

An improved nanocomposite

Technical Field

The present invention relates to an improved nanocomposite, particularly a
5 nanocomposite comprising a carbon nanodot and enzyme, and encapsulated in a metal-organic framework.

Background

Enzymes are commonly used in sensors as they are efficient and selective catalysts. However, their commercial use is greatly limited due to their stability issues and
10 functionality loss outside of optimal conditions, impeding applications that commonly require enzyme reusability. Thus, while enzymes have long been employed as a key recognition element for one-time use sensors such as the well-known glucose sensor, much efforts have been placed into transforming single-use analyte sensors into more reusable, long-term options.

15 In this respect, enzymes have been encapsulated into metal-organic framework (MOF) to improve stability of the enzyme. While the MOF structure provide enzyme protection, the MOF structure resulted in diminished catalytic activity of the enzymes as compared to unencapsulated enzymes since analyte access to catalytic sites of the enzyme was obstructed. Accordingly, such reduced activity would be unsuitable in applications such
20 as electrochemical sensing in which rapid or real-time enzyme response may be required.

There is therefore a need for a way to improve enzyme stability without impacting enzyme activity.

Summary of the invention

25 The present invention seeks to address these problems, and/or to provide an improved nanocomposite, particularly for electrochemical sensing applications.

According to a first aspect, there is provided a nanocomposite comprising a carbon nanodot (C-dot) and an enzyme co-encapsulated in a metal-organic framework (MOF).

The C-dot may be any suitable C-dot. According to a particular aspect, the C-dot may be
30 formed from an amino acid precursor. The amino acid precursor may be a positively

charged amino acid precursor. For example, the amino acid precursor may be, but not limited to, arginine, lysine, or a combination thereof.

The nanocomposite may have a suitable size. For example, the nanocomposite may comprise an average diameter of 10-100 nm. In particular, the average diameter of the
5 nanocomposite may be 20-50 nm.

The nanocomposite may be porous. For example, the nanocomposite may comprise an average pore size of 10-30 nm. In particular, the average pore size may be about 20 nm.

The nanocomposite may be comprised in an electrochemical sensor. According to a particular aspect, the nanocomposite may be comprised in the electrochemical sensor
10 as a sensing layer.

The electrochemical sensor may be any suitable electrochemical sensor. For example, the electrochemical sensor may be an electrochemical biosensor, more particularly a touch-based electrochemical biosensor.

The electrochemical sensor may have a suitable sensitivity for a prolonged period of
15 time. According to a particular aspect, the electrochemical sensor may have $\geq 90\%$ electrochemical sensitivity for at least 1 week. In particular, the electrochemical sensor has 100% electrochemical sensitivity for 1 week.

According to a second aspect, there is provided a method of forming the nanocomposite according to the first aspect, the method comprising:

- 20
- adding carbon nanodots to a solution comprising an organic linker;
 - mixing enzymes with the solution comprising an organic linker and carbon nanodots to form a mixture; and
 - adding metal ions to the mixture to form the nanocomposite.

25 The solution comprising an organic linker may be any suitable solution. For example, the solution may be an aqueous-based solution.

The carbon nanodots added to the solution may have a suitable size. According to a particular aspect, each of the carbon nanodots added may have an average diameter of ≤ 30 nm.

The enzyme added may be any suitable enzyme. For example, the enzyme may comprise, but is not limited to, an oxidoreductase, a hydrolase, or a mixture thereof.

The metal ions may be any suitable metal ions. According to a particular aspect, the metal ions may comprise, but is not limited to, metal ions of transition metals, lanthanide
5 metals, or a combination thereof.

Brief Description of the Drawings

In order that the invention may be fully understood and readily put into practical effect there shall now be described by way of non-limitative example only exemplary embodiments, the description being with reference to the accompanying illustrative
10 drawings. In the drawings:

Figure 1 shows the thermogravimetric analysis (TGA) profile of various composites according to one embodiment;

Figure 2 shows the Fourier Transform Infrared (FTIR) spectra of various composites according to one embodiment;

15 Figure 3 shows the X-ray diffraction (XRD) patterns of various composites according to one embodiment;

Figure 4 shows the Brunauer-Emmett-Teller (BET) surface areas of various composites according to one embodiment;

Figure 5 shows the pore sizes of various composites according to one embodiment;

20 Figure 6 shows the comparison of the encapsulation efficiency of various composites according to one embodiment;

Figure 7 shows the comparison of the enzyme kinetics of various composites according to one embodiment;

Figure 8 shows the average glucose sensitivity over one week on rigid electrodes;

25 Figure 9 shows the percentage change in glucose sensitivity over one week on rigid electrodes;

Figure 10 shows the average lactate sensitivity over one week;

Figure 11 shows the percentage change in lactate sensitivity over one week;

