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# Quantification of morphine in exhaled breath condensate using a double network polymeric hybrid hydrogel functionalized with AuNPs

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## Abstract

**Background** Morphine serves as a foundation for creating other opioid derivatives, such as hydro/oxymorphine and heroin, which possess enhanced pain-relieving properties but are also prone to addiction and abuse. In cases of morphine overdose, it not only affects multiple immune functions but can also cause severe health complications. Given these concerns and the widespread use of morphine, it is crucial to develop efficient, uncomplicated, and precise methods for accurately detecting morphine in various biological and pharmaceutical samples.

**Results** In this investigation, a novel gold nanoparticle (AuNPs)-based double network hydrogel (DNH) nanoprobe has been fabricated for sensitive quantification of morphine in exhaled breath condensate samples. For that, gelatin/agarose DNH was fabricated through a one-step heating-cooling method in the presence of AuNPs, providing not only chemical stability but also prevent the AuNPs aggregation during synthesis process. In this method, the absorbance intensity of the nanoprobe gradually decreased with increasing morphine concentration due to the interaction morphine with AuNPs surface plasmon. The aggregation of AuNPs by addition of morphine was verified by UV-Vis spectrophotometry. The sensor displayed high sensitivity with detection limit of 0.006 $\mu g.mL^{-1}$ in the linear range from 0.01 to 1.0 $\mu g.mL^{-1}$. A reliable performance was attained for the spectrophotometric method for determination of morphine in the real samples.

**Significance**
This article has reported a new advanced optical scaffold based on double network polymeric hybrid hydrogel for the determination of morphine. This is first report of using such composite for determination of opioids in exhaled breath condensate samples.

**Keywords** Double network hydrogel, AuNPs, Morphine, Sensing

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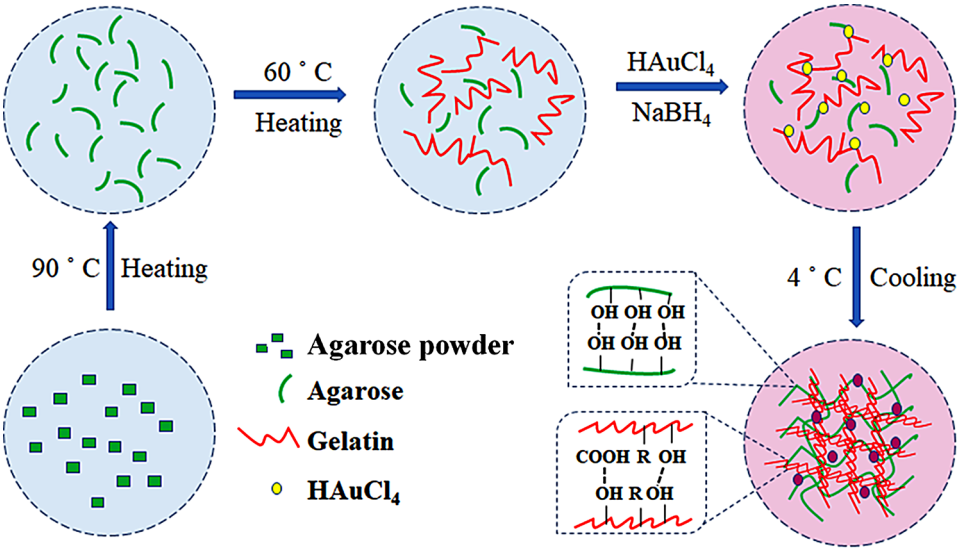
## Introduction

