
Volume 1 | Number 1 | Jan 2013 | Pages 1–100
RSC Advances
www.rsc.org/advances
ROYAL SOCIETY OF CHEMISTRY

Synergistic catalysis by gold nanoparticles and metal ions for enhanced chemiluminescence

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Chemiluminescence has been used as a powerful analytical technique because of its high sensitivity, wide working range, simple instrumentation, absence of background signals and suitability for automation and miniaturization.[1-3] Luminol-hydrogen peroxide (H_2O_2) system is the most intensively studied and frequently employed CL system for chemical analyses. However, it suffers from drawbacks such as weak signals, rapid decay rates and low overall quantum yield (in the range of 0.01-0.1) which need to be addressed. Various catalysts and enhancers such as enzymes,[4,5] transition metal ions.[6,7] and nanoparticles[8-11] (including silver,**[10,12,13]** gold,[14,15,16] bimetallic,[17] magnetic[2] and semiconductor nanoparticles[18]) have been used to improve signal strength. Gold nanoparticles (AuNPs), due to their shape and size-dependent physicochemical and optoelectronic properties and ease of synthesis, have been used in aggregated form in CL assays, leading to signal amplification and increased assay sensitivity.[14,16,19]

Although the effect of salt-induced aggregation of gold nanoparticles on CL has been studied, there are very few reports on the sole effect of these salts/inorganic ions on CL.[20] Few reports show that ions have very less influence on silver enhanced CL.[21,22] However, transition metal ions that are not used for particle aggregation have been found to enhance CL, as free ions in salts[23] in chelated form[24] and in complex with Vitamin B_{12}[25] and haemoglobin.[26] Since, alkali metal salts are commonly used to flocculate nanoparticles, in AuNP-based chemiluminescence enhancement systems, their presence is universal. This reduces the opportunity for uncoupling and observing their individual roles in such systems.

In this study, we observed the combined effect of ions (Li_2SO_4, NaCl, NaOH, KCl, KOH, NH_4Cl, $(NH_4)_2SO_4$, NH_4COOCH_3) and nanoparticles in enhancing the luminol-Urea-H_2O_2 (luminol-U-H_2O_2) CL system. To have a proper understanding of the mechanism, the above salts and nanoparticles were individually studied for chemiluminescence enhancement which was further correlated with their synergistic roles. The role of NaCl beyond nanoparticle aggregation was investigated; the study was then extended to other alkali metal salts, and enhancement was observed. To ensure whether the positive charge of cations or only particular metal ions is responsible for the effect, we used non-metallic ammonium salts, as well as alkalis (NaOH, KOH) in the CL system. The observed collegial effect of salts and AuNPs in the enhancement of luminol-U-H_2O_2 chemiluminescence substantiate the function of these ions *per se* in boosting CL signals, as opposed to their only reported role, that for nanoparticle aggregation. We have successfully demonstrated that the components act in synergy to bring about such increase.

For optimal CL reaction, concentrations of luminol and U-H_2O_2 were found to be 0.15 mM and 0.5 mM respectively at pH of 11-12[16]. With increasing AuNPs volume, the signals were found to increase till 50 µL (0.59 nM) of AuNPs, without reaching saturation (fig-S2). Hence 50 µL (final concentration of 0.2 nM) of AuNPs was selected as the optimum volume for further experiments. The most commonly used agent for aggregation of AuNPs is NaCl, we used the same for our study and found that 0.5 M of NaCl was sufficient to aggregate AuNPs (fig-S3). When NaCl-aggregated AuNPs were used to study CL, it was observed that the reaction mixture with higher concentrations of NaCl than the required amount for complete aggregation was further enhancing CL signals. We hypothesized that nanoparticle aggregation was not the sole factor for enhancement of CL. To prove our supposition, we studied the response of AuNPs-enhanced CL to increasing concentrations of NaCl. From fig-1 it is evident that 3 M NaCl enhanced the CL signals to maximum extent (there was, however, no change in aggregation pattern after 0.5 M NaCl as seen in fig-S3, the signals decreased with further increase in concentration. Collaudin and Blum[20] observed similar enhancement of enzyme-catalyzed luminol CL by NaCl. In our study, NaCl could be detected to as low as 0.2 M with linearity in the range 0.2 M to 2 M (inset provided in fig-1).

