Sensors and Actuators Reports 3 (2021) 100065

Contents lists available at ScienceDirect

Sensors and Actuators Reports

journal homepage: www.sciencedirect.com/journal/sensors-and-actuators-reports

Assessment of $[Ru(bpy)_2]^{3+}$and $[Os(diars)_2(bthp)]^{2+}$ for the electrochemiluminescence detection of gemcitabine and leucovorin toward diagnostic point-of-care sensors within precision medicine

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ARTICLE INFO

Keywords:
Electrochemiluminescence
Precision medicine
Biosensor
Gemcitabine
Leucovorin

ABSTRACT

With advances in medical understanding and pharmaceutical drug development a shift away from the traditional "one size fits all" approaches common place in today's treatment plans have been witnessed. Instead greater emphasis has been placed upon the development of personalised precision medicine, tailored to the individual and their disease characteristics. Although the fundamental knowledge is currently present for such a development, current health care practices do not possess the required resources to see such treatment plans implemented. As such the progress and implementation of precision medicine has stalled. Monitoring of the ADMET properties of a therapeutic species will ultimately aid in the progression of precision medicine in addition to drug development timelines. Within this contribution the development of an electrochemiluminescence (ECL) sensor to this end is discussed. Utilising cancer therapies gemcitabine hydrochloride (GMB) and leucovorin calcium (LV) as model compounds, the ability to use ECL for their detection down to a relevant therapeutic range (6.25–100 μM) via a portable sensing system is shown both within ideal and complex biological matrices. For the first time, GMB and LV are detected via ECL utilising both traditional and non-traditional luminophores, and demonstrated how the employment of alternative luminophores can circumvent competing side reactions preventing detection via the traditional ruthenium luminophores, previously rendering the species as unsuited for ECL monitoring. This approach successfully represents an initial concept proof for the utilisation of ECL sensors for monitoring of therapeutics within complex matrices and lays the initial foundations for wider employment of ECL sensors for medical diagnostics and precision medicine.

1. Introduction

As our understanding of a number of disease diagnosis, staging and treatments has advanced movement away from the traditional "one size fit all" treatment strategy toward personalised medicine has occurred [1-4]. Precision medicine, such as personalised oncology, is predicted to become the next primary strategy in medical diagnostic and treatment plans. Personalised medicine tailor's treatment toward the individual based upon any disease characteristics; boosting treatment effects, minimising unwanted side effects and ultimately improving patient quality of life. Tailored treatments place greater emphasis upon the monitoring of a number of key disease and treatment characteristics; these include real time monitoring of disease specific biomarker expression and circulating blood concentrations of treatment therapies [1-4]. Monitoring of these characteristics allows clinicians to determine therapeutic dosage in real-time minimising side effects upon healthy tissue and cells, made possible through continual adjustment of administered dosage in conjunction with biomarker fluctuations. Whilst the fundamental understanding for the implementation of personalised oncology is present the current healthcare system lacks the resources to implement such ambitious plans across a wide range of disease types, furthered by the ever-growing available treatment therapies all whose absorption, distribution, metabolism, elimination and toxicity (ADMET) properties must be understood prior to implementation into precision medicine treatment plans. As such, the field of personalised medicine has stalled. Development of continuous monitoring sensors would facilitate the real-time monitoring of these ADMET properties and therapeutic concentrations. Development of such a device will ultimately aim to increase the frequency of patient assessment, moving away from the routine interval imaging approaches currently adopted

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https://doi.org/10.1016/j.snr.2021.100065
Received 31 August 2021; Received in revised form 25 October 2021; Accepted 24 November 2021
Available online 1 December 2021

for disease monitoring, minimising patient hospital visits and increasing the likelihood of detecting significant changes in a shorter timeframe.

Electrochemiluminescence (ECL) is a powerful technique, whose employment within the medical device and bio-analytical fields only stands to offer huge benefits including portable instrumentation, improved sensitivity and increased operational simplicity [5-15]. Despite its discovery in the 1960′s ECL has only in recent years seen an increased popularity within the wider analytical community, likely in part owing to an increase acceptance of electrochemical sensors. This is largely correlated to the increase in technology within the last decade which has facilitated the large reduction in instrument size and complexity [5-8,16,17]. Yet the employment of ECL within the pharmaceutical and clinical areas is negligible. To date ECL has been largely based upon the employment of the traditional ruthenium luminophore $[Ru(bpy)_3]^{2+}$; recent advances have seen alternative transition metal luminophores increasingly used [18-24]. One significant limitation facing the wider employment within the analytical community is the inherent lack of specificity which ECL offers. A small fraction of advancements to improve specificity have been made to date via employment of coupled separation strategies [25-29] or pH controlled ECL [30]. However, recent preliminary research indicates that these alternative luminophores present an unique characteristic with different metal complexes possessing different selectivities [31].

