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PAPER

Electrochemiluminescence (ECL) sensing properties of water soluble core-shell CdSe/ZnS quantum dots/Nafion composite films

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Water soluble positively charged 2-(dimethylamino)ethanethiol (DAET)-protected core-shell CdSe/ZnS quantum dots (QDs) were synthesized and incorporated within negatively charged Nafion polymer films. The water soluble QDs were characterized using UV-visible and fluorescence spectroscopies. Nafion/QDs composite films were deposited on glassy carbon electrodes and characterized using cyclic voltammetry. The electrochemiluminescence (ECL) using hydrogen peroxide as co-reactant was enhanced for Nafion/QDs composite films compared to films of the bare QDs. Significantly, no ECL was observed for Nafion/QDs composite films when peroxydisulfate was used as the co-reactant, suggesting that the permselective properties of the Nafion effectively exclude the co-reactant. The ECL quenching by glutathione depends linearly on its concentration when hydrogen peroxide is used as the co-reactant, opening up the possibility to use Nafion/QDs composite films for various electroanalytical applications.

Introduction

The optical and luminescent properties of semiconductor nanocrystals, or quantum dots, make them highly attractive for a large variety of applications in nanotechnology.[1–5] In particular, high fluorescence quantum yields, size-dependent luminescence, and stability against photobleaching make them a very attractive material for biosensing applications.[6–10] Since Bard's first report on the electrochemiluminescence (ECL) properties of CdSe,[11,12] and CdTe nanocrystals,[13] analytical applications of the ECL from QDs have dramatically increased. This is primarily due to the significant advantages of the ECL over conventional spectroscopic techniques, in particular, low background signals and the ability to control accurately, both time and position of the light emitting reactions.[14–18] However, applications of ECL in clinical analysis require the use of QDs compatible within an aqueous environment. While Bard and co-workers demonstrated the ECL of quantum dots in organic solvents,[11–13] other authors have reported the ECL of quantum dots in aqueous solutions.[19–25] Moreover, a remarkable increase in the ECL intensity has been reported for quantum dot composites with carbon nanotubes deposited on electrode surfaces,[26–30] graphene,[31] carbon paste electrodes,[20] and nanoparticles.[30,32] However, the incorporation of QDs within polymer thin films is still relatively unexplored, although PbS QDs have been incorporated into a Nafion membrane for the examination of size dependent radiative emission.[33] Nafion is widely used in electroanalysis due to its exceptional capability to preconcentrate positively charged species.[34,35] Species such as the tris(2,2′-bipyridyl)ruthenium(II) and its derivatives, along with other metal complex (mainly Os, and Ir) derivatives have received a widespread interest in ECL analysis.[36–43] In this paper, we describe for the first time the incorporation of water soluble positively charged core-shell CdSe/ZnS quantum dots within Nafion, the ECL behaviour and the suitability of such composite in electroanalysis for ECL detection. 2-(dimethylamino)ethanethiol (DAET)-protected core-shell CdSe/ZnS quantum dots were synthesized in water. These positively charged QDs were mixed with Nafion and then cast on glassy carbon electrodes. This method allows a remarkable improvement of the ECL signal compared to the ECL of bare QDs deposited on electrode surfaces. We used hydrogen peroxide as a co-reactant for the ECL reaction. To demonstrate the utility of this novel approach, we investigated the ECL of QDs for the detection of glutathione, GSH, due to its relevance in many physiological processes such as cellular oxidative stress[44–46] and Alzheimer's and Parkinson's diseases.[47] Several detection methods have been investigated to detect biological thiols, including fluorescent chemosensors,[48–54]

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with few focusing on the application of ECL. The incorporation of QDs within Nafion is also effective in promoting the elimination of interferences from negatively charged species such as the peroxydisulfate anions. We believe this method could significantly widen and impact the use of QDs in clinical analysis using ECL-based detection methods.

