



Studies on sustained release and neuromodulatory effects of β -cyclodextrin-tyramine complex: Insights from spectroscopic characterization and *Caenorhabditis elegans* behavioural studies

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ABSTRACT

In the present work, the formation of tyramine (TA) and β -cyclodextrin (β -CD) inclusion complex was studied using absorption, FT-IR and ^1H NMR spectroscopic studies. The results obtained from the absorption spectral analysis revealed the formation of a 1:1 inclusion complex of TA and β -CD with a binding constant of $K_{\text{bin}} = 20 \text{ M}^{-1}$. FT-IR and ^1H NMR analysis revealed that TA has complexed within the hydrophobic cavity of β -CD to form an inclusion complex. A blind docking approach using AutoDock Vina was employed to investigate the formation of inclusion complex between TA and β -CD. The inclusion complex of TA and β -CD was then evaluated in *Caenorhabditis elegans* for its effects on TA release-induced immobilization, migration patterns, egg-laying behaviour, and short-term associative memory. The controlled release of TA facilitated by β -CD allowed for effective administration at lower doses compared to direct exposure, ensuring a continuous and stable release profile. The locomotory and behavioural pattern of the worms on treatment with TA and β -CD-TA revealed that treatment with β -CD-TA at a 1:1 concentration did not demonstrate a significant reduction in the migration pattern of the worm and chemotaxis index (CI) of the worm, suggesting the possibility of the slow release of TA. The number of eggs laid was significantly recovered when TA was administered as β -CD-TA inclusion complex, unlike direct TA exposure in egg laying assay. The present study provides a promising strategy for achieving controlled and sustained release of TA, with significant implications for studying behavioural patterns and neuromodulation in biological systems.

1. Introduction

The slow and controlled administration of compounds of therapeutic activity is often challenging as the bioavailability, dosage and stability of the compound is usually compromised on direct application. Cyclodextrins (CD) are starch-derived oligosaccharides consisting of subunits of glucose linked with α -1,4 glycosidic bonds. CD possess a hydrophilic exterior and a hydrophobic interior that allows them to form inclusion complexes with hydrophobic molecules. CD play a major role as complexing agents that increase the aqueous solubility of weakly soluble drugs, increasing their bioavailability and stability [1]. CD-based nanoparticles have also been developed for drug delivery and therapeutics, addressing the drug stability, specificity of drug target and bioavailability [2,3]. β -cyclodextrin (β -CD) has been widely used in

pharmaceutical applications over α -cyclodextrin (α -CD) and γ -cyclodextrin (γ -CD) for its ready availability and suitable cavity sizes for a wide range of drugs [4]. Its structural properties also make it easier to purify and more resistant to enzymatic hydrolysis, contributing to its stability and effectiveness. The slow release of neurotransmitters from the polysaccharide β -CD complex could be a plausible method for assessing neuromuscular activity and behaviour.

Tyramine (TA) is a naturally occurring trace amine with significant physiological effects, predominantly through its interaction with trace amine-associated receptors (TAARs). The TAARs can have a significant impact on various aspects of neurophysiological modulation [5]. TA is known to have sympathomimetic effects in vertebrates [6] and is also known to have catecholamine-releasing properties [7]. TA is found in numerous foodstuffs, most noticeably in aged and fermented food

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products and beverages. Cheeses (notably Parmesan, Cheddar, Camembert, and Emmental), overripe bananas, avocado, canned figs, peanuts, pickled herring, dried and fermented meat products and alcoholic beverages (wine, beer) are found to contain TA [8]. TA triggers vasoconstriction, acceleration of heart rate, and elevates blood pressure, thereby making TA an indirect sympathomimetic compound. On consumption, TA is observed to cause a hypertensive crisis that can result in symptoms such as headache, migraine, nausea, and vomiting, as well as more serious complications such as end-organ damage, intracerebral haemorrhage, and death [9].

The nervous system of model organism *Caenorhabditis elegans*, exhibits significant functional parallels with the human brain, particularly in its use of similar neurotransmitters [10]. Furthermore, the processes of sensory integration, decision-making, and learning in *C. elegans* show notable similarities to the more complex decision-making mechanisms observed in the mammalian brain [11]. Exogenous TA is reported to mediate paralytic effects through the hyperactivation of endogenous TA signalling pathways in *C. elegans* [12]. For instance, *C. elegans* possesses specialized tyraminerbic neurons that are distinct from octopaminergic and dopaminergic cells, both of which are influenced by TA. Moreover, TA has been shown to modulate specific motor programs, including pharyngeal pumping, head twitching, and escape responses, via extra-synaptic release from the RIM neurons in *C. elegans* [12–14]. Studying the physiological role of TA under direct release conditions presents significant challenges. A controlled and sustained release of drugs that target TAARs can be achieved through the use of inclusion complexes. Studies utilizing CD inclusion complexes have been conducted to investigate lifespan extension and assess potential toxicological effects in *C. elegans* [15]. For example, previous research involving *C. elegans* has employed various types of CD, including α -CD, β -CD, and modified-CD poly(anhydride) systems [16]. These inclusion complexes are reported to have minimal inherent interactions with the biological system, thereby facilitating direct and quantifiable assays.

The mechanism and nature of the inclusion complexes of neurotransmitters with CD have been studied extensively. For instance, Roy et al. investigated the inclusion complexes of TA with α -CD and β -CD in aqueous solutions [17,18]. From surface tension, conductivity, UV-visible spectroscopy, and NMR analyses they showed that both complexes exhibit a 1:1 host-guest stoichiometry. The calculated binding constants derived from UV-visible spectroscopy was found to be 1067 M^{-1} for α -CD and 1110 M^{-1} , for β -CD, respectively. In contrast, Ross et al., reported a binding constant value of 70 M^{-1} for β -CD-TA inclusion complex using calorimetric titration measurements [19]. More recently, Aree et al. examined the β -CD-TA complexation using X-ray diffraction (XRD) and density functional theory (DFT) calculations, providing additional insights into the structural properties of the inclusion complex [20]. The theoretical studies revealed that the aromatic ring of TA is relatively large and cannot be fully encapsulated within the cavity of α -CD, rendering the formation of an α -CD-TA inclusion complex energetically unfavourable and unlikely to occur [20]. There are findings suggesting that β -CD exhibits very low acute oral toxicity for β -CD in mice, rats and dogs when exposed to β -CD $> 3,000\text{ mg/kg}$ body weight [21]. All these findings underscore the preference for β -CD in forming stable TA inclusion complexes. Despite these extensive structural studies, the application of inclusion complexes for behavioural assays in *C. elegans* remains unexplored. In view of this, the present investigation systematically examines the β -CD-TA inclusion complex using UV-Vis spectroscopy, FT-IR, and ^1H NMR spectroscopy. The resulting inclusion complex is subsequently evaluated for its effects on neuromuscular activity and behavioural responses in *C. elegans*. This study connects the structural characterization of β -CD-TA inclusion complexes with their functional applications in *C. elegans*, offering new insights into their potential biological relevance.