Gemcitabine (GMB), a nucleoside analogue, is widely employed for the treatment of various carcinomas including non-small cell lung cancer, pancreatic, metastatic breast and recurrent ovarian cancer [32-35]. Monitoring of the concentration of such a antineoplastic drug would be crucial in the implementation of personalised treatment with GMB for the optimisation of the therapy with the management of side effects, to ensure the dosage administered will not cause any toxicity but equally will not be an ineffective treatment [36]. Leucovorin (LV), a reduced derivative of folic acid, is employed alongside methotrexate as an antidote to prevent the toxicity experienced from the high dose required for chemotherapy, alternatively it is administer in high doses as a co-medication to enhance the effectiveness of chemotherapeutic agent 5-fluorourcil (5-FU) [37-39]. Both compounds are of vital importance within the medical and pharmaceutical fields and as a result a number of methodologies have been develop to determine their concentrations both in pharmaceutical preparations and biological samples [40-44]. Both compounds have been previously shown to display the required electroactivity [36,44-48] for electrochemical sensing down to the relevant concentration ranges within pharmaceutical formulations and paired with their well known ADMET properties makes them ideal candidates for employment as model compounds for the development of continuous monitoring sensors.

To this extent within this contribution we investigate the feasibility of ECL sensors for the detection of therapeutic drugs GMB and LV. Utilisation of ECL would provide an innovative new technology and a unique solution to the hurdles facing precision measurement, through the development of a flexible sensor for the detection of cancer therapeutic drugs as a prognostic tool to promote understanding on the effectiveness of drug therapies to individuals. Portable ECL sensors have a proven compatible for the direct detection of a number of drug species within biological matrices, negating the need for sample purification procedures hence making them ideally suited to medical diagnostics. Here we employ both the traditional $[Ru(bpy)_3]^{2+}$luminophore alongside a new luminophore $[Os(diars)_2(bthp)]^{2+}$ to this end for the selective detection of LV and GMB respectively within ideal matrices and human pooled serum, with no sample preparation.

2. Materials and methods

2.1. Reagents & materials

Gemcitabine hydrochloride (GMB), leucovorin calcium (LV), tris (2,2′-bipyridyl) - (dichlororuthenium (II) hexahydrate ($[Ru(bpy)_3]^{2+}$), sodium chloride (NaCl), 117 Nafion (~ 5% mixture of lower aliphatic alcohols) and 20 nm gold nanoparticles (AuNP) in citrate buffer were purchased from Sigma Aldrich. $[Os(diars)_2(bthp)]^{2+}$was prepared as the hexafluorophosphate salts according to the previously published procedure [49,50]. Absolute ethanol was purchased from VWR chemicals. All chemicals were used as received and all solutions prepared in Milli-Q water (18 MΩ cm^{-1}).

2.2. Apparatus

Electrochemical and photoluminescence measurements were performed utilising a combination of a PalmSens 4 potentiostat connected to Hamamatsu H10723-20 photomultiplier tube (PMT), housed within a light tight Faraday cage. Photoluminescence measurements were performed via a specially designed sensor holder which positioned the PMT directly above the working electrode surface. Electrochemical measurements were performed using GSI Technologies carbon screen printed electrodes (SPE) with a 5 mm carbon working electrode, a carbon paste counter electrode and a Ag paste quasi-reference electrode.

2.3. Sensor fabrication

The ECL sensor was prepared via modification of the working electrode surface via drop-casting of the previously optimised $[Ru(bpy)_3]^{2+}$ and $[Os(diars)_2(bthp)]^{2+}$films [30,51-53]. This film contained a final concentration of 0.5 mM of the complex encapsulated within 0.2%w/v Nafion. Once prepared, the film was stored at room temperature under darkness to prevent photodegradation. In order to prevented degradation of the carbon paste working electrode, drop casting of 6 μL of the film to the working electrode surface was immediately followed with the application of heat to evaporate any residual solvent, preventing electrode destruction, while securing the complex to the electrode surface.

2.4. Electrochemical detection of GMB and LV

GMB and LV were diluted with 1 M NaCl in Milli-Q water to obtained varying concentration ranges (6.25–500 μM). For biological matrix samples, GMB & LV dissolved in Milli-Q water were added directly to the corresponding matrices to obtain simulated biological samples. When not in use all samples were stored at 2–8 °C. For pH adjustments 1 M NaOH or HCl were used as required.

3. Results

3.1. Electrochemical behaviour of GMB and LV

Prior studies have previously demonstrated the electro activity of both GMB and LV upon a number of electrode materials.[36,44-46,54] Here, in order to understand the electrochemical behaviour of the therapeutic drugs upon the unmodified carbon paste screen printed electrodes (SPE) utilised within this contribution, cyclic voltammograms (CV) were obtained. Utilising a concentration in excess of the therapeutic dosage utilised in typical patient treatments, both GMB and LV produced the expected anodic peaks. GMB, produces a single oxidation peak at ~ 0.8 V vs Ag, refer to Fig. 1(a). This peak has previously been attributed to the electro-oxidation of the secondary amine present within GMB's structure, highlighted in scheme 1 (a) [44,45]. Oxidation of the secondary amine leads to the generation of the radical cation (b). The irreversible nature of the oxidation is believed to arise from the subsequent dimerisation of GMB via the radicals (c) formed following the rapid decomposition of the unstable radical cation species. This dimerisation forms the new species (d), as such chemically altering the molecule preventing a reversible electrochemical process.

