Wiley
Journal of Nanotechnology
Volume 2025, Article ID 6677970, 8 pages
https://doi.org/10.1155/jnt/6677970

WILEY

Research Article

Controlling Rate of Release of Tsetse Fly Repellent Blend by Encapsulating in β-Cyclodextrin Nanoparticles

Bernadatte M. Ratemo,[1] **Benson M. Wachira**,[2,3] **Eric Masika**,[1] **Margaret M. Ng'ang'a**,[1] **Ahmed Hassanali**,[1] **and Paul O. Mireji**[3]

[1]*Department of Chemistry, Kenyatta University, Nairobi, Kenya*
[2]*Department of Chemistry, Pwani University, Kilifi, Kenya*
[3]*Biotechnology Research Institute, Kenya Agricultural and Livestock Research Organization, Kikuyu, Kenya*

Correspondence should be addressed to Benson M. Wachira; b.wachira@pu.ac.ke and Paul O. Mireji; mireji.paul@gmail.com

Received 17 September 2024; Accepted 24 February 2025

Academic Editor: Karthik Pothiyappan

Tsetse flies are major vectors of African trypanosomiasis, with devastating medical and veterinary consequences in sub-Saharan region of Africa. Insect repellents are promising tool for control of tsetse flies in the region. A four-component tsetse-repellent blend (δ-nonalactone, heptanoic acid, 4-methylguaiacol, and geranyl acetone) previously formulated and optimized was encapsulated in β-cyclodextrin for a slow controlled release. Here, we explored various methods of microencapsulating (kneading, coprecipitation, heating, or freeze-drying) tsetse fly repellent blend in β-cyclodextrin nanoparticles. We assessed release kinetics of the blends and individual compounds using gas chromatography linked with flame ionization detector and evaluated laboratory and field responses (repellence) of the flies by the encapsulated blends. We compared individual performances of releases kinetics of the encapsulated blend relative to nonencapsulated composites. Overall, kneading, coprecipitation, heating, and freeze-drying microencapsulation techniques retained 72.0%, 61.0%, 59.5%, and 57.3% of the blend, respectively. Release rates of blends in 400- and 200-microns thick polythene sachets were 6.73 and 11.82 mg/h, respectively, significantly higher ($p < 0.05$) than that of the kneaded encapsulated blend (5.35 mg/h). Laboratory and field responses of tsetse flies to the unencapsulated native (sachet) and kneaded encapsulated odor blends confirmed our laboratory findings. Microencapsulation technology of repellent odors can be used for controlled release of active molecules in order to give an extended protection period, potentially reducing operational cost in programs for control of tsetse flies and related insect vectors.

Keywords: β-cyclodextrin; controlled release; encapsulation techniques; microencapsulation; nanoparticles; release rate

1. Introduction

Tsetse fly (*Glossina* spp) transmits sleeping sickness (Human African Trypanosomiasis [HAT]) and nagana (Animal African Trypanosmiasis [AAT]) diseases to human and their livestock, respectively, in sub-Saharan Africa [1]. The use of trypanocidal drugs (chemotherapy) is not sustainable due to widespread and increasing resistance of trypanosomes to existing drugs [2], high cost, sporadic availability of drugs in endemic areas, and presence of wildlife trypanosome reservoirs [3]. There are no mammalian vaccines against HAT due to complex mechanism of antigenic variation associated with trypanosome surface coat antigens [4]. Attractant and repellent semiochemical-based control of tsetse (in "pull," "push," and "push–pull" tactics) are considered effective approaches and constitute cornerstone of suppression of tsetse fly populations and transmission of trypanosomosis [5–7]. Studies that expand and enhance the efficacies of semiochemical-based vector control tools are desirable.

Recent characterization of repellent constituents in the tsetse fly refractory waterbuck (*Kobus defassa*)-derived odors and their derivatives have unveiled novel tools for

protecting livestock [7–10]. Currently, most efficacious repellent blend is the four-component (δ-nonalactone, heptanoic acid, 4-methylguaiacol, and geranyl acetone) repellent blend (4-cTRB) [6]. Downstream deployment of the 4-cTRB for routine use necessitates establishment of controlled release of these odors to provide a longer protection of vertebrate host, including livestock against tsetse fly bites. Microencapsulation technology is a vital invention utilized in slow release of active compounds such as drugs and related biomolecules [11]. This technology has been applied in pharmaceutical and biomedical and related fields in packaging particles of finely grounded solids, droplets of liquids, or volatile materials [11]. Cyclodextrin, glucopyranosyl cyclic oligosaccharides [12], with α-, β-, and ɣ-cyclodextrin, are common and widely used natural cyclodextrins encapsulants [13], among which β-cyclodextrin (β-CD) is the most hydrophobic (1.85% w/v solubility in water at 25°C) and most suitable cyclodextrin for encapsulation/entrapment of the generally hydrophobic guests in its cavity [14] to form an inclusion complex [15]. The complex can be achieved using kneading, coprecipitation, heating in sealed container, lyophilization, or spray-drying method for poorly water-soluble, nonwater-soluble, thermostable volatiles, thermolabile/water soluble, and thermostable molecules [16–18], respectively, as informed by physical properties of the guest component [19].

The resultant inclusion complex [15] can potentially improve control release rate of the 4-cTRB relative to conventional polythene sachets, effectively extending the temporal utility 4-cTRB in repellence of tsetse flies from vertebrate hosts, while simultaneously protecting the blend environmental perturbations. The inclusion complex of 4-cTRB with β-CD can be achieved using several methods, as informed by physical property properties of the blend. Kneading, coprecipitation, heating in a sealed container, or freeze-drying techniques are suitable for poorly water-soluble, non-water-soluble, thermostable, and thermolabile compounds, respectively [11].

