Change in spectral properties of dyes upon immobilization on silicon surfaces: a combined theoretical and experimental study

Abhi Mukherjee¹ · Kishor G. Thorat² · Nagaiyan Sekar² · Siddhartha Panda¹

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Abstract

Organic dyes, both in solution phase and in immobilized condition, are useful tools for optical detection. Studies have shown that spectral property of dyes in solution are different from that in the immobilized state. In this work, we have studied the optical properties of dyes in solution as well as in immobilized state with three categories of dyes, mono-carboxylic mono-functional, bi-carboxylic bi functional and bi-carboxylic mono-functional. Two linkers (APTES and PEI) were used for attaching the dyes to the silanol terminated silicon surface. Geometries were optimized using density functional theory. Time dependant density functional theory was used to calculate the absorption spectra of free and attached dyes. B3LYP and CAM-B3LYP hybrid functional were used for the theoretical calculations. Experimental results showed that dyes upon immobilization experienced a large red shift in the absorption maxima. The value of the red shift in absorption maxima was comparable for both the linkers. Theoretical calculations indicated this large shift is not due to bond formation between dye and the linker. This leads to the conclusion that aggregation takes place between dye molecules upon immobilization but not in solution, and this causes the large red shift in the absorption maxima values of the immobilized dyes.

Keywords Dye · Immobilization · DFT · TDDFT · B3LYP.CAMB3LYP · Spectra · Linker · Aggregates

1 Introduction

Conjugated organic dyes, which can react with the desired chemical species with transduction into an optical signal show promise for use in optical sensors and other photonic applications [1–4]. For quick detection of analytes in lab-on-a-chip applications, the dyes need to be attached to a solid platform. The spectral behaviour of dyes attached to a solid platform is different from those in solution and this is attributed to possible changes in the molecular orientation as in some cases formation of J-aggregates or H-aggregates on solid surfaces have been reported [5]. Thus, comparative studies of the change in spectral properties of the dyes in solution to that in an immobilised state are important. The several ways a molecule

can be attached to a surface include physisorption, electrostatic interaction, attachment by affinity interaction and covalent bond formation [6]. Covalent bond formation between the dye molecule and the surface appears to be a better option than other modes of attachments mentioned above, as dyes attached via weaker modes of interaction tends to get removed while washing [5]. Dye molecules are immobilized on solid platforms through some specific functional group on the dye [5, 7–11] and typically carboxylic or phosphoric acid moieties on the dye have served this purpose well [12–15]. Linkers play an important role in tethering the dye molecules to the surface [16, 17]. Thus along with the spectral properties of the dye molecules, the retention of dye molecules on surface also depends on the linker. Upon immobilization, there is

✉ Nagaiyan Sekar, spanda@iitk.ac.in; ✉ Siddhartha Panda, n.sekar@ictmumbai.edu.in | ¹Department of Chemical Engineering, Indian Institute of Technology Kanpur, Kanpur 208016, India. ²Department of Dyestuff Technology, Institute of Chemical Technology, Mumbai 400019, India.

a possibility of denaturation, agglomeration or aggregate formation [18, 19]. The nature of the interaction between the dye molecule and the linker which affects the overall attachment process as well as spectral properties, need to be studied so as to help obtain optimal performance of the optical sensor.

Density functional theory (DFT) has been useful in predicting spectral properties and structure of molecules [20, 21]. Time dependant density functional theory (TDDFT) [22, 23] enables examination of the excited state of the molecules and thereby have better understanding of the changes that occur in the spectral properties of molecules and thus predict the absorption spectra [24, 25]. Predicting the spectra of dye molecules, both in solution as well as those immobilised on a solid surface, provides valuable insights on the ideal nature of spectra and peak positions. These could help understand the experimental results, the degree of aggregation, degradation or distortion of molecular structure upon immobilization.

