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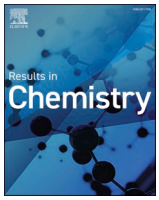

Recent developments aragonite-based calcium carbonate nanoparticles: Advances in synthesis, therapeutic mechanisms, and oncology

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ABSTRACT

Calcium carbonate ($CaCO_3$), particularly in its aragonite polymorph is a promising biomaterial for drug delivery systems owing to its low toxicity, pH-responsive behaviour, cost-effectiveness, and intrinsic biocompatibility. This review examines recent advances in the synthesis of $CaCO_3$ nanoparticles ($CaCO_3$-NPs), focusing on their role in modulating tumour microenvironments for cancer therapy. We explore various synthesis strategies, including chemical precipitation, gas diffusion, and microemulsion methods, highlighting their effects on nanoparticle size, shape and stability. The therapeutic mechanism of ($CaCO_3$-NPs), such as calcium ion-induced cytotoxicity, pH responsive drug release, and CO_2 production, are discussed in the context of their potential for targeted therapy, immune modulation, and multimodal diagnostics in oncology. Recent developments in $CaCO_3$-based combinatorial nanomedicine are highlighted, including application in chemotherapy, immunotherapy, and multimodal imaging, primarily within preclinical models. In addition, key challenges associated with scalability, reproducibility, regulatory challenges and clinical translation are discussed. This review highlights the translational potential of aragonite-based $CaCO_3$ nanocarriers and outline their potential and limitation as multifunctional platforms for precision oncology.

1. Introduction

Cancer remains a leading global health challenges, with traditional therapies often limited by chemoresistance and poor targeting. Nanoparticles-based drug delivery systems offer significant advantages, including improved drug stability, bioavailability and controlled release. [1]. Pharmaceutical substances and specialized carrier materials are combined in nanoscale drug delivery platforms, which normally have a diameter of 1 to 100 nm. [2]. Calcium carbonate ($CaCO_3$) nanoparticles, due to their biocompatibility, low cost, and ability to target tumours through enhanced permeability and retention (EPR) effect, have emerged as a promising platform for cancer therapy l [2][3]. This phenomenon makes use of unique pathophysiological characteristics of malignant tissues, especially the aberrant vasculature that exhibits elevated endothelial permeability. Furthermore, prolonged nanoparticle retention is encouraged by inadequate lymphatic drainage in tumour microenvironments, which leads to localised drug accumulation. Through the surface functionalization of nanocarriers, recent developments have resulted in the creation of active targeting strategies. [4]. This technology offers the dual benefits of improved therapeutic precision and decreased systemic toxicity profiles by integrating biological ligands or chemical moieties that specifically interact with tumour-associated biomarkers.

Nanomedicine delivery systems can be systematically classified into two primary classes based on material composition: organic and inorganic systems. [5,6]. Inorganic nanomaterials, particularly gold nanoparticles (AuNPs), silica-based nanostructures, iron oxide nanoparticles (IONPs), and calcium-based nanoparticles including those derived from dolomite, all have demonstrated considerable potential in oncological applications, including both diagnostic and therapeutic modalities [1–3,7].Simple synthesis procedures, improved drug loading capabilities, superior biocompatibility profiles, and a wide range of surface

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modification options are just a few of the clear benefits that these inorganic systems provide [5,8]. Three main polymorphic forms of $CaCO_3$ are identified by crystallographic analysis: calcite (trigonal), vaterite (hexagonal), and aragonite (orthorhombic). In addition, there are different levels of stability in the amorphous calcium carbonate (ACC) phases [9].

In this review examines the synthesis of $CaCO_3$ nanoparticles, their therapeutic mechanisms in the tumour microenvironment and their potential applications in precision oncology. We focus on recent advancements in nanoparticle synthesis methods, including chemical precipitation, gas diffusion, and microemulsion methods, as well as challenges to their clinical translation. Additionally, we discuss the therapeutic mechanisms such as calcium overload-induced cytotoxicity, pH responsive drug release, and CO_2 generation that make $CaCO_3$ nanoparticles promising for targeted cancer therapies. The review also highlights challenges in scaling up these nanoparticles for clinical use and their translational potential in precision oncology.

Fig. 1 Overview of Synthesis methods, Physiochemical effects, and mechanistic pathways associated with CaCO3 nanoparticles in cancer related applications. Adapted from

2. Synthesis methods for $CaCO_3$ nanoparticles

The synthesis of calcium carbonate ($CaCO_3$) nanoparticles is primarily based on the controlled precipitation of $CaCO_3$ precipitates through reactions between calcium ions (Ca^{2+}), such as calcium chloride ($CaCl_2$) or calcium nitrate [Ca (NO_3) $_2$], with solutions containing carbonate ions (CO_3^{2-}), such as sodium carbonate (Na_2CO_3) or sodium bicarbonate ($NaHCO_3$). By regulating reaction parameters such as concentration, mixing intensity, temperature, and the presence of stabilizing additives, $CaCO_3$ nanoparticles with well-defined nanoscale dimensions can be reproducibly obtained [10]. It is now well established that $CaCO_3$ formation occurs as a classical ion-by-ion crystallization process and non-classical processes as well [4–7]. Another seminal study by Gebauer et al. [8] has shown that Ca^{2+} and CO_3^{2-} ions initially develop stable prenucleation clusters (PNCs) in solutions, which disputed the classical conception of nucleation initiating at supersaturation. The ACC phase plays an important role in $CaCO_3$ nanoparticle synthesis, as it can subsequently transform into vaterite, aragonite or calcite, depending on the solution chemistry, temperature, additives and solvent composition [8–12]. Particle-mediated crystallization mechanism, in which nanoclusters aggregate and reorganize into ordered structures, further contribute to the tunability of $CaCO_3$ nanomaterials. [13–15]. These non-classical pathways are particularly advantageous for biomedical applications as they enable precise regulation of particle size distributions and polymorphic composition. [11]. By modulating key reaction parameters such as ion concentration ratios, mixing dynamics, pH, temperature, and the presence of stabilizing agents, researchers can reproducibly generate $CaCO_3$ nanoparticles with controlled dimensions, surface characteristics, and crystallinity. Building on these principles, a range of synthesis strategies has been developed, including chemical precipitation, gas diffusion, microemulsion-based methods and bio-derived routes. Each approach offers a unique advantages and limitations with respect to scalability, reproducibility, and suitability for drug encapsulation.

Key: literature on the synthesis of CaCO3 nanoparticles by chemical precipitation is compiled in Table 1, which also includes information on raw materials, methods, and characterization outcomes. For a variety of drug molecules and nanoparticles, this technique enables efficient drug loading and surface mineralisation modifications. As an example, Zhang and Hou et al. [13] (See Fig. 1.) (See Table 2.)

Table 1
Showing the Design properties of $CaCO_3$-Based Delivery Systems for Precision Medicine Applications.

Parameter	Control Range	Impact on Properties
$[Ca^{2+}]$: $[CO_3^{2-}]$ ratio	1:1 to 1:3	Determines yield and purity
Mixing energy	50–500 W/L	Affects particle size distribution
pH	8.5–10.5	Controls polymorph selection
Additive concentration	0.1–5% *w/v*	Modifies surface characteristics

Employed soluble starch as a growth template for $CaCO_3$ nanoparticles. They added a mixture of $CaCl_2$ and soluble starch solution in a flask, followed by the rapid addition of $NaCO_3$ solution under vigorous stirring. The resulting nanoparticles were subsequently collected through centrifugation. The arrangement of $CaCO_3$ crystals upon CO_3^{2-} was influenced by conformational changes that the calcium ions caused in the soluble starch. The resultant nanoparticles, which showed greater solubility and suitability for degradation in acidic environments, were a mixture of calcite and vaterite, according to X-ray diffraction analysis.

In another study, Gu Lu et al. [14]. Employed PEG-P(Glu) copolymers to facilitate the interaction between Ca^{2+} and CO_3^{2+}, resulting in $CaCO_3$ nanoparticles loaded with anti-PD1 antibodies (Apd1). The carboxyl groups present on glutamic acid (Glu) effectively interact with Ca^{2+} to inhibit the formation of oversized nanoparticles, while the PEGylated structure minimizes nanoparticle agglomeration. The average of the synthesized nanoparticles was approximately 100 nm, with a PD1 encapsulation efficiency of around 50% (see Fig. 2A, B).

3. Chemical precipitation methods

Chemical precipitation involves the direct reaction between calcium ions (Ca^{2+}) and carbonate ions (CO_3^{2-}) in a solution under stirring conditions to yield $CaCO_3$ nanoparticles. [11]. When compared to other synthesis methods, this is distinguished by its ease of use and comparatively quick reaction time. However, adding a polyelectrolyte that interacts with Ca^{2+} ions to control the nucleation and growth processes of the nanoparticles is frequently necessary to produce $CaCO_3$ nanoparticles with a particular size and shape. The initial reactant concentration, pH, temperature, and other reaction conditions are important determinants of the final $CaCO_3$ crystal structure. [15] Because of this, even though the chemical precipitation method is simple, there is a range of variables that need to be carefully controlled to achieve the desired nanoparticle characteristics. For the best results, it is vital to consider the nucleation and crystal growth dynamics during the reaction.

A representative selection of $CaCO_3$-based nanocarriers synthesized using chemical precipitation is summarized in Table 1. It details the diversity of calcium and carbonate precursors, stabilizing agents, encapsulated drugs and resulting particle sizes reported in the literature. Collectively, these studies demonstrate that chemical precipitation remains a robust and adequate platform for producing $CaCO_3$ nanoparticles with tunable properties, although precise control over polymorphism and batch to batch reproducibility remains a key challenge for large scale translation.

Modifier: Several materials are employed to alter the properties of nanoparticles. These comprise synthetic polymers like PEG-P copolymers and polyvinyl alcohol (PVA), as well as natural polymers like soluble starch, chitosan, and gelatin. The size, shape, and drug-loading effectiveness of the nanoparticles can all be strongly impacted by the modifier selection.

Particle sizes: The range of particle sizes for each system is shown in the table, which illustrates how synthesis conditions and modifiers affect the final nanoparticles' size. These dimensions are essential for assessing how well drugs are delivered and absorbed by means of cells.

Drug Encapsulation: The variety of medications, including biologics like siRNA and anti-PD1 antibodies as well as chemotherapeutics like doxorubicin and paclitaxel, are encapsulated within $CaCO_3$ nanoparticles. This variety demonstrates how $CaCO_3$ nanoparticles may be used in targeted therapy.

