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# Electrochemical Investigation and Molecular Docking Techniques on the Interaction of Acridinedione Dyes with Water-Soluble Nonfluorophoric Simple Amino Acids

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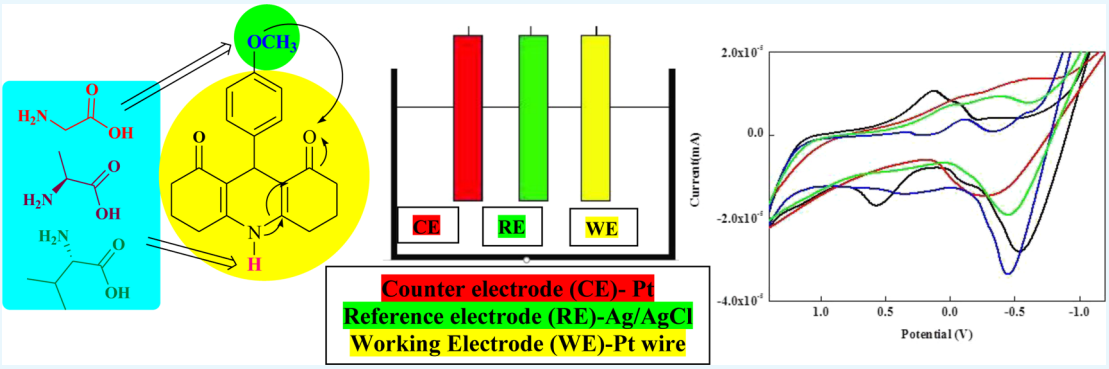


**ABSTRACT:** Electrochemical studies of resorcinol-based acridinedione (AD) dyes with nonfluorophoric simple amino acids, glycine, alanine, and valine, were carried out in water. AD probes are classified into photoinduced electron transfer (PET) and non-PET-based dyes, wherein the electrochemical properties and photophysical and photochemical behavior vary significantly based on the nature of substituent groups and the nature of the solute. The oxidation potential of PET dye (ADR1) to that of non-PET-based dye (ADR2) differs significantly such that the addition of amino acids results in a shift of the oxidation peak to a less positive potential and the reduction peak to a lesser negative potential. The extent of shift of oxidation and reduction potential in PET dye is more pronounced than that of non-PET dye on the addition of valine rather than glycine. The variation in the shift is attributed to the presence of an electron-donating moiety ($OCH_3$) group in the ninth position of ADR1 dye. Consequently, the quenching of fluorescence is observed in ADR2 with non fluorophoric amino acids that are authenticated by the shift of the anodic and cathodic peaks toward a lesser positive potential. Molecular docking (MD) studies of PET and non-PET dye with amino acids portray that neither hydrophobic interactions nor electrostatic or weak interactions such as van der Waals and pi−pi interactions govern the electrochemical nature of dye on the addition of amino acids. Furthermore, the formation of a conventional hydrogen bond between dye and amino acid is established from MD studies. The existence of dye−water−amino acid competitive hydrogen-bonding interactions is presumably well-oriented throughout the aqueous phase as observed through photophysical studies which support our electrochemical investigation.

## INTRODUCTION

Fluorescence spectroscopy of probes coupled with electrochemistry play a significant and important role on the view of chemists and biologists, which is attributed to their photophysical and redox properties.[1,2] The electrochemical studies in combination with fluorescence techniques help us in providing a detailed understanding of local organization in monolayer and multilayer interfaces.[3] However, these studies correlate to the oxidation and reduction properties of the fluorescent probes predominantly in nonaqueous solvents and to a lesser extent in aqueous medium.

Acridinedione (AD) dyes belong to a class of extrinsic fluorescent probes, wherein enormous studies confined to the photophysical studies in the presence of hydrogen-bonding and hydrophobic solutes have been carried out both in

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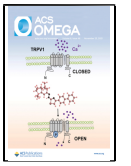

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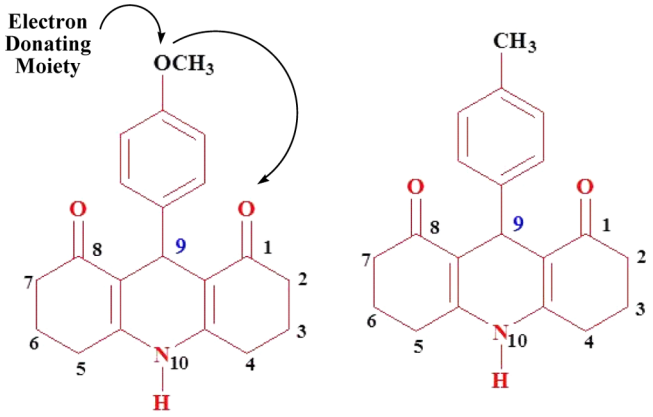
aqueous medium and to a certain extent in nonaqueous solvents. AD dyes act as the host molecule, and the nonfluorophoric solutes such as urea derivatives, guanidine hydrochloride, amides, proteins, amino acids, and sugars behave as the guest molecule.[4−14] Furthermore, these dyes exhibit characteristic adsorption, emission, fluorescence lifetime, and oxidation potential properties that are based on the substituents in the ninth and tenth position [Scheme 1] and are very much solvent-mediated.

