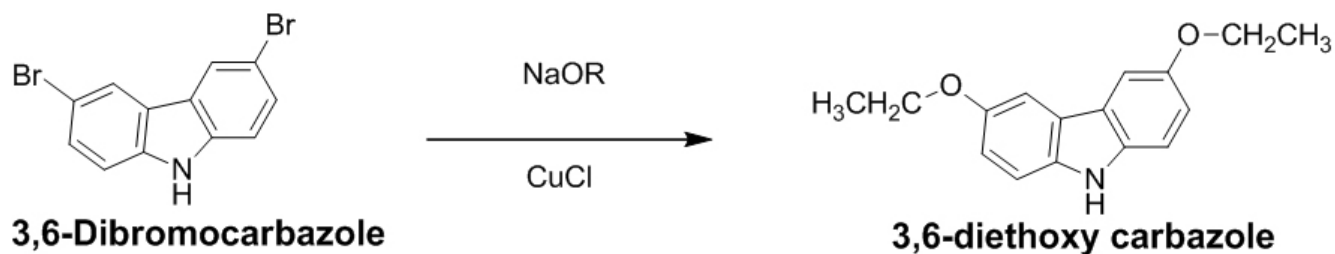
Synthesis of 3,6 - dibromocarbazole (A)

Carbazole 1.67 g (0.01 mol) was dissolved in 15 ml DMF at 0°C with stirring followed by the addition of a solution of NBS 3.63 g (0.02 mol) in 10 ml of DMF. The resulting mixture was stirred at room temp for 2 hr and the solution then poured into 100 ml of water, filtered and washed with water. The crude product was recrystallized from ethanol. Yield: 64%, Appearance: White crystalline solid, Melting point: 204°C. The completion of the reaction was monitored by TLC.

Synthesis of 3,6 - diethoxycarbazole (B)

Into a three-necked flask fitted with a condenser, N₂ inlet, addition funnel, and magnetic stirrer were added 25 ml of dry ethanol. The entire solution was cooled to 0°C with ice water before sodium 2.3 g (0.1 mol) was added gradually. The ice bath was removed and the mixture was stirred until all the sodium had reacted. To this, sodium ethoxide solution was added DMF 12.5 ml, CuCl 3.8 g (0.03 mol), 3,6-dibromocarbazole 1.65 g (0.005 mol) and another

12.5 ml of DMF. The resulting mixture was heated to reflux for 4 hrs under nitrogen. The precipitate was filtered while hot and the filtrate was diluted with 50 ml of water and extracted with chloroform (30 ml x 3). The combined organic layers were neutralized with 5% HCl followed by washing with water and brine, drying by passing through sodium sulphate and concentrating in the vacuum. The residue was recrystallized from methanol. Yield: 60%, Appearance: Pale brown solid, Melting point: 185°C.

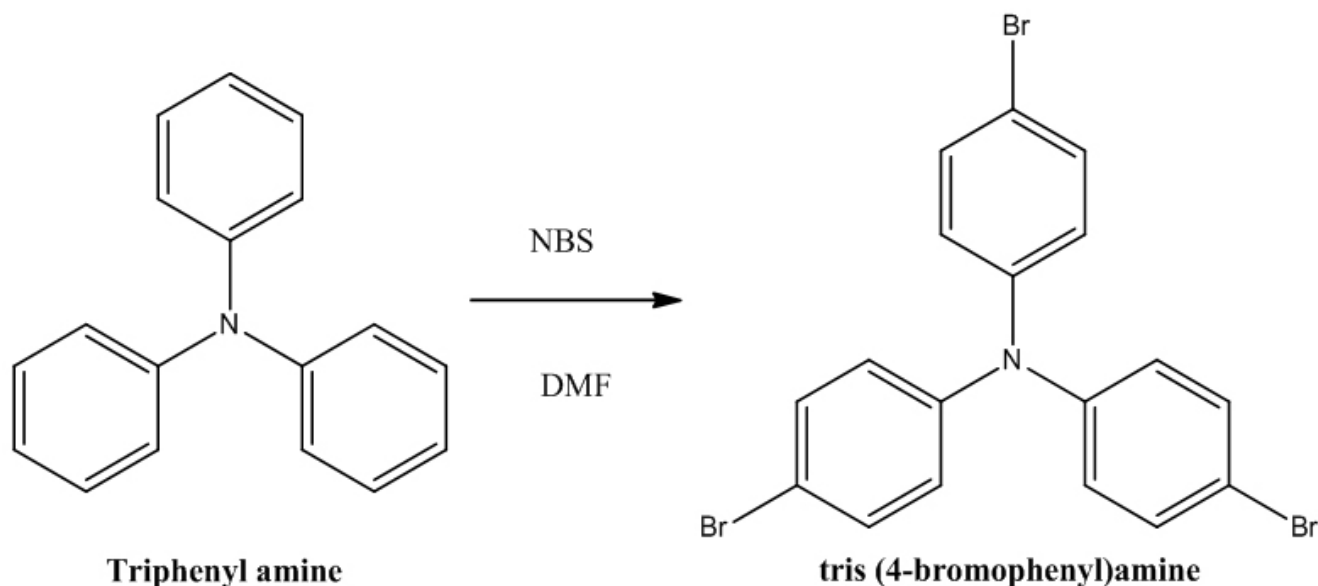


Scheme 2

Synthesis of tris (4-bromo phenyl) amine (C)

Triphenylamine 2.45 g (0.01mol) was dissolved in 20 ml DMF at 0°C with stirring followed by the addition of a solution of NBS 5.34 g (0.03 mol) in 10 ml of DMF. The resulting mixture was stirred at room temp for 2 hr and

the solution then poured into 100 ml of water, filtered and washed with water. The crude product was recrystallized from methanol. Yield: 62%, Appearance: Pale yellow solid, Melting point: 141°C.

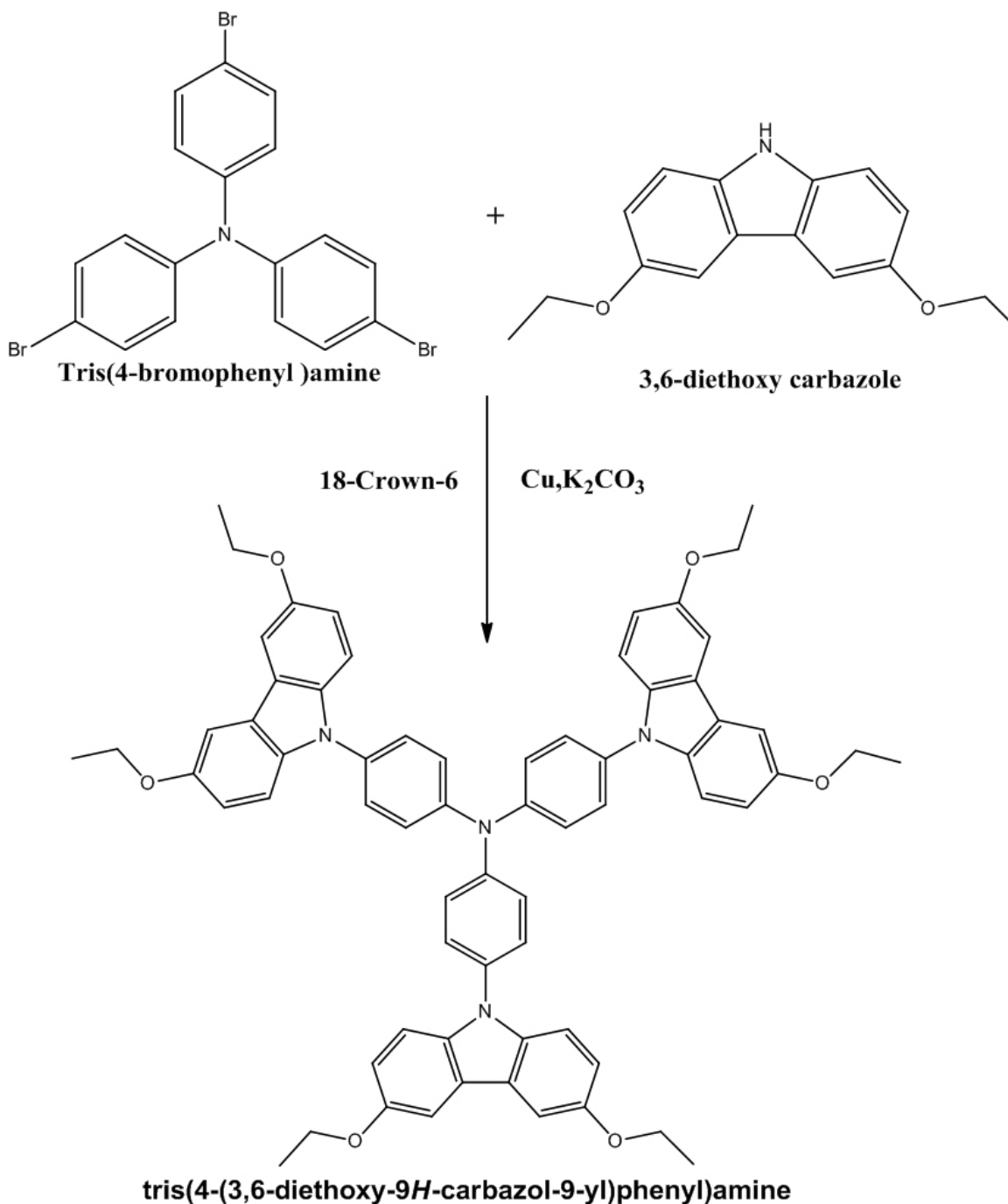


Scheme 3

Synthesis of tris(4-(3,6-diethoxy-9H-Carbazol-9-yl)phenyl)amine (D)

3,6-Diethoxycarbazole 0.7658 g (0.003 mol), tris (4-bromophenyl)amine 0.4820 g (0.001 mol), K_2CO_3 2.65 g(0.02 mol), Copper powder 1.8 g (0.03 mol) and 18-crown-6 200 mg(0.00075 mol) were heated together in 50 ml of orthodichlorobenzene under nitrogen atmosphere and the resulting mixture was refluxed for 24 hrs at 170°C. The

inorganic compounds were removed by filtration and the filtrate was diluted with water. The combined organic layers washed with brine, dried by passing through sodium sulphate and concentrating in vacuum to give the brown residue which was purified by chromatographic column using ethyl acetate-hexane (3:1) as the eluent to obtain brown solid. Recrystallized from acetone. Yield: 54%, Appearance: Brown solid, Melting point: 160°C.



Scheme 4

RESULTS & DISCUSSION

The compound synthesized in every step was subjected to the purification process. The purity of the compound is checked by TLC. The synthesized compounds were characterized by UV-Visible, FT- IR, NMR, and Mass spectroscopic techniques.

Characterization of 3, 6 - dibromocarbazole (A)

UV-Visible spectra (Ethanol, nm): 363, 353, 338, 303, 267.

In UV-Visible spectra, the λ_{max} of 3,6-Dibromo carbazole is observed at 363 nm. The parent compound carbazole shows λ_{max} at 293 nm. The increase in λ_{max} may be due to the substitution of Br with a lone pair of electron.

FT- IR Spectra (KBr, cm^{-1}): 3406, 3068, 1471, 1284, 570

FT-IR gave characteristic peaks 3406 cm^{-1} indicated NH stretching frequency, 3068 cm^{-1} indicated the Ar C-H stretching, 1471 cm^{-1} indicated the Ar C=C stretching, 1284 cm^{-1} indicated the C-N stretching and 570 cm^{-1} indicated the C-Br stretching.

Characterization of 3, 6 - diethoxycarbazole (B)

UV-Visible spectra (Ethanol, nm): 350, 336, 303, 266

In UV-Visible spectra, the λ_{max} of 3,6-Diethoxy carbazole is observed at 350 nm.

FT- IR Spectra (KBr, cm^{-1}): 3409, 1326, 1053

FT-IR gave characteristic peaks at 3409 indicated NH stretching frequency, 1326 cm^{-1} indicated the Ar C=C stretching and the peak at 1053 cm^{-1} is due to C-O stretch.

Characterization of tris(4-bromophenyl)amine (C)

UV-Visible spectra (Ethanol, nm): 315, 290

In UV-Visible spectra the λ_{max} of tris(4-bromophenyl), amine is observed at 315 nm.

FT- IR Spectra (KBr, cm^{-1}): 3068, 2950, 740

The peak at 3068 cm^{-1} and 2950 cm^{-1} indicated aromatic -C-H stretch, the peak at 740 cm^{-1} indicated C-Br stretch

Characterization of tris(4-(3,6-diethoxy-9H-Carbazol-9-yl)phenyl)amine (D)

UV-Visible spectra (Chloroform, nm): 354, 301, 250

In UV-Visible spectra, the λ_{max} of the coupling product is observed at 354 nm. This may indicate that the

coupling reaction makes the compound to bathochromic shift when comparing to the individual compounds.

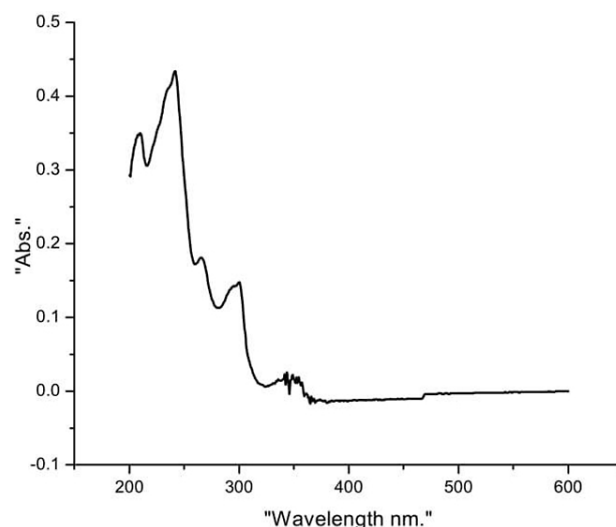


Fig. 1. UV Spectra of the coupling product.

FT- IR Spectra (KBr, cm^{-1}): 3070, 1326.

FT-IR gave characteristic peaks 3070 cm^{-1} indicated the Ar C-H stretching, 1326 cm^{-1} indicated the Ar C=C stretching. The peaks due to N-H stretch and C-Br stretch are absent, this confirms the formation of the coupling product.

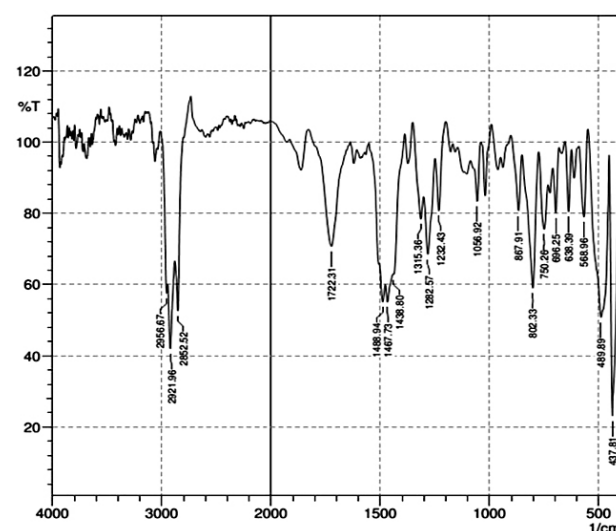
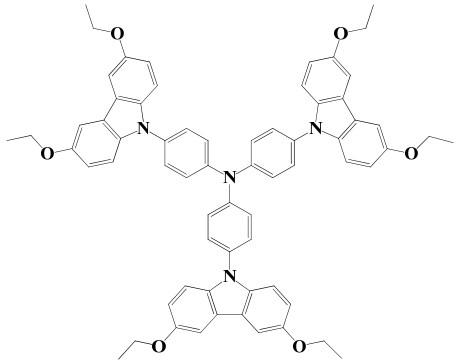
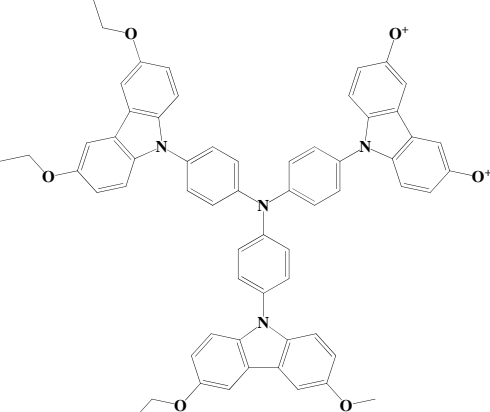
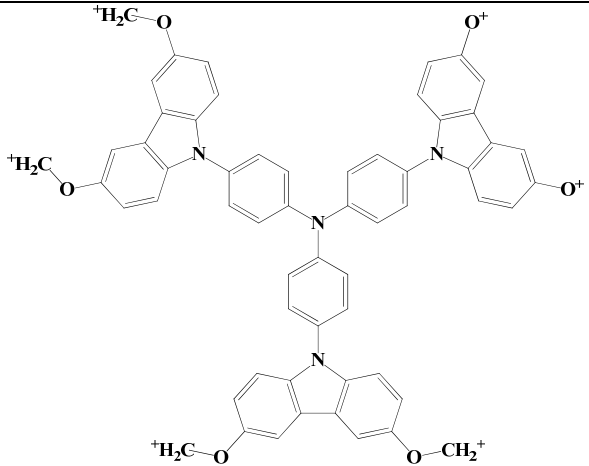
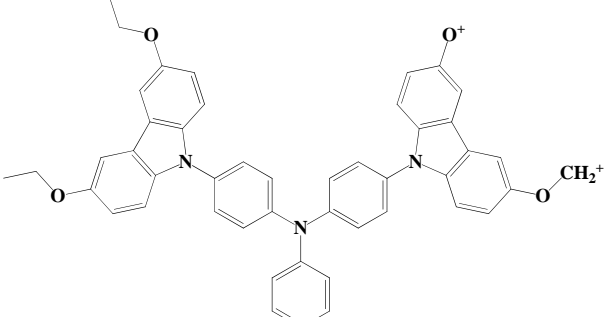


Fig. 2. IR Spectra of the coupling product.

^1H NMR Spectra (CDCl_3 , ppm): 7.1-7.9 (m, 30 H), 3.48 (s, 12H), 1.5 (s, 18 H)

Table 1. Fragmentation pattern.

Sl.No.	Fragment	Molecular Weight	Description
1		1004	Molecular peak (Basic compound)
2		932	Fragmented: 2 Ethyl & 1 Methyl
3		885	Fragmented: 2 Ethyl & 4 Methyl
4		709	Fragmented: 1 Wing, 1 Ethyl & 1 Methyl

5		544	Fragmented:1 Wing, 1 Phenyl& 3 Ethoxy
6		347	Fragmented: 2Wings
7		303	Fragmented:2Wings & 1 Ethoxy

The structure of the synthesized compound tris(4-(3,6-diethoxy-9H-Carbazol-9-yl) phenyl)amine is confirmed from the spectral data.

CONCLUSION

The compound tris(4-(3,6-diethoxy-9H-Carbazol-9-yl)phenyl)amine was synthesized by Ullmann coupling. Various intermediate compounds were synthesized by multistep organic reactions. The synthesized compounds were characterized by UV-Visible, FT-IR, NMR, and Mass spectroscopic techniques. The spectral data confirm the structure of the compound. The synthesized compound is expected to possess a good hole transporting property.

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Microenvironment-sensitive Fluorophore for Recognition of Human Serum Albumin in Aqueous Solution

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Abstract: In this work, the efficacy of a donor-acceptor based red light emitting fluorophore has been assessed for human serum albumin (HSA) detection in aqueous samples. Due to intramolecular charge transfer (ICT) within an extended π -conjugated framework and the positive solvatochromic property, the fluorophore emitted in the far-red region of the electromagnetic spectrum in aqueous buffer samples. The fluorescence intensity was highly sensitive to the microenvironment, which resulted strong turn-on fluorescence response upon encapsulation of the fluorophore within the HSA. The signal was quantitative to the amount of fluorophore-protein complex in the experimental concentration range. The strong association affinity of the fluorophore toward HSA was attributed to the van der Waals and hydrophobic interactions. Such a spontaneous supramolecular association and the subsequent turn-on fluorescence response has potential for medical diagnostic applications.

Key Words: Fluorophore, microenvironment, human serum albumin

INTRODUCTION

Human serum albumin (HSA) is the most abundant protein in blood plasma [1]. It maintains the osmotic pressure of the blood compartment and transports both exogenous and endogenous ligands to various sites [2-4]. It is an important biomarker as different amounts of HSA in body fluids indicate different health conditions, including kidney and cardiovascular damage [5-7]. Therefore, a great amount of resources has been devoted to sensing and the detection of HSA in biological samples. Several techniques such as immunoassay, radioimmunoassay, capillary electrophoresis, spectrometric and fluorimetric techniques, etc., are currently available [8-14]. Among these techniques, the fluorimetric method has attracted much attention recently since it is quick, cost-effective, non-invasive and very accurate [15-17]. Fluorimetric techniques measure the alteration of the emission profile of the fluorophore upon interaction with HSA. If the change of fluorescence signal is significant and correlates with the quantitative amount of protein-fluorophore complex, then a simple fluorophore based sensor can be developed. But, a suitable fluorophore is always required which would undergo

photophysical property change in the presence of HSA and emit light as output signal detectable by standard fluorimeters.

Therefore, an upsurge in the quest for suitable fluorophores for "turn-on" HSA sensors has been noticed. In this regard, environmentally sensitive fluorophores are very promising as their emission intensity significantly changes upon encapsulation within a biomolecule [18-20]. The rigid environment and multiple non-covalent forces reduce the degrees of freedom of the encapsulated fluorophore, hindering non-radiative energy loss and hence increase in fluorescence intensity [18]. The hydrophobic effect also plays a major role in the protein-fluorophore supramolecular complexation and the associated fluorescence enhancement [21]. Lipophilic ligands bind within the HSA's pockets by hydrophobic interactions. As the lipophilicity increases binding affinity increases and the lifetime of the fluorescence lengthens. However, a highly lipophilic compound tends to aggregate heavily in water [22]. If aggregate interactions are stronger than the protein-fluorophore interactions, then the fluorophore becomes unsuitable for the HSA sensing application. Therefore, the fluorophore must remain soluble to some extent in water or at least it should not form strongly clustered aggregates.

EXPERIMENTAL

Herein, we have studied one such environmentally sensitive fluorophore for HSA detection in aqueous buffer samples. A nitrogen donor and three nitrile acceptors are connected by an extended π -conjugation (Figure 1c). The fluorophore emits in the far-red region of the spectrum, indicating the potential for applications with biological samples since it would be free from background emission of other biomolecules [23-24]. It consists of two -OH groups which deliver the required solubility for applications in aqueous solution.

All the reagents were purchased from commercial vendors (Millipore Sigma and Alfa Aesar) and used without further purification. All the solvents were of the spectrophotometric grade. Water was purified using a deionization system.