Results and discussion

Fluorescence and UV-visible measurements were carried out on the QDs before and after the ligand exchange procedure. Fig. 1 illustrates the emission and absorption spectra before and after the ligand exchange with DAET. The absorption spectra show well pronounced peaks at 616 nm and 613nm before and after the ligand exchange, respectively. These peaks correspond to the first discrete energy level of the quantum confinement of the QDs. The gradient of the absorption curves towards shorter wavelength show a less pronounced second and third peak, corresponding to the second and third discrete energy level. The

photoluminescence maximum peak of QDs before and after the ligand exchange are both centred at 646 nm with a line width of about 30 nm. The photoluminescence peak is caused by electrons excited into higher energy states of the QD core. Radiative recombination takes place as band-edge emission from the first discrete energy level into the ground state. For ECL, efficient charge carried injection into the quantum dot core is essential so that efficient radiative recombination occurs. Both the absorbance and emission spectra show similar features indicating successful ligand exchange reaction without QDs aggregation. The emission of the ECL signal is also shown in Fig. 1. While the optically and electrochemically driven peak shapes are similar, the emission maximum for the ECL-derived emission occurs at approximately 10 nm longer wavelength ($\sim$655 nm) than that found for the photo-induced emission. This difference most likely arises from the differences in reorganization energy of the two processes similar to those previously observed in other surface confined ECL processes.[42]

Fig. 1(b) shows the UV-visible spectra of different solutions of Nafion/QDs obtained by varying the amount of Nafion. The absorbance does not show any significant shift compared to the pristine QDs solution. However, it is perhaps important to note that the maximum intensity is observed for the pristine QDs solution, while the decrease in the absorbance intensity is merely a dilution effect that occurs when the concentration of Nafion increases.
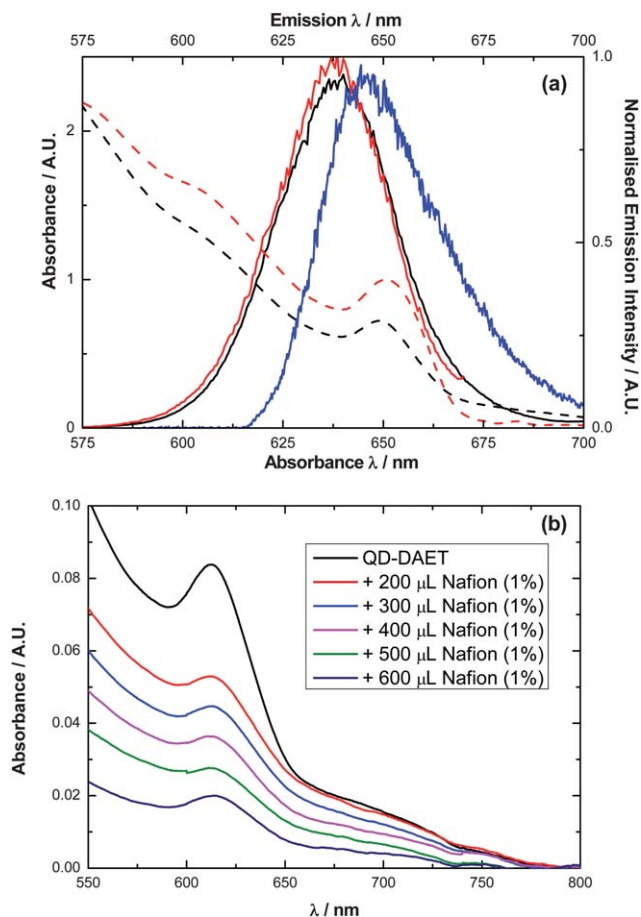