In this work, three categories of dyes were used (i) mono-carboxylic mono-functional, (denoted as dye A) (ii) bi-carboxylic bi-functional (denoted as dye B and dye C) and (iii) bi-carboxylic mono-functional, denoted as dye D and dye E. The effect of the linkers on the spectral properties is also studied by using two different linkers, (i) 3-aminopropyl-triethoxysilane (APTES) and (ii) branched polyetheleneimine (PEI). The spectra of the dyes are simulated using DFT and TDDFT. The combination of theoretical and experimental observations helps us to identify the effects of the covalent bond formation as well as the mode of immobilization on the spectral properties of the carboxylate dyes and the possibility of aggregation and degradation upon immobilization. This study would help design sensors with bi-functional dyes. While the effect of the solution environment [26] and absorption on solid surface [27, 28] have been well studied, most of the work have been based only on experiments, and does not discuss the effect of change of linker molecules in case of covalent attachment of dyes. In this work we have combined theoretical calculations and experimental observations to understand the effects of immobilization on spectral properties of dyes and the shifts due to formation of aggregates. We have also observed the effects of linker molecules by experimentally attaching the given dyes to the surface using different linkers as well as performing theoretical calculations on the attached dye structures. We are not aware of any such studies for acridinediones derivatives, the family of dyes used here, utilising both APTES and PEI as linker for the purpose of immobilization. Also determination of theoretical spectra of given dyes containing more than one dye molecule in immobilized and comparing them with theoretical calculations determining the effect of intermolecular interactions of dyes on spectra, to the best of our knowledge has not been undertaken thus far.

2 Materials

N-type Si wafers of (100) orientation with resistivity of 1–20 Ω cm were obtained from Silicon Materials, USA. Ammonia solution 25%, hydrochloric acid 37%, hydrogen peroxide 30%, toluene 99%, sulphuric acid 98% were obtained from Qualigens Fine Chemicals, India, absolute ethanol 99.9% from S.D. Fine-Chem Ltd, India. DMSO was obtained from Merck Pvt. Ltd, APTES 99% and PEI with a number average molecular weight (M_n) of 60,000 were obtained from Sigma–Aldrich Inc, Germany, de-ionized (DI) water (0–0.5 S/m) from Glen RO+ Systems and zero grade nitrogen from Sigma Gases and Services, India.

Figure 1a(i) provides the schematic of the structure of dye A [4-(3,3,6,6,10-Pentamethyl-1,8-dioxo-1,2,3,4,5,6,7,8,9,10-decahydro-acridin-9-yl)-benzoic acid] which has one carboxylic group capable of reacting with amine terminated surface. Figure 1a(ii) provides the schematic of the structure of dye B [4-[9-(4-Hydroxy-phenyl)-3,3,6,6-tetramethyl-1,8-dioxo-2,3,4,5,6,7,8,9-octahydro-1H-acridin-10-yl]-benzoic acid] which has two carboxylic groups attached to phenyl rings, where one of the phenyl rings is attached to a nitrogen atom while other is attached to a carbon atom. Figure 1a(iii) provides the schematic structure of dye C [4-[10-(3-Carboxypropyl)-3,3,6,6-tetramethyl-1,8-dioxo-1,2,3,4,5,6,7,8,9,10-decahydro-acridin-9-yl]-benzoic acid] which also has two carboxylic groups, one of which is attached to a phenyl ring while the other is attached to an aliphatic chain consisting of three carbons. Figure 1a(iv), (v) provides the schematic of the structures of dye D [4-[9-(4-Hydroxy-phenyl)-3,3-dimethyl-1,8-dioxo-2,3,4,5,6,7,8,9-octahydro-1H-acridin-10-yl]-butyric acid] and dye E [4-[9-(4-Hydroxy-phenyl)-3,3,6,6-tetramethyl-1,8-dioxo-2,3,4,5,6,7,8,9-octahydro-1H-acridin-10-yl]-benzoic acid] respectively, both these dyes have one carboxylic group; in case of the former it is attached to an aliphatic chain consisting of 3 carbon atoms, and in case of the later it is attached to a phenyl ring. The procedure of synthesis of the dyes is given by Thorat et al. [29]. Figure 1b(i), (ii) are schematic (two dimensional) representation of the parts of dye molecule which were used to obtain dihedral angle and bond angle data are using Gaussview5 software.