**Scheme 1. Structure of PET and Non-PET-Based AD Dyes**




The characterization and electrochemical behavior of dimedone and resorcinol-based AD family derivatives are well-established in nonaqueous solvents,[15−17] and to the best of our knowledge, these studies were largely focused on the photophysical and photochemical properties only. A recent report on ADR1 dye in the presence of gum arabic (GA) in aqueous solution reveals that the oxidation potential is governed predominantly through hydrogen-bonding interactions.[9] Cyclic voltammetry (CV) studies portray that all AD dyes undergo irreversible oxidation irrespective of the substitution on the nitrogen atom.[15−17] The significance of ADR1 dye compared to other extrinsic fluorescent probes is that they have a better solubility in water and exhibits both intramolecular charge transfer (ICT) and photoinduced electron transfer (PET) characteristics. These properties impart a significant role in their oxidation and reduction potentials. Herein, the electrochemical studies provide useful information on the current−potential characteristics in the presence of water-soluble hydrogen-bonding solutes, wherein a considerable study on the photophysical properties concerned to the excited state is well-established.

The electrochemical properties of a given surface-bound or physisorbed molecule depend sensitively on its microenvironment,[18] and CV is effective in evaluating physical properties such as electron-transfer kinetics and most probable type of interaction existing at the interface. The purpose of combining fluorescence techniques with electrochemical and molecular docking (MD) studies possesses several advantages. CV is widely used for the measurement and characterization of surface-bound electroactive species[19,20] and provides significant information on the nature of the electroactive species, whereas MD studies provide significant information about the nature of the interaction and the energetics of the host−guest systems. Fluorescence techniques coupled with these methods provide the link between the bulk and interface regions that are ascertained experimentally (CV) and theoretical methods. Since photophysical studies of AD dyes with solutes provide an in-depth information about the nature of interaction in the solvent phase,[8,13] MD methods provide the various molecular interaction of host−guest molecules excluding the water molecule. The electrochemical studies were carried out with AD dyes because knowledge of the electrochemical redox potentials is important in the process of ascertaining laser dye stability and reactivity. This could give an insight into the feasibility of other reactions using these compounds which are mostly nonfluorophore solutes. The products obtained on electrolysis have been isolated and characterized. The chemical oxidation of AD dyes does not yield a single desired product in the case of N-substituted compounds, whereas the electrochemical oxidation yields a single product as reported. The dye ADR1 is a PET-based dye and ADR2 is a non-PET dye.[21] In the present study, a comparison on the electrochemical nature of ADR1 versus ADR2 with glycine, alanine, and valine is investigated in water for which a theoretical approach is provided through MD techniques.[22,23]

## RESULTS AND DISCUSSION

The dyes chosen in the present study undergo irreversible oxidation. Solutions of the dyes in water ($3.0 \times 10^{-5}$ M) were chosen for the CV studies. Substitution in the ninth position of AD dye causes the oxidation peak to shift to a more positive potential, indicating a decrease in the ease of oxidation. Such a behavior has been attributed to steric factors based on the observation made by Stradins et al.[24] in the case of 1,4-dihydropyridines. Similar theories and explanation were presumably extended to AD dyes because the potentials for compounds containing an electron-releasing group in the ninth position are easily oxidized compared to the compounds containing non-electron-releasing group in the ninth position.

The overlay plot of cyclic voltammograms of ADR1 and ADR2 dyes is shown in Figure 1. The variation in the oxidation

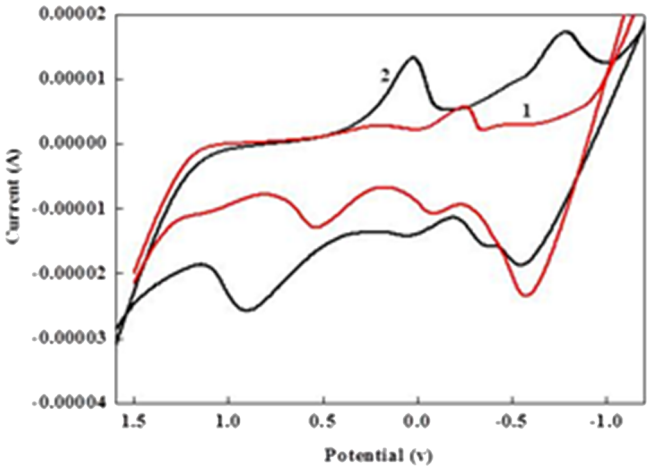



**Figure 1.** Cyclovoltammogram of AD dyes in water. (1) ADR1 and (2) ADR2.

and reduction peaks of PET and non-PET dyes is attributed to the presence of the electron-donating group ($-OCH_3$) in the ninth position. The cyclic voltammogram of ADR1 exhibits one cathodic peak at −0.25 V and two anodic peaks at −0.10 and +0.54 V, respectively. The potential around +0.54 V is of significance, and in our present study, the peak obtained at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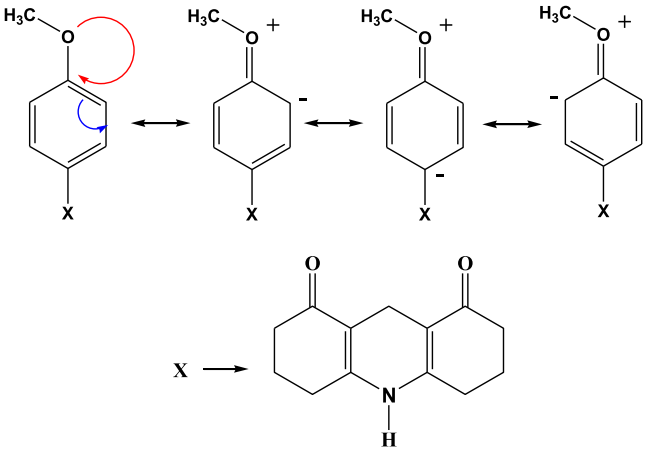
−0.10 V with −1.014 × $10^{-5}$ A is considered as a reverse cathodic peak. A schematic representation resulting in the formation of the oxidation peak in ADR1 dye is provided in Scheme 2. The scheme of ADR1 (PET) depicts the redox

**Scheme 2. Schematic Representation of the Formation of the Oxidation Peak of ADR1 Dye**




behavior of lone pair of electron on the oxygen atom yielding an oxidation peak at +0.54 V and a reduction peak at −0.25 V in aqueous medium.