Figure 12 shows the average glucose sensitivity over one week on flexible printed electrodes;

Figure 13 shows the percentage change in glucose sensitivity over one week on flexible
5 printed electrodes;

Figure 14 shows the enzyme activity of various composites according to one embodiment over a range of temperature;

Figure 15 shows the thermostability over time of various composites according to one embodiment;

10 Figure 16 shows the stability of various composites according to one embodiment in terms of residual enzyme activity after ethanol treatment;

Figure 17 shows the stability of various composites according to one embodiment in terms of residual enzyme activity after trypsin treatment;

Figure 18 shows the percentage change in glucose sensitivity over 50-day storage at
15 37°C;

Figure 19 shows the glucose sensitivity over 50-day storage at 37°C;

Figure 20 shows the calibration plot of a biosensor comprising the nanocomposite according to one embodiment;

Figure 21 shows the interference response of the nanocomposite according to one
20 embodiment;

Figure 22 shows the recovery percentage of sweat glucose;

Figure 23 shows the comparison of biosensor data with commercial assay results; and

Figure 24 shows the stability of glucose readings from repeated use of electrodes over 30-day storage.

Detailed Description

As explained above, there is a need for an improved manner of improving enzyme stability without impacting enzyme activity.

In general terms, the present invention provides encapsulation of enzymes within a metal-organic framework (MOF) which results in improved enzyme stability. In particular, enzymes may be co-encapsulated with carbon nanodots (C-dots) into a MOF, thereby forming a nanocomposite comprising enzyme-carbon nanodot@MOF. The nanocomposite may be highly stable with enhanced electrochemical sensitivity. The sensitivity of the nanocomposite may be enhanced due to C-dot's peroxidase-mimicking activity.

According to a first aspect, there is provided a nanocomposite comprising a carbon nanodot (C-dot) and an enzyme co-encapsulated in a metal-organic framework (MOF).

The C-dot may be any suitable C-dot. According to a particular aspect, the C-dot may be formed from an amino acid precursor or a nucleotide precursor. The amino acid precursor may be any suitable positively charged amino acid precursor. For example, the amino acid precursor may be, but not limited to, arginine, lysine, or a combination thereof. In particular, the C-dot may be formed from arginine. Even more in particular, the C-dot may be Argdot. The nucleotide precursor may be, but not limited to, deoxyadenosine monophosphate.

The nanocomposite may have a suitable size. For example, the nanocomposite may comprise an average diameter of 10-100 nm. For the purposes of the present application, average diameter refers to the average length of the longest dimension of the nanocomposite. In particular, the average diameter of the nanocomposite may be 15-95 nm, 20-90 nm, 25-85 nm, 20-80 nm, 25-75 nm, 30-70 nm, 40-65 nm, 45-60 nm, 50-55 nm. Even more in particular, the average diameter may be 20-60 nm, 28-53 nm, more particularly 28.1-52.6 nm. The average diameter of the nanocomposite decreases as compared to a nanocomposite comprising an enzyme encapsulated in a MOF, as in prior art. For example, the prior art nanocomposites may have an average diameter of 37.1-88.3 nm. The decrease in the average diameter of the nanocomposite of the first aspect may thereby allow for faster analyte diffusion to the enzyme when the nanocomposite is used for sensing applications.

The nanocomposite may be porous. For example, the nanocomposite may comprise an average pore size of 10-30 nm. In particular, the average pore size may be 15-25 nm, 18-22 nm, 19-20 nm. Even more in particular, the average pore size may be about 20 nm.

- 5 The nanocomposite may be of a suitable shape. For example, the nanocomposite may have a definite shape. The shape may be, but not limited to, spherical, hexagonal, or disc-like.

The nanocomposite may have a suitable zeta potential. According to a particular aspect, the zeta potential of the nanocomposite may be lower as compared to the zeta potential
10 of the MOF alone or C-dots encapsulated in MOF without any enzyme. This shows co-encapsulation of the enzyme and C-dots within the MOF structure.

The enzyme comprised in the nanocomposite may be any suitable enzyme. For example, the enzyme may be, but not limited to, glucose oxidase (GOx), lactate oxidase (LOx), cholesterol oxidase, uricase, urease, or a combination thereof. In particular, the
15 enzyme may be GOx, LOx or a combination thereof.

The MOF comprised in the nanocomposite may be any suitable MOF. For example, the MOF may be, but not limited to, zeolitic framework, including zeolitic imidazolate framework (ZIF), copper-based MOF, Material Institute Lavoisier (MIL)-based MOF, or a combination thereof. In particular, the MOF may be, but not limited to, ZIF-8, ZIF-67, ZIF-
20 60, Cu-MOF, MOF-88, Fe-MIL-100, or a combination thereof. Even more in particular, the MOF may be zeolitic imidazolate framework-8 (ZIF-8).