The analysis of opioids has garnered much interest in the quantification and determination of drug abuse in pharmaceutical matrices and human biological fluids [1]. Opioids as potent sedatives are often abused as illicit drugs and cause social issues and health problems besides their medical applications. Opium extracted from Papaver somniferum is a precursor in the construction of some opioids out of which morphine is a strong opiate analgesic and psychoactive drug [2, 3]. Morphine belonging to benzyl-iso-quinolines is used as an initial material for synthesizing other opioids like hydro/oxymorphine and heroin with stronger analgesic effects and addiction regarded as abused medicines [4]. Furthermore, this analgesic drug alleviates chronic and severe pain by regulating the pain receptors in the nervous system. The toxic level and therapeutic dose of morphine in plasma are 0.1 $\mu g.mL^{-1}$ and 0.01–0.1 $\mu g.mL^{-1}$, individually [5]. In the case of overdosing, morphine not only impacts various immune functions but also can lead to dangerous health problems [2]. These issues and the extensive use of morphine require the design of suitable, simple, and selective methods for the accurate sensing of morphine in various biological and drug specimens. This enables monitoring the doses alongside preventing their abuse intoxication induced by overdose. At this state, various kinds of analytical methods have been applied for morphine monitoring in both pharmaceutical and biological samples such as spectroscopy [6, 7], capillary electrophoresis (CE) [8], high-performance liquid chromatography (HPLC)-UV-Vis [9], gas chromatography-mass spectrometry (GC-MS) [10], electrochemical [11, 12], and chemiluminometric [13] procedures. Although these procedures have acceptable sensitivity, some of these techniques are time-consuming or need costly laboratory equipment and several pretreatment steps for drug analytical determination.

Colorimetric nanoprobes based on metallic nanoparticles (MNPs) catch the spotlight among these different procedures owing to their attractive chemical, electronic, and optical features in the field of sensing. These sensors determine different analytes by the oscillation of conduction electrons after the addition of the analyte causing variation in NPs' localized surface plasmon resonance (LSPR) absorption band in the visible region [14]. In some cases, the naked-eye color change is associated with the morphology transitions or surface chemical reactions. In particular, gold nanoparticles (AuNPs) have been investigated for the development of colorimetric sensors, benefiting from their tunable LSPR, rich surface modifiable capability, and better biocompatibility [15, 16]. Nevertheless, synthesizing smaller-size AuNPs and their aggregation probability in complicated biological matrices is challenging [17, 18]. At this state, the need for the development of AuNPs-based colorimetric sensors in alternative media has surged at present [19]. It is found that the amalgamation of NPs into a polymeric hydrogel network could offer a robust solid sensing nanoplatform for synthesizing distributed-smaller NPs and hampering sensor aggregation [20].

Natural polymer-based double network hydrogel (DNH) systems are designed to combine mixed gel of two biopolymers with several nano species with the enhanced mechanical behavior of natural polymer hydrogels. The lower content of natural polymer compared to that of synthetic polymer, necessitate nanocomposite form to improve the mechanical strength of natural polymer hydrogels. Mixing AuNPs with the DNH or merging