In agreement with prior literature LV was observed to produce two anodic peaks (Fig. 1(b)), the first at ~ 0.5 V and the second at ~1.1 V vs Ag. This dual anodic wave system suggests the electrochemical

oxidation of LV (e) proceeds via a two-step process, involving two electrons and two protons ultimately forming the dehydrofolinic (f) acid species. Although the exact mechanism for the electrochemical oxidation of LV has not been described previously in detail its structural similarity to folic acid allows for the logical proposal it will follow a similar electro-oxidation mechanism [55]. This comprises of the oxidation of the bridging -CH_2—NH- group, highlighted in scheme 1(b), as is observed for folic acid within the same positive potential region, with the initial peak attributed to the alkyl radical formation.

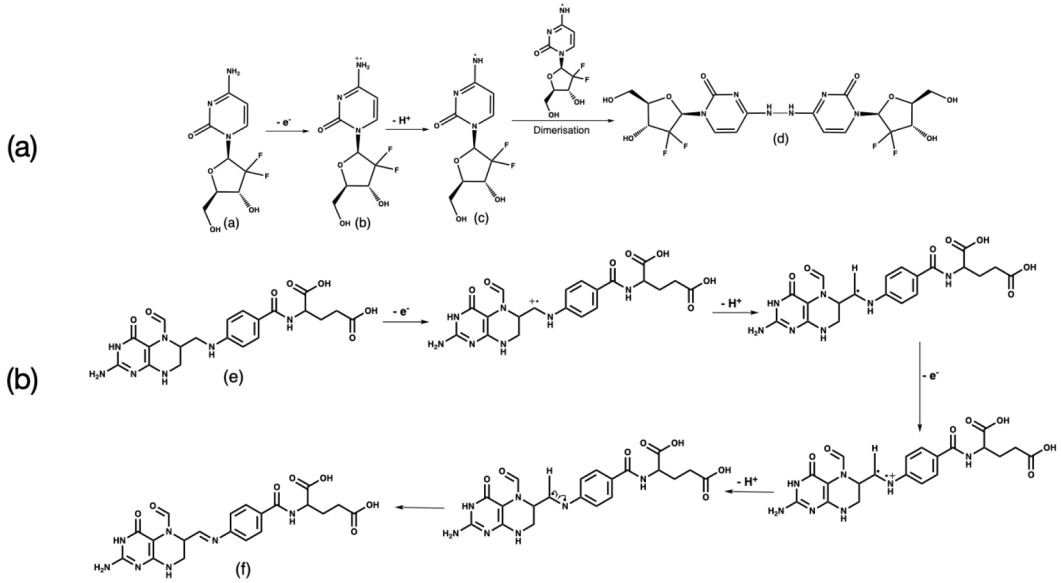
3.2. Preliminary detection of GMB and LV via ECL

The use of ECL for the detection of biologically relevant molecules has grown in recent decades, with a number of proposals on the use of the technique within the medical device arena. ECL is intrinsically suited toward medical or point-of-care devices, owing to its simplicity not only in use but in regards to its instrumentation, offering far less cost intensive systems which are inherently designed for out of lab use. Despite this, the employment of ECL for the monitoring of therapeutic drugs is to date non-existent. Whilst theoretically ECL satisfies a number of the criteria required for live patient monitoring of ADMET properties, it is likely held back due to a poorer understanding and limited use amongst the wider analytical community. Indeed, ECL devices will suffer from a number of intrinsic limitations, such as a lack of specificity, owing to the large number of amine containing species present alongside the target species, all of which will produce signals around similar potentials, however its advantages and continuing advances warrant its further investigation and consideration within this field.

Here we employ ECL for the direct monitoring of GMB and LV for the first time. Although both have been previously studied via traditional electrochemical techniques they have not been screened as suitable ECL co-reactants. To investigate their suitability for detection via ECL, they were investigated with the traditional $[Ru(bpy)_3]^{2+}$ luminophore system. Utilising the previously optimised ruthenium SPE sensing system discussed within our prior publications [30,31,51-53] both GMB and LV were analysed under ideal electrolyte conditions. Fig. 2 shows the obtained ECL signals from GMB and LV. While LV demonstrated a significant ECL signal at ~ 0.9 V, the signal obtained from GMB at ~ 1.0 V was minor in comparison. Both species were analysed initially at significantly higher concentrations than would be present at a therapeutic level, 100 μM. As such the minimal signal observed for GMB indicates its unsuited for ECL monitoring within patient samples, were a typical therapeutic response lies around 26 μM [56].

Consultation of the electrochemical mechanism suggested within scheme 1 for GMB affords some explanation as to the minimal ECL signal observed for this species. Although structurally GMB represents a viable candidate for ECL with its pyrimidine functionality, it is likely that the formation of the excited ruthenium state is in competition with the radical dimerisation reaction observed following the oxidation of GMB. The dimerisation process will consume the radical before it can reduce the Ru(III) species to the desired Ru(II) excited state. As such only a small fraction of the GMB radicals will proceed via the ECL pathway, limiting the concentrations at which GMB can be successfully detected to those in excess of the therapeutic levels. This provided an important consideration for the employment of such a sensing system for the real time monitoring of ADMET properties of early stage therapeutics. Although ECL may theoretically offer a promising technique for a prospective drug candidate, via consideration of its structurally properties (characteristics intrinsically used to determine a species likelihood as a suitable co-reactant) competing side reactions may instead prevent its successful implementation.