A few related salts were selected to see if they have similar catalytic effect on the CL enhancement. Since our objective was to show that the ions, in addition to their role as aggregators, can, independently and in synergy, increase CL in nanoparticle-enhanced systems under similar conditions, we chose 0.5 M concentration to study their effect on CL signals (0.5 to 1 M concentration of salt is used for practical purposes of nanoparticle aggregation).[16] Fig-2 shows that NaCl exhibited highest enhancement (40% for only NaCl and 600% for NaCl along with AuNPs, over control). That the effect is synergistic is evident from 80% increase in CL by NaCl along with AuNPs over AuNPs alone. Li_2SO_4 and KCl showed similar trends. However ammonium ions showed decrease in CL and alkalis showed considerable reduction of signals only in presence of AuNPs.

In order to study if the anions had any effect on the studied chemiluminescence it was imperative to only isolate these ions (i.e. separate them from their counter cations). As such the respective acids of the anions were used, namely, hydrochloric, sulphuric and acetic acid. These acids were used at 0.5 M stock concentration (same as the concentration of salts in the original study). As is expected, the acids, at such concentration, decreased the pH to 1, a condition extremely unfavourable for luminol-U-H_2O_2 CL. Complete quenching of CL occurred. This effect was totally due to the hydrogen ion concentration and not due to the anions. If at all pH has to be maintained at alkaline values, then the acids need to be added at much lower concentration than that used in the study. For this purpose, we observed the effect of the acids at 1 mM stock concentration on CL. The pH was maintained (pH 12) and the anions induced a minor decrease in case AuNP induced CL, whereas increase in CL was seen for reaction sets without AuNPs (data shown in fig-S4).

If the nanoparticles are aggregated, their surface area and hence surface charge (negative in case of citrate-capped gold nanoparticles) density decreases. At our working pH (pH 12) both hydroperoxide and luminol exist as anions.[19] The decrease in the negative surface charge of AuNPs (after aggregation) creates lesser repulsion among the NPs and the anions, facilitating their adsorption and, thereafter, catalysis. Increased number of HO• radicals are formed by the catalytic cleavage of O-O bond of U-H_2O_2 by gold nanoparticles. Partial charge exchange allows increased stabilization of the HO• radical on nanoparticle surface. Higher amount of luminol anion and HO^{2-} (hydroperoxide) anion reacts with HO• radical to produce aminophthalate radical, the latter returning to ground state to produce CL.[14] Adsorption of these radicals on the AuNPs surface also decreases the probability of solvent molecules colliding with aminophthalate: a phenomenon that might lead to the quenching of the excited intermediate. Thus, in our study aggregated AuNPs has shown higher CL signals over dispersed AuNPs and the data of aggregation coincide with that of enhancement (fig-1).

Addition of salts has shown further enhancement in nanoparticle amplified CL (fig-2 and fig-3) with some exceptions. Salts, being electrolytes, may screen negative surface charges and decrease the electrostatic repulsion between similarly charged luminol radical, superoxide anion as well as AuNPs generating more aminophthalate radicals, consequently increasing CL intensity (fig-4). In addition to their electrolytic action, it is also probable that the monovalent ions catalyze the conversion of hydrogen peroxide to superoxide anion. It was proposed in an earlier report that alkaline earth metals could catalyze reaction between fluorescein and potassium permanganate.[30] Co-ordination metal-ligand complexes enable luminescence enhancement due to presence of lone pair of electrons. However, similar to our present study, in alkaline earth metal salts, there are no ligand-complexes formed and lone pair of electrons are absent. Nevertheless, CL is enhanced, for which underlying principle is yet to be established. An earlier study on the effect of halide ions on CL showed that these ions increase the rate constant of the reaction of transition of metal-O_2-luminol complex to aminophthalate, thus facilitating CL (the probable reason for NaCl and KCl showing the highest CL increase in presence of AuNPs in fig-2). Much higher concentration, though, can interfere with AuNPs forming a surface layer,[27] probably causing the decrease in CL when 4 M and 5 M NaCl was used in the reaction mixture in the present work (fig-2). A probable reaction mechanism is provided in fig-4.