We initiated this study to establish (1) most suitable β-CD-based technical approaches among kneading, coprecipitation, heating, and freeze-drying microencapsulation techniques [19] for most effective microencapsulation and release of 4-cTRB and (2) influence of the encapsulation the 4-cTRB on responses (repellence) of *Glossina pallidipes* and *Glossina morsitans morsitans* tsetse flies. Herein, we report our findings.

2. Materials and Methods

2.1. Test Chemicals. Pure (98%–99%) forms of each δ-nonalactone, heptanoic acid, 4-methylguaiacol, geranyl acetone, and β-CD cyclic oligosaccharide nanomolecules were sourced from Sigma-Aldrich, Taufkirchen, Germany. All but β-CD were formulated into the 4-cTRB as described by Wachira et al. [6] under ambient (20–25°C) conditions. A known attractive phenolic blend from a 3-day fermented cow urine was used as negative control in laboratory responses of tsetse flies.

2.2. Tsetse Flies. Three-day-old teneral male *G. pallidipes* and *G. m. morsitans* colony tsetse flies were provided by Biotechnology Research Institute of Kenya Agricultural and Livestock Research Organization (BioRI-KALRO), Muguga Kenya, where the bioassays were performed. Behavioral responses (repellence) of teneral females *G. pallidipes* and *G. m. morsitans* tsetse flies are typically similar to their respective male counterpart [20]. Both colonies were initially started from wild seed puparia sample that originated from Uganda in 1975 and Zimbabwe in 1983. The flies were maintained at 25 ± 2°C, 75 ± 2% RH and 12:12 h LD photoperiod and fed with defibrinated bovine blood through silicon membranes (an artificial feeding system) three times a week as previously described [21].

2.3. Microencapsulation of 4-cTRB by β-CD. We selected four methods (kneading, freeze-drying, coprecipitation, or heating in a sealed container) of encapsulation to generate cyclodextrin–4-cTRB complexes. Due to the hydrophobic nature of the 4-cTRB, β-CD (also hydrophobic) was used for microencapsulation. Kneading technique is suitable for poorly water-soluble guests since the guest slowly dissolves during formation of complex [11]. Kneading affords good yield of inclusion formation but is unsuitable for large scale preparation [13]. First, the liquid or dissolved solid guest is added to slurry of β-CD and kneaded (in a mortar), followed by drying of the paste. The paste is then washed with sufficient solvent to remove free particles on the β-CD surface and then dried under vacuum. Marques [15] reported inclusion complexes of β-cyclodextrins formed by kneading method. The inclusion complexes include ibuprofen, omega-3 fatty acids and essential oils of thyme and European anchovy with β-cyclodextrins.

The 4-cTRB was mixed with β-CD and was separately kneaded, freeze-dried, coprecipitated, or heated in a sealed container [22] to facilitate the encapsulation. In brief, a mixture 2.00 g of β-CD and 1.0 mL of 4-cTRB was transferred in a porcelain mortar (MKCHMP, England) and thereafter kneaded with a pestle for 45 min to obtain the solid paste, washed with 50% EtOH to remove unadsorbed free blend from the inclusion compound, and oven dried at 50°C for 24 h [8]. The β-CD 4-cTRB mixture was alternatively frozen for 24 h (to ice any water vapor present in the cavities of the β-CD) and then freeze-dried for 48 h [13]. In the coprecipitation method, β-CD was dissolved in 50 mL distilled water and added to a beaker containing the 4-cTRB. The mixture was agitated on a magnetic hot plate and stirred for 10 min and then cooled for 20 min. Subsequent crystals formed were suspended, filtered, washed with diethyl ether (to remove free odor blend), and oven-dried at 50°C to yield a powdered sample of microcapsules [22]. To conduct the "heated in a sealed container" method, β-CD and 4-cTRB mixtures were placed in 100 mL beaker, allowed to stand for 2 h to absorb sufficient moisture, sealed with a metallic lid, and heated in an oven through a temperature gradient (43°C–142°C) with a temperature lapse rate of 4°C after every 5 min to obtain powdered microcapsules [23].

2.4. Physicochemical Characterization of the Microcapsules and Assessment of Responses of G. pallidipes and G. m. Morsitans to Encapsulated 4-cTRB in a Laboratory Wind Tunnel. The resultant microcapsules were characterized by Fourier-Transform Infrared (FT-IR; Shimadzu), Ultraviolet-Visible (UV-Vis; Specord 200 plus, Germany), and Gas Chromatography coupled with Flame Ionization Detector (GC-FID; Shimadzu, USA) techniques as per the protocols of Khoshtinat [22]. Responses of *G. pallidipes* and *G. m. morsitans* were evaluated in a cuboidal olfactometer ($195 \times 20 \times 20$ cm) consisting of transparent plexi glass as described by Wachira et al. [8] and Gikonyo et al. [9]. In brief, thirty teneral male *G. pallidipes* and *G. m. morsitans* tsetse flies were separately exposed to 4-cTRB microcapsules, unencapsulated 4-cTRB, β-CD (blank), and a phenolic blend from fermented cow urine as negative control, all conducted in three independent replicates. The test odors, controls, and no-odor controls in dichloromethane carrier solvents were transported through the wind tunnel in pure air dispensed at 12.63 L/min [5, 9]. Tsetse fly behavioral responses in the olfactometer to each test odor were observed for 3 min. Number of tsetse flies departing from the midsection, initial direction of upwind flights, and final tsetse fly landing/resting positions in the wind tunnel were noted. To minimize directional biasness, control and odor treatment arms of the olfactometer were alternated in successive replicates after cleaning the olfactometer with 70% ethanol and pure air after each replicate cycle. Each compound was tested on different days and any residual effects between compounds and replicates monitored using blank (dichloromethane solvent) tests. All tsetse fly exposures in the olfactometer were conducted in the mornings (0900–1200 h) or evenings (1500–1700 h) coincident with tsetse peak activities in the field [24, 25].