Fig. 1 (a) Absorbance (dotted lines) and emission (solid lines) spectra of core-shell CdSe/ZnS quantum dots before (in toluene, black) and after (in water, red) the ligand exchange with DAET. The ECL spectrum of Nafion/QDs composite film (blue line) obtained in the presence of 2 mM H_2O_2 at a potential of -1.5 V vs. Ag/AgCl is also shown. The ECL emission spectrum was smoothed using an eight-point Savitsky–Golay algorithm. (b) Absorbance of pristine QDs (black curve) in solution and of increasing amounts of Nafion.
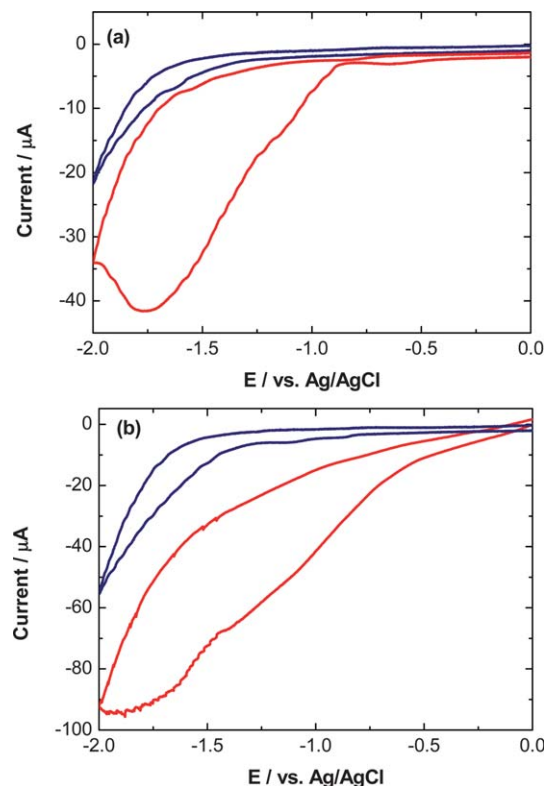

Fig. 2 Current response for (a) QDs film at a scan rate of 100 mVs^{-1} over the potential range -2 V $\leq v \leq 0$ V vs. Ag/AgCl, without H_2O_2 (blue line) and with 2 mM H_2O_2. (b) Current response for Nafion/QDs composite film at a scan rate of 100 mVs^{-1} over the potential range -2 V $\leq v \leq 0$ V vs. Ag/AgCl, without H_2O_2 (blue line) and with 2 mM H_2O_2.

The electrochemical behaviour of QDs and Nafion/QDs composite film was investigated using cyclic voltammetry. Fig. 2 illustrates the CVs of films of bare QDs (a) and Nafion/QDs composite films without and after addition of H_2O_2 (b). The CV of bare QDs in the absence of H_2O_2 does not exhibit any measurable Faradaic peak. However, the addition of H_2O_2 causes a significant change in the electrochemical behaviour and two reduction processes are observed at approximately -0.7 V and -1.75 V $vs.$ Ag/AgCl. The process at -0.7 V is consistent with the electrochemical reduction of H_2O_2 to water and dioxygen,[55] the attribution of the second peak is much more difficult. The reduction of dioxygen in alkaline media may occur through the so called "peroxide pathway"[56] that may involve the concomitant formation of superoxide radical ions, $O_2^{-\bullet}$[57] and further chemical reaction with formation of hydroxyl ions. The CVs of Nafion/QDs composite films are similar to those obtained for the film of bare QDs although the reduction peaks are broader and less well resolved.

Fig. 3 shows the dependence of the ECL intensity for the Nafion/QDs composite on the concentration of H_2O_2. ECL occurs at a potential of approximately -1.5 V $vs.$ Ag/AgCl and increases linearly with an increasing concentration of H_2O_2. The ECL emission corresponds to the following ECL reactions:[19,58]