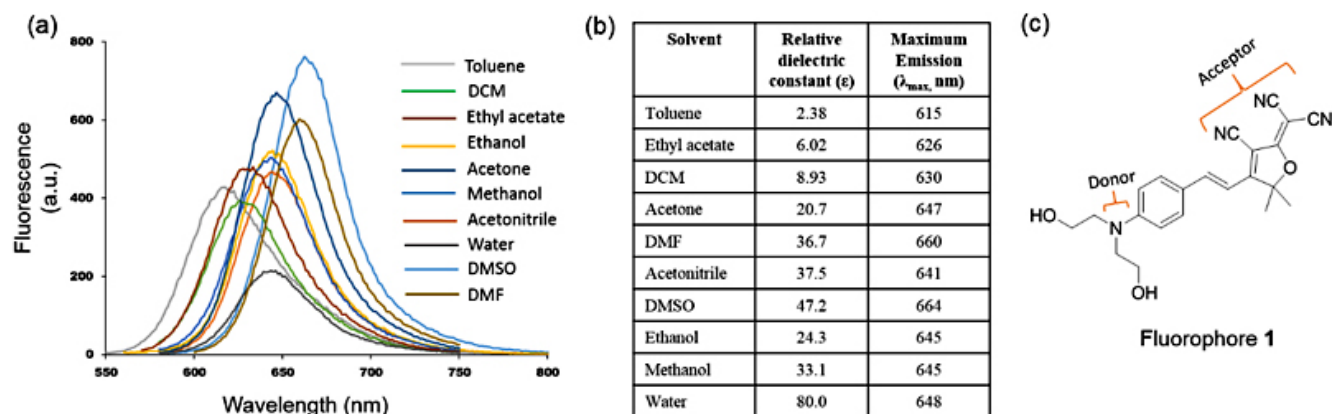


Fig 1. (a) Emission spectra of 1 in various solvents. [1] = 1.0×10^{-5} M; aqueous solution contains 1% DMSO. (b) Positive solvatochromism of fluorophore 1 with polarity of solvents. (c) Structure of fluorophore 1

Absorption spectra were collected on a Shimadzu UV-2501 PC spectrophotometer. Emission spectra were obtained using an LS 55 Luminescence Spectrometer (PerkinElmer) with 5 nm excitation and emission slit widths and 200 nm/min scan speed. Molecular docking was performed on a single CPU Windows-OS computer (64-bit) with 3.30 GHz processor and 8.00 GB of RAM on Vina 1.1.2 program [25]. 3D coordinate structure of the fluorophore (Figure 1c) was constructed and optimized with an MM2 force field on Chem3D. Coordinates of the HSA were obtained from protein crystal structure (PDB code: 2BXC). AutoDock Tools 1.5.6 was used for the preparation of protein and fluorophore input files in pdbqt format, which is a modified protein data bank format containing atomic charges, atom type definitions and, for fluorophore, topological information. Gasteiger partial charges were added to the fluorophore; non-polar hydrogen atoms were merged, and torsional rotatable bonds were defined. All the single bonds were made

rotatable; double and triple bonds were kept as non-rotatable. All other parameters were kept at their AutoDock default values. A rectangular box of dimensions $34 \times 34 \times 32 \text{ \AA}^3$ with 1.0 \AA grid spacing was constructed to encompass the entire site I of HSA. The exhaustiveness of the docking run was set at 20 and the seed was varied randomly as generated by the program.

RESULTS & DISCUSSION

Fluorophore 1 is moderately soluble in water. It is highly soluble in acetone and in halogenated solvents such as chloroform and dichloromethane, as well as in polar aprotic solvents such as dimethyl sulfoxide (DMSO) and dimethylformamide (DMF). For all the spectrometric experiments a DMSO stock of 1 was diluted in 0.1 M phosphate buffer (pH = 7.5) solution. The final DMSO

content in buffer was 1%, and the concentration of **1** was maintained at 10 μ M unless otherwise stated.

Fluorophore **1** exhibited strong fluorescence in DMSO, DMF, and acetone; moderate emission in nonpolar solvents such as toluene and ethyl acetate and very weak emission in deionized water (Figure 1a). All the emission bands were very broad and structureless, which is characteristic of donor-acceptor charge transfer type electronic transition. Emission intensity and position of the bands were very sensitive to the polarity of the solvents,

indicating strong solvatochromism (Figure 1b) [26]. As the polarity of the solvents increased the emission maxima shifted toward lower energy, suggesting a positive solvatochromism [26]. The lowest energy charge transfer emission recorded was in DMSO ($\lambda_{\text{max}}^{\text{em}} \sim 665$ nm). In water emission maxima was at ~ 648 nm with the lowest intensity, most likely due to loss of excited state energy through additional decay channel facilitated by polar protic water through hydrogen bonds.

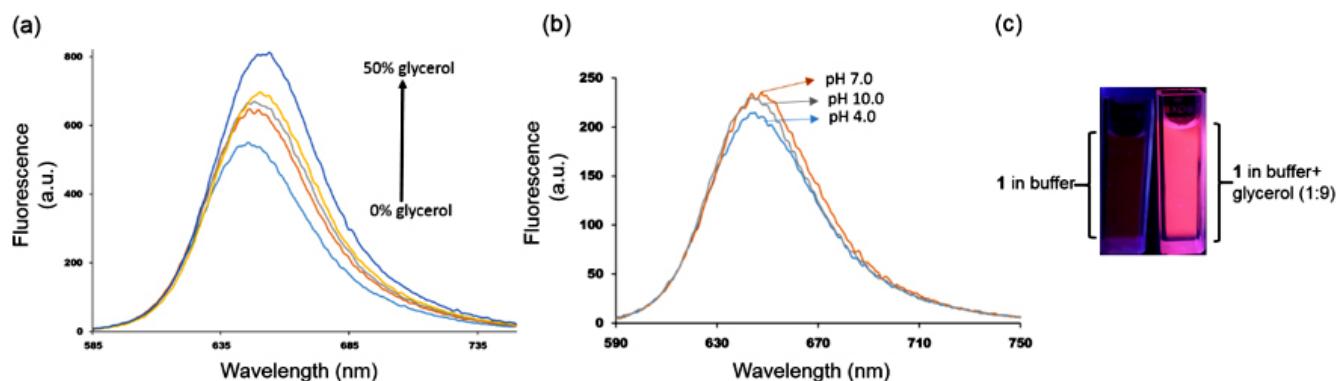


Fig 2. (a) Fluorescence of 1 with added glycerol in methanol. (b) Fluorescence of 1 at different pH values: 4.0, 7.0, and 10.0. (c) The appearance of bright red fluorescence in glycerol/buffer (9:1) mixture.

Studies have revealed that the extent of emission (quantum yield of fluorescence) of the flexible donor-acceptor fluorophores is dependent on the solvent polarity and the identity of the environment [18]. Excited state energy can dissipate via several non-radiative pathways, including rotation of the single bonds. Fluorophore **1** consists of several C-C and C-N single bonds on the main π -conjugated backbone. Therefore, it is not surprising that the fluorescence of **1** is low in low viscous solvents.

Figure 2a shows the effect of the addition of a highly viscous solvent (glycerol, viscosity 950 cP at 25°C) to the fluorescence intensity of the **1**. It showed a broad emission in methanol centered at ~ 645 nm. But, when 10% glycerol was added an increase in intensity was noticed along with the slight bathochromic shift of the peak. As the amount of glycerol was gradually increased –and thus the viscosity of the mixture – enhancement of emission intensity was noticed. At 50% methanol/glycerol mixture (v: v) an almost two times increase in intensity was recorded.

Figure 2c shows the solution of **1** in phosphate buffer and in 9:1 glycerol-buffer (v: v) mixture under UV light. In the latter case, the strong bright red fluorescence of **1** was visible to the naked eyes. Thus both these experiments demonstrate the strong environment effect on the fluorescence of **1**. A heavily viscous microenvironment hinders rotation of the bonds and other local motions, impeding non-radiative decay of the excited state energy.

Therefore, it would be of great interest to study the photophysical property of **1** in the presence of HSA. When a fluorophore binds within a binding pocket of the HSA, it experiences a new microenvironment which is very different from the bulk solvent. Fluorescence intensity and lifetime of the fluorophore change to a significant extent due to the confined geometry of the fluorophore in the protein's pocket. Narrow sterically crowded space and van der Waals interactions restrain the non-radiative decay and significantly increase the fluorescence intensity.

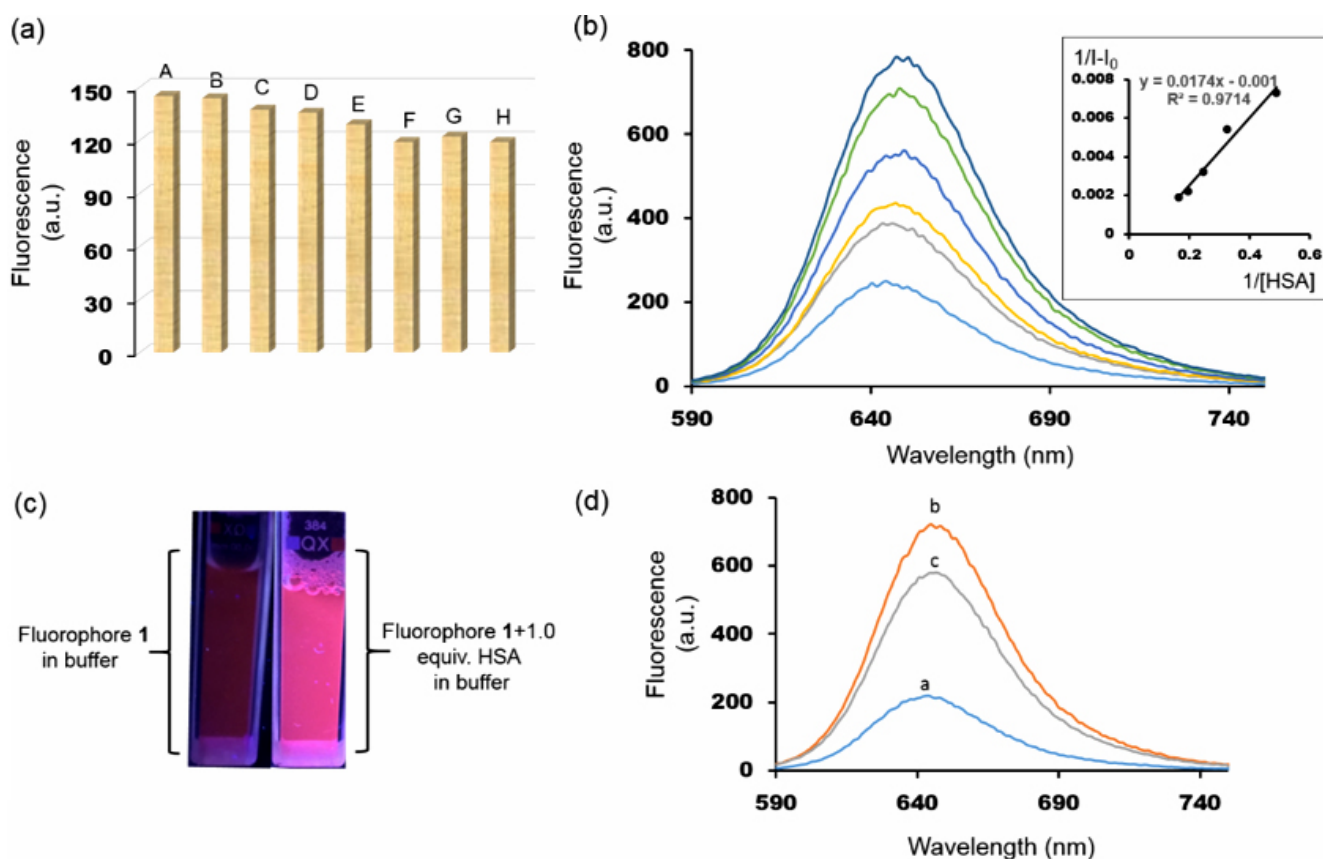


Fig 3. (a) (A) Fluorescence intensity of **1** in phosphate buffer, (B) in presence of Na^+ (1 mM), (C) K^+ (1 mM), (D) NH_4^+ (1 mM), (E) Ca^{2+} (0.5 mM), (F) citric acid (1 mM), (G) lactic acid (1 mM), and (H) uric acid (1 mM). (b) Fluorescence spectra of **1** in presence of varying amount of HSA; $[\mathbf{1}] = 1.0 \times 10^{-5}$ M. Inset: Benesi-Hildebrand plot of $1/(I-I_0)$ vs $1/[\text{protein}]$ (M^{-1}) for binding of **1** with HSA. (c) Appearance of red fluorescence upon addition of HSA, $[\text{HSA}] = [\mathbf{1}] = 10 \mu\text{M}$. (d) Fluorescence spectra of **1** in presence of phenylbutazone in phosphate buffer. (a) Only **1** in buffer, (b) $[\mathbf{1}] = [\text{HSA}] = 10 \mu\text{M}$, (c) $[\mathbf{1}] = [\text{HSA}] = [\text{phenylbutazone}] = 10 \mu\text{M}$.

Since many properties and functions of biological macromolecules are dependent on the pH of the solution, we first studied fluorescence of **1** in a wide range of pH values. No change in emission intensity was noted from pH 4.0 to 10.0, indicating potential compatibility of **1** for biological applications (Figure 2b). Moreover, biological samples usually contain a large variety of cations such as Na^+ , K^+ , NH_4^+ , Ca^{2+} and other molecules such as citric acid, lactic acid, uric acid, etc. Emission intensity of **1** was evaluated in the presence of these interfering ions and compounds. As shown in figure 3a, no significant change in emission was recorded in buffer solutions in the presence of the aforementioned ions and organic compounds. Next, to investigate the effect of HSA on the fluorescence of **1**, titration experiments were carried out in phosphate buffer solutions with 1% DMSO. Emission

intensity increased upon addition of HSA. A linear relationship between concentrations of HSA and fluorescence intensity was observed, suggesting a 1:1 complex formation (Figure 3b).

From this relationship, the binding affinity (K_a) was calculated by using the Benesi-Hildebrand equation [27]. The K_a of **1** was found to be $5.75 \times 10^4 \text{ M}^{-1}$ ($\Delta G = -6.48 \text{ kcal/mol}$). It indicates a strong association of **1** with HSA guided by multiple weak non-covalent interactions and hydrophobic effect. Figure 3c shows the effect of the addition of one equivalent of HSA in a buffered solution of **1** under UV lamp. Negligible fluorescence now becomes visible to the naked eyes. The linear relationship between fluorescence intensity and added HSA and the dramatic increase in fluorescence intensity suggest that **1** can be used to detect HSA in biological samples.

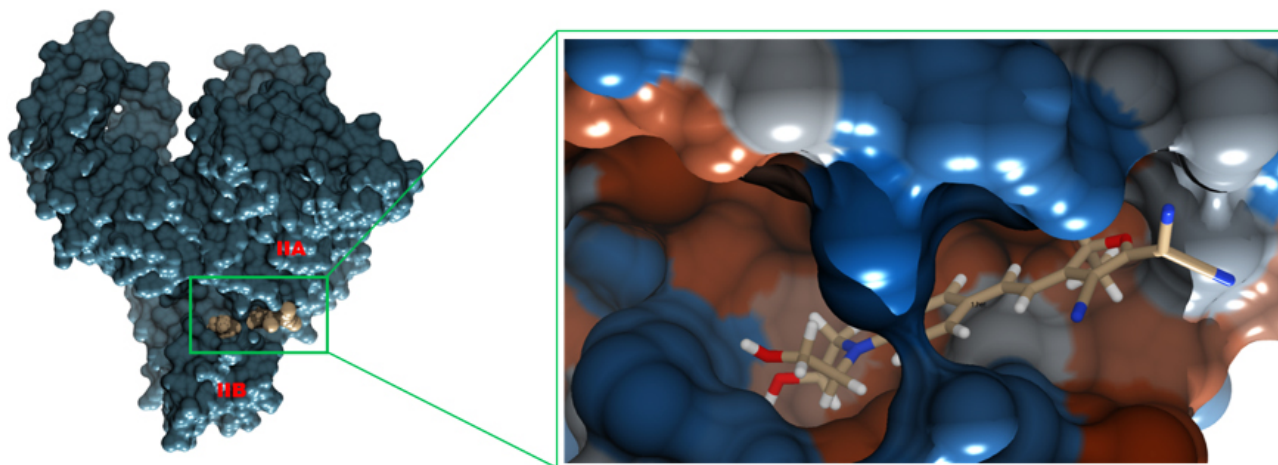


Fig 4. Docking conformation of the HSA-1 supramolecular complex with the lowest binding free energy.

Next, with the help of displacement assay and molecular docking, we investigated the fluorophore binding site in HSA. HSA has three ligand binding domains, and each domain is divided into two subdomains, subdomain A and subdomain B. The hydrophobic drug phenylbutazone binds within domain II (in subdomain IIA) with an affinity of $7.0 \times 10^5 \text{ M}^{-1}$. Addition of equimolar amount of phenylbutazone into a 1:1 solution of HSA-1 reduced the fluorescence intensity by ~20%, which indicates a competition between **1** and the drug for domain II (Figure 3d).

The molecular docking study between HSA and **1** provided additional information about the binding site of **1** in the HSA molecule. As shown in figure 4, **1** binds between the cavity of subdomain IIA and IIB, in one of the fatty acid binding sites [28]. The hydrophobic rings of **1** remain embedded within the deepest part of the cavity whereas the polar groups -OH and -CN are exposed to polar amino acids and water, respectively. Unlike phenylbutazone, **1** does not bind within subdomain IIA probably due to a larger size and polar nature of the functional groups.

CONCLUSIONS

In conclusion, an environmentally sensitive donor- π -acceptor based fluorophore has been assessed for HSA detection in aqueous buffer samples. The fluorophore showed positive solvatochromism. The fluorescence intensity was highly sensitive to the nature of the

microenvironment. Upon binding with HSA the fluorescence intensity increased, displaying a “turn-on” fluorescence response.

Fluorescence response was quantitative for the HSA in aqueous buffer samples, demonstrating a potential for small molecule-based fluorescence sensor for HSA detection in biological samples. The fluorophore was insensitive to pH change and highly tolerant to common ions and organic molecules. It quickly formed a complex with HSA, settling within an area between subdomain IIA and IIB. The high binding affinity was presumably due to strong van der Waals and hydrophobic interactions. The turn-on fluorescence response can be further extended to the diagnosis of HSA protein in human urine samples and protein nanoparticle-based bioimaging.

ACKNOWLEDGMENTS

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Note: The authors declare no conflict of interest.

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Sequential Extraction and HPLC Analysis of Total Anthocyanins of Grape Skin

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Abstract: The objective of the present study was to develop sequential extraction procedures for the anthocyanins obtained from agro-industrial waste material – grape skin using ultrasound-assisted, magnetic stirring and supercritical fluid extraction techniques. A rapid and selective high performance liquid chromatographic (HPLC) method for quantitative determination of the major anthocyanins in obtained organic extracts solutions was developed and validated with respect to robustness, specificity, linearity-range, accuracy, precision, limit of detection (LOD) and quantitation (LOQ) as well. The effect of the operating pressure, the temperature, the extraction time, the flow rate of a supercritical fluid, the composition of solvent's mixture, the sample size, the ultrasound power and the solvent nature used were investigated. The optimal conditions for extraction were found. The calibration curve of the developed HPLC method is linear over a concentration range 0.04-80.0 µg/mL for total anthocyanins expressed as cyanidin chloride ($r^2=0.9999$); the average recovery equals to 95.62 %.

Key Words: Sequential extraction, anthocyanins, high-performance liquid chromatography

INTRODUCTION

Anthocyanins represent a unique subset of phenolic secondary metabolites found in plant tissues. They are one of many compound classes that fall under the flavonoid group, possessing a bi-phenolic structure which can be glycosylated, and the glycosides may be acylated, giving rise to a plethora of potential structural and functional variants [1]. The aglycone flavylium cation is referred to as an anthocyanidin. Anthocyanins differ from the rest of the flavonoid group by a formal positive charge found on the oxygen of the pyran ring (Figure 1). Recently, anthocyanins has attracted considerable attention because of their antioxidant activity [1-2]. They protect against oxidative damage from radical reactive species by various mechanisms that ultimately result in the neutralization of free radicals. Owing to the anthocyanin's positive charge and aromatic hydroxyl groups, these compounds can easily donate protons to the free radicals [3]. This protects cells from oxidative damage that leads to aging and various diseases. Up to 600 different species have been reported [4]. The color of anthocyanins depends on their

structure, the acidity of the environment, and the presence of copigments [5].

Nowadays, various pretreatment techniques followed by chromatographic analysis have been developed to extract anthocyanins from different samples that affect the yield, quality, and composition of targets. Some of them are solid-phase extraction (SPE), conventional extraction (CE), ultrasound-assisted extraction (UAE) and accelerated-solvent extraction (ASE) [6]. Traditional methods including maceration and turbo extraction (high-speed mixing) are very often time-consuming and require relatively large quantities of polluting solvents [7]. Recently, pressurized liquid extraction (PLE) was used to extract anthocyanins from the red grape skins. Ultrasound-assisted extraction (UAE) is often used for the extraction of plant material using a liquid solvent. This extraction process is faster and more complete than maceration, due to the greater surface area in contact between the disrupted solid particles and liquid phase [2]. Several studies show that ultrasound-assisted extraction (UAE) has many advantages over the conventional extraction methods including shorter time, less solvent, and higher extraction efficiency [8]. One major challenge

in the utilization of the UAE procedure for the extraction of anthocyanins from the real sample is the selection of experimental conditions that can provide the optimum recovery [2].

Anthocyanins analysis has been extensively studied but it is still hampered by the limitation of available calibration standards. Classical chromatographic methods, such as planar chromatography and open column chromatography, still play a role in the analytical analysis of anthocyanins, but HPLC is now the standard method for both analytical and preparative applications. HPLC coupled with UV-Vis, photodiode array (PDA), or mass spectrometer (MS) detectors are commonly used [3].

The objective of the present study was to develop and validate extraction procedures of anthocyanins from agro-industrial waste material - grape skin using the ultrasound-assisted extraction (UAE), magnetic stirring extraction (MSE) and the supercritical fluid extraction (SFE) techniques and a rapid and selective HPLC method for quantitative determination of the major anthocyanins in obtained organic solvent extract solutions.

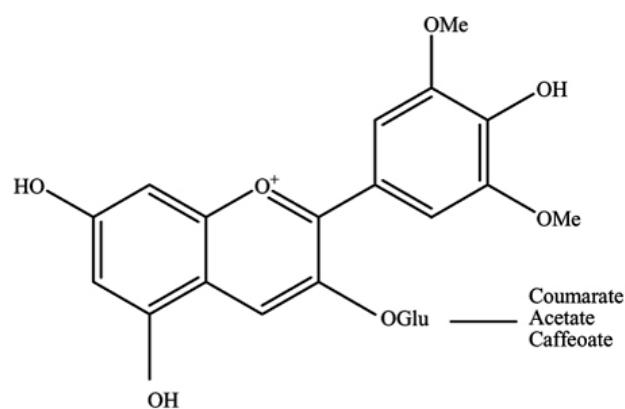


Fig. 1. Structure of the major anthocyanins in red grape [2].

EXPERIMENTAL

Sample material

Grape pomace the harvest (2016) of "Saperavi", red sort of wine grape has been provided by local wine factory from Kakheti region, Georgia. A huge amount of solid waste leftover from wine processing generally consist of pulp, seed, and skin. The fresh waste material is highly perishable especially, after 15-18 days of maceration. Dehydration of waste material is the first step before extraction and a useful means to increase the shelf-life of

grape pomace. The grape skin was dried in the laboratory under controlled conditions (the temperature - 20-25°C and the relative humidity - 30-60 %) and protected from direct sunlight.

Reagent and chemicals

The certified analytical standards of cyanidin chloride and kuromanin chloride, the HPLC grade acetone, ethanol, ethyl acetate, acetonitrile, methanol, the analytical grade hydrochloric acid, formic acid and phosphoric acid from Sigma-Aldrich were used.

Instrumentation

An eco-friendly laboratory supercritical fluid extraction dynamic system (500 mL) was used for sample extraction. The previously weighed sample was loaded in a stainless steel basket of an extractor. The carbon dioxide was compressed and chilled at -5°C. Liquefied CO₂ was pumped continuously into the vessel at the specific extraction conditions. Elmasonic P 300 H ultrasonic bath (Elma Schmidbauer, Germany) was used for ultrasound-assisted extraction. The HF-frequency was 37 and 80 kHz; the temperature was controlled during ultrasonication [9]. IKA C-MAG MS magnetic stirrer (IKA-Werke, Germany) was used for magnetic stirring extraction. The stirring speed was 100 - 1500 rpm and the temperature was 25 - 60°C. The HPLC grade water was prepared using Milli Q Advantage A10 purification system (Merk-Millipore, France).

The chromatographic analysis was performed using an LC-20AD Prominence Shimadzu HPLC system (Japan). Analytical balance ALX-210 (USA) was used for standard and sample preparation. All the measuring equipment was appropriately calibrated and qualified.

Chromatographic system and conditions

The HPLC method was developed using a column - Agilent SB-C18 4.6x250 mm, 5 μm (Agilent Technologies, USA) with isocratic elution of the mobile phase (MP) - a mixture of MP A (a mixture of water and formic acid 90 : 10 v/v) and MP B (a mixture of water, acetonitrile, methanol and formic acid 40:22.5:22.5:10 v/v) 50 : 50 v/v; the flow rate of elution was 1.0 mL/min; The detector wavelength was 535 nm; the injected volume was 20 μL; the temperature of analyte was maintained at 4°C; The column temperature was maintained at 30°C. The mixture of water and 85 % phosphoric acid 90: 10 v/v and the

mixture of methanol and hydrochloric acid 98:2 v/v were used as diluent A and B, respectively.

