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International Journal of Pharmaceutical Research & Allied Sciences, 2025, 14(4):171-183

https://doi.org/10.51847/fdIWdom8xS

Original Article

ISSN : 2277-3657
CODEN(USA) : IJPRPM

Preserving Therapeutic Drug Ratios in Targeted Nanocarriers: A Mechanistic Framework Linking Coencapsulation, Differential Release, Intracellular Processing, and Combination Pharmacology

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ABSTRACT

Coencapsulation of therapeutic agents within targeted nanocarriers can coordinate their biodistribution and improve combination-treatment outcomes. However, the drug ratio established during formulation may change during circulation, tissue accumulation, cellular uptake, intracellular processing, and pharmacological target engagement. This article develops a mechanism-based theoretical account of how these transitions affect the relationship between pharmaceutical drug-ratio preservation and combination pharmacology. Drawing on published experimental, clinical, analytical, and mechanistic research, the analysis distinguishes initial coencapsulation ratios, compartment-specific intact-carrier ratios, released-drug proportions, intracellularly available concentrations, and pharmacologically effective exposure relationships. The central proposition is that coencapsulation creates an opportunity for coordinated delivery, while the preservation of therapeutically relevant ratios remains conditional on drug-specific transport, release, metabolism, sequestration, and temporal exposure. A further distinction separates ratio-dependent synergy from benefits attributable to greater total delivery, improved tolerability, or independent drug actions. The resulting account identifies measurable transitions, competing mechanisms, and discriminating experiments for evaluating combination nanocarriers. It accommodates circumstances in which synchronized exposure is beneficial and situations requiring sequential or spatially differentiated drug action. Therapeutic ratio preservation should therefore be evaluated as a compartment-specific, time-dependent pharmaceutical mechanism whose pharmacological significance requires direct evidence.

Key words: *Combination nanotherapy, Therapeutic drug ratios, Coencapsulation, Differential drug release, Intracellular drug exposure, Combination pharmacodynamics*

Received: 19 July 2025; **Revised:** 22 August 2025; **Accepted:** 27 Augist 2025

INTRODUCTION

Multidrug nanocarriers are increasingly investigated as pharmaceutical systems capable of coordinating therapeutic agents that otherwise exhibit different pharmacokinetic and biodistribution profiles. A comparative analysis of preclinical cancer nanomedicine found advantages for multidrug nanotherapy and, within the investigated systems, for coencapsulation over separately encapsulated combinations [1]. These observations establish an important formulation opportunity. Nevertheless, the translational evaluation of nanomedicines

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requires attention to how physicochemical properties, physiological transport, experimental models, and clinically relevant exposure interact [2]. Improved tumour inhibition or tissue drug accumulation cannot independently identify which intermediate delivery mechanism produced the observed benefit.

A further difficulty arises from the meaning of synergy. Pharmacological interaction is assessed relative to an explicitly defined expectation of noninteraction, which depends on concentration, endpoint, and analytical model [3]. The MuSyC approach illustrates why changes in drug potency and maximal efficacy represent different interaction dimensions and why conventional metrics can classify the same response differently [4]. Consequently, even an experimentally demonstrated favourable combination ratio may lack a stable pharmacological interpretation if the delivered concentrations, their temporal overlap, or the relevant cellular population changes.

The specific theoretical problem addressed here concerns the relation between the drug ratio manufactured into a carrier and the ratio that ultimately matters for biological action. Nanocarriers can encounter blood proteins, membranes, tissue barriers, extracellular environments, intracellular vesicles, metabolic transformations, and molecular targets. Each transition may alter the relative availability of the two agents. The challenge is to determine which changes constitute a meaningful loss of ratio coordination, which represent assay-dependent observations, and which are pharmacologically irrelevant.

This mechanism-based analysis develops operational distinctions between these states and examines their conditional relationships. Its objective is to identify the observations that would support ratio preservation as a causal explanation, the competing explanations that could account for similar outcomes, and the experiments necessary to distinguish them. The article advances testable theoretical propositions without presenting new experimental or clinical results.

Therapeutic drug ratios as a pharmaceutical design problem

The clinical development of CPX-351 provides a consequential example of ratio-directed combination formulation. In a randomized study involving older patients with newly diagnosed secondary acute myeloid leukaemia, liposomal cytarabine–daunorubicin treatment improved clinical outcomes relative to the conventional comparator regimen [5]. The product was designed to coordinate delivery of two chemotherapeutic agents at a specified molar proportion. Its pharmacological rationale incorporates liposomal stability, biodistribution, and tumour-cell uptake characteristics [6]. Although the clinical comparison demonstrates an advantage for the tested treatment regimen, those results do not separately quantify how much benefit originated from preservation of the drug ratio inside individual leukaemia cells.

