Past the Gut, Past the Liver: Quercetin Bilosomes and the Arithmetic of Nose-to-Brain Delivery

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Abstract*—Quercetin is an unusually well-justified candidate for nasal administration, and the justification is quantitative rather than fashionable. In rats given quercetin orally, 59.1% of the dose was absorbed but only 5.3% of unchanged quercetin was bioavailable, because 93.3% was metabolised in the gut wall against only 3.1% in the liver. In humans no free quercetin is detectable in plasma after oral intake at any dose; the circulating species are glucuronides, sulfates and methylated conjugates. The consequence for brain delivery is decisive: the aglycone is the species active in vitro, it is a P-glycoprotein substrate at the blood–brain barrier, and the conjugates penetrate less efficiently still, so rats fed a 1% quercetin diet accumulate conjugates in brain tissue to about 20 pmol g^{-1} at one hour and 40 pmol g^{-1} after a month while the aglycone remains undetectable. An oral dose cannot deliver active quercetin to the brain in principle, not merely in practice, and the nasal route removes exactly the two organs that destroy it. Bilosomes are a rational nasal carrier because the bile salt confers deformability, enhances epithelial permeation and resists the enzymes of the nasal mucosa, and the one published intranasal bilosome precedent reported direct transport percentages above 97%. That figure, and the field's reliance on it, is this review's critical concern. Drug targeting efficiency is a ratio of ratios carrying plasma exposure in its denominator, so a formulation absorbed poorly into the circulation scores well on it; the same study reported intranasal plasma peak concentrations significantly lower than intravenous ones alongside a targeting efficiency of 5451.8%, and published values across the nose-to-brain literature span 117% to 5451.8%, a forty-seven-fold range for what is presented as a physical transport efficiency. Rodent anatomy compounds the problem, the olfactory epithelium occupying some 40 to 55% of the nasal surface in rats against 3 to 10% in humans. For quercetin a further trap applies: plasma is conventionally assayed as total quercetin after hydrolysis while brain tissue is assayed as aglycone, placing numerator and denominator in different chemical units. This review sets out the pharmacokinetic case for the nasal route, the anatomical and formulation constraints it imposes, what bilosomes contribute, and how a targeting claim should be built so that it means what it says.

***Index Terms*—Quercetin; bilosomes; intranasal delivery; nose-to-brain; drug targeting efficiency; direct transport percentage; olfactory pathway; Quality by Design; Box–Behnken design**

I. INTRODUCTION

Most arguments for nasal delivery of a nutraceutical begin with the blood–brain barrier and work backwards. The argument for quercetin can be made the other way round, from its own pharmacokinetics, and it is stronger for it.

Quercetin is the most abundant flavonol in the human diet, present in onions, apples, grapes, berries and tea, and among the most intensively studied phytochemicals of the last three decades [1,2]. Its neuropharmacology is attractive: in animal models of aluminium-induced neurotoxicity it reduces oxidative stress, restores brain-derived neurotrophic factor expression, limits amyloid-β plaque formation and improves spatial memory, and it down-regulates amyloid precursor protein and β-secretase expression [3,4,5].

The obstacle is not that quercetin is poorly absorbed. It is absorbed well. The obstacle is that what is absorbed is not what was administered. In rats, 59.1% of an oral dose crossed the intestinal wall, yet only 5.3% of unchanged quercetin reached the systemic circulation, because 93.3% was metabolised in the gut

against 3.1% in the liver [6]. In humans the endpoint is starker still: no free quercetin is detectable in plasma after oral intake, at any dose studied, and the circulating species are glucuronide, sulfate and methylated conjugates [7,8].

That matters for the brain in a way it does not for every other target, because the aglycone is the species active in the in vitro work on which quercetin's neuroprotective reputation rests, it is a P-glycoprotein substrate at the blood–brain barrier, and its conjugates cross even less efficiently [9,10]. An oral dose therefore fails twice over: it converts the active species into inactive ones before absorption, and the products of that conversion are the ones least able to enter the brain.

Stated that way, the case for the nasal route is specific rather than generic. Intranasal administration removes the intestinal wall and the liver from the absorption path entirely, which is precisely where quercetin is lost, and it offers in addition a direct anatomical connection to the central nervous system through the olfactory and trigeminal nerves that circumvents the barrier the conjugates cannot cross [11,12].

Bilosomes vesicles incorporating bile salts in the bilayer suit this route for reasons examined in Section 4, and one published intranasal bilosome study reports direct transport percentages above 97% [13]. This review takes that literature seriously and then examines the arithmetic behind such numbers, because the indices on which the entire nose-to-brain field rests have structural weaknesses that a careful dissertation can avoid and a careless one will inherit.

II. QUERCETIN: WHY THE ORAL ROUTE CANNOT WORK

2.1 Where an oral dose is lost

Table 1 collects the pharmacokinetic studies and Figure 1A plots the fate of an oral dose.

