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Ex vivo and *in vivo* evaluation of an *in situ* nanogel composite loaded with carmustine: implications for efficient nose-to-brain delivery

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ABSTRACT

In this study, the efficacy of a Nanogel Composite was evaluated and compared to the conventional drug carmustine (BCNU) through *ex vivo* and *in vivo* studies using New Zealand White rabbits. The Nanogel Composite is a thermosensitive, electro-responsive, and mucoadhesive gel containing BCNU-loaded paramagnetic nanoparticles known as Nano-co-Plex (NCP). Following intranasal administration, electrical stimulation (ES) was applied to the rabbit's nasal cavity, initiating the release of BCNU-NCP. Subsequently, a magnetic headband placed on the rabbit's head rapidly attracted the released nanoparticles toward the brain. The *in vivo* results showed high amount of BCNU in cerebrospinal fluid (CSF) and the brain of the rabbit for Nanogel Composite compared to the conventional drug with BCNU concentration values in the CSF, brain and plasma being 0.2571 μg/mL, 0.199 μg/g and 0.0078 μg/mL, respectively, after 30 min of administration with the application of ES and MF; and 0.0087 μg/mL, 0.0076 μg/g and 0.0078 μg/mL without the application. The system enabled a pulsatile 'on-off' release profile, improving drug localization in the brain while minimizing systemic exposure. The applied ES and MF conditions (5 V and 0.4 Tesla) were found to be safe and well-tolerated, indicating the potential of this dual-stimuli-responsive platform for effective, non-invasive, and controlled nose-to-brain drug delivery.

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Introduction

Several therapeutic agents introduced into the nasal mucosa migrate into the systemic circulation rather than traversing directly to the brain (Bonaccorso et al. 2024; Huang et al. 2024). This diversion arises because richly vascularized nasal mucosa exposes compounds to the blood-brain barrier (BBB), impeding direct nose-to-brain transport (Sabir et al. 2020; Samaridou et al. 2020; Qiao et al. 2024). Although numerous reviews document intranasal delivery pathways (Feng et al. 2018; Prabhakar et al. 2022; Ferreira et al. 2025), most formulations lack sufficient targeting to the olfactory bulb. Slow diffusion across the nasal epithelium and drainage into systemic circulation further reduces bioavailability at the olfactory region (Deruyver et al. 2021).

An efficient nose-to-brain delivery system would facilitate rapid transport of therapeutic agents to the olfactory mucosa prior to systemic absorption, thereby maximizing exposure within the central nervous system (CNS). However, current intranasal delivery largely relies on physicochemical properties, passive diffusion, and gravitational forces, which inherently limits effective transport (Tomar et al. 2024). Consequently, any delay in reaching the olfactory mucosa enhances systemic uptake and subsequent clearance by the blood-brain barrier (BBB), significantly reducing brain bioavailability.

Building on our previous work (Akilo et al. 2016; 2019), we developed a nanogel composite: a thermosensitive, electro-responsive mucoadhesive gel encapsulating paramagnetic Carmustine (BCNU)-loaded Nano-co-Plex (BCNU-NCP). *Ex vivo* studies investigated

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a triggered release of BCNU-NCP with Electric Stimulation (ES) and its permeation across rabbit nasal tissue, with and without an external magnetic field (MF). *In vivo* pharmacokinetic and histopathological assessments in New Zealand White Rabbits compared intranasal (IN) administration with ES and MF to conventional intravenous (IV) deliver of BCNU. Rabbits offer advantages over smaller models (e.g. rats) due to larger nasal access and CSF volume, enabling repeated sampling without euthanasia (Bojsen-Moller and Fahrenkrug 1971; Traystman 2003). Electrodes and a magnetic headband provided stimuli, methods previously validated in rabbits (Lerner et al. 2004; Vaiman et al. 2004; Ho 2015). We report on mucoadhesion, permeation enhancement, controlled pulsatile release, CNS delivery efficacy, and local tissue safety.

Carmustine (BCNU) is a potent alkylating agent, but its therapeutic efficacy against brain tumors is significantly limited by rapid systemic clearance and poor penetration across the blood-brain barrier (BBB) following intravenous (IV) administration. The Nanogel Composite was specifically designed to overcome these limitations through intranasal (IN) administration. Its thermosensitive, mucoadhesive gel properties, combined with electrical stimulation (ES) for controlled release and magnetic field (MF) guidance for rapid central nervous system (CNS) delivery, collectively enhance targeted brain drug delivery.

Materials and methods

Materials

Nanogel Composite was prepared using a previous method developed by Akilo et al.[11,12]. Double deionized water was obtained from a Milli-Q water purification system (Milli-Q, Millipore, Billerica, MA, U.S.). All other reagents used were of analytical grade and were employed as purchased. New Zealand White rabbits (2-2.5 kg) were acquired from the Central Animal Service (CAS), University of the Witwatersrand (Wits), Galantamine (Gal), carmustine (BCNU), sodium chloride, potassium chloride calcium chloride, dextrose and sodium bicarbonate were purchased from Sigma-Aldrich (Steinhelm, Germany). UPLC separation was performed using a SunFire™ C18 column (3.5 μm, 4.6 × 75 mm) with an autosampler and 515 pumps (Waters, Milford, MA, USA). Double de-ionized water was used (Milli- Q, Millipore, Johannesburg, South Africa). UPLC solvents were of UPLC grades and all other reagents used are of analytical grades and of the highest purity. Ketamine, Xylazine, Buprenorphine, Carprofen, Isoflurane, Oxygen, Procaine and Sodium pentobarbitone were provided by the Central Animal Service (University of the Witwatersrand).

Ex vivo *evaluation of the nanogel composite*

Preparation of the rabbit nasal tissue for permeation studies. These studies were performed according to method adapted from Kubo et al. 1994. Ringer solution was prepared by mixing 154 mM of sodium chloride, 5.64 mM of potassium chloride, 2.16 mM of calcium chloride, 11.10 mM of dextrose and 2.38 mM of sodium bicarbonate in 1000 ml of deionized water and the pH adjusted to 6.0. The nasal mucosa tissues used in these experiments were obtained from a New Zealand White rabbit. The rabbit was euthanized with an appropriate dose of sodium pentobarbitone. The nasal septum was carefully removed from the bone block and immersed and adequately rinsed in an ice-cold Ringer's solution previously prepared. Two pieces of the nasal mucosa were stripped with care using forceps and surgical scissors from the nasal mucosa ready for the permeation studies.

Ex vivo *evaluation of mucoadhesive strength of the Nanogel Composite.* A Texture Analyzer (TA) (TA.XT plus, Stable Microsystems, Surrey, UK) was employed to determine the mucoadhesive strength of the formulations. This was undertaken by measuring the force required to detach nasal mucosa membrane from the Nanogel Composite. Freshly excised rabbit nasal membrane was attached to the upper probe of the instrument; a 1 ml of Nanogel Composite was placed below the platform inside a temperature-controlled compartment. The temperature was regulated to 34 ± 1 °C. The upper probe containing the membrane was then lowered at a speed of 10 mm/min to touch the surface of the gel. A force of 0.1 N was applied for 2 min to ascertain intimate contact with the gel. The exposed nasal mucosa membrane surface area was 0.785 cm^2.

Determination of the permeation capability of the BCNU-NCP released from the Nanogel Composite across rabbit nasal tissue

One of the important aspects of this work is to investigate how rapidly the nanoparticles released from the gel migrate from the nasal mucosa through the nasal epithelial tissue, to the olfactory region for subsequent delivery to the brain. Thus, *ex vivo* permeation studies would give a better assessment of the permeation of the formulation through nasal tissue. Furthermore, the use of electric and magnetic fields would be assessed to see the effect of these parameters on the release

and subsequent transportation of drug across the nasal epithelial tissue. The permeation studies were performed by employing the experimental setup illustrated in Figure 1. The diffusion cells used were a side-by-side type. This apparatus consists of donor and receptor half-cells of 12 ml volume capacity. The choice of this apparatus was due to the use of a bar magnet that was positioned at the receiver compartment to evaluate the effect of MF on the permeation of the nanoparticles. The apparatus was equipped with a stirrer on each compartment and the system was connected to a thermostat-controlled water jacket maintaining a temperature of 34 ± 1 °C. The apparatus was then mounted on an agitator to aid mixing. The tissue thickness was measured using a micrometer; the tissue was then mounted on a glass disc with round hole of 5 mm diameter with the mucosal surface facing the donor compartment and the serosal side facing the receptor compartment. This was then placed in between the donor and the receptor compartments of the diffusion cell apparatus which contained pre-warmed PBS (pH 6) at 34 ± 1 °C to mimic nasal mucosa physiological conditions. The nasal mucosa was equilibrated in the PBS solution for 1 h to achieve electrophysiological steady state.

