

Strip-based nano-silver biocomposite construct for rapid screening of aqueous mercury

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A simple, rapid and a cost-effective colorimetric method for detection of Hg^{2+} in an aqueous system using silver nanoparticle (AgNP) has been developed in the present study. AgNPs were synthesised using gum kondagogu (GK), a natural biopolymer that acts as both reducing and stabilising agent. The stability of GK-AgNPs composites was determined by zeta potential and was observed to be -30.43 mV. The yellow colour spot on the nitrocellulose membrane turned colourless at Hg^{2+} concentration of 20 ppm in aqueous solution, which can be observed visually. By using inductively coupled plasma mass spectrometry, it was observed that as the concentration of Hg^{2+} increases the Ag concentration decreased. Further, scanning electron microscopy and energy-dispersive X-ray spectroscopy were used to confirm the morphological changes on membrane before and after treating with Hg^{2+} . The interference of other metal ions was also studied to validate Hg^{2+} specificity. This method was successfully employed to screen Hg^{2+} in industrial effluents. In summary, this study entails the development of a nitrocellulose membrane based mercury sensor that has practical applicability for on-site screening of Hg^{2+} contamination in the aqueous system.

1. Introduction: Mercury is one of the most highly hazardous pollutants which recognised accumulative and persistence in the environment [1–3]. The major source of mercury emission is thermal power plant emission, which is a great environmental health hazard as their exposure can lead to damage of brain, kidneys, the central nervous system, the immune system and the endocrine system [4, 5]. In order to prevent the harmful effect of mercury on the world population, the monitoring and observation of mercury contamination are therefore of paramount importance.

The methods currently used for the determination of Hg^{2+} include atomic absorption spectrometry [6], inductively coupled plasma mass spectrometry (ICP-MS) [7], selective cold vapour atomic fluorescence spectrometry [8]. These methods provide excellent selectivity and sensitivity for the detection of Hg^{2+} , however, these are expensive, time consuming and inappropriate for on-site screening applications. Paper-based detection of mercury by using 1,5-diphenylcarbazone has been developed, which is used to test multi-metal ions such as cadmium, chromium, copper, iron, mercury, molybdenum, lead and zinc which can lead to interference of metals (<http://www.guidechem.com/dictionary/538-62-5.html>). To overcome these drawbacks, a novel approach was made to develop a simple biosensor-based procedure for rapid detection of Hg^{2+} in aqueous solution. Organic fluorophores or chromophores [9–12], semiconductor nanocrystals [9, 13–15], polymer-oligonucleotide composites and so on were used [16]. However, these new strategies still suffer from drawbacks like cross-sensitivity with other metal ions and incompatibility with aqueous systems.

Colorimetric probes are a great advantage as these can be detected visibly [17]. Previously, most of the nanoparticles-based colorimetric probes were designed by modification of nanoparticle surface with specific ligands which upon interaction with an analyte changes the dispersion/aggregation state of nanoparticles followed by a change in colour [18–21]. Although these methods have led to successful development of mercury ion sensors, the shortcomings are the high cost of ligands used, difficult in the conjugation of ligands and it is a more time-consuming process. Synthesis of silver nanoparticles (AgNPs) by using gum kondagogu (GK) and its antimicrobial activity nanoparticles exhibited significant antibiofilm activity against gram-negative bacteria compared to

gram-positive bacteria [22]. Silver NPs are preferred over Au NPs due to their higher extinction coefficient [23].

Recently, a ligand-free GK-AgNP for selective determination of Hg^{2+} in various groundwater samples was successfully developed [23]. This method can be effectively used to detect Hg^{2+} in real samples. However, it needs specialised equipment for the analysis and it is intricate to detect Hg^{2+} at onsite sample screening. Hence, presently there is a need to develop a ligand-free, colorimetric protocol which should be simple and easy to carry to on-site for the detection of Hg^{2+} . Previously, we established GK as a matrix to remove toxic metals like lead, cadmium, nickel and total chromium from aqueous solutions. Additionally, it also acts as an excellent reductant for the synthesis of metal nanoparticles [24, 25].