The decisive experiment is that of Chen and colleagues, who administered quercetin to rats orally, intraportally and intravenously, measured parent drug and conjugates in plasma, urine and faeces, and fitted a compartmental model. Total absorption was 59.1%, but the bioavailability of unchanged quercetin was 5.3%; 93.3% of the dose was metabolised in the gut and only 3.1% in the liver, and no significant enterohepatic recirculation of parent or conjugates was observed [6]. The ratio of those two percentages is the finding: the gut wall removes thirty times as much quercetin as the liver does.

The human data agree on the endpoint. In a four-way crossover in healthy volunteers given onion supplement, isolated quercetin-4′-O-glucoside, quercetin-3-O-rutinoside and buckwheat tea, only quercetin glucuronides were detected in plasma and no free quercetin at all [7]. An earlier disposition study in six volunteers given 100 mg intravenously and 4 g orally found protein binding above 98%, an apparent volume of distribution of 0.34 ± 0.03 L kg^{-1}, and urinary recovery of 7.4 ± 1.2% of the intravenous dose as conjugate against 0.65 ± 0.1% unchanged [14]. In a population pharmacokinetic study of subjects taking 500 mg three times daily, oral clearance was high and the terminal half-life averaged 3.5 h, with re-entry peaks in the concentration–time curves consistent with enterohepatic recirculation [15].

Rat data confirm that the conjugation is not solely presystemic: after intravenous quercetin, 93.8% of the dose circulated as sulfates and glucuronides, and after oral administration of either quercetin or rutin the parent compound was not detected in blood at all [16]. Conjugation is therefore both fast and thorough, and an intravenous dose is not a solution either.

Reported bioavailability figures for quercetin vary widely between laboratories, and the reason is instructive rather than merely inconvenient: studies differ in whether they report the aglycone or total quercetin after enzymatic hydrolysis of conjugates. Those are different quantities, differing by one to two orders of magnitude, and a figure quoted without saying which was measured is uninterpretable. The same ambiguity recurs in Section 6 in a form that affects brain-targeting claims directly.

Table 1. Pharmacokinetics and disposition of quercetin. The consistent finding across species is that the aglycone scarcely circulates, with conjugation occurring predominantly in the gut wall rather than the liver.

Study and design	Principal finding	Reported values	Ref.
Rats; oral, intraportal and intravenous quercetin with plasma, urine and faecal assay of parent	The gut wall, not the liver, is the dominant site of presystemic	Total absorbed 59.1%; unchanged quercetin bioavailable 5.3%;	[6]

Study and design	Principal finding	Reported values	Ref.
and conjugates; compartmental model	metabolism; no significant enterohepatic recirculation	metabolised in gut 93.3%, in liver 3.1%	
Twelve healthy volunteers; four-way crossover with onion supplement, quercetin-4′-O-glucoside, quercetin-3-O-rutinoside and buckwheat tea	Only quercetin glucuronides detected in plasma; no free quercetin at any dose	Quercetin circulates in human plasma solely in conjugated form; conjugate antioxidant capacity lower than the aglycone	[7]
Six volunteers; single 100 mg intravenous and 4 g oral doses	Very high protein binding and small volume of distribution; almost all urinary recovery as conjugate	Protein binding > 98%; V_d 0.34 ± 0.03 L kg^{-1}; 7.4 ± 1.2% of the intravenous dose in urine as conjugate, 0.65 ± 0.1% unchanged	[14]
Subjects given quercetin 500 mg three times daily; aglycone and conjugates by LC–MS/MS; population model	High oral clearance with re-entry peaks consistent with enterohepatic recirculation	Terminal half-life averaging 3.5 h; one-compartment model with enterohepatic recirculation best described the data	[15]
Rats; intravenous quercetin and oral quercetin or rutin, assayed before and after β-glucuronidase and sulfatase hydrolysis	Conjugation is rapid and near-complete by either route; the parent compound was not detected after oral dosing	93.8% of an intravenous dose circulating as sulfates and glucuronides; oral absorption rate 53% after dose correction	[16]
Rats fed a purified diet containing 1% w/w quercetin aglycone	Conjugated metabolites accumulate in brain tissue with chronic intake; the aglycone remains undetectable	Brain tissue ≈ 20 pmol g^{-1} at 1 h, ≈ 40 pmol g^{-1} after one month of feeding; aglycone not detected	[10]

