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Comparison between molecularly imprinted polymers and β -cyclodextrin as ionophores, in presence of nanocarbon dots water channels and application in marketed product and environmental samples

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ABSTRACT

Large molecules pass the biological membranes through accessory elements, either carriers (ionophores) and/or water channels. Ionophores shield the charge of the target ion, enabling it to cross the membrane to be released on the other side. This may consume time and some huge structures need a bulky ionophore. However, water channels enable the passage of many ions at a high rate without the need for carriers that may limit the ion transfer rate. Simulation of living cell membrane in engulfing large molecules through channels is a very interesting mechanism to enhance permselectivity of fabricated polyvinyl chloride (PVC) membrane sensors. Green synthesis of carbonaceous nanoparticles to play this role was fulfilled in nanocarbon dots (CD). The polymerization of β -cyclodextrin with acrylates, either imprinted with the target molecule (in molecularly imprinted polymer, MIP) or not (in non-imprinted polymer, NIP) provided a good enhancement of binding selectivity, in comparison with β -cyclodextrin alone as ionophore. Application of both inventions in the fabrication of three sensors (1; CD/ β CD, 2; CD/NIP, and 3; CD/MIP) enabled trace analysis of azoxystrobin fungicide in ranges (10⁻¹¹ -10⁻⁵, 10⁻¹³ -10⁻⁴ and 10⁻¹³ -10⁻⁴ M), respectively, with high selectivity.

Keywords: Carbon dots; Azoxystrobin; Channels; Permselectivity; MIP.

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1. INTRODUCTION

Fungicides play a viable role in food production enhancement to offer crops in a suitable qualitative and quantitative manner with low undesirable effects on the ecosystem, especially by entering waterways [1]. Like other pesticides, fungicides are widely sprayed on agricultural land, especially in wine-growing areas, from where they can move off after application [2]. However, agrochemical residues can remain on crops after harvest and even after some food processing operations, being a major exposure pathway [1]. Strobilurins are one of the widely used agricultural fungicides in a wide range of crops with relatively low toxicity profiles, however, their neurotoxicity profiles are still unknown. Azoxystrobin is one of the bestselling fungicides belonging to the strobilurins family **[1, 2]**.

Azoxystrobin (methyl (E)-2-[2-[6-(2cyanophenoxy)pyrimidin-4-yl]oxyphenyl]-3methoxyprop-2-enoate) (Fig. 1), a white crystalline solid powder was previously detected using different analytical methods such as; LC MS/MS [3-9], immunoaffinity chromatography (IAC) columns coupled to the high sensitivity of ion mobility spectrometry (IMS) [10], liquid chromatography with dual detection using diode array detection (DAD) and electrospray-ion trap tandem mass spectrometry (ESI-IT-MS/MS) [11], GC with electron capture detection [12], HPLC-UV coupled with GC-MS [13], and voltametrically [14, 15].



Fig. 1. Chemical structure of azoxystrobin.

The current maximum residue level (MRL) values for azoxystrobin range from 0.01 (in some fruits and nuts) to 70 mg/kg (in some herbs and edible flowers) [16]. So, it was essential to develop highly sensitive and selective analytical methods to analyze traces of azoxystrobin in different matrices without any interference from other pesticides, fertilizers, and matrix effects.

Electrochemical sensors could offer high sensitivity and selectivity. Their simple preparation and convenient operation, in comparison with routine analytical methods mentioned in the literature, made them a good choice for agrochemical determination [17]. However, selectivity is an inherent bottleneck for detection, which limits sensor-based its application since the existence of various interferants in real samples [18]. Different modifications were then invented to improve sensor selectivity. Ionophores invented were either natural, e.g., nucleic acid, protein, enzymes, and other biomaterials [17, 19, 20], or artificially synthesized, e.g., (MIPs) and molecularly imprinted resin (MIR).

Molecularly imprinted polymers have been used in multiple analytical applications, where they act as a synthetic receptor to host the targeted molecule/substrate in a manner of natural antibody-antigen systems [21]. In most MIP preparation, only one type of functional monomers is involved [22, 23], however many studies have shown that the use of two or more monomers produces a polymer of better selectivity. This is attributed to the complementary function and synergism of diverse monomers in molecular recognition [24].