$$QDs + 1e^- \rightarrow QDs(e^-\ 1_{Se}) \quad (1)$$

$$QDs(e^-\ 1_{Se}) + H_2O_2 \rightarrow QDs + OH^- + OH^\bullet \quad (2)$$

$$OH^\bullet + QDs \rightarrow OH^- + QDs(h^+\ 1_{Sh}) \quad (3a)$$

$$QDs(e^-\ 1_{Se}) + OH^\bullet \rightarrow OH^- + QDs^* \quad (3b)$$

$$QDs(e^-\ 1_{Se}) + QDs(h^+\ 1_{Sh}) \rightarrow QDs^* \quad (4)$$

$$QDs^* \rightarrow QDs + h\nu\ (640nm) \quad (5)$$

During the cathodic scan, electrons are injected into the QDs (eqn (1)), then the electrons-injected QDs (QDs(e$^-$ 1$_{Se}$)) reduce H_2O_2 to produce OH^- and $OH^\bullet$ (eqn (2)). $OH^\bullet$ is the 'key' species that can easily inject a hole into the 1S$_h$ quantum confined orbital of QDs (eqn (3a)) giving to the formation of QDs(h$^+$ 1$_{Sh}$). This process is possible because of the high standard redox potential of the $OH^-/OH^\bullet$ couple.[59] At the same time, the excited states, QDs*, are formed by the reaction of the reduced QDs with $OH^\bullet$ or by the recombination of the injected electrons (e$^-$) with the injected holes (h$^+$) of QDs (eqn (4)).

Both the processes (3b and 4) lead to the formation of the luminophore, QDs*, even though the two processes are mechanistically different, $i.e.$ a co-reactant ECL process the former one and an annihilation process the latter one. QDs* will emit light at a wavelength that depends on the size of quantum dots.[60] It is interesting to note the higher sensitivity (see Inset of Fig. 3) of the Nafion/QDs composite compared to the bare QDs, despite the fact that the stock solution concentration of Nafion/QDs is half that of concentration of the bare QDs. This increased sensitivity suggests that the functionalized QDs are preconcentrated within the Nafion film. In contrast with a previous report from other authors on CdSe QDs,[58] we noted that films of bare QDs deposited on glassy carbon electrodes are much less uniform and stable than Nafion/QDs composite films. This difference is thought to arise because of slow evaporation of water when forming the bare QD films, while the methanol evaporates much more rapidly during Nafion/QDs composite film formation. There is also a significant loss of material due to the solubility of QDs in water, when the electrodes modified with the bare QDs were immersed during the ECL experiments.

To explore the permselectivity properties of the Nafion/QDs composite, we have utilized a negatively charged co-reactant such as the potassium peroxydisulfate species, $K_2S_2O_8$. Although previous work has highlighted the capability of Nafion to quench ECL when negatively charged co-reactants such as oxalate are used, this study examined the possibility of the Nafion film acting as a barrier to particular species.[61] Fig. 4 reports the ECL emission from the bare QDs. As expected, strong ECL is obtained when potassium peroxydisulfate was added as a co-reactant. The ECL signals arising from the QDs when
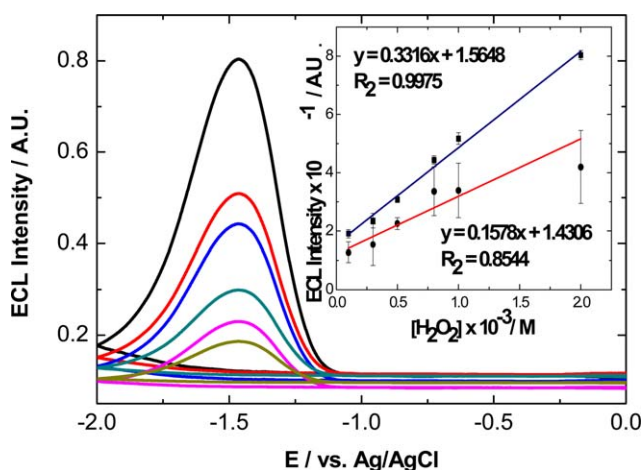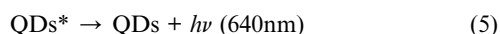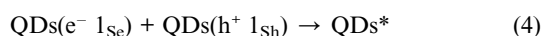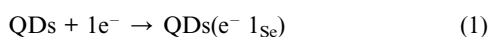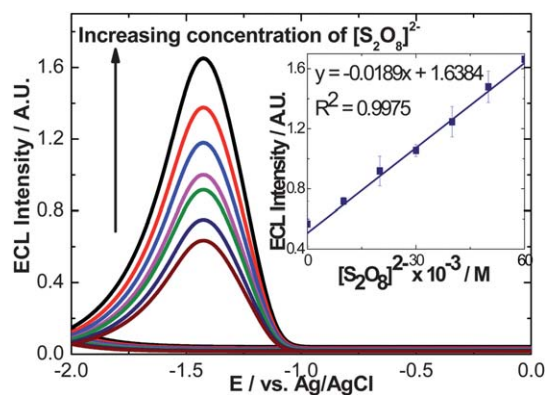

