Does the Salt Really Matter?

Impact of the Counterion Upon ECL Signal

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Abstract

The incorporation of counterions into a variety of substances to control a number of their physiochemical properties is vital within a range of fields. Electrochemiluminescence (ECL) based sensors have grown in popularity in recent years with their employment observed across analytical and bioanalytical applications. ECL is typically concerned with the core electroactive functionality of the species of interest, with structural similarities commonly employed to determine the likelihood of a species ECL capability. However, to date no consideration has been given to the impact of different counterions toward an analytes ECL mechanism. Here we report for the first time how a simple alternation to a co-reactants counterion can significantly impact upon its recorded ECL response. Utilizing the tropane alkaloid scopolamine and its hydrobromide and hydrochloride salt forms, we have seen through interrogation with the traditional ruthenium luminophore, that replacement of the bromide anion for the chloride anion can reduce the electroactivity of the species. Direct comparison between the hydrobromide and hydrochloride salt forms relived differences in respect of their emission potentials and intensities. The impact of the salt form upon the ECL response has here been investigated, in respect to predicted concentrations. Results demonstrated how vastly different concentrations were obtained dependent upon the salt form present within the sample and that which was used to produce the calibration curve. The impact of this discovery will be of interest to the electrochemiluminescent and electroanalytical communities, and in particular forensic practitioners where electrochemical and ECL based sensors are of increasing interest. Ultimately the application of an ECL sensor within an analytical environment relies upon its accuracy and hence a thorough understanding of the phenomenon observed will only stand to widen the acceptance of ECL within the wider analytical community and increase its potential future applications.

1.0 Introduction

Typical reporting of electrochemiluminescence (ECL) mechanisms detail the reactions which occur between the luminophore complex and the core electroactive structure of the target molecule. Structural similarities between ECL co-reactants have long been utilized to determine the mechanistic pathways, predict the potential regions where emission will occur and ultimately the likelihood of a species behaving as a suitable co-reactant.[1-5] Although these considerations have been widely documented since its inception, to date little regard for the counterion of the target analyte has been given.

Counterions are vital within a range of substances but bare particular importance within the pharmaceutical industry, where they are utilized to improve the physiochemistry of active pharmaceutical ingredients.[6, 7] Careful consideration is given to the counterion of choice, to ensure the required bioavailability is achieved, providing control over a drug's solubility, stability and dissolution rate.[6, 7] However, the same consideration has not been given to the counterion during the developmental phase of any electrochemical analytical methodologies to date. Traditionally, the counterion has been considered a bystander, bearing no influence upon the recorded ECL emission. As such discussion surrounding an analytes ECL production has focused upon that of the core electroactive structure of the analyte, with no mention to date of its corresponding salt form. However, any potential differences within the electrochemical behavior of a species as a result of the counterion present will not only bare influence upon the pharmaceutical industry but the forensic arena where common drugs of abuse are available in free base or a variety of salt forms. It therefore become prudent as the

employment of ECL expands, that developed methodologies take into consideration the salt form present of their target analyte.

Scopolamine is a naturally occurring tropane alkaloid, produced by members of the *solanaceous* plant family and one of the earliest identified alkaloids. Since its discovery in 1880 it has been extracted and purified into a number of salt forms, including its hydrobromide and hydrochloride counterparts. Scopolamine has a number of historical uses, such as its employment as a sickness aid during World War II and its use as a 'truth' serum, most notably by the central intelligence agency[8]; more recently it has been used to facilitate sexual assaults[9] and robberies[10-13], in addition to its medicinal applications.[14] Today scopolamine is primarily employed as its hydrobromide salt form, with pharmaceutical manufacturers focusing upon this formulation. Scopolamine has displayed suitable electrochemical behavior facilitating its detection via electrochemical methodologies, with its electroactivity contained within its tropane alkaloid functionality; know to gift structurally related species, including sister tropane alkaloid atropine, quinine and cocaine their electroactivity.[15-25] To date all these species have presented indistinguishable electrochemical behaviors across the same potential region, attributed to their amine functionality. With its variety of salt forms and know electroactive functionality scopolamine presents as an ideal candidate for investigations into the impact a counterion may have upon a species electrochemical behavior.

Prior works have focused upon the use of ECL for the detection of scopolamine in a variety of complex matrices, utilizing the traditional ruthenium luminophore ($[Ru(bpy)_3)^{2+}]$).[16-18] Here the same ruthenium based ECL sensor is used for investigation into the potential impact of

the counterion present. With both the hydrobromide and hydrochloride salt forms of scopolamine containing the electroactive core tropane alkaloid functionality and indistinguishable pKa values, it would be a fair assumption that indistinguishable ECL signals would be expected. Investigation of the potential impact of an analytes salt form, will bare wider significance beyond the ECL community; with significance in the electroanalytical and analytical communities observed. As such, this investigation is prudent at a time where an increased interest in electrochemical sensors exists.