A drug ratio requires an explicit operational definition. The initial formulation ratio, R_F, is the molar amount of encapsulated drug A divided by that of drug B in a specified batch. A carrier-associated ratio, $R_C(c,t)$, concerns amounts retained within intact carriers in biological compartment c at time t. A release ratio, $R_L(c,t)$, may describe either cumulative released amounts or instantaneous release fluxes; these definitions cannot be interchanged. An intracellular availability ratio, $R_I(j,t)$, refers to pharmacologically accessible drug concentrations in cell population j. These variables need not have equal values even when both agents originate within the same nanocarrier. Chemical identity must also be specified whenever an administered precursor produces a different active species. Research on cytarabine–idarubicin liposomes illustrates the relevance of relating dual-drug formulation characteristics to pharmacological experiments [7]. Yet plasma concentrations, total cellular uptake, and unbound intracellular concentrations refer to different molecular pools. The biologically meaningful quantity depends on the drugs' molecular targets, potency, subcellular distribution, and treatment duration. A measured ratio may also be unstable when one component approaches an assay's limit of quantification, making uncertainty in the denominator especially consequential.

Combination-response analysis introduces another level of definition. SynergyFinder provides tools for evaluating multidrug dose-response data under established interaction reference models [8]. Such analyses describe the relationship between administered concentrations and measured experimental responses under defined conditions. They do not independently reconstruct the concentration history of active drug molecules following nanocarrier administration.

A ratio-directed formulation therefore involves at least three distinct design choices: which nominal proportion to encapsulate, which biological exposure relationship is required for treatment, and how the carrier should alter the intermediate transport and release processes. Selecting a favourable ratio in a cell-culture experiment is an informative starting point, provided the corresponding concentration range and exposure schedule are relevant to

the intended delivery environment. Preservation of that nominal ratio becomes a scientifically meaningful design objective only when its connection to active exposure and pharmacological response can be tested.

Current evidence on multidrug nanocarrier systems

The published nanocarrier literature encompasses multiple strategies for coordinating drugs, including receptor-targeted liposomes, polymeric micelles, molecular coassemblies, and anatomically specialized formulations. An anti-CD33 antibody-functionalized liposome carrying cytarabine and daunorubicin investigated how targeting could modify the delivery of a fixed-ratio combination to acute myeloid leukaemia systems [9]. Such targeting introduces a mechanistic distinction between retaining both agents in a shared carrier and increasing carrier uptake by a selected cell population. Receptor binding may improve access while leaving post-internalization drug liberation unresolved.

An investigation of valrubicin–panobinostat micelles combined co-loading with ultrasound- and microbubble-mediated opening of the blood–brain barrier [10]. This system illustrates a different combination of mechanisms: the pharmacological pairing is accompanied by a physical intervention that modifies tissue access. Improved delivery in that setting cannot be attributed exclusively to ratio maintenance because barrier permeability, micellar disposition, and biological susceptibility also influence the observed response.

Coassembly provides a contrasting pharmaceutical strategy. Methotrexate and baricitinib were incorporated into an intermolecular-interaction-mediated nanomedicine investigated for rheumatoid arthritis treatment [11]. Here, molecular association contributes to the formation of the drug-containing structure itself. The relevant development question includes whether the association remains sufficiently stable under physiological conditions and whether its disruption releases the constituent agents in therapeutically suitable proportions.

Drug distribution can also change before a nanocarrier encounters its intended tissue. Experimental research on nanocarrier-mediated repartitioning of fingolimod between blood subcompartments demonstrates how carrier association affects interaction with red blood cells and plasma constituents [12]. Although this is not a dual-drug ratio-preservation experiment, it establishes the relevance of blood partitioning as a possible source of divergence when two coformulated molecules exhibit different affinities for biological components.

Specialized routes further demonstrate that combination delivery should be interpreted through its anatomical context. Inhaled verteporfin–pirfenidone nanoparticles have been investigated in pulmonary fibrosis, where the constituent agents address different pathological processes involving airway epithelium and fibroblasts [13]. Axitinib–telmisartan layered double-hydroxide nanocarriers have likewise been developed for ocular neovascular disease, linking local delivery with complementary vascular targets [14]. The useful exposure relationship in either system may depend on activity across distinct tissue or cellular compartments rather than the maintenance of one uniform intracellular ratio.

More directly relevant to ratio-directed oncology, a mesoporous silica formulation coencapsulating palbociclib and hydroxychloroquine was investigated in pancreatic cancer models. The study related in-vitro combination pharmacology to coordinated intratumour delivery and demonstrated improved preclinical treatment responses against specified controls [15]. This constitutes evidence that rational selection of a combination ratio can contribute to nanocarrier design. Nevertheless, differential intracellular processing and the duration of active molecular exposure remain important for interpreting the mechanism.