In this study, we report a colorimetric method for detection of Hg^{2+} using nanosilver-biocomposite material (GK-AgNP) immobilised on nitrocellulose matrix, which has practical relevance for on-site screening of Hg^{2+} in aqueous samples, with no requirement of specialised equipment. In the presence of Hg^{2+} , the yellow colour of the sensor gets decolourised to leuco colour, which can be observed visually. Furthermore, our method is relatively simple and easy with negligible interference with the other metal ions for detection of Hg^{2+} .

2. Materials and methods

2.1. Synthesis of GK-AgNPs: GK grade-1 was purchased from Girijan Cooperative Corporation, Hyderabad, India. All the solutions were prepared in MilliQ water. GK was powdered in a high-speed mechanical blender (Sumeet, India) and sieved to obtain a material with a mean particle size of 45 μm . GK stock solution (1%) was prepared by adding GK powder to MilliQ water followed by overnight stirring at room temperature. The AgNPs were synthesised by a standard protocol as reported earlier [25].

2.2. UV-visible spectrophotometer: AgNPs synthesised by GK were analysed using UV-visible spectrophotometer (Cary 60 UV-visible spectrophotometer, Agilent, USA) by scan from λ 300–900 nm wavelength. The absorption spectrum of gum was also recorded against MilliQ water blank.

2.3. Zeta potential analysis: The zeta potential of GK and GK-AgNPs was analysed by DelsaMax PRO – Beckman Coulter Life Sciences, USA.

2.4. Transmission electron microscope analysis: The size and shape of GK-AgNP was analysed by transmission electron microscope (JEOL 3010, Tokyo, Japan) operated at 200 kV. The sample was prepared by drop casting of the colloidal solution on a carbon-coated copper grid followed by air drying at room temperature.

2.5. Preparation of GK-AgNP coated nitrocellulose membrane strips: The nitrocellulose membrane disc having 5 mm diameter was punched out using a manual puncher. The discs were immobilised by an adhesive glue stick (Pidilite, Mumbai) on transparent polyester film (thickness 100 μm) with dimensions of 5 $\times$ 1 cm (length $\times$ width). An aliquot of 10 μl of GK-AgNP was coated on the nitrocellulose membrane disc and allowed to dry at room temperature over-night, in an incubator at 40°C. These strips were then used for the detection of mercury in aqueous solutions.

2.6. Scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDAX): The size and morphology of the nanoparticles were determined by SEM (Carl Zeiss EVO 18 special edition, 73447 Oberkochen, Germany). It was equipped with EDAX as attachment with a resolution of 3.5 nm for tungsten filament and 2.5 nm for LaB6 filament and EDAX detector resolution is 133 eV EDAX (INCA Xact, Oxford, UK) and JEOL 3010 (Tokyo, Japan). SEM analysis was carried out on the GK-AgNP coated on nitrocellulose membrane before and after treatment with Hg^{2+} .

2.7. Detection of Hg^{2+} ions: In order to demonstrate the sensitivity of GK-AgNP coated on a nitrocellulose membrane, different concentrations of Hg^{2+} ions were added to the aqueous solution. The binding of GK-AgNP was also tested for dissolution of nanomaterial in ultra-pure water, which served as a reagent control. The content of nanosilver immobilised on the nitrocellulose disc was quantitated using ICP-MS after the dissolution test. The disappearance of yellow colour was monitored after the addition of Hg^{2+} ions at different concentrations (10–1000 ppm). The effect of other metal ions Cu^{2+} , Pb^{2+} , Mn^{2+} , Na^+ , Fe^{2+} and Zn^{2+} were also tested.

2.8. Analysis of real water samples: The real water samples were collected in Hyderabad city at different zones (Nacharam, Uppal and Hussain Sagar Lake) which are exposed to polluted waste water. These water samples were autoclaved at 121°C and 15 kg/cm² pressure and centrifuged at 10,000 rpm for 5 min. The supernatant was used for the detection of Hg^{2+} using GK-AgNP coated on nitrocellulose membrane. The Hg^{2+} concentration in the collected water samples was determined using ICP-MS.