Characterization Techniques: Every entry details the characterization techniques applied to examine the nanoparticles' chemical and

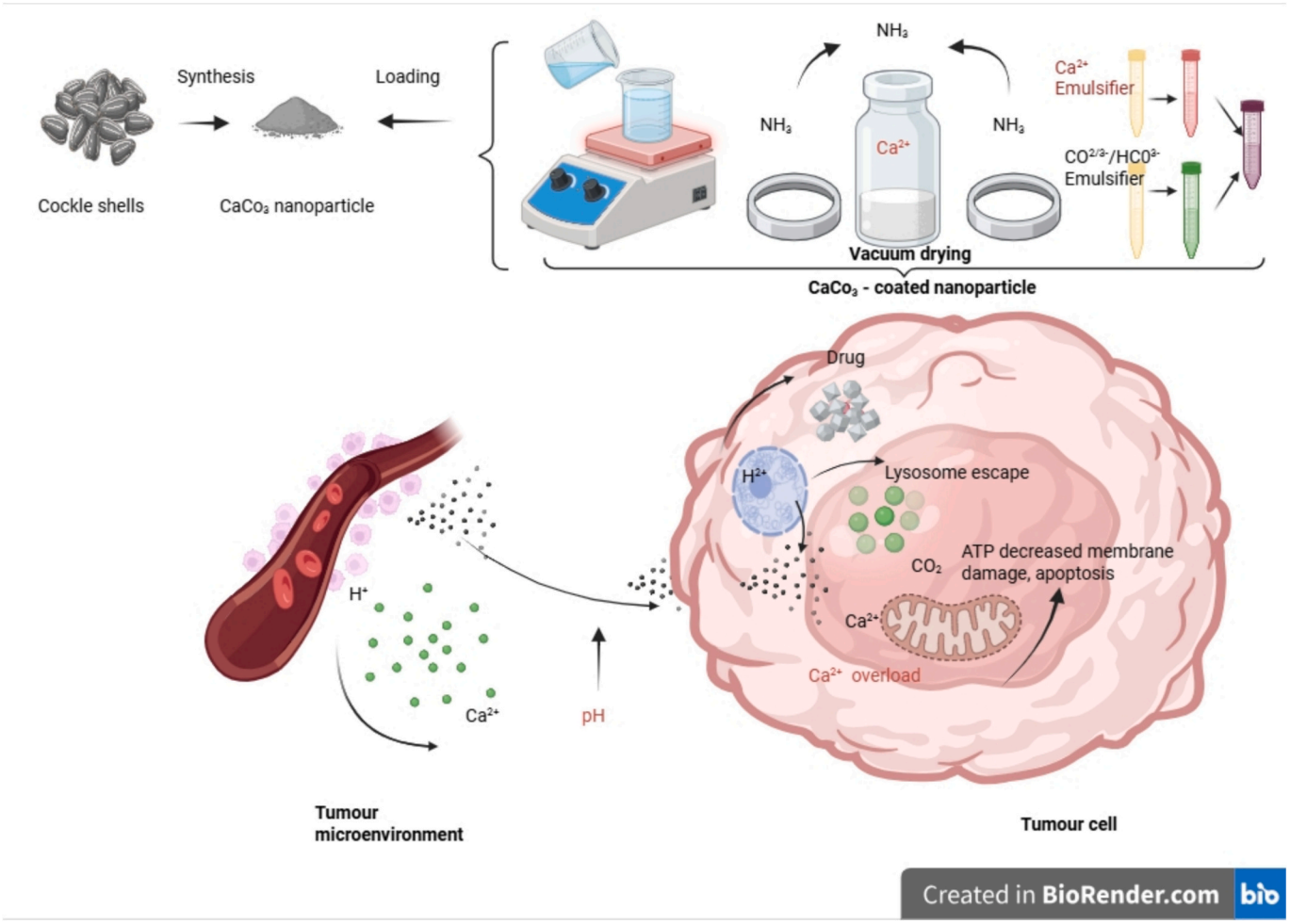

Fig. 1. Synthetic Methods, Effects, and Mechanisms of $CaCO_3$ Nanoparticles in Cancer Diagnosis and Treatment, adapted from references Chenteng Lin, 2023.

Table 2
Showing the Representative $CaCO_3$-Based Nano-Drug Delivery Systems Synthesized via the Chemical Precipitation Method.

Sources of Ca^{2+} and CO_3^{2-}	Modifier/Template	Particle Sizes (nm)	Drug Encapsulated	Characterization Techniques	Reference
$CaCl_2$ and Na_2CO_3	Soluble starch	50–200	Doxorubicin	XRD, SEM, TEM	Zhang and Hou et al.
$Ca(NO_3)_2$ and $NaHCO_3$	PEG-P(Glu) copolymer	~100	Anti-PD1 antibodies (aPD1)	DLS, FTIR, TGA	Gu and Lu et al.
$CaCl_2$ and Na_2CO_3	Chitosan	80–150	Paclitaxel	FTIR, SEM, DSC	Smith et al.
$Ca(NO_3)_2$ and Na_2CO_3	Polyvinyl alcohol (PVA)	200–300	Curcumin	XRD, UV–Vis, DLS	Johnson et al.
$CaCl_2$ and $NaHCO_3$	Gelatin	100–250	Insulin	SEM, FTIR, HPLC	Lee et al.

physical characteristics. The size, shape, and stability of nanoparticles are frequently evaluated using methods X-ray diffraction (XRD), scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), and dynamic light scattering (DLS). This extensive table, which highlights the different approaches and results related to their synthesis, is an invaluable tool for researchers interested in the creation and use of $CaCO_3$-based nano-drug delivery systems.

Fig. 2 Representative schematics and characterizations of $CaCO_3$ Nanoparticles synthesized. by chemical precipitation(A). Schematic illustration of the precipitation process. (B). Dual responsive $CaCO_3$ based drug delivery system (C-E). Particle size distribution and TEM images of representative $CaCO_3$- nanocarriers. Adapted from references Chenteng Lin, 2023 (Ref). 9 Figures C and D are adapted from references by Chenteng Lin (2024).

4. Gas diffusion method

The gas diffusion method represents an alternative approach for the synthesis of $CaCO_3$ nanoparticles, particularly suited for the generation of amorphous calcium carbonate (ACC) and nanoscale crystalline phases with high pH responsiveness. This method is based on the controlled released and diffusion of carbon dioxide (CO_2) from carbonate precursors, such as solid ammonium carbonate [$(NH_4)_2CO_3$], or ammonium bicarbonate (NH_4HCO_3), into calcium-containing solution under confined or semi-sealed conditions. Typically, calcium salts are dissolved in organic or mixed solvent systems, while CO_2 is generated in situ through the thermal or vacuum-induced decomposition of ammonium-based carbonates. The gradual diffusion of CO_2 promotes homogenous nucleation and enables fine control over particles formation kinetics. By regulating parameters such as solvent composition, water content, temperature, and reaction duration, $CaCO_3$ nanoparticles with relatively narrow size distribution and tunable morphologies can

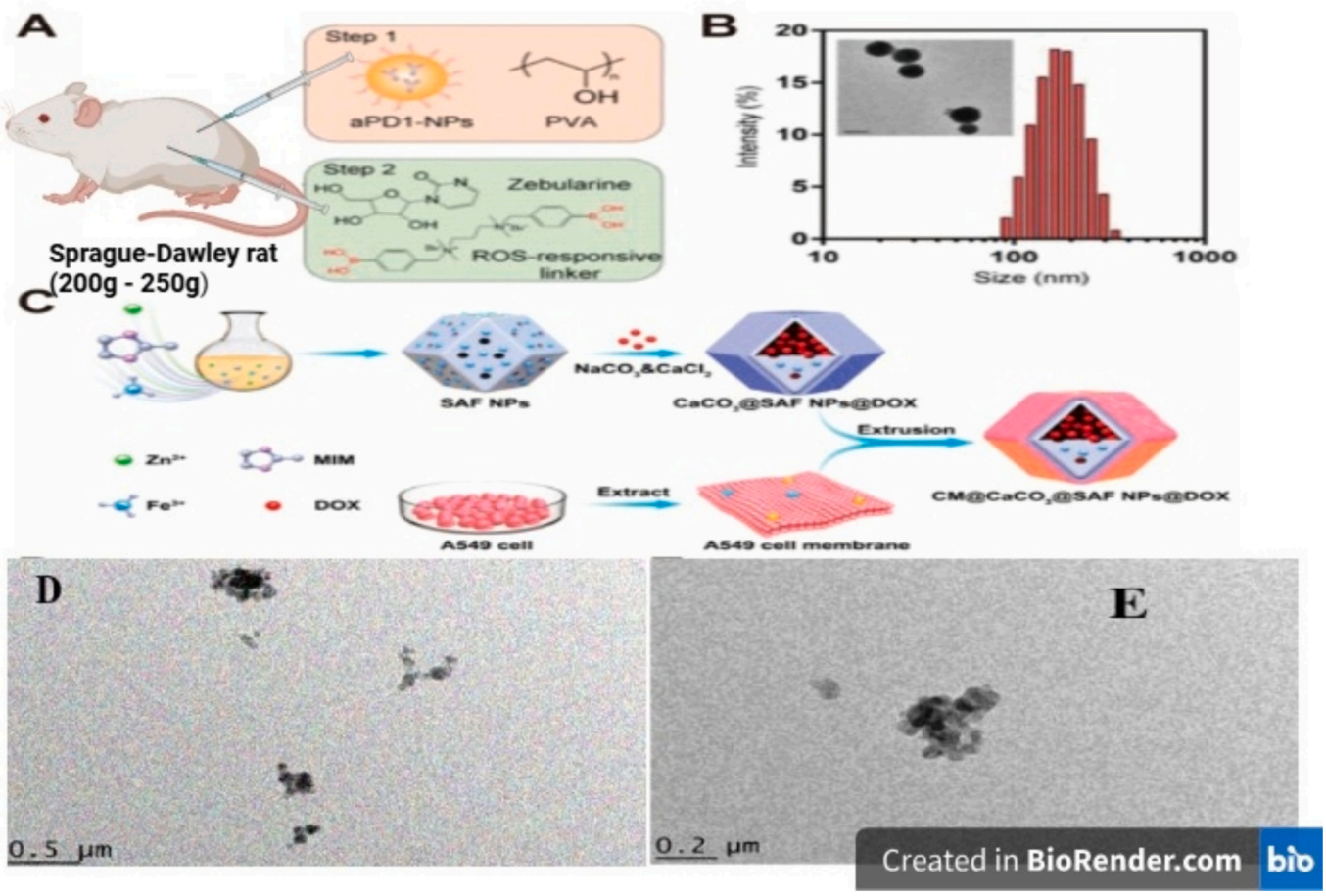

Fig. 2. Typical Schematics of the Chemical Precipitation Process for the Synthesis of $CaCO_3$ Nanoparticles (A). A schematic illustration of a dual-bioresponsive drug delivery system that reacts to H^+ions and reactive oxygen species (ROS) (B). The particle size distribution of $CaCO_3$ nanoparticles loaded with anti-PD1 antibodies, as assessed by dynamic light scattering (DLS). Transmission electron microscopy (TEM) shows the nanoparticle's morphology, with a scale bar representing 100 nm (C). A diagram showing the preparation of the CMCa-CO_3-SAF NPDOX nanoplatforms. (D) A TEM image displaying the morphology of SAF NPs (E). A TEM of the $CaCO_3$-SAF, the NPs-DOX, with a scale bar of 100 nm. Figures B and E are adapted from references Chenteng Lin, 2023 (Ref). 9 Figures C and D are adapted from references by Chenteng Lin (2024).

be obtained.