Combination delivery is also being investigated for diseases such as atherosclerosis, where relevant drug actions and delivery destinations vary with disease biology [16]. Across these settings, coencapsulation, tissue targeting, drug stabilization, and synchronized transport represent partially overlapping pharmaceutical functions. The evidence is strongest when a study provides explicit comparators for the mechanism under discussion. A response comparison alone is insufficient to distinguish all those functions.

Published systems thus differ in the identity of their shared material unit, the destination of their active agents, and the experimental evidence connecting delivery to pharmacology. These distinctions determine which ratio claims can be sustained, as summarized in **Table 1**.

Table 1. Distinct pharmaceutical evidence units in multidrug nanocarrier research and the extent of therapeutic-ratio inference

Pharmaceutical evidence unit	Representative investigated systems	Directly relevant observation	Ratio-preservation interpretation	Remaining mechanistic uncertainty

Fixed-proportion liposomal chemotherapy	Cytarabine–daunorubicin; cytarabine–idarubicin	Defined coformulation, pharmacological evaluation, and clinical or preclinical outcomes	Supports coordinated formulation and exposure as plausible contributors	Relative unbound active-drug exposure at individual cellular targets
Receptor-directed dual-drug liposomes	Anti-CD33 cytarabine–daunorubicin	Targeting and drug co-delivery in leukaemia-related models	Cell-selective carrier access can complement coencapsulation	Drug-specific endosomal escape and intracellular liberation
Barrier-assisted polymeric micelles	Valrubicin–panobinostat with sonopermeation	Delivery and therapeutic response under modified brain-barrier conditions	Physical barrier modification may enhance exposure opportunities	Independent contributions of permeability, micellar release, and ratio coordination
Drug–drug molecular coassembly	Methotrexate–baricitinib	Pharmaceutical assembly and anti-inflammatory treatment effects	Drug association may coordinate carrier composition	Stability and liberation behaviour after biological dilution or processing
Route-specific local nanocarriers	Pulmonary verteporfin–pirfenidone; ocular axitinib–telmisartan	Localized dual-agent delivery and disease-directed responses	Relevant coordination may occur at tissue rather than single-cell level	Exposure distribution among different target-cell populations
Ratio-directed mesoporous nanocarriers	Palbociclib–hydroxychloroquine	Designed co-loading, intratumour exposure, and preclinical combination response	Provides a comparatively direct experimental rationale for ratio-directed delivery	Whether active intracellular ratio trajectories independently mediate treatment benefit

Note. The entries identify evidence-supported system characteristics and conditional mechanistic interpretations. The distinctions between material state, cellular availability, and causal attribution constitute the present nonempirical synthesis. No inference of clinical equivalence across systems is intended.

Unresolved mechanisms governing drug-ratio changes during delivery

The journey from administration to pharmacological action traverses compartments whose transport properties differ substantially. Reviews of solid-tumour nanoparticle delivery describe vascular, stromal, cellular, and material-dependent restrictions on carrier access [17]. Experimental work identifying macropinocytosis as an important endothelial transport pathway demonstrates that carrier movement across tumour vessels can involve active cellular processing [18]. Earlier investigations likewise challenged an exclusively passive vascular-leakage explanation of nanoparticle entry [19]. These findings concern particle disposition. They do not establish that both payloads remain encapsulated during translocation or reach tumour cells in unchanged proportions.

The distinction between carrier movement and active-cargo delivery becomes especially important after internalization. Lipid-nanoparticle studies of RNA delivery identify heterogeneous endosomal processing and demonstrate that cellular uptake can be separated experimentally from successful cytosolic release [20]. RNA and small-molecule drugs have different physicochemical behaviour; therefore, the specific escape results cannot be transferred without qualification. Nevertheless, the broader mechanistic distinction is pertinent: entry of a carrier into a cell is not equivalent to arrival of its payload at a pharmacological target.

Measurements also impose constraints on what can be concluded. Quantitative imaging of intracellular nanoparticle exposure has been associated with predictive analysis of nanotherapeutic effects [21]. Such imaging can provide valuable spatial and cellular information, but the measured label may represent an intact carrier, a drug-associated structure, or a released component depending on probe design. A strongly retained fluorescent marker can therefore report carrier localization even after another component has undergone release, metabolism, or redistribution.

A critical assessment of nanoparticle quantification methods distinguishes functional effects, carrier tracking, and cargo tracking as different approaches to delivery measurement [22]. These approaches answer different questions. Bulk tissue drug analysis can reveal total local abundance while concealing spatial heterogeneity;

carrier imaging may establish cellular access without quantifying free drug; a functional response can indicate biological activity without revealing the concentrations responsible. Combining these measurements requires matched sampling times and explicit identification of molecular state.

Single-cell-resolution imaging across mouse tissues further illustrates how nanocarrier distribution varies within organs and among cellular populations [23]. An organ-average concentration ratio can therefore conceal markedly different local exposures. Even when the two drugs remain associated in circulating carriers, individual tumour cells may encounter different quantities of intact and previously released material.