Two types of ionophores, namely β CD and MIP, were examined to compare the selectivity of the sensor toward the cited fungicide.

Another important of feature, electrochemical sensor development, is its high sensitivity. To increase sensor sensitivity, the passage of molecules across the membrane should be improved. Channel formers enable the passage of many ions at a high rate [25]. Water channel structures develop highly stable, and low-energy membranes, efficient. bv controlling the dynamics of transportation across the membrane. They, either natural or synthetic, can be kept intact and functional by using lipids for the reconstitution of channels and supporting the membrane stability in addition [25]. Various materials are used to modify membrane sensors to get an enlarged sensing surface, including carbonaceous materials [26-28].

Carbonaceous materials, like nanocarbon dots, were tested to enhance the permeability of compounds across the membrane. Carbon dots (CDs) are distinct in structure. They are quasispherical nanoparticles of size less than 10 nm. Other types of CD may be hollow-structured, amorphous, nanocrystalline with sp^{2,} or diamondlike with sp^3 carbons [29, 30]. They, as fluorescent nanoparticles, were accidentally discovered during the purification of carbon nanotubes through preparative electrophoresis in agarose gel and glass bead matrixes derived from arc-discharge soot [31]. In recent years, different inexpensive and simpler synthetic pathways have been proposed [32, 33]. Carbon dots may be used in different applications, e.g., cellular imaging [34], biosensing [35], and also have been used to improve the sensitivity of electrochemical sensors [36]. Despite their advantages, CDs full identification has some problems due to their too wide emission spectrum as a result of the large heterogeneity of synthesis methods [37].

2. Materials and methods

2.1. Apparatus

A Jenway[®] pH meter (3505 UK by Barloworld scientific ltd) pH/mV/°C meter with Aldrich[®] glass reference electrode (Ag/AgCl, double junction, Z113107-1EA). and a thermostatic multiple water bath (model BT-15, Spain) were used. Shimadzu® IRSpirit Fourier transforms infrared spectrophotometer (FTIR). and Philips[®] (PW /830 / 40) X-ray diffraction (XRD) instrument used were for the characterization of synthesized particles. The household mixer was used for the homogenization of real samples. The microwave

oven was used for the synthesis of CD.

JEOL JEM-1400 transmission electron microscope (TEM) was used for particle size determination after subjecting the particles to a carbon microgrid (80 kV) and air drying by Quanta 250 FEG (Field Emission Gun). Scanning Electron Microscope (SEM) (FEI Company, USA) was used for surface imaging of membranes (10.1 mm working distance, with inlens detector, and excitation voltage 20 kV).

2.2. Chemicals and reagents

All chemicals and solvents are of analytical reagent grade unless mentioned.

Azoxystrobin PESTANAL[®], analytical standard; (CAS Number: 131860-33-8) (AZX), trifloxystrobin PESTANAL[®], analytical standard; (CAS Number: 141517-21-7) (TRI), and PESTANAL[®]. analytical difenoconazole standard; (CAS Number: 119446-68-3) (DFC) was purchased from Sigma-Aldrich®. Fungicide Amistar® product. Top (Batch No. GRA4I164D/1, 20 % w/v AZX + 12.5 % w/v DFC) was purchased from Syngenta[®] company agent in Egypt.

Acetonitrile (HPLC grade), chitosan (high molecular weight), polyvinyl chloride (PVC), and ethylene glycol dimethacrylate (EGDMA) were purchased from Sigma-Aldrich[®] (Germany). 2hydroxypropyl-β-cyclodextrin (βCD) was obtained from Fluka Chemie® GmbH (Steinheim, Germany). Dioctyl phthalate (DOP) was purchased from Aldrich[®] (Steinheim, Germany). Benzoyl peroxide, 97% (dry wt.), wet with 25% water, was purchased from Alfa Aesar[®] (China). Tetrahydrofuran (THF) (HPLC grade) was obtained from Fisher® (Germany). Acrylamide was supplied from Loba Chemie® Pvt Ltd (India). Methacrylic acid, hydrochloric acid (HCl), and sodium hydroxide (NaOH) were obtained from Oxford[®] (India). Boric acid, glacial acetic acid (CH₃COOH), potassium chloride (KCl), glycerol, and ammonium renickate (REN) were purchased from Elgomhouria[®] company, Egypt.