The Nanogel Composite containing (5 mg/mL) drug concentration was transferred onto the mounted nasal tissue. The whole system was maintained at 34 ± 1 °C. The formulation solidified immediately after being transferred into the donor compartment containing the nasal tissue. ES of PD (5 V) was applied at time points of 0.5, 1, 2, 4, 6, 8, 16, 20 and 24 h, applied using a potentiostat/galvanostat (PGSTAT302N, Autolab, Utrecht, Netherlands). A permanent magnet of 0.4 Tesla was placed at 1 cm from the receiver compartment after each electrical application. Aliquot samples (1 ml) were withdrawn and replaced with the same quantity of fresh pre-warmed PBS. Sampling was carried out prior and after each ES. The amount of BCNU-NCP permeated was quantified by determining the amount of iron contents in the withdrawn solution as iron formed the core of the BCNU-NCP. All experiments were repeated 3 times. The whole procedure was repeated with ES but no application of the permanent magnet and without both electrical and magnetic stimulation. This was done to evaluate the effect of ES as well as MF on the permeation characteristics of the formulation.

Quantitative analysis of the BCNU-NCP permeated through the rabbit nasal epithelial tissue from the Nanogel Composite. BCNU-NCP permeated through the nasal epithelia tissue of rabbit was determined by following the procedure described by Akilo et al. 2019.

Evaluation of BCNU-NCP permeability. The BCNU-NCP permeability was calculated by ascertaining the cumulative diffusion of BCNU-NCP per unit area of the nasal tissue. The steady state flux (Js) was calculated from the slope of the linear section of the line obtained after plotting the amount of drug permeating nasal epithelium per unit area (mg/cm^2) against time (h) as shown in Equation 1 and the permeation coefficient P calculated using Equation 2.

$$Js = \frac{Qs}{AT} \qquad \text{Equation 1}$$

$$P = \frac{Js}{Cd} \qquad \text{Equation 2}$$

Where Js is the flux (mg/cm^2/h), Qs is the amount of drug (mg), A is the area (cm^2), T is the time interval (h), P permeability coefficient (cm/h) and Cd is the initial concentration (mg/mL) of the sample in the donor compartment.

Enhancement ratios (ER) of BCNU-NCP permeation through rabbit nasal mucosa were calculated based on *P* values, according to Equation 3.

$$ER = \frac{P(\text{Nanogel Composite with ES and MF})}{P(\text{Nanogel Composite without ES and MF})} \qquad \text{Equation 3}$$

Where P(Nanogel Composite with ES and MF) is the permeability coefficient of BCNU-NCP when there is application of electric and magnetic fields, and P(Nanogel Composite without ES and MF) is the permeability coefficient when there is no application of electric and magnetic fields.

Evaluation of the effect of magnetic field on cytotoxicity of the BCNU-NCP on human glioblastoma A170 cells

The effect of MF on cytotoxicity of BCNU-NCP was evaluated by employing the human glioblastoma cell line (A170) and following the method used by Akilo et al.[12] with slight modification. Briefly, two sets of seven plates (A-G) and (H-N) were employed in this experiment, each plate contained similar contents and represented incubation times of 0, 4, 8, 16, 24, 48 and 72 h, respectively for the two sets. The well in each first set of plates was divided into 4 groups (1–4) which were Control, NCP, BCNU-NCP and BCNU group respectively. The second set of seven plates contains only 1 group (group 5), which is group BCNU-NCP (with the application of MF). Predetermined quantities of BCNU-NCP and conventional BCNU were added to

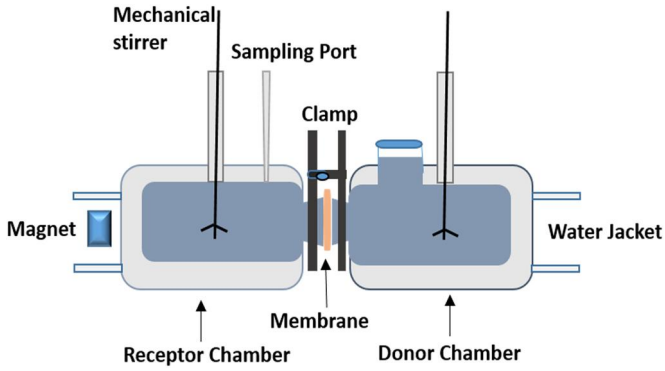

Figure 1. Schematic diagram of side-by-side diffusion cell.

each well as depicted in Table 1. The plates were incubated for the time of incubation they represented. The second set of plates (H-N) was fixed with 0.1 Tesla neo disc magnets (Magnetech, Capetown, South Africa) positioned under the well plates. After incubation, each plate was removed according to the respective time of incubation for assay. The contents distributions in each plate are displayed in Table 1. The cell viability was measured as described by Akilo et al. 2016.

In vivo *studies*

In vivo studies to evaluate the release of BCNU-NCP from the Nanogel Composite on application of ES and Magnetic MF and further evaluation of the BCNU release kinetics from the Nano-co-Plex (NCP) were carried out. The Nanogel Composite delivery system was compared to the conventional drug currently in the market to evaluate the superiority of the new systems in terms of their drug release kinetics.

Sterilization of the Nanogel Composite and the preparation of the conventional BCNU

The Nanogel Composite was sterilized by exposing the formulation to UV light overnight in an aseptic and sterile environment. To compare Nanogel Composite to the conventional drug currently in the market so as to evaluate the superiority of the Nanogel Composite in terms of their drug release kinetics, the BCNU intravenous injection (IV) from the market was prepared by aseptically weighing accurately 5mg of BCNU and then dissolved it in 5mL of sterile 10% ethanol solution to make a concentration of 1mg/mL.

In vivo experimental design and procedure for the studies

Habituation of the experimental animals (New Zealand White Rabbits). The rabbits were purchased and transported from certified suppliers. Appropriate pre-transportation handling, loading, journeying and unloading at the Central Animal services (CAS), University of the Witwatersrand, were accomplished according to CAS standard operating procedures (SOPs). On arrival at CAS the rabbits were habituated, weighed and assessed for any signs of disease, injury or distress and then socialized accordingly before the experimental procedures were conducted (Zhang et al. 2015). Animals were housed at room temperature with a 12-h light/dark cycle (light from 6am to 6 pm) and were fed a standard rabbit diet with water available *ad libitum*.

Experimental design. A total of 100 New Zealand White Rabbits were approved for these studies exclusive of the pilot studies. The rabbits were randomly assigned to 4 groups of 25 rabbits per group, the Placebo, group 1 (drug free Nanogel Composite), the positive control, group 2 (Nanogel Composite without application of 5 V ES and 0.4 T MF), the test, group 3 (Nanogel Composite with application of 5 V ES and 0.4 T MF) and the negative control, group 2 (BCNU IV injection). Each group is subdivided into 5 groups per 2 time points. The experimental design and the assignment of the rabbits into the groups including the outline summary of the *in vivo* studies are illustrated in Figure 2.

Dosing and application of electrodes and magnetic headbands. Rabbits were sedated before dosing by injecting ketamine (50 mg/kg) and xylazine (5 mg/kg), into the marginal vein of the rabbit's ear. Nanogel Composite (200 μL, 0.5 mg/kg) was intranasally administered into the nasal cavity of the rabbit using a 1 ml syringe with rubber needle attached to it to instil 100 μL into each nostril. ES of 5 V potential difference was applied for 1 min using electrodes from a potentiostat/galvanostat (PGSTAT302N, Autolab, Utrecht, Netherlands) on the nose of the rabbits after dosing.

Table 1. Distribution of DPBS, NCP, BCNU-NCP and BCNU contents in individual plate.