The cyclic voltammogram of ADR2 (non-PET) exhibits two oxidation peaks at + 0.05 and +0.90 V. This is similar to that of the voltammograms observed for several non-PET dyes of dimedone- and resorcinol-based AD dyes in nonaqueous solvents.[17] In our present study, the peak obtained at −0.10 V with −1.014 × $10^{-5}$ A is considered as the reverse cathodic peak. The peak at 0.05 V has a minimal anodic current of −1.41 × $10^{-5}$ A. The anodic peak at + 0.90 V is attributed to the oxidation peak due to the loss of electrons from the N−H group and one corresponding reversible reduction at −0.78 V as reported by Srividya et al.[16,17] A similar pattern of oxidation potential in the range of +1.1 to +1.2 V has been established in nonaqueous solvents for several AD dyes.

The CV plots of ADR1 and ADR2 in the presence of amino acids are shown in Figures 2 and 3, respectively. The anodic and cathodic peak potentials and the corresponding peak currents of independent analytes under study are provided in Table 1 and 2, respectively.

The addition of glycine (3.0 × $10^{-2}$ M) to ADR1 resulted in a shift in the oxidation potential toward a less positive value (+0.54 to +0.40 V) and a significant shift in the reduction potential value toward a more negative region (−0.25 to −0.36 V). A similar pattern was also observed in the interaction of a water-soluble food hydrocolloid-like GA to ADR1 dye.[9] The shift in oxidation of potential of ADDR with GA toward less positive potential region indicates that electrostatic interactions also play a crucial role apart from hydrogen-bonding and hydrophobic interactions. Xiao et al.[25] studies on the potential−current studies revealed that the dissociation of amino acids on the Pt surface increases with alkyl substitution and other functional groups of amino acid chain and the self-inhibition of amino acid oxidation originate from the strongly adsorbed cyanide which is oxidized at potentials above 0.2 V against the calomel electrode. The oxidation potential in our study is more positive, and the presence of the potential peak arising around 0.2−0.3 V does not influence the redox

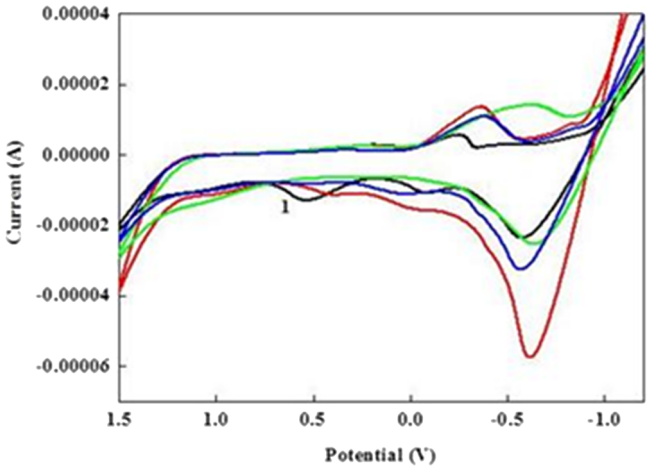



**Figure 2.** Cyclic voltammogram of ADR1 dye with amino acids in water. (1) ADR1 dye alone. Red-, blue-, and green-colored lines represent the voltammogram of ADR1 dye in the presence of glycine, alanine, and valine, respectively.

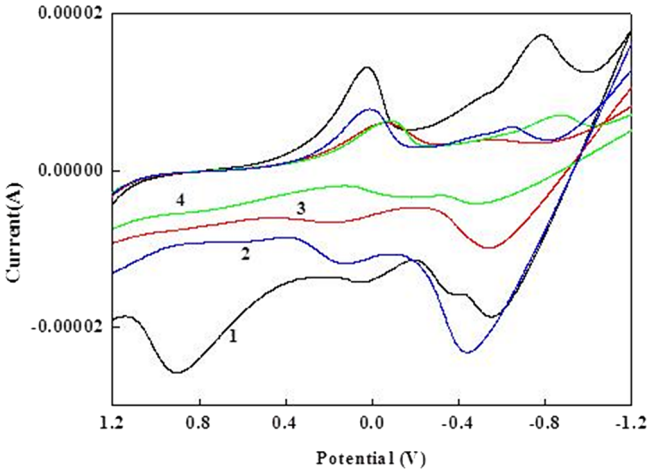



**Figure 3.** Cyclic voltammogram of ADR1 dye with amino acids in water. (1) ADR1 dye alone. (2) ADR1 dye + glycine. (3) ADR1 dye + alanine. (4) ADR1 dye + valine.

properties of ADR1 and ADR2 dyes. Furthermore, there are reports which portray that hydrogen-bonding influences by the solute results in a shift in the oxidation and reduction potential of dyes.[26,27] Similarly, the addition of alanine and valine also resulted in a marked shift in the oxidation potential toward a less positive region (+0.54 to +0.027 V), but this shift is more pronounced than that observed in the case of glycine. The shift in potential is more pronounced in alanine and valine than in the case of glycine, even though the amino acids differ by alkyl group substitution. Interestingly, the peak current corresponding to the oxidation potential of ADR1 dye increases irrespective of the nature of amino acids which reveals that the ET process is facilitated by the introduction of amino acids. The cyclic voltammogram of ADR1 exhibits one cathodic peak at −0.25 V and the anodic peak current increases significantly in the presence of amino acids accompanied with a shift in the potential toward a more negative region (Table 1).