A solution of 10⁻³ M KCl was prepared by dissolving 7.455 mg in 100 mL bi-distilled water. Britton-Robinson buffer was prepared by mixing 0.04 M boric acid, 0.04 M phosphoric acid, and 0.04 M acetic acid that has been titrated to the desired pH with 0.2 M NaOH and 0.2 M HCl **[38]**.

Silver wire (1 mm diameter, 10 cm length), battery and copper wires were purchased from the local market.

2.3. Stock solutions

To prepare a stock solution of AZX, a weighed amount of 40.34 mg AZX was dissolved in the least amount of acetonitrile, and then diluted to 100 mL with bi-distilled water, to get a solution of 10^{-3} M. A serial dilution was made to prepare a calibration set of concentrations ($10^{-4} - 10^{-13}$ M) using bi-distilled water.

Stock solutions of TRI and DFC were prepared by dissolving 40.84 mg TRI and 40.63 mg DFC, separately, in the least amount of acetonitrile. Each solution was diluted to 100 mL with bi-distilled water to get a final concentration of 10^{-3} M from TRI and DFC. Further dilutions with bi-distilled water were made to get different concentrations of both interferants (TRI, DFC).

All prepared stock solutions and reagents were kept refrigerated when not in use, at 4 °C.

2.4. Synthesis of azoxystrobin molecularly imprinted polymers (MIP)

Molecularly imprinted polymers were synthesized from two types of functional monomers: dextrins (β CD) and acrylates (acrylamide and methacrylic acid). Equimolar ratio (1.33: 1.33: 1.33 mM) was added from the three monomers; acrylamide, methacrylic acid, and β CD. Ethylene glycol dimethacrylate was added in 10 mM as a crosslinker, together with 0.15 mM benzoyl peroxide as an initiator. The template molecule AZX was added to the mixture at 1 mM concentration.

All these components were mixed in 5 mL acetonitrile in a screw-capped glass test tube, after degassing with N_2 for 5 min, and finally placed in a thermostatically controlled water bath at 60 °C for a period of 24 h. A similar procedure was done without the template; to prepare NIP **[39]**.

Both polymers (MIP, NIP) were collected, ground with mortar, and then washed with a mixture of methanol/acetic acid (9: 1 v/v), to remove any unpolymerized monomers and template AZX (no peaks detected on UV spectrophotometer). Then, powders were left to dry before use.

A powdered sample was sent for characterization by XRD, FTIR, and SEM.

Molecular modeling software Openeye[®] utilized a virtual library of energy minimized functional monomers using MMFF94 force field, namely β CD, chitosan and bis-acrylamide; to show the binding affinity via fast rigid exhaustive docking (FRED) against the target molecule AZX.

2.5. Synthesis of carbon dots (CD)

Chitosan carbon dots were synthesized via the microwave-assisted green method. 0.1 g chitosan powder was added to 10 mL of a solvent mixture of 1% glacial acetic acid and glycerol (in a ratio of 1:3) with stirring for 1-2 h at room temperature to form a clear pale-yellow solution. 5 N NaOH was added to the solution till neutralized, and a clear slightly tacky gel was formed. A small part of this hydrogel was dissolved in 0.1 M acetic acid, and heated in a microwave for 5 min [40]. The formed carbon dots were collected and characterized by XRD,

FTIR, and TEM.

2.6. Construction of membrane sensors

The membrane was fabricated from a mixture of 190 mg PVC, 0.38 mL plasticizer (DOP), 10 mg ionophore (β CD in sensor 1, NIP in sensor 2, or MIP in sensor 3), and 10 mg ion-exchanger (REN). The water channels forming compound (CD) was added in 10 mg to the membrane mixture. All components were mixed in 5 mL THF in a 5 cm Petri-dish and left to air-dry overnight. The membrane was cut in circles of an appropriate diameter to be pasted on the rubber end of the electrode glass body, with the aid of PVC slurry in THF.

A silver wire of 1 mm diameter and about 10 cm length, was dipped in conc HCl, after connecting to the positive pole of a torch battery with a copper wire connected to the negative one. This helped in the coating of silver wire with a layer of silver chloride to play the role of the internal reference electrode.

