Carbon dots applications in electrochemical and electrochemiluminescence sensors: Some examples of pathogen sensors

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Abstract

The ongoing worldwide pandemic of the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has revealed that simple, fast, low-cost, reliable, and portable sensor systems should be developed immediately. Electrochemical and electrochemiluminescence (ECL) based pathogen sensors are promising alternatives to conventional methods due to several advantages including in situ and fast response time, high sensitivity, low cost, being portable, easy-to-operate and simple to construct using different receptors including, antibody, enzyme, DNA, aptamer, etc. Carbon dots/graphene quantum dots have been used as labels, electrode material, ECL luminophores, electrode modification materials, amplifier, reaction catalysts to increase sensitivity and selectivity of electrochemical and ECL sensors. In this mini-review, the latest applications of carbon dots in electrochemical and ECL based sensors are summarized and some examples of pathogen sensors are given.

Keywords: Carbon dots, electrochemistry, electrochemiluminescence, biosensor, pathogen

1. Introduction

Even today, one of the threats to the life and health system is the pandemic or infection caused by pathogen microorganisms. Approximately 1400 known species, which are primarily water and foodborne microorganisms, are considered as human pathogens [1]. However, novel species may emerge as a worldwide hazard from now to tomorrow. For example, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which has emerged in China in December 2019, now still leads to cause a global pandemic. The ongoing outbreak of SARS-CoV-2 infection throughout the world has revealed that the virus should be diagnosed at the earliest appearance of symptoms. To control or cease the contagion of viruses, viruses should be detected not only in health laboratories but also directly in places where humans circulate and spread the virus such as airports, trains, boats, and any public aggregation places [2]. Therefore, simple, fast, low-cost, reliable, and portable sensor systems should be developed immediately for SARS-CoV-2, which is also an important requirement for all pathogens including bacteria, viruses, and fungi [2]. Because culture-based methods considered as the gold standard for most pathogens are frequently time-consuming and laborious [3]. Similarly, molecular methods including enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) frequently require trained staff to perform the complex assays [4].

The electrochemical sensor, a device detects the concentration of target molecules in real-time by measuring electrical signals generated by biological, chemical, or physical events between target and sensor surface. Various recognition elements including enzymes, proteins, antibodies, MIP, etc. are immobilized onto the electrode via different chemical modification methods [5,6]. The electrical signal monitored by electrode can be current, voltage or impedance, etc. So far, electrochemical sensors have been applied to various fields including clinical, food, environment and drug analysis [7], due to their several advantages such as in situ and fast response time, high sensitivity, low cost, being portable, easy-to-operate and simple to construct [8-10]. Among

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applications of electrochemical sensors, pathogen detection is one of the remarkable applications that enable the before-mentioned advantages as well as shortening of analysis time. Up to now, different electrochemical biosensors were developed for rapid, direct (without sample preparation) and in situ detection of foodborne, waterborne, or human pathogens [11].

With the emergence of nanoscience and nanotechnology, nanomaterial has been used as a signal amplification to increase the sensitivity and selectivity of electrochemical sensors [8, 10]. Among nanoparticles, carbon nanoparticles especially carbon nanotubes and graphene are considered as cornerstone owing to their outstanding properties. Non-toxicity, biocompatibility, high effective surface area, superior electrical conductivity, mechanical strength, and electrocatalytic activity are considered some of these unique properties. Thanks to these properties, they are successfully adapted for the development of electrochemical sensors [10,12]. However, their manufacturing techniques are difficult and expensive [13].

Carbon quantum dots or graphene quantum dots are defined as luminescent nanoparticles with dimensions less than 10 nm [14-16]. Due to their biocompatibility, low toxicity, water/organic solubility, photostability, green and simple synthesis, chemically stability, good disparity, etc., they are assessed as a potential non-toxic alternative to traditional semiconductor quantum dots [17-19]. Recent studies showed that carbon dots and graphene quantum dots, zero-dimensional carbon-based nanostructures, can substitute graphene and graphene oxides because of their own aforementioned superior properties including enhanced electrocatalytic activity [13]. Furthermore, compared to natural enzymes, CDs have broader pH stability and are stable at different temperature ranges. Their large surface area enables them to immobilize a higher ratio of protein, enzymes, and antibodies by simple adsorption or other techniques. Besides, various functional groups on their surface facilitate the attachment of biomolecules. By taking into account all the above considerations, CDs can increase the precision and accuracy of biosensors. Ironically, the utilization of CDs as a signal amplifier or electrode modifiers for the development of electrochemical biosensors has not widely been investigated and still remains undeveloped [13].