A clear understanding on the electrodics of all three amino acids alone illustrates the anodic peak at −0.19, −0.42, and −0.50 V. However, it can be considered as a reverse cathodic peak. Alanine has the highest anodic peak current of 1.12 ×


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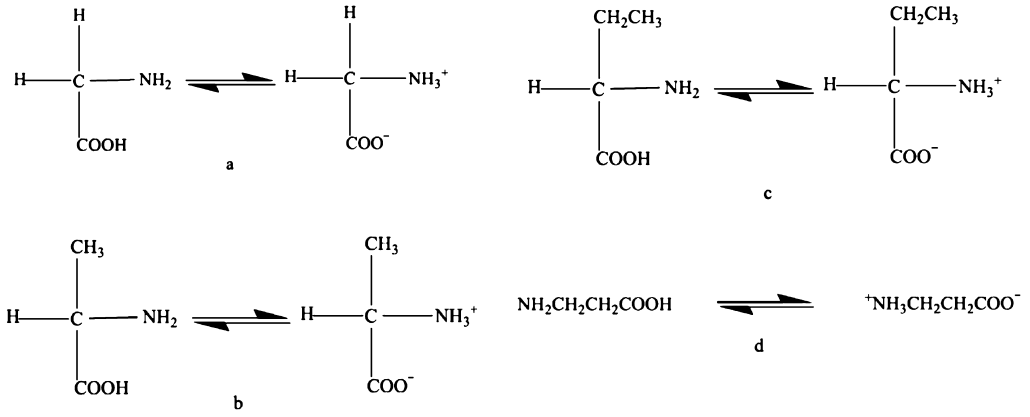
**Table 1. Current−Potential Characteristics of ADR1 Dye with Amino Acids in Water**

| host−guest | dye × $10^{-5}$ M | AA[M] × $10^{-2}$ M | $E_{pa}$ (V) | $I_{pa}$ (A) × $10^{-5}$ | $E_{pc}$ (V) | $I_{pc}$ (A) × $10^{-6}$ |
|---|---|---|---|---|---|---|
| ADR1 | 3.0 | | −0.58 | −2.39 | −0.25 | 5.61 |
| | 3.0 | | −0.10 | −1.014 | −0.84 | 9.16 |
| | 3.0 | | **+0.54** | **−1.28** | | |
| ADR1-glycine | 3.0 | 3.0 | −0.62 | −5.73 | −0.36 | 11.4 |
| | 3.0 | 3.0 | −0.017 | −1.55 | −0.83 | 8.6 |
| | 3.0 | 3.0 | **+0.40** | **−1.15** | | |
| ADR1-alanine | 3.0 | 3.0 | −0.57 | −3.26 | −0.39 | 1.05 |
| | 3.0 | 3.0 | **+0.27** | **−1.07** | | |
| ADR1-valine | 3.0 | 3.0 | −0.63 | 2.52 | −0.62 | 1.42 |
| | 3.0 | 3.0 | **+0.19** | **−2.97** | | |

**Table 2. Current−Potential Characteristics of ADR 2 Dye with Amino Acids in Water**

| host−guest | dye × $10^{-5}$ M | AA[M] × $10^{-2}$ M | $E_{pa}$ (V) | $I_{pa}$ (A) × $10^{-6}$ | $E_{pc}$ (V) | $I_{pc}$ (A) × $10^{-6}$ |
|---|---|---|---|---|---|---|
| ADR2 | 3.0 | | −0.57 | −1.88 | +0.03 | 1.37 |
| | 3.0 | | +0.05 | −1.41 | −0.78 | 1.73 |
| | 3.0 | | **+0.90** | **−2.256** | | |
| ADR2-glycine | 3.0 | 3.0 | +0.55 | | | |
| 0.15 | **−9.70** | | | | | |
| −6.38 | −0.059 | | | | | |
| −0.57 | 5.78 | | | | | |
| 4.27 | | | | | | |
| ADR2-alanine | 3.0 | 3.0 | +0.45 | **−23.3** | 0.025 | 7.91 |
| | 3.0 | 3.0 | **0.12** | | | |
| 0.56 | **−11.9** | | | | | |
| −8.93 | −0.644 | 5.75 | | | | |
| ADR2-valine | 3.0 | 3.0 | **−0.50** | | | |
| 0.11 | **−3.94** | | | | | |
| −3.33 | −0.096 | | | | | |
| −0.86 | 6.43 | | | | | |
| 7.19 | | | | | | |

**Scheme 3. Zwitter Ionic Structure of Simple Amino Acids**




$10^{-4}$ A, and valine exhibits the lowest cathodic peak current of 1 × $10^{-6}$A. The electrochemical process of glycine, alanine, and other amino acids such as serine with the platinum working electrode has been studied vividly and reported in the literature[28−30] which provides us the variation in the properties on adsorption to the surface of the electrode.

Table 2 represents the values obtained from the cyclic voltammogram of ADR2 with the amino acids. The anodic potential at −0.55, −0.50, and −0.45 V can be considered as reversed cathodic potential and it appears for all the nonfluorophores with ADR2 dye. This can be attributed to the hydrogen evolution. This reversed cathodic peak does not contribute to any significant electrochemical process. The addition of glycine, valine, and alanine to ADR2 dye exhibits anodic potential values at 0.15, 0.11, and 0.12 V, respectively. The anodic potential is attributed to the oxidation of functional groups of amino acids or decomposition. Interestingly, for alanine, two anodic peaks at 0.12 and 0.52 V were observed which was not found in the case of glycine.

