INDICATOR DISPLACEMENT ASSAYS (IDAs): AN INNOVATIVE MOLECULAR SENSING APPROACH

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Indicator Displacement Assays (IDAs): An Innovative Molecular Sensing Approach

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FOREWORD

The introduction of diverse indicators to study the molecular recognition (binding/sensing) in various supramolecular receptors opened up a new gateway to mimic nature-based specific and selective host-guest interactions. Taking a step forward from the indicator spacer receptor (ISR) approach involving covalent linkages between receptors and indicators *via* a spacer, the indicator displacement assay (IDA) offers an innovative and powerful sensing approach for various target analytes in the arena of host-guest chemistry. The intense research in the arena of supramolecular analytical chemistry has led to the development of numerous IDA-based sensors for real-world applications.

The authors have chosen the interesting theme of "Indicator Displacement Assays (IDAs): An Innovative Molecular Sensing Approach" and presented a collection of significant topics covering the design, development, and applications of various types of IDAs and sensory arrays operating through IDA. The corresponding author Dr. Rashid Ali and co-author Ishfaq Ahmad Rather have ample experience in the domain of supramolecular chemistry and have published high-impact research articles in reputed journals. Both authors put their efforts to update the knowledge gained in the field of supramolecular chemistry in general and IDA-based systems in particular. The authors have made an effort to cover a wide range of IDA-based sensors developed from diverse supramolecular receptors like pillarenes, calixarenes, calix[4]pyrroles, cucurbit[n]urils, cyclodextrins, tripodals, boronic acid derivatives, *etc*. This authored book is a single source of literature collection on the selected topic. In the future plan of action to design, synthesize, and apply IDA-based sensors for real applications, this book will surely help the scientists worldwide already working and also those who want to enter into this emerging arena.

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PREFACE

Among diverse sensing assays, the indicator displacement assay (IDA)-based competitive experiments employing various indicators are most acknowledged in supramolecular analytical chemistry. IDA experiments have shown a promising role in exploring the interactions between biological hosts (*e.g*., proteins, nucleic acids, *etc*.) and various small molecules of interest, which are otherwise difficult to achieve through other sensing methodologies. This particular competitive technique has great potential to accomplish a rapid analysis of desired analytes because of being easily adaptable for different receptors.

In the first chapter of the book "Indicator Displacement Assays (IDAs): An Innovative Molecular Sensing Approach", we have assembled the background, conceptual details, design, and construction strategies, in addition to the need and urgency to replace indicator spacer receptor (ISR) approach with IDA having numerous advantages. The second, third, and fourth chapter, besides dealing with the molecular recognition of various analytes (cationic, anionic, and neutral), also deal with the design and construction of colorimetric, fluorescence, and metal complexing IDAs, respectively. The fifth chapter deals with various aspects of recent extensions of IDAs like enantioselective indicator displacement assays (e-IDAs), intramolecular indicator displacement assays (I-IDAs), reaction-based indicator displacement assays (R-IDAs), mechanically controlled indicator displacement assays (MC-IDAs), allosteric indicator displacement assays (A-IDAs), dimer dye assembly assay (DDA), and quencher displacement assay (QDA). The sixth chapter discusses conceptual details and various applications of IDA-based sensory arrays. The last chapter of this book deals with the design, fabrication, and applications of IDA-based electrochemical sensors.

This book strives to collectively present the design, construction, and molecular recognition characteristics of IDA-based sensors along with recent advancements, which in turn will provide newer insights to readers as well as young researchers who are willing to work in the field of sensory materials.

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CHAPTER 1

Background and Basic Concepts of Indicator Displacement Assays

Abstract: Taking a step forward from the indicator spacer receptor (ISR) method comprising covalent linkages between receptors and indicators *via* a spacer, the indicator displacement assay (IDA) offers an innovative and powerful sensing approach for various target analytes in the realm of host-guest chemistry. In this chapter, we have assembled the background and conceptual details in order to give essence to the readers about this innovative sensing approach. The photophysical phenomenon and diverse non-covalent interactions involved in the sensing mechanism have been detailed. We have elucidated the need and urgency to replace the ISR approach with IDA, one having numerous advantages. The evolutionary extension of IDA for enzymatic conversion known as supramolecular tandem assays has also been described in this chapter. We believe that the present introductory chapter will give a better understanding to readers who are new to this field.

Keywords: Analyte, Competitive binding, Dynamic, Detection, Enantiomeric excess, Enzyme, Fluorescence quenching, Guest, Host, Indicator displacement assay, Indicators, Macrocycle, Non-covalent interaction, Receptors, Reversible, Recognition, Sensors, Signal, Substrate, Tandem assays.

1. INTRODUCTION

Inspired by nature-based specific and selective host-guest interactions, supramolecular researchers are imitating and exploiting these interactions in molecular recognition and self-assembly phenomena. This in turn gives birth to supramolecular sensors and receptors, which play a significant role in the advancement of biological, industrial, environmental and agricultural sciences [1, 2]. In order to detect and sense a target analyte, these supramolecular sensors and receptors are taking the help of some facile sensing techniques [3, 4]. Among diverse sensing assays, the indicator displacement assay (IDA) centered on competitive experiments employing various indicators are the most acknowledged in analytical and supramolecular chemistry [5, 6]. Studies have revealed that in between receptor and indicator or analyte, both covalent as well as non-covalent

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interactions exist. In fact, covalent bonds of reversible nature are extensively used in IDAs and have found widespread use in the construction of dynamic combinatorial libraries. For example, vicinal diols and aryl boronic acids interact *via* reversible covalent bonds, thereby leading to the formation of cyclic boronate esters. Similarly, dynamic imine centre generation between the host containing carbonyl groups and primary amine also reveals reversible covalent interactions. The various non-covalent interactions between the receptor and indicator or analyte include H-bonding, coordinate bonding, salt bridge, electrostatic interactions, anion-π interactions, cation-π interactions, hydrophobic interactions, and π-π stacking [7]. Although, the credit of introducing IDA based competitive approach in the supramolecular chemistry goes to Inouye and coworkers after carrying out the work on the recognition of acetylcholine in protic media by a supramolecular receptor [5]. Nevertheless, these competitive sensing experiments were promoted by Anslyn and teammates, after their extensive and comprehensive exploration of numerous indicators with diverse receptors for the detection of vital analytes [8, 9]. Though, IDAs have been employed to sense and detect anions as well as cations. Nonetheless, most of the IDAs have been used for anions. This is by virtue of the fact that anions are ubiquitous in nature and play central roles in several phenomena, comprising biological routes like protein biosynthesis, DNA regulation, transport of hormones, and enzyme activity. Taking these facts into consideration, sensing or recognition of anions has become a current objective of molecular recognition. Additionally, these facts have motivated chemists to bestow substantial efforts in the design and construction of real-world chemosensors for the qualitative and quantitative detection of several anions [8]. IDA experiments have shown a promising role in exploring the interactions between biological hosts *(e.g.* proteins, nucleic acids *etc*.) and various small molecules of interest, which are otherwise difficult to achieve through other sensing methodologies [10]. For example, these have been used successfully to measure helicase activity of DNA, characterize double-stranded DNA binding with RecA protein, detect triplex DNA stabilizers, selectively detect amantadine drug in saliva and human urine, and detect cocaine and sensing of caffeine, halides, and histidine [11 - 13]. Moreover, IDAs have also been employed to determine various analytes like inorganic phosphate, citrate, aspartate, gallic acid, nitrate, glucose-6-phosphate, heparin, tartrate, 2,3-bisphospho glycerate and inositol-1,4,5-triphosphate. Interestingly, IDAs also serve to govern the reaction kinetics of simple chemical reactions, thereby helping in understanding the mechanism of a reaction [6]. Thus, bearing in mind the real applicability of IDAs in various domains like supramolecular chemistry, bioorganic chemistry, analytical sciences, environmental and agrochemistry besides keeping in consideration the vast available literature [8, 9, 14 - 18], herein we have discussed the background and basic concepts of IDAs.