2. Materials and methods

2.1. Chemicals and procedure

β -CD and TA were procured from Sigma-Aldrich Chemicals, USA, and utilized without further purification. The UV-visible spectra were recorded using Infinite Mplex, Multimode microplate reader equipped with quartz cuvette port (1 cm) measurement; Tecan, Switzerland. The stock solutions of TA (0.035 M) and β -CD were prepared daily for all experiments. The stock concentration of β -CD (0.030 M) was prepared by dissolving 0.340 g of β -CD in 10 mL distilled water at 40°C until complete dissolution [22]. For the absorption titration experiments, to a fixed concentration of TA (0.5 mM) varying concentrations of β -CD (0–22 mM) was added by adjusting the volume to 2 mL with distilled water. The resulting mixture was allowed to equilibrate for 15 min before spectroscopic measurements. For FT-IR and ^1H NMR analysis, inclusion complex of β -CD and TA was prepared in a 1:1 ratio (15 mM) and the resulting complex was lyophilised to obtain powdered sample. FT-IR analysis was carried out by pelleting the powdered samples of β -CD, TA and β -CD-TA with KBr (JASCO, Japan). ^1H NMR spectra of β -CD, TA and β -CD-TA inclusion complex were acquired in D_2O . ^1H NMR spectra were recorded using Bruker AVANCE 400 MHz equipment. Chemical shifts (δ) are reported in parts per million (ppm) relative to residual protonated solvent signals, with D_2O serving as the internal standard ($\delta = 4.80\text{ ppm}$). Data are presented as chemical shift values.

2.2. TA immobilization assay

The worms were assayed on Nematode growth medium (NGM). The assay plates were prepared on the morning of the experiment by spreading TA (4 mM–100 mM) to the NGM plates [23]. The β -CD inclusion complex intended for slow drug release of TA was made by plating CD-TA inclusion complex (15 mM TA:15 mM β -CD) at (1:1) on NGM plates 2–3 h before the assay. For the assay, animals were placed on a transfer plate for 10 min to minimize carryover and then transferred to fresh test plates and incubated for 1 min before assay. Approximately 10 animals were transferred to assay plates and scored for locomotion every minute over 20 min. Following exposure, inactive ones were scored manually under a stereo-zoom microscope. Animals were scored as immobilized if there was no sustained forward or backward locomotion in a 5 s time period and they did not move even when prodded with platinum wire [12].

2.3. Egg-laying assay

The β -CD inclusion complex intended for slow drug release of TA was made by plating CD-TA inclusion complex (15 mM TA:15 mM β -CD) at (1:1) on plates 2–3 h before the assay. The L4-stage animals were allowed to develop $\sim 29\text{ hrs}$ at $20 \pm 0.5^\circ\text{C}$. For the assay, animals were placed on a transfer plate to minimize carryover, then transferred to test plates and acclimatized for 10 min before the assay [24]. The worms were transferred to assay plates and scored for total no. of eggs laid at 30 min, 60 min and 90 min time periods. The number of egg laid was counted under using a stereo-zoom microscope.

2.4. Locomotion analysis

Locomotion of *C. elegans* was analysed using the Worm Tracking & Analysis Software WormLab 2022 (MBF Bioscience, Williston, VT, USA). All videos were captured in chemotaxis assay plate using the MBF biosciences worm image acquisition system (Fig. S1). The treatment conditions were provided to worms in freshly prepared chemotaxis plates [25]. The videos were captured post-acclimatization in the assay plates for 60 s post transfer. The scale was set for tracking using the calibration scale provided by MBF Biosciences. The bending patterns and locomotion trajectories, moving average speed and idling time from the movies

were analysed between the groups [26].

2.5. Short-Term associative memory (STAM)

Pavlovian reward-based associative training with 10 % butanone as a conditional stimulus was given along with OP50 for 1 h (Fig. 1). The chemotaxis assay was carried out to assess the response towards the associated solvent. The chemotaxis assay was scored based on a comparison of the animals attracted with the non-attracted counterparts to generate chemotactic Index (CI) [27].

For the assay 50–100 numbers of day 1 adult worms were placed at the origin on a 90 mm chemotactic plate with butanone 3 μ L (10 % butanone in ethanol) + 1 μ L NaN₃ at test spot and 3 μ L (90 % ethanol) + 1 μ L NaN₃ at the control spot equidistant from the origin (Fig. 2). The chemotaxis index was calculated as per the formula index = [(n attractant)-(n control)]/[(Total-n origin)] [25].

2.6. AutoDock Vina

Molecular docking studies were conducted utilizing AutoDock Vina (version 1.1.2), developed by The Scripps Research Institute [28]. The three-dimensional structure of β -CD (PDB ID: 3CGT) was obtained from the Protein Data Bank (PDB). Prior to the docking simulations, all protein molecules associated with 3CGT were removed. The crystallographic coordinates of TA were retrieved from PubChem, and its PDB file was prepared using the PyMOL software. Both β -CD and TA structures were converted to PDBQT format through MGLTools (version 1.5.6). To encompass the entire β -CD molecule, a grid box with dimensions of 40 Å $\times$ 40 Å $\times$ 40 Å was defined, centered at the coordinates x = 61.754, y = 11.939, and z = 38.881. The docking was performed using default parameters of AutoDock Vina. Visualization of the resulting docked complexes was performed using the PyMOL software.