Electrochemiluminescence (ECL) is defined as the production of light resulted from the electrochemical reaction that high-energy electron transfer reactions occurred between electron-donors and acceptors generate excited state and then light emission is formed [20]. Since ECL sensors merge the advantages of chemiluminescence and electrochemical sensors, ECL sensors possess many assets such as high sensitivity and selectivity, low background signal, easy reaction control and simple instrumentation, broad working range [21,22]. ECL sensors have been applied to clinical diagnostics, environmental assays, food analysis, etc. until now [23]. Carbon dots/graphene quantum dots show electrochemiluminescence properties [24] and can be used as ECL luminophores [25]. Therefore, integrating graphene quantum dots/carbon dots with ECL sensors platform have led to developing novel biosensors with high sensitivity and selectivity and expanded the applications of carbon dots/graphene quantum dots as well [25].

The aim of this mini-review is to present some examples from the latest applications of carbon dots in electrochemical / ECL based sensors and pathogen sensors.

2. Electrochemical sensors

In electrochemical sensors, carbon dots/graphene quantum dots employed as labels or electrode material can accelerate charge transport and redox reactions, enhance selectivity, conductivity, stability, and reproducibility of sensors. So, they are considered as indispensable components of high performing electrochemical sensors [26]. Recently, instead of carbon dots/graphene quantum dots, their nanocomposites with different structures that are carbon nanotubes, graphene, chitosan, and metal components, etc. are preferred in electrochemical sensor design [27].

Carbon dots/graphene quantum dots are not yet actively integrated with conductometric and potentiometric sensors by virtue of low-sensitivity, less-stability, less-specificity, and inaccuracy. However, their utilization in the voltammetric, amperometric, and impedimetric sensor is common compared with others [28]. Some sensor applications of carbon dots/graphene quantum dots are given in the following.

2.1. Amperometric sensors

Applications of carbon dots in amperometric sensors have recently been explored in the determination of different analytes. Carbon dots/graphene quantum dots have been used in the modification of the carbon paste electrode to increase the electrocatalytic power
of the sensor. For example, carbon dots synthesized from carbon soot were utilized for chronoamperometric detection of adrenaline which acts as a neurotransmitter with an important role in respiration, energy metabolism, etc., in physiological conditions [29,30]. Compared to the bare carbon paste electrode, the modification with carbon dots catalyzed the electrochemical reaction of adrenaline and enabled the simultaneous determination of adrenaline, ascorbic acid, and serotonin via functional groups of carbon dots including hydroxyl groups. Selective determination of adrenaline was achieved with a detection limit of 6 nM [30].

In a study, carbon paste electrode was modified with carbon dots extracted from sugar beet molasses [14] and then the enzymatic detection of neurotransmitter acetylcholine was achieved with this electrode. Carbon dots were used as nanozymes in this amperometric sensor due to peroxidase-mimicking behavior. Hydrogen peroxide formed from acetylcholine in the presence of acetylcholinesterase and choline oxidase enzymes was broken down by carbon dots with similar behavior of peroxidase. In addition, carbon dots provided higher immobilization ratio of enzymes on the electrode. The prepared sensor might easily detect lower level of acetylcholine (LOD: 5 nM) [31].

