Electrochemiluminescence (ECL) based detection methods are of growing importance in several areas of analytical chemistry especially in medical-diagnostics-related applications. However, a key issue is that the sensitivity of these assays is often not sufficient to allow disease biomarkers to be detected at a sufficiently low concentration so as to change clinical practice, e.g., to use cardiac Troponin I (TNI) to diagnose myocardial infarction within the emergency room demand a rapid test with a picomolar limit of detection. ECL can also play an increasing role in the development of biomedical point-of-care devices. Novel high brightness materials including functionalised biocompatible quantum dots (QD), metallopolymers and composites show significant potential to dramatically reduce limits of detection in multi-analyte assays. The objective of this work is to create novel materials and subsequently develop assays for biomedical diagnostics. To fulfil this, the main objectives of the work are to,



**Figure 1.** Typical emission spectra for 100 μM [Ru(bpy)2(PPyBBIM)n]2+ solutions measured in acetonitrile at 298 K. n = 3 (b), 10 (a) or 20 (c). Excitation wavelength of 355 nm was utilised. Samples have been absorption matched at the excitation wavelength.

* Produce polyelectrolytes containing luminophores,
* Develop and characterise Ab-labelled ECL compounds,
* Develop ECL based biomedical sensors for the detection of TNI within point-of-care devices.

Several strategies were used to fulfil these objectives. Firstly, we examined the polyelectrolyte, PPyBBIM or poly[2-(2-pyridyl)-bibenzimidazole], to elucidate the electronic communication between metal centres through a conducting polymer backbone. This was done to examine the possibility of ECL enhancement which would have implications for the development of an ECL biomedical sensor. Unfortunately, the enhanced communication shown by the conducting polymer backbones did not translate to an enhanced communication in the excited state, as can be seen by the dual emission observed, Figure 1. Other conducting polymers were investigated but the retention of the properties of the quantum dots were not retained when modulated in the presence of these polymers.

Secondly, we synthesized dimethylaminoethanethiol (DAET)-capped CdSe/ZnS QDs. The DAET ligand is used as a surfactant which rendered the CdSe/ZnS QDs water soluble and at the same time it prevented the aggregation of individual particles. DAET QDs were then incorporated within the negatively charged Nafion polymer. A major drawback of the ligand exchange reactions is that they are commonly accompanied by a significant decrease in quantum yield. The quantum yield typically decreases to 1%-25%, depending on the quantum yield before the ligand exchange. The quantum yield for the DAET-QD emission is determined to be less than 5%.

The water soluble QDs were characterised using UV-visible and fluorescence spectroscopies. Nafion/QDs composite films were deposited on glassy carbon electrodes and characterised using cyclic voltammetry. The ECL using hydrogen peroxide as co-reactant was enhanced for Nafion/QDs composite films compared to films of the bare QDs, shown in Figure 2. 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. This allowed for further development of this type of sensor, with similar systems examining cholesterol, dopamine and homocysteine all being detectable via this ECL strategy. Of more importance was the achievement of detecting these analytes in whole blood.

The aim of this work was to establish the electrochemical properties and ECL performance of NIR QDs in whole blood and to determine whether they were suitable for incorporation into ECL biosensors under such conditions. The electrochemical characteristics of the QDs are similar in whole blood and buffer, with only one additional process at -1.15 V in the voltammograms of whole blood. The co-reactant free cathodic ECL signal is suppressed in whole blood, due to quenching of ROS by species in solution. ECL with K2S2O8 co-reactant displays a strong reduction peak, which is considerably more intense than that achieved with either 640 or 560 nm QDs, confirming the improved penetrability of 800 nm emission in whole blood, as shown in Figure 3. Co-reactant free anodic ECL of the NIR QDs in blood exhibits an intense oxidation peak at 1.10 V, whereas 640 and 560 nm QDs generate no such detectable response. Both cathodic and anodic ECL were responsive to the addition of reactive molecules, even in this complex matrix.

**Figure 3.** Dependence of the ECL response at -1.35 V with respect to [cholesterol] for the QD/chitosan film in spiked human serum samples. Red line indicates background signal intensity.

**Figure 2.**  ECL response of QDs on the concentration of K2S2O8 at a scan rate of 100 mV s-1 over the potential range -2 V ≤ ν ≤ 0 V vs. Ag/AgCl. Inset shows the dependence of ECL intensity on [K2S2O8] for QDs deposited on GCE. Error bars represent triplicate experiments.

Following confirmation that a detectable, responsive ECL response could be generated in whole blood, a cathodic ECL biosensor for homocysteine and a co-reactant free anodic ECL biosensor for dopamine were developed and exhibited good linearity in the mM and μM range respectively. Significantly, these are the first ECL biosensors to be developed with detection directly from whole blood and have demonstrated the capability and versatility of NIR QD ECL as an option for the development of innovative biosensors with a particular focus on point of care detection.

This work will form the basis of a proof of concept highlighting the ability of ECL sensors to detect target analytes in whole blood. While detection of TNI was not achieved, this work is still significant in that it is the first reported evidence of ECL being utilised to detect analytes without the need for extraction and in the highly complex whole blood matrix. All of the work described here has been submitted or is in preparation for submission to peer-reviewed journals. In addition, the application of point of care diagnostics will have significant socio-economic impact once a first generation device has been created.