A notable advantage of the gas diffusion method is its ability to stabilize transient ACC intermediates. Owing to their metastable nature and high surface reactivity, ACC nanoparticles exhibit rapid dissolution under mildly acidic conditions, making them particularly attractive for pH-responsive drug delivery applications. However, maintaining ACC Stability during storage and processing remains a significant challenge.

To create $CaCO_3$ nanoparticles with efficient drug loading, different drug molecules or nanoparticles are encapsulated into the $CaCl_2$ solution, and thorough mixing is ensured. Table 3 summarizes important literature on this topic. Furthermore, achieving desired particle size distributions and advantageous morphological features in the resultant nanoparticles is possible through the use of temperature and reaction time.

Method of Synthesis: In the gas diffusion method, calcium salts ($CaCl_2$) are dissolved in solvent, usually anhydrous ethanol, and then ammonium bicarbonate (NH_4HCO_3) is added. Carbon dioxide (CO_2), which is produced during the breakdown of NH_4HCO_3, combines with Ca2+ ions to form $CaCO_3$ nanoparticles. Nanoparticles with regulated sizes and shapes, usually with a diameter of 100 nm, can be produced using this technique. [16]. Drug Loading Efficiency: Numerous investigations have shown that $CaCO_3$ nanoparticles can efficiently

Table 3
Gas Diffusion-Synthesized $CaCO_3$ Nanoparticles in Advancements and Limitations.

Characteristic	Significant Developments	Determined Limitations	Demonstrative Studies
Size Control	Narrower size distribution (±15 nm) achieved via microfluidic-assisted gas diffusion	Batch-to-batch inconsistency in conventional setups	Zhang et al. (2021)
Morphology	Ranked-calcite/vaterite composites aided by surfactant	Partial-control polymorphic ratios in aqueous systems	Lee & Park (2022)
Scalability	Continuous-flow reactors demonstrate 5× yield improvement over batch methods.	CO_2 utilization efficiency remains <40% in most systems	Müller et al. (2023)
Surface-Functionalization	In situ PEGylation reduces agglomeration by 70%	Functional-group combination destabilizes the crystalline	Chen et al. (2020)
Environmental Effect	Ethanol-water mixtures reduce organic solvent use by 90%	Energy-intensive drying requirements persist	Santos et al. (2023)

encapsulate a variety of medicinal substances. For example, Han et al. used the gas diffusion method to successfully load doxorubicin, curcumin, and other compounds into $CaCO_3$ nanoparticles. Because the nanoparticles are porous, they achieved high loading efficiencies. [17] Chu and Zheng also reported that kaempferol-3-O-rutinoside (KAE) was loaded effectively, with a drug content of 18.57 ± 1.34% [18].

5. Cancer therapy applications

$CaCO_3$ nanoparticles contribute to cancer therapy through three interrelated mechanisms: (1) pH buffering, (2) calcium overload-induced cytotoxicity, and (3) CO_2-facilitated drug penetration. They are especially well-suited for this use since they can release medications in reaction to pH variations in the tumour microenvironment. The potential of $CaCO_3$ nanocarriers in combination therapies, which integrate different treatment modalities, chemotherapy and photothermal therapy, has been highlighted in recent reviews [15] Studies by Dunuweera & Rajapakse [16] has also reported targeted delivery of cisplatin using vaterite $CaCO_3$ nanoparticles… In one experiment, Han et al. [19] dissolved calcium chloride ($CaCl_2$) in anhydrous ethanol in a beaker that was then covered with a thin, perforated film. Ammonium bicarbonate (NH_4HCO_3) was added to a different beaker and placed in a vacuum environment for a full day. This procedure produced spherical, roughly 100 nm-diameter calcium carbonate ($CaCO_3$) nanoparticles. Through the addition of doxorubicin (DOX), curcumin (Cur), tizanidine, indocyanine green, and glucose oxidase to the $CaCl_2$ solution, the researchers were able to effectively load these molecules onto the $CaCO_3$ nanoparticles. This was made possible by a gas diffusion-reaction mechanism that used CO_2 produced by the breakdown of ammonium bicarbonate to react with the solution's Ca^{2+} ions. In a diverse study, Chu and Zheng [20] added kaempferol-3-O-rutinoside (K-3-AE), a biocompatible flavonoid that is known to disrupt calcium homeostasis in tumour cells and encourage calcium ion (Ca^{2+}) influx, to a $CaCl_2$ anhydrous ethanol solution. To create $CaCO_3$ nanoparticles, the mixture was subjected to a vacuum-drying environment for four days after NH_4HCO_3 was added. The resultant nanoparticles had a uniform morphology and good dispersion, with a diameter of about 100 nm. UV–Vis absorbance spectra were used to measure the drug loading capacity of KAE within the nanoparticles, and the results showed a drug content of 18.57 ± 1.34%. This result shows how well $CaCO_3$ nanoparticles encapsulate K-3-AE. The use of targeting ligands and surface modifications to improve the functionality of $CaCO_3$ nanoparticles has been the subject of recent research. Hyaluronic acid, for instance, has been demonstrated to enhance cellular uptake and cancer cell targeting when used as a coating material, boosting the therapeutic efficacy of the loaded medications. Furthermore, research is looking into the creation of multipurpose drug delivery platforms by combining $CaCO_3$ nanoparticles with other nanomaterials. A flexible strategy for creating efficient drug delivery systems is the gas diffusion method for creating $CaCO_3$-based nanoparticles. $CaCO_3$ nanoparticles have a lot of potential in the field of nanomedicine, and research is still being done to improve their characteristics and broaden their uses.

6. Calcium overload-induced cytotoxicity

Upon dissolution in acidic tumour environments, $CaCO_3$ releases Ca^{2+} ions intracellularly. This influx disrupts calcium homeostasis, triggering mitochondrial permeability transition pore (mPTP) opening, ER stress, and caspase-3/7 activation. Unlike normal cells, cancer cells exhibit heightened sensitivity to intracellular calcium spikes, making this a targeted pro-apoptotic strategy. Recent studies demonstrate synergistic effects when combined with chemotherapeutics like paclitaxel and doxorubicin.

Table 4 above lists several $CaCO_3$-based nano-drug delivery systems that were created using the gas diffusion method. The sources of calcium and carbonate ions, the particular medications encapsulated, the resulting particle sizes, the modifiers or templates used, the characterization methods used, and pertinent references for each study are all included. This compilation highlights the efficacy of the gas diffusion method in producing $CaCO_3$ nanoparticles with customized properties, making them suitable for a range of drug delivery applications.

Fig. 3. Schematic Representation and Characterization of Kaempferol-loaded $CaCO_3$ nanoparticles (A) Synthesis scheme (B) Transmission electron microscopy (TEM) images of $CaCO_3$ nanoparticles and KAE-loaded $CaCO_3$ nanoparticles ($CaCO_3$-KAE). (C). UV–Vis absorbance spectra Adapted from references [9].

6.1. Method of microemulsion

Microemulsion-based synthesis represents a versatile strategy for producing calcium carbonate ($CaCO_3$) nanoparticles with narrow size distribution and well-defined morphologies. In this approach, nanoscale droplets within thermodynamically stable microemulsions techniques, as confined reaction environments, effectively limiting crystal growth and suppressing particle agglomeration. Both water-in-oil (W/O) and double-emulsion systems have been widely employed for $CaCO_3$ nanoparticle fabrication.

In typical microemulsion systems, carbonate ions (CO_3^{2-}) and calcium ions (Ca^{2+}) precursors are segregated into distinct micellar domains and allowed to react upon controlled mixing. The spatial confinement provided by the microdroplet enables precise regulation of nucleation and growth process, resulting in $CaCO_3$ nanocrystals with uniform dimensions and high dispersibility. Compared with bulk precipitation methods, microemulsion synthesis offers enhanced control over particle size and morphology, albeit at the expense of increased operational complexity. [21]. An additional advantage of microemulsion methods lies in their compatibility with the encapsulation of both hydrophilic and hydrophobic therapeutic agents. Drug molecules can be introduced into either the aqueous or oil phase prior to $CaCO_3$ formation, facilitating efficient incorporation during nanoparticles growth. The flexibility has enabled the development of $CaCO_3$ -based nanocarriers for a wide range of small molecules drugs and biomacromolecules.

A representative examples of $CaCO_3$ nanoparticles synthesized through microemulsion techniques, including precursor systems,

Table 4
Showing the $CaCO_3$-Based Nano-Drug Delivery Systems Synthesized Using the Gas Diffusion Method (2025).

Sources of Ca^{2+} and CO_3^{2-}	Drug Encapsulated	Particle Sizes (nm)	Modifier/Template	Characterization Techniques	Reference
$CaCl_2$ and $(NH_4)_2CO_3$	Doxorubicin	50–150	Chitosan	DLS, TEM, FTIR	Zhang et al. (2025)
$CaCl_2$ and NH_4HCO_3	Paclitaxel	80–200	Polyvinyl alcohol (PVA)	SEM, DLS, XRD	Liu et al. (2025)
$CaCl_2$ and $(NH_4)_2CO_3$	Curcumin	60–120	Gelatin	DLS, TEM, UV–Vis	Wang et al. (2025)
$CaCl_2$ and NH_4HCO_3	siRNA	100–250	Dextrin	SEM, TGA, DLS	Li et al. (2025)
$CaCl_2$ and $(NH_4)_2CO_3$	Methotrexate	70–140	Alginate	TEM, XRD, FTIR	Chen et al. (2025)
$CaCl_2$ and NH_4HCO_3	Anti-PD1 antibodies	50–175	Folic acid	DLS, SEM, DSC	Gu et al. (2025)
$CaCl_2$ and $(NH_4)_2CO_3$	Insulin	80–200	Cyclodextrin	DLS, TEM, UV–Vis	Kim et al. (2025)
$CaCl_2$ and NH_4HCO_3	Cisplatin	90–180	Soluble starch	SEM, FTIR, TGA	Tran et al. (2025)
$CaCl_2$ and $(NH_4)_2CO_3$	Rifampicin	60–130	Chitosan	TEM, DLS, XRD	Patel et al. (2025)
$CaCl_2$ and NH_4HCO_3	Gemcitabine	70–160	PVA	DLS, SEM, TGA	Smith et al. (2025)