A mixture of equimolar equivolume of AZX and KCl $(10^{-3} \text{ M}, 5 \text{ mL})$ was added to the electrode as an internal reference solution. Ag/AgCl wire was dipped into the internal reference solution and connected with a copper wire to the Jenway[®] potentiometer.

2.7. Sensor calibration

The fabricated membrane sensors were conditioned in 10^{-3} M AZX solutions overnight. These sensors were then calibrated by immersion in about 50 mL of standard solutions over the concentration range of $(10^{-13}-10^{-4} \text{ M})$ AZX, to follow the Nernstian increase in potential (mV) per decade reduction in concentration, against Aldrich[®] Ag/AgCl reference electrode. The potential readings were recorded after sensor equilibration with stirring and stabilized to ± 1 mV. The procedure was repeated in triplicates and the average potentials were plotted versus the negative logarithmic concentration of AZX, to

construct the calibration curve. The concentration of the unknown sample can be calculated from the corresponding regression equation. It should be known that the electrodes were washed with bi-distilled water before each measurement.

2.8. Effect of pH

The effect of pH over the range 3-10 using Britton-Robinson buffer solutions on the response of the constructed electrodes was studied, using 10^{-8} and 10^{-9} M solutions of AZX.

2.9. Sensor selectivity

The response of the constructed sensors was tested toward different expected interferants having similar structures to calculate the potentiometric selectivity coefficients by using a separate solution method (IUPAC. 2000) [41].

$-\log K_{A,B}^{Pot} = E_A - E_B / (2.303 RT / Z_A F) + (1 - Z_A / Z_B) \log a_A$

where solution A is the solution of AZX and B is the solution of interferant in concentrations of 10^{-7} M, (*E*) is the potential measured, (*Z*) is the charge of the respective ion, (*a*) is the activity of selected ion, and (2.303 *RT*/*Z*_A*F*) is the slope of the investigated sensor calibration curve (mV/concentration decade).

2.10. Determination of azoxystrobin in marketed product

A solution of 10^{-6} M was prepared by diluting 8 mL of the concentrated product Amistar[®] Top (equivalent to 40.33 mg AZX) in the least amount of acetonitrile, and then bi-distilled water was added to prepare 10^{-3} M solution, that was further diluted to get the desired concentration. Then, the developed sensors were tested to determine the concentration directly without any sample pretreatment.

2.11. Determination of azoxystrobin in treated cucumber

Field-grown cucumber was sprayed with a solution of Amistar[®] Top after reconstitution as

indicated in its pamphlet to give a solution of concentration equivalent to 10⁻⁶ M AZX. After 1 hr, 2 Kg cucumber was collected from the field, mixed in a homogenizer with 10 mL acetonitrile and a sufficient volume of distilled water was added to complete the volume to 100 mL.

The sample was collected in a beaker, with sensors 1, 2, and 3 dipped in it separately to determine AZX concentration.

3. Results and discussion

3.1. Characterization of azoxystrobin molecularly imprinted polymers (AZX-MIP)

The synthesis of MIP incorporated the use of two types of functional monomers; dextrins (BCD) and acrylates (acrylamide and methacrylic acid), to obtain the highest adsorption capacity [42]. Molecular modeling software showed that β CD gave the highest binding energy of (Δ G=-5.2767 Kcal/mol), compared to other monomers, chitosan and bis-acrylamide which showed less $(\Delta G = -1.915)$ Kcal/mol) or no docking, respectively (Fig. 2). This confirmed that β CD was better than chitosan in terms of binding and hydrophobic – hydrophobic affinity interactions [43]. The inclusion complex mechanism formed between β CD and the molecule of interest has been previously investigated and applied to serve as a functional monomer [44].



Azoxystrobin docked with Beta-cyclodextrin Azoxystrobin docked with chitosan

Fig. 2. Molecular modeling study showing the better docking of AZX in β CD cavity than that of chitosan long chain.

It was mentioned in the literature that AZX-

MIP was synthesized using hydroxypropyl methacrylate (HPMA) as the only functional monomer, to be utilized in the extraction procedure [45].

Structural elucidation of AZX-MIP using XRD showed no sharp peaks. This proved the amorphous powder entity of the AZX-MIP. FTIR peaks (1161.64 and 1258.68 cm⁻¹) were characteristic of acrylate polymers (**Fig. 3**). A scanning electron microscope image confirmed the presence of binding sites (cavities) for AZX in comparison with the image of NIP which showed a nearly flat surface without cavities (**Fig. 4**).