2.9. ICP-MS analysis: Analysis of metal ions was determined by ICP-MS (Agilent Technologies-7700 Series). Prior to analysis purging was done using plasma gas (15 L min⁻¹), auxiliary gas (1.0 L min⁻¹) and carrier gas (1.0 L min⁻¹). The pressure of argon gas cylinder was maintained in the range of 650–720 kPa; whereas the nebuliser pump was maintained at 0.10 rps. Tuning was done for Li, Y and Tl at no gas, He and H₂ modes whose values matched with the recommended limit values. The analysis was done at spectrum mode with three-point peak pattern, in triplicates at 100 sweeps. The sample uptake was for 30 s with nebuliser pump speed of 0.5 rps. The probe was rinsed with 5% HNO₃ and Milli Q water for 40 s each with nebuliser pump speed of 0.5 rps. Working standard solutions were prepared by diluting the multi-element stock standard solution. The samples were acid digested using concentrated 69% HNO₃ followed by dilution before subjecting it to ICP-MS analysis.

2.10. Detection of Hg^{2+} in simulated effluents: The multiple metal ion solution (Cu^{2+} , Pb^{2+} , Mn^{2+} , Na^+ , Fe^{2+} and Zn^{2+}) was prepared at the concentration of 10 mg L⁻¹. The experiments were conducted to appraise the effect of other metal ions on GK-AgNP coated on nitrocellulose membrane. Different concentrations of Hg^{2+} (20, 60, 100, 200, 500 and 1000 ppm) were used for spiking in simulated multiple metal ion solution.

3. Results and discussion

3.1. Synthesis of AgNPs: A change in colour of the solution was observed when 0.5% of GK was added to 1 mM AgNO₃ solution followed by autoclaving at 121°C and 15 kg/cm² pressure. The colourless AgNO₃ solution changed to a light yellow colour indicating the formation of nanoparticles (Fig. 1B, insert). These GK-AgNP were analysed in UV–visible spectrophotometer. An aliquot of 1 ml of formed GK-AgNP was scanned from λ 300–900 nm wavelength. A strong peak with a maximum absorption around λ 425 nm was observed (Fig. 1B), which corresponds to a typical surface plasmon resonance of conducting electrons from the surface of AgNPs [26]. Zeta potential measurement gives an idea of the surface charge of GK-AgNPs. Measurements of zeta potential were also carried out in order to study the stability of GK-AgNPs as this is extremely important for many applications. The zeta potentials of GK and GK-AgNPs composite were –36.83 and –30.43 mV, respectively [Fig. 1A (i) and (ii)]. This result suggests that the GK AgNPs composite material in solution was stable. These AgNPs were highly monodispersed and circular in shape as visualised by TEM (Fig. 1C). The particle size distribution of GK-AgNP was analysed by using Gattan software by measuring $\sim$ 100 NPs present in TEM image. This particle size analysis indicated a range of 3–11 nm, out of which most of the nanoparticles were of 8 nm in size (Fig. 1D).

3.2. Detection of Hg^{2+} ions using GK-AgNP strips: To evaluate the limit of detection, different concentrations of Hg^{2+} (10, 20, 60, 100, 200, 500 and 1000 ppm) in aqueous solution were subjected to GK-AgNP coated strip (Fig. 2A). The colorimetric response was monitored visually as the yellow colour of GK-AgNP on nitrocellulose membrane turned colourless. There was no change in the colour when the strip was exposed to ultra-pure water which was considered as a negative control [Fig. 2A(a)]. Further, it was observed that there was no significant dissolution of nanosilver in the bio-composite material coated onto a nitrocellulose membrane, as 97% of Ag nano was accounted by ICP-MS analysis. While at a concentrations range of 1000–20 ppm, the yellow spot completely disappeared in 10 min [Fig. 2A(b–g)]. The minimum detectable limit of Hg^{2+} by the strip method was 20 ppm. Similarly, we also

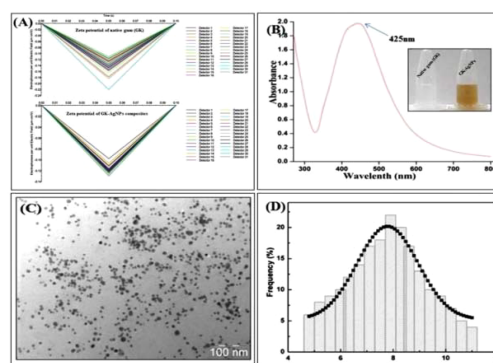


Fig. 1 Characterisation of AgNPs synthesised by using GK
A Zeta potential values of native gum and GK-AgNP composite material.
Visible analysis
B UV–visible absorption spectra of AgNPs
C TEM image of AgNPs synthesised with 0.5% (w/v) GK
D Particle size distribution

