Current Clinical and Medical Education

Received 19 Apl 2026 | Accepted 20 May 2026 | Published Online 22 Jun 2026

vision publisher
Medical Journals

Published By: Vision Publisher

ISSN 2942-0792

CCME 4 (6), 28-37

Development and Characterization of Carboxymethyl Assam Bora Rice Starch-Coated Superparamagnetic Iron Oxide Nanoparticles (CM-ABRS SPIONs) for Magnetically Guided Targeted Delivery of Doxorubicin in Cancer Therapy

Ayush Dayma[1]*, Dr. Vaibhav Verma[2]

[1] M.Pharm Scholar (Enrolment No: PU05224EPG13002), Gyanodaya Institute of Pharmacy, Gyanodaya University, Neemuch, Madhya Pradesh, India

[2] Professor & Supervisor, Gyanodaya Institute of Pharmacy, Gyanodaya University, Village: Suwakheda, Tehsil: Jawad, District: Neemuch, Madhya Pradesh, India

Abstract:
The present study reports the synthesis, physicochemical characterization, and biological evaluation of doxorubicin (DOX)-loaded carboxymethyl Assam Bora rice starch-coated superparamagnetic iron oxide nanoparticles (DOX–CM-ABRS SPIONs) as a novel magnetically guided targeted drug delivery system for cancer therapy. Native Assam Bora rice starch (ABRS) was chemically modified by carboxymethylation using monochloroacetic acid under alkaline conditions in isopropyl alcohol, yielding CM-ABRS with an optimized degree of substitution (DS) of 1.23. The modified polymer was comprehensively characterized by FT-IR, $^{1}H/^{13}C$ NMR, CHN analysis, XRD, DSC, SEM, rheology, swelling index, and mucoadhesive studies, confirming successful introduction of carboxymethyl groups and significant improvement in physicochemical properties — including a ~60-fold enhancement in aqueous solubility compared to native starch. CM-ABRS was subsequently employed as a hydrophilic coating agent for the preparation of SPIONs by co-precipitation. DOX was loaded onto CM-ABRS SPIONs via electrostatic interaction between the positively charged amine group of DOX and the negatively charged carboxylate moieties of CM-ABRS, achieving a drug loading of ~6% (w/w) and entrapment efficiency of 90.24 ± 0.8%. The optimized formulation (dc-7) exhibited a mean particle size of ~205 nm (PDI 0.38) with zeta potential of −26.0 mV, spherical morphology by TEM, and confirmed superparamagnetic behavior (saturation magnetization ~33.5 emu/g) by VSM. In vitro drug release in phosphate-buffered saline (pH 7.4) demonstrated a controlled sustained-release profile (~68.2% in 24 h) best fitted to the Higuchi model (R^2 = 0.937). In vitro magnetic targeting studies in a glass microcapillary confirmed the proof-of-concept for magnetically guided delivery. Cytotoxicity evaluation against MCF-7 breast cancer cells revealed a markedly lower IC_{50} (~5.95 µg/mL) for DOX–CM-ABRS SPIONs compared to conventional DOX solution (~12.16 µg/mL) ($p < 0.05$). Molecular docking against the HER-2 receptor (PDB: 5JEB) demonstrated a more favorable docking score (−13.396) for the DOX–SPION formulation versus free DOX (−12.537). These findings establish CM-ABRS SPIONs as a promising, green, biodegradable, and cost-effective nanoplatform for targeted anticancer drug delivery.

Keywords: Doxorubicin; Carboxymethyl starch; Superparamagnetic iron oxide nanoparticles; Magnetic drug targeting; Targeted drug delivery; Cancer nanotechnology; EPR effect; Cytotoxicity; Breast cancer

Corresponding Author: Ayush Dayma†, M.Pharm Scholar (Enrolment No: PU05224EPG13002), Gyanodaya Institute of Pharmacy, Gyanodaya University, Neemuch, Madhya Pradesh, India

1. INTRODUCTION

Cancer remains one of the most formidable challenges in global health, ranking as the second leading cause of mortality worldwide and projected to surpass cardiovascular disease as the primary cause of death in the coming years (Siegel et al., 2015). Despite advances in surgery, radiotherapy, and conventional chemotherapy, these treatment modalities suffer from significant limitations, including poor tumor selectivity, systemic toxicity, inadequate therapeutic indices, and the development of multidrug resistance. The inability to confine drug action to the tumor microenvironment leads to severe damage to healthy tissues and substantially compromises patient quality of life (Byrne et al., 2008; Shapiro and Recht, 2001).