**Scheme 1** Schematic illustration of DNH-based nanoprobe for detection of morphine

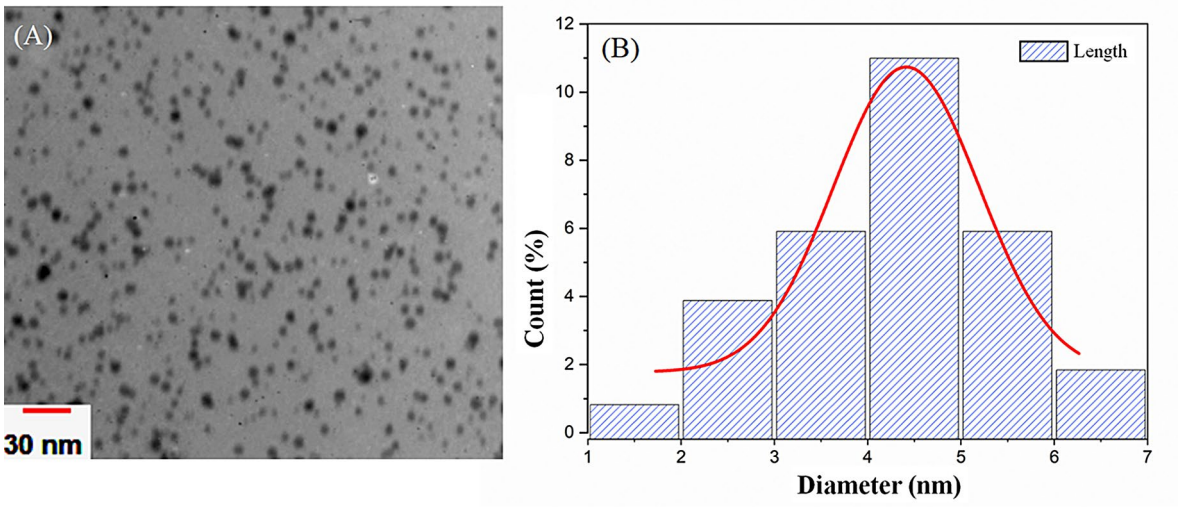



**Fig. 1** TEM image **(A)** and size distribution **(B)** of the synthesized AuNPs-agarose/gelatin DNH

them to cross-link the polymer chains fabricate nanocomposite DNHs with unique and enhanced properties [21]. Endowed with these properties, research indicates that the gelatin/agarose matrices have a promising role in biomedical applications [22]. The capability of holding a high amount of water besides the structural stability, natural origin of agarose and gelatin with hydration properties, and have moved them to the forefront. Put differently, this bilayer matrix brings about appropriate means to insert diverse NPs in layers and synergize the properties of loaded NPs.

Exhaled breath condensate (EBC) has garnered increased attention in recent years as a newly utilized biological sample [23]. It contains both volatile and non-volatile analytes of various sizes, ranging from small metal ions to genes and proteins. Compared to urine, sputum, blood, and plasma matrices, EBC as an alternative biological sample has fewer interfering compounds for drug monitoring [24, 25]. Blood and urine have

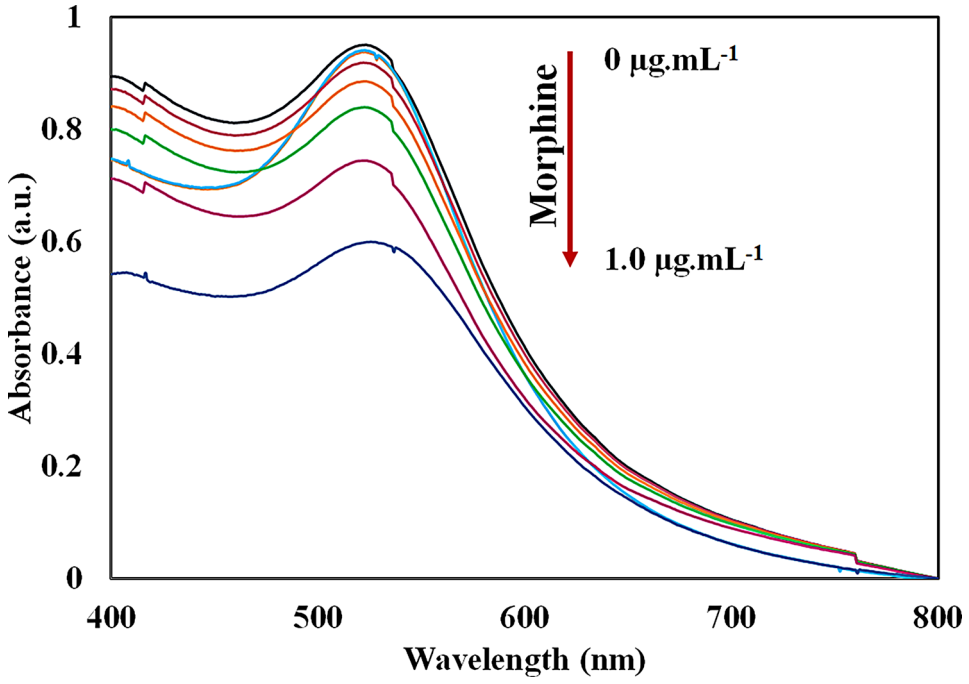



**Fig. 2** UV-Vis spectra of the AuNPs-agarose/gelatin DNH in the absence and presence of morphine at varying concentrations ranging from 0.01 to 1.0 µg. $mL^{-1}$ in EBC sample. Conditions: pH = 5.0, and 150 µL of nanoprobe