2.0 Experimental

2.1 Materials & Reagents

Tris (2,2'- bipyridyl) - dichlororuthenium (II) hexahydrate ($[Ru(bpy)_3]^{2+}$), (-)-scopolamine hydrobromide trihydrate (Sc-HBr), (-)-scopolamine hydrochloride (Sc-HCl), lithium perchlorate ($LiClO_4$), and 117 Nafion (~5% mixture of lower aliphatic alcohols and water) were purchased from Sigma-Aldrich. Absolute EtOH was purchased from VWR Chemicals. All chemicals were used as received and all solutions were prepared in Milli-Q water (18 MΩ cm^{-1}).

2.2 Instrumentation

All electrochemical and photoluminescence measurements were performed utilising the same set-up as described within our prior publications.[16-18] This involved the combination of a CH instrument model 760D electrochemical analyser and Hamamatsu H10723-20 photomultiplier tube (PMT) alongside GSI Technologies electrochemical carbon screen printed electrodes (SPE) with a 5 mm carbon working electrode, a carbon paste counter electrode and an Ag paste quasi-reference electrode. A specially designed sensor holder was employed to position to the PMT directly above the modified working electrode to maximise measurement sensitivity.

2.3 Fabrication of $[Ru(bpy)_3]^{2+}$/Nafion ECL Sensor

The $[Ru(bpy)_3]^{2+}$/Nafion ECL sensor was fabricated utilising the previously optimised methodology.[16-18] In brief this involved drop casting of 7 µL of a 0.5 mM $[Ru(bpy)_3]^{2+}$/0.2% w/v Nafion film, prepared in 50:50 (v/v) EtOH:H_2O, upon the working electrode surface, which was subsequently air dried under darkness for two hours. Measurements were performed

with a maximum of 100 µL sample volume, following three subsequent CV scans within the electrolyte to precondition the electrode surface and ultimately stabilise the resultant signal. All electrochemical measurements were performed across a potential region of 0.5 to 1.36 V vs Ag, with a scan rate of 100 mV s^{-1}, and a sampling interval of 0.001 V.

3.0 Results & Discussion

3.1 Electrochemical Behavior of Scopolamine Hydrobromide and Scopolamine Hydrochloride

The tropane alkaloid scopolamine has previously demonstrated the necessary electroactivity to facilitate its detection via electrochemical methodologies.[15-17] To date focus has been primarily placed upon the hydrobromide salt form of the alkaloid, likely as a result of its wider popularity within medicinal and forensic arenas. The electroactivity of scopolamine is attributed to the tertiary amine functionality contained within its heterocyclic tropane ring system. The amine functionality is widely known within the electrochemical field to gift a number of compounds the necessary electroactivity required to be suited for electrochemical detection.[22, 26-36] Alongside sister tropane alkaloid atropine and fellow alkaloid species quinine and cocaine, scopolamine is observed to undergo an irreversible oxidation, through the oxidative *N*-dealkylation mechanism previously described.[17] To the best of our knowledge no reports concerned with the impact upon the substitution of different counterions exist to date. The assumption that the counterion present would have no significant impact upon the electroactivity of the compound could be forgiven. Afterall oxidation occurs at the tropane ring system, an identical feature of the two salt forms. However, as shown within Figure 1, the hydrobromide salt form does indeed demonstrate a significantly greater electroactivity than its hydrochloride counterpart. In line with the limited electrochemical investigations available with detail the detection of scopolamine, alongside atropine, also know to oxidize within the same potential region, the hydrobromide salt form produced an anodic peak at ~ 1.3 V vs Ag upon a carbon paste screen printed electrode.[15-18] In contrast, even at significantly high concentrations within the mM region, the hydrochloride salt did not display any electrochemical behavior of note.

This difference in electrochemical behavior indicates that the counterion is directly impacting the ability of the tertiary amine contained within the tropane functional group to undergo oxidation. The observed behavior leads to the hypothesis that the hydrochloride (HCl) salt form possesses a higher stability than its hydrobromide (HBr) counterpart. The inability to oxidize the hydrochloride salt suggests its HOMO exists at a lower energy than that of the hydrobromide salt, thus gifting the HCl salt form greater stability and hence a greater resistance to oxidation. As such, to induce electro-oxidation of the Sc-HCl salt far greater positive potentials would be required. This indicates that the hydrochloride counterion is able to stabilize the nitrogen cation, contained within the tropane ring, to a greater degree than the hydrobromide counterion. The larger size of the bromide anion, which gifts it a greater stability, could prevent effective stabilization of the nitrogen cation due to spatial limitations. As such, the probability of the Br anion interacting with the nitrogen cation is reduced compared with the smaller chloride anion. Ultimately providing effective stabilization if the hydrochloride salt form hence reducing its electroactivity.