The emergence of nanotechnology has opened transformative avenues for cancer diagnosis and therapy. Nanocarrier-based drug delivery systems offer several advantages over conventional formulations, including improved pharmacokinetic profiles, enhanced tumor accumulation via the enhanced permeability and retention (EPR) effect, reduced systemic toxicity, controlled drug release, and the possibility of incorporating active targeting moieties for receptor-specific delivery (Misra et al., 2010; Ferrari, 2005; Peer et al., 2007). Among the various nanocarrier platforms explored, superparamagnetic iron oxide nanoparticles (SPIONs) have garnered exceptional interest due to their unique combination of magnetic responsiveness, biocompatibility, biodegradability, and ease of surface functionalization (Gupta and Gupta, 2005; Mahmoudi et al., 2011; Sun et al., 2008).

SPIONs composed of magnetite (Fe_3O_4) or maghemite (γ-Fe_2O_3) possess superparamagnetic properties that enable their external magnetic field-guided accumulation at target sites, forming the basis of Magnetic Drug Targeting (MDT). This active physical targeting strategy — directing drug-loaded SPIONs to tumor sites using an external magnet — can be synergistically combined with passive EPR-mediated targeting, thereby amplifying therapeutic efficacy while minimizing off-target exposure (Anwar et al., 2014; Laurent et al., 2008).

The choice of coating polymer for SPIONs is pivotal in determining colloidal stability, biocompatibility, drug loading capacity, circulation time, and overall therapeutic performance. Natural polysaccharides, in particular, have attracted growing attention as SPION stabilizers owing to their intrinsic biocompatibility, biodegradability, low immunogenicity, and ability to impart hydrophilic stealth properties that prolong circulation (Lemarchand et al., 2004). Starch is one such polysaccharide — widely available, inexpensive, and renewable — and has been previously explored as an SPION coating material (Kim et al., 2003a; Saboktakin et al., 2009).

Assam Bora rice starch (ABRS), derived from Oryza sativa (family Gramineae) and widely distributed in upper Assam, India, has been studied for pharmaceutical applications including matrix tablets, microspheres, and mucoadhesive systems (Zaki Ahmad et al., 2012; Sharma et al., 2013b). However, native ABRS suffers from poor aqueous solubility, high viscosity upon gelatinization, and uncontrolled drug release characteristics, limiting its application in nanoparticle-based drug delivery systems. Chemical modification through carboxymethylation — introducing negatively charged carboxymethyl ($–CH_2COO^-$) groups — substantially enhances solubility, electrostatic drug-loading capacity, and colloidal stability without requiring organic solvents (Mallick et al., 2016; Bhattacharyya et al., 1995).

Doxorubicin hydrochloride (DOX), an anthracycline antibiotic and broad-spectrum chemotherapeutic agent widely used against leukemias, breast, and other solid tumors, was selected as the model drug. Despite its clinical efficacy, conventional DOX formulations are associated with dose-dependent cardiotoxicity and systemic adverse effects (Brunton, 2011). While its PEGylated liposomal formulation (Doxil®) has partially mitigated these issues, adverse reactions such as hand-foot syndrome and cardiotoxicity persist (Barenholz, 2012). Incorporating DOX into a magnetically guided SPION system thus offers the dual advantage of reducing cardiotoxicity while enhancing tumor-specific delivery.

To the best of our knowledge, this is the first report on the synthesis and comprehensive characterization of carboxymethyl Assam Bora rice starch (CM-ABRS) as a green polymeric coating for SPIONs, and the subsequent evaluation of DOX-loaded CM-ABRS SPIONs as a targeted magnetically responsive anticancer drug delivery system. The present study encompasses polymer extraction, modification, and characterization; nanoparticle synthesis and

physicochemical evaluation; in vitro drug release kinetics and magnetic localization; in vitro cytotoxicity; and molecular docking studies, providing a thorough proof-of-concept for this novel nanoplatform.

2. MATERIALS AND METHODS

2.1 Materials

Assam Bora rice (ABR) was collected from local vendors in Neemuch district, Madhya Pradesh, India. Monochloroacetic acid was obtained from Fluka (USA). Glacial acetic acid and sodium hydroxide (NaOH) pellets were procured from S.D. Fine Chem. Ltd., Mumbai, India. Hydrochloric acid (HCl) and methanol (95%) were purchased from Merck, Germany. Ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) and ferrous chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$) were obtained from Sigma-Aldrich, USA. Porcine stomach mucin (Type III) was supplied by Sigma-Aldrich. Potassium dihydrogen phosphate and sodium chloride were from Qualigens Fine Chemicals, Mumbai, India. Doxorubicin hydrochloride was obtained as a research-grade material. All other chemicals and reagents were of analytical grade. Double distilled water (DDW) prepared in the laboratory was used throughout.