These observations motivate a proposed distinction between ratio divergence and ratio irrelevance. Divergence occurs when the relative amount or concentration of the two partners changes between defined material or biological states. Irrelevance occurs when preserving their nominal proportion is unnecessary for the intended therapeutic mechanism, for example because the partners act at different destinations or times. The former requires measurements of transport and release; the latter requires pharmacological comparisons. **Figure 1** separates these questions across the compartments involved in delivery.

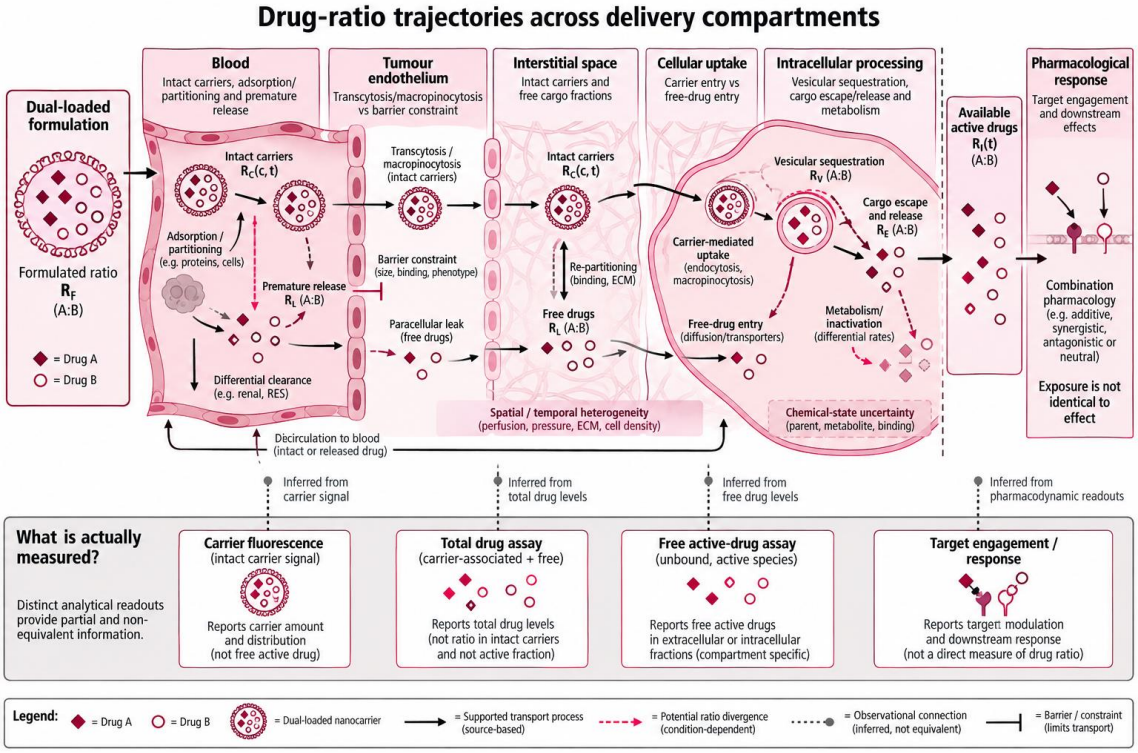


Figure 1. Therapeutic drug-ratio trajectories diverge when carrier movement, payload release, and intracellular availability become uncoupled

Caption and legend. A branching compartment network depicts the possible progression of two coencapsulated drugs from a manufactured carrier through blood, tissue barriers, extracellular compartments, cellular uptake, and intracellular processing. Parallel pathways distinguish intact-carrier retention, premature or differential release, compartment-specific redistribution, and loss of intracellular pharmacological availability. Solid arrows identify transport processes supported by relevant nanocarrier literature, without asserting that each occurs identically for both drugs. Dashed arrows identify conditional ratio-changing relationships advanced for empirical testing. Dotted connectors distinguish analytical observations from the material states inferred from those observations; blunt-ended connectors denote barriers or processes that constrain access. The final exposure state remains distinct from a pharmacological interaction outcome. R_F, formulation ratio; R_C, compartment-specific carrier-associated ratio; R_L, released-drug ratio; R_I, intracellular available-drug ratio. All ratios require defined chemical states, units, compartments, and sampling times.

Relationships between pharmaceutical ratio preservation and combination pharmacology

Pharmaceutical ratio preservation acquires therapeutic significance through the concentration-response relationships of the individual drugs and their combined activity. A carrier may preserve the intended molar proportion throughout circulation yet deliver concentrations that are insufficient for either agent to act.

Conversely, the original ratio may change while the resulting exposures still occupy a favourable region of the combination-response surface. The pharmacologically relevant objective is therefore specific to the interaction being sought.