Unlike GMB, LV does not experience the same competing side reactions and hence presents as a suitable ECL candidate. In contrast to GMB, LV is not reported to undergo the same dimerization reaction observed for GMB. The prior electrochemical analysis of LV detailed the two step electro-oxidation reaction with the abstraction of two electrons and two protons producing the dual peak response observed within its CV (Fig. 1(b)) [46,54,55]. The lack of competing side reactions is further confirmed through the ability to readily detect LV via ECL indicating the radicals produced via the electro-oxidation processes are not consumed prior to proceeding down the ECL pathway as is observed for GMB. Similar to GMB, its structural analysis indicates its high likelihood for successful ECL sensing. The appearance of the peak for LV was observed at a maximum potential of ~ 0.9 V indicates this ECL signal arises from the oxidation of the secondary amine species, corresponding to the second wave within its voltammogram. The direct oxidation of the LV species via the electrode is maintained in spite of the ruthenium modification, as observed within the corresponding CV collected simultaneously with the ECL signal (refer to Figure S1). In contrast, analysis of GMB revealed the oxidation at the electrode surface is minimal in comparison to LV, as noted through an oxidation current 10-fold lower (see Fig. 1). This lower electrochemical activity will also contribute to the lower ECL signal observed for the GMB species. Although direct oxidation is not mandatory for ECL reactions, as mediated oxidation can



Scheme 1. : Proposed electro-oxidation mechanisms for (a) GMB and (b) LV.

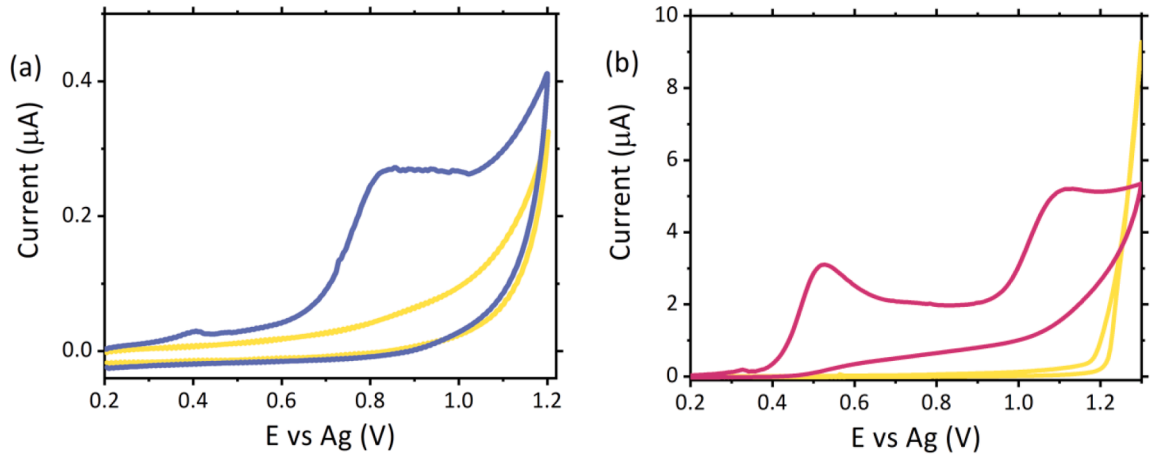


Fig. 1. CV responses of (a) 0.5 mM GMB (purple) and (b) 0.5 mM LV (pink) upon carbon paste SPE in 0.1 M NaCl (yellow) as the supporting electrolyte, collected at a scan rate of 100 mV s^{-1} across a potential range of $0.2 \leq E \geq 1.22$ V vs. Ag.