Based on the shift in the oxidation potentials of ADR1 and ADR2 dye in the presence of amino acids, we correlate the shift to specific binding interactions induced at the electrode interface on the introduction of amino acids. The variation in the current−potential characteristics of both PET and non-


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Table 3. Energetics of ADR1 Dye−Amino Acid in kcal $mol^{-1}$

| conformation | binding energy | ligand efficiency | intermolecular energy | vdW + H bond + desolv energy | electrostatic energy | torsional energy | total internal unbound |
|---|---|---|---|---|---|---|---|
| ADR1V | 96,000 | 4037.5 | 96,900 | 49,100 | 47,900 | 0.6 | 1.35 |
| ADR1A | 99,400 | 4141.67 | 99,400 | 50,900 | 48,600 | 0.6 | −0.58 |
| ADRIG | 86,400 | 3600 | 86,400 | 46,500 | 39,900 | 0.6 | −0.95 |

PET dyes in the presence of amino acids at the electrode interface is presumably attributed to any or all of the following phenomenon that actually exist in the aqueous phase when the dye molecule is surrounded by aqueous solution of amino acid.

**Case 1.** All the amino acids exist in the zwitterionic conformation and they can act as excellent hydrogen-bonding donor and an acceptor, as shown in Scheme 3. The H atom of $^{+}NH_3$ can involve in a hydrogen-bonding interaction with the oxygen atom of water molecules and the $O^-$ atom in the $COO^-$ functional group of amino acid presumably involve in the formation of hydrogen-bonding with the hydrogen atom of water molecules such that an uniform distribution of amino acid−water hydrogen-bonding arrangement exists throughout the phase. This type of hydrogen-bonding pattern is possible for all the amino acids taken in the present study such that amino acid−water hydrogen-bonding exists apart from electrostatic interactions. Since the amino acids exist in the zwitterionic form in water, the forces that contribute involve electrostatic interactions and the presence of methyl group moieties also impart hydrophobic influences such that there exists a combined interaction operating in the aqueous phase

**Case 2.** If the H atom in the $^{+}NH_3$ group of the amino acids had been directly involved in conventional hydrogen-bonding with the oxygen atom ($-OCH_3$) of ADR1 dye, an apparent shift toward the red region would have been resulted irrespective of the nature of the amino acid. Interestingly, a bathochromic shift in the emission spectra of ADR1 dye was observed on the addition of glycine, whereas in the case of absorption spectra addition of glycine or alanine or valine, neither a red nor blue shift was observed [Supporting Information Figures S1 and S2]. The abovementioned observation is well-established and reported in the photophysical studies of amino acids with PET- and non-PET-based AD dyes in water.[11,12] This further authenticates that although the H atom in the $^{+}NH_3$ group of the amino acid is involved in hydrogen-bonding with the $OCH_3$ moiety, it is definitely not responsible for the variation in the PET process in ADR1 dye and could be attributed to observed shift in the oxidation potential values of ADR1 dye. This was authenticated and confirmed based on the shift in the oxidation potential values of ADR2 dye in water on the addition of amino acids. ADR2 dye does not possess any electron-donating moieties in the ninth position and still exhibits a drastic shift in the oxidation potential compared to ADR1 dye in the current−potential characteristics. If the shift had been attributed to the $OCH_3$ moiety, no significant change in the neither oxidation nor reduction properties of ADR2 dye would have been observed. On the contrary, a well-distinguishable property on the electrodics of non-PET dye resulted. The abovementioned studies clearly elucidate that the shift of oxidation potential of ADR1 dye is presumably due to hydrogen-bonding at the oxygen atom of the $OCH_3$ moiety and in the case of ADR2 dye, weak nonconventional hydrogen-bonding through C−H hydrogen of the methyl moiety is probably involved

**Case 3.** It is well-known that the hydrogen atom of water molecules forms a hydrogen bond with the carbonyl oxygen of AD dye and molecular simulation studies establish the existence of such a type of bonding.[10] Similarly, the possibility of hydrogen atoms in the $^{+}NH_3$ moiety involved in the hydrogen bond as observed in the case of water molecules could also coexist. This type of hydrogen-bonding arrangement is possible in both ADR1 and ADR2 dyes such that the shift in the oxidation potential is observed in both these dyes irrespective of the nature of the amino acid

**Case 4.** If the $O^-$ atom in $COO^-$ of all the amino acids had been directly involved in hydrogen-bonding with N−H hydrogen of ADR1 or ADR2 dye molecule, an apparent shift in the absorption or emission maxima would have been resulted irrespective of the nature of the amino acid. A considerable red shift in the emission maxima resulted in the case of addition of glycine, whereas no shift in the absorption spectrum was observed. This is presumably attributed to the large macroenvironment consisting of several water molecules in comparison with that of dye and amino acid moieties. Interestingly, the photophysical studies of alanine or valine with PET or non-PET dye is entirely different from that of glycine such that a blue shift accompanied with fluorescence quenching was observed. The variation in photophysical concepts has been well-established in the concept of amino acids with AD dyes,[11,12] whereas the electrochemical studies provide a different approach on the interface regions when compared to photophysical studies that aligns with the macroenvironment and bulk phenomenon. A shift in the emission maxima toward the red region of ADR1 dye alone resulted on the addition of glycine, whereas no such shift was observed in the case of ADR2 dye. This clearly suggests that the excited-state properties of these dyes are only influenced considerably as reported through photophysical studies and the behavior of individual amino acids in water is found to be entirely different, even though they differ by methyl group substitution

Based on the abovementioned case studies, the possibility of case 1 or 2 or 3 could probably coexist in the aqueous phase or any of these interactions may predominate and presumably influence the electrochemical properties of AD dyes since the hydrogen-bonding formation can be classified into very strong, strong, and weak based on the electronegativity of hydrogen-bonding acceptor and donor groups. The possibility of the type of hydrogen-bonding interaction, hydrophobic interactions, and other weak interactions of ADR1 dye with amino acids was further examined by MD studies.