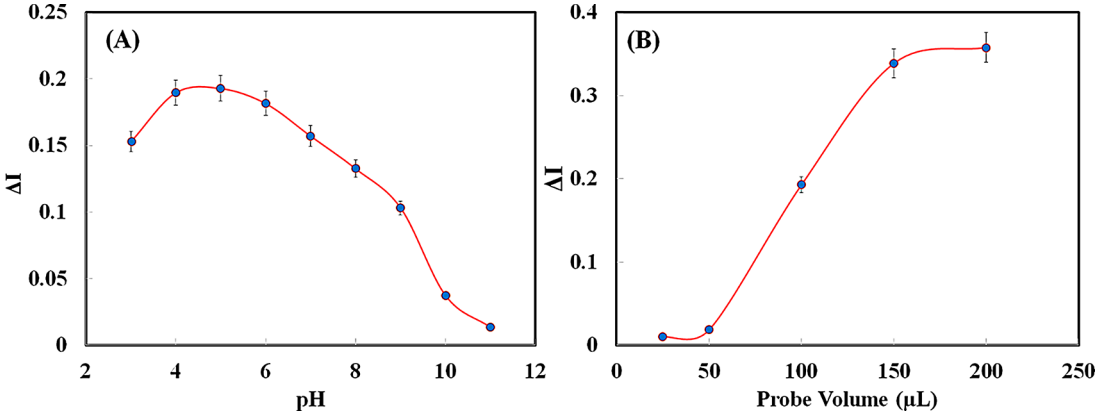



**Fig. 3** Effect of **(A)** pH and **(B)** probe volume on the response of the developed system

sampling challenges, particularly in non-clinical and non-voluntary settings. Additionally, EBC not only overcomes sampling limitations but also avoids tampering and adulteration, which are common issues in diagnosis of abused drugs with blood and urine samples [26].

In this study, we attempt to develop a simple, eco-friendly, and biocompatible nanoprobe based on DNH by incorporating AuNPs in gelatin/agarose biopolymer matrices for morphine monitoring in EBC samples. EBC being an alternative biological sample, contains lower interfering compounds compared to urine, blood, and plasma matrices for drug determination [24]. The morphological transition and aggregation after the addition of morphine bring about a color change in the sample solution and a reduction in the LSPR intensity of AuNPs-based DNH probe.

## Materials and methods

### Reagents and apparatus

Information about utilized reagents and apparatus for synthesis and sensing is provided in the Electronic Supporting Material (ESM) file.

### Synthesis of agarose/AuNPs/gelatin DNH

The integration of Au species with a polymeric network was performed by the growing and synthesis of AuNPs within agarose and gelatin matrices in the presence of $NaBH_4$ as a reducing agent. For this purpose, 10 mL of