Fig. 3 ECL response of Nafion/QDs composite film, on the concentration of H_2O_2 at a scan rate of 100 mV s^{-1} over the potential range -2 V $\leq \nu \leq 0$ V $vs.$ Ag/AgCl. Inset shows the linear dependence of ECL intensity as a function of [H_2O_2] for (blue line) Nafion/QDs composite film and (red line) bare QDs deposited on GCE (without Nafion). Error bars represent triplicate data points.

Fig. 4 ECL response of QDs on the concentration of $K_2S_2O_8$ at a scan rate of 100 mV s^{-1} over the potential range -2 V $\leq \nu \leq 0$ V $vs.$ Ag/AgCl. Inset shows the dependence of ECL intensity on [$K_2S_2O_8$] for QDs deposited on GCE. Error bars represent triplicate experiments.

peroxydisulfate was used as the co-reactant are higher than those obtained with hydrogen peroxide and this required the PMT power supply to be reduced to −650 V. The light emission from the QDs is based on the following mechanism:[62]

$$QDs + e^- \rightarrow QDs^{-\bullet} \qquad (6)$$

$$S_2O_8^{2-} + e^- \rightarrow SO_4^{2-} + SO_4^{-\bullet} \qquad (7)$$

$$QDs^{-\bullet} + SO_4^{-\bullet} \rightarrow QDs^* + SO_4^{2-} \qquad (8)$$

$$QDs^* \rightarrow CdSe + h\nu \qquad (9)$$
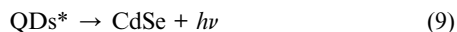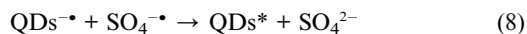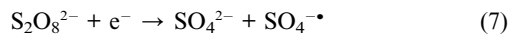
In the case of the Nafion/QDs composite film (Fig. 5) no ECL emission is observed. This result is consistent with the fact that the peroxydisulfate ions, $S_2O_8^{2-}$, are effectively repelled by the SO_3^- groups of Nafion, and are therefore unable to reach the electrode and to form the radical anion $SO_4^{-\bullet}$ that would then react with $QDs^{-\bullet}$. This is a very important result and suggests that Nafion can be effectively used as a barrier to minimise potential interferences from anionic species. This may have a dramatic effect on sensors developed for the detection of GSH as one of the main interferences is ascorbate as it can scavenge H_2O_2 in a similar manner to GSH. The utilisation of a Nafion film which can act as an effective barrier to the ascorbate anion could potentially eliminate this problem with current GSH detection systems. We have investigated the possibility to further explore the use of Nafion/QDs composite film for the ECL detection of a sulfhydryl derivative amino acid such as gluta-thione, GSH. GSH is very abundant in tissues and cells and play a vital role in many physiological processes, since it is an indicator of the oxidative stress of cells along with its oxidation form, GSSG.[44] GSH is also correlated to other cellular functions and its concentration has been related to cancer,[47] diabetes,[63] and neurological diseases.[44]

GSH exerts its properties as an antioxidant due to the reactivity of the sulfhydryl groups as follows:

$$OH^{\bullet} + R\text{-}SH \rightarrow R\text{-}S^{\bullet} + H_2O \qquad (10)$$

$$R\text{-}S^{\bullet} + R\text{-}S^{\bullet} \rightarrow R\text{-}S\text{-}S\text{-}R \qquad (11)$$

From the above mechanisms, it is evident that reaction of the sulfhydryl groups with the hydroxyl radicals would quench the ECL signal with concomitant oxidation of the thiol species. This process is very efficient under physiological conditions with a rate constant between 10^3 and 10^5 mol^{-1} dm^3 s^{-1} being observed.[64,65]