Preparation of kuromanin chloride standard solution

10 mg of standard of kuromanin chloride was weighed and transferred to 100 mL volumetric flask, dissolved in 30 mL diluent A and diluted to volume with the same diluent, mixed well. The obtained solution was filtered through 0.45 μm polyvinylidene fluoride (PVDF) microporous membrane filter, discarding the first 5 mL of the filtrate (Stock solution). 1 mL of this solution was transferred to 10 mL volumetric flask, diluted to volume with diluent B, mixed well (10 $\mu\text{g}/\text{mL}$).

Preparation of cyanidin chloride standard solution

20 mg of standard of cyanidin chloride was weighed and transferred to 50 mL volumetric flask, then dissolved in 30 mL diluent A, diluted to volume with the same diluent, mixed well. The obtained solution was filtered through a 0.45 μm PVDF microporous membrane filter, discarding the first 5 mL of the filtrate (Stock solution). 1 mL of this solution was transferred to 10 mL volumetric flask and diluted to volume with diluent B, mixed well (40 $\mu\text{g}/\text{mL}$).

Preparation of system suitability check solution

4 mL of standard stock solution of kuromanin chloride and 1 mL standard stock solution of cyanidin chloride were transferred to 10 mL volumetric flask, diluted to volume with a diluent, and mixed well (40 $\mu\text{g}/\text{mL}$).

The sample and standard solutions were prepared and stored in dark glassware under refrigeration to prevent any degradation by heat, air, and light.

Calculation formulae

The concentration of total antocyanins - C_u , $\mu\text{g}/\text{mL}$ in the extract sample solution was calculated by the following formula: $C_u = A_u \cdot W_1 \cdot D_1 \cdot P \cdot 1000 / A_s$. Where, A_u - Sum of peak areas corresponding antocyanins obtained with the extract sample solution; A_s - Peak area of cyanidin chloride obtained with the standard solution; W_1 - Weight of cyanidin chloride standard, mg; D_1 - Dilution factor; P - Purity of standard, %.

The content of total antocyanins - X , μg in 1 g of the dried sample (waste material) was calculated by the

formula: $X = C_u \cdot V \cdot D_2 / W_2$. Where, C_u - the determined concentration of total antocyanins in the sample extract solution, $\mu\text{g}/\text{mL}$; V - The volume of extract, mL; D_2 - Dilution factor; W_2 - Weight of dried sample, g.

Method validation

The developed method was validated with respect to robustness - standard solution stability and filter compatibility test, system suitability test, specificity, linearity-range, accuracy, precision, limit of detection (LOD) and quantitation (LOQ) according to ICH guideline and statistical assessment was performed using Microsoft Excel 2010 [9-11].

RESULTS & DISCUSSION

Sequential supercritical fluid extraction

The effect of the operating pressure and the temperature, extraction time, the flow rate of the SC-CO₂, the sample size and the nature of the solvent used was investigated to develop the sequential extraction procedure and establish their optimal parameters.

The effect of pressure on the extraction of anthocyanins was investigated by carrying out the experiments at pressures from 100 to 250 atm. The flow rate of SC-CO₂ was kept constant at 2 mL/min throughout the extraction. The temperature (50°C) was selected to prevent thermal degradation of target analytes.

To investigate the effect of temperature on the extraction the experiment was carried out at the different temperatures - 40, 50, 60 °C; the extraction pressure and the flow rate of SC-CO₂ were kept constant at 200 atm and 2 mL/min, respectively.

The effect of the flow rate of SC-CO₂ on the extraction was investigated at the different flow rates - from 1 mL/min to 5 mL/min. The optimal operating temperature and pressure were established in the previous experiments. Also, the effect of the sample size (grape skin) ranging 10 - 30 g was investigated on the extraction of target analytes.

Hence, the extraction procedure for the supercritical fluid extraction of antocyanins from dried samples was developed. The optimal parameters are: the sample size - 20 g, the extraction pressure - 200 atm, the extraction temperature - 50°C, the equilibrium extraction time - 180 min, the dynamic extraction time - 60 min, the flow rate of SC-CO₂ - 2 mL/min; The obtained colorless residue

indicated complete extraction of target compound. In the experiment, the mixtures – acetone/water, ethanol/water and ethyl acetate/water acidified hydrochloric acid (pH 4-7) were used as co-solvents.

The results of the experiment show that the effect of pressure on the recovery of anthocyanins at a constant temperature is a function of the amount of SC-CO₂. The solubility of target analytes increased with an increase of pressure. The content of total anthocyanins decreases at the higher extraction temperature at a constant pressure, also the recovery of target compounds increases with an increase of the extraction time at a constant temperature as well. Also, the effect of sample size is a function of the extraction time. The less the sample size is, the more is the extraction time and the more the flow rate is, the less is the extraction time. The use of co-solvent – ethanol/water (50:50 v/v) at pH 4 increases the recovery of anthocyanins.

Sequential ultrasound-assisted extraction

The results of the UAE procedure indicate that the effects of the extraction time, the composition of the solvents mixture, as well as the temperature and ultrasound power, are significant for analytes. The effect of the extraction time on the extraction of anthocyanins was investigated by carrying out the experiments during 25, 50, 60 and 90 min. It was observed that the recovery of anthocyanins increased exponentially in 15-30 minutes. Most of the anthocyanins extracted during the 2/3 of total extraction time (30 min), then ultrasound degradation leads to the reduction of the number of anthocyanins due to the side effect of ultrasonication. Thermal effect plays an important role in UAE. At comparatively low ultrasonic power (37 kHz) and temperature (25-50 °C), the thermal effect can be ignored because the heat produced by ultrasound may be completely diffused. The high ultrasonic power (80 kHz) causes the thermal effect on thermally sensitive target substances. In the experiment the mixtures – acetone/water and ethanol/water (from 50: 50 to 90: 10 v/v) were used as solvents. The solvent's mixture was acidified by hydrochloric acid (pH 4). The extraction temperature varied in the range 25-60 °C. It was observed that the influence of the composition of the solvents mixture was important.

Hence, the three-step extraction procedure for ultrasound-assisted extraction of anthocyanins from dried samples was developed. The ultrasonic bath was set at 37 kHz; the optimal parameters are: the sample size – 5 g, the extraction time – 30 min, solvent's mixture – acetone/water (70: 30 v/v) and acetone/water (50: 50 v/v)

(pH 7), the temperature of extraction is 25°C and 50°C, respectively. The obtained organic extracts were transferred to dark glassware and stored under refrigeration. For HPLC analysis the extracts were transferred to a dark volumetric flask and diluted to volume with the diluent, mixed well. The sample extract solutions were filtered through 0.45 µm PVDF microporous membrane filter, discarding the first 5 mL of the filtrate.

Magnetic stirring extraction

The results of MSE procedure indicate that the effects of the extraction time, the extraction temperature and the composition of solvent's mixture are significant for the analyte. The effect of the extraction time on the extraction of anthocyanins was investigated by carrying out the experiments during 20, 30 and 40 min. It was observed that the recovery of anthocyanins increased exponentially in 10-20 minutes. Most of the anthocyanins extracted during the 2/3 of total extraction time (30 min), then photodegradation leads to the reduction of the number of anthocyanins due to the side effect of light. In the experiment, the mixtures – acetone/water, ethyl acetate/water, and ethanol/water (from 50: 50 to 90: 10 v/v) were used as solvents which were acidified by hydrochloric acid (pH 4). It was observed that the influence of the composition of the solvents mixture was important.

Hence, the three-step extraction procedure for MSE of anthocyanins from dried samples was developed. The optimal parameters are: the sample size – 5 g, the extraction time – 30 min solvent – acetone/water (70: 30 v/v) and ethanol/water (50: 50 v/v) acidified hydrochloric acid (pH 4), the temperature of extraction is 60°C. The obtained organic extracts were transferred to dark glassware and stored under refrigeration. For HPLC analysis the extracts were transferred to a dark volumetric flask and diluted to volume with diluent, mixed well. The sample extract solutions were filtered through 0.45 µm PVDF microporous membrane filter, discarding the first 5 mL of the filtrate.

Optimization of chromatographic system conditions

The final chromatographic conditions were determined by optimizing the system operational parameters: the wavelength for detection, the gradient program of the mobile phase, the composition of mobile phase, the flow rate, the nature of stationary phase and the

injection volume. The system suitability parameters – theoretical plates, tailing factor and peak purity – were checked.

METHOD VALIDATION

Specificity

The specificity was checked by injecting the standard solutions, the system suitability check solution, the background control – blank and the sample extract

solution. It has been shown that there is no interference from the blank at the retention time of an analyte's peak. The retention time for principal peaks on the chromatogram obtained with sample extract solution corresponds to that of the respective peaks in the chromatogram obtained with the standard solution. Both principal peaks are pure. Purity factor (993 for kuromanin chloride and 997 for cyanidin chloride) was more than purity threshold (990.0). Figure 2 shows the chromatogram obtained with the system suitability check solution.

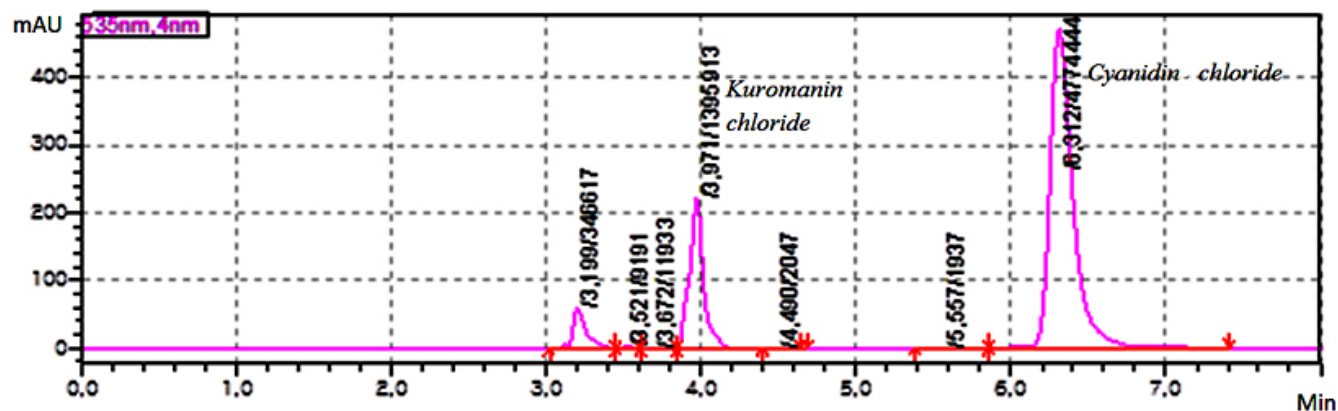


Fig 2. Chromatogram of the system suitability check solution.

Linearity and range

From the standard stock solution, working solutions were prepared at six different concentration levels ranging from 0.08 $\mu\text{g/mL}$ to 40 $\mu\text{g/mL}$ for cyanidin chloride. Three replicate injections ($n=3$) were performed at each concentration level. The linearity was checked by the correlation coefficient (acceptance criteria: >0.990), the square of the correlation coefficient (acceptance criteria: >0.98). The calibration curve was constructed by plotting the response area against the corresponding concentration of the injected solutions. A value closer to the unit of the correlation coefficient indicates a good linearity. The calibration plot and the corresponding statistic parameters of the regression are shown in Figure 3 and Table 1.

Table 1. The linear regression data

Level	Concentration, $\mu\text{g/mL}$	Average peak area
I	40.0	5244659
II	4.0	468994
III	2.0	211816
IV	1.6	205736
V	0.5	71309
VI	0.08	11725
Correlation coefficient (r)		0.99991
Square of correlation coefficient (r ²)		0.99983

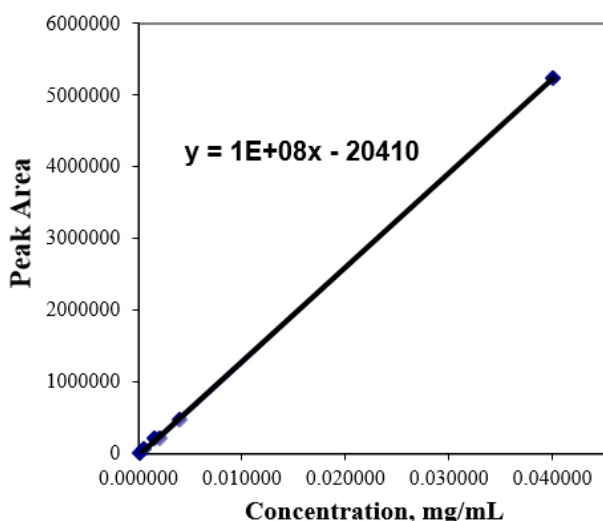


Fig. 3. The linearity graph for cyanidin chloride.

Limit of quantitation (LOQ) and limit of detection (LOD)

The signal-to-noise ratio (S/N) was adopted for the determination of the LOQ. This is estimated to be ten times the s/N ratio; the LOQ is estimated to be three times of S/N ratio (acceptance criteria). The LOQ was achieved by injecting a series of stepwise diluted solutions and precision was established at the specific determined level. The RSD, % of peak areas should not be more than 10 % (acceptance criteria). The determined LOQ and LOD for target analyte are presented in Tables 2. The LOQ of the

method was estimated to be equal to 0.08 µg/mL and 0.02 µg/mL could be considered according to the acceptance criteria.

Table 2. The LOQ and LOD results.

Parameter	Value
LOQ, µg /mL	0.08
LOD, µg /mL	0.02
RSD of peak areas, % for LOQ (n=6)	7.477
RSD of peak areas, % for LOD (n=6)	12.318
s/N for LOQ	22.1
s/N for LOD	5.3

System suitability test

The system suitability test parameters were measured to verify the chromatographic system performance. System suitability was checked by six replicate injections (n=6) of the system suitability check solution. The main parameters including the RSD, % of peaks (acceptance criteria: <2.0 %), the RSD, % of the retention times (acceptance criteria: <1.0 %), the peak tailing factor (the USP coefficient of the peak symmetry) (acceptance criteria: < 2.0), the column efficiency - the number of theoretical plates (acceptance criteria: >2000) and the resolution factor (acceptance criteria: >7) were measured. The results are summarized in Table 3.

Table 3. The results of system suitability test.

Parameter	Acceptance Criteria	Result
Tailing factor	<2.0	For cyanidin chloride: 1.446-1.482 For kuromanin chloride: 0.91-1.01
Column efficiency	>2000 theoretical plates	For cyanidin chloride: 6718-7088 For kuromanin chloride: 3111-3185
Resolution factor	>7.5	>9.8
RSD, % of peak areas (n=6)	< 2.0 %	For cyanidin chloride: 0.215 % For kuromanin chloride: 0.325 %
RSD, % of the retention times (n=6)	< 1.0 %	For cyanidin chloride: 0.192 % For kuromanin chloride: 0.165 %

ACCURACY

The accuracy of the method was assessed by comparing the analyte amount determined versus the

known amount spiked at two different concentration levels with three replicate injections (n=3). The sample solutions were spiked with a standard solution at 40.0 µg/mL, 20.0 µg/mL concentrations of cyanidin chloride. The accuracy is expressed as the percentage of standard

compound recovered from a spiked solution (extract sample solution+standard) with a corresponding RSD, %. The average recovery should be within 85.0 -115.0 % and the RSD, % of the percentage recovery should be <6.0 % for each concentration level of spiked sample solution (acceptance criteria). The recovery - Rec, % for each concentration level of spiked solution was calculated by the following formula: $Rec, \% = (Au_1 - Au_2) \cdot 100/As$. Where, Au_1 - the peak area obtained with the spiked sample solution (endogenous added cyanidin chloride standard), Au_2 - the peak area of obtained with the sample solution (endogenous added cyanidin chloride standard) and As - the peak area obtained with the standard solution. The average recovery equals 95.62 %.

PRECISION

The precision was estimated by measuring repeatability on six individual determinations of total anthocyanins in the extract sample solution at the same concentration. This parameter was checked by the RSD, % of determined concentrations ($\mu\text{g/mL}$) for six individual determinations of target compound which should not be more than 3.0 %; the results are given in Tables 4. The RSD, % of determined concentrations ($\mu\text{g/mL}$) for six individual determinations of total anthocyanins complies with the acceptance criteria which indicate that this method has a good precision.

STANDARD SOLUTION STABILITY

The standard solution stability was studied by injecting standard solutions of cyanidin chloride initially, after 6, 24 and 48 hours against the freshly prepared standard solution. This parameter was checked using two standard solutions and calculated the percentage bias between peak areas of standard solutions stored under refrigeration in dark glassware and freshly prepared which should not be more than 3.0 % (acceptance criteria). The bias in terms of peak area between two standard solutions should be within 0.98-1.02 (acceptance criteria). The standard solution is stable for the period up to 6 hours prepared in refrigerator and dark glassware (2.75 %).

Table 4. The repeatability results.

Solution #	Total Anthocyanins	
	The sum of peak areas	Concentration, $\mu\text{g/mL}$
1	13270008	2.93
2	13238987	2.92
3	13158261	2.91
4	13270668	2.93
5	13233947	2.92
6	13980789	3.09
Average	13358777	2.95
RSD, % (n=6)	2.302	2.330

FILTER COMPATIBILITY TEST

The PVDF membrane filter compatibility was evaluated using standard solution and by calculating the percentage bias between peak areas obtained with standard solutions filtered and non-filtered which should not be more than 0.5 % (acceptance criteria). The result is 0.19 % which gives the confidence that adsorption of target compound does not occur on the used filter.

ESTIMATION OF TOTAL ANTHOCYANINS IN DRIED SAMPLES

The organic extract solutions prepared using the developed supercritical fluid, magnetic stirring extraction, and ultrasound-assisted extraction procedures and were analyzed using the validated HPLC method. Figure 4, 5, 6 show the chromatograms obtained with a korumanin chlorid standard solution, cyanidin chloride standard solution, and extract sample solution, respectively.

The content of total anthocyanins in μg per g of dried grape skin was calculated as well. The results are given in Table 5.

Table 5. The content of total anthocyanins in dried grape skin.

Extraction technique	The content of total anthocyanins, $\mu\text{g/g}$
UAE	2.50 - 51.81
SFE	2.70 - 70.78
MSE	4.06 - 56.00

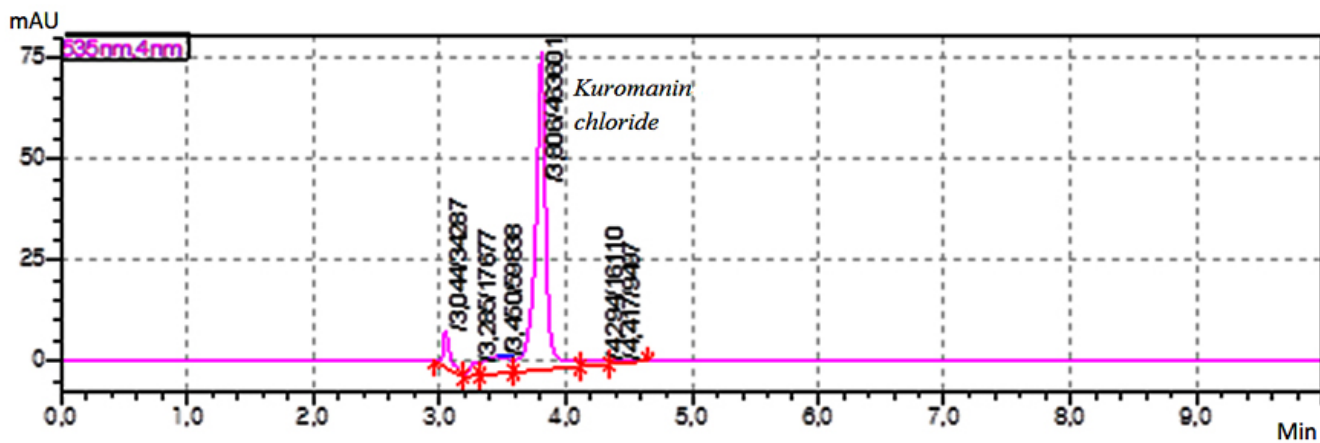


Fig 4. Chromatogram of the korumanin chloride standard solution.

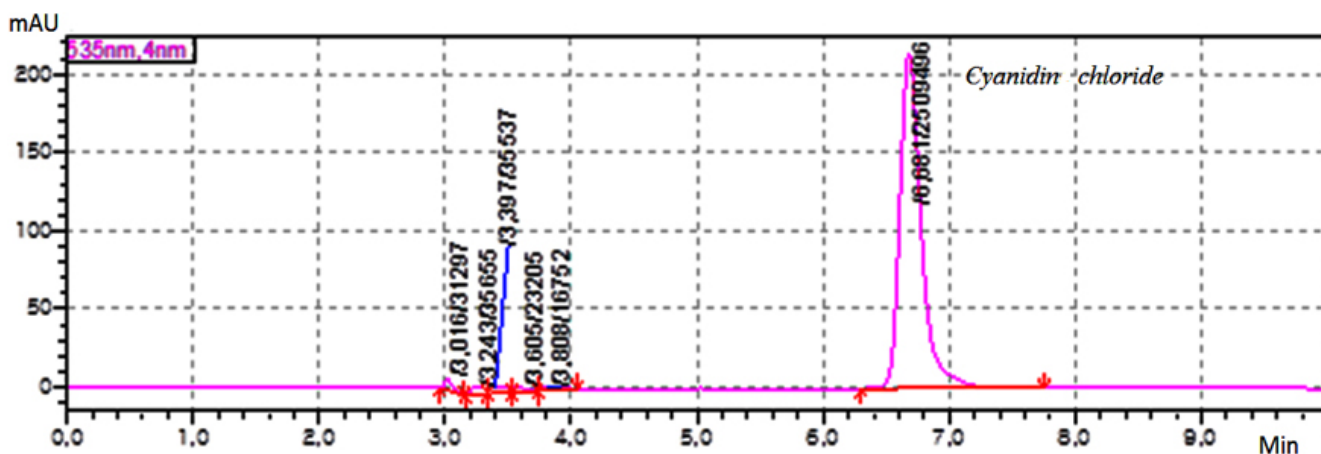


Fig 5. Chromatogram of the cyaniding chloride standard solution.

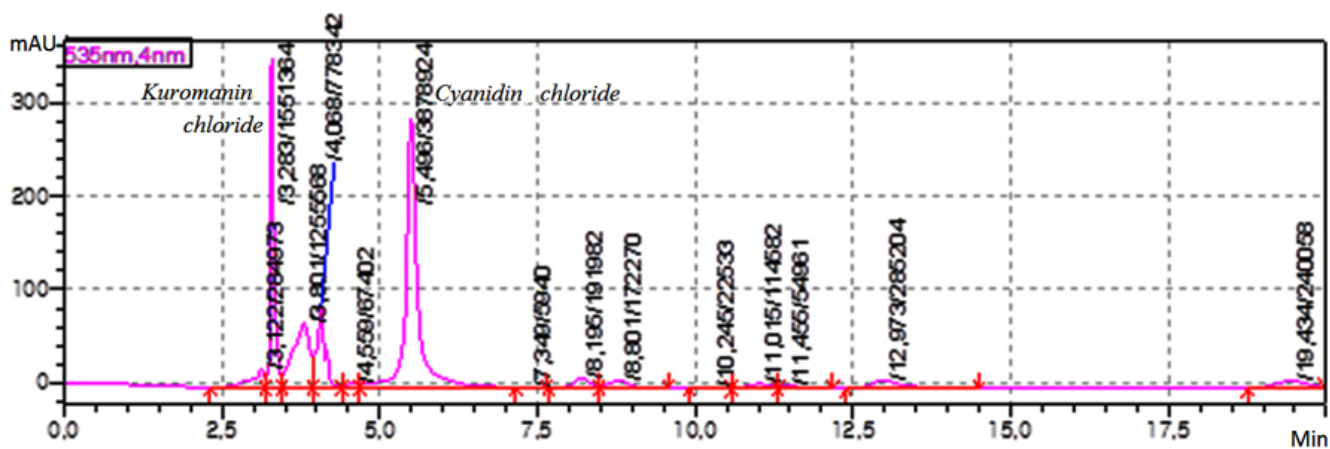


Fig 6. Chromatogram of the extract sample solution.

CONCLUSIONS

Hence, the developed sequential extraction techniques of the major anthocyanins using ultrasound-assisted, magnetic stirring and supercritical fluid extraction are simple, effective, eco-friendly separation procedures, which provide a high quality of target compounds and can be used to develop a standard technological process for utilization of agro-industrial waste material – grape skin. Also, the developed and validated HPLC method for quantitative determination of total anthocyanins is rapid and selective analytical procedure which can be successfully used by scientific and quality control laboratories.