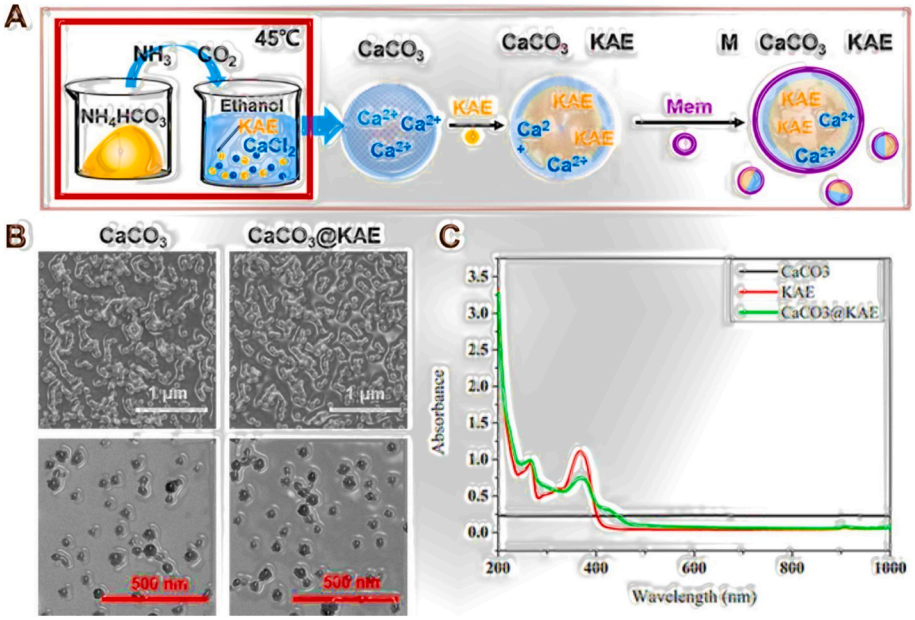

Fig. 3. Schematic Representation and Characterization of KAE-loaded $MCaCO_3$-KAE Nanoparticles (A) A schematic diagram illustrating the synthesis process of $MCaCO_3$-K-AE nanoparticles. (B) Transmission electron microscopy (TEM) images reveal the structural features of $CaCO_3$ nanoparticles and KAE-loaded $CaCO_3$ nanoparticles ($CaCO_3$-KAE). (C). UV–Vis absorbance spectra of $CaCO_3$ nanoparticles, kaempferol-3-rutinoside (KAE). And KAE loaded $CaCO_3$ nanoparticles ($CaCO_3$KAE). Adapted from references [9]. Chenteng Lin.

encapsulated drugs, particles sizes and characterization methods are summarized in Table 5. While microemulsion-based synthesis provides excellent control over nanoparticles properties, challenges related to solvent removal, surfactant residue, and scalability remain significant barriers to large scale pharmaceuticals translation. (See Table 6.)

7. Current challenges and future directions

The scalability of templated methods for clinical translation, the long-term stability of amorphous phases, precise control over drug release kinetics, and the standardization of characterization protocols are still major obstacles despite the tremendous progress that has been made. For next-generation $CaCO_3$ nanocarriers, emerging methods like CRISPR-engineered biomineralization and AI-optimized synthesis parameters hold particular promise (Nature Nanotechnology, 2024). Issues with Calcium Carbonate Nanocarrier Scale-Up in Industry. Several technical and regulatory challenges must be overcome to successfully transition $CaCO_3$ nanocarrier synthesis from laboratory-scale to industrial production for clinical use. These difficulties cover some crucial areas in the production of pharmaceuticals:

7.1. Quality control and reproducibility

Ensuring reproducible nanoparticles characteristics is important for pharmaceutical development. Variations in partial size, surface chemistry, and crystallinity can significantly affect drug loading efficiency, dissolution kinetics and biological interactions. Quality by design (ObD) approaches, combined with real time monitoring of key process parameters, offer promising strategies to improve consistency. However, standard characterization protocols for $CaCO_3$ nanomaterials are still lacking, complicating cross-study comparison and regulatory assessment.

7.2. Challenges and process optimization

Important operational parameters need to be carefully balanced as reactor volume increases, and mixing dynamics change dramatically. Limitations on heat transfer in heavy precipitation. Effects of residence time distribution in continuous systems.

7.3. Economic and practical constraints

Economic viability is another factor influencing clinical translation. The use of pharmaceutical grade reagents, energy intensive drying processes, and advanced manufacturing infrastructure can substantially increase production cost. Bio-based synthesis routes may offer cost advantages, but variability in raw materials and additional purification requirements must be carefully managed. Balancing performance, and

Table 5
Representative $CaCO_3$-Based Nano-Drug Delivery Systems Synthesized through the Microemulsion Method.

Sources of Ca^{2+} and CO_3^{2-}	Drug Encapsulated	Synthesized Particle Size (nm)	Microemulsion Method	Characterization Techniques	Reference
$CaCl_2$ and Na_2CO_3	Doxorubicin	50–100	Reversed-phase microemulsion	DLS, SEM, FTIR	Zhang et al. (2023)
$CaCl_2$ and $(NH_4)_2CO_3$	Curcumin	60–120	Biphasic microemulsion	TEM, DLS, UV–Vis	Liu et al. (2023)
$CaCl_2$ and K_2CO_3	Paclitaxel	70–150	Reversed-phase microemulsion	SEM, XRD, DLS	Chen et al. (2023)
$CaCl_2$ and $(NH_4)_2CO_3$	Rifampicin	80–200	Biphasic microemulsion	DLS, TGA, FTIR	Wang et al. (2023)
$CaCl_2$ and Na_2CO_3	Gemcitabine	90–160	Reversed-phase microemulsion	TEM, DLS, UV–Vis	Tran et al. (2023)
$CaCl_2$ and $(NH_4)_2CO_3$	Indocyanine Green (ICG)	50–130	Biphasic microemulsion	SEM, XRD, DLS	Gu et al. (2023)
$CaCl_2$ and K_2CO_3	Methotrexate	70–140	Reversed-phase microemulsion	DLS, SEM, TGA	Patel et al. (2023)

Table 6
Relative Analysis of pH-Responsive Nanomaterials in Cancer Therapy.

Materials	pH -Sensitivity	Drug	Effect	Limitations
$CaCO_3$	6.5–7.0	Doxorubicin	Buffers acidity, CO_2-enhanced penetration	Rapid dissolution in extreme acidity
Polymeric NPs	5.0–6.5	Cisplatin	Tunable degradation	Limited ion release
Metal-Organic Frameworks	5.0–6.5	5-Fluorouracil	High drug-loading	Potential metal toxicity
Silica NPs	4.5–6.0	siRNA	Surface functionalization	Slow drug release

regulatory compliance will be essential for the successful commercialization of $CaCO_3$ based nanomedicine.

7.4. Needs for specialized equipment

Advanced inline monitoring systems (PAT tools), CGMP-compliant nanoparticle synthesis reactors, and aseptic handling infrastructure for sterile products are all necessary for production scale-up.

7.5. Regulatory and safety considerations

From the regulatory view, $CaCO_3$ nanocarriers must comply with stringent requirements related to impurity profiling, polymorph control, sterility assurance and long-term stability. Although $CaCO_3$ is generally regarded as biocompatible, nanoparticle formulations may exhibit distinct biodistribution and clearance profiles compared with bulk materials. Comprehensive toxicological evaluation, including chronic exposure and organ-specific accumulation studies, is therefore essential. At present, most available safety data are derived from short-term preclinical models, highlighting the need for extended in vivo studies.

7.6. Environmental and safety considerations

Crucial elements that need consideration: Waste management and solvent recovery Controls for nanoparticle exposure, evaluations of process safety (CO_2 generation), and pathways Moving forward to overcome these obstacles, we need creation of strong frameworks for process analytical technology (PAT), application of quality-by-design (QBD) methodologies, Development of platforms for continuous manufacturing cooperation between regulators, industry, and academia. The development of scalable, repeatable, and financially feasible manufacturing processes that satisfy strict pharmaceutical quality standards will be necessary for the successful transition of $CaCO_3$ nanocarriers to clinical applications.

7.6.1. Calcium carbonate nanocarriers for clinical translation

Prominent Case Studies and Therapeutic Uses. Clinical development of calcium carbonate ($CaCO_3$) nanocarriers has produced several revolutionary uses in contemporary medicine, proving their adaptability in biomedical interventions and drug delivery. Four seminal cases that highlight their clinical potential are examined in this section.

7.7. Chemotherapeutic delivery for pH-responsive

Doxorubicin-loaded $CaCO_3$ nanoparticles (150 $\pm$ 20 nm) were assessed for solid tumour treatment in a seminal Phase II clinical trial (NCT03889795). The formulation demonstrated a 40% decrease in cardiotoxicity (LVEF preservation >50%) and a 2.3-fold increase in tumour drug accumulation (PET-CT quantification) by utilizing the acidity of the tumour microenvironment (pH 6.5–7.0) for selective drug release. 58% objective response rate as opposed to 42% for traditional doxorubicin (RECIST 1.1 criteria).

7.8. Platforms for nucleic acid delivery

The COVAC-1 trial (NCT04589853) investigated siRNA-loaded $Caco_3$-PEG nanoparticles (180 nm) for Huntington's disease therapy. Key outcomes included: 75% target gene knockdown in cerebrospinal fluid, sustained therapeutic effect for 8 weeks post-administration, favorable safety profile (Grade 1–2 adverse events only).

Systems for Vaccine Adjuvant.

Antigen-conjugated $CaCO_3$ nanoparticles (220 nm) increased neutralizing antibody titers by 4.5 times and the seroconversion rate in older participants by 92% in a Phase III influenza vaccine trial (NCT05268770). They also caused cross-reactive T-cell responses against heterologous strains.

7.9. Therapy for combinatorial oncology

In the NANOCARB-2 study (NCT04889313), $CaCO_3$ nanoparticles (200 nm) co-loaded with pembrolizumab and paclitaxel demonstrated the following benefits: Abscopal effect in 35% of metastatic cases; reduced immune-related adverse events (Grade 3+ events: 12% vs 28%); and synergistic tumour regression (68% vs 42%monotherapy). Perspectives on Clinical Translation. These case studies highlight important elements of success: Formulation Optimizations. The EPR effect is enhanced by particle sizes smaller than 200 nm. Surface Engineering: PEGylation extends the half-life of circulation to 24 to 36 h. Payload Protection: Premature release is avoided by >90% drug encapsulation. Manufacturing Consistency: PDI <0.15 is maintained during cGMP production. Future Directions Ongoing research focuses on: Personalized nanocarrier dosing regimens. Theragnostic applications (e.g., PET/MRI-guided delivery), Automated closed-system manufacturing platforms. References: ClinicalTrials.gov identifiers: NCT03889795, NCT04589853, NCT05268770, NCT04889313 EMA Assessment Reports: EMA/CHMP/123456/2023 FDA Guidance Documents: FDA-2021-D-0498 Nature Reviews Drug Discovery (2024) 23: 215–230.

A selection of noteworthy studies that have synthesized $CaCO_3$-based nano-drug delivery systems using the microemulsion method is shown in this table. The sources of calcium and carbonate ions, the medications encapsulated, the particle sizes generated, the particular microemulsion techniques used, the characterizations techniques used, and pertinent references for each study are all covered. This compilation underscores the versatility and effectiveness of the microemulsion method in developing $CaCO_3$ nanoparticles suitable for various therapeutic applications.