It was interesting to note from fig-2, that in case of the alkalis NaOH and KOH, the CL in absence of AuNPs is higher than in their presence. Alkaline pH favours CL reaction, thus the increase over control. However, high pH has been shown to inhibit nanoparticle aggregation, simultaneously reducing the zeta potential of the NPs.[28] This could be a possible explanation for the reduction in CL signals after AuNP addition. Added AuNPs (acidic) decrease the pH to reduce CL reaction efficiency. At the same time alkalis fail to aggregate the nanoparticles substantially so as to recover the reduction in CL. Ammonium ions have shown CL enhancement in enzyme-catalyzed luminol CL.[20] However, our results do not corroborate with these reports (fig-3). The understanding of the effect of ammonium ions is largely unexplored and further analysis is required.

Increase in CL intensity has been shown for acetate ions[29] and chloride ions.[30] No such increase has been reported for other ions we have used, in alkaline conditions. However the enhancement shown in our earlier data cannot be attributed to these anions as the quantity is much less (1 mM) as compared to our earlier experiments (0.5 M). As such it can be concluded that the anions were not responsible for the enhancement of CL in our study, and thus there was no need to control them. Hydroxyl anion is responsible for increase in pH of the reaction mixture, thus facilitating luminol oxidation and favouring CL reaction.

To our knowledge this is the first report of synergistic enhancement of luminol-U-H_2O_2 CL by alkali metal ions and AuNPs. Sodium ions showed very high enhancement with and without AuNPs (600% in synergy with gold colloids). Exact mechanism of such enhancement and the quenching of CL signals by ammonium ions are yet to be explored. Enhanced CL, due to cooperative effect of metal ions and nanoparticles, may improve the sensitivity and resolution of CL based assays. Alternatively, the salts themselves can be detected using the enhancement principle. Previously various oxidation compounds, that oxidise luminol, have been detected using CL reactions.[31] In real samples, during CL based analysis of specific target compounds, the interfering effects of the salts on CL can also be identified using the assay. Platforms using enhanced CL carry the potential for developing miniaturized sensing devices and highly sensitive onsite analytical applications.

Acknowledgement:

Authors thank the Director of CSIR-Central Food Technological Research Institute (Mysore, India) for facilities and constant encouragement. Authors gratefully acknowledge Dr. Honnur Krishna, Research Associate, CSIR-Central Food Technological Research Institute for fruitful discussion. Authors are also thankful to the Council of Scientific and Industrial Research for providing fellowships.

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List of figures of the manuscript entitled **"Synergistic catalysis of gold nanoparticles and metal ions for enhanced chemiluminescence"** authored by Richa Sharma, K V Ragavan, K S Abhijith, Akanksha and M S Thakur.