3 Methods

3.1 Immobilisation of dyes to surface

The schematics of the attachment by both covalent (using two different linkers) and non-covalent routes are shown in Fig. 2. Non-covalent route was used to check the possibility of physisorption. DMSO was used as the solvent for preparing the dye solution, and the solution

Fig. 1 **a** Molecular structure of the dyes used. (i) dye A (ii) dye B (iii) dye C (iv) dye D (v) dye E. **b** Nomenclature system for terminal carboxyl group attached to (i) aromatic carbon (ii) aliphatic carbon

was activated by NHS and then by EDC, where the proportion of dye:NHS:EDC was 1:1.5:2. The dye concentration in the solution was 500 ppm. Higher concentration was used to facilitate higher degree of immobilization of dye molecules on the surface. For attachment, the wafers were cleaned and hydrolyzed with piranha solution [30]. Then some wafers were coated with PEI and some with APTES [31], and the rest were kept in the hydrolyzed condition. Then the wafers were dipped in the activated dye solution for 24 h and subsequently washed with DMSO and dried under nitrogen.

3.2 Characterisation

For the spectra of dyes in solution (with absolute ethanol), 10 ppm dye solutions were prepared in DMSO and were characterised by a UV–Vis spectrophotometer (LAMBDA 750 from Perkin Elmer), in the transmission mode with a scan range of 200–800 nm. The spectra of the immobilised samples (non-transparent) were determined by UV–Vis spectrophotometer (Varian 5000 from Agilent) in the reflectance mode, with the scan range 200–800 nm with a scan rate of 600 nm/min at a full slit height.

Fig. 2 Scheme of immobilization of dyes

3.3 Theoretical calculations

Absorption spectra of the dyes were computed using DFT and TDDFT [22–25, 32] with the global hybrid functional B3LYP [33] and the coulomb-attenuated-method (CAM-B3LYP). The basis sets 6-31G(d) and 6-31G(d,p) were used for all the atoms. The fluid atmosphere was created using the CPCM model [34] and calculations were done using Gaussian 09 [35]. The dye molecules, the linkers and the silicon chains were drawn using Avogadro [36]. The silicon structure is approximated by four silicon atoms connected by single bonds in a tetrahedral geometry, the rest of the valences of the terminal silicon atoms are satisfied by hydrogen atoms. The PEI chain is approximated with an oligomer consisting of one primary, two secondary and one tertiary amine group. For the study of interaction of two dyes two units of tetrahedral assembly of silicon atoms and two units of PEI oligomer, as described above, is used. For all the structures, yellow spheres depict silicon, light blue depicts carbon, deep blue-nitrogen, red-oxygen and white depicts hydrogen atoms, the solid lines depict covalent bonds whereas the dotted line depicts the hydrogen bonds. The dihedral angles, bond angles and bond lengths data were extracted from the optimized files using GaussView5 package.

4 Results and discussions

As mentioned in Sect. 2, three categories of dyes are studied here (Fig. 1). Dye A with only one carboxylic group is considered as the control dye. The spectral properties of dye A, in solution and in immobilised state, are discussed in Sect. 4.2. The spectral properties of dye B and dye C are discussed in Sect. 4.3 and that of dye D and dye E are discussed in Sect. 4.4. All these different combinations would help understand the role of the linkers in determining the spectral properties upon adsorption. The non-covalent attachment method showed no retention of dyes on surface upon immobilization. Hence, in the subsequent

sections we discuss only the covalently immobilized molecules.

4.1 Validation of theoretical calculations

A stepwise approach was taken for predicting the absorption spectra of the dyes. Initially B3LYP hybrid function was chosen on the basis of its agreement with experimentally observed values [37] to predict the spectra of the dyes in solution. The DFT calculations were done using B3LYP hybrid functional, using the basis sets 6-31G(d) and 6-31G(d,p). The output files of the DFT calculations were then used as the input files for the TDDFT calculations which provided the theoretical absorption spectra. The calculated values of the maxima of the absorption peaks ($\lambda_{\max}$) obtained using both basis sets differed with the experimentally observed values of the maxima of the absorption peaks by ~60 nm. To obtain results close to the experimental value, we shifted to the CAM-B3LYP hybrid functional [38–41] with 6-31G(d) basis set. The shift in theoretically obtained $\lambda_{\max}$ values from the experimentally observed values, were lesser for CAM-B3LYP compared to those using B3LYP for both the 6-31G(d) and the 6-31G(d,p) basis sets. After determining the optimal density functional, we obtained theoretical spectra of all the dyes using higher basis sets ([6-311+G(d,p)] and [6-311++G(d,p)]) the values, thus obtained were shifted more towards longer wavelength compared to those obtained using 6-31G(d). This prompted us to use CAM-B3LYP with the basis set 6-31G(d) for further calculations involving attached molecules.