2.5. Kinetic Release Studies. Release rate studies of constituents of the 4-cTRB was carried out on blend microencapsulated using kneading technic since this approach most efficient among the microencapsulation method we evaluated. Inclusion complex (2.02 g) obtained was released in an open field (at Kenyatta University), representing the potential actual fields where livestock graze. This study was conducted in three independent replicates in November during the short rains with temperatures of 21°C–27°C, rainfall 110 mm, and humidity 21%–63%. Concentration of individual compounds in the blend was determined at 24 h interval for 7 days by extracting the blend from the microcapsules using dichloromethane solvent and with analysis conducted using GC-FID [26].

2.6. Data Analysis. Mean distances of upwind flight by activated flies in treated or control arms were compared using Chi Square (χ^2) nonparametric tests. Repellency of tsetse flies (*G. pallidipes* and *G. m. morsitans*) was calculated using formulas (C – T)/(C + T)%, where C and T represented number of flies in the control and treated arms of the olfactometer, respectively [5]. The proportional repellency responses of the flies to the test odors were analyzed using Analysis of variance (ANOVA) and means separated uaing Student–Newman–Keuls (SNK) post hoc analyses. Field catches of tsetse flies during field trials were $\log_{10}(n+1)$ transformed to homogenize the variances and normalize the distributions. The effects of day, site, and odor were separated using ANOVA followed by Fisher's least significant difference (LSD) post hoc tests. Statistical analysis was conducted using SPSS Version 22 (SPSS, Inc., Chicago, IL, U.S.A.) with a significance level of 0.05. Mean catches were detransformed, reported, and expressed as a proportion of the mean control catches. Percentage reduction of tsetse fly catches in the field was calculated as difference between fly catches of a trap with treatment odor/blend and without, expressed as a percentage [7, 27].

3. Results

3.1. Spectroscopic Characterization. The tsetse fly repellent blend (4-cTRB) was successfully microencapsulated in β-CD to form β-CD/4-cTRB inclusion complex. This was confirmed by FTIR spectroscopic techniques where the inclusion complex revealed significant overlap with no unique peak between β-CD, β-CD/4-cTRB inclusion complex, and the unencapsulated 4-cTRB (Figure 1A, Supporting file). The FTIR spectra for both raw 4-cTRB and β-CD/4-cTRB microcapsules showed similar peaks, indicating functional groups of the constituent compounds. Both showed a strong and broad O-H stretching (3200–3550 cm^{-1}), δ-lactone C=O stretching (1735–1750 cm^{-1}), C=O stretching of a carboxylic acid (1760 cm^{-1}), and C=O stretching of an aliphatic ketone (1705–1725 cm^{-1}) originating from 4-methylguaiacol, δ-nonalactone, heptanoic acid, and geranyl acetone, respectively. FTIR of β-CD/4-cTRB inclusion complex showed some peaks characteristic to β-CD. They include(C-O-C) glucosydic stretching vibrations at 1152 cm^{-1}. Similar observation was reported by Kariuki et al. [28], where 4-propylguaiacol was encapsulated to various nanoparticles including β-CD, ethyl cellulose, and polyvinylpyrrolidone. The UV-Vis spectroscopic technique also confirmed the microencapsulation where the spectrum of the 4-cTRB in cyclohexane and that of 4-cTRB/β-CD microcapsules (Figure 2A, Supporting file) gave similar absorption bands ($\lambda_{max} = 275$–300 nm) as previously documented by Kariuki et al. [28]. This indicated the presence of 4-cTRB in the inclusion complex. However, levels of the 4-cTRB in the encapsulated microcapsules were lower than that in the unencapsulated (raw) 4-cTRB as shown by lower absorbances of the peaks (Figure 2A, Supporting file). From UV-Vis spectrum, there were obvious bands at 205, 230, and 280 nm, which are attributed to π-π* electronic transitions of the benzyl ring from 4-methylguaiacol. The absorption band at 295 nm was assigned to *n*-π* transitions of the carbonyl substituent group in heptanoic acid, δ-nonalactone, and geranyl acetone. No absorption band was observed for β-CD due to the absence of either π-electrons or nonbonding electrons [29]. The UV-Vis data for β-CD are coincident with that obtained by Eteer [30] where β-cyclodextrin-vanillin inclusion complex was synthesized.

Similar UV-Vis pattern was observed by Oelmann and Meier [31] on encapsulation and phase transfer of Orange II from aqueous to organic phase by polymers.

3.1.1. Supporting Description. Supporting Figures 1A and 2A show that the 4-cTRB was successfully encapsulated in β-CD, forming an inclusion complex. Supporting Figures 1A and 2A are FTIR and UV-Vis spectra, respectively, of the 4-cTRB, β-CD, and β-CD/4-cTRB inclusion complexes.

3.2. Levels of Microencapsulation of 4-cTRB to β-CD. 4-cTRB was microencapsulated in β-CD using different synthetic routes of kneading, heating in a sealed container, freeze-drying, and coprecipitation. The protocols for the various encapsulation methods afforded about 2.0g of microcapsules (percentage yield of > 80%). The concentration of individual compounds in the blend microcapsules is summarized in Table 1.