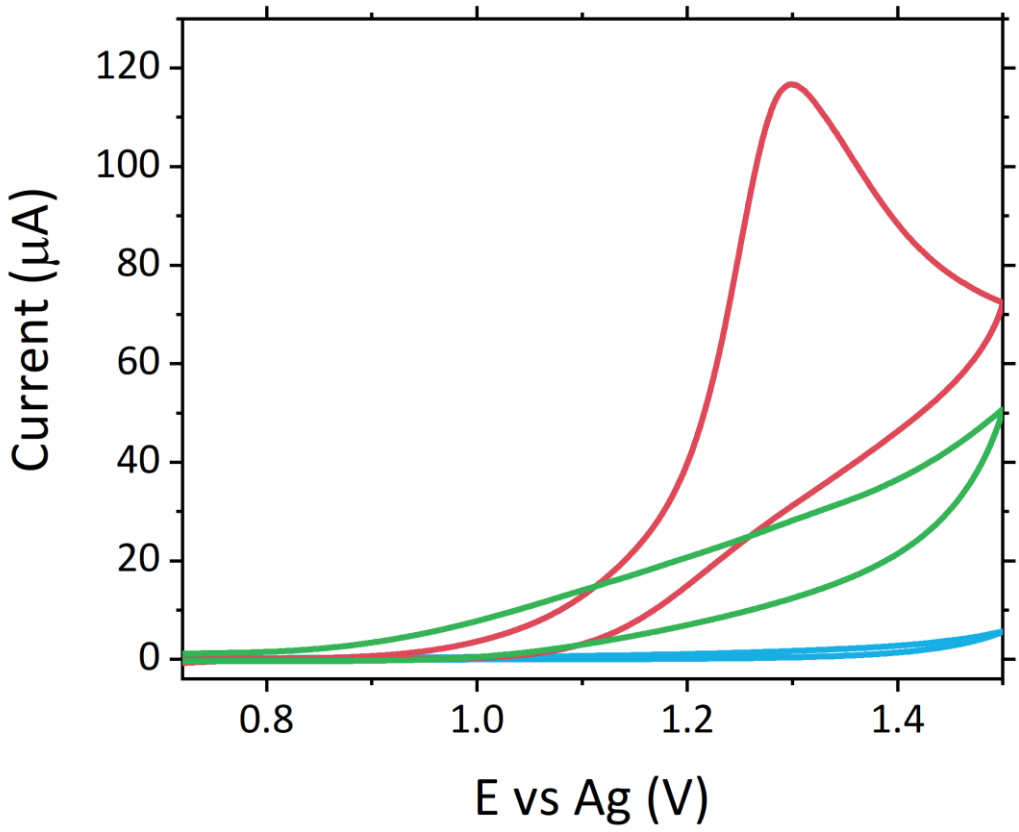

Figure 1: CV of 0.1 M $LiClO_4$ (blue), 2.5 mM scopolamine hydrochloride (green) and 2.5 mM scopolamine hydrobromide (pink) All measurements were collected at a scan rate of 100 mV s^{-1} with a supporting electrolyte of 0.1 M $LiClO_4$.

3.2 ECL of Scopolamine Hydrochloride and Scopolamine Hydrobromide

With interrogation of the CV revealing differing electrochemical behavior between the two salt forms it was not surprising this trend continued through to the luminescence profiles. Figure 2 displays the ECL signals obtained from each of the salt forms when probed with the traditional $[Ru(bpy)_3]^{2+}$ luminophore. In line with the voltammograms depicted in Figure 1, the ECL emission of the hydrobromide salt was observed at a higher signal intensity, attributed to its enhanced electroactivity with a greater number of the corresponding scopolamine radicals, necessary to the form the excited stated from which emission occurs,

available. In contrast to the voltammograms observed following CV interrogation, the hydrochloride salt was observed to produce a measurable ECL signal. The occurrence of this emission signal in the absence of the corresponding voltammogram can likely be explained through consideration of the mediated oxidation process, previously detailed for amine containing compounds when behaving as co-reactants with this ruthenium luminophore.[16-18, 37] The mediated oxidation process proceeds via homogenous electron transfer between the *in-situ* electrogenerated Ru^{3+} species and scopolamine to form the neutral radical, scopolamine•, which subsequently leads to the generation of the excited state and ultimately the observed emission. As such this homogenous oxidative process is the primary mechanism responsible for the formation of the required scopolamine radical within the hydrochloride salt form whilst emission from the hydrobromide salt will primarily arise from the scopolamine radical formed via direct oxidation of the electroactive species at the electrode surface. This trend has previously been observed for the oxidation of tripropylamine (TPrA) in the presence of the $[Ru(bpy)_3]^{2+}$ luminophore by Zu and Bard.[38] Similar to the results observed here it was found that the mechanism by which ECL occurs, either via the catalytical mediated oxidation process or via direct oxidation at the electrode surface, is directly responsible for the intensity of the observed ECL signal.[38] In line with Bard's results, the oxidation of scopolamine which occurs directly at the electrode surface is observed to produce the greatest ECL intensity. This is attributed to the faster oxidation process generating the required scopolamine radical as a result of its direct oxidation at the electrode surface.[38] Although their study investigated the impact of different electrode materials upon the obtained ECL signal intensity, it stands to reason that the ability to oxidize the Sc-HBr salt form directly at the electrode surface is in turn responsible for the enhanced ECL intensity

observed in contrast to the mediated oxidation process of responsible for the emission from the Sc-HCl salt form.