Group	Number of well used	Contents added to each well	BCNU quantity in each well (μg)
Control	15	20 μL of DPBS	0
NCP	15	20 μL of DPBS and 0.5 mg of NCP (drug free Nano-co-Plex)	0
BCNU	15	20 μL of DPBS and 0.088 mg of BCNU	88
BCNU-NCP	15	20 μL of DPBS and 0.5 mg of BCNU-NCP-3	88
BCNU-NCP (with application of a Magnetic Field)	15	20 μL of DPBS and 0.5 mg of BCNU-NCP-3	88

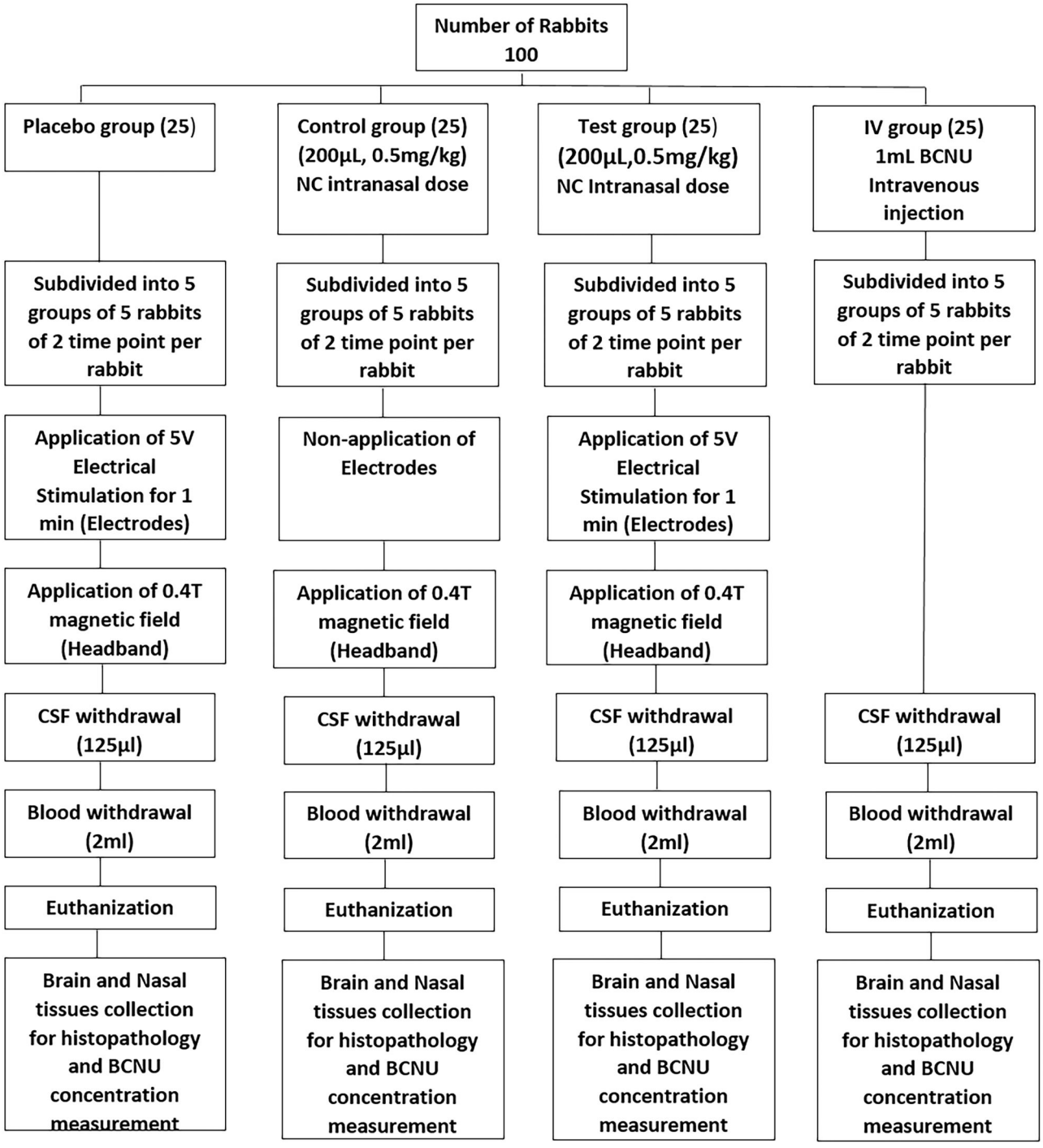

Figure 2. Schematic diagrams showing the outline summary of the *in vivo* animal studies.

A magnetic headband equipped with 0.4 Tesla magnetic strength (Magnetech, Capetown, South Africa) was placed on the rabbit's head. Fluid Sampling was carried out prior and after ES at predetermined time. Blood samples (2 ml) were collected through the marginal vein of the left ear, followed by CSF (125 μL) withdrawal by cisternal puncture through the foramen magnum. Sampling time points were 0, 0.5, 1, 2, 4, 6, 8, 16, 20 and 24 h. The rabbits were then humanely euthanized after the second sampling timeline and the brain removed and placed in 10% phosphate buffered formalin for drug concentration studies. For Placebo group 1, Nanogel Composite was free of BCNU-NCP. For the negative control, group 4, 1 ml of BCNU prepared was intravenously injected very slowly into the rabbits through the marginal ear (Liu et al. 1983; Joshi et al. 2008;).

Blood sample collection. The rabbits were anesthetized prior to blood sample withdrawal. Blood samples (2 ml) were withdrawn directly from the marginal ear vein of the rabbits. Each rabbit in this study were subjected to only 2 sampling timepoints. Sampling time points were 0, 0.5, 1, 2, 4, 6, 8, 16, 20 and 24 h. The withdrawn blood samples were immediately placed in heparinized tubes to prevent clotting. The blood samples were centrifuged at 5000 rpm and the supernatant was transferred into 2 ml Eppendorf tubes and stored in the −80 °C freezer for further analysis.

Cerebrospinal fluid sample collection. The rabbit was anaesthetized prior to the procedure. The back of the neck of the rabbit was shaved to have access to the occipital protuberance and the cranial margins areas of the rabbit. A 23-gauge needle was inserted approximately midway between these two points to have access to the cisterna magna through the foramen magnum. The needle was slowly inserted and directed towards the nose of the rabbit, the needle penetrated the dura and the subarachnoid membranes with a slight 'pop' felt confirming the puncturing of the cisterna magna (Jass et al. 2008). Appearance of CSF at the hub of the needle confirmed correct placement of the needle, the CSF drip from the needle and a 1 ml syringe was used to slowly aspirate 125 μL of CSF. The needle was gently removed, and a mild pressure was applied at the needle exit point for about 15 s. The CSF was quickly transferred into an Eppendorf tube and stored in −80 °C freezer. Sampling points were staggered, and five rabbits were used per subgroup due to the limitation of collecting no more than two samples per rabbit per day. The CSF sampling time point was therefore limited to 2 per rabbit and was well spread to give the rabbits enough recovery time.

Brain tissue collection. The rabbits were humanely euthanized, and the brains were removed. The extraction of BCNU from the brain tissue was performed by a modified method used by Ved and Kim 2011. Briefly, the brain tissue was homogenized employing a VDI-12 homogenizer at 20,000rpm for 15 min, 1 ml of 1.25%($^{v}/_{v}$) absolute ethanol in diethyl ether was added to 100 mg of the brain sample and further homogenized for 2 min. The resultant tissue homogenate was then centrifuged at 5000 rpm for 20 min. The supernatant was measured for BCNU contents, using the extraction methods.

Quantitative chromatographic determination of BCNU in plasma, CSF and the brain tissue of rabbit

Detection and quantification of BCNU in the plasma, CSF and brain samples was carried out on a Waters Acuity™ Ultra Performance Liquid Chromatography (UPLC) (Waters Corporation, Milford, Massachusetts, USA). This was made up of a binary solvent manager, a sample manager and fitted with a photodiode array detector (PAD) synchronously controlled using the Waters Empower 2 software. A SunFire™ C18 column (3.5 μm, 4.6X75mm) (Waters, Milford, MA, USA) auto sampler and 515 oval pumps was used to detect and separate BCNU.