Table 1 lists the theoretically calculated values (of the maxima of the absorption peaks) along with the experimentally obtained values, the experimental spectra of the dyes in the solution showed only a single peak and no aggregate formation was observed in this case. The difference in value of $\lambda_{\max}$ between the experimental values and theoretically calculated values using CAM-B3LYP hybrid function was within 20 nm, and these values are in good agreement with the literature [42, 43].

Table 1 Theoretical and experimental $\lambda_{\max}$ values for absorption spectra of dyes

Dye	Experimental $\lambda_{\max}$ (nm)	Theoretical $\lambda_{\max}$ (nm)					
		B3LYP		CAM-B3LYP			
		[6-31G(d)]	[6-31G(d,p)]	[6-31G(d)]	[6-31G(d,p)]	[6-311+G(d,p)]	[6-311++G(d,p)]
A	381	439	440.7	401	403	411	411
B	385	435	435.5	394.6	395	405	405
C	384	442	444	402	404	413	413
D	384	433	434	399.2	400	410	410
E	372	389	389	382	383	402	402

Once the appropriate hybrid functional was obtained by comparing theoretical and experimental results of free dye molecules in solution, attached dye molecules (described in Sect. 3.3) were subjected to theoretical calculations. The results are described in subsequent sections.

4.2 Spectra of the mono-carboxylic mono-functional dye

The structures and the absorption spectra for dye A are given in Fig. 3. Figure 3a(i) showed that the dye molecule attached to a APTES linker which in-turn is attached to the surface via a Si–O–Si bond and Fig. 3a(ii) represents the dye attached via PEI to the surface, these are depicted as non-interacting structures. Figure 3a(iii) shows two dye A molecules attached via APTES linker to the surface and Fig. 3a(iv) presents the same using PEI as the linker, these two are considered as interacting structures. Figure 3(b) represents the absorption spectra (theoretical and experimental) of dye A. Figure 3(b) presents the absorption spectra (theoretical and experimental) of dye A; Fig. 3b(i) presents the theoretical spectra of the non-interacting dye molecule with both the linkers, APTES and PEI, (i.e. involving one dye molecule and one linker molecule) and the experimental spectra involving both the linkers, Fig. 3b(ii) presents theoretical spectra of interacting (involving two dye molecules in close proximity) and non-interacting dye molecules with APTES linker and the experimental spectra of attached dye molecules with APTES linker, Fig. 3b(iii) presents the theoretical spectra of interacting and non-interacting dye molecules with PEI linker and the experimental spectra of the dye molecules attached via PEI linker. The effect(s) of the linkers by comparing the theoretical spectra and experimental spectra can be observed from Fig. 3b(ii), (iii). Both the linkers provided sufficient moieties in between the surface and the dye to prevent the possibility of surface to dye charge transfer. Once the carboxyl group formed an amide bond with the amine terminated surface, there is no possibility of further attachment with the dye, and hence it is chosen as the control dye. The hydroxylated surfaces kept in the dye solution did not show any characteristic peak suggesting there was no retention of dyes when there was no covalent bonding between the dyes and the groups which are on the surface. The calculated spectra in solution for dye A showed a peak at 401 nm, 20 nm more than that calculated for the dye in solution. Theoretically calculated values of $\lambda_{\max}$ which lie on both the higher and the lower side of experimentally observed values of $\lambda_{\max}$ have been reported in literature depending on the structure of the dyes and the solvent used [44, 45]. The hybrid function as well as the fluid model determines to what extent the theoretical values will match with that of the experimental values. The

difference between the theoretically calculated absorption maxima and experimentally obtained absorption maxima are within the limits reported in literature [43–45].