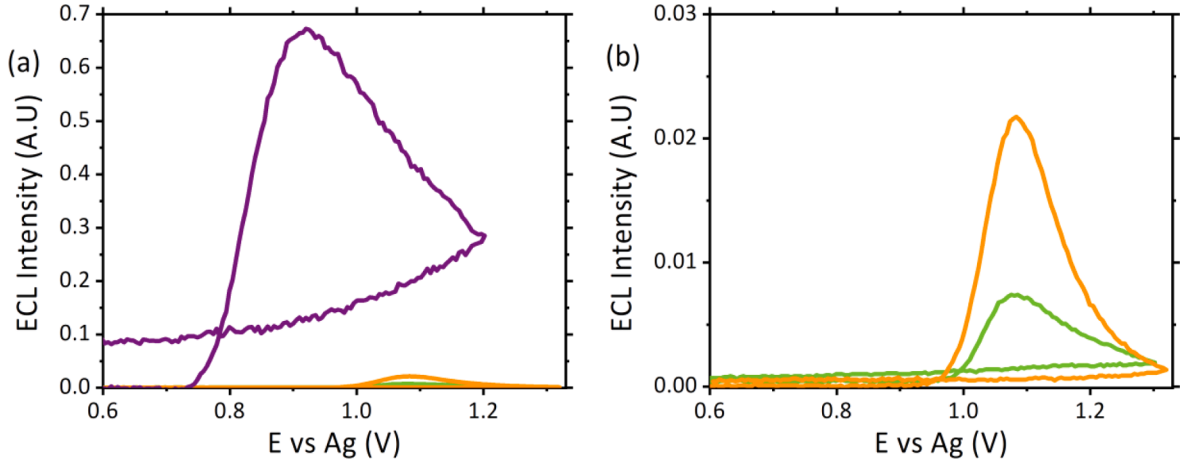


Fig. 2. Typical ECL responses of (a) 0.1 mM GMB (orange) and 0.1 mM LV (purple) upon $[Ru(bpy)_3]^{2+}$ modified carbon paste SPE in 1 M NaCl (green) collected at a scan rate of 100 mV s^{-1} across a potential range of $0.6 \leq E \geq 1.35$ V vs. Ag with a PMT bias of 0.8 V. And (b) expansion of 0.1 mM GMB signal obtained.

proceed via the homogenous oxidation between the Ru(III) species [30, 51,57-59]. Here the mediated oxidation pathway does not appear to occur with the GMB species, indicating that the HOMO of GMB is unable to interact with the LUMO of Ru(III) preventing electron transfer between these species, accounting for its low emission signal.

The analytical performance of this ruthenium-based sensor has been previously established and the same performance was observed during this contribution [30,31,51-53]. The use of the traditional ruthenium luminophore facilitated the ECL detection of LV down to concentrations within the μM region. The association between ECL intensity and LV concentration revealed a linear relationship across the concentration range of 6.25 to 100 μM with a coefficient value of 0.998 refer to Fig. 3, with a detection limit of 6.25 μM established at a level three times the intensity of that of the blank. The typical therapeutic dose of LV is noted to range from 50 mg/m^2 to a high dosage of 500 mg/m^2, these concentrations are noted to produce a typical LV concentration within plasma of a peak of 24 μM and an steady state plasma concentration of 3.2 μM [60,61]. As such it becomes clear that although the peak concentrations lie within the detectable range, the average plasma concentrations are not. This indeed presents a limitation of this current sensor for applications within this field, where although therapeutic doses may be successfully monitored, one must account for the bioavailability of the drugs and resulting concentrations present within a blood or plasma patient sample.

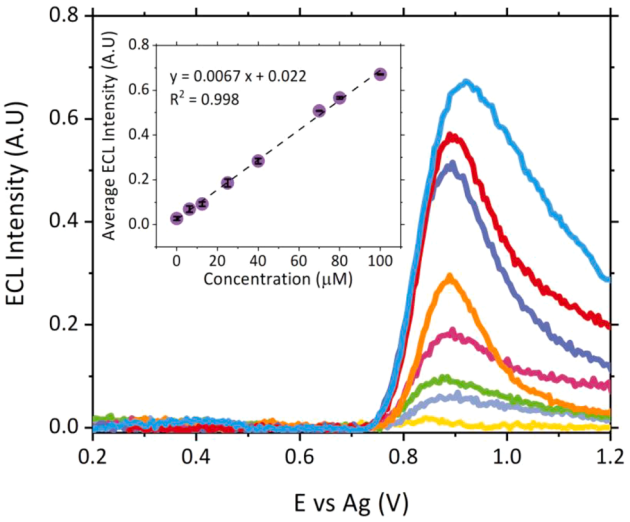


Fig. 3. ECL responses from 6.25 to 100 μM LV with the $[Ru(bpy)_3]^{2+}$ luminophore collected at a scan rate of 100 mV s^{-1} across a potential range of $0.6 \leq E \geq 1.35$ V vs. Ag with a PMT bias of 0.8 V. Insert shows the linear relationship of LV concentration with maximum ECL intensity.

3.3. Alternative ECL methods for GMB and LV detection

The limited portion of GMB molecules able to proceed via the ECL pathway limits the detection ability of this species via the current methodology. Indeed, ECL with the ruthenium sensor was noted down to concentrations of 100 μM, where below this no measurable signal could be detected, refer to Fig. S2. Of further note is the unreliability of the detention of GMB, likely linked to the competing side reaction, which sees varying amounts of GMB proceed down the ECL pathway across different measurements. This was confirmed through the monitoring of the maximum intensity across different electrodes at a GMB concentration of 100 μM, presented in Fig. S3. As such an alternative methodology would be required to facilitate ECL as a feasible detection mechanism for the monitoring of GMB within patient samples. Previously we have investigated the use of alternative luminophores as a result of their unique selectivities toward different co-reactant species. One such species is $[Os(diars)_2(bthp)]^{2+}$, this osmium based luminophore has previously displayed success as a co-reactant for other amine containing species including TPA, atropine, scopolamine and methamphetamine.[19,31] As such, it was believed that the osmium species could be a viable candidate for the generation of ECL from GMB.