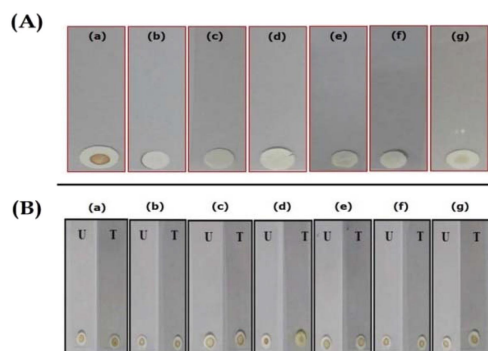


Fig. 2 Colorimetric detection of Hg^{2+} by test strips
 A Colorimetric detection of Hg^{2+} ions using GK-AgNP coated on nitrocellulose membrane: (a) control, (b) 1000 ppm, (c) 500 ppm, (d) 200 ppm, (e) 100 ppm, (f) 60 ppm, (g) 20 ppm
 B Colorimetric response of GK-AgNP on other metal ions at 1000 ppm: (a) copper, (b) sodium, (c) cadmium, (d) iron, (e) manganese, (f) zinc, (g) chromium; (U) – untreated (T) – treated with mercury

carried out GK-AgNP strip experiments to confirm the interaction/detection with other metal ions (Cu^{2+} , Mn^{2+} , Zn^{2+} , Cd^{2+} , Pb^{2+} , Cr^{2+} and Na^+) at higher concentration of 1000 ppm. There was no significant change in the colour of GK-AgNP on nitrocellulose membrane suggesting that this method is highly sensitive and selective to only Hg^{2+} ions (Fig. 2B). Therefore, this method can be used for selectively detecting Hg^{2+} in aqueous solution and also these strips can be easily carried to on-site to test Hg^{2+} contamination in environmental samples.

3.3. SEM-EDAX analysis: The morphological changes of GK-AgNP on nitrocellulose membrane were clearly demonstrated by SEM-EDAX. The circular GK-AgNPs were observed on the membrane which were untreated with Hg^{2+} (Fig. 3A). The GK-AgNP disappeared after treating with Hg^{2+} , only the membrane structures were seen (Fig. 3B). The EDAX analysis was performed before and after treating with Hg^{2+} . A strong Ag peak was observed indicating the presence of Ag on nitrocellulose membrane strip (Fig. 3C), however, when this strip was treated with Hg^{2+} ions, a strong Hg^{2+} peak and a drastically decreased Ag peak were observed (Fig. 3D). This occurs due to the formation of Hg–Ag amalgam. According to Lee *et al.* [21], this is due to the affinity between Ag^0 and Hg^0 . The reduced Hg^0 will be deposited onto the silver surface and this causes the formation of Hg–Ag amalgam. This alloy leaches from the membrane into the solution that ultimately leads to decrease in colour on nitrocellulose

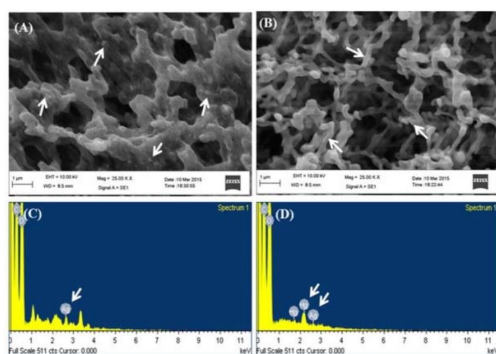


Fig. 3 Morphological changes of GK-AgNP on nitrocellulose membrane
 A Before treating with Hg^{2+}
 B After treating with Hg^{2+} and corresponding EDAX analysis
 C Before treating with Hg^{2+}
 D After treating with Hg^{2+}