**Docking studies.** The MD technique was employed to predict the binding constant and probable binding site of ADR1 dye with glycine, alanine, and valine through different conformers generated through software (Biovia Discovery studio visualizer). The structures were arranged based on their corresponding energetics (kcal $mol^{-1}$) with the formulation of several parameters leading to the dye−amino acid binding. The exegetics related to the formation of these structures and the


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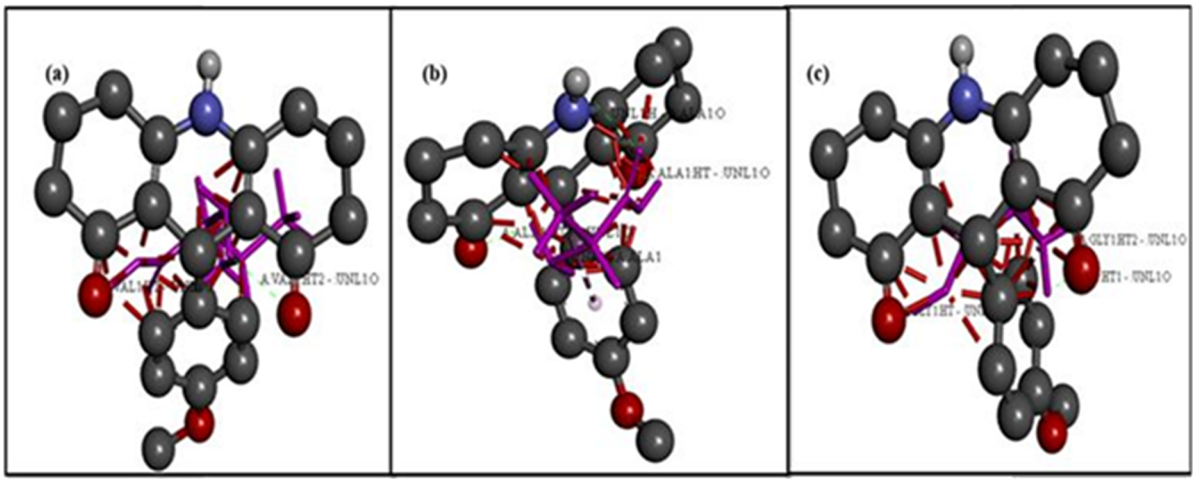


**Figure 4.** Binding interactions of ADR1 dye conformations with amino acids visualized using a Biovia Discovery Studio visualizer: (a) ADR1V; (b) ADR1A; and (c) ADRIG. ADR1 is represented in the scaled ball-and-stick model and colored based on atom colors (C: gray; O: red; N: violet; and H: light gray). Amino acid is represented in pink color.

**Table 4. ADR1−Amino Acid Molecular Interactions**

| conformation | hydrogen-bonding donor−acceptor linkage of amino acid (atom···ligand atom) | hydrogen-bonding distance (Å) | hydrophobic interactions | other interactions |
|---|---|---|---|---|
| ADR1G | (GLY1) N−H···O═C(AD) | 0.918 | nil | no pi−pi and pi−alkyl interactions |
| | (GLY1) N-HT1···O═C(AD) | 1.85 | | |
| | (GLY1)N-HT2···O═C(AD) | 1.62 | | |
| ADR1A | (ALA1) N−H···O═C(AD) | 1.84 | unfavourable | no pi−pi and pi−alkyl interactions |
| | (ALA1) N−H···O═C(AD) | 2.35 | | |
| | (ALA1) N-HT1···O═C(AD) | 2.13 | | |
| ADR1V | (VAL1) N−H···O═C(AD) | 1.29 | unfavourable | nil |
| | (VAL1) N-HT2···O═C(AD) | 2.46 | | |

hydrogen-bonding parameters are provided in Table 3. In general, the most stable conformer formed due to docking of dye with amino acids is correlated to minimum binding energy that is accompanied with a lesser inhibitory constant value (ki). Apart from these parameters, the formation of a most stable structure is favored when the intermolecular energy and the ligand efficiency (dye) values are less negative. The less energetically stable binding structure corresponds to those which have a very high inhibitory constant value. Furthermore, closer examination of these structures with different amino acids also provides the nature of the binding forces leading to the stability of the complexes. ADR1V, ADR1A, and ADR1G represent binding of valine, alanine, and glycine to PET dye, respectively.

The energetics of PET dye with amino acids clearly visualizes that there exists direct binding of dye with amino acids and the formation of these structures in the gas phase is not found to be favorable as observed in the case of PET-based AD dyes in water with several drugs and proteins.[8] The very high inhibitory constant obtained in the case of any amino acid is highly unfavorable for any binding studies and the ligand efficiency values accompanied with an enormous positive binding energy and intermolecular energy signifies that the formation of a complex is least-favored. Since amino acids are comparatively small molecules compared to globular proteins such as BSA, HSA, and ovalbumin, a smaller grid size is used in this study. The grid size is almost 1/16th of the size compared to that of dye docking with BSA.[8] The binding constant values are a clear indication that there exists no direct binding affinity between the amino acid and $OCH_3$ moiety of dye such that these interactions are not favorable. The abovementioned studies confirm the existence of case 3 as postulated. Furthermore, we also rule out the possibility of case 2 and 4 which does not cease to exist. The hydrogen atom of the protonated amino moiety of glycine, alanine, and valine form hydrogen-bonding with the carbonyl oxygen atom situated at the first or eighth position of AD dye. Furthermore, energetically unfavorable hydrophobic interactions existing between the dye and amino acid are established from docking studies, as shown in Figure 4.