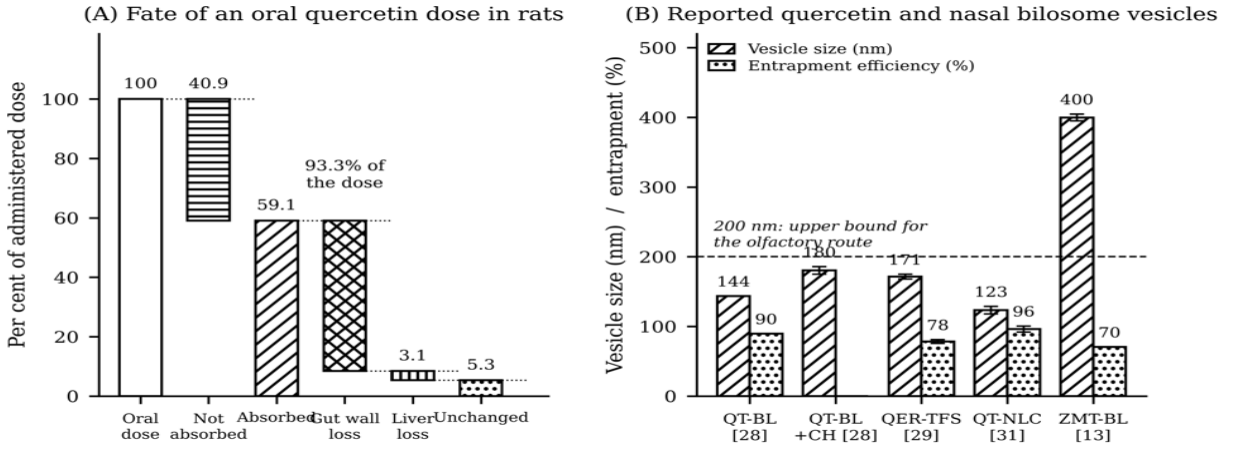


Fig. 1. (A) Fate of an oral quercetin dose in rats. Of the administered dose 59.1% was absorbed across the intestinal wall, but 93.3% was metabolised in the gut against 3.1% in the liver, leaving 5.3% of unchanged quercetin bioavailable [6]. The gut wall removes roughly thirty times as much quercetin as the liver does, which is the pharmacokinetic case for a route that bypasses it. (B) Vesicle size and entrapment efficiency of reported quercetin and intranasal bilosome formulations: quercetin bilosomes and their chitosan-coated variant [28], quercetin transferosomes [29], quercetin nanostructured lipid carriers [31] and the intranasal zolmitriptan bilosome [13]. Values come from different laboratories and methods and are indicative rather than strictly comparable.

2.2 What reaches the brain

The brain data complete the argument and are rarely set alongside the formulation literature.

Rats fed a purified diet containing 1% w/w quercetin aglycone accumulated conjugated metabolites in brain tissue to nearly 20 pmol g^{-1} after one hour, rising to about 40 pmol g^{-1} after a month of feeding, while the parent aglycone remained undetectable [10]. A

chronic dietary intake large enough to be unachievable clinically therefore produces brain concentrations in the tens of picomoles per gram, and none of it is the active species.

Against that, the concentrations at which quercetin acts on its targets in cell culture are micromolar. Five to six orders of magnitude separate what an oral dose achieves in brain tissue from what the in vitro pharmacology requires, and the shortfall is qualitative as well as quantitative, because the molecules that do arrive are the wrong ones.

Reviews of quercetin neuroprotection have made this point with some force: most in vitro work has used the aglycone, which is not detectable in plasma or brain after oral intake, and the metabolites and glycosides appear both less neuroprotective and less able to cross the blood–brain barrier than the aglycone [9]. Where animal models do show benefit, increased barrier permeability in the disease model itself may be contributing [9], which is a caution worth repeating in any thesis that cites efficacy data as evidence of delivery.

The practical conclusion is unambiguous. For a brain indication, a formulation strategy that increases oral absorption of quercetin is addressing the wrong step. The requirement is to deliver the aglycone to brain tissue without passing it through the gut wall, and that is a route problem rather than a solubility problem.

III. THE NASAL ROUTE

3.1 Two pathways, and what they bypass

The nose is connected to the central nervous system by two nerves that breach the blood–brain barrier by anatomy rather than by permeation. The olfactory nerve projects from the olfactory epithelium through the cribriform plate directly to the olfactory bulb, and branches of the trigeminal nerve innervate both the respiratory and olfactory mucosa and enter the brainstem [11,12]. Transport along either may be intracellular, by endocytosis into neurons followed by axonal transport, or extracellular, along perineural and perivascular spaces; the extracellular route is faster and is generally held to account for the rapid appearance of intranasally administered compounds in the central nervous system [12]. Figure 2 sets out the geometry.

A third route operates in parallel and is the source of most of the interpretive difficulty discussed later. The respiratory mucosa is richly vascularised, so a substantial fraction of any intranasal dose is absorbed into the systemic circulation and may subsequently reach the brain across the blood–brain barrier in the ordinary way. This indirect pathway delivers drug to the brain without involving the olfactory or trigeminal nerves at all, and distinguishing the two is the central methodological problem of the field.

What the direct pathways offer quercetin specifically is worth stating plainly, because it is not the same advantage they offer every drug. For a compound limited by blood–brain barrier permeability, the attraction is bypassing the barrier. For quercetin the barrier is the second problem; the first is that the aglycone never reaches the circulation intact. Nasal administration addresses the first by removing the gut wall and liver from the path, and the direct pathways then address the second. The route is therefore well matched to this molecule for two independent reasons, which is a stronger position than most nose-to-brain candidates occupy.