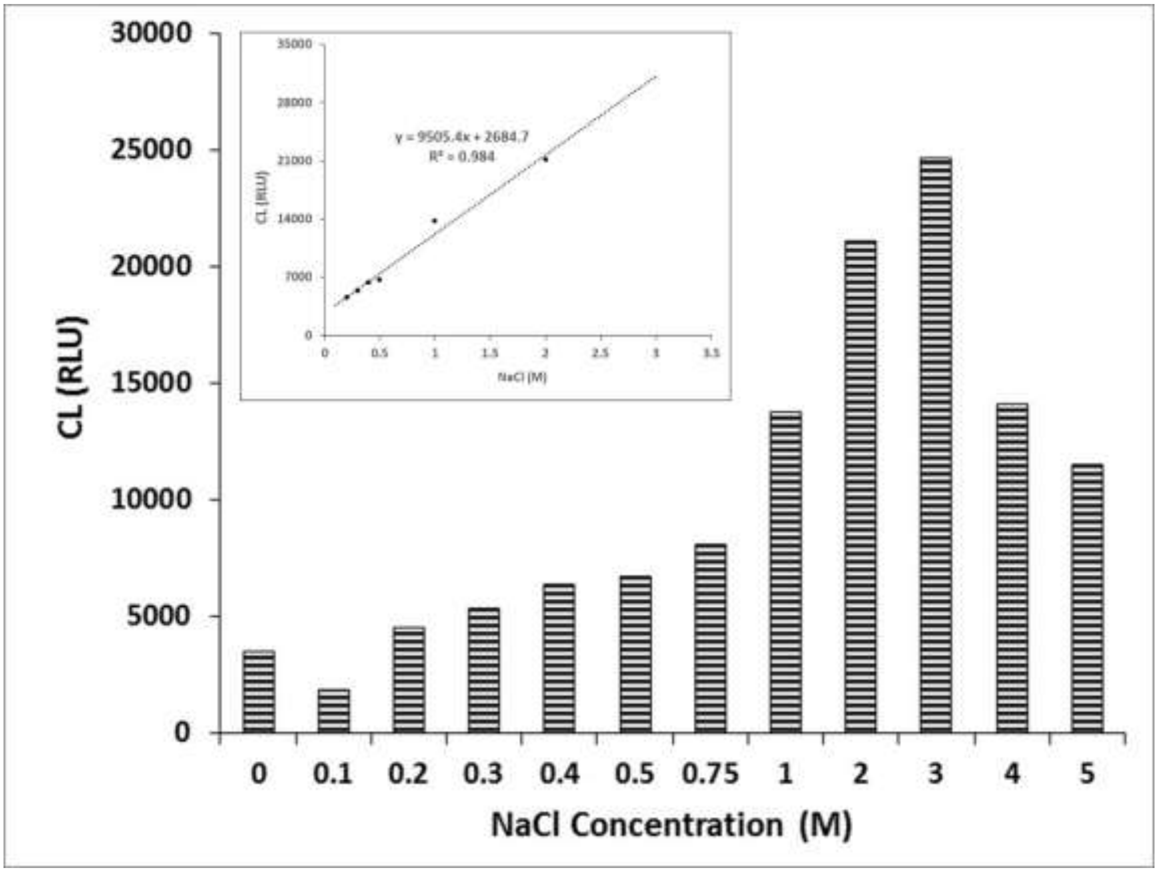


Fig-1: Effect of different concentrations of NaCl (0.1 M to 5 M) on AuNPs-enhanced CL (n=3). The NaCl and AuNP volume was kept constant for all. The maximum enhancement is seen in 3 M. Inset shows the linearity of the enhanced CL data for NaCl (0.2 M to 2 M).