3. Results and discussion

3.1. TA: β -CD as an inclusion complex

UV-Vis spectroscopy is an ideal tool to study the inclusion complex formation of guest molecules with β -CD. The β -CD have nearly no characteristic absorption and as a result, the formation of the inclusion complex of guest molecules can be studied by monitoring the absorption spectral profile of host molecules. As a result, to study the inclusion complex formation the absorbance spectral studies of TA were carried out in varying concentrations of β -CD. As shown in Fig. 3(A) TA exhibits a characteristic absorption maximum at 275 nm owing to the phenyl group [18]. It is apparent that with the increase in the concentrations of β -CD, the absorption intensity of TA at 275 nm significantly increased, while its concentration remained the same. It is to be noted that the observed variations in the molar extinction coefficient of TA at 275 nm upon complexation with β -CD can be attributed to alterations in the polarity of the chromophore microenvironment. Specifically, this shift occurs as the TA molecule transitions from the polar aqueous medium

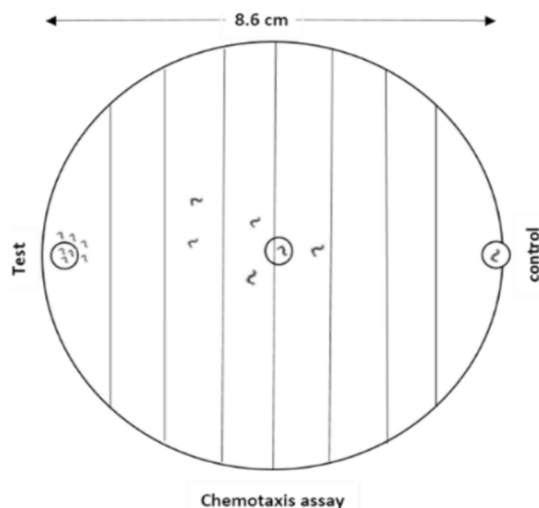


Fig. 2. Pictorial representation of chemotactic assay plate.

into the apolar cavity of the β -CD [29]. Thus, in the present case, the increase in absorption intensity of TA indicates the inclusion complex formation with β -CD. TA with their hydrophobic aromatic ring with phenolic -OH group could readily undergo host-guest interaction with β -CD. The data obtained from the absorption spectral studies was then utilised to construct the Benesi-Hildebrand plot, by using the following equation (1) to determine the binding constant and nature of the binding interaction [30].

$$\frac{1}{\Delta A} = \frac{1}{(\Delta \epsilon)[TA]} + \frac{1}{(\epsilon_1 - \epsilon_0)[TA]K_{bin}} \frac{1}{[\beta - CD]} \quad (1)$$

where, ΔA corresponds to the difference in absorption intensities of TA in the absence and presence of β -CD ($\Delta A = (A - A_0)$, A_0 is the absorption intensity of TA in the absence of β -CD and A is the absorption intensity of TA at a given concentration of β -CD, $[TA]$ is the initial concentration of TA and K_{bin} is the binding constant). As shown in Fig. 3(B), it is apparent that a good linear relationship was obtained for the plot $1/\Delta A$ vs $1/[\beta - CD]$. It has been previously reported that a linear plot is indicative of 1:1 complexation between the TA and β -CD [18]. As a result, from the double reciprocal plot, it can be inferred that the stoichiometry ratio for the formation of inclusion complex between TA and β -CD is 1:1 with a binding constant of $K_{bin} = 20 \text{ M}^{-1}$, which is in good agreement with the previously reported works [19].

The binding constant K_b values of inclusion complexes can be calculated using both linear and nonlinear models. However, linear methods, such as Benesi-Hildebrand, rely on linear regression which involve approximations that limit their accuracy. In contrast, nonlinear models, solved via iterative nonlinear least-squares regression, avoid such assumptions, offering greater accuracy and broader applicability. Yannis et al., proposed a nonlinear model for the calculation of binding constant K_b values for inclusion complex that involves 1:1 binding

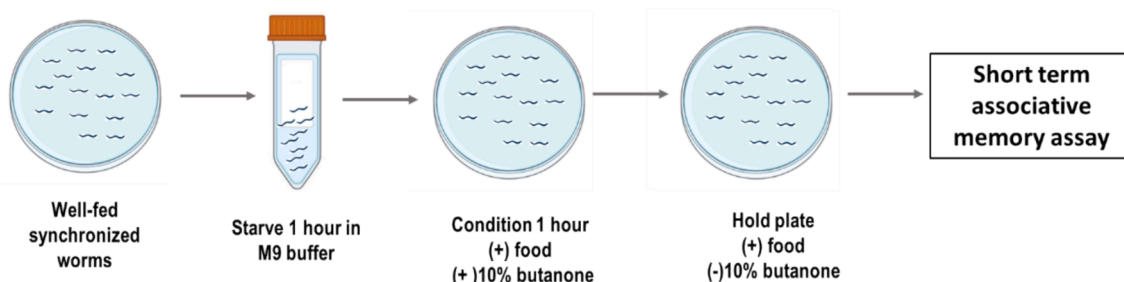


Fig. 1. Schematic representation of short-term associative memory assay (STAM).

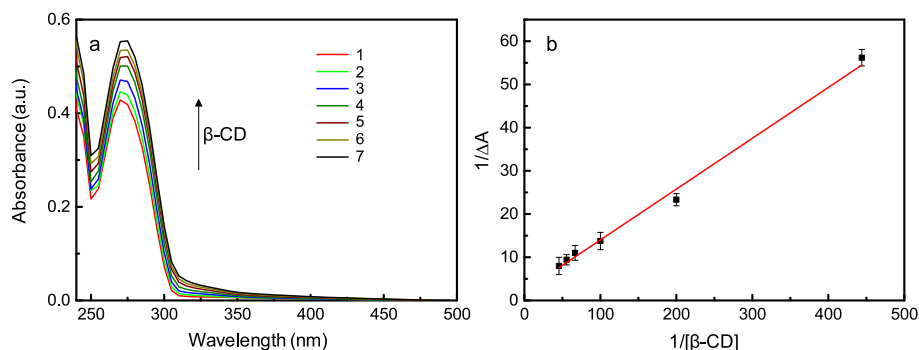


Fig. 3. (A) Absorbance spectral profile of TA in the absence and presence of increasing concentrations of β -CD (1) 0, (2) 2.25, (3) 5, (4) 10, (5) 15, (6) 18 and (7) 22 mM. (B) Benesi-Hildebrand plot for β -CD inclusion complex.

stoichiometry [31]. It is well known that the binding isotherm of the 1:1 complex exhibits a hyperbolic dependence on the free β -CD concentration equation (2).