The encapsulation of the enzyme with C-dots within the MOF enables formation of nanocomposite complexes with optimal size, allowing fast and sensitive detections. In particular, the MOF matrix may be altered resulting in greater pore size and improved
25 unobstructed analyte diffusion to the enzyme. Further, the C-dots have intrinsic peroxidase-mimicking capability which may aid in the downstream catalysation of generated hydrogen peroxidase and subsequent electron transfer in amperometric detection. The C-dots may also stabilise the fragile 3D structure of enzymes by non-covalent binding to impede denaturation.

The nanocomposite may be comprised in an electrochemical sensor. According to a particular aspect, the nanocomposite may be comprised in the electrochemical sensor as a sensing layer.

The electrochemical sensor may be any suitable electrochemical sensor. For example, the electrochemical sensor may be an electrochemical biosensor, more particularly a touch-based electrochemical biosensor. The electrochemical sensor may be used for various sensing applications, such as, but not limited to, sweat tests, saliva tests, tear tests, urine tests, interstitial fluid test, to test for sweat glucose concentration, sweat lactate, uric acid, creatinine, just to name a few.

The electrochemical sensor may have a suitable sensitivity for a prolonged period of time. According to a particular aspect, the electrochemical sensor may have $\geq 90\%$ electrochemical sensitivity for at least 1 week. In particular, the electrochemical sensor has 100% electrochemical sensitivity for 1 week.

The improved sensitivity of the nanocomposite may be as a result of reduced nanocomposite average diameter as compared to enzymes encapsulated in MOF without C-dots, increase in pore size resulting in lowered diffusion barrier, and/or peroxidase-like activity contribution by the C-dots comprised in the nanocomposite. The reduction in the average diameter and increase in pore size may be due to the reduced amount of bonds per unit volume of sensing sample brought about by C-dots interrupting fast biomolecule-induced MOF formation.

The electrochemical sensor comprising the nanocomposites may also have improved stability over a range of temperature. For example, the electrochemical sensor may have stability and improved sensitivity over a temperature range of 40-80°C.

Having now generally described the electrochemical sensor, the same will be more readily understood through reference to the following embodiments which are provided by way of illustration, and are not intended to be limiting.

Characterisation of nanocomposites

Various composites were formed as follows: Argdot@ZIF-8 (i.e. a composite comprising C-dot formed from arginine precursor encapsulated in ZIF-8); GOx@ZIF8 (i.e. a composite comprising enzyme GOx encapsulated in ZIF-8); and GOx-Argdot@ZIF-8 (i.e.

nanocomposite comprising co-encapsulation of enzyme GOx and C-dot formed from arginine precursor in ZIF-8).

Thermogravimetric analysis (TGA) revealed nearly identical weight loss patterns for both GOx@ZIF-8 and GOx-Argdot@ZIF-8 of 74.58% and 74.89% at 800°C, respectively.

5 Similarly, ZIF-8 and Argdot@ZIF-8 had weight loss of 64.65% and 64.37% at 800°C respectively, indicating stable composite formation even with Argdot addition (Figure 1).

FTIR absorption peaks for GOx@ZIF-8 and GOx-Argdot@ZIF-8 largely corresponded to that of a typical ZIF-8. The -C=N- stretching of imidazole was shown at 1590 cm⁻¹, and the imidazole ring stretching was identified at 1415 cm⁻¹. Additional peaks at 1655 cm⁻¹

10 were indicative of amide I bands of protein, mainly from C=O stretching (Figure 2). Thus, the types of bonds present in the composites were largely the same, with the only noticeable difference being a decrease in FTIR peak intensity for GOx-Argdot@ZIF-8. Similarly, XRD data showed that the crystallinity of GOx-Argdot@ZIF-8 was mostly unchanged compared to simulated ZIF-8 (Figure 3).

15 To assess surface area and porosity of the composites, Brunauer–Emmett–Teller (BET) analysis revealed a specific surface area of 198.19 m²/g for GOx-Argdot@ZIF-8 compared to 238.71 m²/g for GOx@ZIF-8 (Figure 4). This agrees with the increased pore size exhibited by GOx-Argdot@ZIF-8 (19 nm), approximately two-fold that of its counterpart GOx@ZIF-8 (Figure 5). Thus, the addition of C-dots leads to increased pore
20 size with comparable specific surface area, a potential contributing factor to improved sensitivity due to the widened channels for analyte diffusion into the nanocomposite.

Compared to enzymes encapsulated in pure MOFs, which are known for their high diffusion barriers, Argdot co-encapsulation lowered the diffusion barrier without increasing composite dimensions, for an overall increased electrochemical sensitivity.