Development of a method for sample analysis employing ultra performance liquid chromatography. UPLC was the technique utilized to determine the concentrations of the drugs in the biological fluid retrieved at different time points through the identification of peaks. A method for the detection and determination of BCNU was explored by the selection of appropriate mobile phases, flow rate, wavelength and injection volume. The mobile phase employed was a mixture of Acetonitrile (ACN) and water. An isocratic method with the ratio of 70:30 (ACN:Water) proved to be the most suitable. Galantamine was chosen as the internal standard (IS) for the quantification analysis. Mobile phases were prepared and filtered through 0.22 μm Millipore filters and placed in the machine with the appropriate solvent flow lines placed in the two mobile phases. The initial step to UPLC analysis is priming the machine which includes washing of the pumps and lines. Two types of washing occurred, a weak wash (90%$^{v}/_{v}$ double de-ionised water and 10%$^{v}/_{v}$ ACN) and a strong wash (90%$^{v}/_{v}$ ACN and 10%$^{v}/_{v}$ double de-ionized water). Once the priming process was completed, the column was equilibrated to the selected separation method.

Extraction of BCNU from rabbit plasma and CSF for UPLC analysis. An effective liquid-liquid extraction method was developed and applied to BCNU in the rabbit plasma (Boon et al. 2006). The frozen experimental plasma samples were thawed at room temperature and allowed to environmentally equilibrate. The samples were then centrifuged at 5000 rpm for 15 min. Centrifugation was repeated twice to release any drug present in the tissues. The supernatant was isolated and 0.2 ml of the supernatant was transferred into Eppendorff tubes. The IS in 10% ethanol Gal; (100 μL, 0.2 mg/mL) was added to each tube, and the tubes were vortexed for 30s. A liquid-liquid phase extraction methodology was employed in extracting the drugs from the studied rabbit plasma. To each of the tube, 0.3 ml of 1.25%($^{v}/_{v}$) absolute ethanol in diethyl ether was added. The tubes were vortexed for 30s and then centrifuged at 5000 rpm for 20 min. The supernatant (ethanol-diethyl ether layer) was then transferred to clean vials and was air dried at room temperature leaving dry residues containing the drugs in the vials. The residues were then reconstituted with 0.5 ml of the UPLC mobile phase (Acetonitrile/Water in ratio 70:30). The solution was filtered through a 0.22 μm Cameo Acetate membrane filter (Millipore Co., Bedford, MA, USA) into Waters® Certified UPLC vial for analysis. The same procedure was performed for the CSF samples and the supernatant samples collected from the brain. Measurements were conducted on all the samples from each time point ($N = 5$). The precision and accuracy of the extraction process was determined by repeating the extraction procedure for 3 consecutive days to allow for the determination of inter-day precision and accuracy ($N = 3$ for each day) and intra-day variability consisting of multiple injections of samples during a 24-h period ($N = 3$).

Determination of BCNU-NCP in the brain tissue employing field emission electron probe micro analyzer (FE EPMA). The presence of BCNU-NCP in the brain was confirmed by evaluating the presence of iron oxide nanoparticles in the brain through the detection of iron content in the brain sample employing Field Emission Electron Probe Micro Analyzer (FE EPMA). Samples of the brain obtained from the euthanized rabbit were homogenized for 15 min, the homogenate was vacuum dried at 40 °C. Sample of the dried brain was placed on aluminium stubs with the aid of a carbon tape. The sample was sputter-coated with gold-palladium and then loaded into the FE EPMA for analysis.

Histopathological evaluation of the nasal mucosa of the rabbits. Samples of the nasal tissues of the rabbits were randomly taken from each group for histopathological examination and analysis. The samples were stained in an automated Haemotoxylin and Eosin (H&E) stainer.

Results and discussion

Ex vivo *evaluation of mucoadhesive strength of the nanogel composite*

Mucoadhesion refers to the phenomenon by which a polymeric system adheres to a mucosal surface, such as the nasal mucosa, thereby prolonging its retention time and enhancing drug delivery efficiency. The strength and durability of this interaction are critical factors determining the performance of intranasal formulations intended for direct nose-to-brain delivery. Mucoadhesion typically involves complex interactions including ionic bonds, covalent bonds, hydrogen bonds, electrostatic forces such as van der Waals forces, hydrophobic interactions, and polymer chain interlocking (Zaman et al. 2015; Dubashynskaya et al. 2024). The intensity and duration of mucoadhesion are further influenced by factors such as the mucus viscosity, polymer chain entanglement within the formulation, and moisture content at the interface.

In this study, chitosan (CS) and hydroxypropyl methylcellulose (HPMC) were specifically selected to prepare the Nanogel Composite due to their well-established mucoadhesive properties (Akilo et al. 2019). The mucoadhesive strength was quantitatively evaluated by measuring the maximum detachment force (MDF) and work of adhesion from the force-distance profiles obtained using texture analysis. The Nanogel Composite exhibited a significant MDF of 0.53 ± 0.07 N and a work of adhesion of 0.27 ± 0.056 mJ. These values compare favourably and exceed typical thresholds published values for mucoadhesive nasal gel. For example, Suhagiya et al. (2023) report detachment force of 0.35 N for in-situ nasal gels. Thus, Nanogel Composite exhibits robust nasal retention, indicative of strong adhesive interactions. These adhesive properties can be primarily attributed to the capacity of the polymers to form extensive hydrogen bonds upon hydration by nasal fluid, subsequently inducing gel formation at the mucosal interface and promoting prolonged retention within the nasal cavity thereby contributing to the overall delivery efficacy of the system.

Assessment of BCNU-NCP permeation across rabbit nasal tissue

Efficient nasal drug delivery involves not only robust mucoadhesion but also effective permeation through the nasal mucosa. The permeation of BCNU-NCP from the Nanogel Composite was systematically evaluated using rabbit nasal epithelial tissue. Three experimental conditions were tested: application of combined ES and MF, ES alone, and no ES or MF (control). As depicted in Figure 3(a), the cumulative permeation profiles clearly illustrate that the application of combined ES and MF significantly enhanced the permeation of BCNU-NCP through the nasal mucosa compared to ES alone or control conditions. This synergistic effect can be explained by electro-actuated controlled release coupled with magnetically-driven nanoparticle transport.

Quantitative analysis of permeation performance included calculation of flux and permeability coefficients, as presented in Table 2. The flux determines the amount of molecules that permeated through nasal epithelial tissue and the permeability coefficient, is a measure of the distance travelled by the drug molecules per unit time (Adeleke et al. 2015). The highest flux and permeability coefficient values ($1.4177 \times 10^{-2} \pm 1.7245 \times 10^{-3}$ mg•cm^{-2}•s^{-1} and 5.6709×10^{-3} cm•s^{-1}, respectively) were observed under combined ES and MF application. Interestingly, the sample treated with ES alone showed moderate permeation, reflecting passive diffusion after electro-actuated release, whereas the control exhibited significantly lower permeation (flux: $2.7654 \times 10^{-3} \pm 7.0929 \times 10^{-4}$ mg•cm^{-2}•s^{-1}, permeability: 1.1062×10^{-3} cm•s^{-1}). The control permeation was attributed primarily to passive leaching of BCNU-NCP from the gel surface.

The enhancement ratios further highlighted the benefits of magnetic field application. Specifically, permeation was enhanced approximately 86-fold with combined ES and MF compared to control and approximately fivefold relative to ES alone (Table 2). These findings underline the significant potential of magnetic guidance in enhancing nasal epithelial transport and subsequent brain bioavailability.

Assessment of the magnetic field influence on the cytotoxicity of BCNU-NCP on human glioblastoma A170 cells

The cytotoxic effectiveness of BCNU-NCP under magnetic field guidance was investigated using human glioblastoma A170 cells. As shown in Figure 3(b), nanoparticle samples exposed to a magnetic field demonstrated significantly increased cytotoxicity compared to those without magnetic treatment. This heightened cytotoxic response can be attributed to improved nanoparticle internalization mediated by magnetophoretic attraction towards the cells, confirming the therapeutic advantage of magnetic field application in nanoparticle-based cancer treatments.

In vivo studies to assess the efficacy of the Nanogel Composite

Drug extraction, UPLC method validation, and assay procedure. Figure 4(a) and (b) shows high resolution chromatographic peaks of the analyte (BCNU) and the internal standard (Gal) as obtained by the UPLC-PDA. The analysis revealed two separated and defined peaks (wavelength 230 nm) at the respective retention times of 1.90 and 15.28 min for Gal and BCNU, respectively, as depicted in Figure 4(c).