Generally, levels of microencapsulation of the blend on kneading was significantly higher ($p < 0.05$) than those of other protocols as indicated by the high concentration of individual compounds (Table 1). The microencapsulation levels of the blend were similar ($p > 0.05$) when heating, freeze-drying, and coprecipitation protocols were used (Table 1). 4-Methylguaiacol demonstrated significantly higher ($p < 0.05$) microencapsulation percentage (83%) as compared with those of δ-nonalactone (78%), heptanoic acid (74%), and geranyl acetone (74%) (Table 1) when the kneading method was employed. The level of microencapsulation on heated microcapsules was not significantly different ($p > 0.05$) from those of freeze-dried and coprecipitated microcapsules.

3.3. Wind-Tunnel Responses of G. pallidipes and G. m. Morsitans to 4-cTRB Encapsulated to β-CD. Tsetse fly upwind flight distances on treated and control arms of the olfactometer and proportions of the flies that rested on either arm were recorded. Tsetse fly behaviors in the olfactometer depended on the presence/absence of odor stimuli [6, 8, 32]. The flies (*G. pallidipes* and *G. m. morsitans*) exhibited upwind flight in the no odor (control) arm whereas in the treated arm, some characteristic 180° turns, zigzag movements (in and out of plume), short hops, and wing fanning against the wall of the tunnel were observed as we had previously observed [6, 8]. Generally, the average distances flown by *G. pallidipes* and *G. m. morsitans* in the control arm were significantly higher ($p < 0.001$) than those in the treated (microencapsulated and non-encapsulated 4-cTRB) arm (Table 2). The final resting choice (repellency) of *G. pallidipes* and *G. m. morsitans* on treatment with microencapsulated 4-cTRB prepared using either method was not significantly different ($p > 0.05$) from each other and those of unencapsulated 4-cTRB (Table 2). The flies (*G. pallidipes* and *G. m. morsitans*) did not exhibit preference for the control or treated arm with β-CD matrix (Table 2). The average tsetse fly distances flown in control and treated arms were similar ($p > 0.05$). The response patterns and behaviors of *G. pallidipes* and *G. m. morsitans* were also similar ($p > 0.05$).

3.4. Release Rate of Individual Compounds in the β-CD-Encapsulated 4-cTRB. Release rates of the constituent compounds are summarized in Figure 1. Average release rates of heptanoic acid, δ-nonalactone, 4-methylguaiacol, and geranyl acetone were 1.23, 1.49, 0.81, and 0.38 mg/hr, respectively (Figure 1). All the constituents of the 4-cTRB had steady release rates in all the 7 days studied (Figure 1).

3.5. Comparative Field Responses of G. pallidipes to 4-cTRB. We validated wind-tunnel laboratory responses of male teneral *G. pallidipes* and *G. m. morsitans* tsetse flies by field responses of *G. pallidipes* (of mixed sexes and ages) to 4-cTRB in ß-cyclodextrin microcapsules and when packed in lay-flat tubing of different thicknesses and the results are summarized in Table 3. Our laboratory results provided us with general pattern on choice preferences of teneral tsetse flies to the volatile odors that could have been affected by the number of generations of the tsetse flies that had been colonized. This may influence the phenotypic behavior of flies but the laboratory response patterns were confirmed by field evaluations using wild flies. The reduction in field catches on dispensing the 4-cTRB on ß-cyclodextrin nanoparticles was similar ($p > 0.05$) to those on 200 and 400 μm lay flat tubing (Table 3). However, the release rate of the odor blend was significantly lower ($p < 0.05$) when dispensed through the ß-cyclodextrin matrix (5.35 ± 0.21 mg/h) as compared to the lay flat tubings (200 and 400 μm lay-flat tubing had mean release rates of 11.82 ± 0.53 and 6.73 ± 0.46 mg/h, respectively) as summarized in Figure 2.

3.6. Release Rate of 4-cTRB Constituent Compounds. Generally, for each release method used (lay flat tubing and microcapsules), release rate of constituent compounds (δ-nonalactone, heptanoic acid, 4-methylguiacol, and geranyl acetone) of 4-cTRB were similar ($p > 0.05$) (Table 4). The rate of release of individual compounds in the ß-cyclodextrin microcapsules was significantly ($p < 0.05$) lower than those of from lay flat tubing (200 and 400 μm) (Table 4).

4. Discussion

The FTIR peaks of the blend and that of the 4-cTRB/β-CD microcapsules overlaid with no new peak in the spectra. This is evident that all the four compounds mixed to produce the optimized tsetse fly-repellent blend, combined well and remained stable. The O-H, -COOH, and C=O functional groups in the 4-cTRB molecules interacted with the β-CD structure by hydrogen bonding and electrostatic interactions, therefore forming an inclusion complex [31]. The FTIR and UV-Vis spectroscopic methods showed perfect microencapsulation of the 4-cTRB to β-CD nanoparticles as described by Wanzala [33] and Kariuki [28]. Encapsulation using the kneading method gave most significant levels of encapsulated compounds, confirming previous observation by Wanzala [33]. This may be attributed to the non-destructive nature of the method to the β-CD cavities.

Table 1: Concentration of individual compounds of the synthesized microcapsules by various methods.