25 An enzyme activity assay was then conducted to ensure successful enzyme encapsulation into MOF, revealing 68.7% and 74.0% encapsulation efficiency for GOx@ZIF-8 and GOx-Argdot@ZIF-8 respectively (Figure 6). Enzyme kinetic characterization revealed that GOx-Argdot@ZIF-8 had slightly smaller K_M to both free GOx and GOx@ZIF-8, indicating an improved enzyme affinity to the analyte (Figure 7).

30 *Electrochemical sensitivity of composites*

Following composite characterization, extended sensitivity studies on electrochemical sensing applications were conducted. To investigate the effect of the GOx-Argdot@ZIF-8 nanocomposites on electrochemical sensitivity compared to typical ZIF-8 encapsulation, the nanocomposites were drop-casted onto screen-printed commercial non-flexible carbon electrodes to test amperometric responses to increasing sweat metabolite concentrations. The responses were tested and averaged over one week. The GOx-Argdot@ZIF-8 nanocomposites produced a significantly increased average response of $9.28 \mu\text{A}/\text{mM}/\text{cm}^2$, which equals 40% improvement as compared to its control GOx@ZIF-8 (Figure 8) for a detection range of up to 7 mM of glucose that well covers the typical range of sweat glucose concentrations.

The limit of detection (LOD) for GOx, GOx@ZIF-8 and GOx-Argdot@ZIF-8 were 21.7, 35.9 and $12.5 \mu\text{M}$ respectively, indicating that GOx-Argdot@ZIF-8 has both superior sensitivity and lower LOD ($\text{LOD} = 3\sigma/\text{S}$). Furthermore, the one-week testing duration also revealed its excellent stability ($\sim 100\%$ retained sensitivity) which was similar to that of GOx@ZIF-8, but in stark contrast to the exponential drop in unencapsulated free GOx sensitivity after the first round of testing (Figure 9).

The improved sensitivity arises from several factors, in particular, the reduced nanocomposite diameters, significantly increased pore size for a lowered diffusion barrier and Argdots' peroxidase-like activity contribution. The changes in composite diameter and pore size could be attributed to the reduced amount of bonds per unit volume of sample potentially caused by Argdot interrupting fast biomolecule-induced ZIF-8 formation, as indicated by the much lower peak intensity of GOx-Argdot@ZIF-8 nanocomposite FTIR.

To further prove that the enhancing capabilities of Argdot is generally applicable to biosensors utilizing different enzymes, more optimization was conducted to synthesize LOx-Argdot@ZIF-8 nanocomposites using the same method of forming GOx-Argdot@ZIF-8 nanocomposite. These similarly demonstrated a 1.4-fold sensitivity increase (Figure 10) for LOx-Argdot@ZIF-8 electrodes ($14.8 \mu\text{A}/\text{mM}/\text{cm}^2$) compared to LOx@ZIF-8 ($10.2 \mu\text{A}/\text{mM}/\text{cm}^2$).

Detection range was tested up to 1.80 mM of lactate, while LODs for all three samples were within $6\text{--}8 \mu\text{M}$. Repeated testing revealed 100% sensitivity for the LOx-

Argdot@ZIF-8 nanocomposites over one week, compared to a 10% drop for LOx@ZIF-8 and a rapid decrease for free LOx (Figure 11).

To test the use of the nanocomposite for wearable applications, additional tests investigated the usage of GOx-Argdot@ZIF-8 nanocomposite and its controls on flexible printed electrodes to assess the transferability of the nanocomposite onto a different electrode support. The 1.4-fold sensitivity increase was similarly exhibited by the nanocomposites on flexible electrodes, with 8.49 $\mu\text{A}/\text{mM}/\text{cm}^2$ sensitivity (Figure 12) and 2.5 mM detection range. An identical trend in sensor stability to the prior tests on commercial electrodes was also generated (Figure 13). Thus, these data proved the versatility and robustness of the GOx-Argdot@ZIF-8 nanocomposites for improving the sensitivity of various biosensing applications.

Stability of nanocomposites

In addition to testing electrochemical sensing stability, enzyme assays were used to further evaluate thermal stability by exposing the nanocomposite and its controls to temperatures from 40°C to 80°C at 5°C intervals for 10 minutes. While free GOx lost 85% of its activity following incubation at 60°C, GOx@ZIF-8 and GOx-Argdot@ZIF-8 maintained full activity for incubation temperatures lower than 70°C. At 70°C, free GOx only retained 3.8% activity, while the encapsulated enzymes displayed over 70% residual activity before degradation at 80°C. In general, GOx-Argdot@ZIF-8's thermostability trend was comparable to that of GOx@ZIF-8 over the tested temperature range with 10 min incubation, confirming that improved sensitivity did not result in compromised stability, unlike most existing MOF-based composites (Figure 14).