Carbon dots have been utilized in nanocomposites with other nanoparticles or compounds in the amperometric sensors to increase the precision and accuracy of sensors. The application of a glassy carbon electrode modified with a nanocomposite composed of aminated graphene quantum dots, thiolated β-cyclodextrin, and gold nanoparticles reported for analysis of quercetin has been reported [32]. The sensor selectively detected quercetin at the linear response range of 1-210 nM with a lower detection limit of 285 pM even in the presence of flavonoids having the similar structure of quercetin [32]. For the detection of glucose, a nanocomposite containing carbon dots and copper oxide was synthesized and used to develop a non-enzymatic amperometric sensor [33]. The copper oxide was used as an enzyme to oxidase glucose. The synergistic effects of copper oxide and carbon dots enhanced the electrochemical performance of the sensor. However, the reported limit of detection (0.2 mM) is not very low.

2.1. Amperometric pathogen sensors
As one of the major global health threats, chronic hepatitis B viruses cause chronic infection, cirrhosis, and liver cancer [34,35]. Yan et. al. [35] developed an amperometric immunosensor for the early diagnosis of hepatitis B viruses by detecting the hepatitis B surface antigen. In this research, a nanocomposite containing nitrogen-doped graphene quantum dots, palladium, gold, copper, and the electroactive polymer was synthesized as a signal amplification platform to design label-free electrochemical immunosensor. Nitrogen-doped graphene quantum dots were used as support material and reducing agents of gold, palladium, and copper. The catalytic activity of nitrogen-doped graphene quantum dots for the decomposition of hydrogen peroxide increased with other metal nanoparticles. Thus, the effects of hindering electron transport resulted from increasing antigen concentration were reduced. Eventually, the study reported the lowest linear range and limit of detection among previously developed electrochemical sensors (Table 1) [35].

Graphene quantum dots can be a promising alternative to horseradish peroxidase having some drawbacks including difficulties in immobilization and high-cost [36,37]. An amperometric immunosensor for the detection of Yersinia enterocolitica which causes foodborne-zoonotic infection via contaminated food and water including especially raw or undercooked meat was fabricated by Savaş and Altintas [37]. In this study, graphene quantum dots laminated on the gold electrode were used as nanozyme instead of horseradish peroxidase due to their peroxidase mimicking properties [37]. The increase in the amperometric signal was arrived at the mA range because of the interplay between gold and graphene quantum dots [37].

2.2. Voltammetric sensors
In literature, applications of carbon dots in voltammetric sensors have been more common compared to amperometric and impedimetric sensors. Carbon dots/graphene quantum dots have been utilized as a signal amplifier with other nanomaterials, conductive polymers, or compounds.

Arumugasamy et al. [38] designed an electrochemical dopamine sensor that graphene quantum dots were incorporated in multiwalled carbon nanotube and this nanocomposite was utilized for rapid, simple, and sensitive detection of dopamine. Carboxyl groups of graphene quantum dots significantly raised electrochemically active surface area of the electrode. The limit of detection (LOD) and the linear range was reported as 95 nM and 0.25-250 μM respectively [38].

In another work, Jahani et al. [39] developed a voltammetric sensor for the simultaneous
determination of norepinephrine and acetylcholine using graphene quantum dots and ionic liquids together. In this research, the combination of graphene quantum dots and ionic liquid dramatically increased the current compared to a single utilization of them. Furthermore, this binary provided two separate oxidation peaks belongs to norepinephrine and acetylcholine. The LOD value was reported as 0.06 μM for norepinephrine [39].

A voltammetric immunosensor was constructed using polyaniline functionalized graphene quantum dots which enables good conductivity was used to determine heat shock protein 70, which is a biomarker of early screening of depression [40]. The detection limit of this method (0.05 ng mL⁻¹) was reported as lower than that of Enzyme-Linked ImmunoSorbent Assay (ELISA) (0.47 ng mL⁻¹) thanks to the higher electrocatalytic activity of polyaniline functionalized graphene quantum dots [40].

Tian et al. [41] fabricated a voltammetric sensor using a nanocomposite containing amino-functionalized graphene quantum dots and conductive polymer for the detection of mercury with low LOD (0.00006 nM) [41].

The accuracy of a sensor depends on the immobilization of receptor molecules on the electrode surface. In this sense, various functional groups of carbon dots/graphene quantum dots facilitate the attachment of receptor molecules. Besides, their large surface area increases receptor loading. In a study reported by Majumdar et al. [42], DNA was immobilized on the surface of carbon dots for the detection of mutagenic, carcinogenic nitrosamines. Carbon dots ensured multiple binding sites for DNA due to larger surface area and various surface functionalities. Selective determination of nitrosamine was achieved with lower LOD (9.9 nM) than those of commercial methods including chromatography (μM) [42].