2.2 Extraction of Assam Bora Rice Starch (ABRS)

ABRS was isolated from Assam Bora rice grains (Oryza sativa, Gramineae) following a modified alkaline steeping procedure. Cleaned, dried rice grains were soaked in 0.01 M NaOH (1:2 w/v) with stirring for 24 hours, with the soaking medium replaced every 2–3 hours. Softened grains were ground with DDW (1:2 w/v) to form a milky suspension, diluted to ~2–3% w/v, and filtered through muslin cloth. The settled starch was washed repeatedly with 0.01 M NaOH followed by DDW until the supernatant was clear, centrifuged at 5000 rpm for 15 minutes, and the sediment was freeze-dried. The dried ABRS was sieved through #125 and #200 mesh sieves and stored in airtight containers.

2.3 Synthesis of Carboxymethyl Assam Bora Rice Starch (CM-ABRS)

Carboxymethylation was performed by the Williamson etherification method. ABRS was dispersed in isopropyl alcohol (IPA), monochloroacetic acid was added, and 8 N NaOH was introduced dropwise over one hour with continuous stirring at 65°C for 2.5 hours. The reaction was terminated by neutralization with 50% glacial acetic acid. The product was washed with 85% ethanol to neutral pH, rinsed with absolute ethanol (99.9%), dried under vacuum at 50°C, and stored in airtight containers. The degree of substitution (DS) was determined titrimetrically using back-titration with 0.05 M HCl after conversion of CM-ABRS (Na) to the acid form CM-ABRS (H), following the standard procedure of Stojanović et al. (2005). Optimized conditions yielded a DS of 1.23.

2.4 Physicochemical Characterization of CM-ABRS

CM-ABRS was characterized by FT-IR spectroscopy (Shimadzu IR Affinity-1; 4000–400 cm^{-1}, KBr pellet method), ^1H and ^{13}C NMR spectroscopy (Bruker Avance 400 MHz; DMSO-d_6/D_2O), elemental CHN analysis (Vario EL III), DSC (PerkinElmer DSC-6; 40–200°C, 10°C/min), XRD (Rigaku Ultima IV; CuKα radiation; 10–80° 2θ), and SEM (EVO® LS10, Carl Zeiss; gold-sputter-coated). Powder flow characteristics (angle of repose, bulk density, tapped density, Carr's index, Hausner's ratio), swelling index, apparent solubility at various temperatures (30, 55, 65, 75°C), rheological behavior (Physica MCR 101 Anton Paar rheometer; 3% w/v gels; 0–1000 s^{-1} shear rate range), textural analysis (TA.XT Plus; cylindrical probe; 7 mm penetration depth), and mucoadhesive properties (TA.XT Plus; mucin gel detachment force) were also evaluated and compared with native ABRS.

2.5 Synthesis of CM-ABRS-Coated SPIONs

CM-ABRS SPIONs (0.8% w/v) were prepared by the alkaline co-precipitation method. $FeCl_3 \cdot 6H_2O$ and $FeCl_2 \cdot 4H_2O$ in a molar ratio of 2:1 were dissolved in DDW under nitrogen atmosphere. CM-ABRS solution (0.8% w/v) was added under vigorous stirring, followed by dropwise addition of 25% NH_4OH to precipitate the iron oxide nanoparticles. The resulting ferrofluid was washed magnetically with DDW to remove excess ions, redispersed, and stored at 4°C.

2.6 Drug Loading and Optimization

DOX was loaded onto CM-ABRS SPIONs by simple incubation. Various amounts of CM-ABRS SPIONs (0.5–2.5 mg) were incubated with a fixed concentration of DOX (120 µg) in PBS (pH 7.4) at room temperature for 2 hours

under gentle agitation. Drug loading was assessed via fluorescence quenching analysis, monitoring the decrease in DOX fluorescence intensity with increasing nanoparticle concentration. The formulation showing maximum quenching without further increase (dc-7: 2.0 mg CM-ABRS SPIONs + 120 µg DOX) was selected as the optimized system. Drug loading capacity (DLC) and entrapment efficiency (EE) were calculated from the difference between total and unloaded drug, quantified by HPLC at 480 nm.

2.7 Physicochemical Characterization of DOX–CM-ABRS SPIONs

Particle size, polydispersity index (PDI), and zeta potential were measured by dynamic light scattering (DLS; Zetasizer Nano ZS, Malvern). Morphology was examined by TEM. FT-IR analysis confirmed drug–polymer interactions. XRD confirmed retention of magnetite crystal structure. Magnetic properties were evaluated by VSM (magnetization vs. applied field curves at room temperature).

2.8 Cryoprotectant Optimization and Lyophilization

The effect of cryoprotectants (lactose, D-mannitol, D-fructose, sucrose, dextrose; all at 5% w/v) on particle size after lyophilization was evaluated. Lyophilized formulations were reconstituted and particle size measured by DLS. D-mannitol (5% w/v) was selected as the optimal cryoprotectant based on minimum particle size increase and lowest PDI.