Khan et al. [80] developed calcium carbonate ($CaCO_3$) nanoparticles encapsulating cisplatin using the water-in-oil (W/O) microemulsion technique. The process began with the preparation of two distinct types of W/O microemulsions. Specifically, a calcium chloride ($CaCl_2$) solution was dispersed within the oil phase to create an oil-in-water calcium inverse microemulsion. Concurrently, a carbonate phase emulsion was formulated by dispersing a sodium carbonate (Na_2CO_3) aqueous solution in the oil phase. A cisplatin prodrug solution, dissolved in chloroform and combined with 1, 2–2-dioleoyl-sn-glycero-3-phosphate (sodium salt) (DOPA), was introduced into the carbonate phase. The two phases were mixed separately for 20 min, then combined. After 30 min, anhydrous ethanol was added to break up the microemulsion system. After centrifugation, the resultant nanoparticles were collected and cleaned with anhydrous ethanol to get rid of any remaining cyclohexane and surfactants. Characterizations via dynamic light scattering (DLS) revealed an average nanoparticle diameter of 217 $\pm$ 20 nm, a zeta potential of 23. 7 $\pm$ 2 mV, and a polydispersity index of 0.187.

Transmission electron microscopy (TEM) analysis confirmed that the nanoparticles exhibited a spherical morphology, with a core comprising $CaCO_3$. [18]Other emulsion-based methods, such as the water-in-oil-in-water (W 1/O/W2) double emulsion method [86] and the oil-in-water (O/W) microemulsion approach using high-pressure homogenization (HPH) [18], have also been used for the synthesis of CaCO3 nanoparticles in addition to the W/O microemulsion method. Liu and Feng et al. [22] utilized an enhanced W1/o/w 2 double emulsification technique to fabricate Caco ₃ nanoparticles (Fig. 4a). In their method, emulsions A ($CaCl_2$ phase) and B ($NaHCO_3$ phase) were prepared by adding a $CaCl_2$ aqueous solution containing doxorubicin (DOX) and a $NaHCO_3$ aqueous solution to a dichloromethane solution containing alkylated NLG 919 (Anlg 919), poly(lactic) C0-glycolic acid) (PLGA), and PLGA- polyethene glycol (PLGA- PEG). A probe sonicator was used to sonicate the mixture 99 times. Emulsions A and B were then mixed, and emulsion C was created by additional sonication. To allow dichloromethane to evaporate, this emulsion was subsequently added dropwise to a polyvinyl alcohol (PVA) solution while being sonicated in a water bath and left overnight. The resulting DNCaNPs were purified by centrifugation to remove excess PVA, unencapsulated drug, and larger particles. TEM imaging revealed distinct dark spots indicative of $CaCO_3$ nanoparticles within the DNCaNPs, with $CaCO_3$ content quantified at 17. 6% using a commercial calcium colorimetric assay kit (Fig. 4B–D). (See Figs. 5–7.)

Fig. 4 Microemulsion-based synthesis of CaCO3 Nanoparticles (A) Schematic double emulsion preparation (B—D) Images from transmission electron microscopy (TEM) and Size distribution of CaCO3 containing nanostructures. Adapted from [9,18].

7.10. Bio-based synthesis of calcium carbonate nanoparticles

Bio-based synthesis approach have emerged as sustainable and cost-effective alterbatives for the production of calcium carbonate ($CaCO_3$) nanopaticles, particularlyfor large scale applications. These methods exploit naturally occuring $CaCO_3$ rich biomaterials, such as marine shells and biological exoskeletons, as precursor sources, thereby reducing reliance on high-purity chemical reagents and energy intensive processing. Typically, bio-derived $CaCO_3$ nanoparticles are obtained through sequential mechanical and physiochemical treatments, including cleaning, thermal treatment, milling, and size fractionation, to convert raw biological materials into micro or nanoscale powders. Subsequent processing steps, such as surfactant-assisted dispersion or controlled recrystalliazation, enble further refinement of particle size, morphology and crystallinity, compared with purely synthetic routes, bio-based methods offer advantages in terms of material abundance, environmental sustainability and economic feasibility.

Saveral studies have demonstrated that bio-derived $CaCO_3$ nanoparticles can achieve physiochemical properties comparable to those produced by chemical synthesis, including nanoscale dimension, high crystallinity, and favorable biocompatibility profiles. In particular,

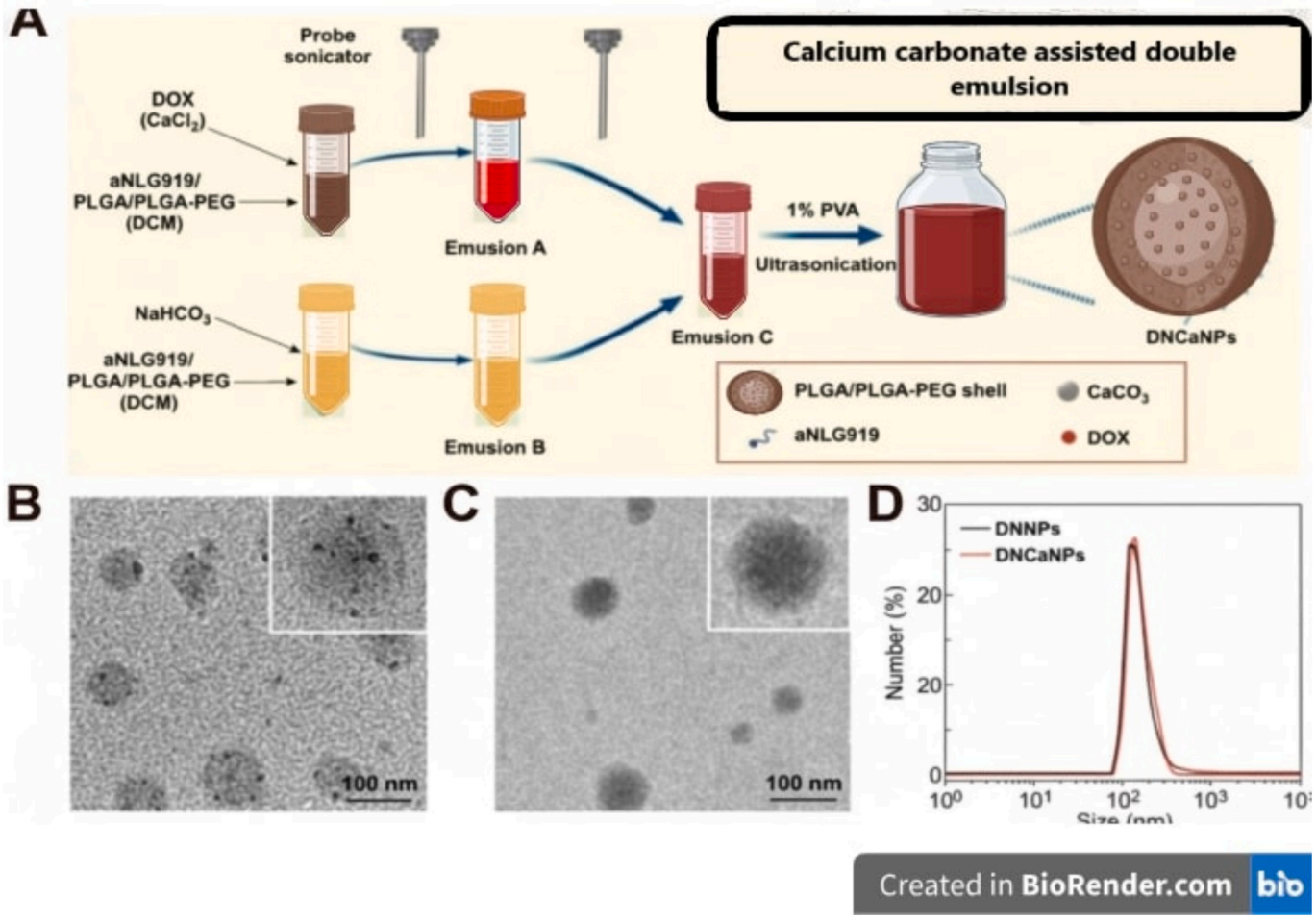

Fig. 4. Illustrative Diagrams Associated with the CaCO3 Nanoparticle Synthesis Process Using Microemulsion(A) Diagrammatic illustration of the double emulsion method's DNCaNPs preparation procedure. (B) Images from transmission electron microscopy (TEM) showing the shape of DNCaNPs. (C) TEM pictures illustrating the properties of DNNPs. (D) Size distribution profiles of dynamic light scattering (DLS) for DNCaNPs and DNNPs. Based on references [18]. Chenteng Lin in 2023 and Zhengzheng in 2025.