The thermal stability test was also extended to 6 hr at a fixed temperature of 55°C. The GOx-Argdot@ZIF-8 nanocomposites maintained 100% activity for the entire duration, in contrast to control GOx@ZIF-8 that lost 18% activity after 3 hr and 34% activity after 6 hr. Meanwhile, free GOx displayed a very drastic loss in activity as expected, with 85% residual activity after 15 min and only 9.8% residual activity after 1 hr (Figure 15).

Thus, GOx-Argdot@ZIF-8 nanocomposites exhibited superior thermal stability over time compared to both control GOx@ZIF-8 and free GOx. While the addition of Argdot to the complex structure did not impact thermal stability over the experimental temperature range for a duration of 10 minutes, its presence greatly improved thermal stability over time at 55°C. For Figure 15, it is possible that the prolonged isothermal heating led to

GOx denaturation in GOx@ZIF-8 after partial decomposition of ZIF-8 structures due to hydrolysis between ZIF-8 ligands and ambient moisture. In contrast, for GOx-Argdot@ZIF-8, denaturation was likely impeded by the incorporation of Argdots which may be able to stabilize the 3D structure of GOx by non-covalent binding.

5 Further stability tests were conducted by exposing samples and controls to ethanol for evaluating composite stability in an organic solvent. GOx@ZIF-8 displayed a residual activity of 70.5%, which was 1.9-fold that of free GOx (Figure 16). GOx-Argdot@ZIF-8 nanocomposites displayed 78.4% residual activity, 8% higher than that of GOx@ZIF-8 and 2.1-fold that of free GOx. As for exposure of the samples to trypsin treatment, both
10 composites maintained 89.1% residual activity, 1.4 times that of free GOx (Figure 17). Thus, both GOx@ZIF-8 and GOx-Argdot@ZIF-8 nanocomposites offer similarly increased enzyme stabilization against organic solvent and trypsin degradation through protective encapsulation.

In addition to assaying enzyme activity, electrochemical tests further explored retention
15 of sensor sensitivity over 50 days after samples were drop-casted onto electrodes and stored at body temperature of approximately 37°C. This test was accomplished by repeated amperometric testing using the same set of electrodes. Both the control GOx@ZIF-8 and GOx-Argdot@ZIF-8 nanocomposites retained full sensing capability for the first 32 days. On Day 35, GOx-Argdot@ZIF-8 nanocomposites had a 73.7% retained
20 sensitivity, while GOx@ZIF-8 had a 66.7% retained sensitivity. Both composites then continued to exhibit a steady decline in retained sensitivity, with GOx-Argdot@ZIF-8 nanocomposites retaining 6% more of its initial sensitivity on average as compared to GOx@ZIF-8. By Day 50, GOx-Argdot@ZIF-8 nanocomposites had 36.8% of its initial sensitivity, while GOx@ZIF-8 had 26.7% of its initial sensitivity. In contrast, free GOx lost
25 35.7% of its activity by the second day and 88.1% of its activity by Day 30 (Figure 18). In short, this proved that GOx-Argdot@ZIF-8 nanocomposites can maintain 100% of its sensing capability for one month, despite long-term storage and repeated measurements at body temperature. This stability is highly crucial for any long-term wearable or reusable sensor applications.

30 More importantly, daily sensitivity values showed that GOx-Argdot@ZIF-8 nanocomposites consistently produced a 1.4-fold sensitivity on average compared to its control throughout the 50-day trial. For the first 30 days, GOx@ZIF-8's average sensitivity was 6.82 $\mu\text{A}/\text{mM}/\text{cm}^2$, while GOx-Argdot@ZIF-8 nanocomposites average

sensitivity was $9.49 \mu\text{A}/\text{mM}/\text{cm}^2$ for a detection range of up to 2.5 mM glucose (Figure 19).

Touch-based sensor for sweat testing

The responses of each of the main samples, free enzyme, control composite GOx@ZIF-8, and GOx-Argdot@ZIF-8 nanocomposites respectively are shown with representative calibration curves in Figure 20. As stated, GOx-Argdot@ZIF-8 nanocomposites produced a significantly improved electrochemical response compared to its control. Its linear range was $12.5 \mu\text{M}$ to $2500 \mu\text{M}$ ($r^2 = 0.998$). Repeated calibrations confirmed its sensor reproducibility. Relative standard deviation (RSD) was calculated to be 3.4% for 5 successive measurements over one week using the same electrode and 4.9% for 5 different electrodes prepared and calibrated separately. To confirm selectivity in addition to stability of GOx-Argdot@ZIF-8 nanocomposites, common interfering compounds in sweat including lactate, uric acid, ascorbic acid, and potassium chloride were added at relevant concentrations. Amperometric tests showed that GOx-Argdot@ZIF-8 nanocomposites based biosensors selectively responded to glucose with negligible response to all other interfering compounds both before and after glucose addition (Figure 21).