Analysis of GMB with an $[Os(diars)_2(bthp)]^{2+}$modified electrode revealed a marked increase in ECL intensity compared with the ruthenium luminophore, refer to Fig. 4(a). In fact, a ~ 50 fold increase in signal intensity was observed. The different chemical properties of the osmium complex likely gifts it this alternative behaviour. With osmium complex dσ* orbitals known to exist at higher energies than the corresponding ruthenium complexes, [62] it is believed that these higher energy levels facilitate the mediated oxidation of the GMB species not previously attainable with the ruthenium luminophores, resulting in an increase of the number of available species to proceed down the luminescent pathway. The employment of this alternative non-conventual luminophore affords to ability to successful utilise ECL for the monitoring of GMB down to the therapeutic range of 26 μM, unachievable with the traditional ruthenium methods. Fig. 4(b) demonstrated the successful detection of GMB with $[Os(diars)_2(bthp)]^{2+}$down to 6.25 μM, with a linear relationship witness between 6.25 and 100 μM. Although the intrinsic signal from this $[Os(diars)_2(bthp)]^{2+}$complex would limit any further reduction in concentrations which could be successfully monitored, it does propose the opportunity to pursue the development of alternative osmium based luminophores which may be more appropriate for GMB and pyrimidine functionalities.

In contrast the same enhancement effect was not observed for LV. Unlike GMB, investigations of LV with the osmium luminophore relieved no notable increase although a decrease in the maximum signal intensity compared with $[Ru(bpy)_3]^{2+}$, refer to Fig. 5. This difference of behaviour between the two complexes is not unexpected, with our previous investigations displaying different behaviours of sister compounds which possess almost identical chemical structures with this $[Os(diars)_2(bthp)]^{2+}$complex. The mechanism behind these extreme differences are not fully understood at this time, although likely linked to the greater stability of the ground and excited states and energies of the complexes HOMO and LUMO levels as a result of the greater orbital splitting from the 5d metal [62]. Further investigations including computational studies are currently underway and hoped to shed light on this observed phenomenon.

The ability to detect both therapeutic drugs down to the desired therapeutic ranges has been successfully achieved via the use of two alternative luminophore species. Using two different luminophores does present a unique opportunity for future investigations, as a direct result of the differing emission colours intrinsic to each species. Spectroscopic data, shown within Figure S4, shows the emission wavelengths of each complex with $[Os(diars)_2(bthp)]^{2+}$emission maximum observed at 596 nm, blue-shifted in comparison to $[Ru(bpy)_3]^{2+}$at 611 nm. These

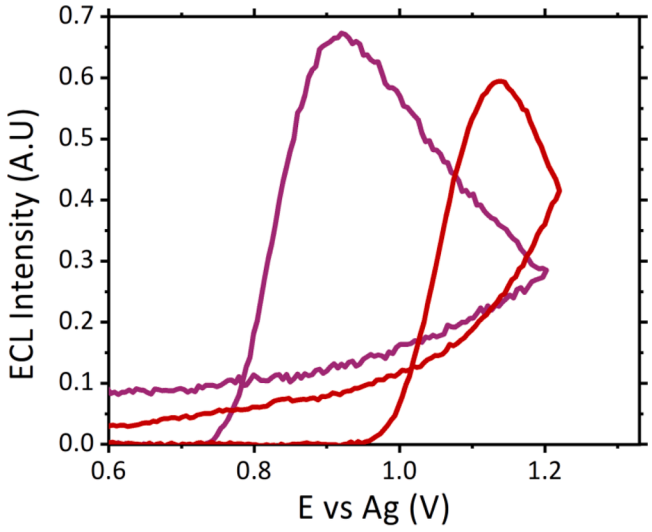


Fig. 5. ECL responses of 100 μM LV with $[Ru(bpy)_3]^{2+}$ (purple) and $[Os(diars)_2(bthp)]^{2+}$ (red), measurements collected at a scan rate of 100 mV s^{-1} across a potential range of $0.6 \leq E \geq 1.25$ V vs. Ag with a PMT bias of 0.8 V.