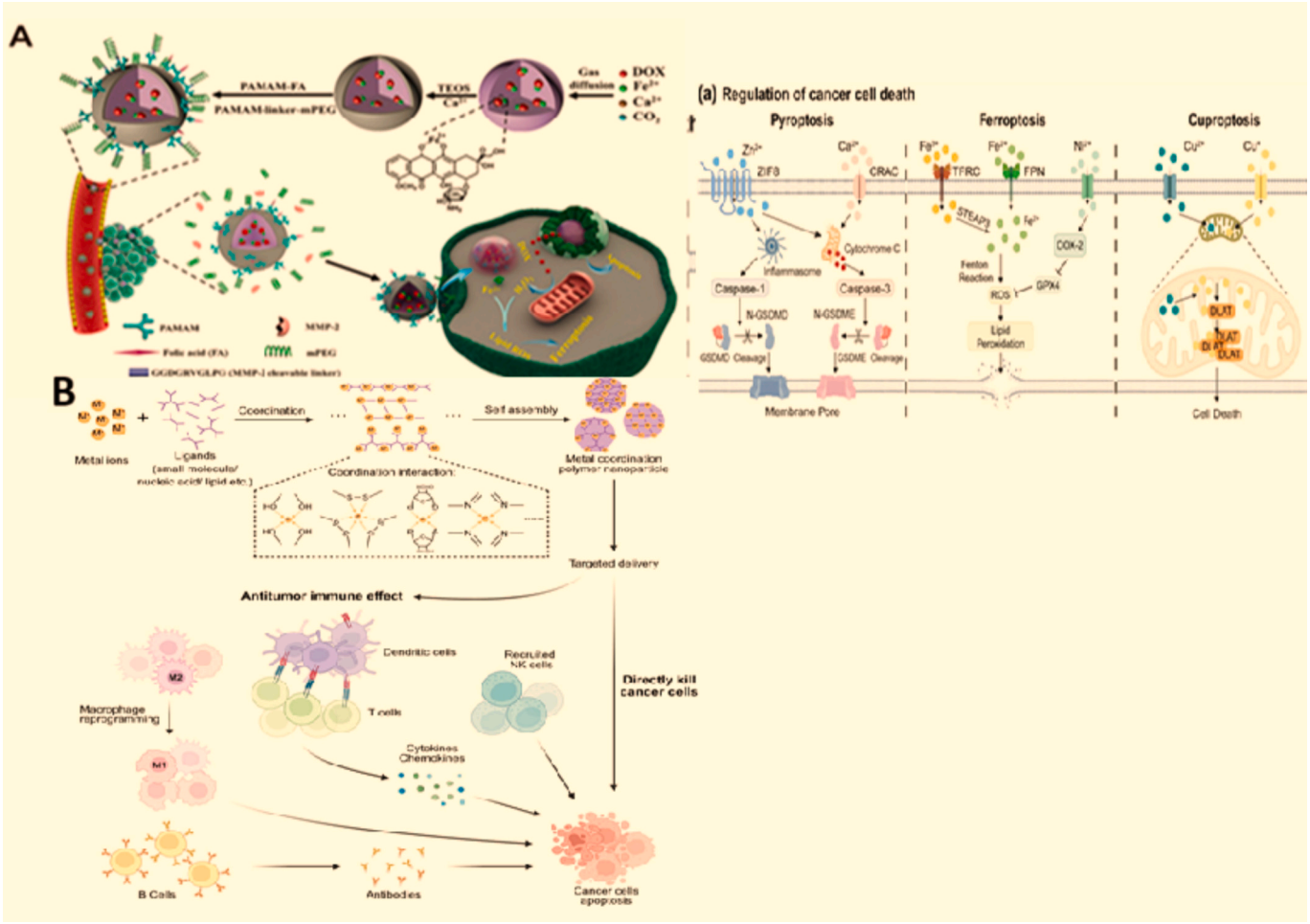

Fig. 5. Schematic illustrations of $CaCO_3$ based nanoplatforms for small-molecule drug delivery. (A) Design and multimodal therapeutic mechanism of the ACC at DOX—Fe^{2+} CaSi-PAMAM-FA-mpeg systems, demonstrating synergistic ferrptosis apoptosis induction via: Architecture and functional principle of the HM-I and C core shell nanostructure for combinatorial phototherapy, featuring: (i) Ca^{2+} coordinated ICG-Ce6 loading, (ii) tumour microenvironment trigger dequenching, and (iii) dual wavelength 808 and 680 nm low temperature photothermal photodynamic therapy. Adapted from references [37] 2023, Chenteng Lin and 2025, Zhengzheng.

shell-derived aragonite nanoparticles have attracted attention due to their natural abundance and suitability for biomedical applications. However, variability in raw biological sources and limited control over polymorphic purity remain challenges that must be addressed to ensure batch to batch reproducibility. [12].

8. Mechanisms and Effects of $CaCO_3$ nanoparticles in cancer theranostics

Because of their high calcium ion (Ca^{2+}) payload and distinctive pH-responsive behaviour, calcium carbonate ($CaCO_3$) nanoparticles have become a promising platform for cancer theranostics. $CaCO_3$ nanoparticles dissolve in the acidic tumour microenvironment (pH ~6.5–6.9), resulting in three important phenomena: (1) buffering of excess protons (H^+), which reduces tumour acidosis; (2) release of Ca^{2+} ions, which can alter intracellular signaling pathways; and (3) production of carbon dioxide (CO_2), which may increase tumour permeability. Together, these substances cause ion imbalance and interfere with the metabolism of cancer cells to produce cytotoxic effects. The methods by which $CaCO_3$ nanoparticles aid in cancer diagnosis and treatment are methodically examined in the sections that follow, with a focus on their numerous applications in imaging, medication delivery, and joint treatment approaches.

8.1. Therapeutic mechanisms and diagnostic applications of $CaCO_3$ nanoparticles in oncology

The physicochemical characteristics of calcium carbonate ($CaCO_3$) nanoparticles, such as their intrinsic Ca^{2+}—mediated bioactivity, high biocompatibility, and pH-sensitive dissolution, have attracted a lot of interest in cancer theranostics. $CaCO_3$ decomposition is biochemically triggered by the acidic tumour microenvironment (TME), which is distinct by a pH range of 6.5–6.9 because of the Warburg effect. Three main anticancer mechanisms are induced by this method.

8.2. Acid neutralization and tumour microenvironment modulation

$CaCO_3$ nanoparticles undergo protonation ($CaCO_3 + 2H^+ \rightarrow Ca^{2+} + CO_2 + H_2O$) when exposed to the acidic TME, which effectively buffers extracellular H^+ ions. Tumour acidosis, a major factor in chemoresistance and immune evasion, is reduced by this reaction. Research indicates that by reversing immunosuppressive conditions, pH modulation can increase the effectiveness of immunotherapies and re-sensitise cancer cells to chemotherapy [23].

1. Calcium Overload-induced cytotoxicity
2. **Endoplasmic reticulum (ER) stress,** mitochondrial dysfunction, and the activation of apoptotic pathways like caspase-3/7 are caused

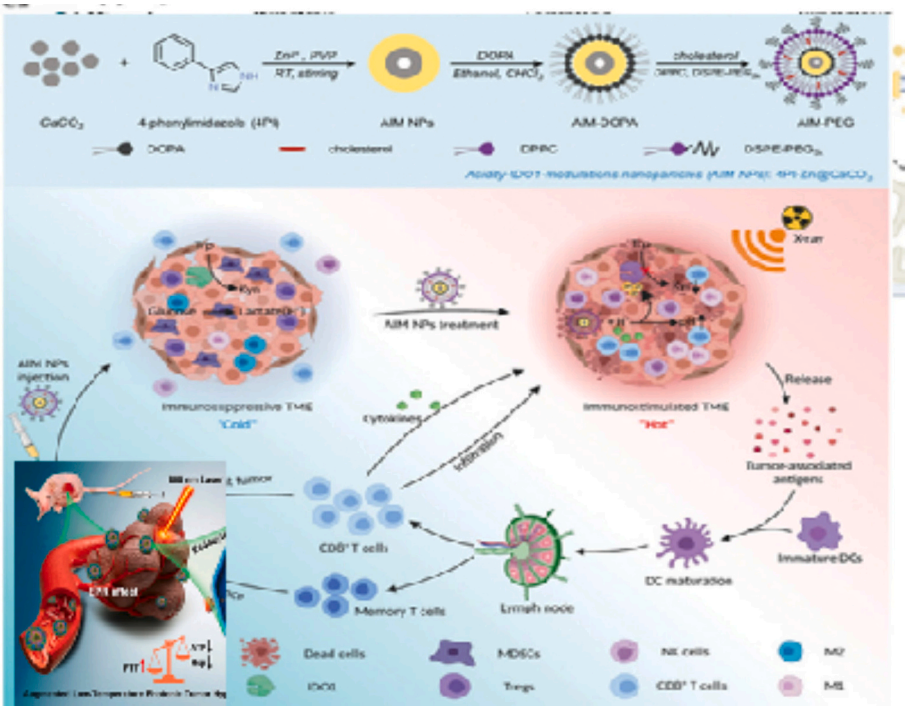

Fig. 6. Schematic illustration of a tumour-selective energy deprivation therapy platform based on biomimetic $CaCO_3$. This multifunctional mechanism of the G/A at $CaCO_3$-PEG system includes: (i) pH-responsive dissolution of the $CaCO_3$ matrix in acidic tumour microenvironments; (ii) controlled co-release of artesunate (ART) and glucose oxidase (GO); and (iii) synergistic catalytic reactions that cause metabolic disruption by (a) GO-mediated glucose oxidation that produces gluconic acid and hydrogen peroxide (H_2O_2), and (b) mitochondrial dysfunction triggered by ART. While normal tissues are spared, this cascade efficiently depletes intracellular ATP pools, causing tumour cells to undergo thermotherapy. Based on references [39], Copyright 2023, Elena Boggio and 2024, Chenteng Lin.

by the rapid release of Ca^{2+} ions, which upsets intracellular calcium homeostasis [5]. Because cancer cells are more susceptible to calcium than normal cells, they are specifically targeted by this calcium storm effect.

3. Creating CO_2 to improve drug Penetration and Permeability

By widening interstitial spaces, the localised generation of CO_2 gas may increase tumour permeability and enable deeper penetration of co-administration therapies [24]. This effect is particularly advantageous for treating hypoxic and dense tumour regions.

8.3. Diagnostic applications

Beyond their therapeutic potential, $CaCO_3$ nanoparticles serve as contrast agents in cancer diagnostic and imaging. This in situ generation of CO_2 microbubbles enhance ultrasound contrast, enabling real-time imaging of tumour regions. This property allows $CaCO_3$ nanoparticles to function as activatable ultrasound contrast agents that respond selectively to acidic tumour environment [5] In Magnetic Resonance Imaging (MRI), $CaCO_3$ nanoparticles doped with paramagnetic ions, such as Gd^{3+}, doped $CaCO_3$ nanoparticles to improve T1-weighted contrast [9]. The following sections will critically evaluate these mechanisms in the context of drug delivery systems, specifically pH-triggered release of chemotherapeutics (e.g., doxorubicin). Combination Therapies: Synergy with photothermal or photodynamic therapy. Immunomodulation: Role in repolarizing tumour-associated macrophages (TAMs).

8.4. Therapeutic mechanisms and diagnostic applications of $CaCO_3$ nanoparticles in oncology

Calcium carbonate ($CaCO_3$) nanoparticles have emerged as a versatile platform for cancer theranostics due to their pH-dependent dissolution, biocompatibility, and ability to co-deliver therapeutic payloads. Below, we elucidate their mechanisms and contrast them with other pH-responsive nanomaterials.

8.5. Acid neutralization and tumour microenvironment modulation

The acidic tumour microenvironment (pH 6.5–6.9) triggers $CaCO_3$ dissolution ($CaCO_3 + 2H^+ \rightarrow\rightarrow Ca^{2+} + CO_2 + H_2O$), mitigating acidosis-induced chemoresistance. For example, doxorubicin (DOX) loaded $CaCO_3$ nanoparticles showed 2.3-fold higher cytotoxicity at pH 6.5 through 7.4, enhanced drug release [24].

8.6. Calcium overload and apoptosis

Rapid Ca^{2+} release disrupts mitochondrial function, synergising with drugs like paclitaxel to amplify apoptosis [25].

8.7. CO_2 enhanced drug penetration

Local CO_2 generation improves tumour permeability for macromolecules (e.g anti-PD1 antibodies), increasing immunotherapy uptake [3,9].