2.9 HPLC Method for DOX Quantification

A validated reverse-phase HPLC method was used for DOX quantification. A linear calibration curve over 1–100 µg/mL (R^2 = 0.9993) with LOD 0.118 µg/mL, LOQ 0.358 µg/mL, accuracy 99.65%, and %RSD 0.45% was established. DOX was eluted at a retention time of ~9.78 min.

2.10 In Vitro Drug Release

Drug release from DOX–CM-ABRS SPIONs was studied in PBS (pH 7.4) at 37°C using a dialysis membrane method. Aliquots were withdrawn at predetermined time intervals and analyzed by HPLC. Cumulative drug release was plotted against time. Release kinetics were evaluated using zero-order, first-order, Higuchi, and Korsmeyer–Peppas models. Correlation coefficients (R^2) were used to identify the best-fit model.

2.11 In Vitro Magnetic Targeting (Localization Study)

The magnetic targeting capability of CM-ABRS SPIONs (0.8% w/v) was demonstrated using a glass microcapillary model. A suspension of CM-ABRS SPIONs was mixed with fluorescently labeled red blood cells to simulate physiological conditions. The mixture was allowed to flow through the microcapillary, and an external permanent magnet was placed adjacent to one side. Localization of SPIONs toward the magnet was observed under the microscope, validating proof-of-concept for magnetically guided targeting.

2.12 In Vitro Cytotoxicity Study (MTT Assay)

Cytotoxicity was evaluated against the MCF-7 human breast cancer cell line using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay. Cells were treated with varying concentrations of free DOX, blank CM-ABRS SPIONs, and DOX–CM-ABRS SPIONs for 48 hours. Cell viability was calculated as the percentage of viable cells relative to untreated control. IC_{50} values were determined from dose–response curves. Statistical significance was assessed by Student's t-test ($p < 0.05$).

2.13 Molecular Docking Studies

Molecular docking of DOX and DOX–CM-ABRS SPIONs against the HER-2 receptor (PDB ID: 5JEB) was performed using the Maestro software package (Schrödinger Suite). The protein structure was prepared using the Protein Preparation Wizard; ligand structures were prepared using LigPrep. Glide XP docking protocol was employed to generate docking scores and binding poses.

3. RESULTS AND DISCUSSION

3.1 Optimization of Carboxymethylation: Effect of Reaction Parameters on DS

Carboxymethylation of ABRS was systematically optimized by varying molar ratio of starch to monochloroacetic acid, solvent system, NaOH concentration, reaction temperature, and reaction time. The results are summarized in Table 1. An increase in molar ratio from 1:1 to 1:1.5 produced a significant rise in DS (0.91 to 1.03), while further increase to 1:2 yielded no additional benefit, suggesting saturation of reactive sites. Among the solvent systems evaluated, IPA provided superior DS (1.12) compared to methanol (1.03) or ethanol (0.94), likely due to better swelling of starch granules. NaOH concentration of 8 N achieved optimal DS (1.19), as higher concentration promoted competing side reactions forming sodium glycolate, reducing availability of the etherifying agent. Reaction temperature of 65°C and duration of 2.5 hours yielded the highest DS of 1.23 ± 0.01, which was selected as the optimal condition. Further increase in time (3 h) showed no significant improvement.

Table 1. Effect of process parameters on degree of substitution (DS) of CM-ABRS (n=3, Mean ± SD)

Formulation	Molar Ratio	NaOH (N)	Solvent	Temp. (°C)	Time (h)	DS (Mean ± SD)
CM-ABRS1	1.0	5	Methanol	50	1	0.91 ± 0.03
CM-ABRS2	1.5	5	Methanol	50	1	1.03 ± 0.04
CM-ABRS3	2.0	5	Methanol	50	1	1.05 ± 0.03
CM-ABRS5	1.5	5	IPA	50	1	1.12 ± 0.02
CM-ABRS8	1.5	8	IPA	50	1	1.19 ± 0.03
CM-ABRS9	1.5	9	IPA	50	1	1.15 ± 0.05
CM-ABRS12	1.5	8	IPA	65	1	1.21 ± 0.02
CM-ABRS15*	1.5	8	IPA	65	2.5	1.23 ± 0.01
CM-ABRS16	1.5	8	IPA	65	3.0	1.23 ± 0.02

**Selected optimized condition (DS = 1.23)*

3.2 FT-IR Characterization

FT-IR spectra confirmed successful carboxymethylation of ABRS. In the spectrum of native ABRS, a broad OH stretching band (3200–3400 cm^{-1}), C–H stretching at ~2920 cm^{-1}, and C–O–C glycosidic linkage peaks (1000–1150 cm^{-1}) were observed. Upon carboxymethylation, new distinctive bands appeared in CM-ABRS at 1580–1610 cm^{-1} and 1410–1450 cm^{-1}, attributable to asymmetric and symmetric stretching of carboxylate ($–COO^-$) groups, respectively. A slight shift in the –OH stretching region also confirmed altered hydrogen bonding. In the DOX–CM-ABRS SPIONs spectrum, reduction of N–H peaks of DOX (wagging ~804 cm^{-1}; bending 1581–1616 cm^{-1}) and a shift in carboxylate stretching (1639 → 1641 cm^{-1}) confirmed interaction of DOX's amine groups with the polymer's carboxylate groups, primarily through electrostatic attraction and hydrogen bonding, confirming stable drug incorporation (Table 2).