Fig. 6 shows the dependence of the ECL intensity on the concentration of GSH. As shown in Fig. 6, the ECL signal showed a rapid intensity decrease as the concentration of the GSH was increased. According to the Stern–Volmer equation:[41,66,67]

$$I_0/I = 1 + K_{SV}[Q] \qquad (12)$$

Where K_{SV} is called the quenching constant, I_0 and I are the ECL intensities before and after the addition of the quencher, GSH, and Q is the amount of GSH, the linear dependence of I_0/I on GSH concentration is determined to be 10 μM–180 μM, (Fig. 7), however, the sensitivity of ECL allows a concentration of approximately 1.5 μM to be easily detected. This value is well below the detection limit of 8.3 μM reported by Jang.[58]

It is interesting to note that no ECL quenching occurs when different concentrations of the oxidized species, GSSG, were added. The reasons for such unusual result are not clear, even though this insensitivity in the ECL for GSSG has already been reported by other authors.[58] A possible reason can be found in the overall charge of GSH under the mild conditions used here (pH 7.4), where the deprotonation of the two carboxylic groups of GSH which bring a charge of −2 is partially balanced by the charge of +1 due to the protonation of the amine group which results in an overall charge of −1 for the GSH, while the overall charge of GSSG is −2.[68] Moreover, it appears that GSSG is more effectively repelled by the Nafion polymer film. However, it is worth mentioning the super acidic characteristics of Nafion:[69] this property may cause the pH within the Nafion film to be lower than the pH measured in solution and therefore, the acidic characteristic of Nafion could be responsible for the protonation of the amino group of GSH and for a limited dissociation of the
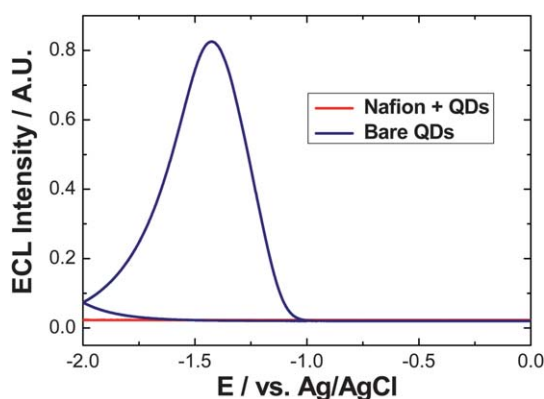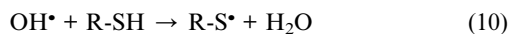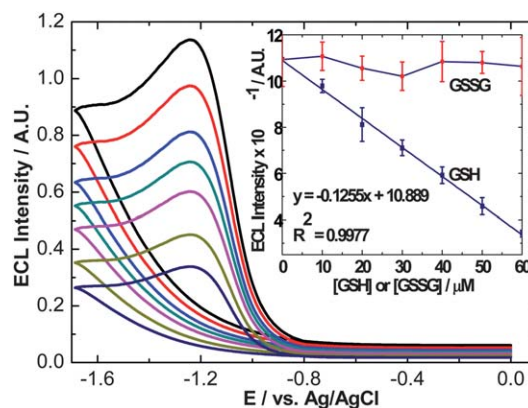

Fig. 5 ECL response of bare QD films (blue line) and Nafion/QDs composite film (red line) at a scan rate of 100 mV s^{-1} over the potential range $-2\,V \leq \nu \leq 0\,V$ vs. Ag/AgCl. The concentration of K$_2$S$_2$O$_8$ is 10 mM.