Figure 4(d–f) show the chromatographic peaks of BCNU and Gal at 230 nm after CSF extraction, with their respective retention times of 1.20 and 16.49 min. These retention times are very similar to those obtained from the plasma analysis. Figure 4 further revealed that there was no change in the retention time when the analyte and the IS were spiked in plasma and CSF individually and when they were spiked in plasma and CSF together. This observation proved the UPLC method adopted to be a suitable and appropriate method for analysing the drug in the samples. The mean recovery percentage of BCNU from the plasma and CSF samples during the liquid-liquid phase extraction implemented was calculated by comparing the peak areas of both low and high extraction calibration standards to those of aqueous standards. Validation of the extraction method indicated that the mean coefficient of variation in the intra-day and inter-day precision and accuracy was determined to be 0.46% and 0.38%, respectively. The results of the extraction procedure of the spiked blank plasma and CSF samples revealed a mean recovery ($N = 5$) range of 91.2-95.7% (SD $\leq$ 3.48), these results were found to be satisfactory.

BCNU concentration in plasma, CSF, and brain tissue of the rabbit model. The concentration of BCNU in plasma, CSF and brain were computed using the calibration curve generated from the area under the curve of BCNU/Gal ($AUC_{BCNU/Gal}$).

Plasma concentration. Figure 5(a) presents the mean BCNU concentration-time profiles in plasma following intravenous (IV) injection and intranasal (IN)

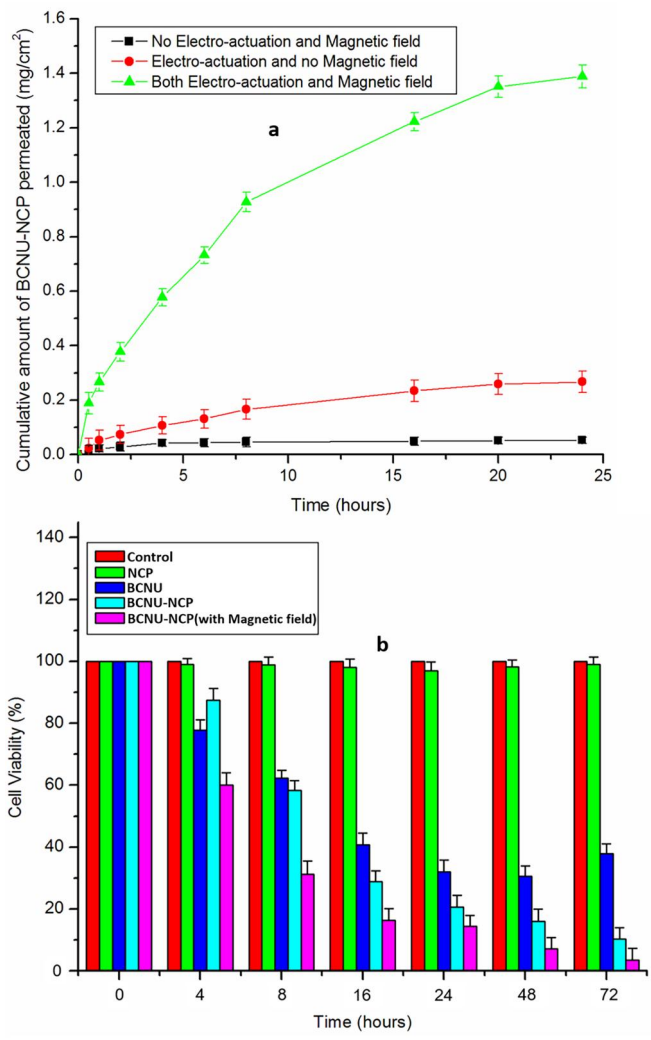

Figure 3. (a) Permeation curve showing the cumulative amount of BCNU-NCP permeated across the nasal epithelial tissue of rabbit at 34 ± 1 °C in a diffusion cell. Data are expressed as mean ± SD ($N = 3$) and (b) the effect on toxicity of human glioblastoma A172 cell line showing the control treatment, treated with NCP, BCNU, BCNU-NCP and BCNU-NCP (with magnetic field) incubated at 37 °C for 72 h.

Table 2. Flux, permeability coefficient and enhancement ratio for permeation of BCNU-NCP through the nasal tissue of New Zealand white rabbit.

Formulation	Flux, Js ($mg.cm^{-2}.h^{-1}$)	Permeability Coefficient, P	Enhancement ratio*	Enhancement ratio**
Nanogel Composite (No application of ES and MF)	$2.7654 \times 10^{-3} \pm 7.0929 \times 10^{-4}$	1.1062×10^{-3}	–	–
Nanogel Composite (with application of ES only)	$1.4177 \times 10^{-2} \pm 1.7245 \times 10^{-3}$	5.6709×10^{-3}	5.13	–
Nanogel Composite (with application of both ES and MF)	$7.3681 \times 10^{-2} \pm 1.6912 \times 10^{-3}$	2.9472×10^{-2}	85.63	5.20

*The enhancement ratio is based on the permeation of Nanogel Composite without the application of ES and MF.
**The enhancement ratio is based on the permeation of Nanogel Composite without application of MF.

administration of the Nanogel Composite, with and without the application of electrical stimulation (ES) and magnetic field (MF). The highest plasma concentration was observed after IV administration, with a C_{max} of 0.5843 μg/mL at 30 min. In comparison, IN administration with ES and MF resulted in a significantly lower C_{max} of 0.0789 μg/mL, and the lowest value (0.0199 μg/mL) was recorded for IN administration without stimulation.

Following IV administration, BCNU levels gradually declined and became undetectable by 6 h. For IN administration with ES, the plasma concentration displayed intermittent peaks, indicative of a pulsatile 'on-off' release mechanism driven by ES. During stimulation, BCNU-NCP was actively released from the Nanogel Composite, elevating plasma levels, which decreased during periods without stimulation. This confirms that ES regulates drug release in a controlled,

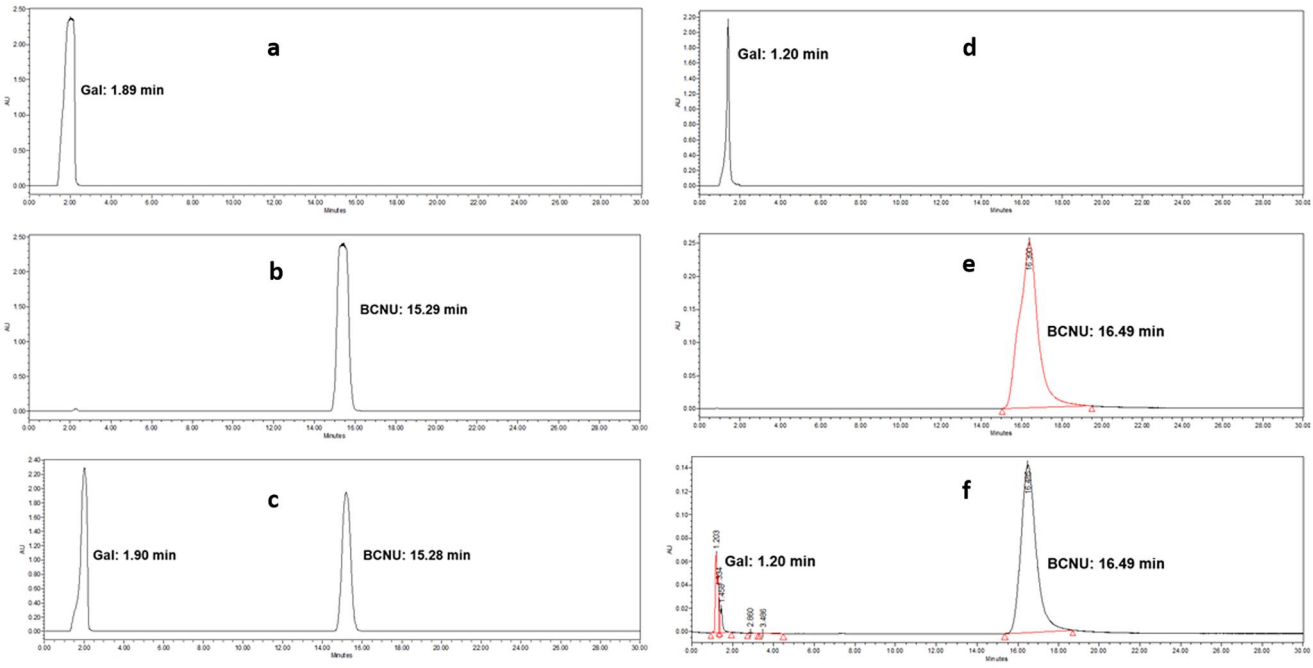

Figure 4. Typical chromatogram displaying (a) the spiking plasma separation of the gal, (b) BCNU and (c) the separation of both gal and BCNU after plasma extraction. Typical chromatogram depicting (d) the spiking CSF separation of the gal, (e) BCNU and (f) the separation of both gal and BCNU after CSF extraction.

responsive manner. In contrast, IN administration without ES showed a steady, minimal release profile, consistent with the absence of an external trigger. Overall, plasma BCNU concentrations following IN administration were more than 10-fold lower than IV, highlighting the reduced systemic exposure associated with the intranasal route.