Table 2. Key FT-IR assignments of ABRS, CM-ABRS, SPIONs, DOX, and DOX–CM-ABRS SPIONs

Assignment	ABRS / CM-ABRS (cm^{-1})	SPIONs (cm^{-1})	DOX (cm^{-1})	DOX-CM-ABRS SPIONs (cm^{-1})
O–H stretch	3200–3400	—	3328	3386
C–H stretch	~2920	2937	2931	2938
COO^- (asym.)	1580–1610	1568	—	—
COO^- (sym.)	1410–1450	1455	—	1456
C=O stretch	—	—	1728	1725
N–H bending	—	—	1614, 1580	1640 (shifted)
Fe–O–Fe	—	520	—	519
C–O–C glycosidic	1000–1150	1080	—	1082

3.3 Physicochemical Properties of CM-ABRS

Carboxymethylation dramatically improved the physicochemical properties of ABRS. Aqueous solubility of CM-ABRS was enhanced approximately 60-fold compared to native ABRS, a critical advantage for drug loading and nanoparticle dispersion. The modified starch showed reduced apparent viscosity and improved powder flow characteristics (lower Carr's index and Hausner's ratio), beneficial for pharmaceutical processing. Swelling index increased significantly, reflecting enhanced hydration capacity. Mucoadhesive strength was also improved, attributed to increased electrostatic and hydrogen bonding interactions through the carboxylate groups. DSC and XRD analyses revealed reduction in crystallinity, consistent with disruption of ordered starch granular structure by carboxymethyl substitution. SEM images showed altered granular morphology with irregular, porous surfaces in CM-ABRS compared to the smooth, oval granules of native ABRS.

3.4 Particle Size, PDI, and Zeta Potential

DLS analysis of CM-ABRS SPIONs (0.8% w/v) revealed an average hydrodynamic size of ~200 nm with PDI of 0.40, indicating reasonably uniform particle distribution. After DOX loading, particle size was ~205 nm (PDI 0.38) — no significant change, confirming structural integrity was preserved post-drug loading. Zeta potential of blank CM-ABRS SPIONs was −28.2 mV, attributed to ionized carboxylate ($–COO^-$) and hydroxyl (–OH) surface groups. Following DOX loading, zeta potential slightly decreased to −26.0 mV, consistent with partial charge neutralization by the positively charged amine of DOX interacting with the anionic polymer surface. Despite this minor shift, the nanoparticles retained adequate negative surface charge for colloidal stability.

3.5 Cryoprotectant Optimization

In the absence of any cryoprotectant, lyophilization caused significant aggregation with Z-average increasing to ~578 nm and PDI of 0.60. Among tested cryoprotectants (all at 5% w/v), D-mannitol was most effective, reducing post-lyophilization particle size to ~205 nm with PDI of 0.38 (Table 3). The superior performance of D-mannitol is attributed to its polyol structure forming a protective hydrogen-bonded matrix around nanoparticles during freezing, preventing irreversible agglomeration. D-mannitol was therefore selected for lyophilization of the final formulation.

Table 3. Effect of cryoprotectants on particle size of DOX–CM-ABRS SPIONs after lyophilization

S. No.	Cryoprotectant	Concentration	Z-average (nm)	PDI
1	None	0% w/v	578.1 ± 0.12	0.60 ± 0.05
2	Lactose	5% w/v	308.4 ± 0.41	0.69 ± 0.01
3	D-Mannitol*	5% w/v	205.6 ± 0.21	0.38 ± 0.02
4	D-Fructose	5% w/v	452.7 ± 0.31	0.60 ± 0.09
5	Sucrose	5% w/v	472.5 ± 0.14	0.85 ± 0.23
6	Dextrose	5% w/v	510.3 ± 0.17	0.55 ± 0.13

**Selected cryoprotectant*

3.6 Drug Loading and Entrapment Efficiency

Fluorescence quenching studies revealed a progressive decrease in DOX fluorescence intensity with increasing CM-ABRS SPIONs concentration, indicating enhanced drug–nanoparticle interaction and binding. Maximum quenching was achieved at formulation dc-7 (2.0 mg CM-ABRS SPIONs + 120 µg DOX), beyond which no further reduction in fluorescence was observed, confirming saturation of binding sites. The optimized formulation dc-7 achieved a drug loading capacity (DLC) of 6.0 ± 0.3% (w/w) and entrapment efficiency (EE) of 90.24 ± 0.8%, demonstrating highly efficient incorporation of DOX into the CM-ABRS SPION system. This is attributable to the high density of anionic carboxylate groups on CM-ABRS that interact favorably with the cationic DOX through electrostatic attraction.