Fig. 6 Quenching effects of concentrations of GSH (0 to 60 μM) on the ECL of Nafion/QDs composite film at a scan rate of 100 mV s^{-1} over the potential range $-1.7\,V \leq \nu \leq 0\,V$ vs. Ag/AgCl. Inset shows the linear dependence of ECL intensity as a function of [GSH] (blue line) and [GSSG] (red line). The concentration of the co-reactant (H$_2$O$_2$) is 30 mM. Error bars represent triplicate data points.
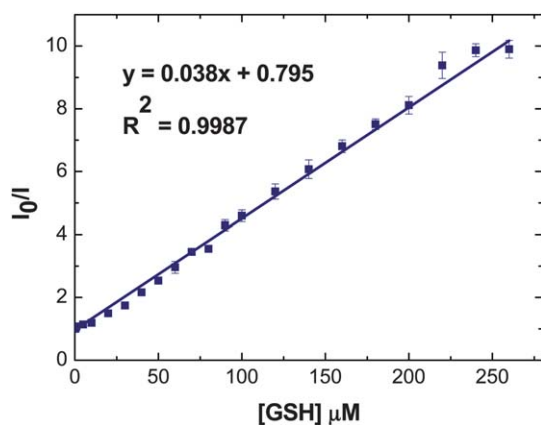
Fig. 7 Linear relationship plot between the relative ECL intensity (I_0/I) and the concentration of GSH from 0.1 to 250 μM. Error bars represent triplicate experiments.

carboxylic group with consequent increase of the net overall charge. The protonation of electroactive species within Nafion films at relatively high pH values has already been observed by other authors.[70,71] This would allow GSH to permeate more effectively within the Nafion films and to react at the electrode/film interface. The stability of this modified film was also investigated. Fig. 8 shows the current and ECL response of a single modified electrode over the course of 30 min. There is a loss in both the ECL intensity and current response within the first few seconds which then reaches a steady state after approximately 450 s. This represents a decrease of ~25% in the ECL intensity over the course of 30 min. However, given that each complete scan is done in under 3 s and each experiment was completed in under 4 min, this decrease is negligible over the time domain of these investigations with reproducibility between electrodes remaining high. Further investigations utilising cysteine, which is another thiol of biological relevance but of smaller molecular size than GSH, will be performed in the future to further elucidate the response of these Nafion/QD composite films.
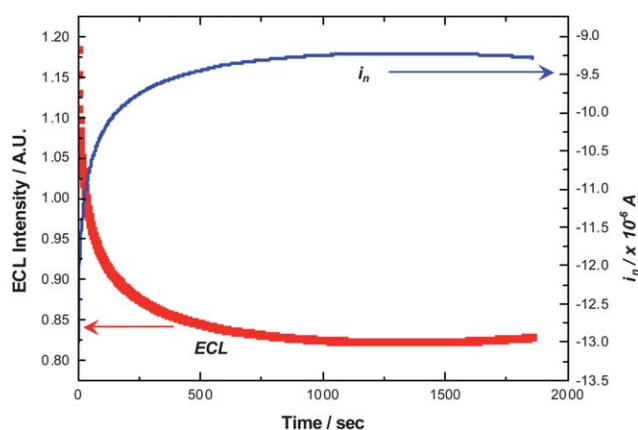

Fig. 8 Typical time response of ECL and current intensity for a Nafion/QDs composite film at a scan rate of 100 mV s^{-1} held at a constant potential of −1.5 V vs. Ag/AgCl for 1800 s.

Conclusions

We have demonstrated that water soluble and positively charged DAET-protected core-shell CdSe/ZnS quantum dots can effectively be incorporated within Nafion films by electrostatic interactions between the negatively charged sulfonic groups of Nafion and the positively charged DAET-protected core-shell CdSe/ZnS QDs. The incorporation of QDs allows the formation of a stable and uniform film on GCE electrode surfaces. Significantly, the emission properties of QDs do not change when they are assembled as a composite material within Nafion.

Nafion/QDs composite films showed strong ECL emission when hydrogen peroxide is used as a co-reactant. In sharp contrast, there is no ECL emission in the case of potassium peroxydisulfate most likely due to permselective exclusion of the co-reactant from the film by the Nafion. The suitability of Nafion/QDs composite film for ECL sensing has been demonstrated using glutathione, GSH, as a model analyte. The quenching of the ECL emission derived from the Nafion/QDs composite film using hydrogen peroxide as a co-reactant, scaled linearly with the concentration of GSH and concentrations up to 68 (±0.7) μM could be detected.