2. COMMONLY USED SYNTHETIC RECEPTORS AND INDICATORS FOR THE DESIGN OF IDAs

In designing IDAs, the synthetic receptors are considered key elements. In order to fine-tune the intrinsic binding features of some familiar recognition motifs, synthetic reforms may offer highly selective and sensitive molecular constructs. Among numerous receptor systems, cyclodextrins (**1**), cucurbit(n)urils (**2**), calix [4]pyrroles (**3**), calix(n)arenes (**5**), tripodals (**6**), and boronic acid derivatives (**7**) are mostly involved in designing IDAs (Fig. **1**). These supramolecular receptors are classified on the basis of capturing analytes (cations, anions, and neutral molecules), interactions (H-bonding, ion pairing/ion dipole, donor-acceptor, and π-interactions) [19 - 21], and structures of the hosts (macrocycles, cavitands, clefts, tweezers *etc.*). Owing to the structural complexity and diverse molecular conformations, these offer a promising role in the domain of molecular recognition, self-assembly, transmembrane transport, drug delivery, polymers, molecular machines, catalysis and waste water treatment [22, 23].

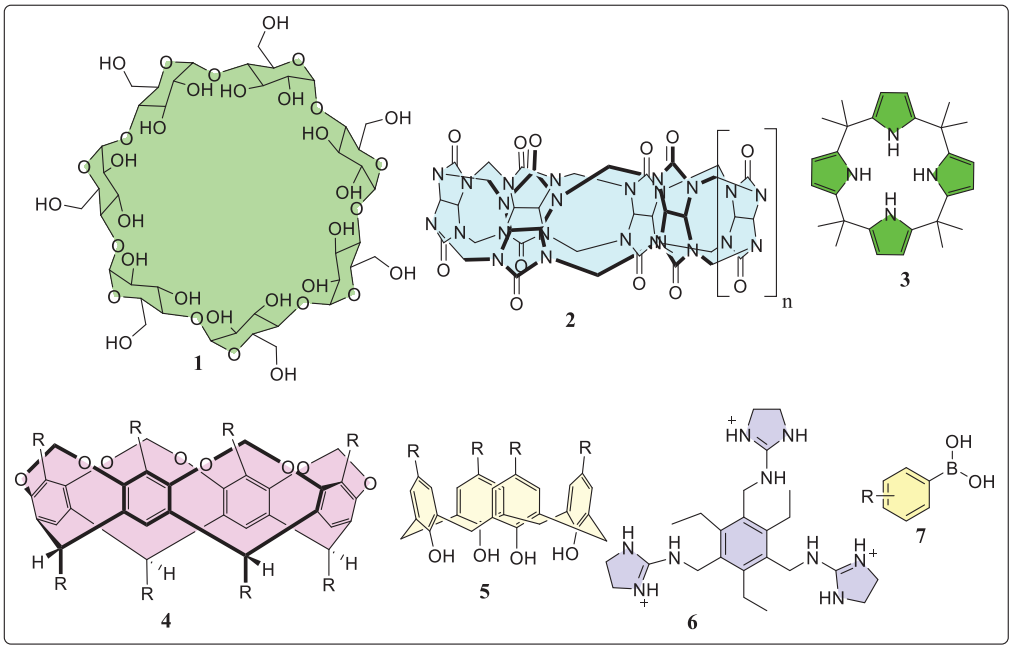

Fig. (1). Some representative receptor systems (**1-7**) used in IDA based phenomenon.

On the other hand, these IDA-based sensors involve various colorimetric and fluorescence indicators besides others in order to signify the successful binding

CHAPTER 2

Colorimetric Indicator Displacement Assays (C-IDAs)

Abstract: Owing to the unique advantages of naked eye color detection in the sensing of various important analytes, colorimetric indicator displacement assay (C-IDA) has received its recognition as the principal evolutionary extension of IDA. This chapter, besides dealing with all conceptual details of C-IDA, also offers basic criteria for the development and classification of chromogenic sensing ensembles. A plethora of examples in order to elucidate the C-IDA sensing mechanism for the detection of anionic, cationic, and neutral analytes *via* prominent UV-vis spectral changes have been revealed. Besides, the recognition of several biologically essential analytes *viz.* phosphate and saccharides have also been discussed in detail through numerous examples. The authors believe that this chapter on C-IDA will offer new dimensions to the design and fabrication of IDA-based colorimetric sensors.

Keywords: Absorption, Analyte, Binding, Binding affinity, Colorimetric indicator displacement assay, Chemosensor, Chromophore, Colorimetric indicator, Colorimetric sensor, Calorimetric titration, Detection, Isosbestic, Naked eye, Quantification, Receptor, Sensing ensemble, Stoichiometry, Signal transduction, Sensing, Saccharides.

1. INTRODUCTION

In general, a chromogenic chemosensor is considered a molecular device that changes its photophysical properties upon the binding of a typical guest molecule and thereby offers a detectable signal. Typically, chromogenic chemosensors contain three moieties *viz*. signaling moieties, recognition moieties, and a linker or spacers connecting signalling moiety with recognition moiety [1, 2]. In between the host and the guest molecule, studies have revealed the involvement of various reaction mechanisms *viz.* protonation-deprotonation, cleavage and construction of covalent bonds, direct/competitive displacement, complexation, and redox reactions. In fact, these types of reactions have been extensively used in the design strategies of various chromogenic probes. So far, various types of chromogenic chemosensors have been developed displaying superior binding properties with different target analytes, and offering detectable signals through

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various sensing mechanisms, in particular, IDA-based competitive approach [3 - 6]. Among various evolutionary extensions of IDAs, the colorimetric indicator displacement assay (C-IDA) is primarily considered a versatile competitive assay for the detection of target analytes. C-IDA involves the use of colorimetric indicators and the absorption phenomenon. It has got inspiration due to the role of naked eye in the detection of diverse analytes. Colorimetric indicators vary in color, depending upon whether the indicator is bound to the receptor or exists in the free-state. It is this color variation, which is held responsible to signify the successful binding event, which in turn modulates the desired optical signals [7 - 12]. The use of the human eye as an optical detector reveals the simplest example of colorimetric analysis, as the human eye possesses a high aptitude to discriminate various colors. In fact, the "naked eye" detection of various target analytes is considered an ancient method for qualitative analysis [13]. These CIDAs have fruitfully been used in the detection of various metal ions, inorganic as well as organic anions, reactive oxygen species *(e.g.* hydrogen peroxide and peroxynitrite), amino acids, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), γ-hydroxybutyrate, and various other biological macromolecules [14 - 20]. In the biomedical realm, these C-IDAs help in the quantification of plasma protamine and are used to monitor the binding events between *α*-thrombin and proflavin [21].

2. CHROMOGENIC SENSING ENSEMBLE

The credit for developing the chemosensing ensemble strategy goes to Inouye, Shinkai, and their coworkers due to their extensive studies in 1994 and 1996. Although until 2000, only very few examples were known in the literature, however, over the past two decades, this area is continuously developing and to date, diverse sensing ensembles have been reported. Anslyn's research group along with various other research groups have fabricated various chemosensing ensemble systems in order to determine various guest entities *viz.* citrate, heparin, gallic acid, phosphate, carbonate, tartrate, short peptide, and amino acid [22 - 25]. In order to design an efficient sensing ensemble-based displacement assay, the following criteria must be taken into consideration.