The hydrogen-bonding parameters are tabulated in Table 4 and reveal that the ADR1 dye acts as the hydrogen-bonding acceptor through the carbonyl oxygen and the amino acid as the donor. H, HT1, and HT2 represent the three hydrogen atoms of the protonated amino group that are labeled in the structure of amino acid. This type of arrangement is also possible in ADR2 dye and this was confirmed based on the shift in the oxidation potential toward a less positive value indicating a hydrogen-bonding interaction. The abovementioned observation authenticates that irrespective of the nature of the substituent in the ninth position in AD dyes, the amino acid involves in a direct bonding with the carbonyl oxygen in addition to other hydrogen-bonding sites.

**Nature of Hydrogen-Bonding between Dye and Amino Acids.** It is well-known that hydrogen bonds are found in protein crystallography indirectly and if a proper


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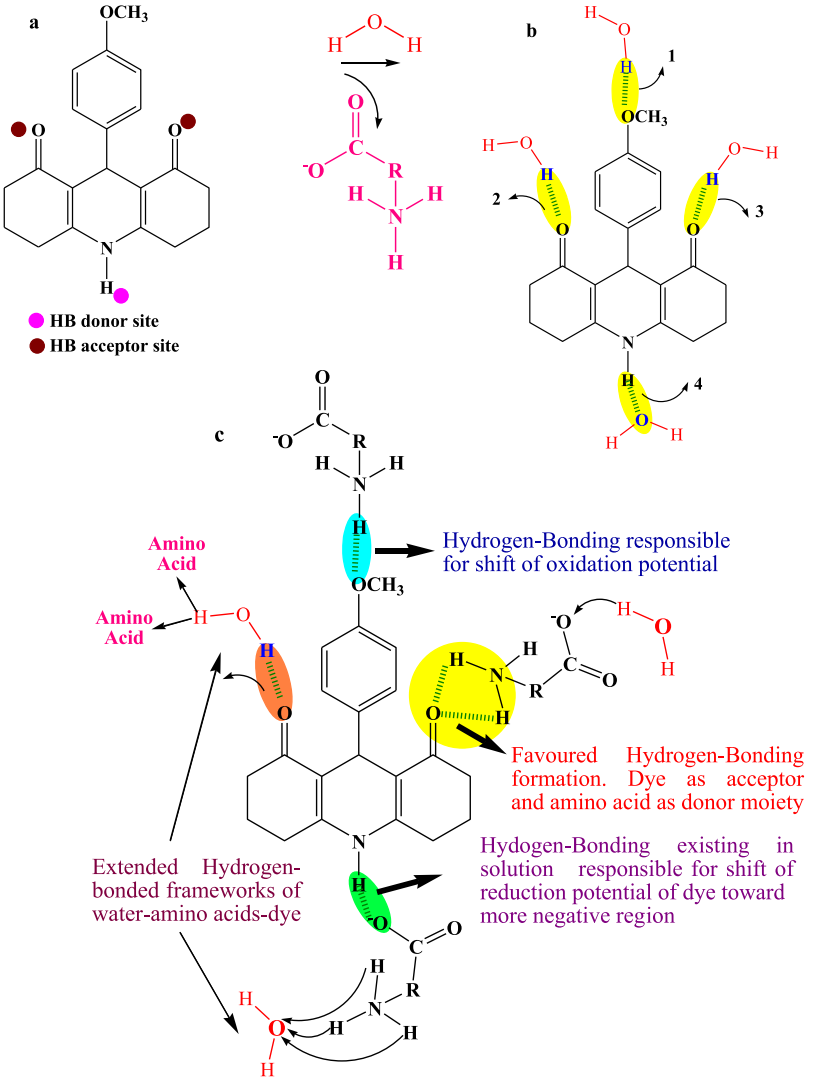
**Scheme 4. Representation of Dye−Amino Acid Assembly in the Aqueous Phase**[a]




[a](a) Hydrogen-bonding donor and acceptor sites in ADR1 dye. (b) Introduction of water molecules results in possible hydrogen-bonding with dye in which sites 1 and 4 are unfavorable positions, whereas 2 and 3 are most favored. (c) Most probable orientation of dye−amino acid in the presence of water molecules. R represents H, $-CH_2$, and $-CH(CH_3)$

hydrogen-bond acceptor−donor pair is within the correct distance, the bond is considered to be a conventional hydrogen bond. This distance is generally in the range of 2.5−3.3 Å, with 3.0 Å being the most common value for protein and water hydrogen bonds. The bond angle is also concerned in determining the strength of the hydrogen bond. The closer the hydrogen bond is to the geometry, the stronger is the bond. Hydrogen bonds often occur in networks frequently with water mediating, and water has the unique property such that it is facile at hydrogen-bonding since it acts as an acceptor and a donor. Even though hydrogen bonds are specific, short-range, and directional nonbonded interactions, they are governed by the nature of the solute and the electronegative atom (usually N, O, or F) involved in bonding.

In the present study, the role of charged ions in the amino acid and the hydrophobic moieties influence the microscopic environment and the local dielectric constant of the surrounding medium also governs the bonding interactions. The hydrogen-bonding in proteins and biomolecules can be categorized into very strong, strong, and weak based on the nature of the donor and acceptor groups. When the acceptor happens to be $O^-$ or $N^-$ or $F^-$ and the donor is $N^+H_3$, $X^+-H$, and F−H hydrogen, the hydrogen-bonding is classified as very strong. Similarly, when the acceptor moieties such as O═C, O−H, N, S═C, and F−H are bound to donors such as $N^+-H$, O−H, and F−H, the resulting hydrogen-bond formation is categorized as a strong interaction. Apart from these moieties, the hydrogen-bonding resulting from C, P, S, Cl, and Br is classified into weaker interactions. Herein, the donor and acceptor atoms are N−H hydrogen and carbonyl oxygen, respectively, and can be broadly classified as stronger interaction. Furthermore, the $O^-$ atom in turn forms hydrogen bonds with a N−H hydrogen atom (tenth position of AD dyes) or with a H atom of another water molecule that forms a well-extended framework of hydrogen bonds. The hydrogen-bonding distance of ADR1 with amino acids results in the order glycine < alanine < valine, and interestingly, the hydrophobicity scale is also of the same order. The variation in the gas-phase properties compared to the aqueous phase nature of amino acid results in difference in the hydrogen-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bonding parameters obtained through docking studies and this could not be correlated to amino acid−dye in the aqueous phase. The number of water molecules surrounding the amino acid, the hydration co-spheres, the number of hydrogen-bonding sites, the electrostatic nature resulting due to charged ions, and the coexistence of hydrophobic interactions in the aqueous phase determine the strength of the hydrogen bond and these combined interactions operating in the aqueous phase differ from that of the gas phase.