Further to the competing oxidation processes gifting the different electroactivity to the salt forms, consideration must also be given to the oxidation by-products generated during the CV scan. Previous reports have identified the ability of electrogenerated aqueous Br_2 to catalytically oxidize primary, secondary and tertiary amine species.[38] This can be seen thorough consideration of the oxidative cleavage of the tertiary amine, of TPrA, via Br_2 generating a secondary amine species via the reaction described within equation (1).[38]

$$Pr_3N + Br_2 + H_2O \longrightarrow Pr_2NH + CH_3CH_2CHO + 2Br^- + 2H^+ \quad (1)$$

As such, it becomes likely that the bromide anion contained within the hydrobromide salt form will undergo oxidation during the positive potential scan forming aqueous bromine. The presence of these electrogenerated bromine molecules will promote the oxidation of scopolamine, in turn increasing its ECL intensity, via the same processes as previously described for TPrA.[38] In contrast the chloride anion was unable to enhance the ECL intensity for either scopolamine or TPrA, likely due to the inability to oxidize the chloride anion within the given potential range, in line with its higher standard potential compared with bromine.

Not only do differences exist in the maximum ECL intensity but also the potential at which this maximum intensity occurs. To ensure this apparent difference was not the impact of the use of the quasi-reference electrode contained within the screen-printed sensor the maximum ECL intensity potential was determined across 7 different measurements for both the hydrobromide and hydrochloride salt. The variation in the maximum intensity potential were observed to be 0.55% and 0.93% for the hydrobromide and hydrochloride salt forms

respectively. As such, the differing potentials between the two salt forms were indeed a characteristic of intrinsic to the differences within their electrochemical behavior and not the result of variation in potential measurements due to the use of a non-isolated reference electrode. The mean potential for each salt form was determined as 0.97 V and 0.88 V vs Ag for the hydrobromide salt and the hydrochloride salt form respectively. It is believed that similarly to the difference observed in ECL intensity the potential differences are also likely linked to the difference in the radical generation mechanisms. The lower emission potential of the hydrochloride salt can be explained through consideration of the generation of the scopolamine radical species. For the HCl salt form, the Ru^{3+} species is firstly required at sufficient concentrations before the emission process can begin. In contrast the hydrobromide salt form is primarily oxidized at the electrode surface in addition to the enhancement effect of the oxidative cleavage due to the electrogenerated bromine. Therefore, the potential of the hydrobromide salt form is observed at the later than the hydrochloride counterpart, associated with the formation of the scopolamine radical through the heterogenous oxidation process.

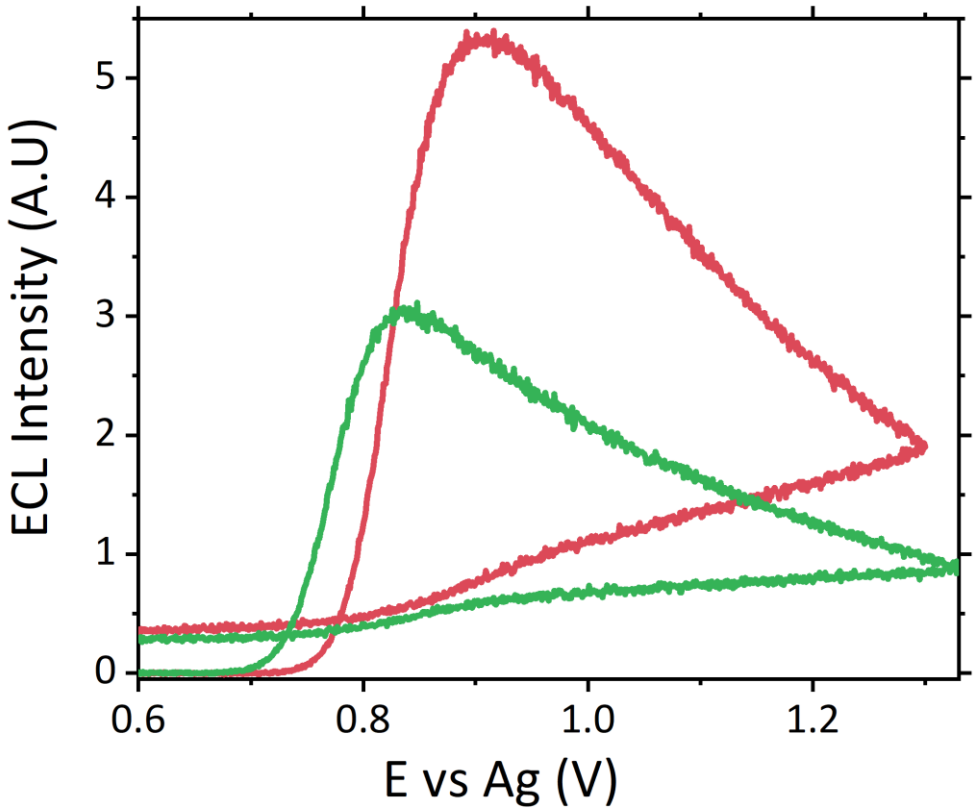

Figure 2: ECL responses of 100 µM scopolamine hydrochloride (green) and 100 µM scopolamine hydrobromide (pink) All measurements were collected at a scan rate of 100 mV s^{-1} with a supporting electrolyte of 0.1 M $LiClO_4$ and a PMT bias of 0.6 V.