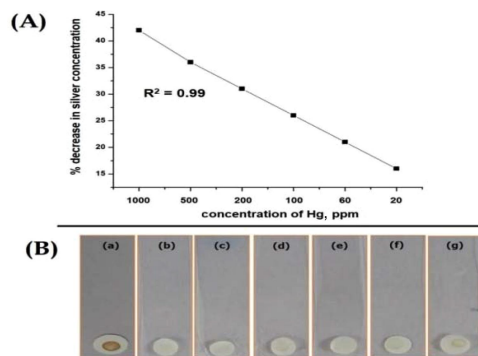


Fig. 4 Quantification of silver on test strip and selectivity of Hg^{2+} detection in the synthetic industrial effluents

A Quantification of silver on nitrocellulose membrane by ICP-MS coated on nitrocellulose membrane at different concentrations (0–1000) ppm before and after exposing of Hg^{2+}
 B Interaction and selectivity of synthetic industrial effluents on GK-AgNP nitrocellulose membrane: (a) after treating with synthetic industrial effluents, (b–g) apiking of Hg^{2+} with different concentration (1000, 500, 200, 100, 60 and 20 ppm) of Hg^{2+}

membrane. A strong mercury peak was observed due to Hg^{2+} on nitrocellulose membrane along with a weak silver peak showing only a few traces of AgNPs (Fig. 3D). In the present study, the EDAX data is well complimented with ICP-MS results.

3.4. Quantification of silver on nitrocellulose membrane by ICP-MS: The sensitivity of GK-AgNP towards Hg^{2+} was determined using ICP-MS. The nanoparticles which were coated on nitrocellulose membrane were quantified by ICP-MS prior to exposure and after exposure to Hg^{2+} . As the concentration of Hg^{2+} increased from 20 to 1000 ppm ($R^2=0.99$), the percent concentration of Ag on nitrocellulose membrane decreased as shown in Fig. 4A, indicating the interaction of AgNPs with Hg^{2+} ions resulting in the formation of amalgam.

3.5. Effect of simulated multi-metal industrial effluents on Hg^{2+} detection: The interaction and selectivity of synthetic industrial effluents on GK-AgNP nitrocellulose membrane were investigated. The colorimetric response was tested in the presence of various metal ions including Cu^{2+} , Mn^{2+} , Zn^{2+} , Pb^{2+} , Cr^{2+} , Fe^{2+} and Na^+ in a solution at the concentration of 100 ppm. The colour of the nitrocellulose membrane strip coated with GK-AgNP remained unchanged after treating with synthetic industrial effluents (Fig. 4B). This indicates that there is no interference of synthetic industrial effluents on the nitrocellulose membrane strip in absence of Hg^{2+} . An attempt was made to study the interference of Hg^{2+} in simulated industrial effluents by spiking with the addition of Hg^{2+} at different concentrations (1000–20 ppm). There was the complete disappearance of colour beyond 60 ppm concentration of Hg^{2+} [Fig. 4B(b–f)]. Complete discolouration of yellow colour was not observed at a concentration of 20 ppm of Hg^{2+} , indicating the limit of sensitivity among the presence of other metal ions [Fig. 4B(g)]. However, the discolouration at 20 ppm of Hg^{2+} was highly significant as compared to negative control [Fig. 4Ba].

3.6. Interference of individual ions on the detection of mercury: The effect of individual metal ions (Cu^{2+} , Mn^{2+} , Zn^{2+} , Pb^{2+} , Cr^{2+} , Fe^{2+} and Na^+) on the GK-AgNP coated on nitrocellulose membrane strip was also investigated. There was no change in the colour of GK-AgNP coated nitrocellulose membrane strips when subjected to individual metal ion interaction, among the tested metals. Further, Hg^{2+} was spiked at three levels (1000, 200, 20 ppm) to individual metal ions (1000 ppm) and subjected

to investigate the interference of Hg^{2+} . The disappearance of colour was observed in all the concentration of metal ions (Cu^{2+} , Zn^{2+} , Pb^{2+} , Cr^{2+} , Mn^{2+} and Na^{+}) when spiked with different concentrations of Hg^{2+} (Fig. 5A). Hence, it can act as good sensing probe for mercury detection. The result obtained confirms that GK-AgNPs were highly selective for Hg. This sensitivity could be due to the smaller the size of nanoparticles as more the surface area available for the interaction, which elicits a faster and more sensitive reaction. Moreover, silver forms instantaneous amalgam with Hg. This property of silver was utilised in designing this nanocomposite material as a Hg sensor on a nitrocellulose strip.