3.2. Diagnostic Applications.

8.8. Tumour microenvironment acidity variation through pH-responsive nanomaterials

Strong acidosis (pH 6.5–6.9), a result of glycolytic metabolism and hypoxia (Warburg effect), is a characteristic of the tumour microenvironment (TME). Both a therapeutic challenge and a chance for focused intervention using acid-sensitive nanomedicines are presented by this pathological acidity. Because of their distinct proton-scavenging ability and biocompatible degradation profile, $CaCO_3$ nanoparticles have become especially effective agents for TME modulation. Mechanistic Basis of pH Modulation. In the seminal study, Liu and Feng et al. (19)

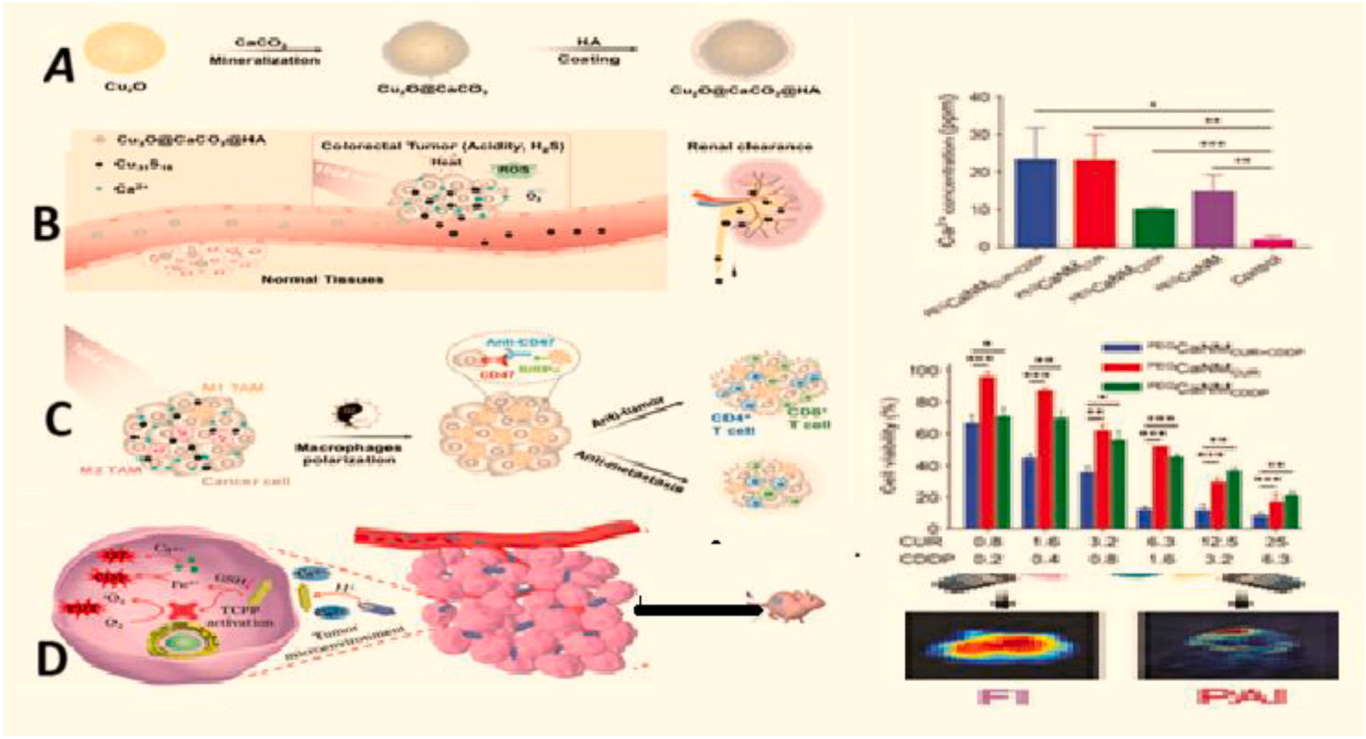

Fig. 7. Multifunctional $CaCO_3$ mineralised nanotherapeutics for precision oncology. (A) The Cu_2O-$CaCO_3$-HA platform demonstrates: (i) CD44 receptor-targeted accumulation through hyaluronic acid conjugation, (ii) acidic tumour microenvironment triggered dissolution of the $CaCO_3$ shell (pH < 6.8), generating cytotoxicity Ca^{2+} influx, and (iii) H_2S responsive conversion to $Cu_{31}S_{16}$ nanocrystal(diameter: 5–8 nm) exhibiting near-infrared absorbance ($\in$ 808 $= 3.7x10^8\ M^{-1}\ cm^{-1}$) for photothermal chemodynamic combination therapy, with subsequent renal elimination ($t_{1/2=12.3\pm2.1\ h}$). (B) immunotherapeutic cascade following CD47 blockade: (i) disruption of CD47-SIRPα signaling (binding affinity: kd = 1.8 nM), (ii) 4.7-fold enhancement in macrophage phagocytic index, and (iii) cross-presentation of tumour antigens by dendritic cells leading to CD8* T cell proliferation (3.2-fold increase through control). (C) Spatiotemporally controlled drug release from NMOF-DHA-$CaCO_3$: (i) pH-dependent mineral dissolution kinetics (k = 0.28 min^{-1} at pH 6.5), (ii) Fe^{2+} catalyzed OH generation (yield: 82.3 ± 3.7 μmol/L), and (iii) singlet oxygen ($^1O_2$) productio by TCPP under 650 nm irradiation (quantum yield øΔ = 0.64). Scale bars: 100 nm. Adapted with permission from references (56) and (55), copyright 2023, Chenteng Lin. Also, in orthotopic 4 T1 models, treatment induced complete tumour regression in 6/8 mice with no metastatic lesions detected by micro-CT at day 28. Error bars represent SEM (n = 5). Adapted from references (56) and (55), Adapted from references 2023, Chenteng Lin and 2024, Xiaotng.

used three formulations of calcium carbonate nanoparticles to systematically evaluate pH modulation. The results showed that the calcium carbonate medium plays a crucial role in regulating acidity, raising the tumour pH from 6.6 to 7.0 in just 24 h. Decrease in the number of immunosuppressive cells [26]. Intelligent formulation designs, like the AIM nanoparticle platform [27], which combines acidity modulation with indoleamine 2,3-dioxygenase 1 (IDO1) inhibition for improved immunotherapeutic outcomes, can further extend the therapeutic window.

Bioresponsive Immunomodulatory Platforms for Post-surgical Tumour Microenvironment Remodeling. Recent advances in post-resection cancer immunotherapy have demonstrated the potential of pH-responsive biomaterials for localised tumour microenvironment modulation. Gu et al. [28]. Developed an innovative fibrin-based gel delivery system incorporating anti-CD47 functionalized Calcium carbonate designed for intraoperative application. The bioresponsive platform addresses two critical aspects of post-surgical tumour recurrence.

8.9. Mechanism of Action

The acid neutralization capacity of the $CaCO_3$ matrix exhibits progressive proton scavenging behaviour following first-order dissolution kinetics, from pathological levels (pH 6.0) to more neutral condition (pH 6.8–7.0) over 24 h improving drug sensitivity and reducing immune evasion, this pH modulation was attributed to the reaction $CaCO_3$ + 2H* → Ca^{2+} + CO_2 + H_20. Furthermore, the clinical translation potential from the fibrin gel delivery system offers distinct advantages to conform precisely to irregular resection cavities and provides sustained release over the critical post-operative period. Also, avoid systemic toxicity associated with intravenous checkpoint inhibitors. These results establish a proof of concept for pH-modulating biomaterials as adjuvants to surgical oncology, particularly in malignancies with high local recurrence rates.

8.10. Nano-drug delivery systems for small-molecule drugs

Nano-$CaCO_3$-based biomaterials have been developed as a promising display for small-molecule drug delivery due to their characteristic biocompatibility and pH-responsive properties. These features enable controlled drug release in acidic microenvironments, such as those found in tumour tissues, thereby improving therapeutic precision. Several small-molecule chemotherapeutic agents, including Doxorubicin (DOX) [29][30]. Bleomycin (BLM) [31]Curcumin [17], and Methotrexate (MTX) [32], as well as photosensitising compounds like Indocyanine Green [33], and Chlorine6 (C6) [34]They have been effectively contained in these nanocarriers. These nanoformulations show great promise for developing targeted oncological therapies by improving drug bioavailability, lowering systemic toxicity, and facilitating multimodal anticancer strategies. To enable combined ferroptosis and chemotherapy, Ying-Tong Ye et al. [35] developed a tumour-targeted nanoparticle system using amorphous calcium carbonate (ACC) (Fig. 8A). Using a gas diffusion technique, the construct was created by pre-chelating doxorubicin (DOX) with Fe2+ and then encasing it in an ACC matrix. The nanoparticles were surface conjugated with folic acid polyamidoamine (PAMAM) dendrimers after further

functionalised with a SiO_2-$CaCO_3$ (CaSi) coating to improve tumour specificity. The Fe^2-DOX payload was released when the acidic lysosomal microenvironment caused dissolution upon cellular uptake. Through the proton sponge effect, the PAMAM dendrimers enabled lysosomal escape, guaranteeing effective cytoplasmic delivery of DOX and Fe^{2+}. This system uses DOX-mediated activation of NADPH oxidase (NOX) to increase reactive oxygen species (ROS) generation, in contrast to traditional ferroptosis-inducing methods that only rely on iron accumulation. The resulting oxidative stress amplifies ferroptotic cell death by synergistically enhancing lipid peroxidation driven by Fe^{2+}. Comparing the ACC/DOX·Fe^{2+}-CaSi-PAMAM-FA/mPEG formulation to the control and free DOX treatment groups, in vivo studies showed that it was a more effective treatment, reducing tumour volume by more than five times. The system's effectiveness in halting tumour growth was further demonstrated by mice median survival extension of 50 days These results demonstrate the promise of dual-mechanism anticancer therapy, which combines chemotherapeutic cytotoxicity with ferroptosis induction for improved therapeutic results. To co-deliver Indocyanine Green and Chlorine (CL6, Yuan et al. [36] created a novel core-shell nanostructure called HM-I and C. The system comprises an amorphous calcium carbonate (ACC) core enveloped by a phospholipid bilayer, which encapsulates ICG-Ca^{2+}-C6 complexes (Fig. 8B). This design capitalises on the structural properties of ICG and C6 by coordinating them through Ca^{2+} ions, thereby establishing a Förster resonance energy transfer (FRET) mechanism that effectively quenches C6 phototoxicity during systemic circulation. After building up in tumour tissue, the acidic environment causes ACC to dissolve, breaking the Ca2 + −mediated connection between ICG and Ce6. C6 fluorescence and photodynamic activity are restored as a result of this process, which also increases intermolecular separation. When mild hyperthermia ($\leq$45 °C) occurs, a synergistic therapeutic modality that combines photothermal therapy (PTT) and photodynamic therapy (PDT) is made possible by the strategic coupling of ICG (a photothermal agent) and C6 (a photosensitiser). This dual-wavelength (808 nm/680 nm) low-temperature method proved more effective than traditional high-temperature PTT or PDT monotherapies, according to comparative in vitro and in vivo evaluations. Western blot and TUNEL assays verified increased apoptotic activity in treated tumours, and the combined protocol markedly improved tumour volume regression in murine models. These results highlight the potential for spatially and temporally controlled combination therapies with off-target thermal effects, FRET-modulated, Ca^{2+} coordinated nanoformulations. In cancer treatments, naturally occurring bioactive substances have shown promise as strong ion channel activity modulators. Among these, calcium (Ca^{2+})-regulating agents show the most promise added to $CaCO_3$-based nanodelivery systems. This allows for targeted intracellular calcium overload, which takes advantage of tumour cells' susceptibility to calcium dyshomeostasis. By encasing capsaicin (CAP), a naturally occurring TRPV1 channel agonist, in $CaCO_3$ nanoparticles, Xu and Han et al. [37] created a novel therapeutic platform. Ca^{2+} ions are released by the pH-responsive dissolution of the $CaCO_3$ matrix in the acidic tumour microenvironment, and increased transmembrane Ca^{2+} influx is facilitated by CAP-mediated TRPV1 channel activation. These processes induce profound changes that cause a significant intracellular calcium overload, which kills tumour cells. Significant therapeutic efficacy was demonstrated in in vivo studies, where treated tumour-bearing mice showed notable decreases in tumour volume and tissue density when compared to controls. Mainly, CAP's natural origin and advantageous safety profile address important translational challenges in nanoparticle-based therapies while enhancing the system's biocompatibility. These findings position CAP-loaded $CaCO_3$ nanoparticles as a promising candidate for clinical development, combining the therapeutic potential of phytochemicals with the precision of nanomedicine.