The impact of sample pH upon ECL intensity is widely known within the field. Amine containing species in particular are widely recognized to produce the greatest emission intensities close to their pKa values.[26, 29, 39, 40] This is attributed to the non-pronated and hence oxidizable form of the species dominating under these pH conditions. The relationship between ECL intensity and pH with the scopolamine co-reactant has been extensively studied and reported previously.[16, 17] The pKa values of scopolamine hydrobromide and scopolamine hydrochloride have been reported as 7.53 and 7.56 respectively.[41] These almost indistinguishable values are attributed to the identical trends observed for both salt forms

with variations in pH. Both salt forms are observed to produce an increasing ECL intensity between pH 5 and 6, followed by a slight decrease at pH 7, before reaching their maximum intensity at pH 8, refer to Figure 3. This trend is as expected given the pKa of the species lying close to pH 8 and is in line with alternative amine containing compounds investigated. [26, 29, 39, 40] As the pH of the electrolyte solution is altered the stability of the ruthenium luminophore remains unaffected. Confidence in the stability of the ruthenium luminophore across the pH range investigated is observed from the consistent voltammograms of the $Ru^{2+/3+}$ redox couple obtained alongside the negligible ECL signal observed within the blank electrolyte, at the varying pH values investigated, refer to Figure 4. As such the trends and difference observed with pH alterations are solely attributed to the effects of pH upon the co-recants dissociation mechanism. The only notable difference was observed at pH 11, where emission of scopolamine hydrochloride was observed to increase again, whilst the ECL intensity of the hydrobromide salt continued in a downward trend. The observed increase at pH 11 has been previously observed for sister tropane alkaloid atropine and attributed to the alkaline catalyzed degradation of the species forming tropine and tropic acid.[18] Where tropine can also behave a suitable co-reactant species.[18] As such, it tracks that here the HCl salt is undergoing the same alkaline catalyzed degradation processes, generating a second emitting species in the form of scopine. At present the mechanism gifting the HBr salt forms resistivity toward the alkaline catalyzed degradation is not well understood, although may be linked to competition from the oxidative cleavage of the tertiary amine within the hydrobromide salt due to the presence of aqueous bromine.

With comparable behavior with changes in sample pH, the intrinsic behavior responsible for the ECL emission are observed to be largely similar between the two salt forms. As such

indicating that alterations in sample pH would affect both salt forms equally and allow for either salt to be readily identified across a variety of sample matrices with varying pH values.