Sensor accuracy was also evaluated by running a current baseline and then spiking in a known concentration of glucose ($1000 \mu\text{M}$) dissolved in artificial sweat. Stabilized amperometric responses were recorded and used to calculate analyte concentration with the earlier calibration curves. When spike-in tests were run on the same day as calibration, the average detected concentration for GOx-Argdot@ZIF-8 nanocomposites was $1016 \pm 35 \mu\text{M}$ (in triplicates), equivalent to 101.6% recovery. On the same day, free GOx produced 94.7% recovery and control GOx@ZIF-8 produced 111.0% recovery. After repeating the spike test again exactly one week after the calibration, the new current responses were used with the initial calibration to calculate analyte concentrations. In this case, GOx-Argdot@ZIF-8 nanocomposites recovery percentage remained sufficiently stable at 102.9%, while GOx@ZIF-8 recovery increased to 113.8% and GOx recovery dropped to 55.4%, impacting the accuracy of Day 7 readings (Figure 22). GOx's significant decrease in % recovery over a week was similarly reflected above in Figure 22, which reflected an even greater loss due to repeated testing throughout the week. Hence, the data validates the improved stability, accuracy and capability of the GOx-Argdot@ZIF-8 nanocomposite biosensor to detect glucose spiked-in concentration.

After testing with artificial sweat, three pre-collected sweat samples from different individuals were used to measure real sweat glucose concentrations. The sweat concentrations were determined with a commercial glucose assay kit prior to amperometric testing. GOx-Argdot@ZIF-8 nanocomposite biosensor readings were compared with assay values and found to produce an accuracy of 106.9%, 102.2% and 104.4% for each of the samples, respectively (Figure 23), confirming the biosensor to be accurate and applicable in real sweat sensing.

To further evaluate GOx-Argdot@ZIF-8 nanocomposites functionality, touch-based fingertip sweat glucose detection was also designed for non-invasive, resting state measurement despite low sweat volume. A simple, porous PVA hydrogel was optimized to provide interfacing and sweat-collecting capability, followed by a series of mini clinical trials to obtain sensor data from 3 volunteers over 30 days. GOx-Argdot@ZIF-8 nanocomposite electrode biosensors were prepared as previously indicated, dry-stored at ambient temperature and reused for weekly testing over a period of 30 days. For every test, each electrode had a fresh PVA hydrogel (1 cm diameter, 0.5 mm thickness) placed over for sweat collection. Amperometric readings were first recorded to obtain a baseline current. Concurrently, sweat was allowed to accumulate on the volunteers' cleaned fingertips, an ideal testing location due to high sweat gland density releasing 50-500 nL $\text{cm}^{-2} \text{min}^{-1}$ of sweat for sufficient biofluid volume. Sweat accumulation was followed by direct contact of fingertips with the PVA hydrogel, allowing diffusion of collected sweat towards the enzymes. A second set of amperometric readings were taken, and the increase in current magnitude compared to the baseline was used to determine glucose concentrations with the calibration data.

Sensing data from 3 volunteers were collected over 30 days and averaged to determine stability of readings. Since all readings occurred consistently an hour after lunch for a month, minimal fluctuation of glucose concentrations can be assumed at the same time of the day for healthy individuals. GOx-Argdot@ZIF-8 nanocomposites produced a highly consistent set of data, with the entire set of readings over 30 days remaining within 94% to 104% of the mean. As for GOx@ZIF-8, there was a noticeable drop in readings, with the last reading being 76% of the original reading. As expected, free GOx had an even more drastic drop of 55% by the second reading on Day 7 (Figure 24). Thus, the above trials proved the superior capability of GOx-Argdot@ZIF-8 nanocomposites as a highly stable enzyme nanocomposite for long-term repeated use in non-invasive biosensor applications.

According to a second aspect, there is provided a method of forming the nanocomposite according to the first aspect, the method comprising:

- adding carbon nanodots to a solution comprising an organic linker;
- mixing enzymes with the solution comprising an organic linker and carbon
5 nanodots to form a mixture; and
- adding metal ions to the mixture to form the nanocomposite.

The solution comprising an organic linker may be any suitable solution. According to a particular aspect, the solution may be an aqueous-based solution. For example, the
10 solution may comprise, but is not limited to, water, phosphate buffered saline, tris-buffer saline, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer, or a mixture thereof.

The organic linker may be any suitable organic linker. For example, the organic linker may be a conjugate base of a carboxylic acid or may comprise anions such as
15 organophosphorus compounds, salts of sulfonic acid, heterocyclic compounds, and imidazoles. In particular, the organic linker may comprise, but is not limited to, 2-methylimidazole, 4,4'-bipyridine, trimesic acid, terephthalic acid, or a mixture thereof.