2.1. Thermodynamic Equilibrium

It includes two broad parameters *viz.* binding constant (K_a) and the Gibbs free energy change (ΔG). The binding constant (K_a) between the indicator/guest and the target receptor can successfully be determined through supramolecular titration based on either changes in absorbance/fluorescence or shifting in NMR signals. Prior to quantification of K_a, it is necessary to know the stoichiometry

between the host (receptor) and the guest with the help of continuous variation method (Job plot), mass analysis, structure of host-guest complex, and experimental clues like isosbestic/isoemissive points [26]. Out of various thermodynamic equilibria, 1:1 host-guest equilibrium and relevant K_a calculation are most commonly studied. Additionally, complicated complex structures involve either 1:2 or 2:1 host-guest equilibrium. Besides $K_{a,}$ the ΔG with the aid of equations (1 and 2) has also been used in the prediction and design of an efficient sensing ensemble based on displacement assays.

$$\Delta G = -RTlnKa \quad \textbf{(1)}$$

$$\Delta G = \Delta H - T\Delta S \quad \textbf{(2)}$$

Where ΔH is the change in enthalpy, ΔS is the change in entropy, R is the ideal gas constant, and T is the absolute temperature.

On the other hand, the addition of a guest to the host (calorimetric titration) to an isothermal calorimeter leads to an increase in enthalpy. These calorimetric titrations not only help in the quantification of ΔG from K_a values but also determine changes in the enthalpy (ΔH) *vis-à-vis* change in entropy (ΔS). Interestingly, isothermal titration calorimetry (ITC) is regarded as a powerful method to determine stoichiometry and binding constant (K_a).

2.2. Molecular Design Setup and Environmental Factors

In order to design a displacement assay for the detection of a target analyte, the indicator as well as receptor should be designed and chosen carefully by taking the following criteria into consideration:

(i) Suitable and well-suited binding affinity to interested target analytes.

(ii) Suitable absorption/emission wavelengths in order to remove interference.

(iii) Determination of required concentration and limit of detection (LOD).

(iv) Water solubility

(v) Optimized screening strategy in order to save time.

Besides these criteria, various experimental conditions like concentration, solvent polarity, pH, viscosity, and temperature enable tuning to obtain the desired binding constant (K_a) values for the indicator and/analyte with receptor [27, 28].

CHAPTER 3

Fluorescence Indicator Displacement Assays (F-IDAs)

Abstract: Owing to the ease in their usage and versatility, fluorescent chemosensors have attracted the remarkable attention of researchers across the globe. In fact, the dawn of supramolecular chemistry has begun a new journey in the design, construction, and development of diverse fluorescent chemosensors. Fluorescent indicator displacement assays (F-IDAs) in principle utilize fluorescent indicators and emission phenomenon for the construction of various IDA-based Turn-ON/OFF fluorescent sensors. Particularly, F-IDAs have been found valuable in pattern-based recognition, where slightly different multiple sensors are constructed from diverse receptors simply by swapping fluorescent indicators in and out of receptors or even varying the concentration ratio. These F-IDAs offer huge potential to develop proficient optical sensors from numerous supramolecular receptors and imply the effective molecular recognition event *via* competitive assay of receptors with an indicator and an analyte. Besides conceptual and mechanistic details, authors have ensured the significance of F-IDAs in the recognition of biologically and environmentally essential cationic, anionic, and neutral analytes through various examples.

Keywords: Aggregation-induced emission, Amino acids, Biomarker, Drug, Explosive, Emission, Enhancement, Fluorescent indicator displacement assay, Fluorophore, Fluorescent indicator, Fluorescent chemosensor, Quenching, Quencher, Receptor Turn-ON, Receptor, Sensor, Selective, Sensitive, Turn-OFF.

1. INTRODUCTION

Within the broad domain of chemosensors, fluorescent chemosensors involving supramolecular chemistry for sensing purposes have attracted tremendous attention of chemists over the past several decades [1, 2]. These fluorescent chemosensors are designed in a way to bind target analytes through non-covalent interactions, thereby offering discernible variation in an emission profile of incorporated fluorophore. In fact, the emergence of supramolecular chemistry has started a new chapter in the design and construction of diverse fluorescent chemosensors. In simple terms, fluorescent chemosensors are compounds containing a fluorophore, a binding site, and an established mechanism of

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communication between a binding site and a fluorophore [3]. These fluorescent chemosensors are recognized as chemodosimeters in cases where the chemical reactions at binding sites are irreversible. Goppelsroder in 1867 first time developed a fluorescent chemosensor for the recognition of aluminium ion (Al^{3+}). Consequently, this led to the development of ample fluorescent chemosensors for diverse metal ions. As a matter of fact, the metal ion binding is easier in water as compared to the binding of anions and neutral molecules, the researchers in early days have mainly concentrated on the development of cation sensors. However, since 1980, explosive development is being witnessed in the domain of fluorescent chemosensors for different analytes, with a pioneering and inspirational work of de Silva and Czarnik, being considered as the fathers of modern fluorescent chemosensors [4]. Since then, the scope of fluorescent chemosensors has extended with particular applicability on biological analytes including cations, anions, neutral molecules, biomolecules *viz.* proteins and DNA. As far as analyte detection by means of these fluorescent chemosensors is concerned, various photophysical phenomena *viz.* photoinduced electron transfer (PET), intramolecular charge transfer (ICT), chelation induced enhanced fluorescence (CHEF), aggregation induced emission (AIE) *etc*. govern the mechanism of sensing [5, 6]. Due to their high sensitivity and applicability for *in vivo* imaging, fluorescent chemosensors have found widespread uses in biology, pharmacology, physiology, and environmental sciences [7, 8]. Fluorescent indicator displacement assays (F-IDAs) involve the usage of fluorescent indicators and emission phenomenon. These IDAs possess vast potential to develop efficient optical sensors from diverse supramolecular receptors and signify the successful molecular recognition event upon the swapping of a fluorescent indicator from a target receptor by a competitive analyte of particular intrigue (Fig. **1**) [9].

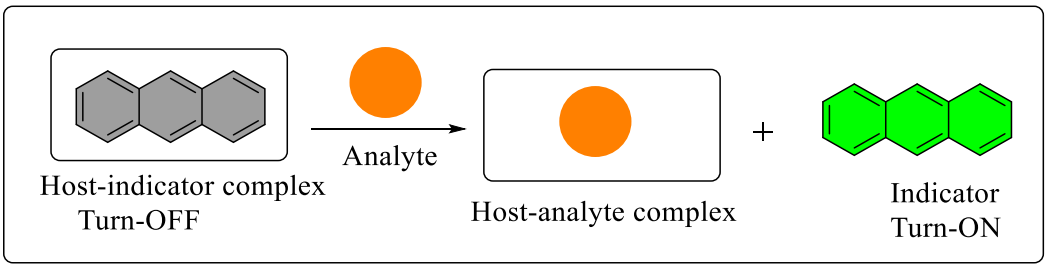

Fig. (1). Schematic representation of fluorescent indicator displacement assay (F-IDA).