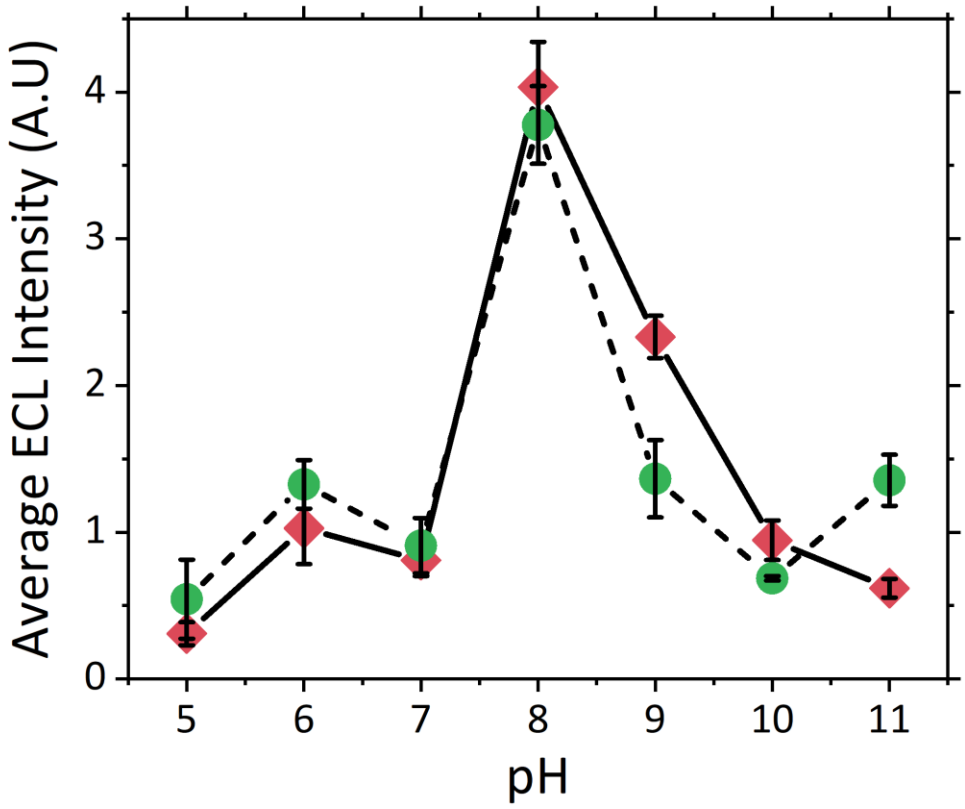

Figure 3: Comparison of ECL intensity with variations in pH for the hydrobromide salt form (pink diamonds) and the hydrochloride salt form (green circles). Both species are present at 50 µM and were prepared in 0.1 M $LiClO_4$ as the supporting electrolyte adjusted to the desired pH. Measurements were collected at a scan rate of 100 mV s^{-1} across $0.5 \leq E \leq 1.36$ V vs Ag at a PMT biased of 0.6 V. Each point represents the mean of the maximum ECL intensity at n=3 with error bars comprising of +/- 1SD across these measurements.

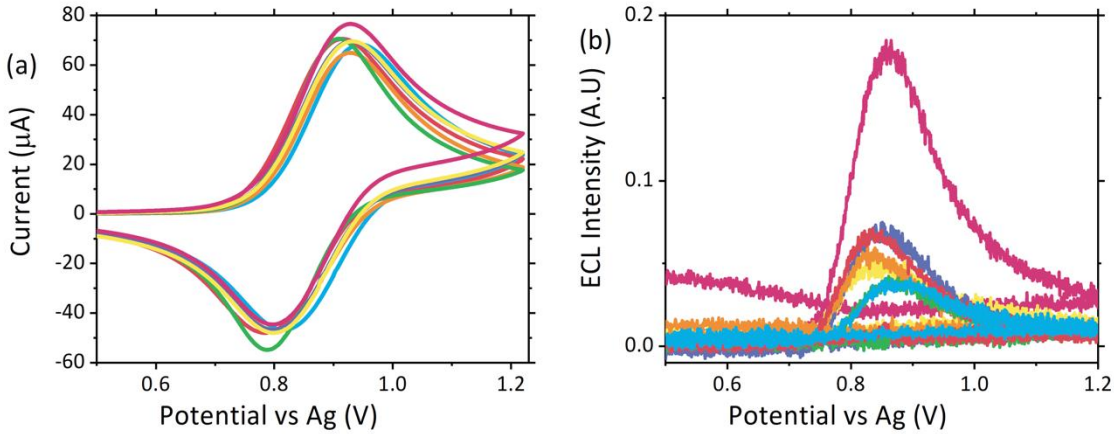

Figure 4: (a) voltammograms show the stability of the ruthenium based sensor with comparable $Ru^{2+/3+}$ redox couples obtained in 0.1 M $LiClO_4$ at all pH ranges investigated. (b) shows the corresponding ECL signals obtained during CV measurements. Measurements were collected at a scan rate of 100 mV s^{-1} across $0.5 \leq E \leq 1.36$ V vs Ag at a PMT biased of 0.6 V.

3.3 Potential Consequences

Typical use of ECL based sensing for analytical applications relies upon detection of the functional group responsible for the electrochemical behavior. To date no one has considered the impact different salt forms may have upon the analytical performance of an ECL based sensor. To assess the wider impact of salt forms upon the analytical sensor performance, assessment was made through predicted concentrations of scopolamine present within samples as determined via the ECL sensor. The analytical performance of the sensor has been extensively discussed in prior works and established to meet the necessary prerequisites for use as an analytical based sensing platform, including a sensor reproducibility of 1.9% and detection limits of 0.418 and 0.136 µM for Sc-HBr and Sc-HCl respectively.[16-18, 42] Scopolamine concentrations present were calculated utilizing the calibration curves constructed within Figure 5. A calibration curve for each salt form was constructed, between 0.625 µM to 100

µM, with both salt forms displaying a linear relationship between ECL intensity and concentration, with linear coefficient values (R^2) of 0.997 and 0.999 for Sc-HCl and Sc-HBr respectively.