Table 2 presents the theoretically calculated and the experimentally observed values of $\lambda_{\max}$ for dye A, both in the solution and in the immobilised state. Upon immobilisation, we observed a blue shift in the theoretical absorption spectra with both the linkers, which indicates that the gap between the energy levels increases upon immobilisation. This further implies some other factor(s) play(s) a role apart from the covalent attachment. Figure 3b(i) depicts the spectra of immobilized dye molecules, both the theoretically calculated and the experimentally obtained ones. The experimental spectra have different distinct peaks, of which the peaks around 460 nm are of interest. Both the linkers, APTES and PEI, contain aliphatic amines. For APTES the nature of the aliphatic amine is primary whereas PEI contains primary, secondary as well as tertiary aliphatic amine groups. Some of these groups react with the dye molecules while others remain free, and the peak in the experimental spectra in the range of 320–325 nm is attributed to these free aliphatic amine groups. The peaks observed in both the experimental spectra in the range of 320–325 nm, arises due to aliphatic amine, present in the linkers, which is also reported by Kumar et al. [46]. The peak around 230–240 nm could be due paramagnetic defects on silicon surface as reported by Rahman et al. [47] and Kumar et al. [46]. To determine whether presence of another dye molecule in the neighbourhood affects the spectra, theoretical calculations were carried out with two immobilized dye molecules in close proximity, for both the linkers. The structures used for theoretical calculations is described in Sect. 3.3.

There are conflicting results reported in literature regarding the nature and magnitude of the shift in the absorption peaks from solution to the immobilised state. Some authors [48, 49] reported about 50 nm of blue shift upon immobilization while others [5, 50] have reported simultaneous red and blue shift upon immobilisation. The experimental spectra for the solution and the immobilised state in this work showed a red shift and this is in contradiction with the expectation from the theoretical results. Dyes immobilised to metal surfaces show red shift upon immobilisation due to metal-dye charge transfer, but introduction of a linker negates that effect [51]. In this study the presence of the linker to attach the dye to the substrate rules out the possibility of such a charge transfer. This implies some other factors are stabilising the energy levels. A possible explanation of this could be that the carboxyl group is an acceptor group in the donor–acceptor chromophore we have used, with the donor being the nitrogen atom (which contains a lone pair of electrons). During immobilisation, there is a covalent bond between

b i **b ii** **b iii**

Fig. 3 **a** CPK model structure of dye A for theoretical calculations (**a**) attached using (i) APTES- (ii) PEI. **b** Spectra of dye A in the immobilised state involving experimental and theoretical spectra of (i) non-interacting dye molecule with both linkers (ii) interacting and non-interacting dye molecules with APTES linker (iii) interacting and non-interacting dye molecules with PEI linker

the dye and the surface, and therefore the nature of the carboxy group is modified—it becomes a stronger acceptor. It is known that in a Donor–Acceptor chromophore, increasing donor as well as acceptor capacity would lead to a red shift and this is what has happened here.

The double humped peak in the experimental spectra indicates that aggregate formation has taken place as similar phenomenon has been reported by other authors [5, 50, 52]. The dihedral and bond angle values were extracted from the optimized structures. The purpose of this exercise is to determine whether the dye structure is distorted upon attachment or not. If the dihedral angle and bond angle values differ significantly from normal values, expected from the nature and hybridized state of atoms involved, then there is a possibility that there might be degradation of the dye structure upon attachment. The 2D representation of the moieties, from which the data was extracted, are shown in Fig. 1b(i), (ii). The theoretically calculated dihedral angle $\alpha(\text{O}=\text{C}_1-\text{C}_2-\text{C}_4)$ is 179° where as that of the immobilized dye using silane and PEI are 156° and 158° respectively. This closeness of the values indicated that nature of the linkers used here affect the covalent bond formation in a similar manner. This fact is also observed while calculating the bond angle of $\angle\text{O}=\text{C}_1-\text{C}_2$, where the value is 121.4° for the free molecule, while immobilized molecules had values of 122.4° and 121.5° using silane and PEI as the linker, respectively. The values are close to what is expected from a double bonded carbon atom (120°). The theoretical absorption spectra also showed that the linker molecules affected the spectra in a similar manner as indicated by the dihedral angle and the bond angle values of the free and the immobilized molecules. The theoretical calculations indicated blue-shift in the absorption maxima while the experimentally observed results showed a large red shift. This contradiction in the results can be explained by incorporating the effect of neighbouring molecules in the theoretical calculations. Theoretical calculations involving covalent bond formations between the amine terminated surface and a single dye molecule (Fig. 3a(i), (ii)) did not capture the effect of the interaction between the dye molecules in close proximity. To overcome this shortcoming, theoretical calculations involving two attached dye molecules in proximity were carried out (Fig. 3a(iii), (iv)). The λ_{max} values obtained from such calculations with APTES as the linker was found to be 428 nm and that with PEI as linker was

431 nm. The values obtained from these calculations of two immobilized molecules in proximity were found to be closer to the experimental values compared to that obtained from calculations of a single immobilized molecule. The dye–dye interactions could have stabilized the ground states thereby resulting in the red shift. This shows that dye–dye interaction affects the spectra and theoretical calculations involving single immobilized molecules were not able to capture this effect. Due to limitations of available facilities we could not go beyond two molecules, never the less these calculations contributed in strengthening our conjecture that dye–dye interaction caused the large red shifts obtained in experimental spectra of immobilized dyes. The interaction study was carried out only with dye A as single dye molecule systems followed the same trend it was expected two molecule system will do the same.