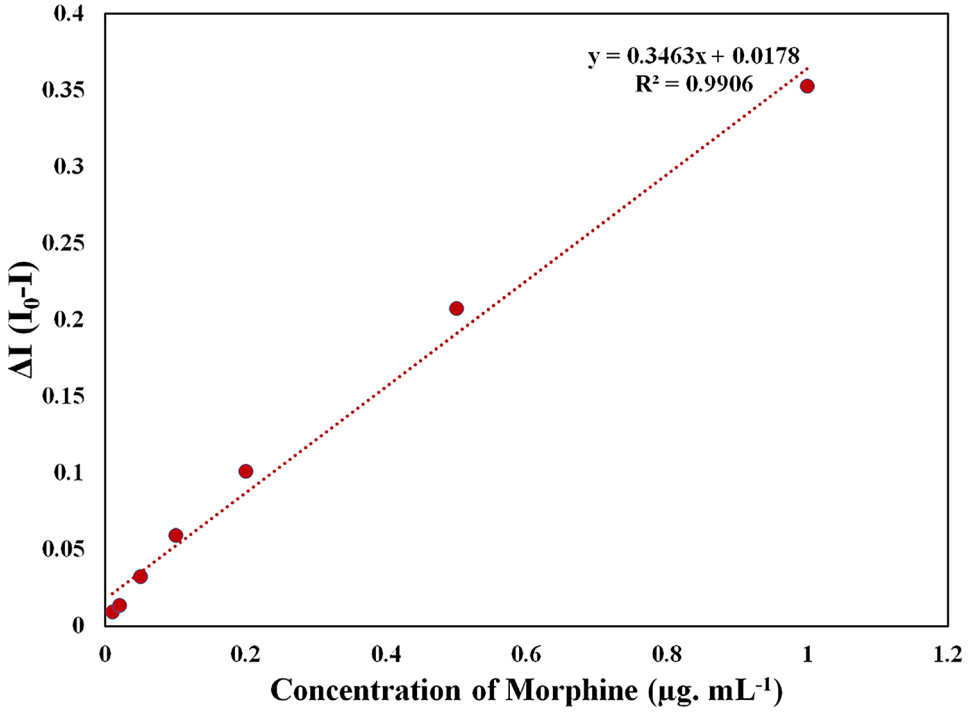



**Fig. 4** Calibration curve for AuNPs-agarose/gelatin DNH response toward different concentrations of morphine

**Table 1** Comparison of analytical characteristics for determination of morphine

| Method | Sample | LOD ($\mu g\ mL^{-1}$) | Linear range ($\mu g\ mL^{-1}$) | Reference |
|---|---|---|---|---|
| Voltammetry | Urine | 0.002 | 0.014–92.72 | [29] |
| Voltammetry | Aqueous solution | 0.102 | 0.57–85.59 | [30] |
| Colorimetric dipstick immunoassay | Blood, urine, and saliva | 0.005 | 0.001–1.0 | [31] |
| Colorimetric immunoassay | Oral fluid | 0.001 | < 0.001–0.002 | [32] |
| Spectrophotometry | Urine | 0.002 | 0.025–2 | [33] |
| Spectrophotometry | Spiked serum and urine | 0.15 | 1.33–33.29 | [34] |
| Raman spectrometry | Urine | < 1.0 | - | [35] |
| Fluorescence assay | Plasma | 0.016 | 0.02-2.0 | [36] |
| Fluorescence immunoassay | - | $2.7\times10^{-4}$ | $3.2\times10^{-4}$ – 1.0 | [37] |
| AuNPs-agarose/gelatin DNH based spectrophotometric assay | EBC | 0.006 | 0.01-1.0 | **This work** |

agarose solution (5% W/V) and 10 mL of gelatin solution (5% W/V) heated up to 90°C for 30 min were transferred to a 50 mL flask during vigorous stirring. Then, 150 µL of $HAuCl_4$ (50 $mmol.L^{-1}$) was added to the mixture. Afterward, 0.002 g in 2 mL $NaBH_4$ was dropped as a reductant to notice the color change representing the successful synthesis of AuNPs. Finally, the obtained nanoprobe was reached to the room temperature to form DNH and was kept at 4 °C. The DNH was diluted with deionized water at a ratio of 1:100 in preparation for additional experiments.

### Samples preparation

A lab-made setup was used to collect EBC samples by trapping exhaled air blown [27]. To validate the applicability of the synthesized DNH as a nanoprobe, EBC samples were collected from healthy volunteers. The real samples were obtained and frozen from patients receiving morphine after signing a consent form authorized by the Ethics Committee of the Tabriz University of Medical Sciences (IR.TBZMED.VCR.REC.1401.140). The "informed" consent to participate was obtained from all of the participants in the study.