2.2.1. Voltammetric pathogen sensors
Similar to Majumdar et al. [42], Xiang et al. [43] developed a voltammetric sensor for the detection of hepatitis B viruses in which single-stranded DNA was immobilized on graphene quantum dots based on the strong interaction between graphene quantum dots and single-stranded DNA, as a probe. In this study, the signal of electroactive indicator (K[Fe(CN)]₃) decreased when the probe was strongly bound to graphene quantum dots. However, the signal increased when the hepatitis DNA was bound to probe. By taking into account the increase in signal, the concentration of the virus was detected with the detection limit of 1 nM (Table 1) [43].

Doping of carbon dots/graphene quantum dots with different compounds leads to new ways for novel applications. For example, Abazar and Noorbakhsh [45] constructed an impedimetric aptasensor for the detection of insulin using a nanocomposite composed of thiol doped graphene quantum dots and silver nanoparticles in immunosensor for the hepatitis C virus (Table 1). To load more antibodies, silver nanoparticles were covalently bound to thiol doped graphene quantum dots on the glassy carbon electrode. In this research, thiol doped graphene quantum dots utilized as intermediator to immobilize antibody owing to their large surface area and biocompatibility [44].

2.3. Impedimetric sensors
Carbon dots/graphene quantum dots are used for immobilization of receptors including aptamers. For example, Abazar and Noorbakhsh [45] constructed an impedimetric aptasensor for the detection of insulin using a nanocomposite composed of carbon dots and chitosan. In this study, carbon dots were dispursed in chitosan to prevent agglomeration. The synthesized nanocomposite was used as a platform for covalent immobilization of aptamer and fabrication of insulin sensor. A detection limit of 106.8 pM was reported with a linear range of 0.5-10 nM [45].

In recent years, an increase in the usage of antibiotics for nonbacterial infections as feed additives has created antibiotic resistance, threatening the world. Antibiotic resistance is an important issue in both the management of infections and food safety. Ye et al. [46] developed an impedimetric biosensor for rapid and sensitive detection of bacterial response to antibiotics. In this research, graphene quantum dots were conjugated with Salmonella antibody and immobilized on the alumina membrane. This electrochemical sensing platform was used to capture bacteria. After then, the antibiotics, enrofloxacin, and ampicillin were contacted on the sensor. Bacterial response to antibiotics was determined rapidly according to the change in the impedance signal. The authors reported that graphene quantum dots provided enhanced sensitivity and low detection limit (pM level) [46].

2.3.1. Impedimetric pathogen sensors
An impedimetric imnosensor was constructed for the detection of the hepatitis E virus, causing acute viral hepatitis worldwide. Nitrogen and thiol doped graphene quantum dots and gold-embedded
polar polyaniline wires were deposited on the glassy carbon electrode (Table 1). Nitrogen and thiol doped graphene quantum dots ensured to conjugate antibodies using EDC-NHS chemistry to amplify electrochemical activity and to provide their adsorption onto gold nanoparticles. The sensor determined the hepatitis E virus selectively at the femtogram level in the presence of other viruses, serum, and even in the culture medium [47].

3. ECL sensors

Owing to their unique properties, especially electrochemical properties, carbon dots/graphene quantum dots can be used as ECL luminophores (Fig. 1), electrode modification materials, amplifier, reaction catalysts, etc. in the development of ECL biosensors [21].

![Figure 1](image)

**Figure 1.** Illustration of carbon dots utilized as ECL luminophore in the presence of persulfate.

ECL properties of carbon dots were discovered in 2009 by Zhu et al. [48]. In this research, carbon dots were synthesized from glucose and polyethylene glycol (PEG-200) via the microwave pyrolysis method. After discovery, ECL sensors were developed using carbon dots/graphene quantum dots for the detection of metals ions, protein, DNA/RNA, folic acid, nitrite, atrazine, lincomycin, tetracycline, etc. [21,27,49-54]. In addition, carbon dots/graphene quantum dots based ECL sensors were fabricated for the determination of cancer cells, enzyme activity, or pathogen microorganisms [21,55,56]. Some examples of ECL sensors published in 2020 are given as follows.