3.7 XRD and Magnetic Properties (VSM)

XRD diffraction patterns of CM-ABRS SPIONs and DOX-loaded CM-ABRS SPIONs displayed characteristic peaks consistent with inverse spinel Fe_3O_4 (magnetite) crystal structure. Importantly, drug loading did not alter the

crystalline structure of the magnetite core, confirming structural integrity. A gradual decrease in average crystallite size was observed from pristine magnetite (~17 nm) to CM-ABRS SPIONs (~12 nm) and DOX-CM-ABRS SPIONs (~9 nm) by Scherrer analysis, reflecting the influence of polymer coating. All crystallite sizes fall within the 4–18 nm superparamagnetic range (Laurent et al., 2008). VSM analysis revealed that both CM-ABRS SPIONs and DOX-loaded SPIONs displayed superparamagnetic behavior, with saturation magnetization (Ms) values of ~31.2 emu/g and ~33.5 emu/g, respectively, with negligible coercivity and remanence. The slight reduction in Ms compared to pristine magnetite is attributed to the non-magnetic CM-ABRS coating. These properties confirm the suitability of the system for magnetic drug targeting.

3.8 In Vitro Drug Release and Release Kinetics

The in vitro release profile of DOX from DOX–CM-ABRS SPIONs in PBS (pH 7.4) demonstrated a biphasic pattern: an initial burst release of ~10.6% within the first 2 hours, attributable to surface-associated drug molecules, followed by a sustained, controlled release reaching ~68.2% cumulative release at 24 hours. This sustained profile is highly desirable for prolonging drug availability in systemic circulation and enhancing tumor accumulation via the EPR effect. Release kinetics modeling (Table 4) revealed the best fit with the Higuchi model (R^2 = 0.937), indicating diffusion-controlled release from the polymeric matrix. The Korsmeyer–Peppas release exponent (n) suggested a combination of Fickian diffusion and polymer relaxation, consistent with the hydrophilic polymeric nature of CM-ABRS.

Table 4. Release kinetics model fitting for DOX release from CM-ABRS SPIONs in PBS pH 7.4

Model	Equation	Regression Equation	R^2
Zero-order	$M_0 - M_t = K_0 t$	y = 0.0102x + 0.1451	0.826
First-order	$\ln(M_0/M_t) = K_1 t$	y = −0.0079x + 1.9480	0.929
Higuchi*	$M_t = K_h\sqrt{t}$	y = 0.1031x − 0.0552	0.937
Korsmeyer–Peppas	$M_t/M_\infty = kt^n$	y = 0.8720x − 1.5290	0.865

**Best-fit model*

3.9 HPLC Method Validation

The HPLC method employed for DOX quantification demonstrated excellent analytical performance. The calibration curve was linear over 1–100 µg/mL with a correlation coefficient of 0.9993 (y = 22934x – 30037). The LOD and LOQ were 0.118 µg/mL and 0.358 µg/mL, respectively. Method accuracy was 99.65% with %RSD of 0.45% (n=6), confirming the reliability of the quantification method. The consistent retention time of ~9.78 min confirmed chromatographic reproducibility (Table 5).

Table 5. Validation parameters of the HPLC method for doxorubicin quantification

Parameter	Result
Linearity range	1–100 µg/mL
Regression equation	y = 22934x – 30037
Correlation coefficient (R^2)	0.9993
LOD	0.118 µg/mL
LOQ	0.358 µg/mL
Retention time	~9.78 min
Precision (%RSD, n=6)	0.45%
Accuracy (n=3)	99.65%

3.10 In Vitro Cytotoxicity (MTT Assay)

The anticancer efficacy of DOX–CM-ABRS SPIONs against MCF-7 breast cancer cells was markedly superior to that of conventional free DOX solution. The IC_{50} of DOX–CM-ABRS SPIONs (~5.95 µg/mL) was approximately 2-fold lower than that of free DOX solution (Doxutec®, ~12.16 µg/mL), and the difference was statistically significant ($p < 0.05$). Blank CM-ABRS SPIONs showed negligible cytotoxicity at equivalent concentrations, confirming the biocompatibility of the carrier. The enhanced cytotoxicity of the nanoformulation can be attributed to improved cellular internalization via endocytic pathways, sustained intracellular drug release, and the combined effect of passive EPR-based tumor accumulation. These results demonstrate that encapsulation within CM-ABRS SPIONs significantly potentiates the therapeutic efficacy of DOX while potentially reducing the dose required for effective tumor cell killing.