3.7. Studies on Hg^{2+} spiking to real polluted water samples: Water quality monitoring (WQM) generally relied on a manual approach for water sampling and analysis, where a human user would travel to a water source, take one or more samples of the water, and transport these samples to a laboratory for subsequent analysis. Over the period of time, innovative technologies were introduced to address the limitations to detect different water quality parameters in situ. The use of sensors for WQM is particularly appealing due to the low cost of the sensors and time reduction. In this study, we designed a simple, rapid and a cost-effective visual method for the detection of Hg^{2+} in an aqueous system using GK-AgNPs. The real polluted water samples were analysed to test the potential and practicality of GK-AgNPs coated nitrocellulose membrane strip for the determination of Hg^{2+} in these polluted samples. The samples were collected from different areas of Hyderabad [Hussain Sagar lake, Uppal drain, Nacharam drain (industrial area)] Telangana, India. The samples were analysed by using ICP-MS for quantification of Hg^{2+} . The concentration of Hg^{2+} was 16, 14.2 and 6.5 ppb in Hussain Sagar lake, Uppal and Nacharam areas, respectively. These real water samples were analysed for the detection of Hg^{2+} by using GK-AgNP coated nitrocellulose membrane strip, before spiking there was no change in the colour of GK-AgNP after exposure of strips for 10 min (Fig. 5B). The test strips did not show any colorimetric response, since the real water samples had Hg^{2+} concentration lower than the detection limit of 20 ppm, as reported in the present study. In order to test the efficacy of the method, various concentrations of Hg^{2+} (20, 200, 1000 ppm) were spiked to real polluted water samples. Complete discolouration was observed in spiked real polluted water samples spiked with 200 and 1000 ppm of Hg^{2+} , collected from Hussain Sagar lake, Nacharam and Uppal area (Fig. 5B). However, complete discolouration was not observed at the spiked level of 20 ppm Hg^{2+} ,

in all the three real polluted water samples tested, which can be attributed to the presence of non-metal contaminants present in the real polluted water samples collected, affecting the sensitivity of detection.

4. Conclusions: In conclusion, a simple, cost-effective and rapid colorimetric solid sensing method for detection of Hg^{2+} using GK-AgNP coated on nitrocellulose membrane strip has been developed in this study. The presence of mercury in aqueous solution was monitored based on the disappearance of yellow colour due to GK-AgNP. The reported method here showed an excellent selectivity for Hg^{2+} even in presence of other metal ions in simulated industrial effluents, indicating non-interference of other metal ions, tested. Further, the biosensor was also sensitive in detecting spiked mercury in real polluted water samples collected both from the industrial area and domestic sources. The reported method being user-friendly and cost-effective may find field application for screening environmental mercury contamination in aqueous samples of industrial origin and water bodies in the mining area.

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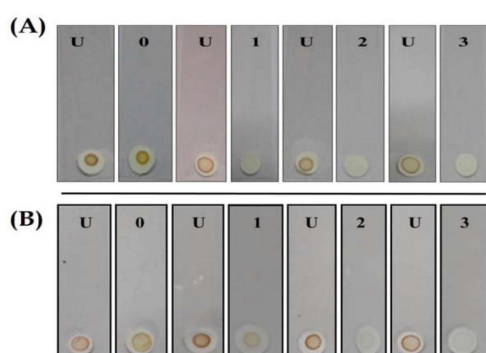


Fig. 5 Colorimetric response of test strips to various metal ions and real polluted water sample, spiked with Hg^{2+} ions.

A Typical profile of individual metal ions effect (Cu, Mn, Cd, Cr, Na, Zn and Fe) on the GK-AgNP coated on nitrocellulose membrane strip; (U) – untreated with Hg^{2+} , (0) no added Hg^{2+} , (1) 20 ppm Hg^{2+} , (2) 200 ppm Hg^{2+} , (3) 1000 ppm Hg^{2+}
 B Typical profile of spiked Hg^{2+} in real polluted water sample collected from different areas of Hyderabad. (U) – untreated with Hg^{2+} , (0) no added Hg^{2+} , (1) 20 ppm Hg^{2+} , (2) 200 ppm Hg^{2+} , (3) 1000 ppm Hg^{2+}

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