3.2 Constraints the formulation must respect

The nasal cavity is an unforgiving site, and the constraints are quantitative enough to drive formulation decisions directly.

Volume is the first. Only about 25 to 200 µL can be administered per human nostril, so the dose is bounded by the product of that volume and the achievable drug concentration [17,18]. For a compound as poorly water-soluble as quercetin this is a severe limit, and it is the single strongest argument for a carrier with high entrapment efficiency rather than a solution or suspension.

Mucociliary clearance is the second. Nasal mucus is cleared at intervals of roughly ten to twenty minutes, with a mucociliary transport rate in humans of about 6 mm per minute, which sets the residence time available for absorption [12,17,19]. A formulation that is not retained is swallowed, at which point the dose re-enters the gastrointestinal tract and the entire rationale collapses. Mucoadhesion and in situ gelation are therefore not refinements but requirements, and the relevant measurement is mucociliary transit time rather than viscosity.

Regional deposition is the third and the least tractable. The olfactory epithelium lies high in the nasal cavity above the superior turbinate, in a region reached by very little of the inspired airflow, and conventional nasal sprays deposit predominantly in the anterior cavity and on the respiratory mucosa [17,18,20]. Drug that lands on respiratory mucosa are absorbed systemically, not transported directly.

The fourth constraint is the one that most effects how preclinical results should be read, and it is anatomical rather than pharmaceutical. The olfactory epithelium occupies approximately 40 to 55% of the nasal surface area in rats and mice but only about 3 to 10% in humans [12,18,20,21]. One detailed comparison put the human olfactory region at 2097 mm², or 10.5% of the nasal cavity, against 1294 mm² but 55.6% in the rat [21]. Nasal cavity volume differs comparably, of the order of 15 to 25 mL in humans against roughly 0.3 mL in the rat [17,22]. A rat is therefore a structurally favourable model for this route, and a direct transport percentage measured in rats should be expected to overstate what the same formulation would achieve in a person. This is not a reason to avoid the rat; it is a reason to say so when reporting the number.

Two further constraints deserve brief mention. The nasal mucosa contains cytochrome P450 isoforms, glutathione S-transferases and carboxylesterases, so it is not a metabolically inert surface, although its conjugating capacity is far below that of the intestinal wall. And ciliotoxicity is a genuine safety endpoint for any formulation containing a permeation enhancer, bile salts included, since damage to the ciliated epithelium would compromise a protective function of the nose.

IV. BILOSOMES AS A NASAL CARRIER

Table 2 compares the vesicular options for this route. Bilosomes were introduced in 2001 by Conacher and colleagues, who formulated peptide and protein antigens in lipid vesicles incorporating bile salts for oral immunisation, the bile salt being added to stabilise vesicles against the bile and enzymes of the gut [23]. For the nasal route that original rationale is largely irrelevant there is no bile in the nose and the properties that matter are different ones, which is worth stating because bilosome papers frequently carry the oral justification over unexamined.

Three properties do transfer. The bile salt acts as an edge activator, lowering the energy cost of bilayer curvature so that vesicles deform rather than rupture when passing through a constriction, with reported deformability following the order sodium deoxycholate ≥ sodium cholate > sodium taurocholate [24]. It acts independently as an epithelial permeation enhancer, affecting tight junctions and the apical membrane [25,26]. And it confers a negative surface charge that influences interaction with the mucus layer and with the epithelium.

Vesicle size is a design constraint specific to this route. The olfactory pathway is generally held to admit particles below roughly 200 nm [18], which is a real target rather than the conventional preference for small particles, and it argues for an upper bound on vesicle size rather than simple minimisation.

The directly relevant precedent is the work of El Taweel and colleagues on intranasal zolmitriptan-loaded bilosomes, which appears to be the first study of drug-loaded bilosomes administered nasally for brain targeting. Bilosomes were prepared by thin-film hydration under a Box–Behnken design with the amount of sodium deoxycholate and the amount and molar ratio of the cholesterol–Span 40 mixture as factors, then loaded into a mucoadhesive in situ gel of poloxamer 407 and hydroxypropyl methylcellulose. The gel had a soul–gel transition temperature of 34.03 °C and an extended nasal mucociliary transit time of 22.36 min, and systemic and brain kinetics were compared in rats against an intravenous solution [13]. The study is well constructed and its findings are discussed in Section 6, where the targeting indices it reports are examined in detail.

Rizatriptan-loaded bilosomes have since been developed for the same route, the authors noting that only a few papers on bilosomes for intranasal brain targeting had been reported [27].

For quercetin itself, bilosomes have been developed for the oral route rather than the nasal one. Alruwaili and colleagues prepared surface-modified quercetin bilosomes by solvent evaporation and optimised them

with a three-factor three-level Box–Behnken design using lipid, span 60 and sodium deoxycholate as factors; the optimised formulation had a vesicle size of 143.51 nm, polydispersity index 0.256, zeta potential −15.4 mV and entrapment efficiency 89.52%, with a chitosan-coated variant at 180.32 ± 5.27 nm [28].