CSF concentration. Figure 5(b) shows the BCNU concentration-time profiles in CSF for all three administration methods. IN administration with both ES and MF achieved the highest BCNU concentration in CSF, characterized by a stable profile and pulsatile release behavior. The average concentration of BCNU released per electro-actuation was 0.2819 μg/mL. This enhancement is attributed to the action of ES in triggering drug release and MF in facilitating nanoparticle transport through the olfactory route.

For IV administration, the BCNU concentration in CSF was significantly lower. This is likely due to the blood-CSF barrier (BCB), which limits drug permeation from the systemic circulation into the CSF, reducing central bioavailability. Although some BCNU still crossed into the CSF, the levels remained much lower compared to the intranasal approach with stimulation. IN administration without ES and MF resulted in negligible BCNU levels in the CSF, indicating that passive diffusion or spontaneous release of BCNU-NCP was insufficient without external stimulation. Figure 5(c) illustrates the percentage of BCNU released into the CSF over a 24-h period following IN administration with ES and MF.

Brain concentration. Figure 5(d) displays the BCNU concentration-time profile in brain tissue. The highest brain concentrations were achieved with IN administration of the Nanogel Composite combined with ES and MF. The average BCNU concentration per electro-actuation was 0.1968 μg/g and drug levels remained approximately constant throughout the 24-h observation period. This sustained profile indicates continuous delivery and improved brain bioavailability, likely mediated by ES-triggered release and MF-enhanced navigation of BCNU-NCP to the brain *via* the olfactory route.

In contrast, IV administration showed a rapid spike in brain concentration, with a C_{max} of 0.00776 μg/mL reached in less than 2 min, followed by a steady decline to undetectable levels. IN administration without stimulation produced the lowest and clinically insignificant brain concentrations, consistent with limited release of BCNU-NCP in the absence of ES.

Together, these findings underscore the advantage of using the electro- and magneto-responsive Nanogel Composite for nose-to-brain delivery, achieving sustained therapeutic concentrations in both the CSF and brain while minimizing systemic exposure.

BCNU is a chemotherapeutic agent that inhibits cancer cell proliferation (Urbschat et al. 2017).

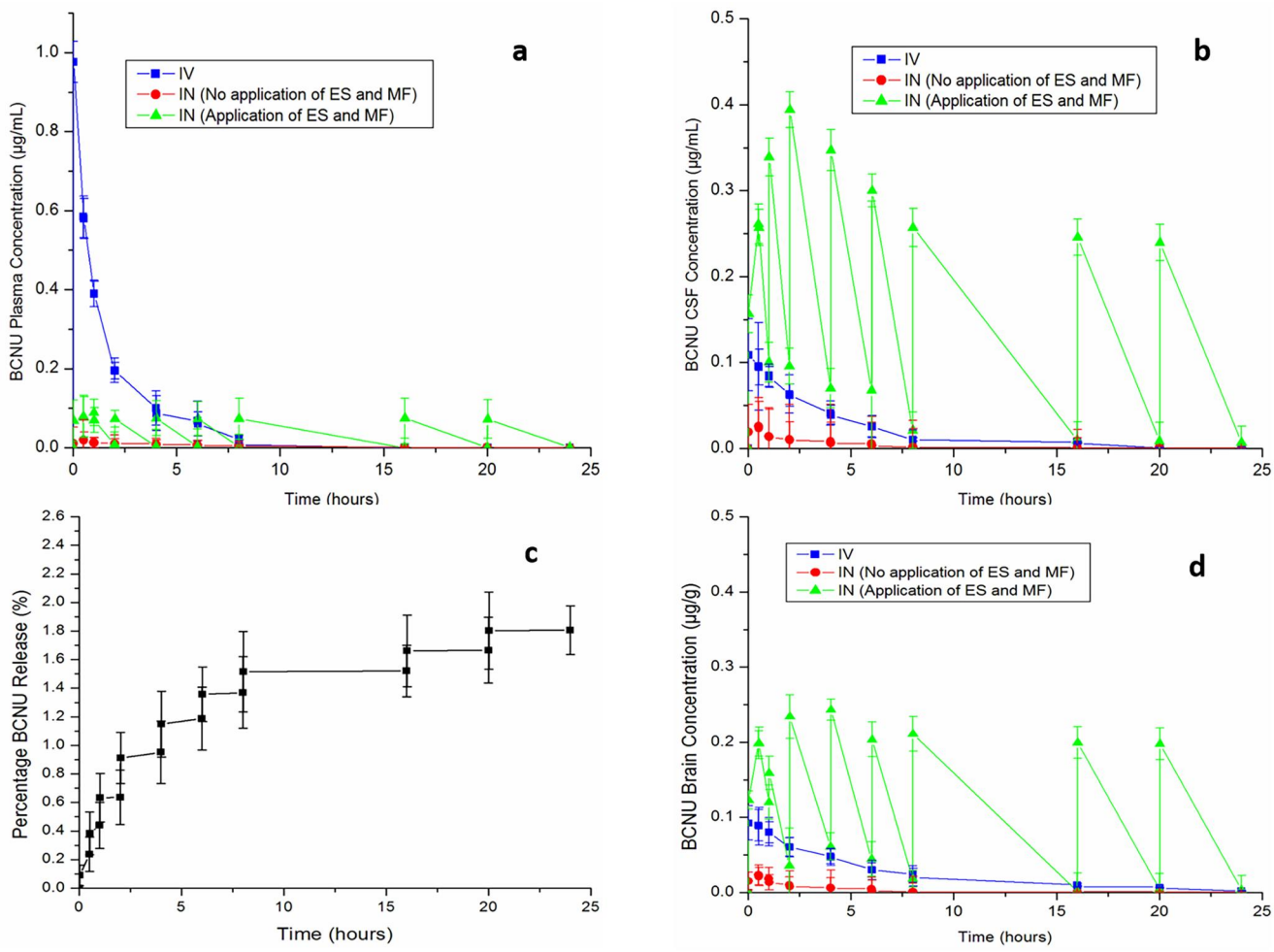

Figure 5. (a) Mean BCNU concentration-time profile in plasma after IV injection of the conventional BCNU, IN administration of Nanogel Composite without application of ES/MF and IN administration of Nanogel Composite with the application of ES/MF (SD≤ 0.053; $N = 5$ at each time point). (b) Mean BCNU concentration–time profile in CSF after IV injection of conventional BCNU, IN administration of Nanogel Composite without application of ES and IN administration of Nanogel Composite with the application of ES and MF at 1 mg/kg dose in rabbits. (SD≤ 0.051; $N = 5$ at each time point). (c) Percentage of BCNU released in CSF following IN administration. (SD≤ 0.051; $N = 5$ at each time point) and (d) Mean BCNU concentration–time profile in brain after IV injection of the conventional BCNU, IN administration of Nanogel Composite without application of ES and IN administration Nanogel Composite with the application of ES and MF at 1 mg/kg dose in rabbits. (SD≤ 0.05; $N = 5$ at each time point).

However, intravenous administration is associated with several systemic side effects, including nausea, vomiting, diarrhoea, nephrotoxicity, and bone marrow suppression. This study aimed to demonstrate the superiority of the Nanogel Composite over conventional BCNU by improving brain and CNS delivery through non-invasive, controlled IN administration with ES and MF.

To confirm this, BCNU levels in plasma, CSF, and brain were compared across three delivery routes: IV, IN with ES/MF, and IN without ES/MF. As shown in Figure 6(a), IV administration resulted in high plasma concentrations but low CSF and brain levels—approximately nine-fold lower due to the blood-brain barrier. In contrast, Figure 6(b) shows that IN administration with ES and MF produced significantly higher BCNU levels in the CSF (0.2571 µg/mL at 30 min; 0.2395 µg/mL at 24 h) and brain (0.199 µg/g at 30 min; 0.1979 µg/g at 24 h), while maintaining low plasma exposure (0.0078 µg/mL and 0.0072 µg/mL, respectively).