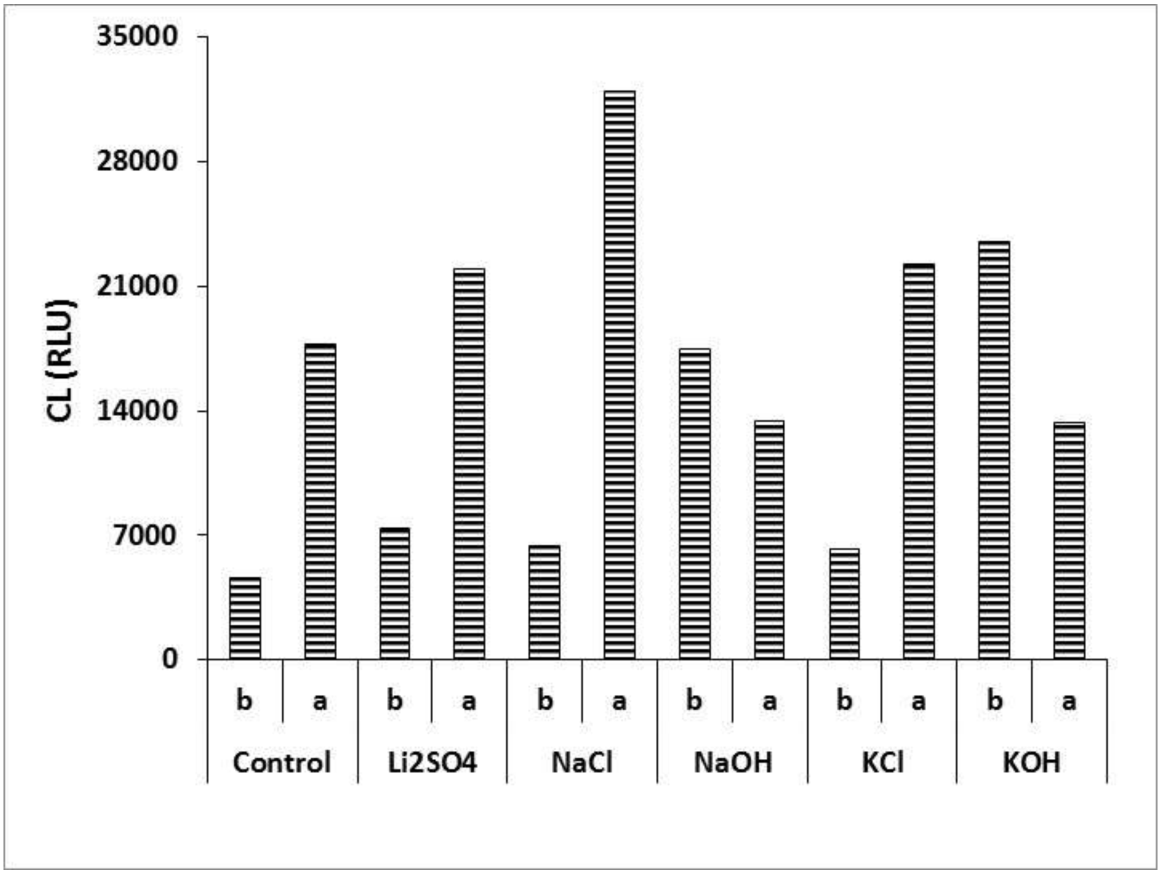


Fig-2: The effect of different salts and alkalis on CL (n=3). The bars represent the CL observed on addition of all reaction components. Control contains only luminol, U-H2O2 and AuNPs. All others have the respective salt (or alkali) in addition. (b): The CL signals before addition of AuNPs. (a): The CL signals after addition of AuNPs.