Other vesicles have been taken nasally with quercetin. Quercetin-loaded transferosomes in an in situ gel, optimised by Box–Behnken design over lipid amount and edge activator amount and type, gave vesicles of 171.4 ± 3.4 nm with 78.2 ± 2.8% entrapment and a zeta potential of −32.6 ± 1.4 mV, with brain localisation demonstrated by computed tomography of gold-labelled formulations [29] and an antidepressant effect shown in a companion study [30]. Quercetin-loaded nanostructured lipid carriers in a Lutrol F127 in situ gel reached 123.3 ± 5.46 nm with 96.1 ± 4.40% entrapment and 83.74 ± 1.40% release [31]. Figure 1B plots these alongside the bilosome data.

The gap this leaves is specific and is the opening for the present work: quercetin bilosomes exist but have been evaluated orally, and intranasal bilosomes exist but have been evaluated with triptans. The combination has not been reported, and the pharmacokinetic argument of Section 2 suggests quercetin is a better-motivated payload for the nasal route than a triptan, whose oral bioavailability is already moderate.

Table 2. Carriers reported for nasal brain targeting of flavonoids and small molecules, with the property each contributes and its limitation for this route.

Carrier	Composition	Contribution to the nasal route	Limitation
Solution or suspension	Drug with cosolvent or suspending agent	Simple; no excipient burden on the mucosa	Cleared within the mucociliary interval; no retention, and poorly soluble drug cannot be dosed in 25–200 µL
Niosome	Non-ionic surfactant with cholesterol	Chemically stable, inexpensive, encapsulates lipophilic actives well	Rigid bilayer; no intrinsic permeation enhancement; still cleared rapidly without a gel
Transferosome	Phospholipid with an edge activator	Ultra-deformable; reported for quercetin nasally at 171 nm with 78% entrapment	Phospholipid oxidation on storage; edge activator choice governs performance and is often fixed rather than optimised
Nanostructured lipid carrier	Solid and liquid lipid blend	High entrapment (reported above 96% for quercetin) and controlled release	Solid matrix limits deformability; drug expulsion on storage as the matrix recrystallises
Bilosome	Non-ionic surfactant, cholesterol and a bile salt in the bilayer	Deformability from the bile salt as edge activator; epithelial permeation enhancement; the one published intranasal precedent reported direct transport above 97%	Bile salt above an optimum solubilises the bilayer; permeation enhancement is non-specific, so ciliotoxicity must be assessed
Vesicle in a mucoadhesive in situ gel	Any of the above dispersed in poloxamer 407 with a mucoadhesive polymer	Gels at nasal temperature and extends mucociliary transit time; reported 34.03 °C sol–gel transition and 22.36 min transit	Added viscosity may impair spray atomisation and shift deposition away from the olfactory region

V. FORMULATION VARIABLES AND SYSTEMATIC OPTIMISATION

Table 3 sets out the variables, the attributes each governs and the trade-off that makes each a candidate design factor.

Bile salt identity and concentration carries the most mechanistic weight, with the deformability ranking above giving a rational basis for selection [24] and sodium deoxycholate the usual choice [13,28]. Concentration is bounded in both directions: too little and the vesicle is a niosome with incidental bile salt, too much and the bile salt solubilises the bilayer into mixed micelles. For the nasal route a third bound applies that does not apply orally ciliotoxicity and it

should be measured rather than assumed to lie outside the design space.

Non-ionic surfactant and cholesterol content together determine bilayer packing, with cholesterol working directly against the bile salt: it improves retention and entrapment while stiffening the bilayer and removing the deformability the bile salt was added to provide. The cholesterol-to-bile-salt relationship is better handled as an interaction term than as two independent factors.

Vesicle size should be treated as a bounded target rather than minimised, for the reason given above: the olfactory pathway favours particles below about 200 nm [18], while the formulation must still carry an adequate dose in 25 to 200 µL.

The gelling system is a second formulation layer with its own variables, and for this route it is not optional. Poloxamer 407 concentration governs the sol–gel transition temperature, which must sit below nasal mucosal temperature so that gelation occurs on administration but above ambient so the preparation remains sprayable; the zolmitriptan work achieved 34.03 °C [13]. A mucoadhesive polymer such as hydroxypropyl methylcellulose governs retention, and the response that matters are mucociliary transit time, measured directly.

The Quality by Design framework of ICH Q8(R2) provides the structure a quality target product profile, critical quality attributes derived from it, a risk assessment, and a design space within which performance is robust [32,33] and a Box–Behnken design estimates a full quadratic surface in fifteen runs for three factors including centre-point replicates [34,35], as applied in each of the vesicle studies cited above.