IN administration without ES and MF (Figure 6(c)) resulted in substantially lower BCNU levels: 0.0078 µg/mL (plasma), 0.0087 µg/mL (CSF), and 0.0076 µg/g (brain). These minimal values likely reflect passive leaching of surface-associated BCNU-NCP rather than active release, emphasizing the importance of ES-triggered electro-actuation.

The ability of this delivery system to maintain stable BCNU concentrations in the CSF and brain for 24 h is a major advantage over conventional IV delivery.

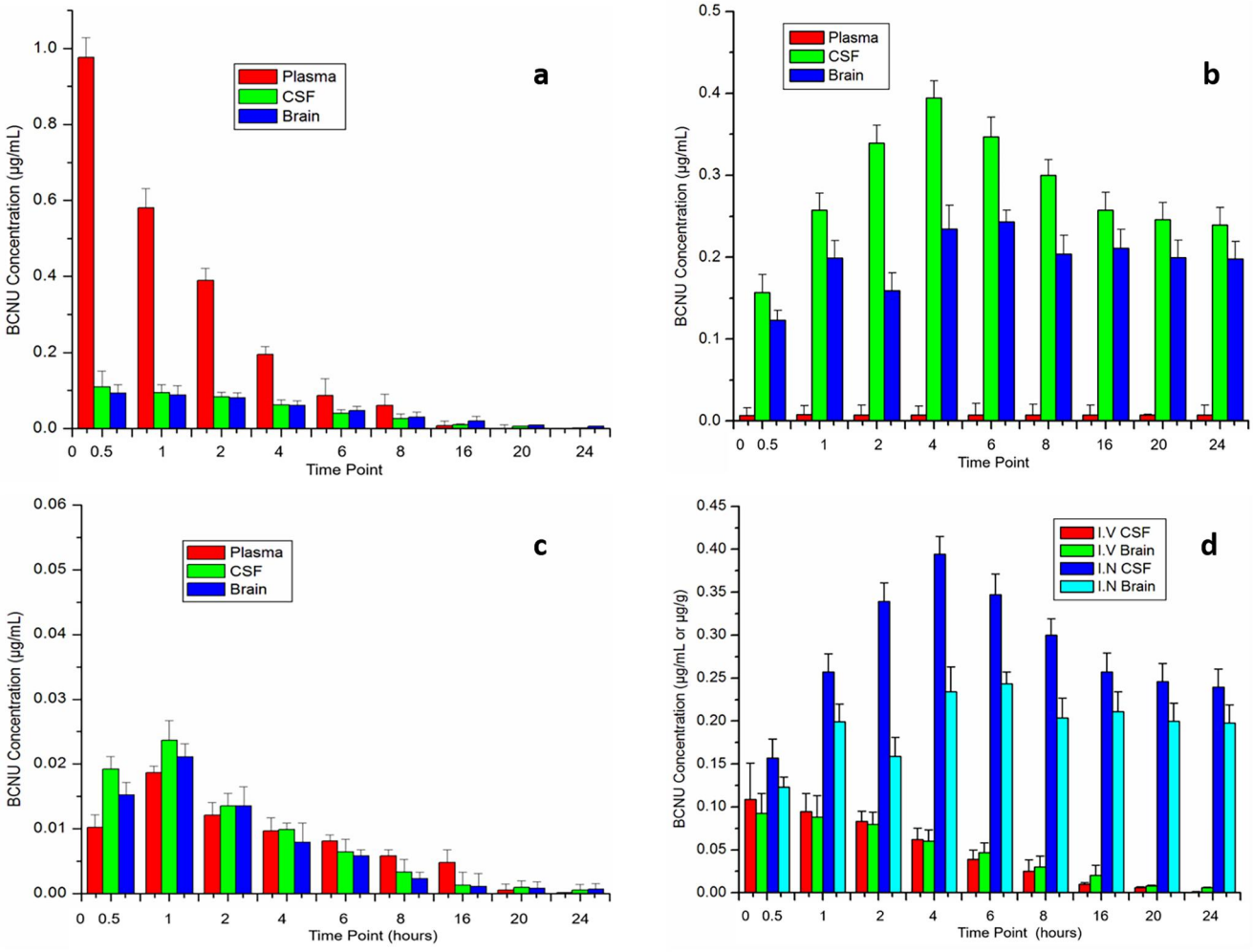

Figure 6. (a) BCNU concentration in the plasma, CSF and brain tissue after IV injection of conventional BCNU. (b) BCNU concentration in the plasma, CSF and brain tissue after IN administration of Nanogel Composite with application of ES and MF. (c) BCNU concentration in the plasma, CSF and brain tissue after IN administration of Nanogel Composite without application of ES and MF, and (d) comparison of the concentration of BCNU in CSF and brain following IV of conventional BCNU and IN administration of Nanogel Composite with application of ES and MF.

Figure 6(d) highlights this benefit, showing sustained CSF and brain concentrations with IN administration of the Nanogel Composite under ES and MF, in contrast to the declining profile seen with conventional IV administration. Notably, the average BCNU concentration in CSF was approximately six times higher than that achieved with IV dosing.

Assessment of BCNU-NCP in the brain tissue employing field emission electron Probe micro analyzer (FE EPMA). Qualitative analysis using Field Emission Electron Probe Micro Analyzer (FE-EPMA) provided clear spectral confirmation of iron, the core material of BCNU-NCP, within rabbit brain tissue post-IN administration (Figure 7). The presence of distinct iron peaks at 0.6 and 6.4 keV conclusively indicated successful nanoparticle transport to the brain *via* the olfactory pathway.

Histopathological examination of nasal epithelia of the rabbit. Histological evaluations (Figure 8) confirmed the biocompatibility of the Nanogel Composite and the safety of ES and MF application. The placebo group exhibited normal epithelial structure without lesions. Control and experimental groups showed **moderate inflammation**, characterized by focal epithelial disruption and mild immune cell infiltration, without significant necrosis or irreversible tissue damage. This confirms that the Nanogel Composite, when administered intranasally with ES and MF application, does not exert significant adverse histopathological effects on nasal tissues.

Mechanism of ES-triggered 'on-off' drug release from the Nanogel Composite. Upon application of electrical stimulation (ES), the Nanogel Composite composed of an electro-responsive polymeric matrix undergoes

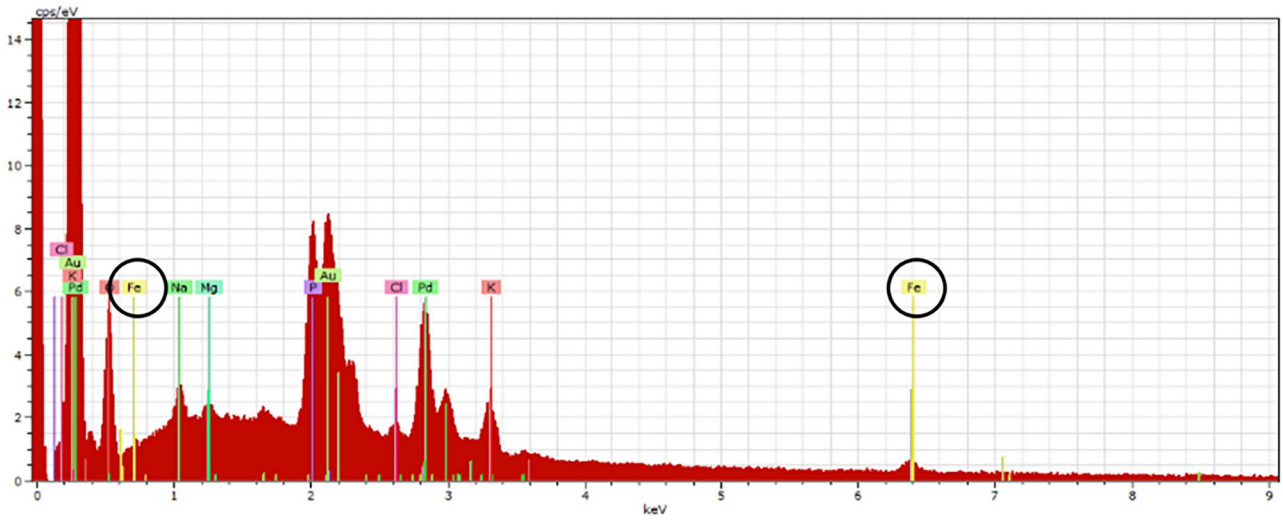

Figure 7. FE EPMA spectra of brain tissue of rabbit after IN administration of Nanogel Composite for Fe detection.