Yang et al. [57] developed a sandwich-type ECL sensor for the detection of prostate-specific antigen, a significant biomarker of prostate cancer. In this research, two antibodies for the recognition of antigens were used. The primary antibody (Ab1) was immobilized through an amide bond between the antibody and the nanocomposite (1) composed of poly (indole-6-carboxylic acid)/flower-like gold nanoparticles on the glassy carbon electrode.

Secondary antibody (Ab2) was attached to the nanocomposite (2) containing gold nanoparticles, graphene quantum dots on the poly (etherimide)-graphene oxide. When these two antibodies were bound to a prostate-specific antigen, ECL signal was formed through luminescent graphene quantum dots under applied voltage to the glassy carbon electrode. ECL signal was amplified very strong thanks to the synergistic effects between the nanocomposite (1) and the nanocomposite (2). The strong ECL signal gave an improvement in the sensitivity of the sensor with the detection limit of 0.44 pg mL\(^{-1}\) [57].

Raju et al. [58] developed an ECL sensor for the detection of copper ions in which phosphorus-doped carbon dots was utilized as a luminophore. The authors reported that phosphorus-doped carbon dots gave a strong ECL signal compared to bare carbon dots. The detection limit was measured as 0.27 nM for this sensor with the range of 1-1000 nM [58].

In another study, ECL immunosensor for okadaic acid, being diarrhetic shellfish poison was developed using phosphor and sulfur-doped carbon dots [59]. Co-doped carbon dots showed the best ECL performance among bare, mono-doped carbon dots. The linear working range and detection limit were reported as 0.01-20 ng mL\(^{-1}\) and 0.005 ng mL\(^{-1}\), respectively [59].

Apart from the utilization of carbon dots/graphene quantum dots as luminophore, Kalaiyarasan et al. [60] used aminated carbon quantum dots as co-reactant for Ru(bpy)\(^{2+}\). While ECL intensity of Ru(bpy)\(^{2+}\) decreased with butein, a plant polyphenol, in the presence of aminated carbon dots there was no change in the case of commercial co-reactants [60].

3.1. ECL pathogen sensors

*Escherichia coli* O157:H7 as one of the major foodborne pathogens, causes severe and fatal diseases including hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS) especially in young and immunocompromised peoples. Chen et al. [61] reported an easily prepared ECL sensor for the detection of *Escherichia coli* O157:H7. In this report, nitrogen-doped graphene quantum dots were synthesized instead of bare dots to enhance ECL efficiency (Table 1) [21]. *Escherichia coli* O157:H7 recognition and binding were performed with polyaniline wires decorated polymer synthesized via electropolymerization method. In this approach, nitrogen-doped graphene quantum dots labeled with *E. coli* O157:H7 polyclonal antibody used as luminophore to give strong ECL activity with co-
reactant (potassium persulfate) on the glassy carbon electrode [61].