F-IDAs have been found more sensitive in comparison to C-IDAs, as they involve changes in emission properties of fluorescent indicators. As a matter of fact, they can determine the concentrations upto one million times smaller in comparison to

C-IDAs utilizing absorption method. They also help in measuring the concentration of short-lived reactive nitrogen species (RNS), reactive oxygen species (ROS), and reactive sulphur species (RSS) [10, 11]. Not only this, they are also convenient in the recognition of hydrogen sulfide, food additives, and new ligands, possessing the ability to bind with diverse target molecules [12 - 14]. The quenching and recovery of fluorescence inherent in emission phenomenon are very operative in the effective screening of various ligands capable of binding with RNA. For instance, by using fluorescent indicators like thioxanthone and 2,7-diaminoalkoxy xanthone, interactions between RNA and ligands have successfully been studied through F-IDA. These indicators experience quenching phenomenon upon interacting with the target RNA. However, fluorescence recovery has been observed upon the swapping of fluorescent indicator from the target RNA by a competitive ligand [15 - 20]. On the other hand, F-IDAs have been used to study the interactions between biotin and avidin, which are two common agents employed in histochemistry and purification process of various biological samples [21]. Moreover, F-IDAs with the aid of thiazole orange as a fluorescent indicator help to monitor the activity of Rec. A protein of *Escherichia Coli* with double-stranded DNA [22]. High throughput selection of small sized molecules capable of binding with DNAs have also been achieved with the aid of F-IDAs [23].

2. F-IDA BASED ANION SENSORS

2.1. Phosphate Anion F-IDA Sensors

In the early years of the development of F-IDAs, Anslyn and teammates have reported the detection of polyanionic secondary messenger known as inositol-1,4,5-triphiosphate (**2**) *via* F-IDA [24]. They have used receptor **1** which possesses guanidinium moieties in addition to three units of 1,3,5-trisubstitute--2,4,6-triethylbenzene and used 5-carboxyfluorescein (**3)** as a fluorescent indicator (Fig. **2**). The competitive binding of **2** towards receptor **1** leads to the displacement of indicator **3** from receptor **1** with an observed binding constant (Ka) of 1.0 $\times 10^8$ M^{-1}. In another study, Akkaya and teammates have developed F-IDA for the recognition of ATP using tetracationic calixpyridinium receptor **4** and 8-hydrox--1,3,6-pyrenetrisulfonate (**5**) as the fluorescent indicator (Fig. **2**) [25]. Suitable ion-pairing interactions have been noticed between the receptor **4** and indicator **5**, leading to the formation of indicator receptor complex. Upon the addition of ATP, there occurs the displacement of **5** from receptor **4** by a competitive binding of ATP (Ka = 2.87 $\times 10^4$ M^{-1}).

CHAPTER 4

Metal Complexing Indicator Displacement Assays (M-IDAs)

Abstract: As a sub-class of colorimetric indicator displacement assays (C-IDAs) as well as fluorimetric indicator displacement assays (F-IDAs), metal indicator displacement assays (M-IDAs) involve primarily the coordination of metal ions with a target receptor and subsequent coordination of colorimetric or fluorimetric indicator with complexed metal ions and also with the target receptor. The chronological addition of an interested analyte brings the displacement of the colorimetric or fluorimetric indicator from the metal ion and the receptor, which in turn illustrates the binding affair either through colorimetric or fluorescence Turn-ON/OFF response. The authors have illustrated the role of M-IDAs in the detection of various indispensable analytes through a vast number of examples and deduced their need in various domains from future perspectives. The authors are of the opinion that this chapter will bring new dimensions to metal coordination chemistry in general and sensor development in particular.

Keywords: Aminoacid sensing, Analyte, Anion sensing, Biomolecules, Binding, Biothiol recognition, Colorimetric response, Complexation, Detection, Fluorescence response, Fluorescence recovery, Interference, Metal indicator displacement assay, Metal-coordination, Macrocyclic receptors, Quantification, Quenching, Sensing ensemble, Selective detection.

1. INTRODUCTION

Metal ion coordination chemistry has engrossed significant attention of the research community in the design and construction of novel functional materials, holding great promise in the biological, medicinal, and sensory material world [1, 2]. In the design of diverse sensors, indicator displacement assays (IDAs) exploit metal ion coordination chemistry and hence lead to the fabrication of novel sensors for the analytes of significant interest [3, 4]. This in turn offers a new class of IDAs known as metal indicator displacement assays (M-IDAs), which is regarded as a sub-class of C-IDAs and F-IDAs. Notably, the metal ion binds first with the receptor to form a typical metal-receptor complex. In the subsequent step, the colorimetric or fluorimetric indicator coordinates with a complex metal

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ion and target receptor. The sequential addition of an analyte of interest results in the displacement of a colorimetric/fluorimetric indicator from the metal ion and the receptor, which signifies the binding affair either through colorimetric or fluorescence Turn-ON/OFF responses [5]. Usually, these M-IDAs function in extremely polar solvents and offer higher affinity for anionic analytes [6]. The credit for the development of M-IDAs goes to Fabbrizzi and teammates, as they first time developed M-IDA for selective recognition of glutamate, histidine, carbonate, and pyrophosphate in aqueous solutions *via* fluorescence response [7 - 10]. For the detection of carbonate in water, they have used Cu(II) complex based receptor **1** and fluorescent coumarin indicator **2** (Fig. **1**). It has been revealed that the two Cu(II) centers of polyamine cage receptor **1** get bridged with the help of the carboxylate moiety of indicator **2**, which in turn leads to the fluorescence quenching of **2**. Upon the addition of carbonate anionic analyte possessing higher binding affinity with receptor **1**, there occurs the displacement of **2** from receptor **1** by carbonate anion and hence leads to fluorescence recovery of **2** [7]. On the other hand, they have used polyamine cage type receptor **1** and fluorescence indicator 6-carboxytetramethyl rhodamine (**3**) for the selective recognition of neurotransmitter glutamate in aqueous solutions at neutral pH *via* M-IDA (Fig. **1**) [8]. Owing to partially filled 3d orbitals, Cu(II) ion acts as a fluorescence quencher and hence signifies the selective molecular recognition event of glutamate among various other interfering amino acids, for example, glycine, alanine, aspartate, *etc* [11]. Interestingly, Severin and teammates have reported M-IDA for the selective detection of histidine and methionine-possessing peptides sequentially in an aqueous solution at neutral pH [12]. In another study, Lippard and coworkers have reported the selective and sensitive recognition of nitric oxide *via* M-IDA based fluorescence response and taking the help of rhodium metallated macrocyclic receptor [13]. Looking at the diversified role of M-IDAs in the recognition of critical analytes, we have sub-classified the applications of M-IDAs in this chapter as follows:

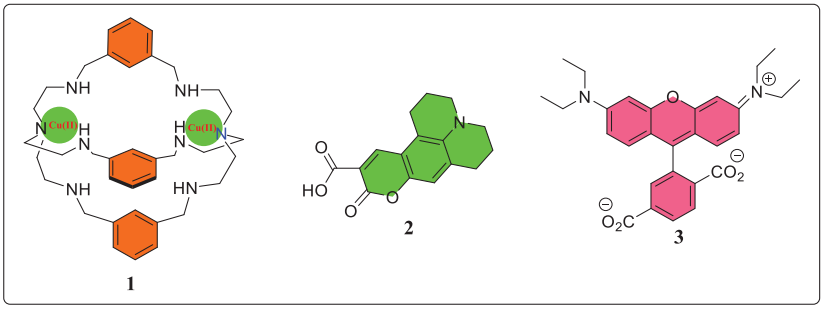

Fig. (1). Structures of polyamine cage receptor **1** and fluorescent indicators (**2** and **3**) used for detection of carbonate and glutamate *via* M-IDA by Fabbrizzi's group.

2. M-IDA BASED SENSORS FOR PHOSPHATE AND OTHER VITAL ANIONS

Over the past several decades, scientists have devoted much attention towards the selective and sensitive detection of pyrophosphate (PPi), as it plays an essential role in several biological processes like DNA polymerase reaction and ATP hydrolysis [24, 25]. In this regard, Fabbrizzi and coworkers have established M-IDA for the selective detection of pyrophosphate using macrocyclic cage type Cu(II) complexed receptor (**4**) and fluorescent indicator eosin (**5**) (()Fig. **2**) [9]. Results have revealed that the pyrophosphate has higher binding affinity with receptor **4** in comparison to monophosphate by virtue of coordination with two Cu(II) centers. In another study, Anslyn and coworkers have reported M-IDA for the detection of phosphate by using C_{3v}-symmetric Cu(II) coordinated receptor (**6**) and fluorescent indicator 5-carboxyfluorescein (**7**). It has been revealed that Cu(II) and peripheral guanidinium units in receptor **6** enhances the binding of phosphate with a binding constant (Ka) of 1.5×10^{4} M^{-1} [14, 15].