The weakness in the published designs is again the choice of responses. Vesicle size, polydispersity index, zeta potential and entrapment efficiency are conventional and easily measured, and none of them bears directly on brain delivery. For this route two responses would: ex vivo permeation across excised nasal mucosa, and mucociliary transit time. Both are harder than particle sizing and both are closer to the claim being made.

Table 3. Formulation and process variables for a quercetin bilosome nasal system, and the trade-off that makes each a candidate design factor rather than a fixed value.

Variable	Attributes affected	Trade-off specific to the nasal route
Bile salt identity and amount	Deformability; permeation enhancement; zeta potential; entrapment	Deformability follows deoxycholate ≥ cholate > taurocholate, but permeation enhancement is non-specific, so the upper bound here is ciliotoxicity rather than entrapment
Non-ionic surfactant type and amount	Bilayer packing and permeability; vesicle size; entrapment	A higher transition temperature retains drug better but stiffens the bilayer and may exceed the deformability needed for the olfactory pathway
Cholesterol content	Membrane rigidity; leakage on storage; entrapment	Works directly against the bile salt; best modelled as an interaction term rather than an independent factor
Vesicle size	Olfactory pathway access; surface area; deposition behaviour	A bounded target rather than a minimum: the olfactory route favours particles below about 200 nm, while dose must still fit in 25–200 µL
Drug: lipid ratio	Payload; unentrapped drug; physical stability	The nasal volume limit makes payload unusually important, but excess loading risks crystallisation of free quercetin
Poloxamer 407 concentration	Sol–gel transition temperature; gel strength	Must gel at nasal mucosal temperature yet remain sprayable at ambient temperature
Mucoadhesive polymer type and amount	Mucociliary transit time; residence at the application site	Longer retention raises exposure but added viscosity can impair atomisation and shift deposition away from the olfactory region
Sonication or extrusion energy	Vesicle size and polydispersity; lamellarity	Prolonged sonication heats the dispersion and risks degrading a thermolabile polyphenol

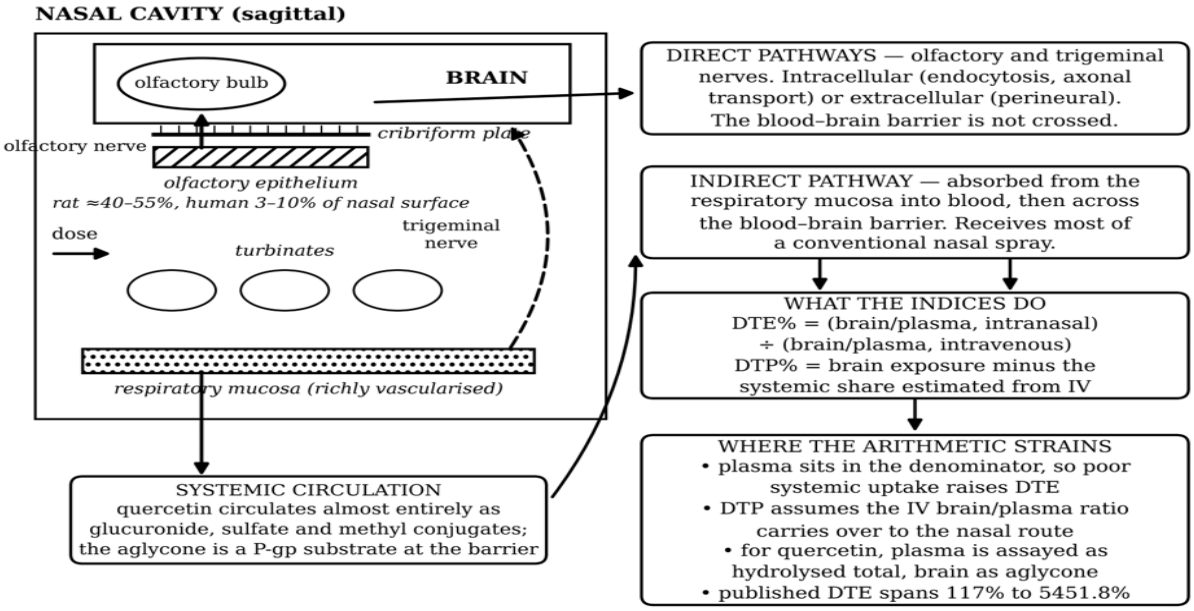


Fig. 2. Routes from the nasal cavity to the brain, and the point at which the targeting indices are computed. A dose deposited on the olfactory epithelium, high in the cavity above the superior turbinate, may be carried along the olfactory nerve through the cribriform plate to the olfactory bulb, either intracellularly by endocytosis and axonal transport or extracellularly along perineural spaces; branches of the trigeminal nerve innervate both olfactory and respiratory mucosa and enter the brainstem. These are the direct pathways, and they do not cross the blood–brain barrier. A dose deposited on the richly vascularised respiratory mucosa, which receives most of a conventional nasal spray, is absorbed systemically and may then reach the brain across the barrier in the ordinary way: the indirect pathway. Drug targeting efficiency compares the brain-to-plasma exposure ratio after intranasal dosing with the same ratio after intravenous dosing, and direct transport percentage subtracts an estimate of the indirect contribution derived from the intravenous arm. Neither index observes the direct pathway; both infer it. The olfactory epithelium occupies some 40–55% of the nasal surface in rats but only 3–10% in humans, so a rat result is an upper bound. Simplified; mucosal metabolism and lymphatic drainage are not shown.