Constituent compounds	Mean concentrations in mg/mL ± SE (% encapsulation)			
	A	B	C	D
Heptanoic acid	0.21 ± 0.01 (74)[a]	0.18 ± 0.00 (64)[b]	0.17 ± 0.00 (60)[b]	0.17 ± 0.00 (60)[b]
4-Methylguaiacol	0.14 ± 0.00 (83)[a]	0.112 ± 0.00 (67)[b]	0.109 ± 0.01 (65)[b]	0.106 ± 0.00 (63)[b]
δ-Nonalactone	0.32 ± 0.01 (78)[a]	0.28 ± 0.00 (68)[b]	0.276 ± 0.00 (67)[b]	0.26 ± 0.01 (63)[b]
Geranyl acetone	0.05 ± 0.00 (74)[a]	0.038 ± 0.00 (57)[b]	0.04 ± 0.00 (60)[b]	0.037 ± 0.00 (58)[b]

Note: A: kneading, B: heating in a sealed container, C: freeze-drying, D: coprecipitation inclusion complexes; means followed by same capital letter in a row are not significantly different ($p > 0.05$, SNK test).

Table 2: Wind-tunnel behavioral responses of tsetse flies to 4-cTRB encapsulated by various methods.

Test material	*G. pallidipes*			*G. m. morsitans*		
	Average distance of upwind flight (cm ± SE)		Final resting choice (repellency)	Average distance of upwind flight (cm ± SE)		Final resting choice (repellency)
	C	T		C	T	
β-CD[¥]	44.17 ± 4.04	50.11 ± 5.03	0.43 ± 0.32[b]	48.39 ± 2.04	46.08 ± 3.05	1.31 ± 0.87[b]
Cow urine↗	28.76 ± 2.03	78.71 ± 3.55***	−67.11 ± 1.15[a]	26.33 ± 3.23	82.87 ± 3.04***	−68.07 ± 1.89[a]
A	73.64 ± 3.73	28.43 ± 3.83***	87.04 ± 0.22[c]	72.67 ± 5.16	26.04 ± 4.12***	88.63 ± 1.44[c]
B	72.82 ± 3.25	21.85 ± 4.67***	87.89 ± 1.86[c]	69.95 ± 3.84	22.83 ± 3.06***	89.04 ± 1.02[c]
C	68.26 ± 5.61	26.94 ± 4.07***	89.22 ± 1.37[c]	71.16 ± 3.06	20.82 ± 3.04***	88.77 ± 1.06[c]
D	70.37 ± 2.86	25.67 ± 2.78***	88.24 ± 1.22[c]	69.32 ± 3.89	23.67 ± 4.46***	89.67 ± 0.83[c]
4cTRB	75.45 ± 2.12	24.24 ± 4.31***	89.02 ± 1.02[c]	71.16 ± 4.41	20.33 ± 3.05***	88.13 ± 0.21[c]

Note: Number of tsetse flies used in each test ($N = 30 \times 3$); C = control arm; T = treated arm; A: kneading, B: heating in a sealed container, C: freeze-drying, D: coprecipitation inclusion complexes; 4cTRB: four-component tsetse repellent blend (without encapsulation). Each pair of average distance of upwind flight in C and T were compared by χ^2 (*** $p < 0.001$); means followed by the same letter in final resting choice are not significantly different ($p > 0.05$, SNK test).
↗Three-day fermented cow urine (negative control).
[¥]Blank control.

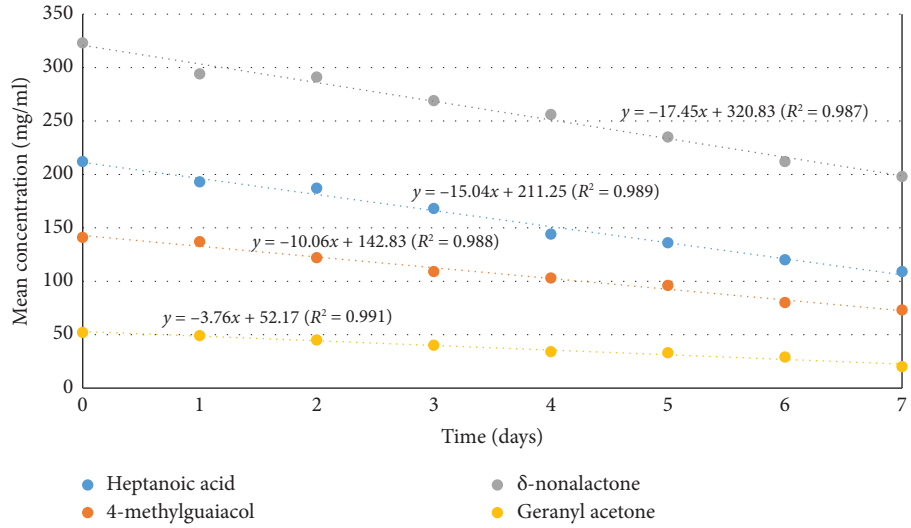

Figure 1: Time-course levels of the constituents of the repellent blend from kneaded microcapsules of β-cyclodextrin.

Different compounds are encapsulated at different levels in the β-CD cavities depending on the structure and nature of the molecule [34].

From the laboratory assays, it is evident that responses of *G. pallidipes* and *G. m. morsitans* to β-CD microcapsules of the 4-cTRB in all the inclusion methods were similar to those of unencapsulated blend. Behavioral responses of the tsetse flies to the microencapsulated tsetse fly-repellent blend and the raw blend (un-encapsulated) at the wind tunnel experiments were similar since the active molecules were released from both test materials. Similar responses of *G. pallidipes* and *G. m. morsitans* were attributed to similar olfactory apparatus as previously observed by Wachira et al. [8]. The similar laboratory responses of *G. pallidipes* and *G. m. morsitans* to the odor blend were ascribed to common mechanism of chemoreception. There is need for a follow-up

Table 3: Mean catches of *G. pallidipes* (males + females) to baited NG2 G traps in presence of optimized 4-cTRB through various media.