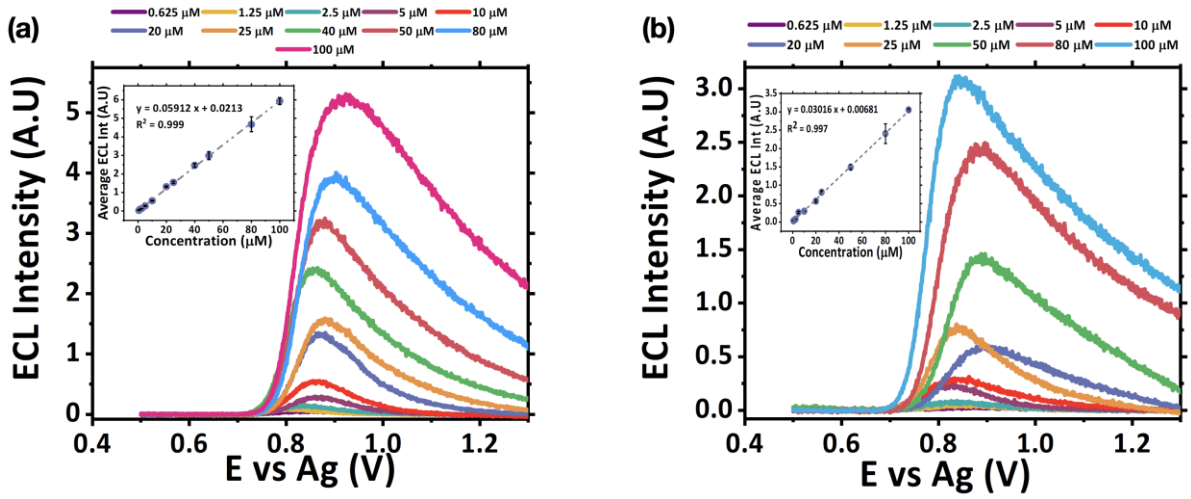

Figure 5: Dependance of ECL intensity with (a) scopolamine hydrobromide and (b) scopolamine hydrochloride concentration between 0.625 to 100 µM. All samples were prepared in 0.1 M $LiClO_4$ as the supporting electrolyte. Measurements were collected at a scan rate of 100 mV s^{-1} across 0.5 ≤ E ≤ 1.36 V vs Ag at a PMT biased of 0.6 V.

To establish the impact of the salt form upon the predicted concentration, samples containing 50 µM Sc-HBr, 50 µM Sc-HCl and a mixed sample containing 50 µM of each salt form, giving a total scopolamine concentration of 100 µM, were analyzed refer to Figure 6. For each sample the predicted concentration of scopolamine present was determined through the use of the equivalent calibration curve, the results of which are summarized within Table 1. As can be seen with consultation of the precited concentrations, when each salt form was calculated using its corresponding calibration curve, good recoveries were observed at 99% for Sc-HBr and 104% for Sc-HCl, both within the typical analytical standard expected of 90-110%. Conversely when the alternative salt calibration curve was used the concentration of

scopolamine calculated was either significantly overestimated or underestimated. This apparent trend was further confirmed through analysis of scopolamine at 10 µM and 100 µM. Investigations over this wide concentration range would help establish the consistency of the observed response variation with change in salt form, and whether it is concentration dependent. All concentrations investigated displayed the same trends with overestimation or underestimation of scopolamine concentration when the analyte salt form and that used to construct the calibration curve salt were contrasting, refer to Table 1. When the salt forms were complementary however, the recoveries observed all lay within the typical analytical standard of 90 - 110%. Interestingly, this demonstrated that despite the total concentration of scopolamine, and consequently the electroactive species, remaining constant, when the salt form was changed the electroactivity of the species was subsequently altered. The impact of such a characteristic, could ultimately lead to significant errors in the predicted concentrations of samples when differing salt forms are used for construction of the calibration curve and the sample under analysis. As such, the values determined would be significantly different from that of the true analyte concentration present. Such scenarios would ultimately have huge consequences upon the potential analytical employment of the sensor. Of particular concern would be unknown samples, were the identity of the analyte itself and thus the salt form present may be unknown, such as encountered within forensic or biomedical applications. Further to this when a mixed sample containing multiple salt forms was analyzed, such as performed here at a final concentration of 100 µM, neither of the calibration curves constructed hold. Instead, the Sc-HBr curve is observed to underestimate the concentrations present, at 75.4 µM, while the Sc-HCl curve overestimates the concentration, at 148 µM. As such, both calibration models and ultimately the ECL sensor

fail to provide the required accuracy necessary for the detection and quantification of analytes for employment within the analytical environment.