### General procedure for morphine determination

Using a batch procedure, the monitoring of morphine by UV-Vis was organized in a 2 mL vial. At this state, 10 µL of phosphate buffer (0.1 $mol.L^{-1}$, pH 5.0) and 150 µL of DNH probe were added to the microtube containing 100 µL of EBC samples spiked with different concentrations of morphine (0.01–1.0 $\mu g.mL^{-1}$). Using deionized water, the vial's final volume was fixed to 0.5 mL. The spectrophotometer response ($\Delta I = I_0 - I$) of the nanoprobe toward morphine was recorded at 532 nm at room temperature. Where the sensor absorption response in the presence and absence of morphine is represented by $I$ and $I_0$, respectively.

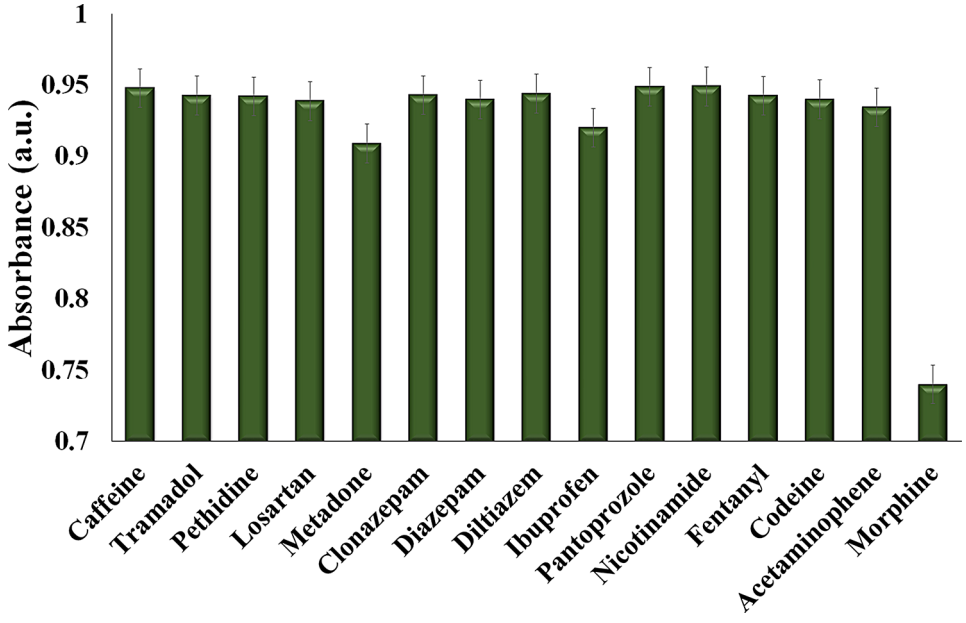



**Fig. 5** The investigation of interferences on the developed probe by employing various potential interfering substances with concentrations of 2.0 µg.$mL^{-1}$ and morphine with concentration of 0.5 $\mu g.mL^{-1}$

**Table 2** Determination of morphine in EBC samples by validated probe of AuNPs-agarose/gelatin DNH

| No. | Co-administrated drugs | Added ($\mu g \cdot mL^{-1}$) | Found ± SD ($\mu g \cdot mL^{-1}$) | Recovery (%)[a] |
|---|---|---|---|---|
| 1 | - | - | < LOQ[b] | - |
| | | 0.5 | 0.508 | 101.6 |
| 2 | - | - | < LOQ 0.498 | - |
| | | 0.5 | | 99.6 |
| 3 | Methamphetamine | - | < LOQ 0.488 | - |
| | | 0.5 | | 97.6 |
| 4 | Opium | - | < LOQ 0.492 | - |
| | | 0.5 | | 98.4 |
| 5 | Methadone | - | < LOQ 0.505 | - |
| | | 0.5 | | 101.0 |
| 6 | - | - | < LOQ 0.482 | - |
| | | 0.5 | | 96.4 |

[a] Recovery (%) = [morphine concentration in samples (after spiking – before spiking)/Added] × 100

[b] Lower than limit of quantification

## Results and discussions

### Structural characterization of AuNPs-agarose/gelatin DNH

A novel nanosensor based on AuNPs-agarose/gelatin DNH matrix was developed herein. The general layout is schematically represented in Scheme 1. TEM analysis was used to investigate the particle size and morphology of AuNPs-agarose/gelatin. As depicted in Fig. 1A, AuNPs were well-dispersed throughout the entire polymeric matrix with no obvious aggregation. With the size distribution histogram of the nanomaterial achieved from TEM images, the nanoparticles size was found to be around 4.13 nm by studying 30 particles (Fig. 1B). Additionally, the zeta potential values informing about the surface charge on nanoparticles were obtained negative in acidic, neutral, and basic media.