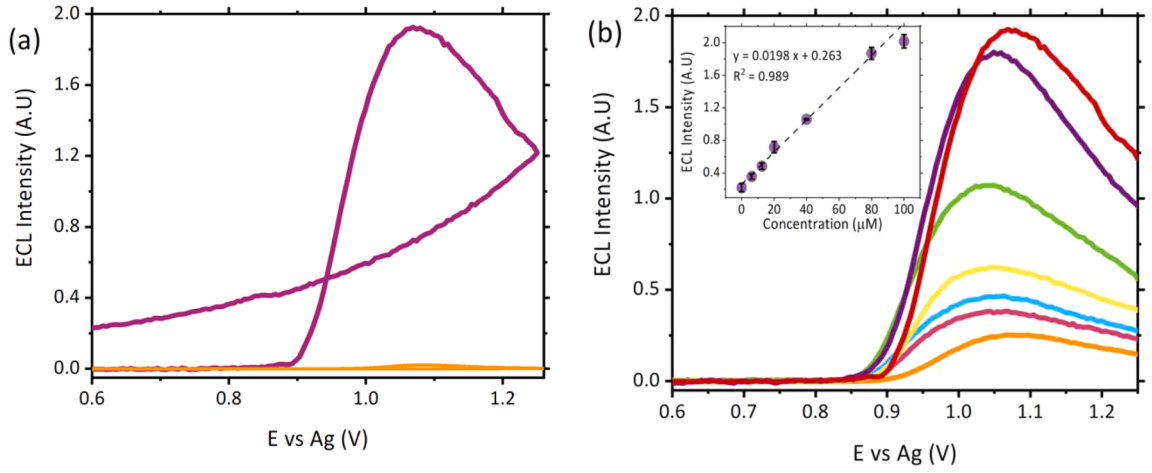


Fig. 4. ECL responses of (a) 100 μM GMB with the [Ru(bpy)3]2+ luminophore (orange plot) and the [Os(diars)2(bthp)]2+ luminophore (purple plot) and (b) responses from 6.25 to 100 μM GMB with the [Os(diars)2(bthp)]2+ luminophore, inset shows linear relationship between ECL intensity and concentration. All measurements collected at a scan rate of 100 mV s^{-1} across a potential range of $0.6 \leq E \geq 1.28$ V vs. Ag with a PMT bias of 0.8 V.

differing emission wavelengths could afford opportunities to utilise a combination of both luminophores within a single sensing device permitting multicolour ECL.

3.4. Application to biological matrices

Through the use of two alternative luminophores the ability to detect GMB and LV down the clinically relevant ranges was achieved. This fulfils one necessary criteria for the employment of ECL as a technique for the monitoring of ADMET properties required for precision measurement. However, one further criterion which required investigation is the compatibility of the developed methods for detection within biological matrices. ECL has been more widely employed for analysis within complex matrices in recent years, with detection achieved within a range of biological matrices, both directly and after extraction and purification strategies [51,52,63,64]. For real-time monitoring of ADMET properties the desire would be for sensors which negate the need for extraction and purification strategies due to their additional requirements in regard to cost, time and experience needed to perform. As such, assessment was made for the detection of both species at therapeutic concentrations within human pooled serum.

Serum samples were spiked with either GMB or LV and measured directly via the ECL sensors modified with either the ruthenium or osmium luminophore, the resulting signals can be seen in Fig. 6. GMB was spiked into serum at a concentration of 26 μM and LV at 24 μM. Inspection of Fig. 6 demonstrates how the signal observed is impacted in comparison to the ideal matrices (see Fig. 4). This is not entirely surprising given the complexity of serum. Not only does serum possess its own intrinsic signal due to the varying ECL active compounds present including amino acids widely documented to behave as suitable co-reactants with the $[Ru(bpy)_3]^{2+}$luminophore, as well as having demonstrated a notable signal with this sensor in our prior works [52]. But it's physical make-up also impacts the detected signal, with a higher viscosity and lack of electrolyte the mass transport of the species to the electrode surface where the luminophore is housed will be hindered with slow diffusion and migration now contributing to the mass transport of the species. The impact of serum upon the detected signal is shown with consultation of the predicted concentrations, calculated using the calibration curves for each species respectively. Table 1 summarises these findings and highlights the impact the matrix will have upon ECL sensors. Where the intrinsic serum signal results in an overestimation of the LV concentration present through measurement with the $[Ru(bpy)_3]^{2+}$luminophore. However, the ability of the $[Os(diars)_2(bthp)]^{2+}$to negate these interference effects allows for the calculated concentration of GMB to lie within the tolerance limit for analytical methods. The same behaviours however of the species with the luminophores was maintained, with GMB still not producing a notable single with the ruthenium luminophore and underestimate of the LV concentration with the osmium luminophore. As an initial concept proof, the ability to detect these signals within 15% is promising. What's more, as demonstrated via the osmium complex, the ability to negate the serum signal and selectively detect GMB in the presence of other co-reactants is a significant finding. This will not only improve the sensitivity of the methodology by reducing the background interference, it will also lead to the improvement of ECL selectivity, a widely known limitation of the technique, increasing its attractiveness to the biomedical field.

Table 1
Summary of the calculated GMB and LV concentrations within pooled serum.

Drug Species	Luminophore	Concentration Added(μM)	Calculated Concentration (μM)	Difference in theoretical and actual concentrations (%)
GMB	$[Ru(bpy)_3]^{2+}$	26	ND	ND
GMB	$[Os(diars)_2(bthp)]^{2+}$	26	25.4	98%
LV	$[Ru(bpy)_3]^{2+}$	24	26.8	111%
LV	$[Os(diars)_2(bthp)]^{2+}$	24	14.3	60%