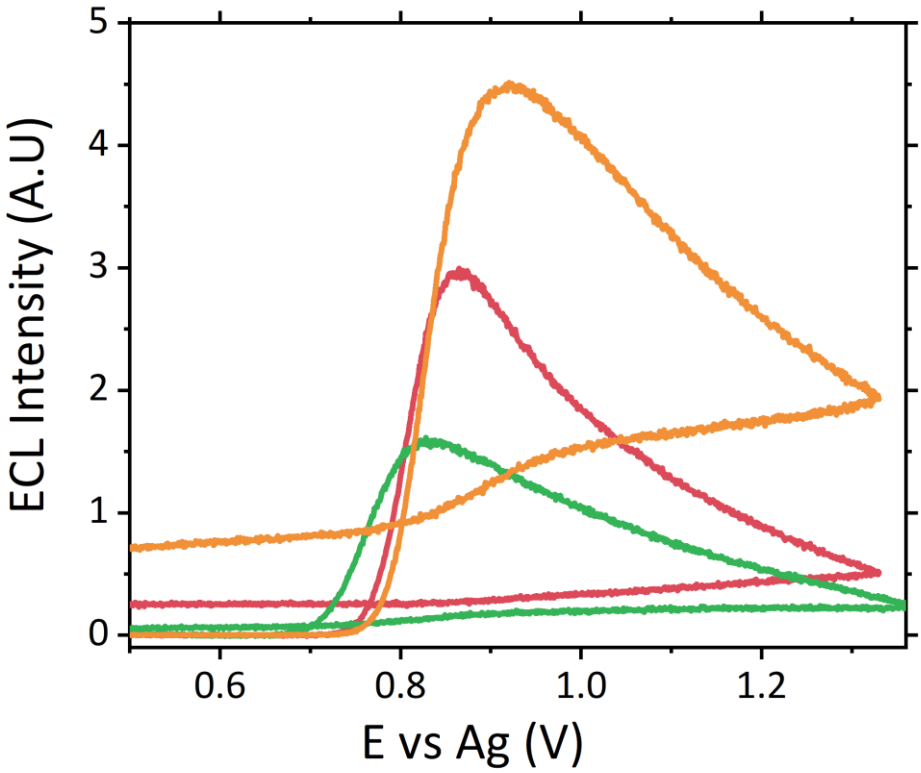

Figure 6: Comparison of ECL responses of 50 µM Sc-HBr (pink), 50 µM Sc-HCl (green) and 50 µM Sc-HBr and 50 µM Sc-HCl (giving a total Sc concentration of 100 µM) (orange). Both species were prepared in 0.1 M $LiClO_4$ as the supporting electrolyte adjusted to pH 8. Measurements were collected at a scan rate of 100 mV s^{-1} across $0.5 \leq E \leq 1.36$ V vs Ag at a PMT biased of 0.6 V.

Table 1: Summary of actual and predicted scopolamine concentrations for each of the salt forms, Sc-HBr and Sc-HCl and a final mixed sample of Sc-HBr and Sc-HCl. Where concentrations were calculated from the specified calibration curve.

Sample	[Sc] Added (µM)	ECL Intensity (A.U)	Calculated [Sc] from Sc-HBr Cal. (µM)	% Recovery	Calculated [Sc] from Sc-HCl Cal. (µM)	% Recovery

Sc-HCl	10	0.282	4.38	44	9.12	91
Sc-HBr	10	0.639	10.4	104	21	210
Sc-HCl	50	1.58	26.3	53	52.1	104
Sc-HBr	50	2.95	49.5	99	97.6	195
Sc-HCl	100	3.07	51.6	52	101.6	102
Sc-HBr	100	5.35	90.1	90	175.8	176
Mixed HBr/HCl	100	4.48	75.4	75	148.3	148

4.0 Conclusions

Within this work we have identified the impact and potential consequences which the counterion can have upon species identification and quantification via ECL. ECL emission is intrinsically linked to the electroactive functionality of the target analyte however, to date no consideration of the counterion and its influence upon ECL emission has been made. Although often considered as bystander ions, and hence not previously considered, we have shown here that the salt form present can indeed have a significant impact upon the ECL signal obtained, even when analyzed upon the same electrode material. Here we have shown, through consideration of the tropane alkaloid scopolamine, how altering the hydrobromide counterion to the hydrochloride counterion can dramatically reduce the electroactivity of the tropane alkaloid functionality of the analyte. This results in the loss of a measurable electrochemical oxidation via cyclic voltammetry interrogation, and a reduction in ECL intensity paired with a shift to less positive potentials. These results indicate the counterion directly influences the electroactivity of the tropane functionality, making it less favorable toward oxidation. This is likely to be the result of a number of factors, including the stabilization of the scopolamine cations, and the formation of aqueous bromine within the hydrobromide salt form. Complementary to prior works, it was seen that if promotion of direct oxidation can be made, the oxidation current and ECL signal intensities are enhanced. Here we see that the hydrobromide salt form undergoes primarily direct oxidation at the electrode surface, promoted by the oxidative cleavage of the tertiary amine within the tropane ring system by the presence of the electrogenerated aqueous bromine. In contrast the hydrochloride salt form, which appears to be more stabilized due to the small spatial requirements of the chloride anion and the inability of chloride ions to promote oxidation and hence enhance ECL intensity, is observed to be less electroactive than the hydrobromide

counterpart. This is in spite of the electroactive behavior attributed to the identical tropane alkaloid groups within both salt forms. This intrinsic characteristic will therefore impact all analytical applications of ECL based sensors where various salt form of the target analyte are available. A such, the reliability of the concentrations calculated via ECL sensors is question with either overestimation or underestimation observed when contrasting salt forms are present within calibration models and samples for analysis. This would have a detrimental effect upon the employment of ECL sensors for future analytical applications, particularly within environments where the salt form may not be known prior to measurement. A complete understanding of why the salt form has such a compounding effect upon the electroactivity of the functional group would require in further in-depth computational studies to assert the mechanism leading to this behavior and confirm the hypothesis proposed within this contribution. Furthermore, computational methods would ultimately aid in the determination of whether this phenomenon is intrinsic across all co-reactants, solely to the amine functional group or specific to scopolamine itself. The importance of such behavior will be of great interest to the general electroanalytical community in addition to the wider analytical community. The importance of consideration of analyte salt forms have been highlighted within contribution and it is now suggested that different salt forms should be incorporated during method development of ECL and electroanalytical methodologies and address the impact were appropriate, particular those intended for use within the forensic and pharmaceutical fields.

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