VI. THE ARITHMETIC OF BRAIN TARGETING

A formulation reaches the brain either directly, along the olfactory and trigeminal nerves, or indirectly, by systemic absorption followed by passage across the blood–brain barrier. Because the two cannot be separated by sampling brain tissue, the field relies on two derived indices. Understanding what they can and cannot support is the most useful thing a thesis on this subject can do.

Drug targeting efficiency compares the brain-to-plasma exposure ratio after intranasal dosing with the same ratio after intravenous dosing, expressed as a percentage. Direct transport percentage then estimates the share of brain exposure attributable to the direct pathway by subtracting the contribution predicted from systemic absorption [36,37,38]. Values above 150% for targeting efficiency and above 20% for direct transport are commonly treated as evidence of meaningful direct delivery [39].

Four difficulties attach to these indices, and the first is the most consequential.

The denominator problem. Plasma exposure sits in the denominator of the targeting efficiency ratio, so any formulation that is absorbed poorly into the systemic circulation scores highly whether or not more drug reaches the brain. The zolmitriptan bilosome study illustrates this in its own data: peak plasma

concentrations after intranasal administration of the bilosomes and of the gel, 108.58 ± 1.94 and 86.66 ± 4.13 ng mL^{-1}, were significantly lower than after the intravenous solution, and the direct transport percentages were 97.2% and 98.1%, with a targeting efficiency for the gel reported as high as 5451.8% [13,39]. The authors themselves attribute the gel's higher value partly to its longer residence at the application site raising the area under the curve [13]. A reader should hold both facts together: the formulation performed well, and a large part of the index's magnitude reflects the denominator.

The implausible range. Published targeting efficiencies across nose-to-brain nanocarriers range from about 117% to 5451.8% [39], and direct transport percentages cluster between 60% and 99% almost regardless of carrier type [36,37,40]. Figure 1B gives a sense of the spread. A genuine physical transport efficiency does not plausibly vary forty-seven-fold across broadly similar lipid nanocarriers delivered to the same organ in the same species. What varies that much is a quotient with a small and variable denominator.

The embedded assumption. Direct transport percentage is computed by subtracting an estimate of the systemic contribution to brain exposure, and that estimate is obtained from the brain-to-plasma relationship measured after intravenous administration. The step assumes that the relationship transfers unchanged to the intranasal route — same plasma protein binding, same transporter activity, same time course of barrier presentation. It is a reasonable approximation and it is an approximation, and direct transport percentage is accordingly a model-derived quantity rather than a measurement.

The chemical-units problem, which is specific to quercetin and is a genuine trap. Plasma quercetin is conventionally assayed as total quercetin after enzymatic hydrolysis of glucuronide and sulfate conjugates, because the aglycone is undetectable otherwise [7,8,16]. Brain tissue, by contrast, is usually assayed for the aglycone. A targeting efficiency computed from a hydrolysed plasma denominator and an unhydrolysed brain numerator is not a ratio of like quantities, and it will be inflated by however much of the plasma signal was conjugate. Any quercetin brain-targeting study must state which species was measured in each compartment and, ideally, measure both in both.

None of this means the indices should be abandoned; they are the best summary measures available and they are what reviewers expect. It means they should be reported with their inputs visible. Four practices would make a targeting claim considerably more robust.

Report the absolute brain concentration, in nanograms per gram or picomoles per gram, alongside the ratios, and compare it with the concentration at which quercetin is active in vitro. A twenty-fold improvement on an undetectable baseline may still be pharmacologically irrelevant, and only the absolute figure reveals that. Given the dietary-exposure data of Section 2.2, this is the single most informative number such a study can produce.

Measure the aglycone and the conjugates separately in both plasma and brain. For this molecule that is not a refinement; the aglycone is the active species and the conjugates are the ones the body actually makes.

Include an arm that constrains the direct pathway independently of the arithmetic. Sampling cerebrospinal fluid alongside plasma and brain, or comparing olfactory bulb against a distal brain region, gives evidence of direct transport that does not depend on the intravenous comparator at all. A steep olfactory-bulb-to-cortex gradient early after dosing is harder to explain by systemic redistribution than any targeting efficiency value.

State the species caveat explicitly. A direct transport percentage obtained in rats, whose olfactory epithelium is five to fifteen times larger as a proportion of nasal surface than a human's [12,18,20,21], should be presented as an upper bound on what the formulation would achieve clinically.