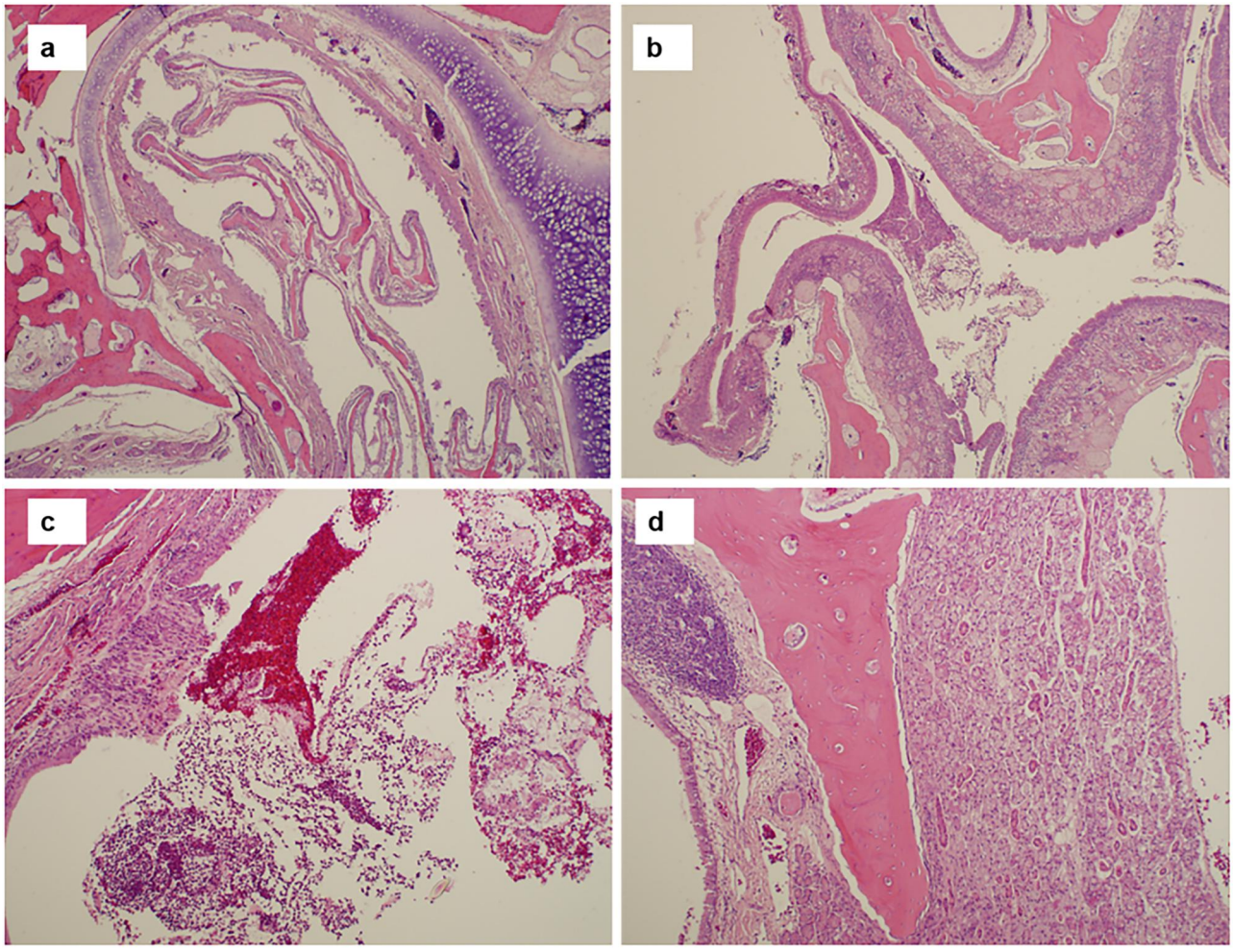

Figure 8. Histological evaluation of nasal epithelia tissue samples from rabbits showing light digital microscopic images of the H&E stained slides of (a) normal nasal epithelia tissue, (b) placebo sample, (c) control group sample and (d) experimental group sample. Magnification is x10.

structural and physicochemical changes that mediate a controlled 'on–off' release of the encapsulated therapeutic, in this case, BCNU-NCP. This phenomenon, broadly described as electro-actuation, is driven by electric field-induced ion migration, electro-osmotic swelling, and reversible network deformation within the nanogel matrix. Specifically, when a 5 V direct current is applied across the nasal mucosa, the electric field interacts with the charged functional groups present in the Nanogel Composite chains ($–SO_3^-$, $–COO^-$, or $–NH_3^+$). This leads to the electrophoretic migration of mobile counterions within the gel, disrupting the internal ionic balance and generating localized osmotic pressure gradients. As a result, water influx occurs within the polymer network, causing swelling of the gel matrix and loosening of the polymer chains. This conformational relaxation enhances the diffusivity of the encapsulated nanoparticles, effectively triggering the 'on' phase of BCNU-NCP release. Conversely, upon cessation of the electric field (i.e. the 'off' phase), the driving force for ion migration is removed, and the nanogel matrix begins to re-establish its internal equilibrium. Electrostatic interactions between the polymer chains are gradually restored, and water is expelled from the Nanogel Composite, resulting in re-shrinking or contraction of the network. This leads to a significant reduction in BCNU-NCP diffusivity, thereby halting further release of BCNU-NCP. The system thus operates as a switchable delivery platform, in which pulsatile or intermittent drug release can be precisely modulated by external ES input. Importantly, this mechanism is highly reversible and repeatable, allowing multiple cycles of 'on–off' release in response to sequential stimulation, without compromising the structural integrity of the Nanogel Composite. The ability to control BCNU-NCP release in real time enhances both targeting precision and dosing efficiency-attributes particularly valuable in central nervous system (CNS) delivery strategies where tight control over drug exposure is critical.

Conclusions

This study demonstrates the potential of a novel electro-responsive, magnetically guided Nanogel Composite system for efficient nose-to-brain delivery of carmustine (BCNU). The Nanogel Composite, exhibited strong mucoadhesive and stimuli-responsive characteristics, enabling on-demand, pulsatile drug release under the influence of electrical stimulation (ES). When combined with an external magnetic field (MF), the system significantly enhanced BCNU transport to the cerebrospinal fluid (CSF) and brain *via* the olfactory pathway, while minimizing systemic exposure.

In vivo pharmacokinetic data clearly showed that intranasal administration of the Nanogel Composite with ES and MF resulted in substantially higher concentrations of BCNU in the brain and CSF compared to both conventional IV administration and IN delivery without stimulation. The electro-actuation enabled a controlled, sustained release pattern, while the MF guided the nanoparticles effectively to the target site. Additionally, the applied electrical (5 V) and magnetic (0.4 Tesla) fields were well-tolerated, confirming the safety and comfort of the system. The microscopic examination of the nasal epithelial tissue of the rabbits revealed moderate lesions on the tissue. These lesions were localized, non-severe, and reversible, supporting the overall safety and translational potential of the Nanogel Composite system with appropriate optimization.

These findings underscore the therapeutic promise of the Nanogel Composite platform for non-invasive, targeted brain delivery, offering a viable strategy to circumvent the blood-brain and blood-CSF barriers. This approach holds potential not only for BCNU but also for a broad range of central nervous system (CNS) therapeutics where precise, localized delivery is critical.

The success of this study lays the groundwork for translating the Nanogel Composite system into clinical applications. Future efforts would focus on validating the safety and efficacy of the system in higher animal models and eventual human trials. Miniaturization of the stimulation device into a user-friendly wearable form will be essential for patient compliance.

Expanding the platform to deliver other CNS-targeted agents, including biologics, would significantly broaden its therapeutic scope. Further mechanistic and biodistribution studies, along with the integration of feedback-controlled release systems, may enhance targeting precision and therapeutic outcomes. Collectively, these advancements will help position the Nanogel Composite as a promising tool for non-invasive, targeted nose-to-brain drug delivery.

Animal ethics clearance

Animal ethics clearance for these *in vivo* studies was obtained from the Animal Ethics Screening Committee (AESC) of the University of the Witwatersrand with Clearance Number 2015/05/C.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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