$$\frac{\Delta A}{b} = \frac{[TA_t]K_b(1:1)\Delta\epsilon(1:1)[\beta-CD]}{1 + K_b(1:1)[\beta-CD]} \quad (2)$$

where, $[TA_t]$ is the initial total concentrations of TA, $[\beta-CD]$ is the free/non-complexed β -CD concentration, b is the path length of the cell and $K_b(1:1)$ is the binding constant. When assuming $[\beta-CD] \approx [\beta-CD_t]$ (valid under high excess of $[\beta-CD_t]$ compared to $[TA_t]$), Eq. (2) simplifies to the Benesi-Hildebrand linear form (Eq. (1)). However, to avoid this assumption, a more rigorous model was developed by substituting Eq. (3) using the β -CD mass balance in equation (2).

$$[\beta-CD] = [\beta-CD_t] - \left(\frac{\Delta A}{\Delta\epsilon(1:1)} \right) \quad (3)$$

The resulting Eq. (4) eliminates the need for approximations, providing a generalized mathematical model for accurate estimation of binding constant.

$$\Delta A = \frac{[TA_t]K_b(1:1)(\Delta\epsilon(1:1)[\beta-CD_t] - \Delta A)}{\Delta\epsilon(1:1) + K_b(1:1)(\Delta\epsilon(1:1)[\beta-CD_t] - \Delta A)}\Delta\epsilon(1:1) \quad (4)$$

By utilising the relationship between the absorbance difference (ΔA) and the initial total concentrations of $[TA_t]$ and $[\beta-CD_t]$, unknown parameters, $K_b(1:1)$ and $\Delta\epsilon(1:1)$ can be determined through nonlinear least-squares regression analysis. Subsequently, data obtained from the absorption titration experiments of β -CD-TA was subjected to non-linear fitting using equation (4). The nonlinear method yielded a K_b value of 30 M^{-1} , indicating that both the linear and non-linear models produced comparable estimates for K_b . The calculated binding constant K_b of 30 M^{-1} for the β -CD-TA inclusion complex in this study aligns well with a previous report of 70 M^{-1} determined using calorimetric titration [19]. The slight variation can be attributed to differences in the analytical techniques employed. However, the K_b value deviates significantly from that reported by Roy et al., likely due to the differing concentrations of TA ($50 \mu\text{M}$) and β -CD used in their study, which may have influenced the equilibrium conditions. In general, K_b reflects the stability of inclusion complexes, which typically ranges from 50 to 2000 M^{-1} [32]. It has been reported that complexes with $K_b < 50$ dissociate readily in aqueous solutions, whereas those with high K_b resist dissociation, limiting the release of the host molecule. Despite low K_b values, inclusion complexes have enhanced physicochemical and pharmacological properties of drugs [33]. For instance, a 1:1 2-phenylethanol: β -CD complex prepared by co-precipitation had a K_b of 97 M^{-1} [34]. In a recent study, a 1:1 complex of 2-phenylethanol in β -CD prepared via spray drying yielded a K_b of 36.8 M^{-1} [35].

3.2. FT-IR and ^1H NMR spectral studies

FT-IR and ^1H NMR spectral studies of TA, β -CD and β -CD-TA were carried out to ascertain the inclusion complex formation. In previous studies, FT-IR and ^1H NMR studies were used to establish the formation of inclusion complexes of various guest molecules and neurotransmitters with α -CD and β -CD, respectively [17,36]. As shown in Fig. 4(A), the FTIR spectrum of TA exhibits a broad band at 3106 cm^{-1} corresponding to the vibration of amine group, while the band at 1598 cm^{-1} is assigned to the C=C bending vibrations of the aromatic ring. The aliphatic C-N

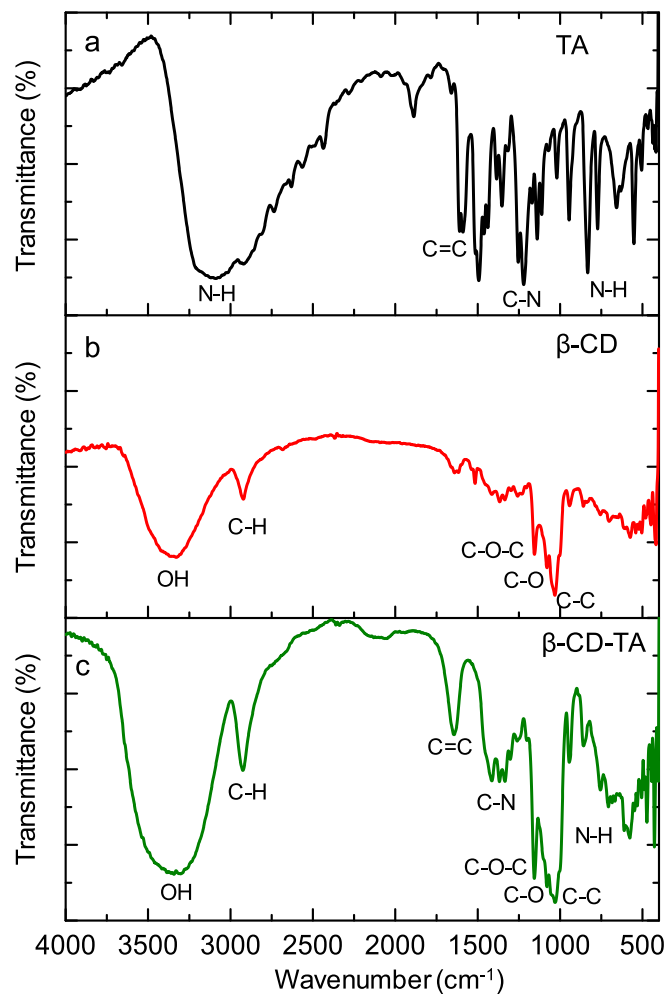


Fig. 4. FTIR profile of (A) TA alone, (B) β -CD alone, (C) β -CD-TA inclusion complex respectively.

stretch appears between $1250\text{--}1020\text{ cm}^{-1}$ and the bands between $832\text{--}620\text{ cm}^{-1}$ correspond to the N-H wag. The FT-IR profile of β -CD shown in Fig. 4(B) exhibit bands at 3352 and 2921 cm^{-1} corresponding to the O-H stretching and C-H stretching vibrations, respectively. The asymmetric stretching vibration of the C-O-C glycosidic bridge appears at 1154 cm^{-1} .