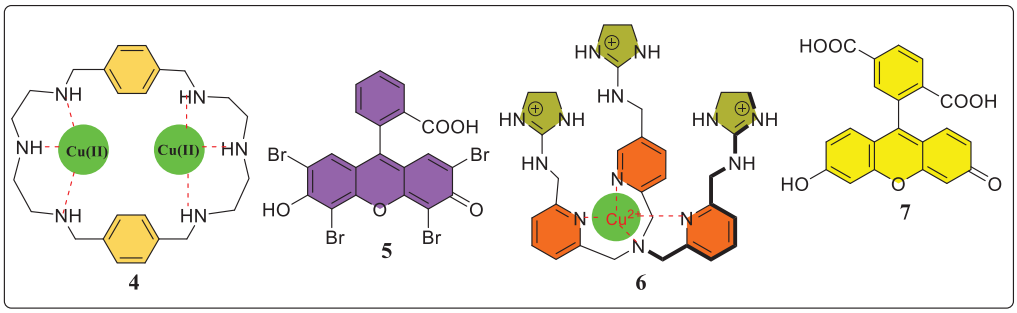

Fig. (2). Structures of macrocyclic receptors **(4** and **6)** fluorescent indicators **(5** and **7)** used in the detection of pyrophosphate and phosphate *via* M-IDA.

In another event, Tuntulani and coworkers have testified M-IDA for the selective colorimetric detection of PPi using dinuclear Zn(II) complex (**8**) as the receptor and methylthymol blue (**9**) as the colorimetric indicator (Fig. **3**) [16]. The large tetramine moieties in **8** offer a cleft to adapt **9**, thereby resulting in the construction of indicator-receptor complex (**8@9**). The successive addition of PPi possessing higher binding affinity with **8**, carries out the displacement of indicator **9** from the receptor **8** and offers a naked-eye color variation from blue to violet. Furthermore, computational and spectroscopic studies have exposed 2:2 binding stoichiometry of target analyte (PPi) with **8** upto a detection limit of 0.3 μM (Fig. **3**).

CHAPTER 5

Recent Extensions of IDA-based Sensing Protocols

Abstract: With the advancement of supramolecular chemistry in the realm of sensing, researchers across the globe have developed various evolutionary extensions of IDAs from time to time. These comprise enantioselective indicator displacement assays (e-IDAs), intramolecular indicator displacement assays (I-IDAs), indicator catalyst displacement assays (ICDA), reaction-based indicator displacement assays (R-IDAs), mechanically controlled indicator displacement assays (MC-IDAs), allosteric indicator displacement assays (A-IDAs), catalytic chemosensing assay (CCA), dimer dye assembly assay (DDA), and quencher displacement assay (QDA). All these evolutionary extensions of IDAs have been discussed in detail from concept to applications along with the possible future outcomes. The authors are of the viewpoint that this chapter will develop a better understanding of the researchers worldwide in the context of the advancement of IDAs.

Keywords: Analyte, Allosteric, Chiral analyte, Catalyst, Catalyst, Coordination, Dye, Displacement, Extensions, Enantiomer, Enantiomeric excess, Intramolecular indicator, Mechanical force, Mechanical force, Optical signal, Quencher, Receptor, Recognition, Sensing, Signal modulation.

1. INTRODUCTION

The traditional IDAs like C-IDAs, F-IDAs, and M-IDAs are regarded as supramolecular ensembles, comprising an indicator (optical guest) being reversibly bound with a host (synthetic receptor) [1, 2]. The presence of a competitive binding analyte leads to the displacement of the indicator from the host. In order to acquire complementary binding of the indicator with a synthetic host, a proper understanding of supramolecular chemistry is essential so as to identify the suitable charge, size, and hydrophobicity of the indicator. At a particular concentration, the target analyte should necessarily have a higher binding affinity with the synthetic host in comparison to the chosen indicator. The synthetic hosts must have the provision of various non-covalent interactions in order to form a suitable complex either with the indicator or target analyte. Upon the exposure of the indicator-receptor complex to a target analyte, the release of indicator (chromophore) should be easily detectable either through UV-vis and

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emission spectroscopy or *via* electrochemical measurements [3, 4]. With no prerequisite of synthetic effort and being cost-effective in nature, IDA-based molecular sensors are progressively replacing traditional molecular sensors. The credit for the development of traditional IDAs goes to Anslyn, Shinkai, and Inouye, through their seminal work on various synthetic receptors [5]. These seminal works on IDAs have inspired researchers across the globe to develop several extensions of this innovative idea of molecular sensing. These led to the development of enantioselective indicator displacement assays (e-IDAs), intramolecular indicator displacement assays (I-IDAs), indicator catalyst displacement assay (ICDA) reaction based indicator displacement assays (R-IDAs), mechanically controlled indicator displacement assays (MC-IDAs), allosteric indicator displacement assays (A-IDAs), catalytic chemosensing assay (CCA), dimer dye assembly assay (DDA), and quencher displacement assay (QDA). These extensions in original IDA sensing phenomenon have evolved due to the continuous development of synthetic receptor scaffolds and exploitation of synthetic/natural dyes. Here, in this chapter, we have discussed the basics of all the IDA extensions and have exemplified them by means of several examples, highlighting various applications. The discussion on various extensions of IDAs will illuminate the readers about the current advancements of IDAs in varied domains of science and technology.

2. ENANTIOSELECTIVE INDICATOR DISPLACEMENT ASSAYS (E-IDAs)

In nature, chirality is a basic feature offering great significance in the medical as well as life sciences [6]. For example, chiral enantiomers of DNA, amino acids, proteins, and saccharides govern the operational mechanism of life. In several cases, one chiral isomer is advantageous in terms of acting as a corner stone of life, while another isomer might be impractical or even detrimental for life [7]. Notably, for the discrimination of chiral enantiomers, it is necessary to develop simple yet effective methodologies. To this regard, researchers have developed various chiral sensory platforms based on organic compounds, inorganic nanoparticles, and polysaccharides for the detection of enantiomers of typical bioactive compounds [8, 9]. The quantification of enantiomeric excess (*ee)* through UV-vis spectroscopy is well established. Usually a chiral analyte binds with a chiral receptor/host and offers a detectable variation in spectroscopic signatures. In various cases, the chiral receptor requires several steps in synthesis before its efficacy gets proved through spectroscopic means. This is usually unfavourable approach in cases where a poor spectroscopic signal is observed and hence needs the synthesis of target receptors afresh, which is a cumbersome task and hence appears as a bottleneck in the determination of *ee.* In sharp contrast,

enantioselective indicator displacement assays (e-IDAs) are very expedient in the detection of diverse chiral enantiomers and offers an excellent strategy in the discrimination of chiral compounds possessing a wide range of functional groups [10 - 14]. It has been noticed that colorimetric and fluorescent indicators have the potential to tune both the sensitivity and chiral resolution of e-IDAs. Typically in e-IDAs, a non-fluorescent receptor-dye complex is generated once a fluorescent indicator binds with an enantiomerically pure target receptor. However, the high-affinity competitive binding of chiral analyte results in the displacement of a fluorescent indicator from the preformed receptor-dye complex, which in turn offers signal modulation (Fig. **1**) [10, 15 - 18].