4. Conclusion

Within this contribution we have successfully demonstrated the compatibility of ECL for the monitoring of therapeutic ADMET properties utilising cancer therapeutics GMB and LV. For the first time we report on the successful detection of GMB and LV via ECL and have shown the ability to selectively detect each compound via the exploitation of different behaviours offered through the employment of alternative metal luminophores. Here we have shown how both compounds could be directly detected down to therapeutic concentration ranges, utilising $[Ru(bpy)_3]^{2+}$for GMB and $[Os(diars)_2(bthp)]^{2+}$for LV within both ideal and more importantly biological matrices. Through the employment of $[Os(diars)_2(bthp)]^{2+}$we were able to circumvent the competing side reactions which prevented the successful detection of GMB via ECL previously, as a result of its poor electroactivity and minimal oxidation at the electrode surface. The employment of this non-traditional luminophore offered improved selectivity, a limitation known to plague ECL research to date. The ability to use $[Os(diars)_2(bthp)]^{2+}$for GMB detection within human serum achieved a

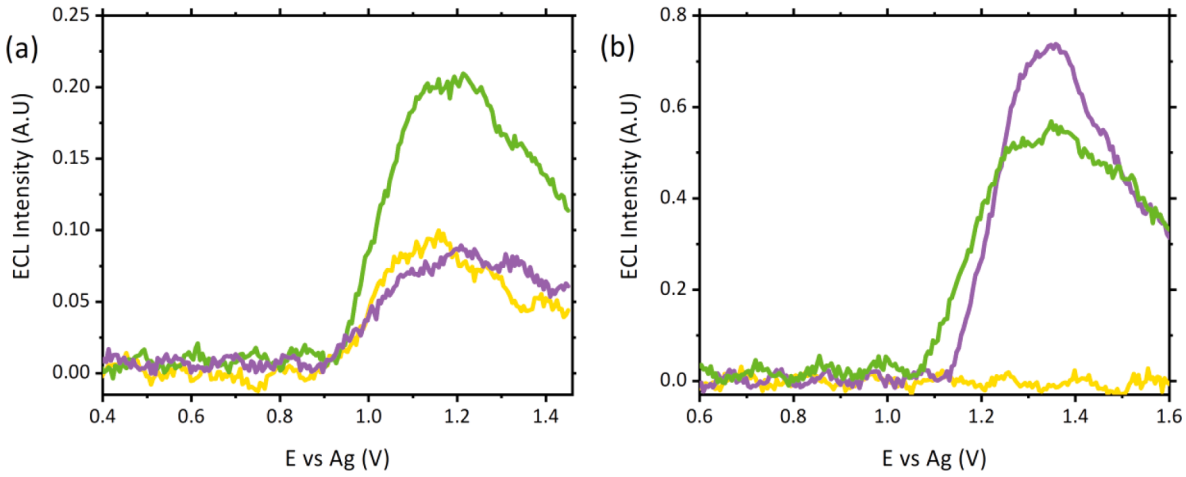


Fig. 6. ECL responses of 26 μM GMB (purple) and 24 μM (green) and serum (yellow) with (a) $[Ru(bpy)_3]^{2+}$ and (b) $[Os(diars)_2(bthp)]^{2+}$All measurements were collected at a scan rate of 100 mV s^{-1} across a potential range of $0.6 \leq E \geq 1.3$ V vs. Ag with a PMY bias of 0.8 V.

recovery of 98%, within the tolerance of 95 to 105% established for GMP analytical methods. This provides a strong concept proof for the suitability of this portable ECL sensor for the monitoring of ADMET properties and as a tool for future precision medicine. Previously the use of the traditional ruthenium luminophore for the direct detection of species within human serum samples was hindered by the intrinsic background response of the compounds present within serum [52], mainly the amino acid groups, who amine functionality gifts them suitability as co-reactants. Here we demonstrated the complete removal of this background signal, which will ultimately not only increase ECL selectivity but also improve sensitivity through the reduction of background interference. Although this contribution provides a promising start far greater research, understanding and information is required should ECL be actively considered as a monitoring tool for precision measurement. Indeed, we have demonstrated a superior specificity than previously shown, however this study was limited to these two specific therapeutics and hence a wider group of compounds would need to be considered, this would include a variety of naturally occurring amino acids, other therapeutic drugs which may also be present including common non-prescription drugs such as paracetamol or buscopan in addition to commonly encountered species such as caffeine or nicotine. The current lack of a thorough understanding as to different luminophores alternative behaviours and ultimately their selectivities is required to aid in the expansion of such a methodology. Through this understanding new luminophores could be coined specifically targeting a range of functionalities or individual functional groups to offer the desired selectivity and sensitivity required. This would be a critical step should the proposed sensor be considered as a tool for use within the drug development timeline or as a real-time patient monitoring device. However, the promise of this initial step should not be diminished by the current limitations it faces, instead consider an initial proof for the successful development of techniques required to fill the current deficit in the health care system, stalling the progress of precision medicine.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors would like to thank the University of Strathclyde STEM Equals scheme for financially supporting this research (grant EP/S012133/1). They would also like to extend their thanks to the Royal Society of Chemistry for provision of the Mobility Grant (RM1802-1666) which made the research visits and collaboration with Deakin University possible. All data underpinning this publication are openly available from the University of Strathclyde KnowledgeBase at https://doi.org/10.15129/bd4e96da-9510–4e5b-a69a-22b6e48b030d.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.snr.2021.100065.

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