Conflict of interests

The authors declare no conflicts of interests.

ACKNOWLEDGMENTS

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Preparation and Characterization of Adsorbents from Physic Nut Plant (*Jatropha Curcas* L)

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Abstract: *Jatropha curcas* is a drought-resistant perennial species that grows well in marginal or poor soil. The use of the plant as raw material for producing porous carbon was investigated in this work. Physic nut (*Jatropha curcas* L.) stem, root and seed coat (plant samples) were modified and used to prepare activated carbon. Activated carbons were prepared by carbonizing the plant samples at 450 °C and soaking the chars in 75% H₃PO₄. Modifications of the plant samples were performed by drying, grinding and further treatment with citric acid (C₆H₈O₇). All the samples were labeled accordingly as MDS, MDR, MDC, ACS, ACR, and ACC. The physicochemical properties, such as Iodine value, percentage moisture, ash content, pH and bulk densities of all the samples, were investigated. The surface morphology of all the samples was determined using the scanning electron microscope (SEM). The pores of char activated by H₃PO₄ materially modified by citric acid was found to vary in shapes and sizes, and the macropores appeared to be linked to mesopores in the case of activated H₃PO₄. Elemental analysis of the samples by XRF showed K, Ca, Fe, and I as predominant elements, which indicates that the samples cannot be used for the removal of these elements.

Key Words: Physic Nuts, Activated carbon, SEM, Elemental Analysis, Modified samples, Mesopores, Macropores

INTRODUCTION

The presence of inorganic and organic pollutants in wastewater is increasing and several of them are not efficiently removed by conventional wastewater treatment processes and pose re-occurring and persistent problems such as odor, toxicity, and foaming. Several studies have been conducted on the use of plant and animal materials for the removal of heavy metals from aqueous solution by adsorption [1-11]. Activated carbons and modified natural materials have been found to be excellent adsorbents materials, and thus are used to purify, decolorize, detoxify, filter or remove dissolved substances. They are also used as catalysts in industrial processes [12]. Activated carbon has long been serving the industrial need of decolorizing and removal of unwanted dissolved impurities from aqueous and non-aqueous media [13]. Activated carbons made from conventional raw materials such as coconut shell, bituminous coal, peat, and lignite, however, are expensive [14]. Activated carbons are prepared by

physical and chemical activation methods. There are two important advantages of chemical activation over physical activation. Lowering of temperature, and the yield of the chemical activation [15]. Acid activation followed by the thermal treatment increases the adsorption capacity to a large extent due to the increased surface area and pore volume [16, 17].

Many researchers have worked on the production of adsorbents materials from renewable resources using low-cost methods and materials, with emphasis to decontaminate water in the environment. Agricultural and industrial waste material is used as adsorbents by different researchers for the removal of heavy metals, such as coconut husk, palm pressed fibers, coconut shell activated carbon, wood-dust coal activated carbons [18-20], and used rubber tire carbon [21]. Cactus, olive stone, wool, charcoal and pine needles [22], rice husk carbon [12], *Cequisetifolia* leaf carbon [22], bagasse and fly ash [23], hazelnut shell [24], husk of Bengal gram [25], sugarcane bagasse [15], rice bran [26], and rice husk (Hindu) have been reported in literature.

Jatropha curcas is a multi-purpose non-edible oil yielding perennial shrub and a drought tolerant plant, with their seeds used to produce renewable energy such as bio-diesel. Indian railways use 2 million kg diesel per year. Indian Government has decided to use bio-diesel at 5% level in the regular diesel. It has started planting *Jatropha* along both sides of the railway tracts, which will cover an area of 2500 km². Many researchers grow these plants for biodiesel production supported by several funding agencies.

This investigation addresses the preparation of a number of adsorbents using different acids, such as citric acid and orthophosphoric acid. All the chemicals used are of analytical reagent grade.

EXPERIMENTAL

Materials and Methods

The root, stem and seed coat of *Jatropha Curcas* was obtained from Agbo-oba area in Ilorin, Kwara State, Nigeria. The root, stem, and seed coat of the *Jatropha curcas* plant were chopped into smaller sizes and exposed to outdoor sunlight (drying) for about 2 days. The dried samples were then divided into two portions for the modification and carbonization processes. The portion for modification was ground to a smaller size using mortar and pestle.

Preparations

Citric Acid Modified Samples: The air dried and ground samples were soaked in excess 0.3 M HNO₃ for 24 hrs at room temperature. The mixture was purified with de-ionized water until pH of washing mixture was 6.8. The crystals were filtered and air dried with the samples steeped in 0.6 M citric acid overnight. The citric acid modified crystals were severally surface cleaned with distilled water to remove excess citric acid, and finally air dried and kept in a closed container [13].

Activated Carbon: The dried roots, stem and seed coat from the second portion were carbonized each separately in a muffle furnace at a temperature of 450° C for 4hrs. The carbonized samples were ground and further sieved to 150 µm. The filtered charcoal was treated with 75% orthophosphoric acid (H₃PO₄) at a ratio of 1:1 by weight of charcoal-H₃PO₄. The mixture of H₃PO₄ and charcoal were dried in an oven for 24 hrs at 60° C. Each of the samples

was washed with deionized water until the pH of the solution reached 6.8 - 7.0. The samples were filtered and the residue (Charcoal) mixed with 0.1 HCl in a beaker and stirred for 1 hr on a magnetic stirrer [27], with the mixture finally purified with hot deionized water until the pH of washing solution reached in the 6.9 - 7.0 range. The washed activated carbon was dried and kept in a closed container for adsorption experiment [27, 28].

The citric acid modified samples and carbonized samples were labeled as shown in Table 1.

Table 1. Acronyms for sample identity.

Citric acid Modified stem	MDS
Citric acid Modified root	MDR
Citric acid Modified coat	MDC
The activated carbon of stem	ACS
The activated carbon of root	ACR
The activated carbon of coat	ACC

Ash Content: Sample ash content was performed by weighing, 1 g MDS, MDR, MDC, ACS, ACR, and ACC. These were placed in a crucible of known mass and heated in a furnace (*Fisher-Thermo Scientific, USA.*) at a temperature of 600°C for 4 hrs. The heated samples were placed into desiccators for cooling and reweighed. The ash content of each sample was calculated from the weight of the sample before and after heating [29].

$$\text{Ash content} = \frac{W_3 - W_2}{W_2 - W_1} \times 100 \quad (i)$$

W1 = Weight of crucible

W2 = Initial weight of crucible with sample

W3 = Final weight of crucible with the sample.

pH: 1 g each of MDS, MDR, MDC, ACS, ACR, and ACC was boiled in a beaker containing 100 ml of distilled water for 5 min. These solutions were diluted to 200 ml with distilled water and cooled to room temperature. The corresponding measured pH was performed using a pH meter (model ATPH-6, USA.) [30].

Iodine Number: The iodine number (iodine value) of each of the prepared samples was determined by adding 1 g of the sample to 20 ml of 5% HCl and boiled. The solution was allowed to be cooled down to room temperature. 100 ml of 0.1 N Iodine solution was added and stirred for 30 min using a magnetic stirrer; the solution was filtered and 20 ml each of filtrates was titrated with sodium thiosulphate (Na₂S₂O₃) solution in the presence of a starch indicator. The iodine value of each sample was

determined from the reading of titration containing the sample and that of the blank [31]. Blank titration using 0.1 N iodine solution prepared was titrated with 0.1 N $\text{Na}_2\text{S}_2\text{O}_3$ and the readings obtained were used as the blank.

$$\text{Iodine Number} = \frac{B-A}{W} \quad (ii)$$

B = Volume of Blank solution

A = Volume of a solution of the sample

W = Mass of sample

Moisture Content: Samples of MDS, MDR, MDC, ACS, ACR, and ACC were placed into the crucible of known mass and placed inside an oven of 80°C for 3 hrs. Samples were removed and cooled over silica in a desiccator and weighed and reheated at an oven temperature of 105°C for an additional 30 mins. This process was repeated 5 - 7 times for 15 minutes until a constant mass was achieved. The percentage moisture contents of each sample were calculated from the readings obtained [29].

$$\text{Moisture content} = \frac{W_3 - W_2}{W_2 - W_1} \times 100\% \quad (iii)$$

W_1 = Weight of crucible

W_2 = Initial weight of crucible with sample

W_3 = Final weight of crucible with the sample.

Bulk Density: The bulk densities of each of samples were determined by using Archimedes' principle. This was determined by weighing an empty measuring cylinder, versus the mass of cylinder containing each sample, versus the mass of cylinder filled with water to the brim. The mass of each sample was determined from the difference in mass of the filled and empty measuring cylinder. The volume of water in the container was determined by taking the difference in mass of the empty and filled measuring cylinder. The bulk densities were determined by dividing the mass of each by the volume of the measuring cylinder [32].

$$\text{Bulk density} = \frac{W_2 - W_1}{V} \quad (iv)$$

W_1 = Initial mass of the cylinder

W_2 = Final mass of cylinder filled with sample

V = Volume of cylinder

RESULTS & DISCUSSION

Scanning Electron Microscopy

The surface characteristics of MDS, MDR, MDC, ACS, ACR and ACC were analyzed using scanning electron

microscopy in order to visualize the samples surface morphology by magnifying their pore sizes, using The JSM-7400F (USA) field emission scanning electron microscope having a 1 nm resolution at 15 kV and a 1.5 nm resolution at 1 kV. This allows operation in a wide range of beam energies from 0.1 keV to 30 keV and affords various operation modes, including secondary electron imaging (SEI), backscattered electron imaging, gentle beam mode, and imaging with energy filters.

The surface morphology of MDS, MDC, MDR, ACR, ACS, and ACC was visualized via scanning electron microscopy (SEM), the corresponding SEM micrographs were obtained at $\times 2000$ (2 kx) and $\times 500$ magnifications. Photographic images of SEM micrographs of MDS, MDC, MDR, ACR, ACS, and ACC showed spongy or coarse areas of the surface of the carbon and the micropores were identifiable. It is generally understood that pore structures development is influenced by many factors, such as inorganic impurities, and the initial structure of the carbon precursor. For the SEM images of MDS and ACS, see Figures 1 (a-b) and 2 (a-b), respectively.

The surfaces showed relatively smooth regions as well as cracked and pitted morphology. The presence of small pores on the surface indicated that the adsorbents had developed an elementary pore network. It can be shown that chemical activation resulted in a porous structure opening of the sample surfaces of MDS and ACS. According to the International Union of Pure and Applied Chemistry (IUPAC) [33] pores on activated carbons are classified by their sizes into three groups: macropores having an average diameter > 50 nm, mesopores with a diameter of 2-50 nm, and micropores having an average diameter of < 2 nm.

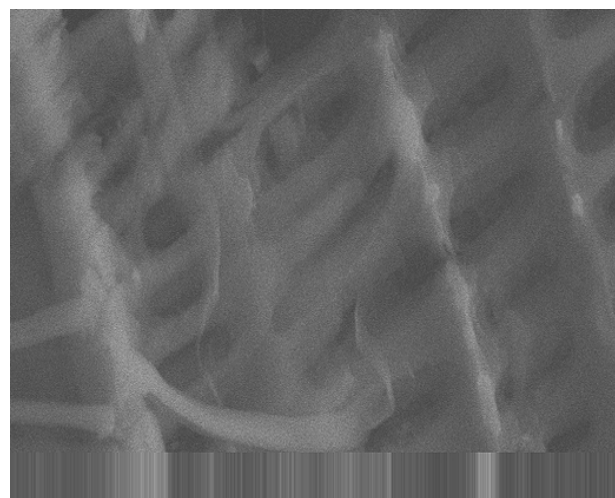


Fig. 1(a) SEM of MDS $\times 2000$ magnification.

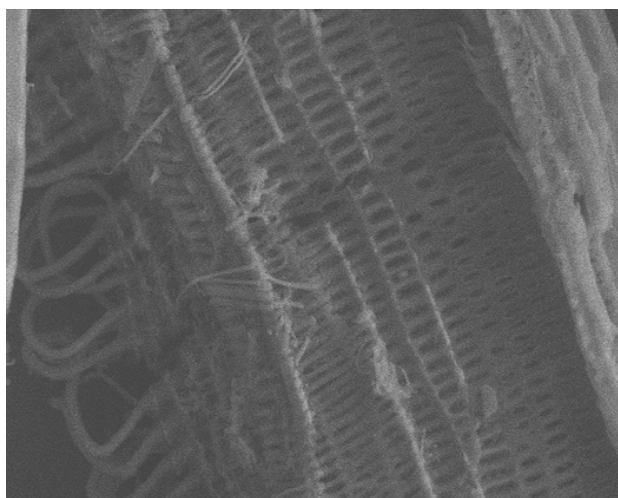


Fig. 1(b) SEM of MDS ×500 magnification

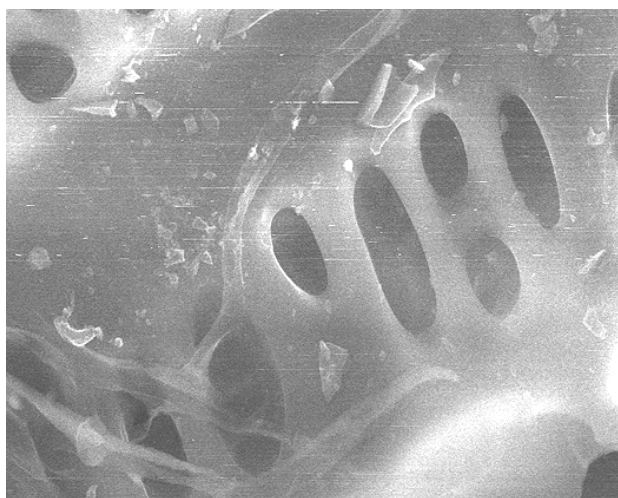


Fig. 2(a) SEM of ACS ×2000 magnification

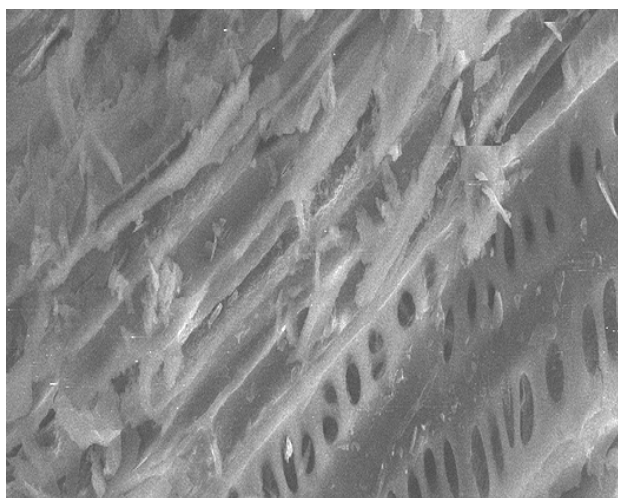


Fig. 2(b) SEM of ACS ×500 magnification

Physicochemical Properties of the Samples

The moisture content, which is the amount of water present in a sample, suggests that extensive porosity has been introduced during the acid treatment in the structure of all carbons. The moisture content of the samples was found to be 4.2, 4.7, 5.1, 4.8, 5.6, and 5.2% [Table 2]. These do not affect the adsorptive power of the sample. It was observed that if the moisture content of adsorbent is high, it will limit the action of the samples and necessitate utilizing some extra loads of the carbon [34]. Similar characteristics are observed in the moisture content of *Ricinus Communis*, found to be 2.5 [29], and the moisture content of *Jatropha Husk* activated with H_3PO_4 is 15 [35], which indicated that the result obtained was consistent with the same data found in other studies. Sample data obtained from this study is tabulated in Table 2.

The iodine number is a relative indicator of porosity in a carbonaceous material and may be used as an approximation of surface area for some types of carbon; higher iodine numbers reflect the better development of the microporous structure and higher adsorption abilities for low-molar-mass solutes [36, 37]. The iodine number of the samples MDS, MDR, MDC, ACS, ACR, and ACC was 244.9, 208.5, 237.3, 233.1, 229.6 and 232.5, respectively [Table 2]. This is consistent with the results obtained in *Jatropha Husk* activated with H_3PO_4 , HCl, $ZnCl_2$, NaOH, H_2SO_4 and steam (81, 157, 91, 138, 301 and 112) [35]. These indicate that the sample prepared by acid activation has a good adsorptive capacity and can be considered for adsorption of heavy metals and organic pollutants. Iodine number of samples are usually high at a temperature of 400°C because of the incomplete carbonization process at 400°C. The tar left over also contributed to iodine absorption [38]. It was clear that the iodine number of ACR, ACS and ACC is high due to the phosphoric acid destroying the aliphatic and aromatic species present in plants, therefore swiftly removing the volatile matter during the carbonization process [39].

The ash content generally provides information about the inorganic constituents associated with carbon. Ash content of adsorbents usually increases with an increase in carbonization temperature. This was believed to be due to reduced volatile matter (VM). The percentage ash contents of MDS, MDC, MDR, ACR, ACS, and ACC is 7.8, 8.4, 3.6, 7.4, 8.3 and 8.2% [Table 2]. The ash content of adsorbents increased with a decrease in percentage of volatile matter, which indicates that ash is non-volatile [40].

The percentage ash content of *Jatropha* Husk activated by NaOH, H₂SO₄, H₃PO₄, HCl, ZnCl₂, and steam were 7.4, 12.5, 10.2, 7.2, 0.5, 31.8% [35]; another report indicates the percentage ash content of *Ricinus Communis* as 6.5 [33]. All of these indicate that the ash content of MDS, MDC, MDR, ACR, ACS, and ACC is in the normal range [41, 42].

The pH of adsorbent is the degree of acidity or basicity of that adsorbent and it depends on the methods of preparation, inorganic matter and chemically active oxygen groups on its surface, as well as the kind of treatment to which the adsorbent had undergone [43]. The pH of the solution after mixing MDS, MDC, MDR, ACR, ACS, and ACC with distilled water is 6.5, 6.8, 6.7, 6.8, 6.7 and 6.6, respectively. These results correspond to the results of *Jatropha* Husk activated by NaOH, H₂SO₄, H₃PO₄, HCl, ZnCl₂ and steam, which are 7.8, 6.98, 6.74, 6.33, 6.01 and 8.11 [35], and *Ricinus Communis* which is 6.90 [29]. These data indicate that the adsorption of H⁺ ions

from solution or desorption of OH⁻ ions from the sorbent surface [44].

Bulk density is an important parameter when carbon is removed by filtration. It determines how many pounds of carbons can be contained in a filter of a given solid capacity and how much treated liquid is retained by the cake filtrate. Carbons with adequate density also help to improve the filtration rate by forming an even cake on the filter surface. The American Water Work Association has set a lower limit on bulk density at 0.25 gm/ml for Granular Activated Carbon (GACs) to be of practical use [26, 33]. The bulk density of the prepared sample used for this work is within that limit, which is 0.52 gm/ml. The bulk densities of MDS, MDC, MDR, ACR, ACS and ACC were 0.109, 0.120, 0.224, 0.152, 0.21 and 0.37 g/m³. These results imply that the outcome in this experiment corresponds well with the expected and standard value.

Table 2. Summarized physicochemical properties of Physic nut (*Jatropha curcas* L) samples.

	MDS	MDR	MDC	ACS	ACR	ACC
Ash content (%)	7.8	3.6	8.4	7.4	8.3	8.2
Bulk density (g/m ³)	0.109	0.120	0.224	0.152	0.21	0.37
Iodine number	244.9	237.3	208.5	233.1	229.6	232.50
Moisture content (%)	4.2	5.1	4.7	4.8	5.6	5.2
pH	6.5	6.7	6.8	6.8	6.7	6.6
Percent Yield (%)	-	-	-	87	85	85
Particle size (nm)	150	150	150	150	150	150

Table 3. Results of Samples Elemental Analysis.

Elements & Symbol	(mg/kg)					
	MDS	MDR	MDC	ACS	ACR	ACC
Potassium (K)	1648	1164	1279	1573	1677	1892
Calcium (Ca)	767	1576	5188	2148	2541	854
Vanadium (V)	-	7	16	28	25	17
Barium (Ba)	92	-	-	-	102	41
Chromium (Cr)	87	17	12	22	4	40
Manganese (Mn)	117	43	26	36	33	110
Iron (Fe)	642	224	400	629	582	868
Nickel (Ni)	3	-	-	-	-	1
Copper (Cu)	15	6	3	3	-	9
Zinc (Zn)	312	451	183	83	11	68
Selenium (Se)	18	17	2	4	2	17
Strontium (Sr)	109	61	35	38	18	50
Bromine (Br)	24	11	4	3	2	14
Iodine (I)	200	463	849	249	267	57

Elemental Analysis of MDS, MDC, MDR, ACR, ACS, and ACC

The XRF results of MDS, MDC, MDR, ACR, ACS, and ACC showed that K, Ca, Fe and Zn are the major elements present, while Sr is also high in MDS, respectively (as seen in Table 3).

Other elements present are either too low at ultra-trace levels or not detected. This shows that MDS, MDC, MDR, ACR, ACS, and ACC cannot be used as adsorbents in the removal of metals like K, Ca, Fe and Zn, as well as I, which is a non-metal due to the adsorption process taking place, leading to an increase in the amount of these elements instead of their removal.

CONCLUSIONS

Preparation of adsorbents from pyrolysis and modification of physic nut plant was performed on a laboratory-scale. Data obtained indicate that the adsorbents with favorable physicochemical properties may be produced using several methods. A number of adsorbents were prepared by activation with acids such as $ZnCl_2$, H_3PO_4 , and NaOH, and characterized using standard methods. The MDS, MDR, MDC, ACS, ACR, ACC in this study has shown to have excellent physicochemical properties and the SEM showed they had constructed or organized surface morphology structures. The iodine number of the adsorbents is in the range 244.9, 208.5 - 244.9, which is in agreement with the result obtained in *Jatropha* Husk activation with H_3PO_4 . These showed that they could be used as effective adsorbents for the removal of contaminants from water. As the raw material of the carbons is disposed of as waste, applications of the MDS, MDR, MDC, ACS, ACR, ACC adsorbents to the water treatments are expected to be low cost and effective.

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Recent Advances and Perspectives in the Asymmetric Reformatsky Reaction

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Abstract: Over the last five years much progress has been made in the catalytic asymmetric Reformatsky reaction. This reaction has been used since then in modern organic synthesis, especially in the synthesis of complex target molecules. In this review, both enantioselective and diastereoselective Reformatsky reactions were achieved using novel chiral ligands in combination with different metals such as Zn, Sm, Cr and Sn are described. Recent achievements in the total synthesis of natural products using diastereoselective Reformatsky reaction are also described and covers literature from 2013 to 2017.

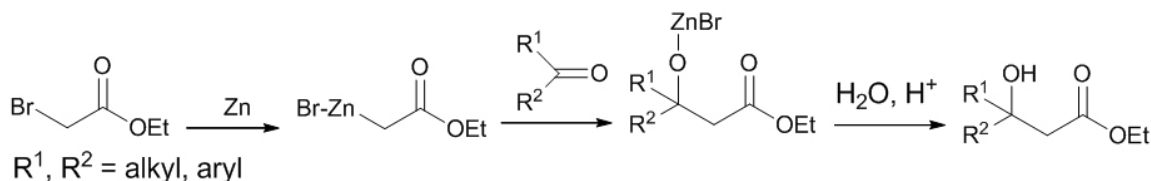
Key Words: Reformatsky reaction, Asymmetric reaction, C-C bond formation, Metal-catalyzed reaction, Chiral auxiliary

INTRODUCTION

The classical Reformatsky reaction was discovered by Sergei Nikolayevich Reformatsky in 1887 [1]. It consists of a zinc-induced reaction between α -haloesters and aldehyde or ketone to produce β -hydroxy esters (Scheme 1), which are valuable precursors in natural product synthesis and pharmaceuticals. Generally, in this reaction, the enolate is formed by the oxidative addition of a metal or low-valent metal salt or complex into a carbon-halogen bond, activated by a vicinal carbonyl-derived group, followed by a reaction between enolate and appropriate electrophile [2]. It was reported that other metals like Sm, Cr, Sn, Ti, Co, In or Fe in low oxidation states are also suited for this reaction [3]. An aqueous metal-free electrochemical Reformatsky reaction is also known [4]. The solvents used are generally ethers such as Et₂O, THF, and 1,4-dioxane. The most important application is that this reaction takes place in neutral conditions, whereas the aldol reaction is a base or acid catalyzed reaction. The disadvantages are lower yield and stereoselectivity. Much

progress has been made in this field to enhance the stereoselectivity by using chiral ligands. Furstner *et al.* introduced the catalytic redox cycle of this reaction [5]. Cozzi highlighted the possibility of catalytic enantioselective and diastereoselective Reformatsky reaction with different electrophiles [6]. A recent review describes the highly diastereoselective Reformatsky reactions using various chiral auxiliaries, either on the electrophile or on the nucleophile, in presence of different metals, which gave rise to high yield products [7]. In the same year, another review on highly enantioselective asymmetric Reformatsky reaction using chiral catalysts was also published [8].