The carbon nanodots added to the solution may have a suitable size. According to a particular aspect, each of the carbon nanodots added may have an average diameter of
20 ≤ 30 nm. In particular, the carbon nanodots may have an average diameter of ≤ 10 nm. The carbon nanodots may have an average diameter of 1-30 nm, 5-25 nm, 10-20 nm, 12-15 nm.

The carbon nanodots may be any suitable carbon nanodots. For example, the carbon nanodots may be as described above in relation to the first aspect.

25 The enzyme added may be any suitable enzyme. For example, the enzyme may comprise, but is not limited to, an oxidoreductase, a hydrolase, or a mixture thereof. In particular, the enzyme may be, but not limited to, oxidase, peroxidase, dehydrogenase, amidohydrolase, or a mixture thereof. Even more in particular, the enzyme may be, but not limited to, glucose oxidase, lactate oxidase, cholesterol oxidase, uricase, sarcosine
30 oxidase, lactate dehydrogenase, creatininase, creatinase.

The metal ions may be any suitable metal ions. According to a particular aspect, the metal ions may comprise, but is not limited to, metal ions of transition metals, lanthanide metals, or a combination thereof. For example, the metal ions may comprise, but not limited to, Zn^{2+} , Cu^{2+} , Fe^{3+} , Zr^{4+} , Co^{2+} , $\text{Eu}^{3+}/\text{Gd}^{3+}$.

- 5 The adding metal ions to the mixture may be performed at any suitable temperature. For example, the adding metal ions to the mixture may be at a temperature of 4-37°C. In particular, the temperature may be about 25°C.

The adding metal ions to the mixture may preferably be with stirring for a pre-determined period of time. The stirring may be at a suitable speed. For example, the stirring may be
10 at 150-600 rpm. In particular, the stirring may be at 200-550 rpm, 250-500 rpm, 300-350 rpm. Even more in particular, the stirring may be at about 300 rpm. The pre-determined period of time may be any suitable period of time. For example, the pre-determined period of time may be 10 minutes to 24 hours, 30 minutes, to 12 hours, 1-10 hours, 2-8 hours, 3-5 hours, 4-6 hours. In particular, the pre-determined period of time may be about
15 30 minutes.

The method may further comprise centrifuging the formed nanocomposite following the adding metal ions to the mixture. The centrifuging may enable the formed nanocomposite to be separated from the reaction product following the adding metal ions to the mixture. The centrifuging may comprise centrifuging the formed nanocomposite at a suitable
20 speed. In particular, the centrifuging may be at a speed of 3000-10000 rpm, 4000-9000 rpm, 5000-8000 rpm, 6000-7000 rpm. Even more in particular, the centrifuging may be at a speed of 7500 rpm. The centrifuging may be at a suitable temperature. For example, the temperature may be 4-37°C. In particular, the temperature may be about 25°C.

The method may further comprise washing the nanocomposite. The washing may be for
25 a suitable number of times and with a suitable solvent. The solvent may be an aqueous-based solvent. For example, the solvent may be, but not limited to, water, phosphate buffered saline, tris-buffer saline.

The method may further comprise depositing the nanocomposites onto an electrode surface to form a nanocomposite layer. The depositing may be by any suitable method.
30 For example, the depositing may be by, but not limited to, drop-casting, ink jet printing, ink dispensing, dip-coating, or a combination thereof.

The electrode may be any suitable electrode. For example, the electrode may be a rigid, flexible, or stretchable electrode.

The method may further comprise providing a protective layer over the nanocomposite layer. The protective layer may comprise one or more layers. Each of the one or more
5 layers may be the same or different from each of the other layers comprised in the protective layer. The protective layer prevents the leaching of the nanocomposites from the nanocomposite layer over time and repeated use of the electrode.

According to a particular aspect, the protective layer may comprise a first protective layer comprising a combination of polymers. The polymers may be, but not limited to, chitosan,
10 Nafion, or a mixture thereof. The protective layer may comprise a second protective layer provided above the first protective layer and further from the nanocomposite layer. The second protective layer may comprise a sweat-collecting gel. The sweat-collecting gel may comprise polyvinyl alcohol (PVA).

As can be seen, the method of forming the nanocomposite is a simple method. Further,
15 the method may be a one-pot method. Subsequent steps may be taken to form an electrode comprising the nanocomposites.

Having now generally described the method, the same will be more readily understood through reference to the following examples which are provided by way of illustration, and are not intended to be limiting.

20 *Synthesis of carbon nanodots*

10 mL of amino acids (150 mM) dissolved in ultrapure water was added into a Teflon vessel and autoclaved for 12 hr at 180°C. The carbon dots were then filtered using a 0.2 µm membrane filter. Immediately before GOx-Argdot@ZIF-8 synthesis, carbon nanodots were further ultrafiltered with a 10 kDa MWCO centrifugal filter unit for 20 min at 8,000
25 rpm. Amino acid precursors used included arginine, asparagine and aspartic acid.