3.2. Characterization of chitosan carbon dots (CD)

Carbon dots were found to be highly crystalline as concluded from different sharp peaks shown in the XRD pattern. FTIR showed a broad peak of (OH) at 3500 cm⁻¹, a forked peak of (C=O) at 1600-1700 cm⁻¹, and a broad peak at 1050 cm⁻¹ of (C-O-C) [46] (Fig. 3). Images of TEM showed that a random sample of CD powder showed that the particles were in a size range of around 10 nm which proves the characteristic feature of CDs among other carbonaceous matters (Fig. 5).

3.3. Sensor calibration

Regarding ionophore, the incorporation of β cyclodextrin in the polymerization procedure, either imprinted or not, increased the sensor sensitivity than β -cyclodextrin alone. This may be due to the great increase in binding sites available for the targeted molecule.

On the other side, CDs were more preferred in electrochemical biosensing due to their intrinsic low toxicity, high solubility in many solvents, excellent electronic properties, robust chemical inertness, large specific surface area, abundant edge sites for functionalization, great biocompatibility, low cost, and versatility (**Table** 1) [47]. Fig. 6 showed SEM images for membranes in sensors 1 and 3. It showed the crystals of CDs arranged in groups embedded

within the PVC matrix to construct the water channels for large molecule passage.



Fig. 3. X-ray diffraction patterns and FTIR charts of MIP and CD.



Fig. 4. Scanning electron microscope images of (a) non-imprinted polymer (NIP), and (b) azoxystrobin molecularly imprinted polymer (AZX-MIP) show the arrangement of polymer around the cavity for AZX binding in MIP, however, no cavities on the surface of NIP.



Fig. 5. Transmission electron microscope images of carbon dots CD.



Fig. 6. Scanning electron microscope images for the membranes fabricated; (a) sensor 1, (b) sensor 3.

| Table 1. | Results | obtained | over a p | oeriod (| of two | months fo | or the | e suggested | three sensors |
|----------|---------|----------|----------|----------|--------|-----------|--------|-------------|---------------|
| | | | - · · | | | | | | |

| D (| Sensor 1 | Sensor 2 | Sensor 3 | |
|---------------------------------|----------------------|----------------------|----------------------|--|
| Parameters | CD/βCD | CD/NIP | CD/MIP | |
| Slope (mV/decade) [a] | 59.43 | 29.70 | 59.97 | |
| Intercept (mV) | 1897 | 1766.2 | 2044.3 | |
| LOD (M) ^[b] | 10 ⁻¹² | 10^{-14} | 10^{-14} | |
| Response time (s) | 30 | 40 | 30 | |
| Working pH range | 6-8 | 6-9 | 4-7 | |
| Concentration range (M) | $10^{-11} - 10^{-5}$ | $10^{-13} - 10^{-4}$ | $10^{-13} - 10^{-4}$ | |
| Stability (weeks) | 8 | 8 | 8 | |
| Avg. recovery $\% \pm SD^{[a]}$ | 100.00 ± 1.101 | 100.13±1.796 | 99.99±0.467 | |
| Repeatability ^[c] | 1.300 | 1.650 | 1.032 | |
| Intermediate precision [c] | 1.670 | 1.980 | 1.090 | |
| Robustness ^[c] | 2.030 | 2.980 | 1.890 | |
| Correlation coefficient | 0.9978 | 0.9986 | 0.9998 | |

^[a]Average of seven determinations for sensor 1, ten for sensors 2 and 3.

^[b] Limit of detection was measured by interception of extrapolated arms of calibration curves.

^[c] Precision parameters expressed in RSD%.

The sensor response time of less than 1 min, together with the high membrane stability of about 8 weeks were recorded. The results obtained showed the Nernstian linearity with slopes of about 60 mV for sensors 1 and 3, and

30 for sensor 2.

Seven molar concentrations $(10^{-11}-10^{-5} \text{ M})$ were used in the construction of the calibration curve for sensor 1, however, ten $(10^{-13} - 10^{-4} \text{ M})$ were used for sensors 2 and 3.