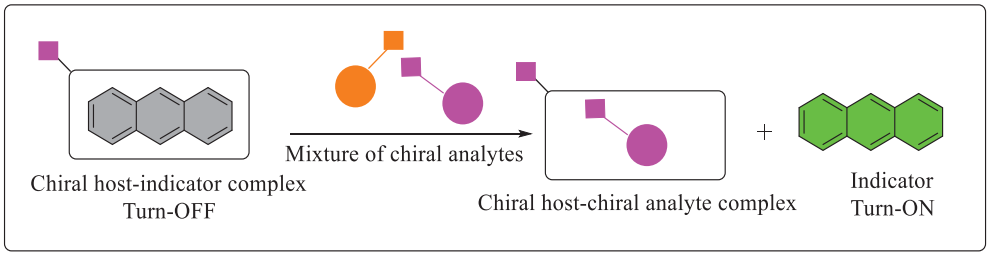

Fig. (1). General mechanistic illustration of enantioselective indicator displacement assays (e-IDA).

In a typical experiment, Anslyn and coworkers have employed e-IDA in the quantification of *ee* as well as an unknown concentration of various chiral analytes *viz.* α-hydroxycarboxylates and vicinal diols (Fig. **2**). They have implemented several boronic acid-based chiral receptors (**1-12**) besides various colorimetric/fluorescent indicators (**13**, **21**, and **24**) (Fig. **2**). Interestingly, fluorescent indicator 4-methyl esculetin (**24**) and colorimetric indicators like alizarin complexone (**13**) and pyrocatechol violet (**21**) have been found more fruitful to detect chiral analytes *via* e-IDA. These colorimetric/fluorescent indicators construct indicator-receptor complexes and high-affinity competitive binding of chiral analytes (**14-17**) results in the displacement of indicators from chiral receptors. The inclusive binding event offers either fluorescence Turn ON/OFF response or colorimetric response (Fig. **2**) [16]. On the other hand, quite recently, the same group have developed e-IDA for the determination of *ee* and the concentration of unknown chiral primary amines in a high throughput screening manner [19]. It has been noticed that the concentration of primary amine chiral analytes is determined *via* the construction of preformed non-fluorescent imine probe (**27**) generated through Schiff base reaction between aldehyde (**25**) and 2-naphthalamine (**26**) (Fig. **3**). The addition of chiral primary amine analyte to **27** offers transimination and hence results in the displacement of **26** fluorophore perceived through a Turn-ON fluorescence response. After the

CHAPTER 6

IDA-based Differential Sensory Arrays and Assays

Abstract: For the pattern-based recognition of various metal species and bioanalytes, nature has stimulated an emergent supramolecular domain of synthetic receptor arrays and assays. It is not always necessary for a synthetic receptor to be selective for a particular analyte in a differential receptor array, but the inclusive signal response from a typical sensory array must be diagnostic for the target analyte. This new category of molecular recognition is rapidly advancing with various groups constructing novel array platforms as well as receptors. Owing to easy operation and better selectivity, the sensory array has gained significant attention in the realm of complex system analysis. Besides the conceptual background, the authors have elaborated on the applications of various differential arrays through numerous examples. The authors believe that this chapter devoted to IDA-based differential sensory arrays and assays will bring a new episode of IDA-based chemosensors for target analytes.

Keywords: Analyte, Anion sensing, Complex mixtures, Colorimetric indicator, Differential sensory array, Diagnostic patterns, Detection, Differential sensing, Differential anion sensing, Fingerprint response, High-throughput screening, Immobilized sensory, Molecularly imprinted polymers, Molecular recognition, Optical signatures, Supramolecular receptors, Sensitive, Selective, Supervised pattern recognition protocols, Small molecules.

1. INTRODUCTION

Optical molecular probes have the ability to recognize target analytes *via* physical or chemical interactions. These interactions in turn induce variation in the optical signals of molecular probes [1]. The conventional "1 *vs* 1" probe *i.e.* one molecular probe for the recognition of only one target analyte has several advantages *viz.* good selectivity/sensitivity, easy operation, and great space-time resolution (Fig. **1**). Nevertheless, the design and construction of such optical molecular probes are highly complex besides facing the difficulty of low yield and hence fail to fulfill the requirements of complex system analysis. It is always a daunting task to develop specific receptors for complex analytes and mixtures present in the solution phase. In order to find a solution to this particular problem, researchers are inspired by nature employing arrays of receptors to sense taste and

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smell. This leads to an emerging field in the supramolecular chemistry to detect diverse analytes in the solution phase by employing synthetic as well as freely available receptors in an "*N vs N*" sensory array setup *i.e*. multiple molecular probes for multiple target analytes, designed and constructed using a number of chromophores and recognition motifs of various molecular probes (Fig. **1**) [2 - 4]. This in turn reduces the difficulty level of the synthesis of a molecular probe and can simultaneously recognize multiple analytes in a complex mixture. It is not mandatory that every receptor in an array possesses selectivity for a target analyte. However, overall fingerprint response signifies a visual diagnostic pattern for an analyte of interest [5 - 7]. Over the past several decades, this new field of molecular recognition is rapidly advancing with various research groups constructing a novel array of receptors for pattern-based recognition of the analytes [8 - 10]. For instance, Wang *et al*. developed an IDA-based supramolecular sensory array *via* lanthanide-doped nanoparticles for the sensitive detection of glyphosate and proteins [11]. Similarly, Zhong *et al.* constructed an immobilized sensory array based on cucurbit[n]uril-based macrocyclic receptor for the detection of small metabolic analytes *viz.* spermine, cadaverine, and amantadine [12]. On the other hand, Anslyn and teammates have reported C-IDA in combination with an artificial neural network (ANN) for the detection of citrate anion along with calcium using a tri-guanidinium based pinwheel receptor and a colorimetric indicator known as xylenol orange [13]. Quite recently, Mahram *et al.* employed IDA-based sensory arrays in the simultaneous detection and quantification of phosphate and sulfate [14]. In another event, Feng's research group exploited a colorimetric IDA-based sensory array for the colorimetric determination of tetracyclines [15]. Differential sensing allows the recognition of analytes of complex nature and a mixture of analytes in the solution phase. For real-world applications, differential arrays have found uses in detecting bioterrorism agents, discrimination of medicinal compounds, medical diagnostics, recognition of chiral compounds, environmental analysis and various other societal demands [5, 16 - 19].

2. IDA OPERATED SINGLE ANALYTE SENSING VERSES DIFFERENTIAL SENSING

Keeping in consideration the well-known lock and key hypothesis, conventional IDAs involve the design of one receptor for one particular analyte in order to accomplish an increase in selectivity and a decrease in interference from structurally similar analytes. In addition to the design of receptors, other factors like solvents, indicators, and pH also play a crucial role in fine-tuning of the selectivity towards the target analytes. Over the past several decades, tremendous progress has been made in the realm of sensing for small molecules *via* selective

receptors. Nevertheless, it still remains a challenge for supramolecular chemists to achieve sensing of macromolecules (proteins and nucleic acids) through selective molecular receptors. Besides, it is also difficult to investigate complex mixtures by means of selective receptors through IDA. Thus, selective receptors are not always beneficial and have been substituted with better alternatives. In this direction, natural protein receptors of differential nature have guided chemists to employ an array of receptors for numerous desired analytes. Within the realm of supramolecular and combinatorial chemistry, differential sensing by virtue of synthetic receptors has flourished over the past several decades. Differential receptors have the ability to bind or sense a series of analytes with different affinities [2, 3]. In sharp contrast to single analyte sensing, a dearth of selectivity and specificity is always anticipated for differential receptors. A differential sensing array can be made from multiple differential receptors, multiple analytes and multiple indicators, which in turn operate multiple IDAs at one time. The signals generated from multiple IDAs are recognized through the pattern recognition protocols like artificial neural networks (ANN) and principal component analysis (PCA). The resultant fingerprint offers distinctive diagnostic patterns either for individual analyte or for complex mixtures entailing multiple analytes [5].