Odor blend	Mean catch ± SE	Catch index[#]
200 µm lay-flat tubing	51.22 ± 6.67^{a}	0.19
400 µm lay-flat tubing	58.54 ± 9.26^{a}	0.22
Encapsulated♦	64.57 ± 11.35^{a}	0.24
Control[¥]	267.12 ± 21.34^{b}	1.00

Note: Means followed by different letters are significantly different ($p < 0.05$, LSD post hoc analyses).
[¥]NG2G trap baited with fermented cow urine, acetone, and phenol sachets.
♦Repellent blend encapsulated with ß-cyclodextrin by the kneading process.
[#]Total mean catch expressed as a proportion of that of the blank control trap (reference value).

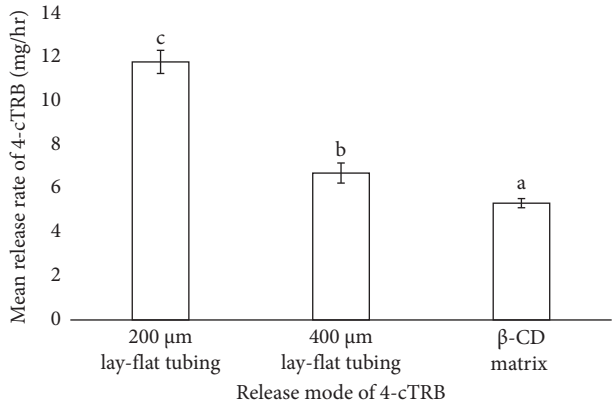

Figure 2: Mean release rate of 4-cTRB in various delivery systems (means with different small letters are significantly different ($p < 0.05$), ANOVA, SNK post hoc analyses).

Table 4: Field mean release rate of individual constituent compounds of the 4-cTRB.

Constituent compounds in the blend	Mean release rate (mg/hr)		
	200 µm lay-flat polythene tubing	400 µm lay-flat polythene tubing	Encapsulated blend
Heptanoic acid	2.83 ± 0.03^{c}	1.57 ± 0.01^{b}	1.38 ± 0.01^{a}
4-Methylguiacol	2.74 ± 0.02^{c}	1.61 ± 0.00^{b}	1.33 ± 0.00^{a}
δ-Nonalactone	2.46 ± 0.02^{c}	1.62 ± 0.01^{b}	1.30 ± 0.01^{a}
Geranyl acetone	2.43 ± 0.01^{c}	1.68 ± 0.01^{b}	1.27 ± 0.00^{a}
p value	> 0.05	> 0.05	> 0.05

Note: Means followed by different letters in a row are significantly different ($p < 0.05$, LSD post hoc analyses).

on gene expression and genomics studies to establish e associated genes and receptors in order to give insight on the molecular interaction of the odors with the insect.

The β-CD did not affect responses (repellence) of tsetse fly by blend odor; thus, it is an affective polymeric shell for microencapsulation. Microencapsulation allows real-time effective controlled-release in downstream deployment of the tsetse-repellent blend. Use of β-CD as the polymeric shell allows the tsetse-repellent blend to be used in the field by farmers to protect humans and their livestock from African trypanosomiasis. Release rate of the odor constituents was constant and followed the zero-order kinetics, correlating with findings reported by Wanzala [33]. The rate at which the constituent compounds of 4-cTRB are released from the microcapsules are relatively the same indicating that the initial proportions of the compound are maintained. This was similarly observed by Ishiguro et al. [35], where the release of fragrances from β-CD complexes was at constant level.

β-CD is considered environmentally safe (due to its biodegradable nature), nontoxic, and reusable (cost efficient) and is used in many applications, including water treatment, pesticide removal, larvae control, food safety analyses, and drug delivery [36, 37]. The kneading method of microencapsulation was the most effective one over other method.

5. Conclusion

Microencapsulation of 4-cTRB in the β-CD complex improves controlled release rate of the blend, which offers prolonged protection against bites by tsetse fly. The β-CD is a promising release tool for volatile molecules for an increased longevity in its activity. This technology provides a potential tool for dispensing tsetse and other related

arthropod vectors' (such as ticks and mosquitoes) repellents on hosts to protect them from diseases transmitted by respective vectors.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Disclosure

The content is solely the responsibility of the authors and does not necessarily represent the official views of the Bioinnovate Africa Program and/or National Institutes of Health.

Conflicts of Interest

The authors declare no conflicts of interest.

Author Contributions

Bernadatte M. Ratemo and Benson M. Wachira are co-first authors. Other contributions are as hereunder stated. Conceptualization: Paul O. Mireji and Ahmed Hassanali. Data curation: Eric Masika, Benson M. Wachira and Bernadatte M. Ratemo.Formal analysis: Eric Masika, Benson M. Wachira and Bernadatte M. Ratemo.Funding acquisition: Paul O. Mireji. Investigation: Eric Masika, Benson M. Wachira and Bernadatte M. Ratemo.Methodology: Paul O. Mireji, Benson M. Wachira and Bernadatte M. Ratemo.Project administration: Paul O. Mireji.Resources: Paul O. Mireji. Supervision: Paul O. Mireji, Eric Masika, Margaret M. Ng'ang'a and Ahmed Hassanali. Visualization: Eric Masika, Benson M. Wachira and Bernadatte M. Ratemo.Writing — original draft: Benson M. Wachira and Bernadatte M. Ratemo.Writing — review & editing: Paul O. Mireji, Benson M. Wachira, Eric Masika, Margaret M. Ng'ang'a.