The bands located at 1080 , and 1039 cm^{-1} are assigned to the C-O stretching vibration, and C-C stretching vibration of β -CD, respectively. It is obvious from Fig. 4(C) that the FT-IR spectrum of the inclusion complex of β -CD-TA exhibits characteristic absorption bands at 3348 , 2920 , 1156 and 1037 cm^{-1} corresponding to the O-H, C-H, C-O-C glycosidic and C-O stretching vibrations of β -CD, respectively. It is to be noted that in the FT-IR spectrum of the inclusion complex, the C = C stretching vibrations of TA exhibited a shift from 1598 cm^{-1} to 1647 cm^{-1} . Furthermore, bands corresponding to the bending vibrations of aliphatic C-N group of TA showed a shift to 1253 cm^{-1} . It is apparent from the FT-IR analysis that the presence of characteristic absorption bands corresponding to the N-H group ($800\text{--}600\text{ cm}^{-1}$) and the aromatic C=C bending vibrations of TA strongly indicate the formation of inclusion complex.

The ^1H NMR spectrum of TA, as depicted in Fig. 5(A), displayed a pair of interacting doublets at 7.14 ppm and 6.82 ppm, which correspond to the aromatic protons on the benzene ring. Additionally, a pair of triplets observed at 3.15 ppm and 2.84 ppm were attributed to the protons of the ethyl group in the aliphatic side chain of TA. Fig. 5(B) shows the ^1H NMR profile of β -CD exhibiting distinctive signal for H-1 at 5.00 ppm, while the chemical shifts corresponding to the protons H-2, H-

4 and H-6 are located at 3.60 , 3.52 and 3.81 ppm, respectively. The chemical shifts at 3.79 , 3.81 , and 3.90 ppm are due to the H-5, H-6 and H-3 protons which are located in the inner hydrophobic bonding surface of β -CD, respectively [37]. The ^1H NMR spectrum of β -CD-TA inclusion complex displayed in Fig. 5(C) reveals that all the protons corresponding to β -CD exhibit upfield shift changes. The chemical shift at 5.00 ppm pertaining to H-1 remained the same without any obvious change. In the presence of TA, the chemical shifts at 3.59 and 3.80 ppm corresponding to the H-2 and H-6 protons present in the outer surface of β -CD showed a marginal upfield shift. Notably, ^1H NMR peaks located at 3.88 and 3.76 ppm corresponding to the H-3 and H-5 protons positioned inside the cavity of β -CD showed a significant shift. The ^1H NMR spectrum of the β -CD-TA inclusion complex, depicted in Fig. 5(C), demonstrates upfield shifts for all protons associated with β -CD. Notably, the chemical shift at 5.00 ppm, corresponding to the H-1 proton, remained unchanged. In the presence of TA, minor upfield shifts were observed for the chemical shifts at 3.59 ppm and 3.80 ppm, corresponding to the H-2 and H-6 protons located on the outer surface of β -CD. Notably, the ^1H NMR signals at 3.88 ppm and 3.76 ppm, attributed to the H-3 and H-5 protons residing within the hydrophobic cavity of β -CD, exhibited pronounced shifts, suggesting the inclusion of TA. Furthermore, the proton signals of β -CD and TA in the spectra of the inclusion complex (β -CD-TA) exhibit no overlap or interference, thereby facilitating the assignment of each proton in TA. Moreover, the characteristic aromatic protons (7.12 and 6.81 ppm) and aliphatic side chain protons (3.14 and 2.85 ppm) of TA showed upfield shift changes as compared to that of pure TA. The upfield shift of β -CD cavity protons and protons of guest molecule TA, is a clear indication of β -CD-TA inclusion complex and it is consistent with the literature reports [18,38]. Thus, from the FT-IR and ^1H NMR analysis, it can be inferred that TA has complexed within the hydrophobic cavity of β -CD to form an inclusion complex.

3.3. Computational studies

A computational analysis based on AutoDock Vina was performed to investigate the inclusion complex formation between TA and the β -CD receptor [39]. In this docking study, the conformer with the lowest binding energy was selected for detailed examination. Fig. 6(A) illustrates the conformer exhibiting the lowest binding energy for the TA and β -CD inclusion complex. The docking results demonstrate that TA is positioned within the central cavity of β -CD, with a binding energy of -15.78 kJ/mol (-3.77 kcal/mol), indicating a favourable interaction between TA and β -CD. The docked pose, as shown in Fig. 6(B), reveals that TA adopts a twisted linear conformation when bound within the hydrophobic cavity of β -CD. Notably, the amine group of TA is oriented toward the secondary rim of β -CD, allowing enhanced interaction with the hydroxyl groups, while the aromatic ring of TA remains embedded within the hydrophobic core of the β -CD cavity. These interactions highlight the structural compatibility of TA within the β -CD host, facilitating the formation of a stable inclusion complex.

3.4. Effect of β -CD-TA slow release on *C. elegans* mobility

To assess the influence of TA on *C. elegans* the worms were exposed to various concentrations of TA at 0 , 5 , 10 , 20 and 30 mM , respectively. The results depicted in Fig. 7(A) suggest that the worms showed complete 100% paralysis at higher concentrations of TA with direct exposure treatment. TA is reported to hyperactivate metabotropic receptor SER-2 and ligand-gated ion channel-55 (LGC-55) causing immobilization [12,14]. TA is reported to be released extrasynaptically from RIM neurons to the nerve ring influencing the behaviours [12,13]. However, there is a dose-dependent reduction in paralysis of the worms at lower concentrations of TA (20 , 10 and 5 mM). For instance, at 20 mM TA the worms tend to have a mean of 81% paralysis by the 20^{th} min while the 10 mM treatment resulted in only 51.6% mean paralysis by the 20^{th} min. The paralysis trend was limited on the treatment of 5 mM TA where