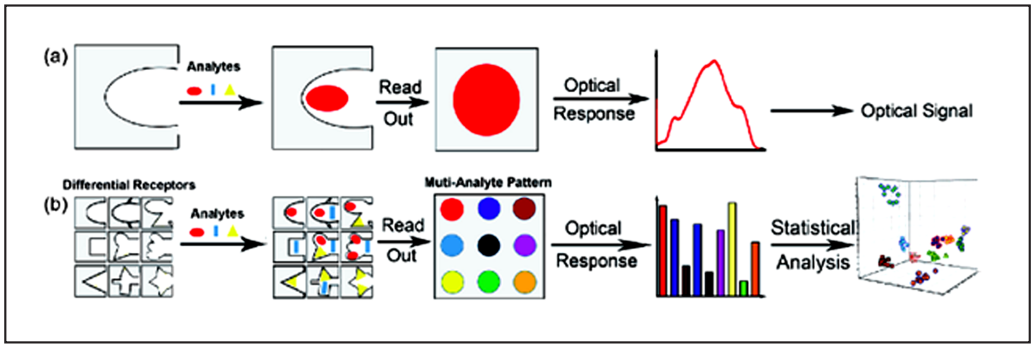

Fig. (1). Schematic illustration of the sensing mechanism of "1 *vs* 1" probe (**a**) sensing mechanism of "*N vs N*" sensory array (**b**). (Reproduced with permission from ref [3], Copyright 2018 Science China Press and Springer-Verlag GmbH Germany).

3. SENSORY ARRAY EXPERIMENTAL DATA ANALYSIS

Studies have revealed that the experimental data in sensory arrays is analyzed through supervised and unsupervised pattern recognition protocols. Unsupervised pattern recognition protocols are also known as clustering methods and are used to handle random samples whose characteristics and regularities are figured *via* mathematical calculation. For multivariate data, they mainly include hierarchical

CHAPTER 7

Electrochemical Sensors Based on Indicator Displacement Assays

Abstract: Owing to the low cost, fast response, easy miniaturization, portable instrumentation, and multiple analyte detection capabilities, electrochemical sensors stand out in the realm of sensing and thus occupy a prominent place in analytical supramolecular chemistry. Over the past several decades, the recognition of biologically and environmentally vital analytes *via* electrochemical responses (increase or decrease in current density), has attracted much attention from supramolecular chemists. The fabrication of electrochemical sensors through a competitive sensing phenomenon known as indicator displacement assay (IDA) has made them more realistic for target analyte detection. In this chapter, besides discussing various types and techniques of electrochemical sensors, we envisioned discussing diverse IDA-based electrochemical sensors for saccharides, biomarkers, neurotransmitters, and various other analytes. The authors are of the viewpoint that this chapter will meet the needs of the researchers working on the design, fabrication, and application of IDA-based electrochemical sensors.

Keywords: Analyte, Biomarker, Current density, Detection, Electrochemical sensor, Guest, Host, Indicator displacement assay, Indicators, Macrocycle, Recognition, Signal, Signal modulation, Teceptors.

1. INTRODUCTION

In essence, an electrochemical sensor belongs to a class of chemical sensors, whereby in the presence of an analyte, the electrode acts as a transducer element. These sensors typically operate through the variation of electrical potentials at the electrodes, which is due to the occurrence of chemical reactions in an electrolyte solution [1, 2]. The usage of electrochemical sensors began in the 1950s with the monitoring of industrial oxygen. Presently, paradigms involving electrochemical sensor research and development allow for the investigation of novel materials, application of various samples, new manufacturing procedures, and tactics to improve selectivity and detection limits. Electrochemical sensors of modern type employ several properties for the detection of various analytes having physical, chemical, and biological nature [3]. These are widely used in the detection of toxic gases in the environment at ppm level. In fact, these sensory systems offer

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precise and quick analytical tools for the selective as well as sensitive electrochemical detection of environmentally dangerous samples [4]. Moreover, they have also been found ideal and effective in the monitoring of environmental pollutants present in the environment in very small amounts or volume [5, 6]. In order to fabricate electrochemical sensors for diverse applications, several design concepts are being taken into consideration for the accomplishment of a particular goal [7 - 9]. Among these, the competitive sensing paradigm known as indicator displacement assay (IDA) is considered one of the versatile design concepts for the construction of electrochemical sensors. In this tactic, the electrochemical response modulates *via* the binding competition between the redox active indicator and the target redox active analyte towards a receptor of specific interest. Due to the higher binding affinity of the target analyte with the receptor in comparison to the indicator, the indicator receptor complex formed initially disintegrates leading to the generation of the desired analyte-receptor complex detected either through an increase or a decrease in the current density. Over the past few decades, researchers have developed a lively interest in the design and development of IDA-based electrochemical sensors, owing to their low cost and high selectivity as well as sensitivity (lower limit of detection) for a typical analyte [10, 11]. In this particular chapter, we have disclosed various findings related to the design, fabrication, and various sensing applications of IDA-based electrochemical sensors. This will in turn serve as a guide for the researchers to trace the development of IDAs in the electrochemical sensing realm.

2. TYPES OF ELECTROCHEMICAL SENSORS

Electrochemical sensors are broadly categorized into three main types: 1) Potentiometric sensors, 2) Amperometric sensors, and 3) Conductometric sensors.

2.1. Potentiometric Sensors

In essence, a potentiometric sensor can measure the voltage, and because of their low cost and simplicity, these are widely used in practical applications. These are further classified as follows:

2.1.1. Ion-Selective Electrodes (ISEs)

These are indicator electrodes having the ability to selectively measure the activity of desired ionic species. The pH electrodes are the most widely employed potentiometric devices, and the general electrochemical measurement setup employing the usage of ISEs is depicted in Fig. (**1**). Interestingly, Stradiotto and

teammates have fabricated ISE-based potentiometric sensors for the Cl^- ions based on acyclic Ru(II) bipyridyl complexes (**1** and **2**) (Fig. **2**) [12]. Currently, immense interest is being devoted by electrochemists to the development of solid-state membrane-based ISEs. In this direction, single crystals, cocrystals, and polycrystalline pellets have shown a promising role in developing ISEs for several anions (F^-, Cl^-, Br^-, I^-, & SCN^-, *etc*.) and cations like Cd^{2+}, $Pb^{2+,}$ and Cu^{2+} [13].

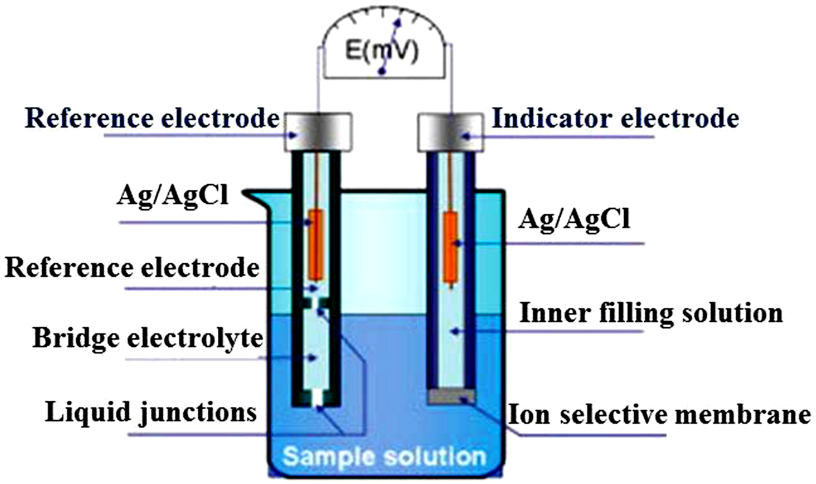

Fig. (1). General electrochemical measurement setup of ISEs. (Reprinted with permission from ref [14], copyright 2013 Elsevier).