The first example of an enantioselective Reformatsky reaction was reported in 1973 by using (-)-sparteine as the reagent [9]. In 1991, Soai reported an enantioselective Reformatsky reaction with the use of a chiral amino alcohol ligand (Table 1, entry 1), gave rise to the product with 91% yield and 75% enantiomeric excess (*ee*) [10]. This aroused great interest in the minds of organic chemists and led to the development of different chiral ligands for asymmetric Reformatsky reaction (Table1) [11].



Scheme 1. The classical Reformatsky reaction.

Table 1. Different chiral ligands used for asymmetric Reformatsky Reaction [12-18].

Entry	Chiral Ligand	ee (%)
1.	S-(+)-Diphenyl(1-methylpyrrolidin-2-yl)methanol ¹²	75
2.	Trifluoromethylaminoalcohol ¹³	90
3.	(-) DAIB ¹⁴	93
4.	D-Glucosamine derived tertiary amino alcohol ¹⁵	74
5.	Mn salen complex ¹⁶	63
6.	Chiral indolinylmethanol ¹⁷	81
7.	(R)-Binol ¹⁸	85

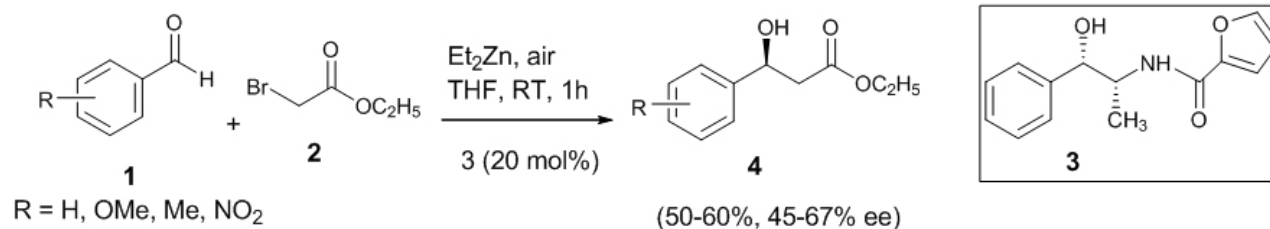
ENANTIOSELECTIVE REFORMATSKY REACTION

Enantioselective Reformatsky reactions have been developed using chiral ligands for the formation of new C-C bonds.

Asymmetric Reformatsky reaction involving aldehyde

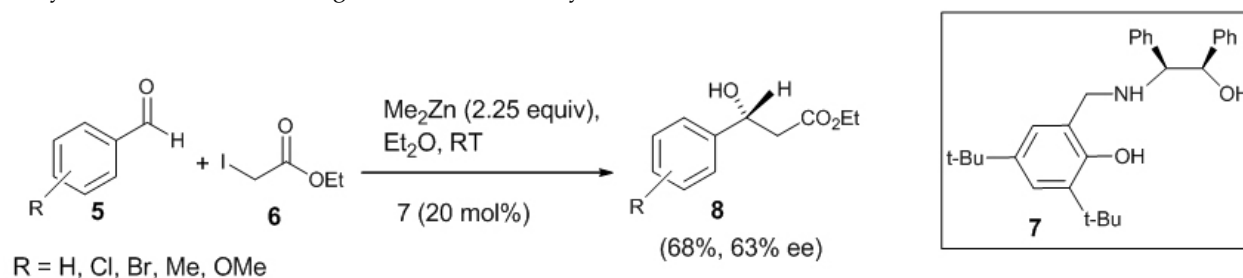
A chiral amide ligand **3** was developed from (1*S*, 2*R*)-(+)-norephedrine and 2-furoic acid and applied in

Reformatsky reaction which enhanced the enantioselectivity of the product. The asymmetric Reformatsky reaction was carried out between various substituted benzaldehydes **1** and α -bromomethyl acetate **2** in presence of chiral ligand **3** (10-30 mol%) mediated by Et₂Zn, which gave the corresponding β -hydroxy ester **4** in 50-60% yield and 45-67% ee (enantiomeric excess) (Scheme 2) [19]. Benzaldehydes with electron withdrawing group gave the product with good yield and enantioselectivity.

**Scheme 2. Asymmetric Reformatsky reaction by chiral amide ligand.**

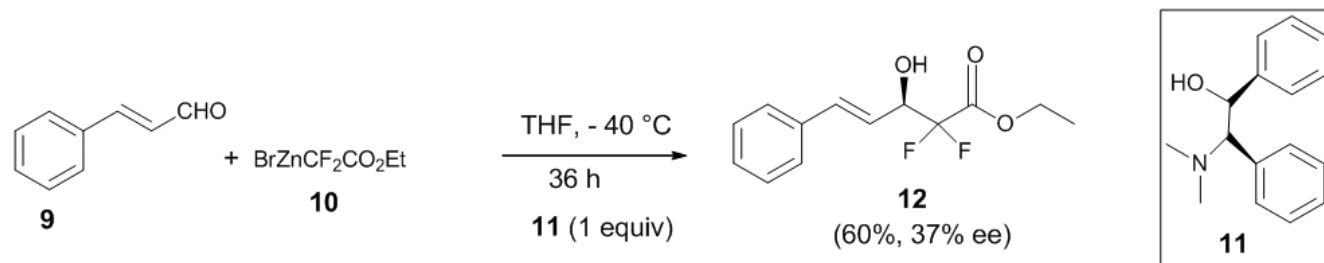
A novel chiral tridentate β -amino alcohols **7** synthesized from a mixture of 2,5-di-tertbutyl salicylaldehyde and chiral (1*R*, 2*S*)-2-amino-1,2-diphenylmethanol was used as a catalyst for the asymmetric Reformatsky reaction [20]. Here, the reaction was carried out with variously substituted aldehydes **5** and ethyl iodoacetate **6** to obtain good enantioselectivity

of the desired product **8** (Scheme 3). Both electron withdrawing and electron donating groups attached to the benzaldehyde gave enantioselectivity in the range 60-63% ee. It was found that 1-naphthaldehyde and 2-naphthaldehyde gave the best enantioselectivity with 81% and 80% ee, respectively.

**Scheme 3. Asymmetric Reformatsky reaction by chiral tridentate β -amino alcohol.**

Wu *et al.* performed an asymmetric Reformatsky reaction between Cinnamaldehyde **9** and ethylbromodifluoroacetate **10** in the presence of the chiral

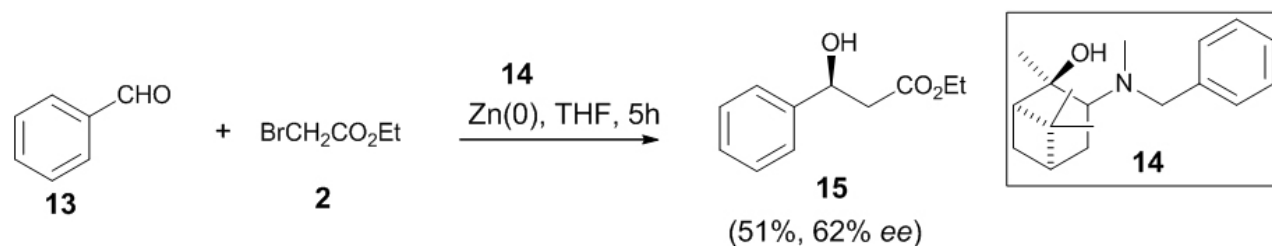
ligand (1*S*, 2*R*)-*N,N*-dimethyl-2-amino-1,2-diphenylmethanol **11** to afford the desired product **12** in 37% *ee* (Scheme 4) [21].



Scheme 4. Enantioselective Reformatsky reaction mediated by chiral amino alcohol.

Morita *et al.* utilized the tertiary amino alcohol **14** obtained from α -pinene as a ligand for the asymmetric Reformatsky reaction between benzaldehyde **13** and 2-

bromoethylacetate **2**, which yielded the corresponding β -hydroxy ester **15** in 51% yield and 62% *ee* (Scheme 5) [22].

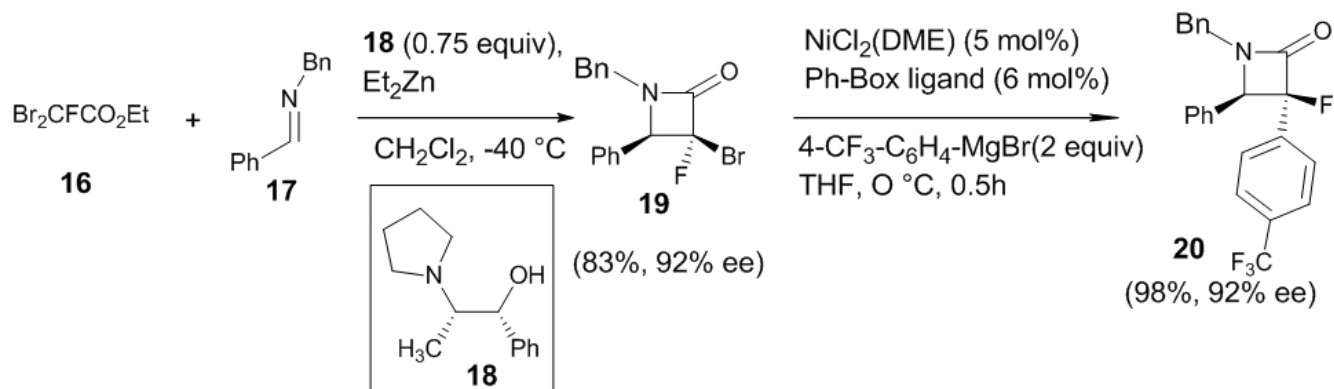


Scheme 5. Reformatsky reaction using tertiary amino alcohol derived from α -pinene.

Asymmetric Imino-Reformatsky Reaction

The incorporation of fluorine atoms to β -lactams enhanced its antibiotic activity. In 2014, Ando *et al.* reported the asymmetric Reformatsky reaction of dibromofluoroacetate **16** with imine **17** using stoichiometric amount of ligand **18** (1*R*,2*S*)-1-phenyl-2-(1-

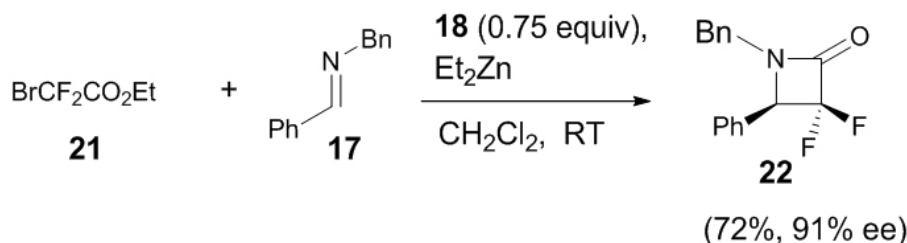
pyrrolidinyl) propan-1-ol (0.75 equiv) in Et_2Zn (3.5 equiv.) at -40°C producing α -bromo- α -fluoro β -lactam **19** with 83% yield and 92% *ee* (Scheme 6) [23]. The synthetic utility of the reaction was demonstrated by the aryl functionalization of **19** to α -aryl- α -fluoro- β -lactam **20** without any reduction in the optical purity.



Scheme 6. Asymmetric Reformatsky reaction of dibromofluoroacetate with imine and its synthetic utility.

In the same year, Ando and co-workers also investigated a similar synthetic approach for the formation of α,α -difluoro- β -lactam from bromodifluoroacetate **21** with the same imine **17** and catalyst **18** at room temperature, which gave **22** in moderate yield (72%) and good enantioselectivity (91% *ee*) (Scheme 7) [24]. Imines which

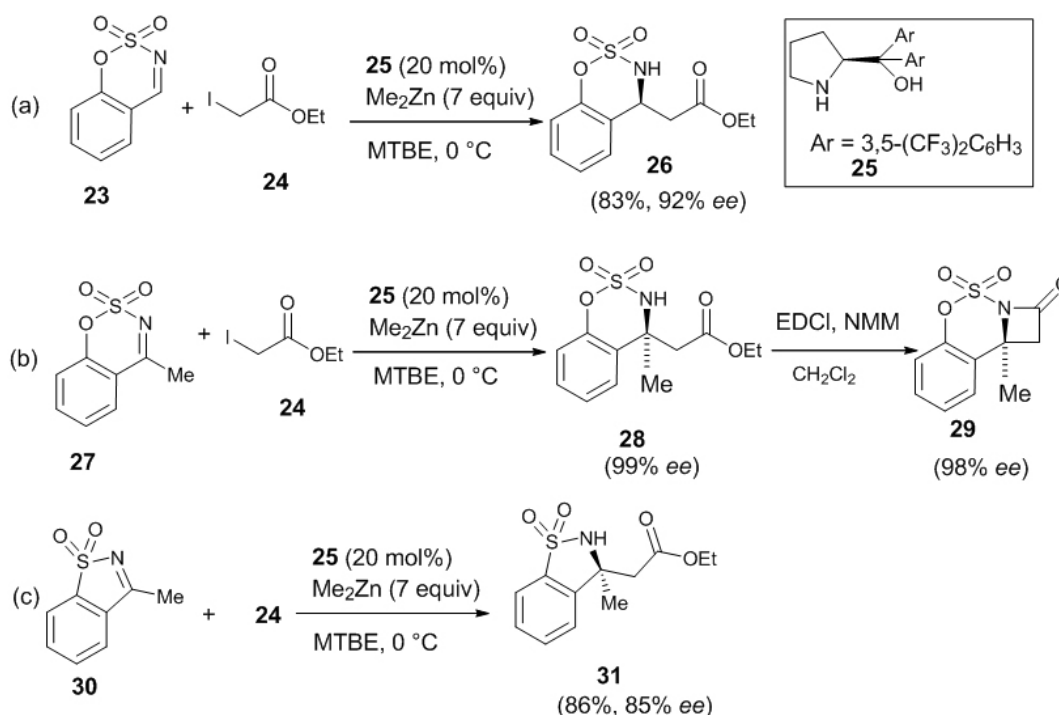
contain an electron withdrawing group as substituent in the aromatic ring, gave the corresponding products with high enantioselectivity in the range (94-99% *ee*) and moderate yield (45-74%), whereas electron donating group exhibited lower enantioselectivity (86-90% *ee*).



Scheme 7. Asymmetric Reformatsky reaction of bromodifluoroacetate with imine.

In 2016, De Munck *et al.* reported the first enantioselective aza-Reformatsky reaction of ethyliodoacetate with cyclic imines (aldimines and ketimines) forming chiral β -amino esters, which are valuable precursors for β -lactams, one of the most important classes of antibiotics [25]. Here, the reaction between cyclic benzo[*e*][1,2,3]-oxathiazine-2,2-dioxide **23** and ethyliodoacetate **24** in the presence of ligand, (*S*)- α,α -bis[3,5-bis(trifluoromethyl)phenyl]-2-pyrrolidinemethanol **25** (20 mol%) mediated by Me_2Zn

afforded the product β -amino ester **26** in good yield (83%) and enantioselectivity (92%) [Scheme 8(a)]. When the amount of ligand was reduced to 10 mol%, the yield and enantioselectivity were reduced to 89% and 87%, respectively. Both electrons withdrawing and electron donating groups attached to the 6-position of the phenyl ring of the cyclic imine gave rise to high enantioselectivity (85-92% *ee*) and good yield (70-95%). Naphthyl ring substituted to the phenyl ring of cyclic imine gave excellent enantioselectivity (90-93% *ee*).

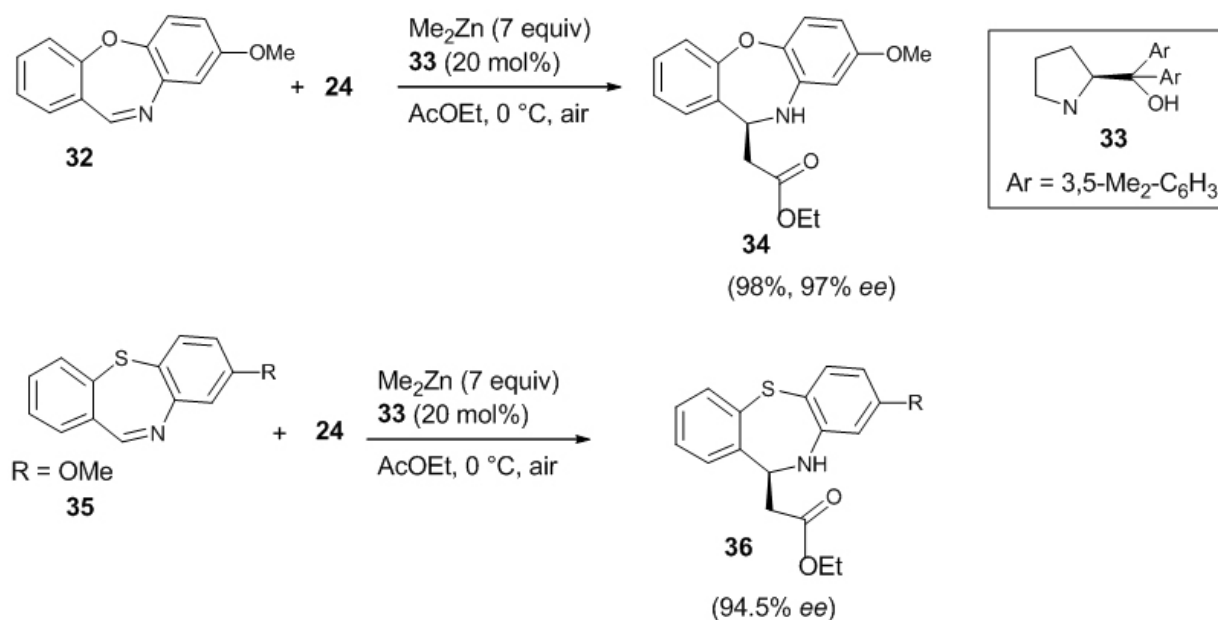


Scheme 8. Enantioselective aza-Reformatsky reaction between ethyliodoacetate and cyclic aldimines and ketimines.

They also reported the enantioselective aza-Reformatsky reaction between cyclic ketimines like 4-methyl-benzo[*e*][1,2,3] oxathiazine-2,2-dioxide **27** and ethyliodoacetate **24** with the same ligand **25** and reaction condition as in aldimines [Scheme 8(b)]. The chiral β -amino ester **28** thus formed bears a quaternary stereocentre with excellent enantioselectivity (99% *ee*). Even when the amount of the ligand was reduced to 10 mol%, the enantioselectivity was 97% *ee*. The chiral β -amino ester can be used for the synthesis of β -lactam **29** without any reduction in the optical purity (98% *ee*). The reaction was extended successfully in the synthesis of

chiral β -amino ester **31** (86%, 85% *ee*) from the five-membered cyclic N-sulfonyl ketimine **30** under the same reaction conditions [Scheme 8(c)].

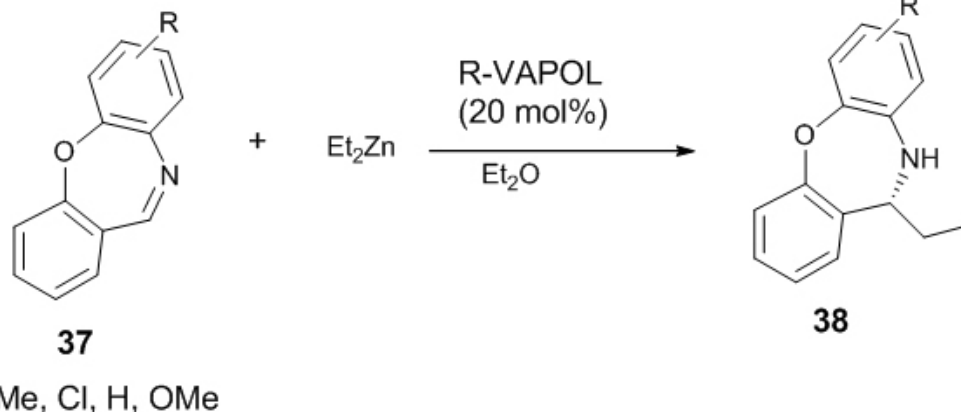
Very recently, De Munck *et al.* published the enantioselective Reformatsky reaction between seven-membered cyclic imine like dibenzo [*b, f*] [1,4] oxazepine **32** and ethyliodoacetate **24** in the presence of ligand **33** (20 mol %) in Me_2Zn and air, and the corresponding β -amino ester **34** was obtained in 98% yield and 97% *ee* [26]. They also conducted similar Reformatsky reaction between dibenzo[*b, f*][1,4] thiazepine **35** and **24**, affording β -amino ester **36** in 95% yield and 94.5% *ee* (Scheme 9).



Scheme 9. Reformatsky reaction between oxazepine/thiazepine derivatives with ethyliodoacetate.

Later on, De Munck *et al.* extended their work and disclosed the first enantioselective addition of Et_2Zn to cyclic imines **37** in presence of (*R*)-VAPOL-Zn (II) complex

as the catalyst [27]. The product **38** was obtained with moderate enantioselectivity (up to 70% *ee*) and good yield (up to 76%) (Scheme 10).



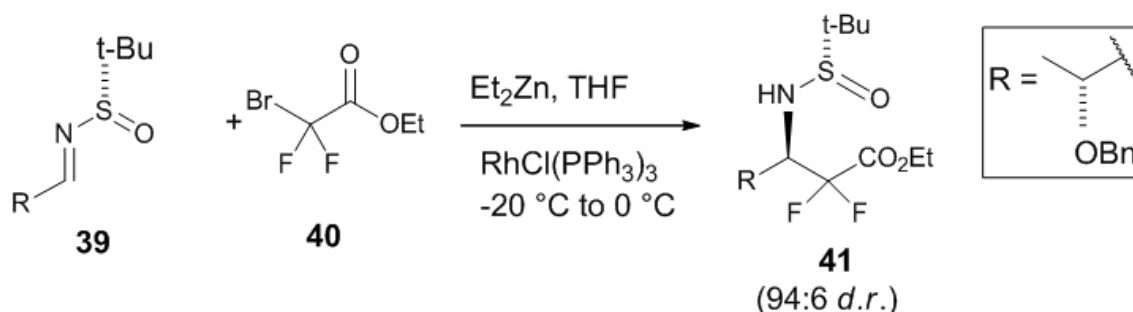
Scheme 10. Enantioselective Reformatsky reaction between cyclic imines and Et_2Zn .

DIASTERESELECTIVE REFORMATSKY REACTION

Diastereoselective Reformatsky reactions are widely used to perform the synthesis of cyclic and acyclic moieties using a large variety of chiral auxiliaries which control the stereochemistry of the desired products.

Reformatsky reaction on chiral imines

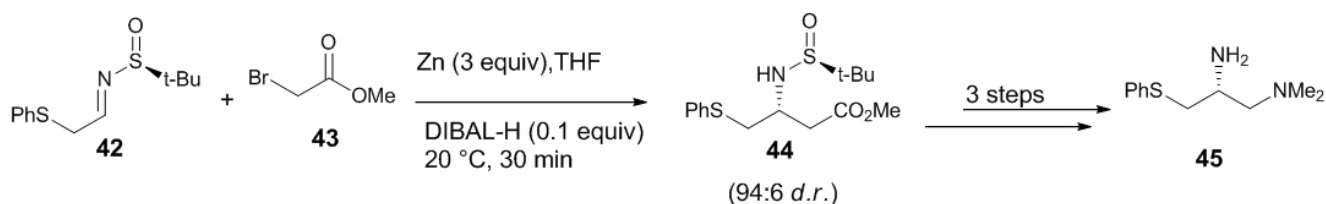
Reformatsky reaction between α -oxygenated sulfinylimine **39** and ethyl bromodifluoroacetate **40** has been reported using Honda-Reformatsky condition which afforded the desired product **41** with excellent diastereoselectivity (>94:6) (Scheme 11) [28]. The stereoinduction of the product was determined by the configuration of the chiral auxiliary.