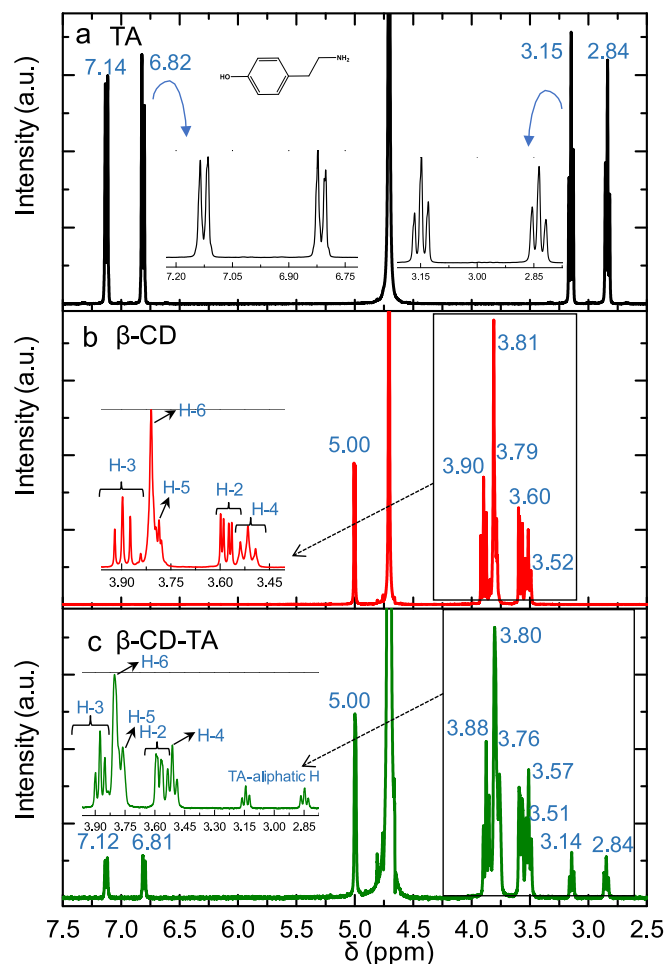


Fig. 5. NMR profile of (A) TA alone, (B) β -CD alone, (C) β -CD-TA inclusion complex respectively.

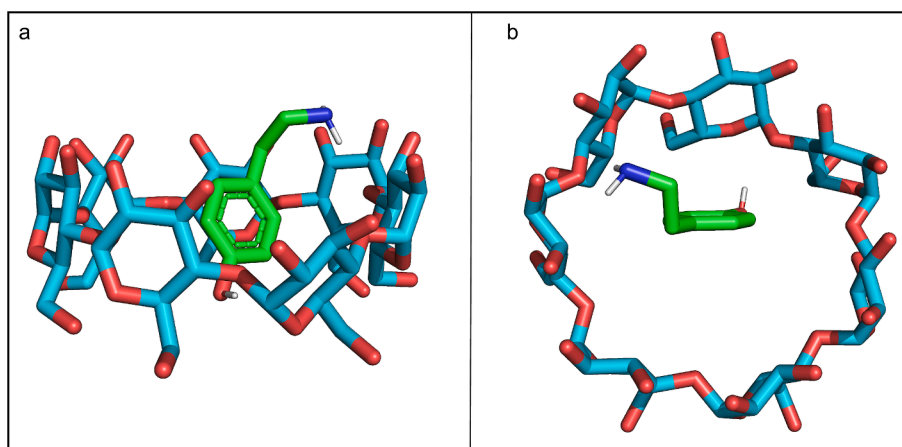


Fig. 6. The minimum energy docking conformation of the β -CD-TA inclusion complex in two distinct poses: (A) side view and (B) front view. The structural visualization was performed using the PyMOL software package (Schrödinger, academic version).

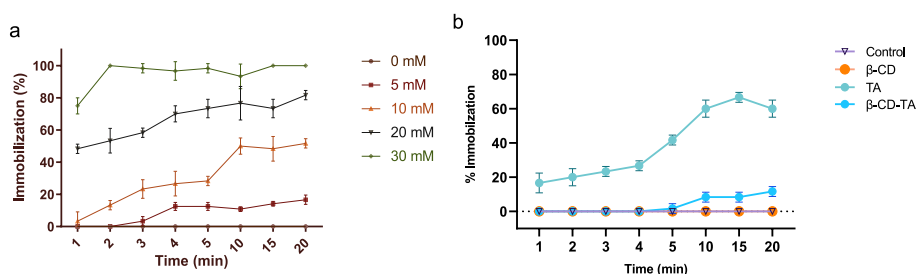


Fig. 7. (A) Immobilization of wild-type worms at different concentrations of exogenous TA at 0, 5, 10, 20 and 30 mM concentrations respectively. ($n \geq 25$ worms). (B) Immobilization pattern of Wild-type N2 worms as control, TA, β -CD and β -CD-TA ($n \geq 30$ worms).

only 16.6 % paralysis was observed by the 20th min. In order to ensure there was a slow release of TA to the worms, β -CD-TA inclusion complex was used in the subsequent studies [40]. We evaluated the effects of varying concentrations of β -CD in concentrations ranging from 0 to 30 mM (Fig. S2). Throughout the observation period, the worms demonstrated 100 % mobility, indicating active movement without any signs of toxicity associated with the tested concentrations. The worms also exhibited normal reflex responses suggesting no adverse effects of β -CD exposure.

The studies on *C. elegans* were performed in the presence of β -CD-TA inclusion complex (1:1), TA (15 mM) and β -CD alone (15 mM). It was apparent from Fig. 7(B) that at 15 mM concentration of TA there is 50 % immobilization by the 8th min and by the 20th min a 60 ± 5 % immobilization was observed. The β -CD alone did not show any immobilization and was comparable to the control with normal reflex response and mobility Fig. 7(B). Interestingly, in the presence of 15 mM β -CD-TA inclusion complex (1:1), the worms exhibited a low paralysis trend. It is to be noted that for the immobilisation studies an optimum concentration of 15 mM β -CD-TA inclusion complex (1:1) was chosen, owing to the fact that elevated concentrations of TA favours immobilization of the worms within 2–3 min of exposure [12]. It is apparent from Fig. 7(B) that as compared to the TA treatment, the immobilization pattern of *C. elegans* in the presence of β -CD-TA inclusion complex showed a lowered immobilization course than direct exposure (11.67 ± 2.8 %) by 20th min. Interestingly, the slow-paced immobilization is the result of the slow release of TA from the β -CD inclusion complex.