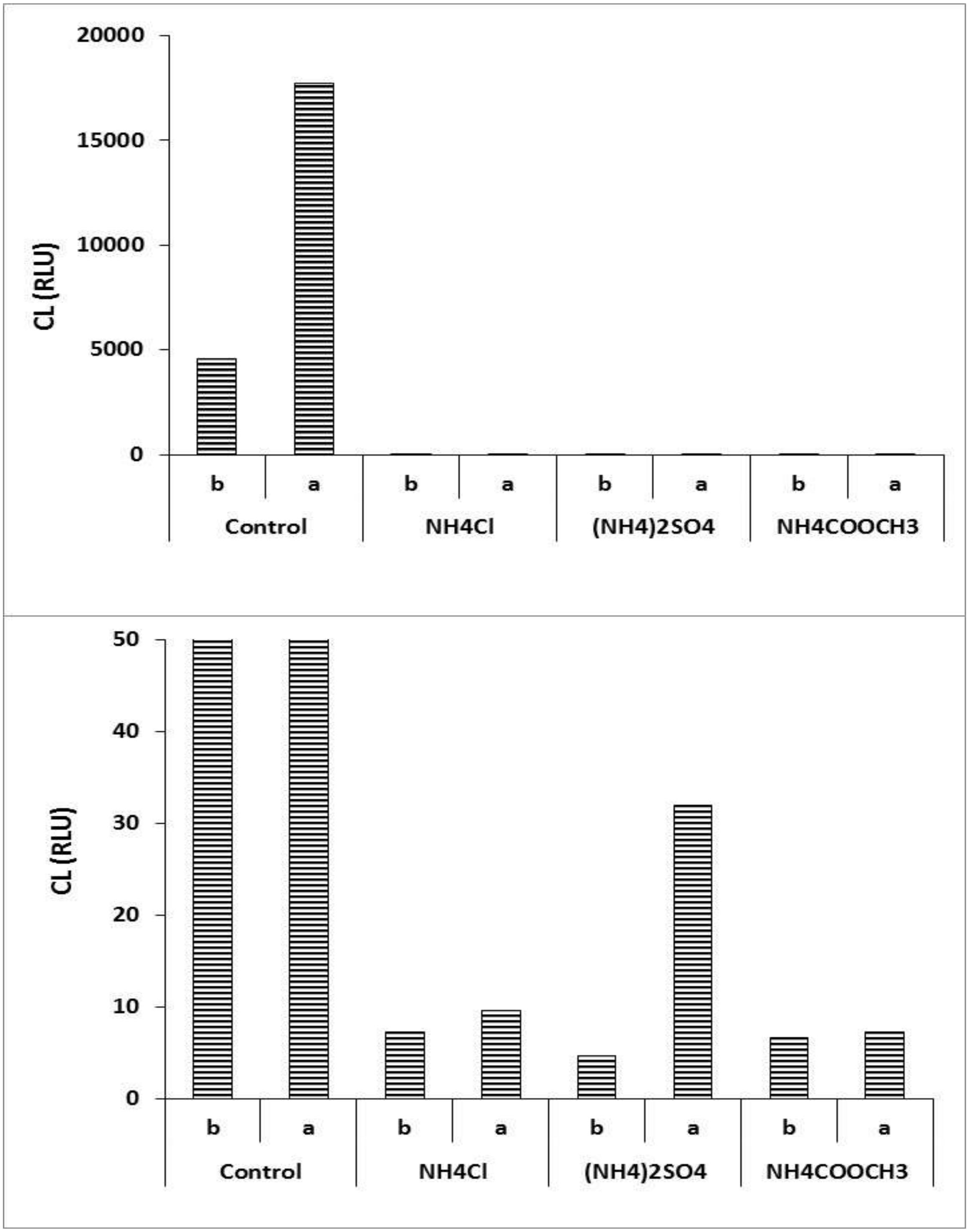


Fig-3: The effect of ammonium salts on CL (n=3). Very low CL signals as compared to control necessitated representation in a separate graph, with a lower CL scale range (bottom panel).