Scheme 11. Reformatsky reaction between α -oxygenated sulfinylimines and ethyl bromodifluoroacetate.

Laclef *et al.* reported a diastereoselective aza-Reformatsky reaction for the synthesis of the key fragment diamine present in anti-apoptotic Bcl-2/Bcl-xL protein inhibitors, used in anticancer therapy [29]. They performed a large-scale synthesis of the diamine with fewer purification

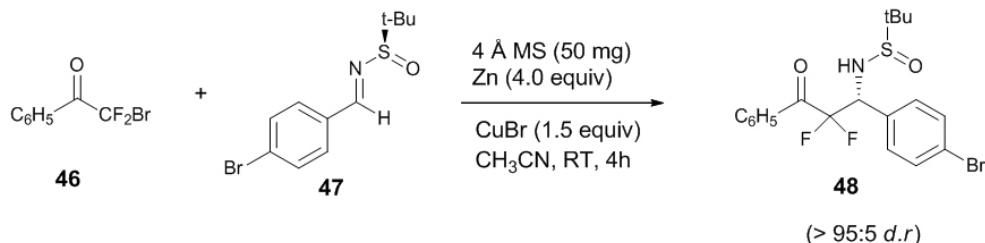
processes. The Reformatsky reaction between sulfinimine **42** and excess of methylbromoacetate **43**, afforded the corresponding product **44** with a high diastereomeric ratio (>94:6) which was further transformed to give the diamine fragment **45** with 95% HPLC purity (Scheme 12).



Scheme 12. Aza-Reformatsky reaction for the synthesis of diamine fragment of anti-apoptotic protein inhibitor.

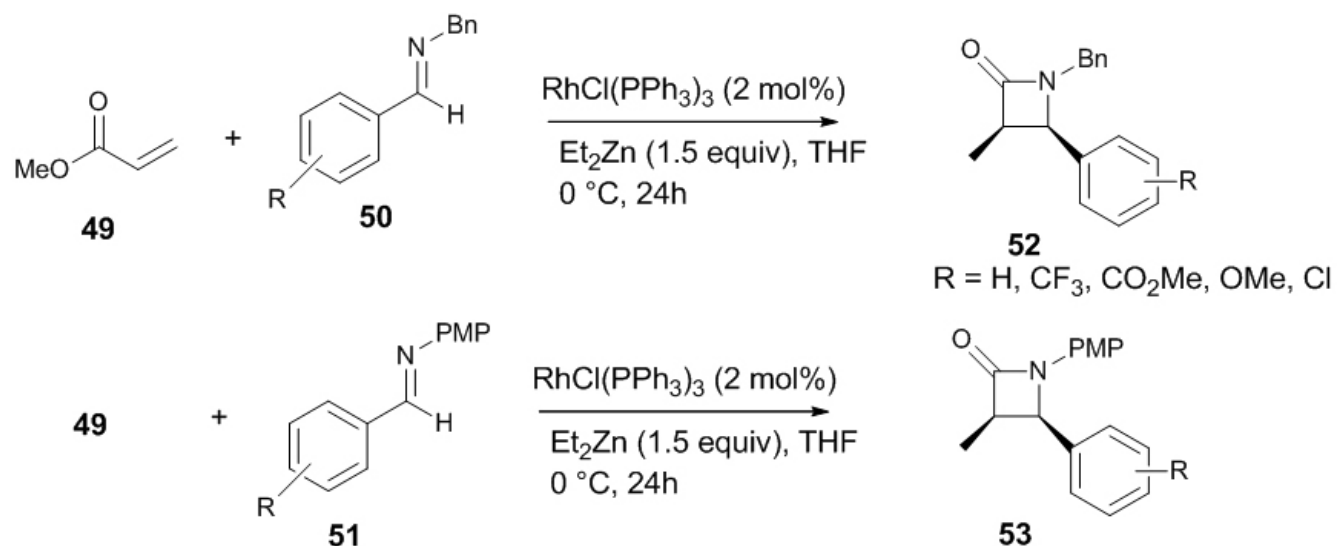
An asymmetric Reformatsky reaction between bromodifluoromethyl phenyl ketone **46** and various substituted chiral ketimines **47** in presence of 4 Å molecular sieves (MS) was reported (Scheme 13) [30]. Both

electron withdrawing and electron donating groups on the chiral ketimine afforded the product **48** with excellent diastereoselectivity (>95:5 d.r.) which has many synthetic applications.

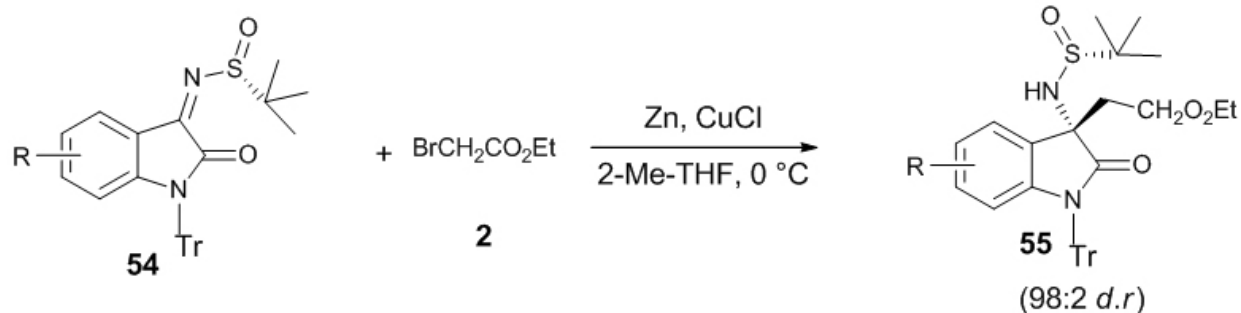


Scheme 13. Asymmetric Reformatsky reaction in presence of molecular sieves.

Rhodium enolate based Reformatsky reaction in presence of Wilkinson catalyst and Zn has also been developed [31]. Here, the reaction was conducted on α,β -unsaturated esters **49** and various imines like N-benzyl substituted imines **50** and N-*para*-methoxy phenyl imines



A highly efficient diastereoselective asymmetric Reformatsky reaction between isatin-derived chiral N-sulfinyl ketimines **54** and ethyl bromoacetate **2** in presence of the solvent 2-methyltetrahydrofuran and mediated by Zn-CuCl has been developed in which optically pure 2-oxindolonyl $\beta,3$ -amino acid ester **55** was obtained with excellent diastereoselectivity (98:2 *d.r*) (Scheme 15) [32].



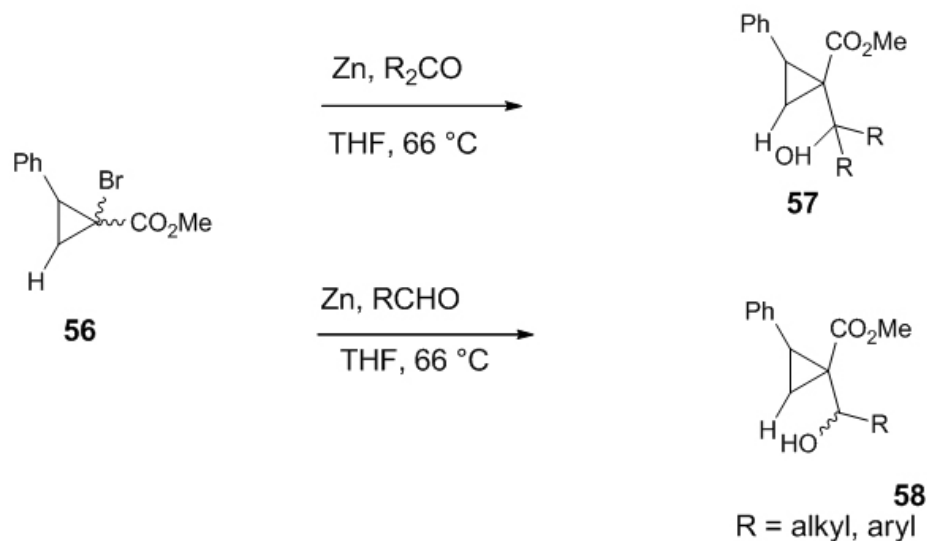
Reformatsky reaction involving chiral auxiliary on cyclopropane ring

Nishii *et al.* developed a Reformatsky reaction between α -bromocyclopropanecarboxylate **56** and ketones, in presence of zinc affording *trans* adducts **57**

51 resulting in *syn* β -lactam **52** and **53** as the products (Scheme 14). Both electron withdrawing and electron donating groups in the benzene ring produced the *syn* β -lactams in better yields. This method can be used for the one-pot synthesis of spiro β -lactams from ketimines.

The reaction was performed with different ketimines and concluded that both electrons withdrawing and electron donating groups in the benzene ring of the ketimine gave rise to better yield and excellent diastereoselectivity. The β -amino acid so obtained was used for the synthesis of gastrin/cholecystokinin-B receptor antagonist AG-041R.

(99:1 *d.r*) in high yield [33]. When the same reaction was carried out in presence of aldehydes, the product **58** was obtained with excellent *trans* selectivity at the α -position (99:1 *d.r*) and moderate diastereoselectivity at the β -position (62:38 *d.r*) (Scheme 16).



Scheme 16. Synthesis of *trans* adducts from α -bromocyclopropanecarboxylates by Reformatsky reaction.

APPLICATIONS OF REFORMATSKY REACTION IN THE TOTAL SYNTHESIS OF NATURAL PRODUCTS

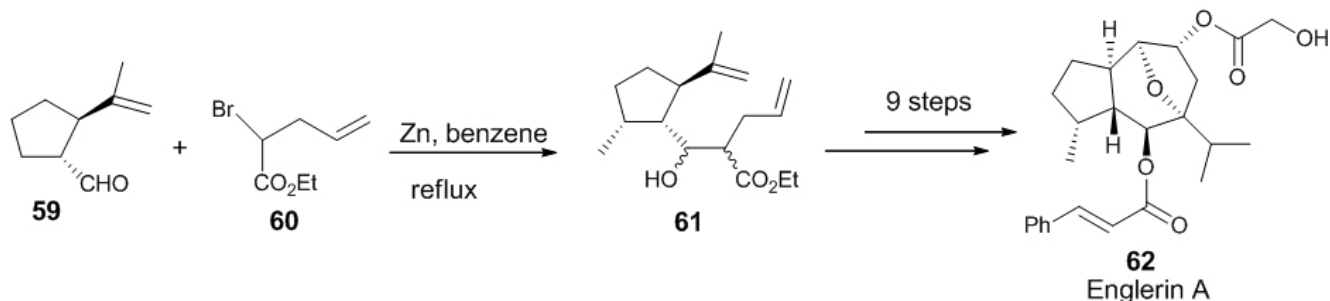
Reformatsky reaction has been applied in a large number of total synthesis of natural products. We have categorized the reactions based on the metal complex used.

Zinc-mediated Reformatsky reaction

Different Zn complexes will show different reactivities, for example, the weak nucleophilic Me_2Zn was found to be less reactive than Et_2Zn [34]. Zinc reacts slowly with halo compound due to the formation of the oxide coating on Zinc. This problem was solved to some

extent by using activated Zn like Rieke-Zn [35] or Cu-Zn alloy [36]. Later, Knochel developed a practical method for the activation of Zn. It was reported that for the synthesis of β -hydroxyesters, ZnCl_2 or SnCl_2 reduced with sodium in liquid ammonia was used. Some additives in combination with zinc are also used for this reaction which includes tantalum, vanadium binuclear complex, Hg_2Cl_2 , triethyl boron, cerium (III) salts, etc.

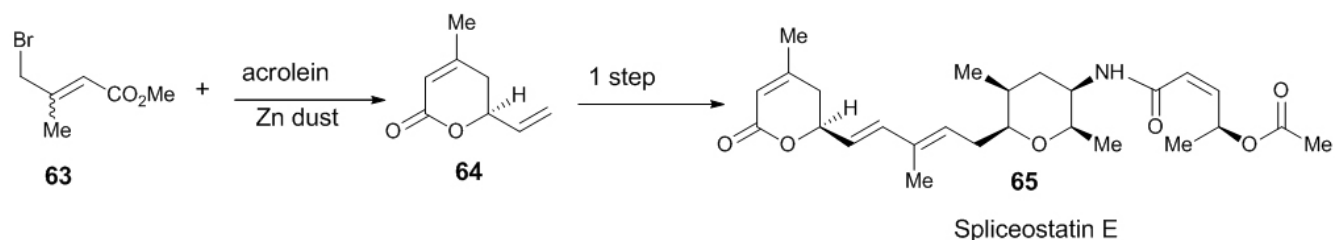
A short synthetic route for the natural product, (-)-Englerin A (active against renal cancer cell lines) **62** was reported with 12 steps in 16% yield [37]. The first step in the construction of the hydroazulene framework was a zinc-mediated reaction between photocitral A **59** and bromoester **60** under mild conditions, affording the product **61** in good yield as a diastereomeric mixture (Scheme 17).



Scheme 17. Zinc-mediated Reformatsky reaction for the total synthesis of (-)-Englerin A.

Ghosh *et al.* performed zinc-mediated reaction for the formation of racemic dihydropyranone from methyl-3-bromomethylcrotonate **63** and acrolein as one of the steps in the synthesis of Spliceostatin E **65**, which has potent

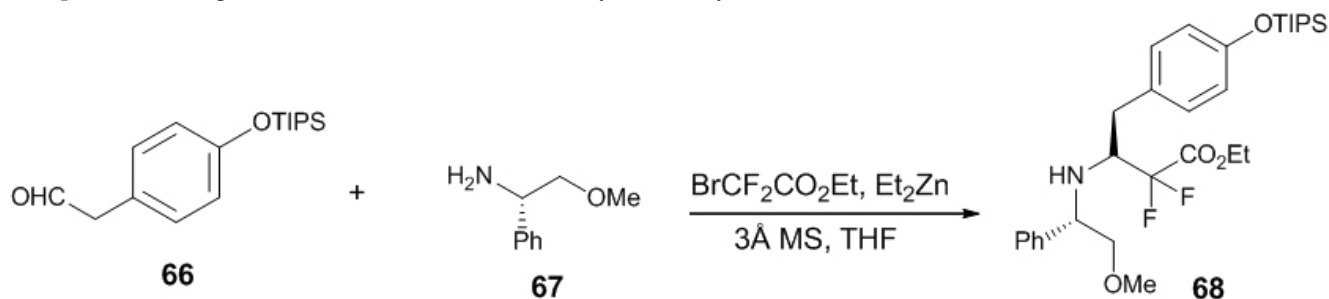
antitumor activity (Scheme 18) [38]. The racemic mixture was resolved by chiral HPLC and the (*S*)-dihydropyranone **64** was used for the synthesis.



Scheme 18. Synthesis of (*S*)-dihydropyranone by Reformatsky reaction for the eventual synthesis of Spliceostatin E.

The synthesis of Tyr^{1-ψ} [(*Z*) CF=CH]-Gly² Leu-enkephalin fluorinated peptidomimetics have been reported using Reformatsky reaction [39]. In the key step, fluorine was incorporated through a diastereoselective Reformatsky-

Honda reaction between TIPS protected phenyl acetaldehyde **66** and chiral amine **67** using molecular sieves, and the desired product **68** was obtained in good yield (Scheme 19).



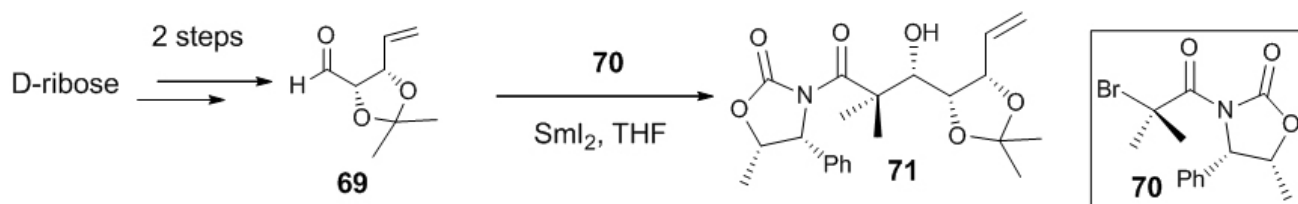
Scheme 19. Incorporation of fluorine through Reformatsky-Honda reaction.

Samarium-mediated Reformatsky reaction

High diastereoselective products can be obtained by using a one-electron reducing agent, Samarium diiodide, due to its moderate oxidation potential and high oxophilicity. Samarium-mediated Reformatsky reaction proceeds through an intramolecular reaction yielding medium to largely sized carbocycles [1(b)]. Metallic Samarium can be used in combination with Cadmium (II) chloride or Bismuth (III) Chloride to produce β-hydroxy

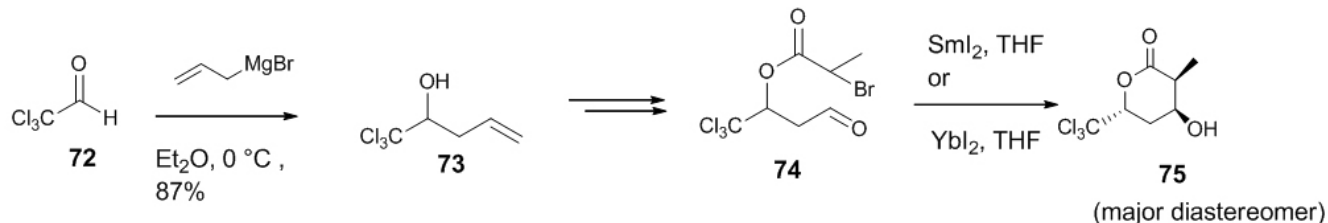
ketones in moderate to good yields from α-bromoacetophenone and aldehydes in THF-H₂O.

The key step for the synthesis of the eastern fragment of PI-3- a jatrophone diterpene used in the treatment of cancerous conditions, swellings and warts was a diastereoselective SmI₂-mediated Reformatsky reaction [40]. Here, D-ribose was converted to an unsaturated aldehyde **69** which was further reacted with bromoacyl oxazolidinone **70** to afford the oxazolidinone product **71** as a single diastereomer with *S*-configured hydroxyl group (Scheme 20).



Scheme 20. SmI₂-mediated Reformatsky reaction in the total synthesis of the eastern part of PI-3.

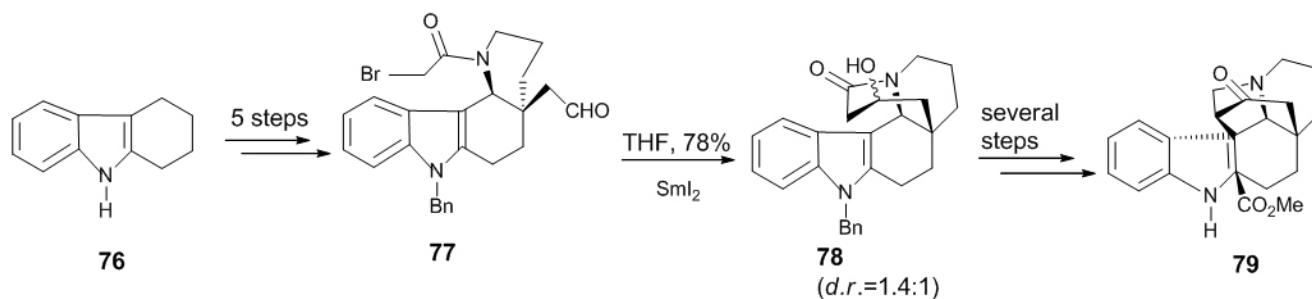
The importance of substituted lactones in the synthesis of natural products encouraged Schulze *et al.* to synthesize substituted valerolactones by the intramolecular SmI_2/YI_2 -mediated Reformatsky reaction [41]. The synthesis involved the preparation of intermediate **73** from allyl magnesium bromide and chloral **72**, which was



Scheme 21. Synthesis of β -hydroxy- δ -trichloromethyl- δ -valerolactone by Reformatsky reaction.

An indole alkaloid, Methyl N-decarbomethoxychanofruticosinate **79**, used in the treatment of pharyngitis, tonsillitis, rheumatoid arthritis, etc., has been synthesized using Reformatsky reaction [42]. The commercially available 1, 2, 3, 4-tetrahydro-4-

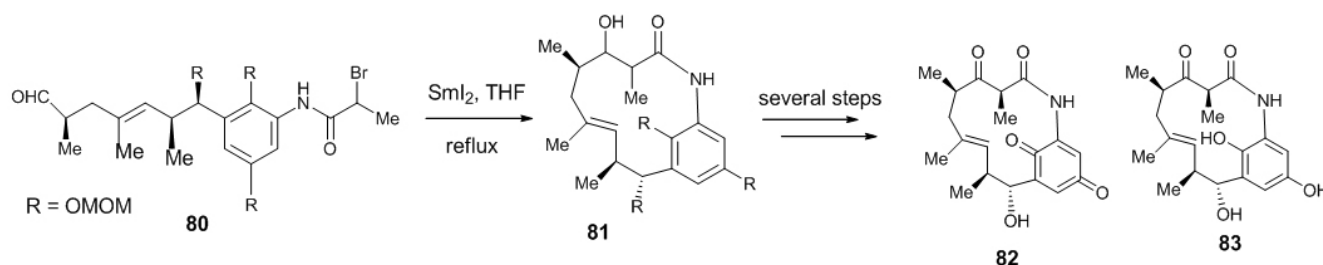
oxocarbazole **76** was transformed to aldehyde **77**. In order to create a seven-membered ring, an intramolecular SmI_2 -mediated Reformatsky reaction was performed to give **78**, which underwent several steps to afford the product as a mixture of two diastereomers in good yield (Scheme 22).



Scheme 22. Synthesis of indole alkaloid using SmI_2 -mediated Reformatsky reaction.

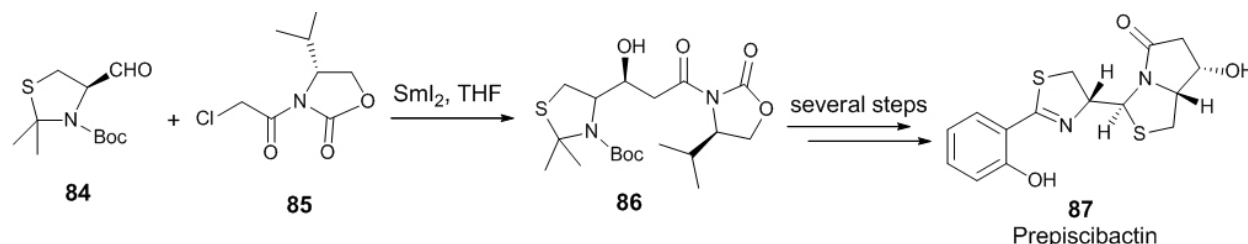
One of the key steps in the total synthesis of Ansamacrolactams (+)-Q-1047H-A-A **82** and (+)-Q-1047H-R-A **83** (class of macrolactam bacterial metabolites, used for the synthesis of some natural products) was the SmI_2 -

mediated intramolecular Reformatsky reaction for the formation of macrolactam ring **81** from the intermediate **80** (Scheme 23) [43].



Scheme 23. SmI_2 -mediated intramolecular Reformatsky reaction for the synthesis of ansamacrolactams.

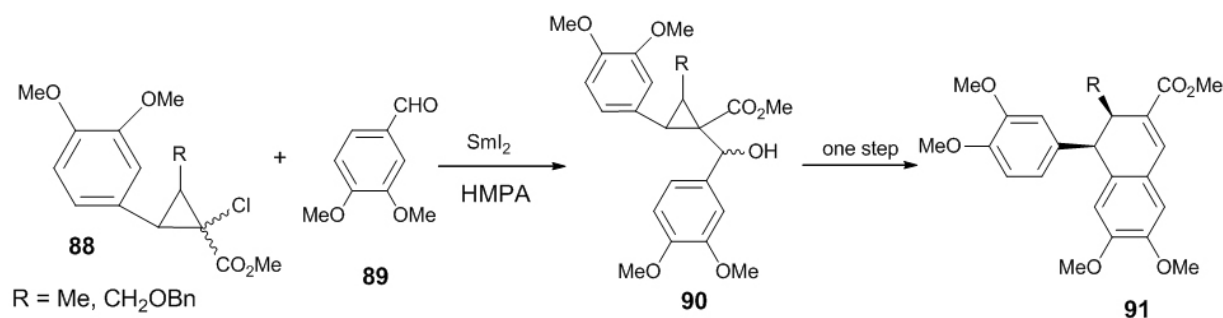
The first total synthesis of Prepiscibactin **87**, the key intermediate for the biosynthesis of piscibactin (siderophore seen in bacterium *Photobacterium damsela*) was realized by SmI_2 -mediated Reformatsky reaction [44].