3.5. The effect of β -CD-TA on the locomotory pattern of the worms

Image-based analysis on quantifiable traits exploring key locomotory parameters of animals allows the understanding of locomotory patterns and migration traits. The migration patterns and the mean idling time,

which can conclusively allow us to measure the immobilization effect of TA on *C. elegans* were analysed and the results are depicted in Figs. 8 and 9. Fig. 8 shows the pattern of migration of worms post-treatment with control, TA, β -CD and β -CD-TA respectively. It is obvious that the control worms exhibited active migration around the plate exhibiting normal body bends and natural displacements and movement speed (Fig. 8 (A)). As shown in Fig. 8(B), upon treatment with the β -CD the worms exhibited normal migration patterns confirming the biocompatibility of the carrier. The worms exhibited no significant difference in mobility idling time, which accounts for the average duration the worms remained idle during the assay. It is obvious from Fig. 8(C) that the worms treated with 15 mM TA showed a noticeable reduction in migration pattern. Interestingly, treatment with β -CD-TA at a 1:1 concentration did not demonstrate a significant reduction in the migration pattern of the worm, suggesting the possibility of the slow release of TA (Fig. 8 (D)).

The idling time of the worm post-treatment with TA, β -CD and β -CD-TA complex was displayed in Fig. 9. The worms treated with β -CD alone showed an identical idling time as that of the control worms. However, upon exposure to TA, the worm displayed a rise in mobility idling time and the changes in movement patterns can be attributed to the interaction of TA with the nervous system, which activates inhibitory circuits and leads to paralysis [12]. Interestingly, the treatment of worms with β -CD-TA inclusion complex showed only a marginal increase in mobility idling time, which was not statistically significant compared to the control worms. This assessment conclusively demonstrates the biocompatibility and effectiveness of β -CD as a carrier for the slow release of the TA.

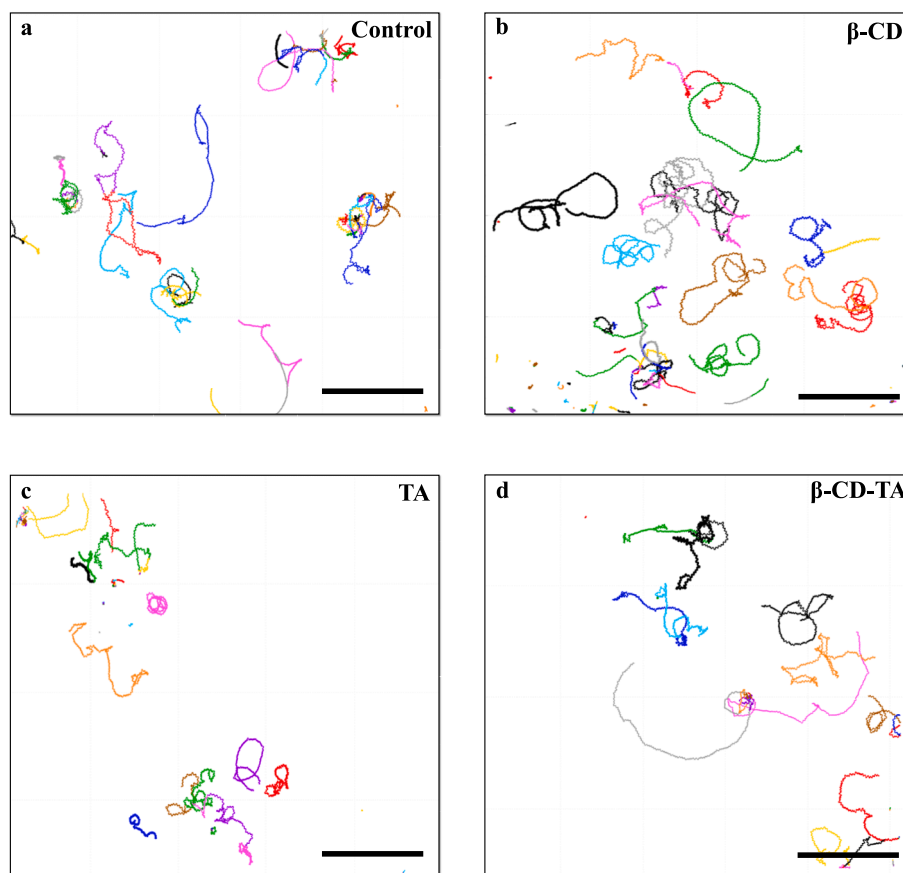


Fig. 8. The locomotory pattern of wildtype N2 worms analyzed using wormlab (A) Control (B) β -CD (C) TA (D) β -CD-TA respectively ($n \geq 14$ worms). (Scale bar represents 10 mm).

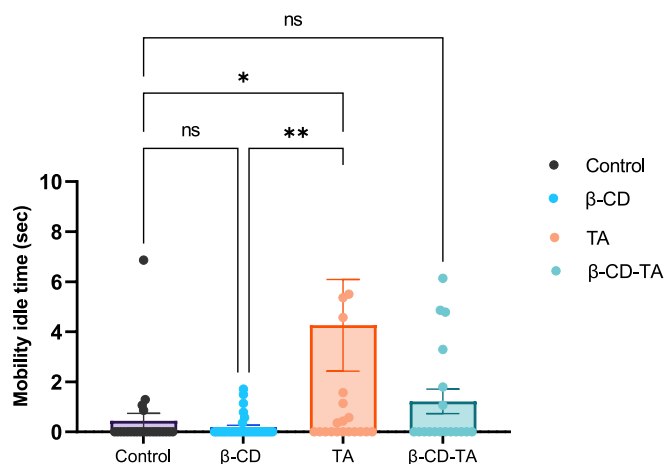


Fig. 9. The mobility idle time of Wildtype N2 worm as control, TA, β -CD and β -CD-TA respectively ($n \geq 14$ worms).

3.6. Effect of β -CD-TA complex on egg laying and associative memory in *C. elegans*

The interaction of TA with the *C. elegans* nervous system also highlights its role in regulating not just locomotion but also reproductive functions. The inhibition of egg-laying by TA involves complex signalling pathways, stressing its importance in the survival and reproductive vigour of the organism [13,41]. To assess the influence of TA on the neuromodulation of egg-laying circuit, we performed egg laying assay. The uv1 cells in *C. elegans* are known to track the movement of eggs

through the vulva and TA is released to inhibit egg laying. This circuit of activation and inhibition of egg laying is mediated by TA through LGC-55 channels in HSN neurons of the organism [14,41].