4.3 Bi-carboxylic Bi-functional dyes

Dye B and dye C are bi-functional dyes with two carboxylic moieties available for attachment. The structures of the dyes used for theoretical calculations and the absorption spectra (both theoretically calculated and experimentally obtained) are shown in Figs. 4 and 5 respectively.

Figure 4a(i), (ii) represents the structure of dye B attached to APTES via two different carboxylic groups which are at two ends of the dye molecule (details are provided in Sect. 2). Figure 4a(iii), (iv) represent the structure of dye B attached to the amino group of PEI via two different carboxylic groups. The different regions of connectivity were chosen to investigate whether or not there is an extended conjugation with the linker. Since the carboxylic groups are different, any extended conjugation with the linkers will affect the spectra differently. Figure 4(b) presents the combined spectra of all the theoretical and experimental findings. The λ_{max} values are given in Table 3.

Figure 5a(i), (ii) represent the structure of dye C attached to APTES by two different carboxylic groups. Figure 5a(iii), (iv) represent the structure of dye C attached to the amino group of PEI via two different carboxylic groups. Figure 5(b) represents the combined absorption spectra of the theoretical and the experimental findings. Table 3 presents the λ_{max} values of the theoretical and the experimental spectra for dye B and dye C, both for solution and immobilised state. Theoretical calculations of the λ_{max} values for dye B show that when the dye is attached to a linker via two different carboxylic groups (denoted as the C end and the N end), the λ_{max} values are quite close (380 nm and 381 nm for APTES). This is true for PEI as well (381 nm and 383 nm). Theoretical calculations for dye C also yields similar results. This leads us to believe that covalent bonding of the terminal carboxylic group does not

Table 2 Theoretical and experimental $\lambda_{\max}$ values of absorption spectra for dye A

Dye	Experimental $\lambda_{\max}$ in solution (nm)	Experimental $\lambda_{\max}$ in immobilised state (nm)		Theoretical $\lambda_{\max}$ in immobilised state for non-interacting system (nm)		Theoretical $\lambda_{\max}$ in immobilised state for interacting system (nm)	
		APTES	PEI	APTES	PEI	APTES	PEI
A	381	465	463	380	381	428	431

Fig. 4 CPK model structure of dye B for theoretical calculations **a** attached using (i, ii) APTES via two distinct carboxylic groups (iii, iv) PEI via two distinct carboxylic groups. **b** Theoretical and experimental spectra of dye B in the immobilised state

Fig. 5 Attached CPK model structure of dye C for theoretical calculations **a** (i, ii) APTES via two distinct carboxylic groups (iii, iv) PEI via two distinct carboxylic groups **b** Theoretical and experimental spectra of dye C in the immobilised state

affect the conjugation to a large degree. These dyes also show a blue shift upon immobilisation in the theoretically simulated spectra while experimental spectra on the other hand show a large red shift as was the case with dye A. The $\lambda_{\max}$ value considered here are 470 nm for APTES as linker and 466 nm for PEI as linker. The origin of the rest of the

peaks are explained in Sect. 4.2. Considering that the theoretical spectra indicates that only covalent bond formation does not cause such magnitude of red shift in absorption maxima, a possible reason is formation of aggregates [28] which was indicated in the case of dye A. Formation of such aggregates upon immobilization of carboxylate dyes

Table 3 Theoretical and experimental $\lambda_{\max}$ values of absorption spectra for dye B and C

Dye	Experimental $\lambda_{\max}$ in solution (nm)	Experimental $\lambda_{\max}$ in immobilised state (nm)		Theoretical $\lambda_{\max}$ in immobilised state (nm)	
		APTES	PEI	APTES	PEI
B-carbon end	385	470	466	380	381
B-nitrogen end				381	382
C-carbon end	384	468	471	380	383
C-nitrogen end				381	382

on different surfaces has been reported by Mulhern et al. [28] and Choi et al. [53]. So formation of aggregates on the surface in our case is not an exception. The red shift (bathochromic shift) indicates formation of J-aggregates.