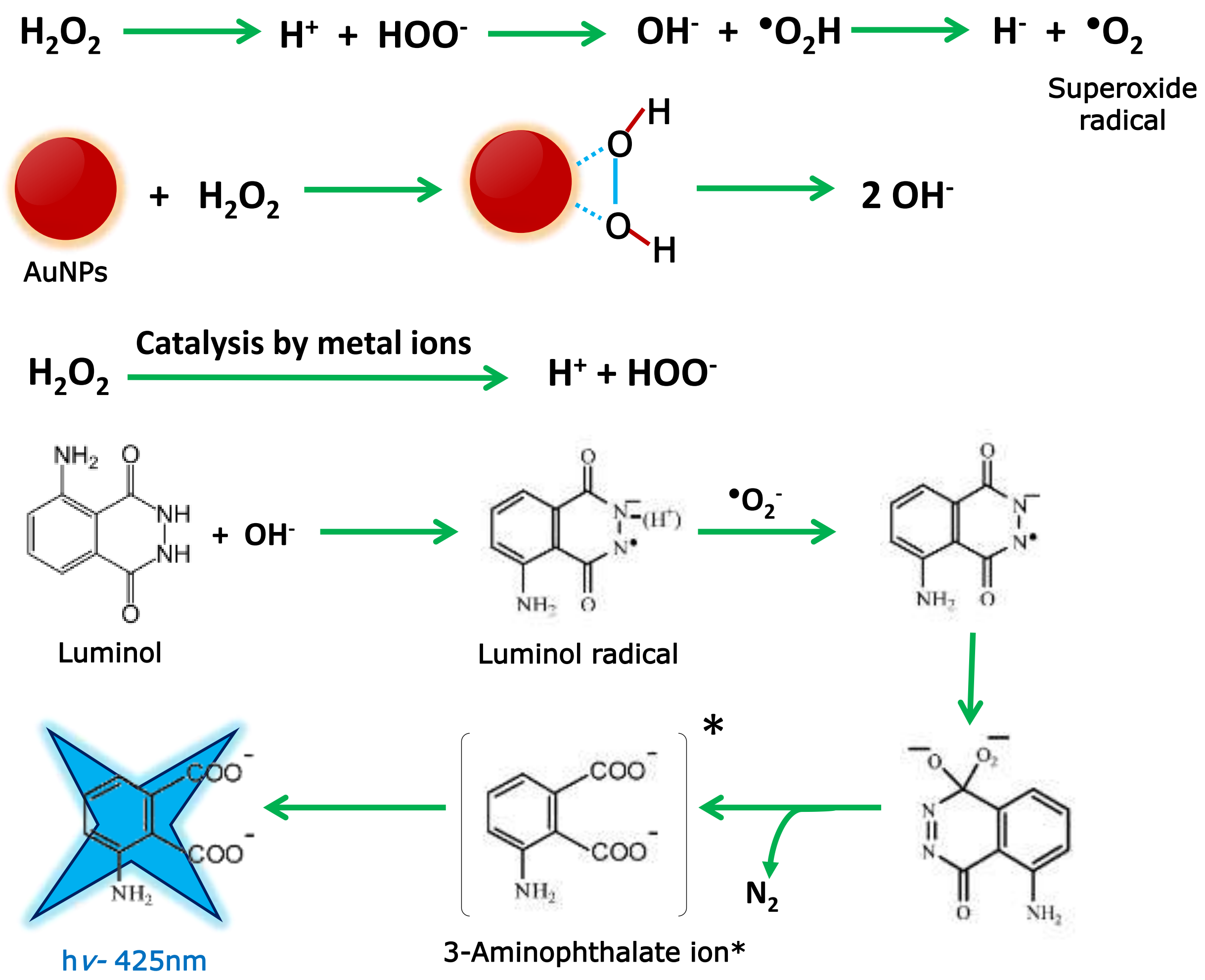
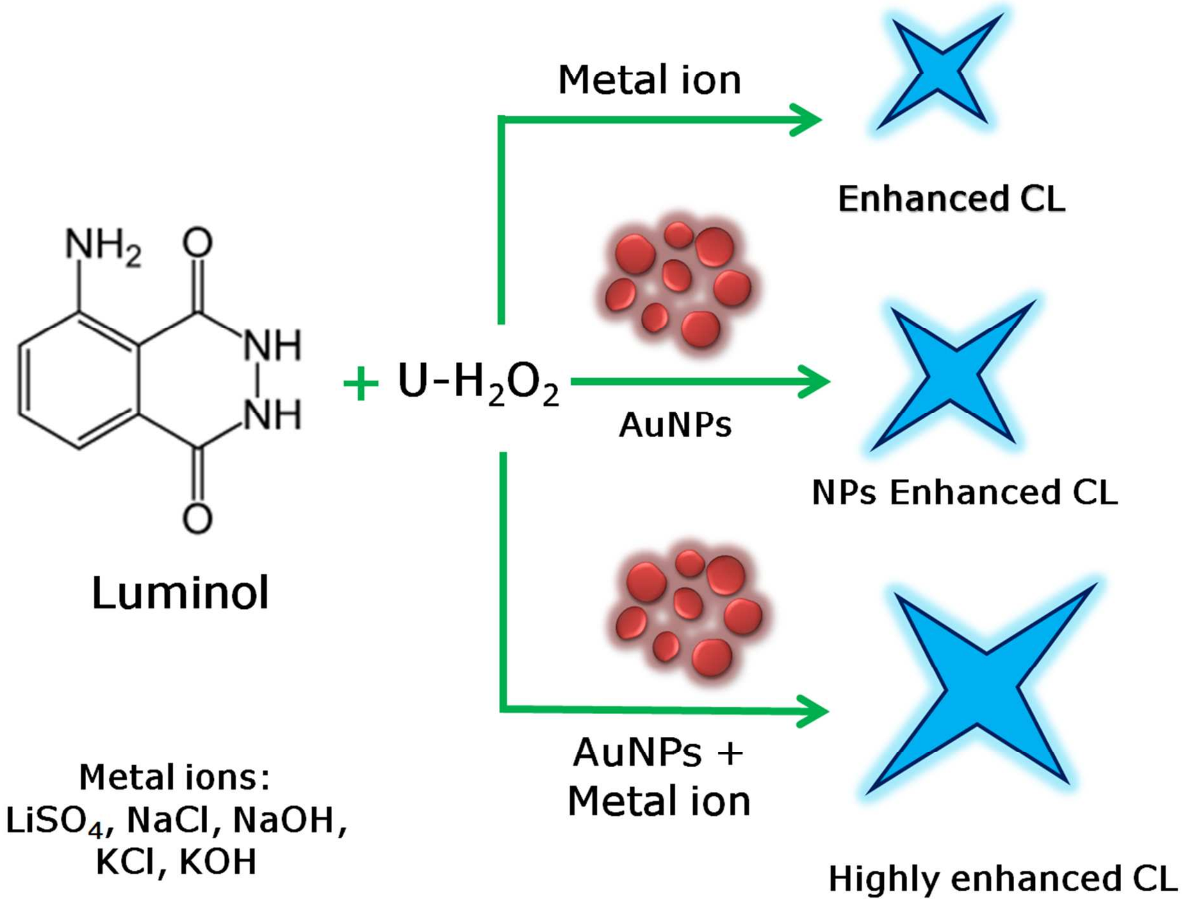


Fig-4: The probable mechanism for CL enhancement by salts in synergy with gold nanoparticles. The alkali metal salts catalyze the breakdown of hydrogen peroxide. Aggregated gold nanoparticles adsorb more hydroperoxide radicals thus catalyzing the CL reaction. The electrolytic action of the salts further reduce electrostatic repulsion between luminol radical, gold nanoparticles and superoxide anion. Thus reaction rates are increased, more aminophthalate ions are generated enhancing chemiluminescence.

TOC Graphic:



Keywords: *Gold nanoparticles, enhanced chemiluminescence, metal ions, synergistic catalysis, luminol-hydrogen peroxide.*

Novelty:

The first report of individual and synergistic roles of alkali metal ions and gold nanoparticles in the enhancement of luminol-Urea-H_2O_2 chemiluminescence. A maximum of 600% increase was evident for NaCl in synergy over gold nanoparticles.