3.11 Molecular Docking Studies

Molecular docking analysis against the HER-2 receptor (PDB: 5JEB) was performed to understand the molecular basis of enhanced anticancer activity. Free DOX exhibited a docking score of −12.537, while the DOX–CM-ABRS SPION formulation showed a more favorable score of −13.396, representing stronger binding interactions at the receptor active site. The binding orientation of DOX remained largely conserved between the two systems; however, additional stabilizing interactions conferred by the polymer-coated nanoparticle matrix contributed to the improved binding affinity. These computational findings corroborate the enhanced in vitro cytotoxicity and support the superior molecular-level performance of the nanoparticle system.

4. CONCLUSION

A novel, green, and sustainable magnetically targeted drug delivery system was successfully developed by loading doxorubicin onto carboxymethyl Assam Bora rice starch-coated superparamagnetic iron oxide nanoparticles (DOX–CM-ABRS SPIONs). Native ABRS was chemically modified to CM-ABRS with an optimized degree of substitution of 1.23, yielding a polymer with ~60-fold enhanced aqueous solubility, improved flow, rheological, and mucoadhesive properties. CM-ABRS served as an effective green coating material for SPIONs, enabling electrostatic drug loading with high entrapment efficiency (90.24 ± 0.8%) and drug loading (~6% w/w). The optimized formulation exhibited a particle size of ~205 nm, appropriate zeta potential (−26.0 mV), spherical TEM morphology, confirmed cubic Fe_3O_4 crystal structure, and superparamagnetic behavior. In vitro drug release was controlled and sustained (~68.2% over 24 h), best described by the Higuchi diffusion model. Magnetic targeting was validated in a microcapillary model, and superior in vitro anticancer efficacy against MCF-7 cells (IC_{50} ~5.95 µg/mL vs. ~12.16 µg/mL for free DOX, $p < 0.05$) was demonstrated. Molecular docking supported stronger receptor-binding interactions. Overall, DOX–CM-ABRS SPIONs represent a promising, cost-effective, and eco-friendly nanoplatform combining active magnetic and passive EPR-based targeting strategies for improved cancer therapy with potential for reduced cardiotoxicity.

5. FUTURE PERSPECTIVES

Future research should focus on long-term colloidal stability studies under physiological conditions and assessment of any aggregation tendencies beyond 600 seconds to ensure safety for in vivo use. In vivo pharmacokinetic, biodistribution, and tumor regression studies in appropriate cancer models will be essential to fully establish therapeutic efficacy and safety. Investigation of hyperthermia-based therapy using an alternating magnetic field to induce localized heat from the magnetic core could further augment anticancer activity. Surface functionalization of CM-ABRS SPIONs with active targeting ligands (e.g., folic acid, hyaluronic acid, antibodies) could enhance receptor-mediated cellular uptake. Additionally, the excellent physicochemical properties of CM-ABRS make it a promising excipient for formulations beyond nanoparticles, including tablets, capsules, sachet-based dosage forms, topical products, and body powders, meriting further pharmaceutical development.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgments

The authors sincerely acknowledge Dr. Vaibhav Verma, Professor and Supervisor, Gyanodaya Institute of Pharmacy, Gyanodaya University, Neemuch (M.P.), for his expert guidance, supervision, and continuous encouragement throughout this research work. The authors also thank Gyanodaya Institute of Pharmacy, Village: Suwakheda, Tehsil: Jawad, District: Neemuch, Madhya Pradesh, for providing the necessary infrastructure, instruments, and experimental facilities for conducting this work.

REFERENCES

[1] Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. CA Cancer J Clin. 2015;65(1):5–29.

[2] Byrne JD, Betancourt T, Brannon-Peppas L. Active targeting schemes for nanoparticle systems in cancer therapeutics. Adv Drug Deliv Rev. 2008;60(15):1615–1626.

[3] Shapiro CL, Recht A. Side effects of adjuvant treatment of breast cancer. N Engl J Med. 2001;344(26):1997–2008.

[4] Misra R, Acharya S, Sahoo SK. Cancer nanotechnology: application of nanotechnology in cancer therapy. Drug Discov Today. 2010;15(19–20):842–850.

[5] Ferrari M. Cancer nanotechnology: opportunities and challenges. Nat Rev Cancer. 2005;5(3):161–171.

[6] Peer D, Karp JM, Hong S, Farokhzad OC, Margalit R, Langer R. Nanocarriers as an emerging platform for cancer therapy. Nat Nanotechnol. 2007;2(12):751–760.

[7] Gupta AK, Gupta M. Synthesis and surface engineering of iron oxide nanoparticles for biomedical applications. Biomaterials. 2005;26(18):3995–4021.

[8] Mahmoudi M, Sant S, Wang B, Laurent S, Sen T. Superparamagnetic iron oxide nanoparticles (SPIONs): development, surface modification and applications in chemotherapy. Adv Drug Deliv Rev. 2011;63(1–2):24–46.