To further strengthen our hypothesis, two different bi-functional mono carboxylic dyes were subjected to investigation.

4.4 Bi-functional mono carboxylic dyes

Dye D and dye E are bi-functional dyes but they have only one carboxylic group to connect with the linker. The –OH groups present can form hydrogen bond with the neighbouring molecule and aid in aggregate formation. The structures used for theoretical calculations and the absorption spectra are shown in Figs. 6 and 7 respectively.

Figure 6a(i) or 7a(i) shows the dye structure attached to the surface via a silane linker. Figure 6a(ii) or 7a(ii) shows the dye structure attached via PEI to the surface. Figure 6b or 7b represents the combined theoretical and experimental spectra of dye D/dye E. Table 4 shows the values of $\lambda_{\max}$ for theoretical and experimental spectra for dye D and dye E for solvated and immobilised states. The theoretical spectra for dye D show a blue shift on immobilisation as expected from the behaviour of the dyes studied earlier. In the case of the experimentally observed spectra, the expected red shift upon immobilisation is observed, indicating aggregation upon immobilisation had a role to play even when the dyes contained only one carboxylic

Fig. 6 Attached CPK model structure of dye D for theoretical calculations **a** (i) APTES (ii) PEI. **b** Theoretical and experimental absorption spectra of dye D in the immobilised state

Fig. 7 Attached CPK model structure of dye E for theoretical calculations **a** (i) APTES (ii) PEI. **b** Theoretical and experimental spectra of dye E in the immobilised state

Table 4 Theoretical and experimental $\lambda_{\max}$ values of absorption spectra for dye D and E

Dye	Experimental $\lambda_{\max}$ in solution (nm)	Experimental $\lambda_{\max}$ in immobilised state (nm)		Theoretical $\lambda_{\max}$ in immobilised state (nm)	
		APTES	PEI	APTES	PEI
D	384	474	472	383	386
E	372	463	458	379	384

group. The –OH groups in this case might have helped in aggregate formation via hydrogen bonding.

The results for all the dyes reveal that the degree of red shift is comparable. As the value of $\lambda_{\max}$ for the dyes

in solution are very close, this observation leads to the conclusion that degradation of dyes did not occur upon immobilisation or was negligible and only aggregation has affected the spectra. Thingna et al. [54] have created silicon assemblies for theoretical calculations, Carciato et al. [55] used the ONIOM approach for the same purpose has been used by but such approach is beyond the scope of this study. A comparison of similar works is provided in Table 5.

The synthesized knowledge from this study can be utilized to fabricate optical or colorimetric sensors. One carboxyl group can be used to immobilise the dye on the surface and with the other carboxyl group we can attach affinity proteins, antibody and use them as optical sensors [56].

Table 5 Comparison of results with works from other authors

Authors	Surface	Linker	Observation
Mark et al. [18]	TiO ₂	–	Preferential formation of H-aggregates
Alderman et al. [4]	Si	Diol (2 to 11 carbon chain)	Preferential monomer formation
Liu et al. [27]	TiO ₂	–	Preferential monomer formation
Mulhern et al. [28]	TiO ₂	–	Preferential aggregate formation
Our work	Si	APTES, PEI	Preferential aggregate formation

5 Conclusions

We have successfully attached a set of carboxylic dyes upon silanol terminated silicon surface, using two linkers (APTES and PEI). The linkers, though similar in terminal group, were different from each other in structure. This difference in structure, contrary to expectation, caused similar red shift in the absorption maxima of the attached dyes. The spectra of the dyes in solution as well as that attached to the surface were also calculated using DFT and TDDFT. It was found that covalent bond formation was not the reason for causing such a large red-shift in spectra of the dyes. From the theoretically obtained spectra of bi-functional dyes it was indicated that conjugation within the dye was affected to smaller extent by covalent bond formation. The theoretical calculations did not take into account effect of neighbouring molecules. These information and nature of the experimental peaks lead us to conclude that formation of aggregates influenced the real life spectra of the attached dyes and caused a large red shift. Another observation was that hardly any degradation occurred upon immobilisation of the dyes.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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