Based on the electrochemical and photophysical nature of the ground-state interactions of dye−amino acid and MD techniques, we have postulated a scheme that presumably exists in the aqueous phase which satisfies most of our theories proposed. The schematic representation of the existence of amino acid−dye−water at the interface region is postulated in Scheme 4 representing ADR1 dye. The possible donor and acceptor moieties in AD dyes are 1 and 2, respectively. In addition, ADR1 dye has one more acceptor site in $OCH_3$, as shown in Scheme 4, and in the case of ADR2 dye, a similar pattern of hydrogen-bonding is possible except in position 1 (marked in b of Scheme 4).

The scheme also provides the possibility of bonding of dye−amino acid through a spacer, wherein the water molecule fits as a linker or serves as an extended conjugative molecule with host−guest molecules such that the existence of case 1 or case 3 is justified. The schematic representation of the dye−water−amino acid network, as shown in Scheme 4, for AD dyes depends on the microenvironment and the number of water molecules bound to the amino acid. The extent of the hydrogen-bonding network definitely depends on the nature of the "R" group of amino acid and the ease of the formation of number of microscopic environments that consist of several competitive hydrogen-bonding microenvironments such as water−water, amino acid−water, dye−water, dye−amino acid, dye−amino acid−water, and dye−water−amino acid frameworks.

## MATERIALS AND METHODS

Glycine, alanine, and valine of purity above 99% were obtained from Merck chemicals and used as procured. ADR dyes were synthesized as reported in the literature.[21] Cyclic voltammogram studies were recorded using a CH INSTRUMENT CH1604E electrochemical analyzer. A platinum electrode with a surface area of 0.0314 $cm^2$ was employed as the working electrode and Ag/AgCl as the reference electrode. A platinum wire was employed as the auxiliary electrode. A supporting electrolyte (0.01 M KCl) was used in all our electrochemical experiments for recording voltammograms. The voltammograms of ADR dyes with varying concentration of amino acids (ranging from $3.0 \times 10^{-4}$ to $3.0 \times 10^{-1}$ M) were recorded at various scan rates ($50 > \nu < 500$ mV $s^{-1}$). The solution of dyes and amino acids was prepared using triply distilled water. The bulk concentration of AD dyes involved in our studies was $3.0 \times 10^{-5}$ M.

**MD Techniques.** ADR1 and ADR2 dye structures were drawn and optimized using Chemsketch and saved in MDL-mol format and converted to pdb format using open babel molecular converter program. The SMILES format was generated using Chemsketch. The PET and non-PET dye properties were calculated using Molinspiration tool.[22] The polar hydrogen and kollman charges were added and saved in the pdbq format. The ligand structures (amino acids) were uploaded; center node and torsional bonds were selected and saved in the pdbqt format. In grid preparation, the host−guest system was saved in the pdbqt format; later grid spacing was set as 0.560 Å with a grid box size of 16 × 16 × 16 Å. Lamarckian genetic algorithm was applied in docking studies. Ten (10) genetic algorithm (GA) runs were performed with the following parameters: a population size of 150, a maximum number of $2.5 \times 10^6$ energy evaluations, and a maximum number of 27,000 generations, and other parameters were default. The region of the most populated of the first 10 clusters was selected as the probable binding region which is universally accepted. The resulting conformations were clustered using a root-mean-square deviation of 2.0 A and the clusters were ranked in the order of increasing binding energy of the lowest binding energy conformation in each cluster. Energies were calculated based on the auto-dock scoring function.[23] Dye−amino acid binding was visualized using a Biovia discovery studio visualizer and analyzed for hydrogen-bonding, hydrophobic, and van der Waals interactions.

## CONCLUSIONS

All the amino acids effectively influence the redox properties of both PET and non-PET dye resulting in variation in the potential and current characteristics in aqueous solution. A characteristic and strong hydrogen-bonding interaction existing between the dye and amino acid governs the oxidation and reduction potential of AD dyes irrespective of the nature of the substitution in the ninth position. Dye acts as the acceptor, whereas amino acid as the donor moiety is established from MD techniques. The hydrogen-bonding influences predominate and govern the binding interaction between dye and amino acid, wherein highly unfavorable hydrophobic interactions also coexist along H-bonding. The combination of electrochemical techniques in association with photophysical process and with the aid of MD studies is used as an efficient tool in establishing the behavior of an electrochemically active fluorophore with an amino acid both at the bulk and the interface regions of a macroenvironment containing a large number of water molecules.

## ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.1c03172.

Absorption spectra of PET-based AD dye: ADR1 with glycine and alanine, absorption spectra of ADR1 dye with glycine in water, ADR1 dye alone, ADR1 dye +0.6 M glycine, ADR1 dye +0.9 M glycine, and ADR1 dye +1.2 M glycine and absorption spectra of ADR1 dye with alanine, 0.60 M alanine, 1.2 M alanine, ADR1 dye alone, ADR1 dye + 0.60 M, and ADR1 dye + 1.2 M alanine (PDF)

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## Notes

The authors declare no competing financial interest.

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