Computational approaches to drug synergy illustrate both the value and the limits of formally relating paired exposures to treatment responses. A unified predictive model based on drug and cellular representations can estimate interaction patterns across combination datasets [24]. Such predictions remain conditional on the training data, concentration ranges, biological context, and endpoint definitions. They cannot independently establish the concentrations achieved after nanocarrier processing. Experimental screening platforms provide complementary information by measuring drug responses under controlled exposure conditions and allowing candidate combinations to be compared systematically [25].

These studies support a distinction between three related quantities: the nominal formulation ratio, the time-dependent pharmacologically accessible concentration ratio, and the combined response relative to a specified noninteraction model. The last of these also depends on absolute concentration. For example, two experiments can maintain an identical molar ratio while differing substantially in potency or maximal response because the total concentrations differ. Likewise, the temporal relationship between the drugs may change the interaction even when their cumulative exposure ratio is unchanged.

The consequences of differential release depend on the pharmacological mechanism. Where both agents must engage targets simultaneously within the same cell, substantial temporal separation could weaken an intended interaction. For a combination in which the first drug sensitizes cells to a later intervention, staggered exposure may instead be desirable. Implantable dual-drug depots designed to sequentially release a STING agonist and an apoptosis-inducing agent provide an experimental example of therapeutically purposeful exposure timing [26]. Such a system does not establish the optimal sequence for unrelated combinations, but it challenges the assumption that continuous ratio preservation is universally necessary.

Spatial separation raises a related question. A drug acting on tumour cells may be paired with an agent acting on immune or stromal cells. The relevant combination relationship may then involve coordinated exposure across different cellular populations. An identical intracellular drug ratio in every cell would offer limited mechanistic information if the two drugs engage distinct compartments.

A useful pharmacological interpretation consequently begins by specifying the intended treatment mechanism. For simultaneous intracellular interactions, evidence should establish overlapping active concentrations and an interaction response within a defined time window. For sensitization or cascade mechanisms, sequence and interval may be more informative than constant ratio. For multicellular mechanisms, local exposure across the relevant populations may be the critical variable.

Table 2 distinguishes these mechanisms through the ratio transitions they involve, their possible consequences for drug interactions, and the comparisons needed to determine whether preservation is causally relevant.

Table 2. Ratio-state transitions, pharmacological mechanisms, and comparisons capable of identifying the consequences of differential exposure

Ratio-state transition	Mechanistic variable	Potential interaction consequence	Discriminating comparison	Interpretation status
Formulated proportion to circulating carrier-associated proportion	Drug-specific leakage, retention, and blood partitioning	Altered availability before tissue delivery	Matched initial loading with measured carrier retention and plasma drug states	Proposed ratio-specific causal test
Carrier-associated proportion to extracellular released-drug proportion	Differential release kinetics and local clearance	Change in concentration pairing at the tissue site	Matched carrier accumulation with independent extracellular drug-time measurements	Mechanistically plausible; pair-specific validation needed
Extracellular exposure to intracellular active-drug proportion	Transport, vesicular sequestration, liberation, and chemical processing	Loss or restoration of simultaneous intracellular activity	Matched total uptake with measurements of chemically active intracellular drug	Proposed mechanism-discriminating test

Stable molar proportion at different absolute concentrations	Concentration-response shape and interaction-model assumptions	Different measured potency or efficacy interactions despite identical nominal ratios	Full concentration-response surfaces at fixed ratios and varying total concentration	Supported pharmacological principle
Simultaneous versus temporally staggered active exposure	Order, overlap, duration, and cellular response memory	Synchronization may help or hinder the intended combination mechanism	Concurrent and staggered regimens with comparable cumulative active exposure	Conditional; sequence-dependent mechanisms documented
Same-cell versus different-cell exposure	Spatial target distribution and multicellular signalling	A uniform intracellular ratio may be unnecessary for therapeutic cooperation	Cell-resolved exposure and pharmacodynamic assessments across relevant populations	Proposed generalization requiring system-specific evidence

Note. The rows distinguish measurable pharmaceutical states, established principles of concentration-dependent interaction, and proposed mechanistic comparisons. Predictions concerning particular drug pairs are conditional and must not be interpreted as experimentally validated by this article.

The resulting conceptual distinction is consequential for pharmaceutical development. A formulation that maintains a nominal ratio should be considered pharmacologically optimized only when the preserved exposure relationship is appropriate to the drugs' mechanisms and has been assessed against relevant alternatives. For some combinations, synchronized intracellular availability is a meaningful design target. In others, timed release, compartment-selective delivery, or unequal drug exposure may produce a more appropriate therapeutic interaction. The next section develops the causal relationships needed to distinguish these possibilities.

Development of the proposed mechanistic framework

The preceding evidence supports a mechanism-based account in which therapeutic ratio preservation depends on the movement and transformation of two drugs through distinct pharmaceutical states.