Funding

Research reported in this publication was supported by Bioinnovate Africa Program under Award Number BA/3/2022/05. The research was also supported by the Fogarty International Center (FIC) and National Institute of Allergy and Infectious Diseases (NAID) of the National Institutes of Health under Award Numbers D43TW011813 and R01AI169503, respectively.

Acknowledgments

We thank Mr. Paul Thande and Mr. Patrick Obore (technicians in Entomology Division) of BioRI for providing facilities and technical support in laboratory and field studies. We also thank Ms. Catherine Njeru of Kenyatta University (Department of Chemistry) and Ms. Joanna Auma (BioRI) for providing laboratory space for microencapsulation studies and managing the project respectively.

Supporting Information

Additional supporting information can be found online in the Supporting Information section. *(Supporting Information)*

Figures 1A and 2A are FTIR and UV-Vis spectra, respectively, confirming 4cTRB was successfully microencapsulated in β-CD, forming an inclusion complex.

References

[1] J. G. Grootenhuis and R. O. Olubayo, "Disease Research in the Wildlife-Livestock Interface in Kenya," *Veterinary Quarterly* 15, no. 2 (1993): 55–59.

[2] N. Baker, H. P. de Koning, P. Mäser, and D. Horn, "Drug Resistance in African Trypanosomiasis: the Melarsoprol and Pentamidine Story," *Trends in Parasitology* 29, no. 3 (2013): 110–118.

[3] K. I. Kasozi, G. Zirintunda, F. Ssempijja, et al., "Epidemiology of Trypanosomiasis in Wildlife—Implications for Humans at the Wildlife Interface in Africa," *Frontiers in Veterinary Science* 8 (2021): 621699.

[4] M. Murray, J. D. Berry, W. I. Morrison, R. O. Williams, H. Hirumi, and L. Rovis, "A Review of the Prospects for Vaccination in African Trypanosomiasis," *Part II* (1980).

[5] B. M. Wachira, J. M. Kabaka, P. O. Mireji, et al., "Characterization of a Composite with Enhanced Attraction to Savannah Tsetse Flies From Constituents or Analogues of Tsetse Refractory Waterbuck (Kobus defassa) Body Odor," *PLoS Neglected Tropical Diseases* 15, no. 6 (2021): e0009474.

[6] B. M. Wachira, J. M. Kabaka, P. O. Mireji, S. O. Okoth, G. A. Murilla, and A. Hassanali, "Blending Studies With Selected Waterbuck Odor Constituents or Analogues in the Development of a Potent Repellent Blend against Savannah Tsetse," *Acta Tropica* 211 (2020): 105597.

[7] M. K. Bett, R. K. Saini, and A. Hassanali, "Repellency of Tsetse-Refractory Waterbuck (*Kobus defassa*) Body Odour to Glossina Pallidipes (Diptera: Glossinidae): Assessment of Relative Contribution of Different Classes and Individual Constituents," *Acta Tropica* 146 (2015): 17–24.

[8] B. M. Wachira, P. O. Mireji, S. Okoth, et al., "Responses of Glossina Pallidipes and Glossina Morsitans Morsitans Tsetse Flies to Analogues of δ-octalactone and Selected Blends," *Acta Tropica* 160 (2016): 53–57.

[9] N. K. Gikonyo, A. Hassanali, P. G. N. Njagi, and R. K. Saini, "Responses of Glossina Morsitans Morsitans to Blends of Electroantennographically Active Compounds in the Odours of Its Preferred (Buffalo and Ox) and Non-preferred (Waterbuck) Hosts in a Two-Choice Wind Tunnel," *Chemistry and Ecology* 29 (2003): 2331–2345.

[10] N. K. Gikonyo, A. Hassanali, P. G. N. Njagi, P. M. Gitu, and J. O. Midowo, "Odor Composition of Preferred (Buffalo and Ox) and Non-Preferred (Waterbuck) Hosts of Some Savanna Tsetse Flies," *Journal of Chemical Ecology* 28 (2002): 961–973.

[11] N. V. N. Jyothi, P. M. Prasanna, S. N. Sakarkar, K. S. Prabha, P. S. Ramaiah, and G. Y. Srawan, "Microencapsulation Techniques, Factors Influencing Encapsulation Efficiency," *Journal of Microencapsulation* 27, no. 3 (2010): 187–197.

[12] H. J. Schmann and E. Schollmeyer, "Applications of Cyclodextrins in Cosmetic Products: A Review," *Journal of Cosmetic Science* 53 (2002): 185–191.

[13] H. Marques, J. Hadgraft, I. Kellaway, and W. Pugh, "Studies of Cyclodextrin Inclusion Complexes. II. Molecular Modelling and 1H-NMR Evidence for the Salbutamol-β-Cyclodextrin

Complex," *International Journal of Pharmaceutics* 63, no. 3 (1990): 267–274.

[14] A. R. Hedges, W. J. Shieh, and C. T. Sikorski, "Use of Cyclodextrins for Encapsulation in the Use and Treatment of Food Products," in *Encapsulation and Controlled Release of Food Ingredients*, eds. S. J. Risch and G. A. Reineccius (Washington, DC: American Chemical Society, 1995), 60–73.

[15] H. M. C. Marques, "A Review on Cyclodextrin Encapsulation of Essential Oils and Volatiles," *Flavour and Fragrance Journal* 25, no. 5 (2010): 313–326.

[16] H. M. C. Marques, "Structure and Properties of Cyclodextrins. Inclusion Complex Formation," *Revista Portuguesa de Farmacia* 44 (1994): 77–84.