<table>
<thead>
<tr>
<th>Target Pathogen</th>
<th>Method</th>
<th>Receptor</th>
<th>Working electrode/ ECL luminophore</th>
<th>Synthesis of carbon dots</th>
<th>Linear working range</th>
<th>LOD</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatitis B</td>
<td>Amperometry</td>
<td>Surface antigen</td>
<td>A nanocomposite composed of nitrogen doped graphene quantum dots, palladium, gold, copper and electroactive polymer on glassy carbon electrode</td>
<td>Hydrothermal method using citric acid and dicyandiamide (180 °C- 12 h)</td>
<td>10 fg mL$^{-1}$ - 50 ng mL$^{-1}$</td>
<td>3.3 fg mL$^{-1}$</td>
<td>[35]</td>
</tr>
<tr>
<td>Yersinia</td>
<td>Amperometry</td>
<td>Monoclonal antibody</td>
<td>Graphene quantum dots laminated on the gold electrode</td>
<td>Commercial</td>
<td>1- 6.23 x10$^8$ cfu mL$^{-1}$</td>
<td>5-cfu mL$^{-1}$ for milk</td>
<td>30-cfu mL$^{-1}$ for serum</td>
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<tr>
<td>Hepatitis B</td>
<td>Voltammetry</td>
<td>Single stranded DNA</td>
<td>Graphene quantum dots modified with glassy carbon electrode</td>
<td>Pyrolysis using citric acid (200 °C)</td>
<td>10-500 nM</td>
<td>1 nM</td>
<td>[43]</td>
</tr>
<tr>
<td>Hepatitis C</td>
<td>Voltammetry</td>
<td>Antibody</td>
<td>A nanocomposite of thiol doped graphene quantum dots and silver nanoparticles on glassy carbon electrode</td>
<td>Acid oxidation using multiwalled carbon nanotube</td>
<td>0.05 pg mL$^{-1}$ - 60 ng mL$^{-1}$</td>
<td>3 fg mL$^{-1}$</td>
<td>[44]</td>
</tr>
<tr>
<td>Hepatitis E</td>
<td>Impedimetric</td>
<td>Antibody</td>
<td>Nitrogen and thiol doped graphene quantum dots and gold-embedded polyaniline wires on the glassy carbon electrode</td>
<td>Hydrothermal method using citric acid and thiourea (160 °C- 4 h)</td>
<td>10$^{-12}$ - 10$^{-15}$ g mL$^{-1}$</td>
<td>8 x 10$^{-15}$ g mL$^{-1}$</td>
<td>[47]</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>ECL</td>
<td>Antibody</td>
<td>A nanocomposite composed of graphene quantum dots and silver nanoparticles used as luminescence enhancer</td>
<td>Hydrothermal method using citric acid and ammonia</td>
<td>10$^{-10^7}$ cfu mL$^{-1}$</td>
<td>8 cfu mL$^{-1}$</td>
<td>[61]</td>
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<tr>
<td>O157:H7</td>
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<td>Escherichia coli</td>
<td>ECL</td>
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<td>A nanocomposite composed of graphene quantum dots and silver nanoparticles used as luminescence enhancer</td>
<td>Hydrothermal method using citric acid and ammonia</td>
<td>10$^{-10^7}$ cfu mL$^{-1}$</td>
<td>5 cfu mL$^{-1}$</td>
<td>[62]</td>
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</tbody>
</table>

In another study issued by Li et al. [62], a smartphone-based ECL system was developed for the detection of Escherichia coli using an antigen-antibody relationship. Silver nanoparticles were adsorbed on the graphene quantum dots to amplify the ECL signal and this nanocomposite was used as a luminescence enhancer in tris(2,2′-bipyridyl)ruthenium (II)/tripropylamine (Ru(bpy)$_3^{2+}$/TPA) assay (Table 1). Generated ECL signal was caught with the smartphone camera and was measured by image analysis application during analysis [62]. The researchers pointed out that the portable device constructed in the research can be suitable for point of care testing (POCT) [62].

4. Summary and future works

Nowadays, infections and pandemics caused by pathogens are continuing to threaten the world. Besides, a novel pathogen may emerge as a global threat at any moment as in coronavirus pandemic. The ongoing outbreak of SARS-CoV-2 global infection has proved the need for reliable, selective, rapid, easily accessible, portable, and low-cost diagnostic methods for the detection of pathogens. Electrochemical and electrochemiluminescence (ECL) based pathogen sensors are promising alternatives with respect to conventional methods because they offer several advantages including in situ and fast response time, high sensitivity, low cost, being portable, easy-to-operate and simple to construct. As rising stars of carbon-based nanoparticles, carbon dots/graphene quantum dots have been used as labels, electrode material, ECL luminophores, electrode modification materials, amplifiers, and reaction catalysts to increase sensitivity and selectivity of electrochemical and ECL sensors. This mini-review highlights the applications of carbon dots in the electrochemical and ECL pathogen sensors and tries to draw attention to this subject, for future studies as well.
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