The relevant causal relationship begins with coencapsulation, continues through drug-specific disposition and cellular processing, and becomes pharmacologically meaningful only when the resulting active exposures interact within an appropriate biological context. Precision nanocarrier research establishes that material properties, physiological barriers, and cellular transport jointly influence delivery [27]. These processes must therefore be represented as conditional dependencies.

Let $A_j(t)$ and $B_j(t)$ denote the pharmacologically accessible concentrations of two active agents at their relevant sites in cellular population j and at time t. Their concentration ratio describes one aspect of joint exposure, while the individual concentration trajectories retain information concerning total exposure, temporal overlap, and drug-specific clearance. A pharmacological response can be conceptualized as a function of both trajectories, their interaction with cellular state, and the selected observation window. This expression is theoretical; it neither specifies a validated functional form nor assumes that identical concentration ratios produce identical responses.

Three mechanisms connect formulation design to this exposure relationship. First, coordinated carrier disposition can restrict differences in tissue arrival, provided the payloads remain sufficiently associated with the carrier. Second, release and intracellular processing determine whether shared arrival becomes simultaneous active availability. Third, the pharmacodynamic interaction determines whether the resulting exposure relationship is beneficial. A change at one level may be compensated, amplified, or rendered irrelevant by processes at another.

The resulting theory yields competing predictions. When intracellular target concentrations govern an interaction, formulations producing similar active exposure histories should generate similar combination responses after accounting for biological variability. If one formulation remains superior despite such matching, an additional mechanism—including spatial redistribution, altered cellular signalling, or a carrier-associated effect—must be considered. Conversely, equivalence of bulk tissue uptake cannot exclude meaningful differences in active intracellular exposure.

An essential methodological requirement is that each hypothesized transition be observable independently of the outcome used to judge its value. Pair-correlation microscopy offers one approach to studying intracellular molecular transport and barriers using fluorescence fluctuations [28]. However, a fluorescent tracer reports active-drug transport only when its correspondence with the relevant chemical species has been validated. Carrier

localization, molecular transport, pharmacological exposure, and target engagement should consequently retain separate evidentiary identities.

The proposed relationships are assembled in **Figure 2**. Their central implication is that coordinated delivery can influence the probability of achieving a useful exposure relationship, while pharmacological benefit remains conditional on concentration, timing, target accessibility, and the biological mechanism of interaction.

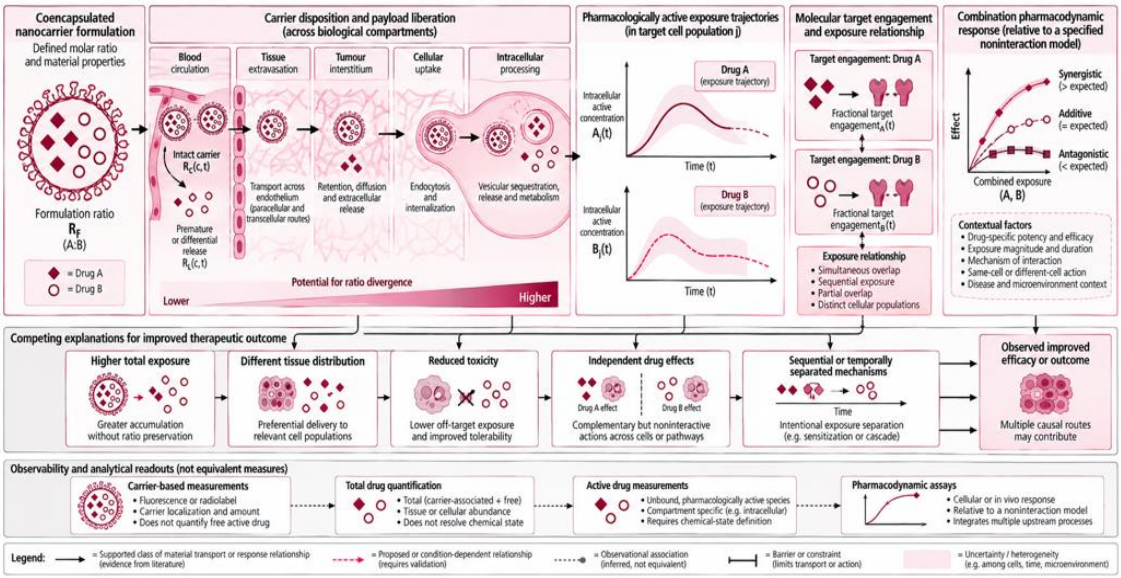


Figure 2. Coupled exposure trajectories determine when coordinated nanocarrier delivery becomes pharmacologically consequential