The egg-laying assay was performed by testing the worms with 15 mM TA, β -CD and inclusion complex of TA with β -CD at a 1:1 ratio Fig. 10(A). The number of eggs laid was counted at 0th min, 30th min, 60th min, and 90th min respectively. The number of eggs laid by wild-type worms, β -CD-treated worms, and β -CD-TA treated worms remained constant until the 60th min is shown in Fig. 10(A). It is apparent from Fig. 10(A) that there was a significant reduction in number of eggs laid in 15 mM TA treated group. While in the case of β -CD-TA treated groups a reduction was observed in number of eggs laid post 60th min. However, the number was significantly higher when β -CD-TA inclusion complex was used to deliver TA to the worms. The recovery in the presence of β -CD allowing the slow release of exogenous TA could have allowed the worms to exhibit egg-laying patterns higher when compared to 15 mM TA-treated groups. These results suggest that the slow release could facilitate the prolonged release of drugs in a dose-dependent manner. The reduction in egg-laying in the presence of extrasynaptic TA was reported earlier [41]. Direct exposure to exogenous TA may influence egg-laying behaviour by directly penetrating the HSN neuronal circuitry. These findings underscore the significant impact of slow-released TA on *C. elegans*, providing insights into the potential effects of sustained TA exposure in higher organisms that consume monoamine-rich foods, such as those containing TA and octopamine (OA) [5,42]. It is to be noted that the egg-laying pattern in worms returned to the normal range after 90 min, even in the presence of TA. This suggests that TA functions as a short-lived neurotransmitter in nematodes. A similar recovery from touch stimulation was reported by Donnelly et al., and the present findings are consistent with their observations [12].

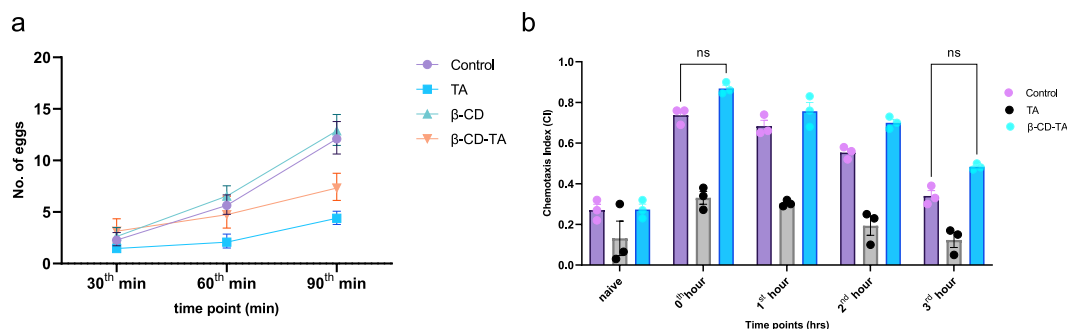


Fig. 10. (A) Wild-type N2 worms were observed for egg laying in the order control, TA, β-CD and β-CD-TA respectively ($n \geq 25$ worms). (B) Short-term associative memory in wild-type N2 treated control, TA, β-CD and β-CD-TA respectively. ($n \geq 53$ worms).

In order to test the adaptive learning memory in the worms pavlovian model classical conditioning paradigm was performed using 1/10 butanone as conditional stimuli (Fig. 10 (B)) [25,27]. As shown in Fig. 10(B), a significant increase in the 0th h CI post-conditioning was observed in Wildtype (N2) ($p < 0.0001$) worms and the memory was retained till 2nd hour and at 3rd hour memory was flattened to the naïve levels. We analysed the memory dependent CI in the presence of exogenous TA as the direct extra synaptic application of TA at 15 mM generally resulted in paralysis and inhibition of mobility. As shown in Fig. 10(B), the resulting short-term memory was diminished in wild-type N2 due to reduced CI in the 0th h, 1st h, 2nd h and 3rd h, respectively. When subjecting worms to short-term training, it was observed that in the presence of β-CD-TA, a slightly increased retention of memory was observed compared to the N2 control in 0th h, 1st h, 2nd h and 3rd h, respectively. However, the increase was not statistically distinct.

The slow release of TA from inclusion complex could facilitate the worm to have normal chemotaxis memory and related migrations, similar to control. Thus, confirming that the β-CD-TA based extra synaptic additions may not be affecting the migration pattern and learning capability of the worm, compared to TA alone. Similar kinds of observations were found in rodents with administrations of endogenously produced TA having effects on the central nervous system which include modification in food intake and sleep pattern composition and yields pro-learning and anti-amnesic effects [43]. TA and OA is analogous to epinephrine and norepinephrine respectively, and they are formed by decarboxylase and hydroxylase action [6,13]. Studies in *Drosophila melanogaster* have highlighted the role of these monoamines in regulating starvation responses, lifespan and other metabolic traits [44]. We demonstrate that TA could extrasynaptically reconfigure the migration patterns, learning and memory through hyperactivation of TA receptors.

4. Conclusion

In summary, the present study demonstrates the efficacy of using β-CD as a complexing agent to achieve controlled and sustained release of TA. The absorption spectroscopy, FT-IR, and ¹H NMR studies revealed that TA is effectively encapsulated within the hydrophobic cavity of β-CD. The binding constant value of $K_{bin} = 20 \text{ M}^{-1}$ further supports the stability of this inclusion complex. The experimental results were further corroborated by blind docking simulations using AutoDock Vina, which demonstrated that TA assumes a twisted linear conformation when bound within the hydrophobic cavity of β-CD. The behavioural assays of *C. elegans*, including assessments of immobilization, migration patterns, egg-laying behaviour, and short-term associative memory, indicated that the administration of the β-CD-TA inclusion complex at a 1:1 ratio did not result in a significant reduction in the migration or behavioural patterns of the worms suggesting the likelihood of a controlled, slow release of TA when delivered through the β-CD complex. Furthermore, the egg-laying assay demonstrated a notable increase in the number of eggs laid by the worms treated with the β-CD-TA complex compared to

those treated with TA alone. From the short-term associative memory assay, it is evident that TA have a role in the learning and migration of *C. elegans*. These findings indicate that the β-CD-mediated delivery system enhances the bioavailability of TA, promoting its neuro-modulatory effects in a more sustained and efficient manner than direct administration. The present findings open avenues for further behavioural studies using this model organism, showcasing the potential of β-CD as effective tools for enhancing drug delivery at the host-drug interface.

CRediT authorship contribution statement

Amal Wilson Varghese: Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Arumugam Selva Sharma:** . **Swathy S. Nair:** Methodology, Data curation. **Anoopkumar Thekkuveetil:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.molliq.2025.127151>.

Data availability

Data will be made available on request.

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