[17] W. Saenger, "Cyclodextrin Inclusion Compounds in Research and Industry," *Angewandte Chemie International Edition in English* 19 (1980): 344–362.

[18] Y. Nakai, K. Yamamoto, K. Terada, and D. Watanabe, "New Methods for Preparing Cyclodextrin Inclusion-Compounds.1. Heating in a Sealed Container," *Chemical and Pharmaceutical Bulletin* 35 (1987): 4609–4615.

[19] B. Cheirsilp and J. Akmai, "Inclusion Complex Formation of Cyclodextrin With its Guest and Their Applications," *Biology, Engineering and Medicine* 2 (2016): https://doi.org/10.15761/BEM.1000108.

[20] S. J. Torr, A. Chamisa, G. A. Vale, M. J. Lehane, and J. M. Lindh, "Responses of Tsetse Flies, Glossina Morsitans Morsitans and Glossina Pallidipes, to Baits of Various Size," *Medical and Veterinary Entomology* 25, no. 4 (2011): 365–369.

[21] S. K. Moloo, "An Artificial Feeding Technique for *Glossina*," *Parasitology* 63 (1971): 507–512.

[22] K. Khoshtinat, M. Barzegar, M. A. Sahari, and Z. Hamidi, "Encapsulation of Iranian Garlic Oil with β-cyclodextrin: Optimization and its Characterization," *Journal of Agricultural Science and Technology A* 19, no. 1 (2017): 97–111.

[23] S. Junco, T. Casimiro, N. Ribeiro, M. Nunes, D. Ponte, and M. H. Cabral, "A Comparative Study of Naproxen–Beta Cyclodextrin Complexes Prepared by Conventional Methods and Using Supercritical Carbon Dioxide," *Journal of Inclusion Phenomena and Macrocyclic Chemistry* 44 (2002): 117–121.

[24] N. Asfaw, B. Hiruy, N. Worku, and F. Massebo, "Evaluating the Efficacy of Various Traps in Catching Tsetse Flies at Nech Sar and Maze National Parks, Southwestern Ethiopia: An Implication for Trypanosoma Vector Control," *PLoS Neglected Tropical Diseases* 16, no. 12 (2022): e0010999.

[25] J. Brady, "Spontaneous, Circadian Components of Tsetse Fly Activity," *Journal of Insect Physiology* 18, no. 3 (1972): 471–484.

[26] J. Wang, Y. Cao, B. Sun, and C. Wang, "Physicochemical and Release Characterisation of Garlic Oil-β-Cyclodextrin Inclusion Complexes," *Food Chemistry* 127, no. 4 (2011): 1680–1685.

[27] R. K. Saini and A. Hassanali, "A 4-Alkyl-Substituted Analogue of Guaiacol Shows Greater Repellency to Savannah Tsetse (Glossina spp.)," *Journal of Chemical Ecology* 10 (2007): 1–11.

[28] M. W. Kariuki, A. Hassanali, and M. M. Ng'ang'a, "Nano-encapsulation of 4-Propylguaiacol in β-Cyclodextrin, Ethyl Cellulose, and Polyvinylpyrrolidone," *Journal of Chemistry* 2022, no. 1 (2022): 6590850.

[29] X. Wang, Z. Luo, and Z. Xiao, "Preparation, Characterization, and Thermal Stability of β-Cyclodextrin/Soybean Lecithin Inclusion Complex," *Carbohydrate Polymers* 101 (2014): 1027–1032.

[30] S. A. Eteer, "UV-vis Spectroscopic Characterization of β-Cyclodextrin-Vanillin Inclusion Complex. Mediterr," *Journal of Chemistry* 12, no. 175 (2022): 10–13171.

[31] S. Oelmann and M. A. R. Meier, "Synthesis and Unimolecular Micellar Behavior of Amphiphilic Star-Shaped Block Copolymers Obtained via the Passerini Three Component Reaction," *RSC Advances* 7, no. 71 (2017): 45195–45199.

[32] N. K. Gikonyo, A. Hassanali, G. N. Njagi, and R. K. Saini, "Behaviour of Glossina Morsitans Morsitans (Diptera: Gossinidae) on Waterbuck *Kobus defassa* Ruppel and Feeding Membranes Smeared with Waterbuck Sebum Indicates the Presence of Allomones," *Acta Tropica* 77 (2000): 295–303.

[33] W. Wanzala, N. F. K. Sika, S. Gule, and A. Hassanali, "Attractive and Repellent Host Odours Guide Ticks to Their Respective Feeding Sites," *Chemoecology* 14, no. 3–4 (2004): 229–232.

[34] A. C. da Rocha Neto, A. B. D. O. da Rocha, M. Maraschin, R. M. Di Piero, and E. Almenar, "Factors Affecting the Entrapment Efficiency of β-cyclodextrins and Their Effects on the Formation of Inclusion Complexes Containing Essential Oils," *Food Hydrocolloids* 77 (2018): 509–523.

[35] T. Ishiguro, Y. Sakata, H. Arima, et al., "Release Control of Fragrances by Complexation with β-cyclodextrin and its Derivatives," *Journal of Inclusion Phenomena and Macrocyclic Chemistry* 92 (2018): 147–155.

[36] J. Zhou, J. Jia, J. He, J. Li, and J. Cai, "Cyclodextrin Inclusion Complexes and Their Application in Food Safety Analysis: Recent Developments and Future Prospects," *Foods* 11, no. 23 (2022): 3871.

[37] T. Urooj, M. Mishra, and S. Pandey, "Unlocking Environmental Solutions: a Review of Cyclodextrins in Pollutant Removal," *Discover Environment* 2 (2024): 65.