Caption and legend. A coupled exposure–effect model separates carrier-mediated delivery, drug-specific active exposure, and combination pharmacodynamics. The upper analytical field represents the transition from coencapsulated drug quantities to time-dependent exposure trajectories. A parallel pharmacological field represents drug-specific target engagement and interaction-dependent biological responses. Cross-links connect exposure timing, concentration magnitude, cellular location, and effect interpretation. Solid connectors indicate established classes of transport or pharmacological relationships; dashed connectors indicate proposed relationships requiring drug-pair-specific validation. A lower alternative-explanation field distinguishes ratio preservation from increased total delivery, altered tissue distribution, and independent drug actions. Different line patterns, boundary enclosures, and textual evidence-status markers communicate relationship types without reliance on colour. The figure expresses qualitative mechanisms and contains no fitted pharmacokinetic or pharmacodynamic parameters.

Implications for nanocarrier formulation design and translational evaluation

The pharmaceutical design objective should follow the required therapeutic interaction. When simultaneous exposure is essential, coencapsulation and carrier stability may be selected to coordinate tissue arrival, while release characteristics must support overlapping intracellular availability. When sequential or compartmentally differentiated activity is desired, deliberately unequal release may be more appropriate.

A sphingolipid-derived paclitaxel nanovesicle demonstrates the potential of integrating drug conjugation, transport engineering, and combination delivery. In preclinical breast and pancreatic cancer models, surface modifications designed to enhance transport and limit phagocytic clearance contributed to therapeutic performance when paclitaxel delivery was combined with other anticancer agents [29]. The result supports engineering delivery around multiple physiological constraints, although the benefit cannot be assigned exclusively to an unchanged drug ratio.

A different architecture has been demonstrated using dual asparagine-depriving nanoparticles. Extracellular asparaginase release was coupled with intracellular rotenone delivery to suppress complementary sources of asparagine availability [30]. This mechanism illustrates why two therapeutic components may require distinct sites of action and release conditions. A single same-cell ratio would inadequately describe their coordinated contribution.

Translation therefore requires evidence connecting critical material attributes with the relevant active exposure and pharmacological endpoint. **Table 3** distinguishes the design questions raised by different ratio-sensitive mechanisms and the corresponding measurements that could support a development decision.

Table 3. Conditional formulation-design decisions and evidence requirements for ratio-sensitive combination nanocarriers

Pharmaceutical design decision	Mechanistic requirement	Critical measurement	Informative comparator	Conditional development principle
Establish dual-drug loading	Both agents present in defined chemical states	Drug-specific loading and carrier-level distribution	Separate-drug and alternative-ratio formulations	Control individual carrier composition when heterogeneous loading changes delivery
Select carrier retention properties	Maintain suitable association before relevant tissue arrival	Intact-carrier and released-drug fractions across time	Formulations with differing retention	Maximize retention only when premature liberation undermines intended exposure
Determine release architecture	Match liberation kinetics to drug-specific action	Time-resolved release and active-species analysis	Simultaneous versus staggered release	Choose coordinated or differential release according to interaction mechanism
Optimize target access	Deliver the required agents to biologically relevant compartments	Compartment- and cell-resolved uptake and exposure	Targeted versus otherwise matched untargeted systems	Interpret targeting through active payload access, not carrier accumulation alone
Establish pharmacological relevance	Reproduce the exposure relationship associated with benefit	Active concentration trajectories and interaction response	Exposure-matched free-drug or alternative-carrier combinations	Require ratio-specific evidence before attributing benefit to preservation
Evaluate translation	Retain relevant product behaviour across biological and manufacturing contexts	Stability, release, exposure, safety, and pharmacodynamic comparability	Relevant biological models and manufacturing variants	Assess the causal attributes necessary for the intended clinical mechanism

Note. The final column contains original conditional design principles derived from the reviewed evidence; it is not a set of experimentally validated universal rules. No quantitative release limits, acceptable concentration ranges, or clinical equivalence criteria are proposed.

Mechanism-discriminating evidence and testable predictions

The proposed account is most useful when experiments can distinguish ratio-dependent effects from alternative delivery advantages. Such testing requires matched comparators, time-resolved measurements, and explicit pharmacological endpoints.

One discriminating experiment would compare two formulations with similar bulk tissue drug accumulation but different release and intracellular processing profiles. Measuring both chemically active agents at matched sampling times would test whether a change in intracellular ratio predicts treatment response after accounting for absolute concentration. A related experiment would preserve the same nominal drug ratio while varying the duration of intracellular exposure overlap. The resulting pharmacological classification should be assessed using complete concentration-response information.

Chemical activation presents an additional challenge. A dual-targeted SN38–JQ-1 nanoparticulate system employing conjugated prodrugs illustrates how engineered chemical linkages can influence the conversion of administered material into active agents [31]. For such systems, a direct mechanistic test would quantify intact

precursors, conversion products, and active-drug concentrations separately. Agreement between precursor loading and pharmacological response would be insufficient to establish the intervening mechanism.