The suggested sensors were accurate with recoveries $(100 \pm 1.101, 100.125 \pm 1.796, and 99.988 \pm 0.467)$ for sensors 1, 2, and 3. Different factors were controlled to keep the method precise and robust as expressed in terms of RSD%.

3.4. Effect of pH

Resistance of membrane sensors against

different pH changes was observed (**Fig. 7**) over acidic or basic sides. Sensor 1 showed potential stability in pH range (6-8) and sensor 2 (6-9), however sensor 3 was stable over wider pH range (4-7). This was attributed to the presence of channels that helped target molecules pass across the membrane. The poor water solubility of azoxystrobin together with unionization led to the minimization of buffering solution to specific pH.



Fig. 7. pH profile of the suggested sensors.

3.5. Sensor selectivity

Table 2 showed that there was no interference from TRI, which is similar in structure and from the same class of AZX, and DFC which was introduced as a co-treatment for fungal plant infections in the marketed product Amistar[®] Top.

Table 2. Selectivity coefficients (expressed in Log K) of the proposed sensors in presence of other interferences

| Interferents [a] | Sensor 1 CD/βCD | Sensor 2 CD/NIP | Sensor 3 CD/MIP |
|--------------------------|--------------------|--------------------|--------------------|
| Trifloxystrobin (TRI) | 2.98 | 3.67 | 3.85 |
| Difenoconazole (DFC) | 4.89 | 5.40 | 6.18 |

^[a] Average of three determinations

3.6. Determination of AZX in marketed products and treated cucumber

The suggested sensors could determine the concentration of AZX in marketed products and fresh cucumber without any interference from additives or matrix (**Table 3**).

3.7. Statistical analysis

Two types of statistical comparisons were tested: between each sensor against a reported HPLC-UV method **[48]** in t-student test and Ftest without any significant difference between developed methods and reported one (**Table 4**). This means that the innovation of different fabrication techniques had a great performance in determination of AZX in environmental samples.

Conclusion

Advanced nanomaterials from carbon sources play important role in opening water channels that facilitate the permeability of lipophilic large organic molecules across the PVC membranes. Thus, the sensitivity may be increased to detect AZX in its maximum residue levels, and selectivity may be enhanced to determine AZX in presence of combined fungicides or other strobilurins.

The incorporation of β CD polymerized with acrylates (acrylamide and methacrylic acid) is more premium than β CD alone, regarding selectivity. However, the imprinting has no

effect.

| Average recovery \pm SD ^[a] | | | | | | | |
|--|----------|----------|------------|--------------------|-----------|----------|-----------|
| Marketed product Amistar® | Sensor 1 | Sensor 2 | Sensor 3 | Cucumber | Sensor 1 | Sensor 2 | Sensor 3 |
| Тор | CD/βCD | CD/NIP | CD/MIP | | CD/βCD | CD/NIP | CD/MIP |
| 10 ⁻⁶ M | 92.3 | 94.8 | 99.2 | 10 ⁻⁶ M | 90.1 | 89.4 | 92 |
| 10 14 | ± 1.3 | ± 1.5 | ± 0.49 | | ± 3.2 | ± 4.3 | ± 2.5 |

Table 3. Determination of azoxystrobin in marketed product

^[a]Average of three determinations

Table 4. Statistical t-student test and F-test of the results obtained from the seven suggested sensors with a reported HPLC-UV method

| Statistical test | Sensor 1 | Sensor 2 | Sensor 3 | Reported method* |
|------------------|-----------------------|-----------------------|----------------------|------------------|
| | CD/βCD | CD/NIP | CD/MIP | HPLC-UV |
| Mean | 100.00 | 100.12 | 99.99 | 126.01 |
| Variance | 1.413 | 3.586 | 0.242 | 2191.263 |
| Observations | 7 | 10 | 10 | 7 |
| t-student test | -1.470 | -1.462 | -1.471 | |
| t-tabulated | 2.447 | 2.447 | 2.447 | |
| F test | 6.45x10 ⁻⁵ | 1.64×10^{-3} | 1.1×10^{-4} | |
| F critical | 0.237 | 0.296 | 0.296 | |

*Reported method [48]

Declarations

Ethics approval and consent of participation

Not applicable

Consent of publication

Not applicable

Data and materials availability

All data produced or analyzed throughout this study are included in the current manuscript.

Competing interests

No competing interests were found between the authors.

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