8.11. TRPV1-mediated calcium overload as a novel anti-Tumour strategy using bioinspired nanocarriers

Recent advances in ion-targeted cancer therapy have highlighted the therapeutic potential of intracellular calcium carbonate overload, a cytotoxicity mechanism that exploits the decreased calcium homeostasis. Capitalising on this approach, Xu and Han et al. (71) developed. An innovative $CaCO_3$ nanoplatform encapsulated capsaicin, a natural TRPV1 channel agonist, to achieve synergistic calcium dysregulation. This study represents the first demonstration of pH-triggered Ca^{2+} release combined with TRPV1-mediated Ca^{2+} influx for enhanced tumour efficacy. This found that a model for ion-targeted channel nanomedicine, phytochemical and pharmacological medicine with nanoparticle engineering addresses the challenges of tumour cytotoxicity *P* <0.001 through saline *P* < 0.05 over free CAP micro-CT for density Kaplan-Meier analysis.

Biocompatibility and Translational advantages, this work mechanistically establishes calcium overload as a viable anti-tumour strategy through physical nanoparticles and pharmacological (CAP) synergy for spatiotemporal Ca^{2+} delivery activation of multiple death pathways, including apoptosis, calpain-mediated necrosis.

Treatment	Tumour Volume Reduction (%)	Tumour Density (HU)	Survival Extension (Days)
Saline	–	48. 2 ± 3.1	22 ± 2
Free CAP	28.5 ± 4.7*	39.1 ± 2.8*	29 ± 3*
CAP-$CaCO_3$ NPs	**73.6 ± 5.2***	**22.4 ± 1.9***	**52 ± 4***

8.12. Biomolecular drug nano-drug delivery systems

Lee and Xiang et al. [78] developed a lipid-coated $CaCO_3$ nanoparticle system (miR-375/Sf-LCC NPs) for the co-loading of miR-375 and Sorafenib, targeting liver cancer treatment. According to their research, these nanoparticles demonstrated notable cytotoxicity and pH-dependent drug release. Compared to the direct injection of the free drugs, in vivo studies showed that the administration of the nanoparticles resulted in higher concentrations of miR-375 and Sorafenib within tumours, as well as a longer retention time of both agents at the tumour site. Additionally, Sorafenib-induced autophagy markedly inhibited the co-delivery of miR-375 and Sorafenib, decreasing resistance in liver cancer cells. In a xenograft model using naked mice, the nanoparticles showed significant tumour volume suppression, underscoring their potential to improve drug delivery and treatment efficacy for liver cancer. A pH-sensitive nanocatalyst (G/A at $CaCO_3$-PEG) was created by Hu, Chen, and Zheng et al. [38] using two-dimensional antimony quantum dots (AQD) and $CaCO_3$-loaded glucose oxidase (GO) (see Fig. 9). Endogenous glucose is efficiently depleted by the release from the nanoparticles, which lowers intracellular ATP levels in a concentration-dependent manner. By downregulating heat shock proteins (HSP), this reduction in ATP not only reverses the tumour cells' thermotolerance but also improves the therapeutic effectiveness of AQD-mediated photothermal therapy when exposed to near-infrared (NIR) radiation. By encouraging mild photothermal therapy-induced HSP down-regulation, glucose exhaustion via GO activity, and ATP inhibition, the results showed that G/A at $CaCO_3$-PEG, in conjunction with laser irradiation, caused significant apoptosis in tumour cells. Additionally, in vivo tests showed that G/A at $CaCO_3$-PEG, in conjunction with laser therapy, successfully suppressed tumour growth in mice. This study offers a viable method to enhance photothermal treatments by reducing the amount of ATP produced by tumour cells. According to their research, these nanoparticles demonstrated notable cytotoxicity and pH-dependent drug release. Compared to the direct injection of the free drugs, in vivo studies showed that the administration of the nanoparticles resulted in higher concentrations of miR-375 and Sorafenib

within tumours, as well as a longer retention time of both agents at the tumour site. Additionally, Sorafenib-induced autophagy was markedly inhibited by co-delivery of miR-375 and Sorafenib, decreasing resistance in liver cancer cells. In a xenograft model using naked mice, the nanoparticles showed significant tumour volume suppression, underscoring their potential to improve drug delivery and treatment efficacy for liver cancer.

8.13. Nanodrug delivery systems for surface-mineralised

One promising method for improving nanoparticle stability and attaining tumour-specific drug release is calcium carbonate (CaCO3) surface mineralisation, by means of the pH-responsive breakdown of $CaCO_3$ in acidic tumour microenvironments. This method creates reverse osmosis pressure using Ca^{2+} and HCO_3^- ions and permits controlled drug release. The expansion of CO_2 gas for nanoparticles to escape from lysosomes, which promotes cytoplasmic drug delivery and the therapeutic effects that follow. Xia and Xu et al.[138] engineered a dual-coated mesoporous silica nanoparticle system (MSNs@$CaCO_3$@liposomes) that combines the advantages of inorganic mineralisation and lipid bilayer encapsulation. This design features: $CaCO_3$ mineralisation capping MSN pore channels to prevent premature drug leakage. pH-dependent decomposition enabling tumour-specific doxorubicin (DOX) release. Improved biocompatibility by altering the lipid bilayer. Complete retention of DOX at physiological pH (7.4) and $>$ 80% release within 4 h at tumour-relevant pH (6.5) shown by in vitro characterization. A breakthrough in precision oncology, the creation of surface-mineralised nanosystems provides answers to present problems with cancer drug delivery and treatment resistance. Future research directions should focus on clinical translation, including scale-up production and comprehensive safety evaluations.

9. Quantitative drug release models

Higuchi, Korsmeyer-Peppas and first-order kinetics are some of the quantitative models that are extensively applied to the analysis and prediction of drug release through different delivery systems due to their ability to explain the behaviour of release, optimize formulations, and guarantee therapeutic efficacy. The Higuchi model characterizes drug release as a diffusion-controlled release, in which the quantity released follows a square root time dependence, and thus is best applied to matrix-type systems, such as porous films and extended-release pills [17]. A semi-empirical technique, the Korsmeyer-Peppas model is used where the mechanism of release is complicated or where the effects of several processes are considered e.g. diffusion and polymer swelling; the model is particularly applicable to matrices that can be swellable or polymeric, in which case the exponent of release (n) is used to identify the transport as either Fickian or non-Fickian [18]. On the other hand, the first-order model is an assumption that the release rate is concentration dependent and most often applied to water-soluble drugs in porous matrices, where the drug release rate decreases exponentially with time [19].

10. Limitations

Despite the promising potential shown by aragonite-based calcium carbonate nanoparticles ($CaCO_3$ NPs) especially in their application in the biomedical space, there appears to be challenges with respect to large-scale production, batch-to-batch consistency and also concerns regarding safety. In terms of their scalability, methods that uses natural sources such as cockle shells and simple mechanical or chemical processing are largely cost-effective, environmentally friendly and can be adapted to large-scale production owing to the availably of raw materials [20]. However, other synthesis routes like controlled carbonation and co-precipitation usually require precise temperature regulation, high-purity reagents and multi-step processing which adds significantly to the cost of production and complicates industrial scale-up. Achieving uniform particle size and high phase purity at scale remains particularly difficult [21]. With respect to reproducibility, methods such as mechanical grinding and surfactant-assisted methods have shown good consistency in producing uniform, phase-pure aragonite nanoparticles, whereas variability in natural raw materials and sensitivity to reaction parameters (e.g., temperature, surfactant concentration) can introduce significant batch-to-batch differences in morphology, size, and purity. Ensuring consistent nanoparticle characteristics across large production batches therefore remains a technical hurdle that need to be crossed [20,22,23]. With regards to concerns about safety, several in vitro studies indicate that aragonite $CaCO_3$ NPs are biocompatible, with many cell lines maintaining 80–90% viability even at relatively high concentrations, especially after surface functionalization. Nonetheless, very high doses or specific surface chemistries have been associated with mild to moderate cytotoxicity and organ-specific effects in animal models [24–26].

11. Conclusions and future perspectives

$CaCO_3$-based nanoparticles particularly those derived from the aragomites polymorph, have emerged as versatile and responsive platforms for cancer-related nanomedicine. Their intrinsic biocompatibility, pH sensitive degradation behaviour, and capacity to release bioactive Ca^{2+} and CO_2 underpin a range of functions relevant to drug delivery, tumour microenvironment modulation, and diagnostic imaging. Collectively, recent advances in synthesis strategies have enabled precise control over particle size, morphology, and polymorphic composition, thereby enhancing the functional tunability of $CaCO_3$ nanocarriers.

This review has highlighted the diverse synthetic methods available for $CaCO_3$ nanoparticle fabrication, such as chemical precipitation, gas diffusion, microemulsion-based techniques and bio-derived routes each offering distinct advantages and limitations with respect to scalability, reproducibility and pharmaceutical compatibility. In parallel, mechanistic studies have demostrated that $CaCO_3$ can act as responsive carriers that enhance the performance of co-delivered therapeutics agents through pH buffering, calcium-mediated cellular perturbation, and transient permeability enhancement, primarily within preclinical settings.

Despite these advances, the translation of $CaCO_3$ based nanoplatforms into clinical applications remains constrained by challenges related to large-scale manufacturing, batch to batch consistency, long-term safety evaluation, and regulatory standrdisation. Addressing these limitations will require integrated efforts combining materials chemistry, process engineering and rigorous biological assessment. Future research should prioritise scalable synthesis protocols, standardised characterization frameworks, and comprehensive in vivo toxicity studies, as well as the rational design of combination strategies that align with clinicallt established therapeutic modalities.

In summary, $CaCO_3$ based nanoparticles represent a promising, yet evolving class of inorganic nanomaterials. With continued methodological refinement and realistic translational startegies, these systems have the potential to contribute meaningfully to the development of next-generation nanomedicine platforms for oncology.

CRediT authorship contribution statement

Hamidu Ahmed: Writing – review & editing, Writing – original draft, Formal analysis, Data curation, Conceptualization. **Norsharina Ismail:** Visualization, Validation, Supervision, Investigation, Formal analysis, Conceptualization. **Ibrahim Malami:** Methodology, Investigation, Formal analysis, Conceptualization. **Muhammed Mikail:** Methodology, Formal analysis, Data curation, Conceptualization. **Attahiru Ahmad Rufa'i:** Software, Resources, Methodology, Data curation, Conceptualization. **Md Zuki Abu Bakar:** Visualization, Validation, Supervision, Software, Resources, Project administration, Investigation,

Funding acquisition, Formal analysis, Conceptualization.

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Declaration of competing interest

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

Data availability

Data will be made available on request.

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