Experimental

Materials and methods

Core-shell CdSe/ZnS quantum dots (red Lumidots™ 640, 5 mg ml^{-1}) in toluene and Nafion 117 solution (5% w/v mixture of low molecular weight alcohols) were purchased from Sigma-Aldrich. Phosphate buffered saline (PBS, pH 7.4), hydrogen peroxide, potassium peroxydisulfate, and 2-(dimethylaminoethanthiol) (DAET) were all from Sigma-Aldrich and used as received. All other chemicals were of reagent grade quality and used as received.

Glassy carbon electrodes (3mm diameter) were purchased from IJ Cambria (UK). Glassy carbon electrodes were cleaned by successive polishing using 0.3 and 0.05 μm alumina slurry, followed by sonication in acetone, ethanol, and water, respectively, for 15 min. Absorbance and photoluminescence spectra were recorded using a Shimadzu UV-240 spectrophotometer and a PerkinElmer LS-50 luminescence spectrometer, respectively. Measurements involving simultaneous detection of light and current utilized a CH instrument model 760B connected to an Oriel 70680 photomultiplier tube (PMT). The PMT was biased at −850 V by a high voltage power supply (Oriel, model 70705) and an amplifier/recorder (Oriel, model 70701) was used in all the experiments, with the exception of potassium peroxydisulfate. In this specific case and due to the higher sensitivity, the PMT bias was reduced to −650 V. During the experiments, the cell was kept in a light-tight box in a specially designed holder configuration where the working electrode was positioned in a direction opposite to the fibre optic bundle, the other end of which was coupled to the PMT. All electrochemical experiments were carried out using a 3 mm diameter glassy carbon working electrode in a conventional three-electrode assembly. Potentials are quoted versus Ag/AgCl using a platinum flag as counter and all measurements were made at room temperature. An Oriel model IS520 gated intensified CCD operated at −20 °C, coupled to an Oriel model MS125 spectrograph, was used to acquire ECL

spectra. Where necessary, thin film emission spectra were smoothed using an eight-point Savitsky–Golay algorithm.[41]

All measurements were made at room temperature (20 °C). In all the ECL experiments H_2O_2 (30 mM) and $K_2S_2O_8$ (10 mM) in 0.1 M saline phosphate buffer, PBS, at pH 7 were used as the co-reactants. All other reagents used were of analytical grade, and all solutions were prepared in milli-Q water (18 mΩ cm).

Synthesis of water soluble core-shell CdSe/ZnS quantum dots

The ligand exchange reaction was carried out using a procedure similar to that of Woelfle and Claus.[72] Briefly, a 0.5 M DAET solution in methanol was prepared for the reaction. 0.25 mL of the original CdSe/ZnS quantum dots in toluene (5mg ml^{-1}) were mixed with 0.5 mL of methanol containing DAET. The solution was stirred and stored under nitrogen atmosphere in the dark at room temperature overnight. Core-shell CdSe/ZnS quantum dots were precipitated with an excess of acetone, followed by centrifugation at 3000 rpm for 6 min. The excess liquid was decanted and the precipitate was subsequently suspended in 0.25 mL deionized water and stored in the fridge in the dark. The final concentration of the QDs after the ligand exchange was calculated using the method of Peng[73] and was 0.15μM. We will generally refer to DAET-QDs as QDs for simplicity.

Preparation of core-shell CdSe/ZnS quantum dots Nafion composite film

Aliquots of commercial stock solution of Nafion were diluted 1 : 4 (v/v) with methanol. Then, the Nafion/QDs composite was formed by mixing aliquots of water soluble QDs with the diluted Nafion solutions in a 1 : 1 (v/v) ratio. Finally, 15 μL of Nafion/QDs composite solution was cast on glassy carbon electrodes and allowed dried for 5–6 h in the dark at 4 °C. In a similar way, water soluble QDs were deposited on glassy carbon electrodes by casting 15 μL of QDs on glassy carbon electrodes.

Acknowledgements

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