### Detection mechanism

After successful synthesis of the nanoprobe, the response and performance of the system was studied by the gradual addition of different concentrations of morphine to decide about the interactions between analyte and nanoprobe. Based on the Fig. 2, AuNPs-based nanoprobe exhibits a distinct LSPR peak at 532 nm in their UV-Vis spectrum. The absorption intensity of the nanoprobe was quenched and shifted by the addition of morphine along with a color shift from pink to blue representing the interaction between morphine and AuNPs. Morphine contains -OH and amine groups in its structure. The aptitude of morphine functional groups to the AuNPs form strong interactions, leading to aggregation [28]. The decrease in the LSPR absorption band in the visible region and the change in color intensity are directly related to the amount of morphine added, suggesting a novel approach for monitoring morphine concentration in EBC samples.

### Optimization of reaction conditions

To acquire an efficient analytical response for the determination of morphine, main reaction parameters including pH, the concentration of nanoprobe, and incubation time were optimized. For that, the difference in absorption intensity of the probe in the presence and absence of the analyte with the concentration of 1.0 $\mu g.mL^{-1}$ was investigated. Due to the sensitivity of the probe to pH, the pH effect on analytical response was considered in the range of 3.0–11.0 (condition: morphine concentration, 1.0 $\mu g.mL^{-1}$; EBC, 100 µL; probe, 100 µL; and final volume of the solution, 0.5 mL). According to Fig. 3A, the nanoprobe's maximum response was achieved at pH 5.0 where the response was decreased by increasing the pHs to higher values. Furthermore, a color change from pink to blue was observed presenting the aggregation of AuNPs at higher pHs. Based on the zeta potential analysis, it can be observed that the surfaces of the nanoprobe carry a negative charge. Additionally, the reported $pK_a$ value for morphine is 8.21. Consequently, at a pH lower than 8.2, morphine exists in a positively charged form, enabling it to readily interact with the surface of the nanoparticles. Therefore, pH 5.0 was selected as the optimal value. According to the plot of $\Delta I$ value and volume of DNH (Fig. 3B), 150 µL of AuNPs-agarose/gelatin provided the highest response in which the response reached a plateau at higher values. To reach effective conditions for the considered nanoprobe, the impact of exposure time was examined and found that time had no significant effect on the response. Lastly, variation of $\Delta I$ value in the presence of morphine was calculated over temperatures from 0 to 30 ℃. The results showed that the absorption response was nearly unchanged. Hence, the experimentations were performed at room temperature (25 ℃).

### Analytical figures of merit

Under optimum condition, the analytical behavior of the AuNPs-agarose/gelatin probe toward morphine in EBC samples was evaluated by study of limit of detection (LOD), linear range, selectivity, precision, and accuracy. As illustrated in Fig. 4, the calibration curve showed a linear relation in the range of 0.01 to 1.0 $\mu g.mL^{-1}$ with an equation of $\Delta I = 0.3463C + 0.0178$ ($R^2 = 0.9906$) where $C$ was morphine concentration. Based on the 3 $Sb/m$ equation (in which $m$ and $Sb$ were calibration slope and blank's standard deviation, individually), the LOD for the validated system was obtained 0.006 $\mu g.mL^{-1}$ for morphine in EBC samples. Additionally, the precision of the probe was measured using relative standard deviations ($RSDs$ %) of the repeated analysis of morphine

with 0.05 μg.mL$^{-1}$ on the same and different days. According to the results, *RSDs* % for inter- and intraday were obtained 3.4% and 2.1%, respectively. The respectable reproducibility and repeatability of the confirmed method confirmed the applicability of the nanoprobe for morphine monitoring. The analytical comparison of the validated method with other reported procedures in the literature for the determination morphine is given in Table 1. The results of current work exhibited comparable sensitivity compared with other systems.