Scheme 24. Total synthesis of Prepiscibactin utilizing SmI_2 -mediated Reformatsky reaction.

Nishii *et al.* reported the total synthesis of Cyclogalgravin and its dicarboxyl analog due to its biological importance such as antineoplastic cytotoxicity and apoptosis-inducing activities [45]. One of the key steps in this synthesis was a SmI_2 -mediated Reformatsky

reaction between α -chloroester **88** and veratraldehyde **89**. An excellent *trans* selective product **90** (*trans/cis* = 99/1) was obtained in good yields which on ring expansion gave Cyclogalgravin **91** (Scheme 25).

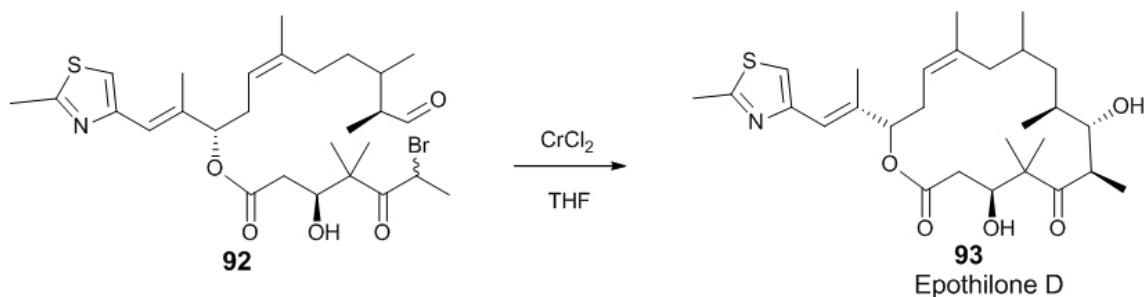


Scheme 25. Total synthesis of Cyclogalgravin and its dicarboxyl analog using SmI_2 -mediated Reformatsky reaction.

Chromium-mediated Reformatsky reaction

Chromium is rarely used in the Reformatsky reaction. A chromium-mediated Reformatsky reaction is a powerful tool for the formation of the asymmetric C-C bond due to its unusual stereospecificity and chemoselectivity in macroaldolization. In this reaction, when aldehydes are

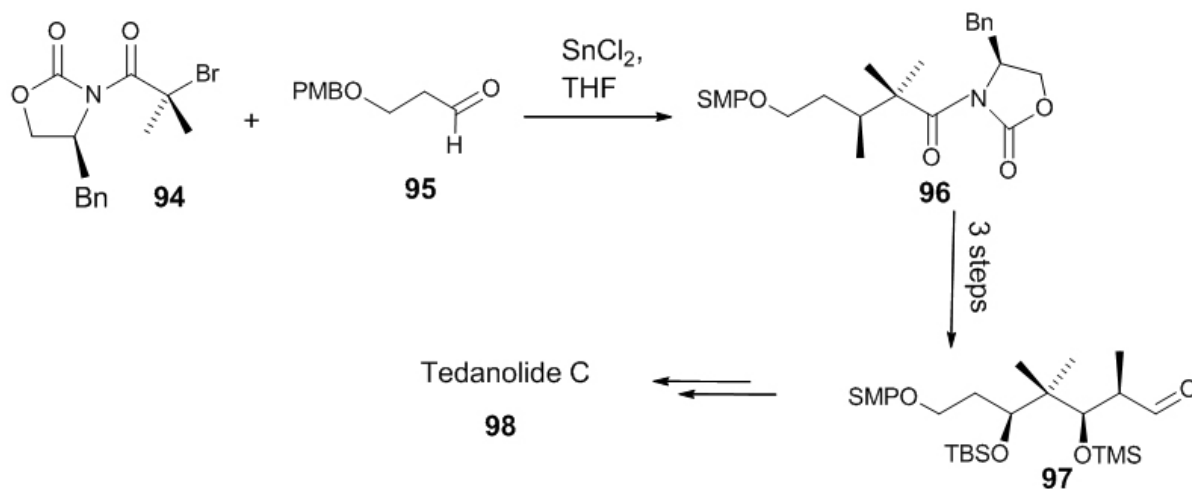
used as electrophiles, diastereoselective *syn* products are obtained whereas *anti* products are obtained with chiral auxiliaries. A chromium-mediated Reformatsky reaction was used in the total synthesis of Epothilone D **93** [46]. The required *syn* selective C6-C7 bond of this compound was prepared from the precursor **92** in presence of CrCl_2 . (Scheme 26).



Scheme 26. Chromium-mediated Reformatsky reaction used in the synthesis of Epothilone D.

Tin-mediated Reformatsky reaction

Tedanolide C **98** is a marine natural product isolated from a Papua New Guinea sponge which showed excellent potency towards colorectal cancer cell line and is also a protein synthesis inhibitor. The starting material **97**



Scheme 27. Tin-mediated Reformatsky reaction for the synthesis of Tedanolide C.

CONCLUSIONS

Asymmetric Reformatsky reaction facilitates the formation of a C-C bond between a halide and an aldehyde which leads to the formation of a chiral alcohol. This review covers the asymmetric Reformatsky reaction using different transition metals and various chiral ligands. Both enantioselective and diastereoselective Reformatsky reactions are discussed. In addition to Zinc, other transition metals such as Sm-, Sn-, and Cr-mediated Reformatsky reactions have also evolved, affording the products in excellent yields due to their improved reactivity. The incorporation of fluorine into β -lactam has become a major research interest due to the enhanced antibiotic activity. Much progress has been observed in the intramolecular Reformatsky reaction using SmI₂, which was also included in this review.

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Oxidation Behavior of Permanganate Functions Supported on 4-Vinylpyridine Based Polymers

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Abstract: Divinylbenzene (DVB), ethylene glycol dimethacrylate (EGDMA), and N,N'-methylene-bis-acrylamide (NNMBA)-cross-linked poly[4-vinyl(pyridine-co-acrylamide)] polymers (5-20 mol%) were synthesized and permanganate functionalities were incorporated. The polymers were characterized by their characteristic IR peaks. With the nature of crosslinking, the permanganate capacity of the polymers varies in the order: NNMBA- > DVB- > EGDMA-cross-linked system. The oxidizing ability of various permanganates was investigated with primary, secondary alcohols and aldehydes. Primary and secondary alcohols were oxidized to aldehydes plus ketones. Polymer-supported permanganate reagent accomplished selective oxidation of primary alcohols to aldehyde. Oxidation of aldehydes resulted in the corresponding carboxylic acid. The influence of different reactions such as the nature of the solvent, molar concentration of the reagent, temperature and time on the rate of the reaction were investigated using the oxidation of benzoin to benzil as the model reaction.

Key Words: Polymer-supported reagents, Oxidation, 4-Vinylpyridine, acrylamide

INTRODUCTION

The chemistry of polymer-supported reagents has been an active field of research in recent years [1-3]. A polymeric-supported organic reagent can provide an effective alternative to its low molecular weight counterpart. Nowadays, the environmental and economic pressure is forcing the chemical community to search for more efficient ways of performing chemical transformations in a single operation by reusable catalysts without toxic and costly reagents. Reactions using solid-supported reagents and scavengers have created considerable interest among synthetic chemists around the world [4-6].

Major advantages of polymer-supported reagents are the possibility of reducing the potential of pollution stemming from chemical research and industrial chemical processes, potential for automated synthesis and testing using high-throughput screens, simplified product isolation, and recovery, the use of excess reagent to drive reactions to completion and can be easily recycled and reused [7-9]. Due to several advantages in terms of yield,

purity, and selectivity, supported reagents have been used under solvent-free conditions for the synthesis of various important synthetic intermediates. Another attractive feature is that the polymeric reagent is non-volatile and the reduced volatility, increases the shelf life of the reagent so that, the supported analog can be stored under ordinary conditions for a long period. Polymer-supported reagents have been developed for use in functional group transformations, in peptide synthesis, as stoichiometric reagents, and catalysts, in ion-exchange chromatography and immobilization of enzymes [10-13].

Oxidation is an important class of reactions from both the industrial and academic points of view. Polymer-supported oxidizing agents have attracted the attention of many scientists due to their various unique features and have been used for a variety of industrial reactions [14-17]. Polymer-supported hypobromites, iodates, periodates, and permanganate were reported as efficient recyclable oxidizing agents [18, 19].

In organic chemistry, potassium permanganate is widely used as one of the most versatile of the commonly used oxidants. Oxidation of organic substrates with low molecular weight potassium permanganate poses many

problems. Most organic solvents are either readily oxidized by potassium permanganate or they do not dissolve the permanganate ion in appreciable amounts. These difficulties led to the use of heterogeneous conditions for permanganate oxidation

The present paper is based on the development of a new class of various cross-linked poly(4-vinylpyridine) based polymers, their synthetic applications and studies on the role of the polymer support on the reactivity of this reagent. The main advantage of cross-linked polymer-supported solid-phase organic reagents over their monomeric counterparts in organic synthesis is the ease of separation of the excess reagent from the desired reaction product.

EXPERIMENTAL

Materials

4-vinylpyridine, acrylamide, ethylene glycol dimethacrylate, N, N'-methylene-bis-acrylamide, and divinylbenzene were commercial products availed from Aldrich Chemical Company, USA. All low molecular weight compounds were commercially available samples and were purified by distillation under reduced pressure immediately before use or recrystallization unless otherwise stated. IR spectra were recorded on Shimadzu FT-IR-8400 S spectrophotometer. UV spectra were recorded on Shimadzu UV-2450 spectrophotometer. Melting points were measured using Buchi-530 melting point apparatus.

Methods

Preparation of 5-20 mol% DVB-, EGDMA- and NNMBA-cross-linked poly[4-vinyl(pyridine-co-acrylamide)s] based permanganates

For the preparation of 5-20 mol% DVB-, EGDMA- and NNMBA-cross-linked poly[4-vinyl(pyridine-co-acrylamide)s], the monomers 4-vinylpyridine (5.1228 ml), acrylamide (3.3763 g) and required an amount of the crosslinking agent was dissolved in methanol (20 ml). The initiator AIBN (100 mg) was added and the mixture heated to 60°C and stirred. Heating and stirring continued for 6 h. It was cooled to room temperature. The product was filtered, washed with water, acetone, dichloromethane and finally with methanol.

Incorporation of permanganate Function into cross-linked polymers: General procedure

To a suspension of the cross-linked polymer (10 g) in water (40 ml), 1N H₂SO₄ (10 ml) was added and stirred for 1 hour. A solution of KMnO₄ (12 g) in water (100 ml) containing 1N H₂SO₄ (10 ml) was added to the suspension with vigorous stirring at 0°C. The mixture was stirred for 1 h at 0°C and 2 h at room temperature. The product was filtered at the pump and washed with water until the filtrate was completely free from permanganate ions. The polymer was then washed with a little methanol and dried in vacuum to yield a dark powder.

Estimation of permanganate in the functionalized polymers: General procedure

A definite amount of (0.1 g) of the functionalized polymer was accurately weighed and suspended in excess of 2N H₂SO₄ (10 ml) for 1 h. A known excess of standard ferrous ammonium sulfate solution was added and stirred until the dark color of the polymer disappeared. The unreacted ferrous ammonium sulfate solution was titrated against the standard KMnO₄ solution. From the titer value, the permanganate equivalent was calculated.

Oxidation reaction using polymer-supported permanganates: General procedure

To a five-fold molar excess of the reagent moistened with water, a solution of the substrate in a suitable solvent (20 ml) was added and stirred at refluxing temperature of the solvent. The progress of the reaction was followed by TLC. After 24 h, the reaction mixture was filtered and the polymer was washed with solvent. The filtrate was dried over anhydrous sodium sulfate and the product was isolated by evaporating the solvent. Oxidation of benzoin to benzil was carried out to find out the dependence of temperature, solvent, molar excess of the polymeric reagent and time duration on oxidation reaction.

Investigation of the effect of the reaction condition on the extent of oxidation

In order to investigate the dependence of various parameters like solvent, molar excess of the reagent, temperature and time on the oxidation by polymer-supported permanganates, benzoin to benzil oxidation was taken as a model reaction.

a. Effect of solvent

To study the effect of various solvents on the oxidation, the oxidation of benzoin to benzil was carried out in solvents of varying polarity. Five-fold molar excess of the reagent was used and oxidations were carried out at refluxing temperature of the solvent. In each case, the extent of oxidation was followed by TLC. The various solvents used were chloroform, cyclohexane, dioxane-water, and dichloromethane.

b. Effect of molar excess of the reagent

A calculated quantity of the reagent (for the appropriate molar excess based on capacity) was weighed and made wet with water added to benzoin (100 mg) dissolved in cyclohexane and refluxed. The extent of oxidation was followed by TLC.

c. Effect of temperature

Five-fold molar excess of the reagent was added to benzoin (100 mg) in cyclohexane at room temperature (30°C) and the extent of oxidation was followed by TLC. The same experiment was repeated at temperature 50°C and refluxing temperature of the solvent.

d. Effect of time

Five-fold molar excess of the reagent was added to benzoin (100 mg) in cyclohexane and refluxed. The extent of oxidation was followed by TLC at regular intervals of time.

Recycling and Reuse

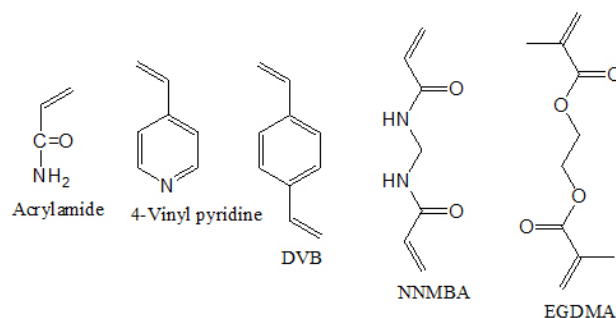
The spent polymeric reagent was stirred for 2 h with 2 N (50 ml) of ferrous ammonium sulfate in the presence of 2 N H₂SO₄ (20 ml) to remove all oxides of manganese. The polymer was filtered, washed with water and methanol, and dried. The regenerated polymer was functionalized as mentioned earlier. The recycled polymer was used for oxidations as mentioned earlier.

RESULTS & DISCUSSION

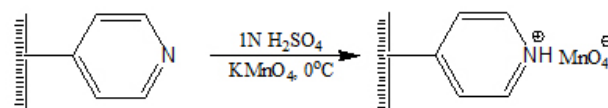
In the present study oxidation behavior of permanganate functions supported on cross-linked poly(4-vinylpyridine-co-acrylamide) are detailed along with the dependence of oxidation behavior on the nature of polymer support and nature of crosslinking.

Preparation of cross-linked poly[4-vinyl(pyridine-co-acrylamide)]-type supports

Cross-linked poly[4-vinyl(pyridine-co-acrylamide)] based supports were prepared by free radical initialized solution polymerization using AIBN as the initiator. The monomers and crosslinking agents used are given in Scheme 1. The polymer was then functionalized with permanganate to afford a dark powder (Scheme 2).



Scheme 1. Monomers and crosslinking agents.



Scheme 2. Incorporation of permanganate function into poly(4-vinylpyridine) based polymers.

The permanganate capacity of the new functionalized polymer was determined by excess-back titration with standard ferrous ammonium sulfate solution in the presence of dilute sulphuric acid. Permanganate capacities of various cross-linked poly(4-vinylpyridine) based polymers are shown in Table 1. The FT-IR spectrum of the permanganate incorporated polymers showed intense absorptions at 420, 790, 820 and 900 cm⁻¹. The absorption at 900 cm⁻¹ is characteristic of the permanganate ion.

Table 1. Permanganate capacities of various cross-linked poly(4-vinylpyridine) based polymers.

Crosslink density (%)	Permanganate capacity (mequ/g)		
	DVB	NNMBA	EGDMA
5	1.97	2.44	1.93
10	1.85	2.05	1.74
15	1.81	1.84	1.67
20	1.75	1.80	1.63

OXIDATIONS REACTIONS USING POLY(4-VINYLPYRIDINE-CO-ACRYLAMIDE) – PERMANGANATE POLYMERS

The oxidizing ability of poly(4-vinylpyridine-co-acrylamide)-permanganate polymers was investigated in detail for its ability to oxidize alcohols to carbonyl compounds. Primary alcohols were converted to aldehydes, secondary alcohols to ketones and aldehydes to corresponding acids. The details of oxidation reactions of different alcohols and aldehydes with the polymer are

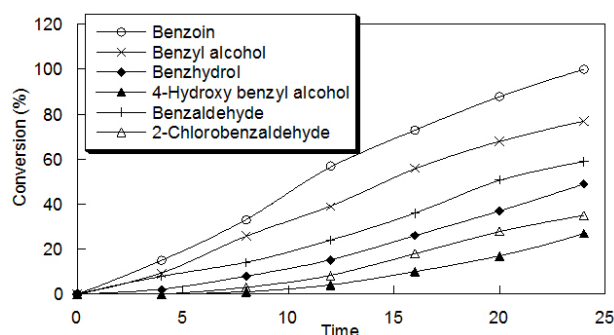


Fig. 1. Oxidation of different alcohols and aldehydes with 5 mol% DVB-cross-linked poly[4-vinyl(pyridine-co-acrylamide)]-permanganate.

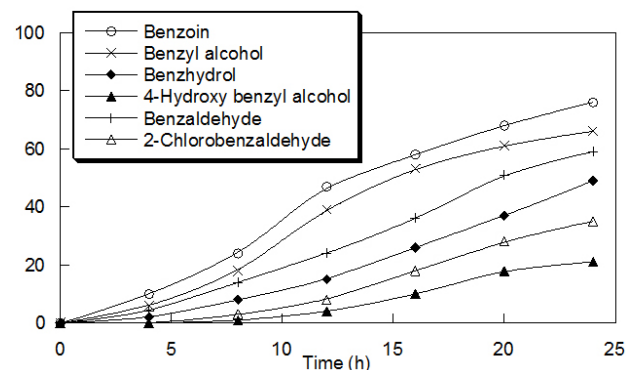


Fig. 2. Oxidation of different alcohols and aldehydes with 5 mol% NNMBA-cross-linked poly[4-vinyl(pyridine-co-acrylamide)]-permanganate.

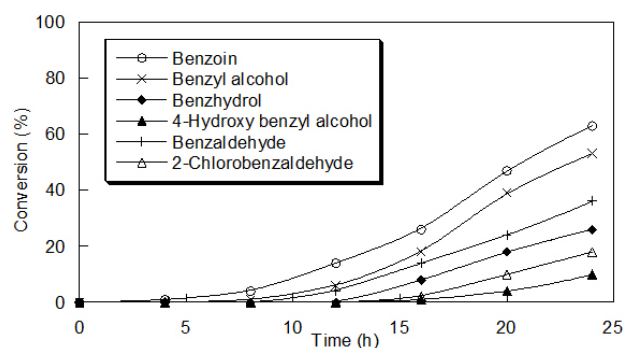


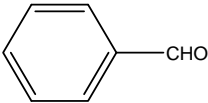
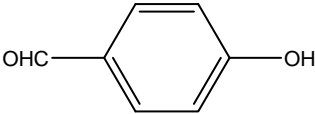
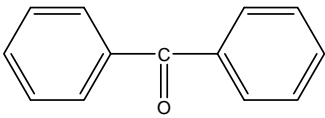
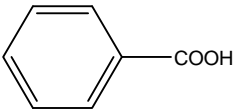
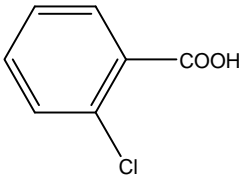
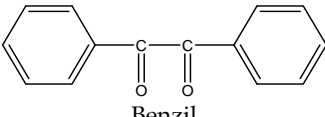
Fig. 3. Oxidation of different alcohols and aldehydes with 5 mol% EGDMA-cross-linked poly[4-vinyl(pyridine-co-acrylamide)]-permanganate.

In order to investigate the dependence of various parameters like solvent, temperature, and molar excess of the reagent on oxidation benzoin to benzil was carried out as a model reaction.

EFFECT OF VARIOUS PARAMETERS ON OXIDATION REACTION

given in Table 2. The oxidation conditions involve stirring of the reaction mixture in cyclohexane and refluxed for the indicated period. In all these cases of oxidation wetting of the polymer with water was found to be necessary. The progress of the reaction was followed by TLC. After 24 h, the reaction mixture was filtered and the polymer was washed with solvent. The filtrate was dried over anhydrous sodium sulfate and the product was isolated by evaporating the solvent. The products were characterized by melting point/boiling point, IR, and UV-vis. Spectra.

Table 2. Characterization of oxidation products.

Sl. No.	Product	UV-vis. (nm)	IR (cm ⁻¹)	M.P/B.P (°C)
1	 Benzaldehyde	280	1700 (-CHO)	178
2	 4-Hydroxybenzaldehyde	275	1660 (-CHO)	118
3	 Benzophenone	260	1670 (-CO)	48
4	 Benzoic acid	260	2800(-OH) 1690 (-CO)	122
5	 2-Chlorobenzoic acid	265	2900(-OH) 1705 (-CO)	139
6	 Benzil	277	1650 (-CO)	94.5

EFFECT OF SOLVENT

The oxidation reactions were investigated in solvents of different polarity like dioxane, tetrahydrofuran, chloroform, cyclohexane, dichloromethane and dioxane-water mixture (1:1). Because of the hydrophilic nature of the acrylamide, the reagent could not swell in any other solvent than dioxane: water mixture (1:1). Hence all oxidations were carried out in dioxane: water mixture.

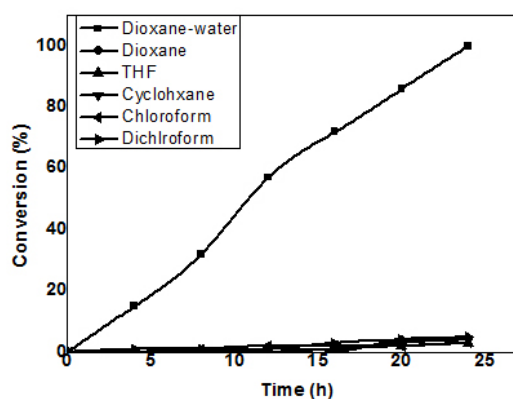


Fig. 4. Effect of solvent on the oxidation of benzoin by 5 mol% DVB-cross-linked poly[4-vinyl(pyridine-co-acrylamide)]-permanganate.

EFFECT OF MOLAR EXCESS OF REAGENT

Oxidation of benzoin was carried out by varying the molar excess of poly[4-vinyl(pyridine-co-acrylamide)]-permanganate, keeping other experimental conditions the same. The oxidation reactions of 1 mmol of benzoin with a different molar excess of polymeric reagent are studied. With increasing substrate to reagent ratio, the extent of oxidation increased. The results are presented in Figure 5.

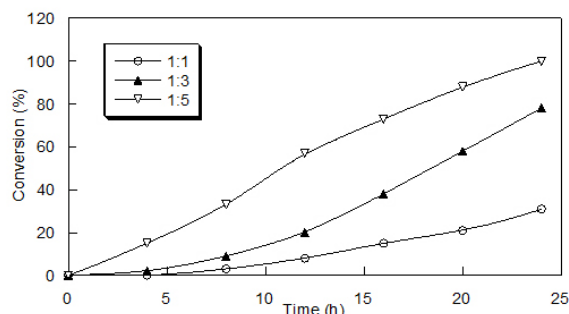


Fig. 5. Effect of molar excess of reagent on the oxidation of benzoin by 5 mol% DVB-cross-linked poly[4-vinyl(pyridine-co-acrylamide)]-permanganate.

Effect of temperature

Temperature is one of the major factors influencing the rate of the polymer-supported oxidation reaction. To study the effect of temperature on the reactivity of poly[4-vinyl(pyridine-co-acrylamide)]-permanganate, benzoin to benzil oxidation was conducted at various temperatures ranging from room temperature to 90°C. Figure 6 shows the extent of conversion increased as the temperature increased.