[9] Sun C, Lee JSH, Zhang M. Magnetic nanoparticles in MR imaging and drug delivery. Adv Drug Deliv Rev. 2008;60(11):1252–1265.

[10] Anwar M, Asfer M, Prajapati AP, Mohapatra S, Akhter S, Ali A, Ahmad FJ. Synthesis and in vitro localization study of curcumin-loaded SPIONs in a micro capillary for simulating a targeted drug delivery system. Int J Pharm. 2014;468(1–2):158–164.

[11] Laurent S, Forge D, Port M, Roch A, Robic C, Vander Elst L, Muller RN. Magnetic iron oxide nanoparticles: synthesis, stabilization, vectorization, physicochemical characterizations, and biological applications. Chem Rev. 2008;108(6):2064–2110.

[12] Lemarchand C, Gref R, Couvreur P. Polysaccharide-decorated nanoparticles. Eur J Pharm Biopharm. 2004;58(2):327–341.

[13] Kim DK, Mikhaylova M, Wang FH, Kehr J, Bjelke B, Zhang Y, et al. Starch-coated superparamagnetic nanoparticles as MR contrast agents. Chem Mater. 2003a;15(23):4343–4351.

[14] Saboktakin MR, Maharramov A, Ramazanov MA. Synthesis and characterization of superparamagnetic nanoparticles coated with carboxymethyl starch (CMS) for magnetic resonance imaging technique. Carbohydr Polym. 2009;78(2):292–295.

[15] Zaki Ahmad M, Akhter S, Anwar M, Singh A, Ahmad I, Ain MR, et al. Feasibility of Assam Bora rice starch as a compression coat of 5-fluorouracil core tablet for colorectal cancer. Curr Drug Deliv. 2012;9(1):105–110.

[16] Sharma HK, Lahkar S, Nath LK. Formulation and in vitro evaluation of metformin hydrochloride loaded microspheres prepared with polysaccharide extracted from natural sources. Acta Pharm. 2013;63(2):209–222.

[17] Mallick S, Sharma S, Banerjee A, Ghosh S. Development and evaluation of carboxymethyl starch-based nanoparticles for controlled drug delivery. Int J Biol Macromol. 2016;84:229–236.

[18] Bhattacharyya D, Singhal RS, Kulkarni PR. Physicochemical properties of carboxymethyl starch prepared from corn and waxy amaranth starch. Carbohydr Polym. 1995;27(3):167–169.

[19] Brunton LL. Goodman & Gilman's The Pharmacological Basis of Therapeutics. 12th ed. New York: McGraw-Hill; 2011.

[20] Barenholz YC. Doxil®—the first FDA-approved nano-drug: lessons learned. J Control Release. 2012;160(2):117–134.

[21] Stojanović Ž, Jeremić K, Jovanović S, Lechner MD. A comparison of some methods for the determination of the degree of substitution of carboxymethyl starch. Starch-Stärke. 2005;57(2):79–83.

[22] Hanahan D, Weinberg RA. The hallmarks of cancer. Cell. 2000;100(1):57–70.

[23] Matsumura Y, Maeda H. A new concept for macromolecular therapeutics in cancer chemotherapy: mechanism of tumoritropic accumulation of proteins and the antitumor agent smancs. Cancer Res. 1986;46(12):6387–6392.

[24] Davis ME, Chen ZG, Shin DM. Nanoparticle therapeutics: an emerging treatment modality for cancer. Nat Rev Drug Discov. 2008;7(9):771–782.

[25] Mura S, Nicolas J, Couvreur P. Stimuli-responsive nanocarriers for drug delivery. Nat Mater. 2013;12(11):991–1003.

[26] Mohapatra S, Siddiqui AA, Anwar M, Bhardwaj N, Akhter S, Ahmad FJ. Synthesis and characterization of novel carboxymethyl Assam Bora rice starch for the controlled release of cationic anticancer drug based on electrostatic interactions. AAPS PharmSciTech. 2017;18(4):1268–1281.

[27] Khalil MI, Hashem A, Hebeish A. Carboxymethylation of maize starch. Starch-Stärke. 1990;42(2):60–63.

[28] Korhonen O, Raatikainen P, Harjunen P, Nakari J, Suihko E, Peltonen S, et al. Starch acetates—multifunctional direct compression excipients. Pharm Res. 2000;17(9):1138–1143.

[29] das Neves J, Bahia MF, Amiji MM, Sarmento B. Mucoadhesive nanomedicines: characterization and modulation of mucoadhesion at the nanoscale. Expert Opin Drug Deliv. 2011;8(8):1085–1104.

[30] Amstad E, Textor M, Reimhult E. Stabilization and functionalization of iron oxide nanoparticles for biomedical applications. Nanoscale. 2011;3(7):2819–2843.