Around these, the supporting work follows the standard pattern, with two additions specific to the nasal route. Release data are fitted to zero-order, first-order, Higuchi and Korsmeyer–Peppas models [42,43]; analytical methods are validated to ICH Q2(R1) [44], with separate calibration for aglycone and hydrolysed total quercetin for the reason given above; and accelerated stability testing follows ICH Q1A(R2) [45], monitoring vesicle size, zeta potential, drug leakage and, for the gelled system, sol–gel transition temperature. The two additions are nasal tolerability ciliary beat frequency in excised mucosa or histology after repeated dosing and isotonicity and pH, since a formulation outside the physiological nasal range of roughly 5.5 to 6.5 will itself impair ciliary function [41].

VII. GAPS AND OUTLOOK

Several gaps follow directly from the preceding sections.

The payload gap. Intranasal bilosomes have been evaluated with triptans, whose oral bioavailability is already moderate and whose limitation is speed of onset rather than presystemic destruction. Quercetin, which loses 93.3% of an oral dose in the gut wall, is a better-motivated payload for a route that bypasses the gut wall, and the combination has not been reported.

The species-measured gap. Studies of quercetin brain delivery rarely state whether brain and plasma assays measured aglycone, total quercetin after hydrolysis, or both. For this molecule that single methodological detail determines whether the result means anything.

The absolute-concentration gap. Brain targeting is almost always reported as a ratio. Set against the finding that a chronic 1% dietary intake produces brain concentrations of tens of picomoles per gram, with no detectable aglycone [10], the useful question is not how many times better a formulation is but whether it reaches the micromolar range at which quercetin acts. Almost no study answers it.

The deposition gap. Nose-to-brain formulations are evaluated for particle size, entrapment and release, and almost never for where in the nasal cavity they land, although regional deposition determines whether the direct pathway is engaged at all [20]. Deposition is measurable, by gamma scintigraphy or in a nasal cast, and it is the step between the formulation and the mechanism.

The ciliotoxicity gap. Bile salts are permeation enhancers acting non-specifically on the epithelium [25,26], and the nasal epithelium's ciliary function is a protective mechanism rather than merely a clearance nuisance. A ciliotoxicity assessment, by ciliary beat frequency in excised mucosa or histology after repeated dosing, costs little and is rarely reported.

The chronic-dosing gap. Neurodegeneration is a chronic indication, and nasal formulations for it are almost always evaluated after a single dose. Whether a bile-salt-containing vesicle can be given nasally twice daily for months without cumulative mucosal change is the question that would decide clinical viability, and it is open.

The translational gap. No quercetin nasal formulation appears to have reached clinical evaluation. Given quercetin's dietary ubiquity and benign safety record, and given that human pharmacokinetic baselines exist against which a nasal formulation could be compared [7,14,15], an early human study measuring plasma aglycone after nasal administration would be a modest and informative step, even without brain sampling.

VIII. CONCLUSION

Quercetin is a better-justified candidate for nasal administration than most compounds pursued by that route, and the justification is specific. Its losses are not absorptive but metabolic and they are overwhelmingly intestinal: 59.1% of an oral dose crosses the gut wall, but 93.3% is conjugated there against 3.1% in the liver, and only 5.3% of unchanged quercetin becomes bioavailable. In humans no free quercetin circulates at all. Because the aglycone is the active species, is a P-glycoprotein substrate at the blood–brain barrier, and gives way to conjugates that cross even less readily, oral dosing fails the brain twice over: a chronic 1% dietary intake in rats yields tens of picomoles per gram of conjugate in brain tissue and no detectable aglycone. The nasal route removes the two organs responsible and adds a direct anatomical connection to the central nervous system.

Bilosomes suit the route, though for different reasons than they suit the gut. Stability against bile is irrelevant in the nose; deformability, permeation enhancement and a vesicle size compatible with the olfactory pathway are what carry over, and they must be set against constraints the nasal cavity imposes without negotiation 25 to 200 µL per nostril, mucociliary clearance every ten to twenty minutes, and an olfactory region that receives little of the administered dose. Those constraints make high entrapment efficiency and in situ gelation requirements rather than refinements, and they make mucociliary transit time a more informative response than polydispersity index.

Where such a study can contribute most is in how it supports its central claim. Drug targeting efficiency and direct transport percentage are the field's currency, and they carry plasma exposure in the denominator, rest on an assumption transferred from the intravenous arm, come from an animal whose olfactory epithelium is proportionally five to fifteen times larger than a humans, and for quercetin specifically risk placing a hydrolysed plasma denominator against an unhydrolysed brain numerator. Reported values spanning 117% to 5451.8% should be read in that

light. Report the indices, but report the absolute brain concentration beside them and compare it with the concentration at which quercetin actually works; measure aglycone and conjugate separately in both compartments; add an olfactory-bulb gradient or a cerebrospinal fluid arm that constrains the direct pathway without relying on the arithmetic; and state the species caveat rather than leaving it to the reader. These are modest additions to a conventional protocol, and together they would turn a targeting number into a result.

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