### Interference study with coexisting substances

To investigate the selectivity and specificity of the developed nanoprobe, studying the impact of possible co-administrated drugs with morphine under optimum conditions is required. In this regard, the absorbance intensity of the system toward the 2.0 μg.mL$^{-1}$ of potential interfering substances and morphine (0.5 μg.mL$^{-1}$) in EBC were measured. Based on Fig. 5, the exitance of each interference such as caffeine, tramadol, pethidine, losartan methadone clonazepam, diazepam, diltiazem, ibuprofen, pantoprazole, nicotinamide, fentanyl, codeine and acetaminophen had negligible effect on absorbance intensity even in higher concentrations, revealing the promising selectivity of a probe to morphine determination.

### Real samples analysis

To verify the applicability of the developed system, the determination of morphine was accomplished in EBC samples of six patients receiving morphine. The volunteers who participated in our study were recruited from a hospital setting, where they received morphine as part of their standard medical care for pain management. Before enrolling in the study, participants were provided with detailed information about the study's objectives, procedures, and potential risks and benefits, and they provided written informed consent. Although the obtained values were lower than LOQ of method, for investigation of matrix effect, the morphine measurement was investigated in the spiked real EBC samples. As stated in Table 2, the satisfying recovery percentage in the range of 96.4–101.6% indicates high accuracy and independency of matrix effect for morphine determination in EBC sample. It should be noted that the method can be applied to cases involving morphine abuse or overdose. Adapting the method or employing complementary techniques such as extraction techniques may be necessary to obtain accurate and reliable results for low level of morphine concentration.

## Conclusion

A successful development of a reliable colorimetric AuNPs-agarose/gelatin DNH nanoprobe has resulted in the determination of morphine in EBC samples. This chemical sensor based on Au NPs with size of <5 nm has demonstrated selectivity, rapidity, and ease of use due to its superior LSPR properties, high extinction coefficient, and chemical stability. Notably, the utilization of agarose and gelatine as a DNH in this nanoprobe has provided stability. In this method, the absorbance intensity of the nanoprobe gradually decreased with increasing morphine concentration from 0.01 to 1.0 μg.mL$^{-1}$. At this stage, morphine with -OH and amine groups could interact with the surface of AuNPs, leading to aggregation. The validated method exhibits several desirable properties, including low LOD of 0.006 μg.mL$^{-1}$, fast response, minimal interference from other substances, and high sensitivity for the determination of morphine in EBC samples. This system is effectively employed for the determination of morphine in EBC samples, with recoveries ranging from 94.5 to 104.7%. With these advantages, a hydrogel-based matrix could potentially allow for the visualization of concentration-dependent analytical responses in biological samples such as EBC from healthy individuals or those addicted to morphine. Moving forward, the agarose/gelatin DNH presents new possibilities for diverse applications through the incorporation of various nanofillers, thereby creating diverse opportunities for the development of innovative probes in drug testing and sensing technologies.

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13065-024-01299-9.

Supplementary Material 1

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This study was approved by the ethics committee at Tabriz University of Medical Sciences.

#### Author contributions

ZK: Investigation and writing. AJ: Supervision, review & editing, MK: Investigation. V J-G: Data curation. ER: Data curation, Writing – review & editing,

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#### Data availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

## Declarations

#### Ethics approval and consent to participate

This study was approved by the ethics committee at Tabriz University of Medical Sciences. Patients receiving morphine signed a consent form

authorized by code of IR.TBZMED.VCR.REC.1401.140. The "informed" consent to participate was obtained from all of the participants in the study.

**Consent for publication**
Not applicable.

**Competing interests**
The authors declare no competing interests.

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