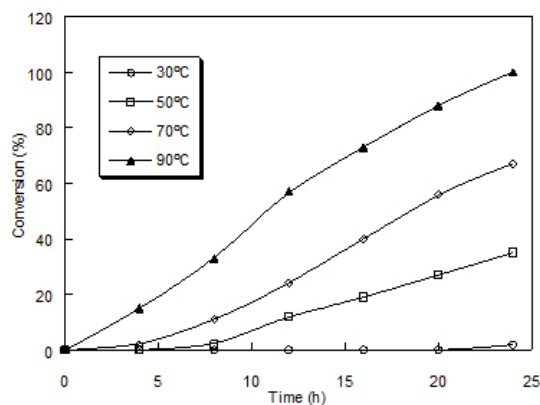


Fig. 6. Effect of temperature on oxidation of benzoin by 5 mol% DVB-cross-linked poly[4-vinyl(pyridine-co-acrylamide)]-permanganate.

Effect of the nature and degree of crosslinking on the reactivity of various cross-linked poly[4-vinyl(pyridine-co-acrylamide)]-permanganates

In order to investigate the oxidizing ability of permanganate functions supported on 5–20 mol% DVB-, NNMBA- and EGDMA-cross-linked poly[4-vinyl(pyridine-co-acrylamide)], oxidation of benzoin to benzil, was selected as a model reaction. As shown in Figure 7, in all cases the 5 mol% cross-linked system has high reactivity and decreased further with increasing crosslinking. The observation of the decrease of the rate of oxidation of functionalized polymer with an increase in crosslink density is due to the decreased availability of the reactive sites buried within the crosslinks. For an effective oxidation to occur, the functional group should be more accessible to the reagent in the solvent phase. As the degree of crosslinking increases, the availability of the reactive moiety decreases even in the presence of good solvents.

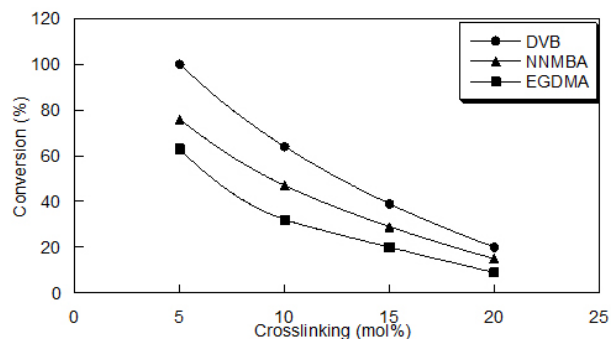


Fig. 7. Effect of the nature and degree of crosslinking on oxidation of benzoin by DVB-, NNMBA- and EGDMA-cross-linked poly[4-vinyl(pyridine-co-acrylamide)]-permanganates.

The nature of the crosslinking agent does influence the kinetics and extent of functional group conversions in polymer-aided reactions. Thus in the present case, the reactivity decreased in the order: DVB- > NNMBA- > EGDMA-cross-linked system. Even though the permanganate capacity of the NNMBA-cross-linked system is high its low reactivity than the DVB-cross-linked system is due to the non-compatibility between the hydrophilic support and the hydrophobic substrate in the dioxin-water medium. The oxidizing ability of permanganate functions supported poly (4-vinylpyridine) is higher than the corresponding poly[4-vinyl(pyridine-

co-acrylamide)]. This is due to the extreme hydrophilicity and inter-chain hydrogen bonding of the amide groups present in the copolymer. This hinders the swelling of the polymer matrix in organic solvents from hydrophilic-hydrophobic imbalance resulting in lower reactivity. The dioxane-water mixture, the best solvent for the copolymer is not a favorable medium for oxidation.

RECYCLING & REUSE OF THE SPENT REAGENT

An important advantage of the polymeric reagent over their low molecular weight counterpart is that the polymer bound reagent could be regenerated and reused. The regeneration was achieved by removing all the manganese oxides by treating with acidic ferrous ammonium sulfate solution in Table 3. The recycling process was repeated several times without appreciable loss of activity, and the resulting polymer could be used for subsequent oxidation reactions.

Table 3. Regeneration of various cross-linked poly(4-vinylpyridine) based permanganates.

No. of Cycles	Permanganate capacity (mequ/g)		
	DVB	NNMBA	EGDMA
1	1.97	2.44	1.93
2	1.97	2.44	1.93
3	1.95	2.44	1.91
4	1.93	2.43	1.90
5	1.92	2.43	1.90

CONCLUSIONS

Permanganate functions supported on cross-linked poly(4-vinylpyridine-co-acrylamide) polymers could oxidize primary and secondary alcohols to aldehydes and ketones, and aldehydes to corresponding carboxylic acids. Among the various cross-linked systems, the oxidation capacity decreased in the order: DVB > NNMBA > EGDMA. Even though the permanganate capacity of the NNMBA-cross-linked system is high, it has a lower reactivity than the DVB-cross-linked system is due to the non-compatibility between the hydrophilic support and the hydrophobic substrate in the dioxane-water medium. The observation of the decrease in the rate of oxidation of functionalized polymer with an increase in crosslink density is due to the decreased availability of the reactive

sites within the crosslinks. The various reaction parameters already as noted were investigated. The suitable solvent for this polymer is dioxane: water (1:1) due to the hydrophilic nature of the acrylamide. The yield of the product increases with increasing temperature. Polymeric reagent could be used in excess to get a good yield without causing any separation problem and over oxidation products. It is observed that a high molar excess of polymeric reagent resulted in an enhanced rate of reaction.

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CIVIC CHEMISTRY: HELPING COMMUNITIES ADDRESS CHALLENGES

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Introduction

In this brief, I argue that chemists can play an important role in democratic societies by getting more involved with citizen science and especially civic science. From the perspectives of both science and democratic self-rule, we live in interesting times. This is especially true for chemists and chemical educators who are interested in scientific literacy and the public perception of science. Many scientists have little appetite for advocacy, but considering the global lack of chemical expertise, helping communities address their chemistry-related concerns and challenges, especially underserved communities, is a great way for chemists to become more productively engaged. Improving the understanding, engagement, and well-being of our fellow citizens is difficult to argue with, as is helping other species and ecosystems, so every chemist should be aware of the benefits of civic science.

Abstract: This brief argues that chemists can play an important role in democratic societies by getting more involved with citizen science and especially civic science. Many scientists have little appetite for advocacy, but helping communities address their chemistry-related concerns and challenges, especially underserved communities, is a great way for chemists to become productively engaged. Civic science, which arises from local community concerns rather than academic research, offers an excellent framework to help. In addition to providing expertise that can directly improve the health or peace-of-mind of local communities, there are potentially beneficial synergies with policy and education that can occur when chemists become more involved in civic science. The implications for chemistry education are particularly noteworthy, as civic science assignments and projects could play an important role in connecting departments to their communities and attracting and engaging a broader range of students to the field.

Key Words: Civic chemistry, community, citizen science.

Challenges

Unprecedented increases in global population, consumption, and urbanization have led to a range of environmental challenges at scales from local to global. As they often involve incomplete data and value conflicts, many of these challenges are described as "wicked" problems, which democratic societies are not always able to effectively address [1]. One of the ongoing issues associated with such challenges is an unbalanced or unfair distribution of the benefits and harms our lifestyles or technologies create. It has been argued here [2] and elsewhere that mutual comprehension and trust are critical to forming effective bridges from science to the

community. Unfortunately, we are in the midst of an era of ideological polarization, mistrust of science and other institutions, and an apparent inability to effectively deliberate when politically contentious issues involve science, as so many do. For better or worse, chemistry does not have celebrity experts that the public trusts. As a result, when people become concerned with the potential effects of pollutants or wonder if certain chemicals are to blame for changes in their health or local environments, there are no obvious, reliable sources of information for them to turn to.

Solutions

It's worthwhile to consider the range of ways that the public can engage with science. I find it helpful to consider civic-citizen science as a spectrum. In general, citizen science projects are top down; that is, designed by and for researchers. These can often be considered as a kind of crowd-funded science [3], and examples abound, from the venerable Christmas bird count to game-like Foldit (<https://fold.it/portal/>) for analyzing protein structure. Civic science, conversely, arises not from scientists, but rather from community concerns and issues. Such concerns may not be on the radar of government and academic researchers, or can even conflict with them, as the messy and shameful Flint water crisis illustrated. Not surprisingly, pollution often disproportionately affects communities that lack political or economic power, making the lead pollution in Flint a worthwhile case study for chemists interested in public health regulation or environmental justice [4]. Both civic and citizen science research projects are growing, and advances in sensor, computing, and communications technology are making air and water monitoring increasingly practical for home and small community use. For example, a basic AirPi weather station (airpi.es/) is inexpensive, and associated modules can record and upload information including carbon monoxide, nitrogen dioxide, and smoke levels. Civic and citizen science projects will continue to see an explosion of available data from such sensors, in a range of environments.

Considering civic-citizen science as a spectrum – rather than a dichotomy – is more helpful, and community concerns can be guided by the interests and expertise of local scientists as well as vice versa. For many members of the public worldwide, chemicals are invisible and often worrisome, and chemists can help relieve concerns or focus them on the most relevant risks. For instance, one research project incorporated soil, water, and vegetable samples from trained local residents who were concerned about gardening near a mining site [5]. Research like this can involve reciprocal dialogue between researchers and the community; in a follow-up article, the researchers described the process as "co-created citizen science", and highlighted the reciprocal links throughout the process [6]. Whatever it is called, and wherever it ends up on the civic-citizen science spectrum, similar research could benefit many communities worldwide. To help increase the quantity and quality of such partnerships, chemists can take the initiative to seek out community networks and leaders. Many individuals may be hesitant to approach academics, or unaware of how to. Chemists can offer to help examine and address local issues of concern, and provide expertise, advice, direction, training, and even equipment or lab time. Such work can provide direct, immediate help, and is especially relevant for disadvantaged or neglected communities that lack the resources to hire experts themselves.

Synergies

In addition to providing expertise that can directly improve the health or peace-of-mind of local communities, there are some potentially beneficial synergies with policy and education that can occur when more chemists become involved in civic science. It certainly doesn't hurt to have chemists (and other scientists) become more democratically engaged. This does not imply advocacy for specific issues; rather, it involves working within our social systems and government, particularly at the local level, to help individuals and communities make more

sound decisions. Chemists have the potential to help at the individual, institutional, and political levels. For instance, you might join citizen science projects such as Florida Lakewatch (<http://lakewatch.ifas.ufl.edu>), which has been using volunteers to monitor water quality for over 30 years. In addition to simply collecting samples, chemists can help with skills, education, expertise, and even provide role models for others. You might deliberately engage with your community leaders or local politicians, offering to help with issues they feel are important. Or you might encourage your business, association, or society to become more engaged in similar ways.

Implications for Chemistry Education

One of the reasons we need more chemists to become involved with civic science is that our educational systems, here and abroad, have not done a good job of producing graduates with the range of qualities needed to effectively recognize and address the considerable environmental challenges they will face [7]. Tweaking the curriculum to cover basic socioenvironmental literacy, rather than more focused career training, is part of the ongoing debate of the merits of a liberal arts education. Incorporating civic science projects into chemistry education will help students see the real-world implications of their studies and expand their view of what careers in chemistry might entail. Chemistry programs often make the links to industry explicit; indeed, the notion of undergraduate education becoming more akin to job training is at least as controversial now as it has been in the past. Yet many programs are not effective at making the links to policy and community as relevant and do little to help students build interest and skills in these areas. Chemistry education programs could do a lot more by including civic science projects as service learning [8] or as part of ongoing partnerships with local community groups. Once again, there are many underserved communities that could benefit greatly from well-designed, long-term educational partnerships with chemistry programs. Such programs could draw a more diverse group of students into the field.

None of these are magical solutions. As popular as superheroes are these days, you probably won't be donning a lab coat and swooping in to save lives (although don't rule it out!). We shouldn't need to be superheroes [9]. Good governance, especially when wicked problems must be addressed, is not easy. It takes real work, the best information available, careful deliberation, and ultimately compromises by people whose values often conflict. One of the systemic problems of democracies and other forms of self-rule is a mutual distrust between citizens and important societal institutions [6]. By engaging more with civic science projects, by encouraging policy that facilitates such engagement, and by incorporating civic science into chemistry education, chemists can help improve these relations, and therefore the resilience and adaptability of the communities and societies they live in. These are important benefits in a changing world. By leveraging citizen and especially civic science, chemists can make a real difference.

Biography:

Bryan Nichols is an assistant professor of science and environmental education, a behavioral ecologist, and a science journalist.

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RECOGNIZING & TEACHING THE SCIENTIFIC METHOD ACROSS ALL FIELDS, NOT JUST THE NATURAL SCIENCES

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Introduction

Science fairs have long been used as vehicles to spark students' interest in science, providing an opportunity to apply classroom concepts to a real-world project. In the spring of 2017, my son Hart participated in his school's STEM fair. Leading up to the fair, the school made it clear (with both its use of the STEM label and written instructions on how to participate) that student-led projects applying science principles were encouraged across a variety of areas. Hart describes his project below.

"In 4th grade, I participated in my school's STEM fair. For my STEM project, I chose to find out which gum had the longest-lasting flavor. The reason I chose this question was because I wanted to know which brand could actually claim "we have the longest-lasting gum." There were three brands in my project: Orbit mint gum, Extra watermelon gum, and Trident bubble gum.

To collect data, I tested 12 people, asking each the same question about how long the initial burst of flavor lasted. I gave them each of the three pieces of gum to chew, timed them with a stopwatch, and recorded the times on a paper. After collecting all the timing data, I made some graphs of my results and presented them on a big poster board. I learned nobody really liked Trident best and it had the quickest times of all of them. The winner was Orbit, the mint gum.

Also, my dad and I did a display of a chewed gum timeline. We chewed gum in 15-second intervals and put the gum on the poster board. We wanted to study the chewed gum and see if it changed in color or appearance when it was being chewed – but it didn't.

If I were to do this experiment again, I would keep all the gum flavors the same. I realized afterwards that maybe many people preferred the mint gum because of the flavor, not the brand. Also, I would hide the brand so people don't know which gum they're chewing."

Abstract: Science education in American schools conflates two disparate definitions of "science," thereby leading students to assume science and related concepts are the exclusive domain of the natural sciences. Ultimately, natural sciences are privileged in schools as real sciences and social sciences are often marginalized with very real consequences. This article argues schools should teach students about the application of scientific methods broadly across fields in both the natural and social worlds around them to see all as legitimate pursuits of knowledge.

Key Words: Science Fair, STEM, Social sciences, Interdisciplinary, Model-based inquiry

Discussion

Being a social scientist, I (Michael) was pleased to learn of Hart's interest in applying scientific inquiry to a squishier question of consumer preferences and I encouraged him in the process. Yet, his project was an outlier in the STEM fair, where most projects focused on magnets, growing plants, or electrical currents. Many students and parents browsing the completed projects in the STEM fair and asking questions of participants also did not immediately see why this question was a science project. Through this experience, it was clear to me that science as currently taught in our schools omits some useful details about the broad pursuit of knowledge.

Schools often conflate two disparate definitions of the word "science" to students' detriment. Courses in elementary and early secondary schools that teach content from the natural sciences are generally referred to

simply as “science” courses. Yet, another definition of “science” refers to systematized knowledge in any domain, which includes a number of fields both inside and outside of the natural sciences.

A common result of using the same word for two different ideas is that students mistakenly assume that “science” and related concepts like the scientific method are the exclusive domain of the natural sciences. Typically, courses in social science fields are not introduced until late in secondary grades or later and, consequently, many students are likely missing formal instruction in broader conceptualizations of science. This late or missing exposure to other scientific fields outside of the natural sciences is certainly related (whether as cause or effect is unclear) to the hierarchical categories often applied to distinguish the “hard” and “soft” sciences [1]. In effect, natural sciences are privileged in schools as real sciences and social sciences are often marginalized with very real consequences in public perception (and funding).

Modern approaches to science learning and application do not appear to correct this omission. The current interdisciplinary focus of STEM initiatives also fall prey to a similar narrowness, where the “S” in the ubiquitous acronym is generally considered to refer to natural sciences exclusively, not social sciences. Similarly, the Next Generation Science Standards label the conventional natural sciences (physical science, life science, earth and space science) along with engineering and technology applications as Disciplinary Core Ideas.

Even the methods of inquiry themselves are not neutral. The scientific method—with a heavy reliance on developing a hypothesis, testable predictions, and experimentation—is generally prioritized as the primary means to produce scientific knowledge. Yet, schools’ use of the scientific method has previously been criticized as an overly narrow construct to produce evidence; instead, model-based inquiry is arguably more broad and appropriate for school-age students to directly apply [2]. Further, testable predictions and direct experimentation are less commonly used in social sciences for a number of reasons, thus favoring model-based inquiry in schools would help to show how scientific inquiry can be broadly applicable across many fields inside and outside natural sciences.

Final Thoughts

I argue schools should teach students sooner about the application of scientific methods broadly across fields to help them see how scientific principles can elucidate both the natural and social worlds around them. Students currently learn social studies and history, though the scientific pursuits that created this knowledge are often not explicitly stated, nor is it clear that many of these methods may be shared with the natural sciences. I encourage scholars in science education to train teachers, write textbooks, and develop curricula in ways that help students connect these concepts across many scientific domains.

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2. Windschitl M, Thompson J, Braaten M. Beyond the scientific method: Model-based inquiry as a new paradigm of preference for school science investigations. *Sci. Educ.*, 2008, 92(5), 941-967. DOI: 10.1002/sce.20259



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The AIC Code of Ethics

Approved by the AIC Board of Directors, April 29, 1983



The profession of chemistry is increasingly important to the progress and the welfare of the community. The Chemist is frequently responsible for decisions affecting the lives and fortunes of others. To protect the public and maintain the honor of the profession, the American Institute of Chemists has established the following rules of conduct. It is the Duty of *The Chemist*:

1. To uphold the law; not to engage in illegal work nor cooperate with anyone so engaged;
2. To avoid associating or being identified with any enterprise of questionable character;
3. To be diligent in exposing and opposing such errors and frauds as *The Chemist's* special knowledge brings to light;
4. To sustain the institute and burdens of the community as a responsible citizen;
5. To work and act in a strict spirit of fairness to employers, clients, contractors, employees, and in a spirit of personal helpfulness and fraternity toward other members of the chemical profession;
6. To use only honorable means of competition for professional employment; to advertise only in a dignified and factual manner; to refrain from unfairly injuring, directly or indirectly, the professional reputation, prospects, or business of a fellow Chemist, or attempting to supplant a fellow chemist already selected for employment; to perform services for a client only at rates that fairly reflect costs of equipment, supplies, and overhead expenses as well as fair personal compensation;
7. To accept employment from more than one employer or client only when there is no conflict of interest; to accept commission or compensation in any form from more than one interested party only with the full knowledge and consent of all parties concerned;
8. To perform all professional work in a manner that merits full confidence and trust; to be conservative in estimates, reports, and testimony, especially if these are related to the promotion of a business enterprise or the protection of the public interest, and to state explicitly any known bias embodied therein; to advise client or employer of the probability of success before undertaking a project;
9. To review the professional work of other chemists, when requested, fairly and in confidence, whether they are:
 - a. subordinates or employees
 - b. authors of proposals for grants or contracts
 - c. authors of technical papers, patents, or other publications
 - d. involved in litigation;
10. To advance the profession by exchanging general information and experience with fellow Chemists and by contributing to the work of technical societies and to the technical press when such contribution does

not conflict with the interests of a client or employer; to announce inventions and scientific advances first in this way rather than through the public press; to ensure that credit for technical work is given to its actual authors;

11. To work for any client or employer under a clear agreement, preferable in writing, as to the ownership of data, plans, improvements, inventions, designs, or other intellectual property developed or discovered while so employed, understanding that in the absence of a written agreement:
 - a. results based on information from the client or employer, not obtainable elsewhere, are the property of the client or employer
 - b. results based on knowledge or information belonging to *The Chemist*, or publicly available, are the property of *The Chemist*, the client or employer being entitled to their use only in the case or project for which *The Chemist* was retained
 - c. all work and results outside of the field for which *The Chemist* was retained or employed, and not using time or facilities belonging to a client or employer, are the property of *The Chemist*;
12. Special data or information provided by a client or employer, or created by *The Chemist* and belonging to the client or employer, must be treated as confidential, used only in general as a part of *The Chemist's* professional experience, and published only after release by the client or employer;
13. To report any infractions of these principles of professional conduct to the authorities responsible for enforcement of applicable laws or regulations, or to the Ethics Committee of The American Institute of Chemists, as appropriate.

Manuscript Style Guide

The Chemist is the official online refereed journal of The American Institute of Chemists (AIC). We accept submissions from all fields of chemistry defined broadly (e.g., scientific, educational, socio-political). *The Chemist* will not consider any paper or part of a paper that has been published or is under consideration for publication anywhere else. The editorial office of *The Chemist* is located at: The American Institute of Chemists, Inc. 315 Chestnut Street Philadelphia, PA 19106-2702, Email: aicoffice@theaic.org.

Categories of Submissions

RESEARCH PAPERS

Research Papers (up to ~5000 words) that are original will only be accepted. Research Papers are peer-reviewed and include an abstract, an introduction, up to 5 figures or tables, sections with brief subheadings and a maximum of approximately 30 references.

REPORTS

Reports (up to ~3000 words) present new research results of broad interest to the chemistry community. Reports are peer-reviewed and include an abstract, an introductory paragraph, up to 3 figures or tables, and a maximum of approximately 15 references.

BRIEF REPORTS

Brief Reports (up to ~1500 words) are short papers that are peer-reviewed and present novel techniques or results of interest to the chemistry community.

REVIEW ARTICLES

Review Articles (up to ~6000 words) describe new or existing areas of interest to the chemistry community. Review Articles are peer-reviewed and include an abstract, an introduction that outlines the main point, brief subheadings for each section and up to 80 references.

LETTERS

Letters (up to ~500 words) discuss material published in *The Chemist* in the last 8 months or issues of general interest to the chemistry community.

BOOK REVIEWS

Book Reviews (up to ~ 500 words) will be accepted.

Manuscript Preparation

RESEARCH PAPERS, REPORTS, BRIEF REPORTS & REVIEW ARTICLES

- **The first page** should contain the title, authors and their respective institutions/affiliations and the corresponding author. The general area of chemistry the article represents should also be indicated, i.e. General Chemistry, Organic Chemistry, Physical Chemistry, Chemical Education, etc.
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- **Abstracts** explain to the reader why the research was conducted and why it is important to the field. The abstract should be 100-150 words and convey the main point of the paper along with an outline of the results and conclusions.
- **Text** should start with a brief introduction highlighting the paper's significance and should be understood to readers of all chemistry disciplines. All symbols, abbreviations, and acronyms should be defined the first time they are used. All tables and figures should be cited in numerical order.
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References should be cited as numbers within square brackets [] at the appropriate place in the text. The reference numbers should be cited in the correct order throughout the text (including those in tables and figure captions, numbered according to where the table or figure is designated to appear). The references themselves are listed in numerical order at the end of the final printed text along with any Notes. Journal abbreviations should be consistent with those presented in Chemical Abstracts Service Source Index (CASSI) (<http://www.cas.org>) guide available at most academic libraries.

- **Names** and initials of all authors should always be given in the reference and must not be replaced by the phrase *et al.* This does not preclude one from referring to them by the first author, et al in the text.
- **Tables** should be in numerical order as they appear in the text and they should not duplicate the text. Tables should be completely understandable without reading the text. Every table should have a title. Table titles should be placed above the respective tables.

Table 1. Bond Lengths (Å) of 2-aminophenol

- **Figure legends** should be in numerical order as they appear in the text. Legends should be limited to 250 words.

Figure 1. PVC Melt Flow Characterized by Analytical Structural Method

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Booth DE, Isenhour TL. *The Chemist*, 2000, 77(6), 7-14.

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Turner GK in *Chemiluminescence: Applications*, ed. Knox Van Dyke, CRC Press, Boca Raton, 1985, vol 1, ch. 3, pp 43-78.

- **Patents** should be indicated in the following form:

McCapra F, Tutt D, Topping RM, UK Patent Number 1 461 877, 1973.

- **Reports and bulletins, etc.** - For example:

Smith AB, Jones CD, *Environmental Impact Report for the US*, final report to the National Science Foundation on Grant AAA-999999, Any University, Philadelphia, PA, 2006.

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Smith AB. Presented at the Pittsburgh Conference, Atlantic City, NJ, March 1983, paper 101.

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Jones AB, Ph.D. Thesis, Columbia University, 2004.

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Jones AB, presented in part at the 20th American Institute of Chemists National Meeting, Philadelphia, PA, June, 2004.

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