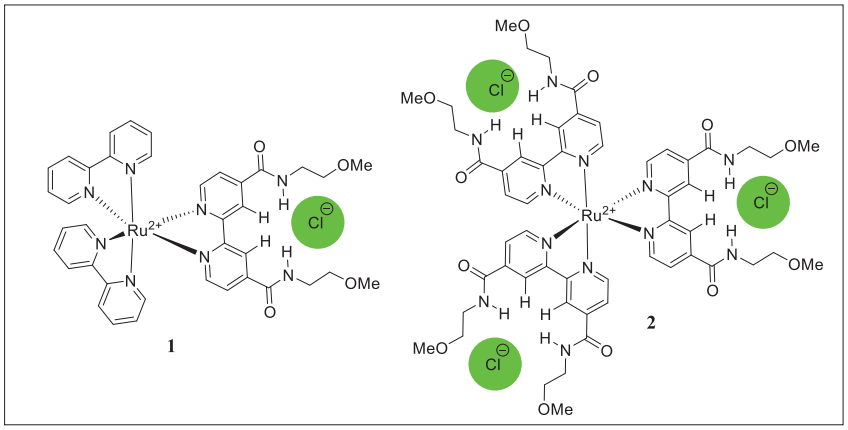

Fig. (2). Ru(II) bipyridyl complexes (**1** and **2**) based ISE potentiometric sensors for Cl^- ions.

LIST OF ABBREVIATIONS

AIE	Aggregation-Induced Emission
Ach	Acetylcholine
ARS	Alizarin Red S
A-IDA	Amperometric Indicator Displacement Assay
AMP	Adenosine Monophosphate
ABAM	*N*-(4-(aminomethyl)benzyl)-1-(anthracen-9-yl)methanamine
AMA	Amantadine
A-IDA	Allosteric Indicator Displacement Assay
ANN	Artificial Neural Network
BPR	Bromopyrogallol Red
BRB	Butyl Rhodamine B
BODIPY	Boron Dipyrromethane
BHQ	Black Hole Quencher
BPHA	*N,N-bis*(pyridin-2-ylmethyl)hexan-1-amine
BSA	Bovine Serum Albumin
BINOL	1,1′-bi-2-Naphthol
C4Ps	Calix[4]pyrroles
CT	Charge Transfer
C-IDA	Colorimetric Indicator Displacement Assay
CHEF	Chelation Induced Enhanced Fluorescence
CCA	Catalytic Chemosensing Assay
DPV	Differential Pulse Voltammetry
DDA	Dimer Dye Assembly Assay
ET	Energy Transfer
ESIPT	Excited-State Intramolecular Proton Transfer
EET	Electron Energy Transfer
e-IDA	Enantioselective Indicator Displacement Assay
FRET	Fluorescence Resonance Energy Transfer
FET	Field Effect Transistors
F-IDA	Fluorescence Indicator Displacement Assay
GO	Graphene Oxide
GCE	Glassy Carbon Electrode

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GAG Glycosaminoglycan

GSH Glutathione

HOMO Highest occupied molecular orbital

Hcy homocysteine

HCA Hierarchical Cluster Analysis

HiPS Human Induced Pluripotent Stem

HTS High-Throughput Screening

ISR Indicator Spacer Receptor

IDA Indicator Displacement Assay

ICT Intramolecular Charge-Transfer

ICDA Indicator Catalyst Displacement Assay

I-IDA Intramolecular Indicator Displacement Assay

LUMO Lowest Unoccupied Molecular Orbital

LE Locally Excited

LOQ Limit of Quantification

LOD Limit of Detection

LDA Linear Discrimination Analysis

MB Methylene Blue

MLCT Metal-Ligand Charge Transfer

M-IDA Metal Indicator Displacement Assay

Mu Murexide

MC-IDA Mechanically Controlled Indicator Displacement Assay

MRSA Methicillin-Resistant Staphylococcus Aureus

MIPs Molecularly Imprinted Polymers

NIR Near Infrared

NAG *N*-acetyl-*β*-D-Glucosaminidase

PET Photoinduced Electron Transfer

Phe Phenylalanine

PV Pyrocatechol Violet

PPi Inorganic Pyrophosphate

PA Picric Acid

PFOS Perfluorooctane Sulfonate

PFOA Perfluorooctanoic Acid

PAR 4-(2-pyridylazo) Resorcinol

PCA Principal Component Analysis

PLS-DA Partial Least Squares Discriminant Analysis

Q_{zz} Quadrupole Moment

QDA Quencher Displacement Assay

ROS Reactive Oxygen Species

RNS Reactive Nitrogen Species

RSS Reactive Sulphur Species

RhB Rhodamine B

Rh-6G Rhodamine 6G

R-IDA Reaction Based Indicator Displacement Assay

SA Sialic Acid

ST Safranine T

SVM Support Vector Machine

SARS-CoV-2 Severe Acute Respiratory Syndrome Coronavirus 2

TICT Twisted Intramolecular Charge-Transfer

Trp Tryptophan

Tyr Tyrosine

TBAF Tetrabutylammonium Fluoride

ThT Thioflavin T

TEA Tetraethyl Ammonium

TNT 2,4,6-Trinitrotoluene

TPE Tetraphenylethene

UC Photon Upconversion

UD Uranine Dye

$\alpha_{\|}$ Molecular Polarizibility

SUBJECT INDEX

A

B

C

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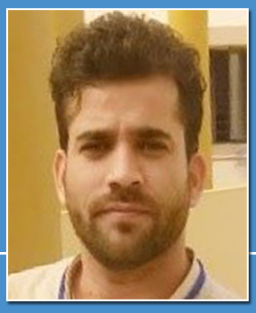

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Dr. Ishfaq Ahmad Rather obtained Ph.D. in 2023 from Jamia Millia Islamia (a Central University), New Delhi, India. His research work was focussed on synthetic organic and supramolecular chemistry in general and design, synthesis and applications of functionalized Calix[4]pyrroles in particular. Then He worked as an Institute Postdoctoral Fellow (IPDF) in the Department of Chemistry, Indian Institute of Technology (IIT) Kanpur, Uttar Pradesh, India, and worked on fluorescence spectroscopy in Prof. Pratik Sen's research group. In 2024, he joined the research group of Prof. Anthony P Davis in the School of Chemistry, University of Bristol where he is currently working on biomimetic carbohydrate recognition in general and towards glucose-responsive switches with tailored properties in particular. He has been awarded with junior research fellowship (CSIR-JRF and UGC-JRF), senior research fellowship (CSIR-SRF), research associate (CSIR-RA), and national postdoctoral fellowship (SERB-NPDF).

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Dr. Rashid Ali is a pioneering researcher engaged in the area of organic & supramolecular chemistry. He received his doctorate degree from the Indian Institute of Technology Bombay (IITB), Mumbai, India. He has more than 13 years of research experience, around one year spent at Sookmyung Women's University, South Korea, as a post-doc fellow. He is a holder of more than 70 original research papers published in the reputed journals like Nature Chemistry, JACS, Chem. Commun., Green Chemistry, Coord. Chem. Rev. etc. Besides, he has edited two books, and contributed ten book chapters in the books published by different publishers. Presently, he is working as an assistant professor in the Department of Chemistry, Jamia Millia Islamia, New Delhi, India.