Manufacturing provides another source of potential divergence. Comparative research on cytarabine–daunorubicin dual-encapsulation liposomes addresses how preparation procedures influence formulation properties and performance [32]. If manufacturing variants show different treatment responses despite matched administered amounts, a test should determine whether carrier composition, release behaviour, or biological distribution explains the difference.

Three predictions follow. First, when simultaneous intracellular interaction is genuinely ratio-dependent, active-exposure concordance should explain response variation better than formulation ratio alone. Second, where improved outcome results primarily from increased total exposure, matching pharmacologically accessible concentrations should diminish the apparent advantage of the delivery strategy. Third, where sequential or spatially separated mechanisms dominate, measures of exposure timing or compartment-specific target engagement should be more informative than constant same-cell drug-ratio preservation.

These predictions are falsifiable but not universally applicable. Each requires an experimentally specified drug pair, biological system, concentration range, time window, and response definition. Negative findings would delimit the mechanism rather than automatically invalidate co-delivery as a therapeutic strategy.

Discussion, competing explanations, and research priorities

The major implication of this analysis is that preservation of the manufactured drug ratio cannot independently explain the therapeutic performance of a multidrug nanocarrier. The preclinical benefits observed across coencapsulation studies provide a substantive pharmaceutical rationale, but they also raise questions of mechanism identification [1]. An improved response may result from coordinated active exposure, increased total delivery, altered biodistribution, improved tolerability, or interactions among these processes.

The first competing explanation is an exposure-magnitude effect. Improved efficacy may arise because both drugs reach higher concentrations even if their ratio becomes less stable. The second is a distribution effect: preferential delivery to responsive cells could improve treatment without preserving the original drug proportion throughout circulation. The third is a pharmacological interpretation effect, because synergy classification depends on the experimental response model [3]. The fourth is a complementary-action mechanism, in which different drugs act at different biological sites or times.

These explanations should be investigated through comparisons that isolate specific variables. Research priorities include drug-specific measurements of carrier retention, time-resolved active intracellular concentration, chemically differentiated drug-state analysis, and exposure-matched pharmacodynamic experiments. Cell-resolved and compartment-specific observations are particularly important when organ averages conceal heterogeneity.

The theory also challenges the assumption that maximizing all dimensions of delivery is always desirable. Greater carrier stability can restrict premature release but may delay pharmacologically useful liberation. Stronger targeting may favour one cellular population while reducing exposure elsewhere. More synchronized delivery may benefit a simultaneous interaction yet interfere with a sequential therapeutic mechanism.

A central unresolved question is how much molecular and spatial resolution is required for a reliable development decision. The answer depends on the hypothesized mechanism. When active-drug colocalization is causally necessary, cell- and time-resolved measurements become important. When the drugs act across different compartments, their separate target engagement and integrated biological response may constitute more appropriate evidence.

Limitations and context-dependent boundaries

This article provides a nonempirical mechanistic synthesis. It does not establish a universal mathematical relationship between formulated and active drug ratios or validate the proposed dependencies against a new dataset.

The evidence spans different payload classes, delivery materials, experimental models, administration routes, and therapeutic indications. Transport findings involving nucleic-acid carriers cannot automatically be extrapolated to small-molecule combinations. Likewise, preclinical tumour observations, pulmonary treatment, and ocular delivery differ in anatomical constraints and pharmacological requirements.

Several relevant states remain difficult to measure directly. Fluorescent labels may report carrier-associated material rather than free active molecules. Total-drug assays may include inactive, bound, or sequestered species.

Intracellular concentration measurements may not resolve the local abundance at molecular targets. These limitations can create apparent agreement or disagreement between ratio measurements without establishing their causal importance.

Pharmacodynamic conclusions also depend on concentration range, exposure duration, cellular state, and interaction definition. The account should therefore be applied as a set of conditional, experimentally testable relationships, with its predictions revised when direct mechanistic measurements disagree.

CONCLUSION

Coencapsulation can coordinate the delivery of therapeutic agents, but the preservation of a manufactured drug ratio does not guarantee preservation of pharmacologically relevant exposure. Transport, differential release, intracellular processing, and drug-specific activity can alter the relationship between formulation and effect.

The therapeutic importance of ratio preservation depends on the intended interaction mechanism and the concentrations, timing, and cellular destinations required for that interaction. Evidence linking these intermediate states to treatment response is necessary to distinguish ratio-dependent synergy from other benefits of nanocarrier delivery.

Mechanism-based formulation development should therefore evaluate the exposure relationship required by the drugs, the material processes capable of producing it, and the competing explanations for observed therapeutic outcomes.

ACKNOWLEDGMENTS: None

CONFLICT OF INTEREST: None

FINANCIAL SUPPORT: None

ETHICS STATEMENT: None

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