Synthesis of GOx@ZIF-8 and GOx-Argdot@ZIF-8

For GOx@ZIF-8 synthesis, 2 mg of enzyme GOx (or BSA control) was added into 1 mL 2-methylimidazole (600 mM in water) and solubilised. 1 mL of Zn(OAc)₂ (40 mM in water) was then added and vortexed to combine. The solution was shaken at 300 rpm for 30
30 min at 23°C, allowing composite formation, and centrifuged at 3000 rpm for 10 min.

GOx@ZIF-8 formed a pellet after centrifugation, allowing for the removal of supernatant and subsequent washing with ultrapure water. After three washes, the pellet was resuspended in 700 μ L of 50 mM Tris-HCl buffer at pH 8.8 in preparation for electrochemical testing.

- 5 The same procedure was used for the synthesis of GOx-Argdot@ZIF-8, with an additional 4 mg of Argdot solubilised with 2-methylimidazole prior to Zn(OAc)₂ addition. Encapsulated enzymes were similarly resuspended and stored at 4°C until drop cast. All of the above was applied for LOx@ZIF-8 and LOx-Argdot@ZIF-8 synthesis using 10 mg of LOx enzyme with the same concentrations for all other reagents.
- 10 The formed nanocomposites were subjected to further characterisation as described above in relation to the first aspect.

Sensor Preparation

- 3 μ L of BSA@ZIF-8, GOx@ZIF-8, GOx-Argdot@ZIF-8 and free GOx was drop casted onto a working electrode of the commercial C710 screen printed electrode with a diameter of 4 mm. Next, 3 μ L of SWCNT-Chitosan (2 mg/mL SWCNT suspended in 1% Chitosan dissolved in 2% Acetic Acid) was drop casted over the enzyme to act as a protection layer. An additional protection layer of 3 μ L Nafion (1 wt% Nafion in water) was drop casted over the chitosan layer. The same protocol was used for preparation of screen printed flexible electrodes (diameter = 3mm), with an additional step of drop casting of 3 μ L Prussian Blue (30 mM) prior to sensor functionalization. Electrodes were stored at 4°C.
- 15
- 20

- Whilst the foregoing description has described exemplary embodiments, it will be understood by those skilled in the technology concerned that many variations may be made without departing from the present invention.
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Claims

1. A nanocomposite comprising: a carbon nanodot (C-dot) and an enzyme co-encapsulated in a metal-organic framework (MOF).
- 5 2. The nanocomposite according to claim 1, wherein the C-dot is formed from an amino acid precursor.
3. The nanocomposite according to claim 2, wherein the C-dot is formed from a positively charged amino acid precursor.
- 10 4. The nanocomposite according to claim 2 or 3, wherein the C-dot is formed from: arginine, lysine, or a combination thereof.
5. The nanocomposite according to any preceding claim, wherein the
15 nanocomposite comprises an average diameter of 10-100 nm.
6. The nanocomposite according to any preceding claim, wherein the nanocomposite comprises an average pore size of 10-30 nm.
- 20 7. The nanocomposite according to any preceding claim, wherein the nanocomposite is comprised in an electrochemical sensor.
8. The nanocomposite according to claim 7, wherein the electrochemical sensor is an electrochemical biosensor.
- 25 9. The nanocomposite according to claim 8, wherein the electrochemical biosensor is a touch-based electrochemical biosensor.
10. The nanocomposite according to any of claims 7 to 9, wherein the
30 electrochemical sensor has $\geq 90\%$ electrochemical sensitivity for at least 1 week.
11. The nanocomposite according to claim 10, wherein the electrochemical sensor has 100% electrochemical sensitivity for 1 week.

12. The nanocomposite according to any of claims 7 to 11, wherein the nanocomposite is comprised in the electrochemical sensor as a sensing layer.

13. A method of forming the nanocomposite according to any preceding claim, the
5 method comprising:

- adding carbon nanodots to a solution comprising an organic linker;
- mixing enzymes with the solution comprising an organic linker and carbon nanodots to form a mixture; and
- adding metal ions to the mixture to form the nanocomposite.

10

14. The method according to claim 13, wherein the solution comprising an organic linker is an aqueous-based solution.

15. The method according to claim 13 or 14, wherein each of the carbon nanodots
15 has an average diameter of ≤ 30 nm.

16. The method according to any of claims 13 to 15, wherein the enzyme is: an oxidoreductase, a hydrolase, or a mixture thereof.

20 17. The method according to any of claims 13 to 16, wherein the metal ions comprise transition metal ions, lanthanide ions, or a mixture thereof.