Eugenol, Emulsified: QbD Nanoemulgels of Clove Essential Oil

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***Abstract*—Localised inflammatory conditions are treated for months or years, and the oral anti-inflammatory drugs that treat them accumulate gastrointestinal, renal and cardiovascular risk over exactly that timescale. Herbal essential oils offer a mechanistically broad alternative for superficial targets: clove oil, dominated by eugenol at 70 to 90 per cent, inhibits cyclo-oxygenase-2 and 5-lipoxygenase, prevents nuclear translocation of nuclear factor-kappa B, scavenges reactive oxygen species and blocks voltage-gated sodium channels, while the β-caryophyllene present in the same oil is a selective cannabinoid CB2 agonist — four mechanistically distinct anti-inflammatory routes in one generally-recognised-as-safe material. What prevents its pharmaceutical use is not pharmacology but physical chemistry: essential oils are volatile, poorly water-soluble, unstable to light, heat and oxygen, strongly odorous, and irritant when applied neat. Nanoemulsions resolve several of these at once because the oil is its own internal phase, so no separate solubilising oil is needed; the droplets shield the volatiles from oxidation, present an enormous interfacial area for partitioning into the stratum corneum, and allow a much smaller total quantity of oil to be used. Their low viscosity then makes them unsuitable for direct application, and incorporation into a hydrogel — a nanoemulgel — supplies the rheology, residence time and cosmetic acceptability a topical product needs. This review assembles the phytochemistry and mechanisms of clove oil, examines why a self-emulsifying essential oil is a special case among topical nanocarriers, sets out the formulation variables that govern globule size and permeation, and argues that the value of a Quality by Design treatment lies in the design space it documents rather than in the single optimised batch it produces.**

***Index Terms*—Clove oil; eugenol; nanoemulsion; nanoemulgel; transdermal delivery; anti-inflammatory; Quality by Design; Box–Behnken design; design space**

I. INTRODUCTION

Inflammation is a protective response that becomes a disease when it persists. Its physiological origins and roles are well characterised [1], and the prostaglandin arm of the arachidonic acid cascade that drives much of its clinical expression — pain, swelling, heat — is among the best-understood pathways in pharmacology [2]. The non-steroidal anti-inflammatory drugs that act on it do so by inhibiting cyclo-oxygenase, a mechanism established half a century ago [3].

The difficulty is duration. Localised inflammatory conditions — osteoarthritic joints, soft-tissue injury, inflammatory skin disease — are managed over months and years, and systemic exposure to a cyclo-oxygenase inhibitor over that period carries gastrointestinal, renal and cardiovascular costs that scale with time and with patient age. For a target lying a few millimetres beneath the skin, delivering the drug systemically in order to reach it is a poor trade, and this reasoning is what has driven topical anti-inflammatory therapy from a marginal option to a mainstream one [13].

Herbal essential oils are attractive in that setting for a reason beyond tradition: they are mechanistically promiscuous in a useful way. Volatile oils from Syzygium aromaticum, Eucalyptus globulus, Zingiber officinale and Cymbopogon citratus contain phenylpropanoids, monoterpenes and sesquiterpenes that act at several points in the inflammatory cascade

simultaneously, and that additionally possess antioxidant and local analgesic activity [4,5]. A single-target inhibitor can be defeated by a parallel pathway; a multi-target mixture is harder to route around.

Clove oil is the best-characterised member of the group. Its dominant constituent eugenol has been reviewed from ethnobotany to industrial use [7] and its anti-inflammatory and antinociceptive activity demonstrated in standard animal models [10]. Yet the properties that make essential oils biologically interesting — volatility, lipophilicity, chemical reactivity — are precisely those that make them pharmaceutically awkward.

Nanoemulsions address that awkwardness with an elegance specific to this class of actives: the oil is the carrier. Where a conventional lipid nanocarrier must dissolve a drug in an inert excipient oil, an essential oil nanoemulsion uses the active itself as the internal phase, eliminating the excipient and its associated loading limit entirely. Converting the resulting low-viscosity dispersion into a nanoemulgel then provides what a topical product actually requires [26,27].

This review sets out the pharmacology that justifies the choice of oil, the physical chemistry that governs the formulation, the variables that must be controlled, and the Quality by Design framework within which they are best explored.

II. CLOVE OIL AND EUGENOL

2.1 Composition and the standardisation problem

Essential oils are complex, volatile, lipophilic secondary metabolites obtained by hydrodistillation, steam distillation or cold expression. A single oil may contain twenty to sixty constituents, of which two or three are present at high concentration and largely determine the biological activity; the principal classes are the monoterpenes, sesquiterpenes and phenylpropanoids [4,5]. Table 1 compares the candidate oils for an anti-inflammatory indication.

Clove oil, from the flower buds of Syzygium aromaticum, is dominated by eugenol (4-allyl-2-methoxyphenol) at typically 70 to 90 per cent, with eugenyl acetate and β-caryophyllene as the other major constituents; its chemical composition and biological activity have been reviewed [6]. Eugenol is listed as generally recognised as safe as a flavouring agent, which is a meaningful regulatory advantage for a topical product intended for chronic use.

The composition is not fixed, and this matters more for an essential oil than for a purified drug. Geographical origin, harvest time, plant part and distillation conditions all shift the constituent profile, so two batches of "clove oil" may differ materially in eugenol content. Gas chromatography–mass spectrometry against retention indices is the standard method for characterising such mixtures [42], and it should be applied to every batch of oil entering the formulation rather than once at the outset. A formulation whose active is defined only as "clove oil" is not standardised, and the quantitative relationships that a Quality by Design study seeks to establish cannot survive an input whose composition drifts between batches.

Table 1. Candidate herbal essential oils for topical anti-inflammatory therapy and their principal constituents. Constituent percentages are typical ranges and vary with origin, harvest and distillation conditions.

Essential oil (botanical source)	Family	Major constituents	Reported anti-inflammatory basis
Clove oil (Syzygium aromaticum, flower buds)	Myrtaceae	Eugenol 70–90%, eugenyl acetate, β-caryophyllene	COX-2 and 5-LOX inhibition; suppression of NF-κB nuclear translocation; antioxidant; sodium channel blockade giving local analgesia; CB2 agonism via β-caryophyllene
Eucalyptus oil (Eucalyptus globulus, leaves)	Myrtaceae	1,8-Cineole 60–85%, α-pinene, limonene	Reduces TNF-α, IL-1β and leukotriene B_4; counter-irritant and rubefacient action

Essential oil (botanical source)	Family	Major constituents	Reported anti-inflammatory basis
Ginger oil (Zingiber officinale, rhizome)	Zingiberaceae	Zingiberene, β-bisabolene, α-curcumene	Dual cyclo-oxygenase and lipoxygenase inhibition; suppression of PGE_2 and nitric oxide
Lemongrass oil (Cymbopogon citratus)	Poaceae	Citral (geranial + neral), myrcene	Inhibits NF-κB and inducible nitric oxide synthase; reduces IL-1β and IL-6

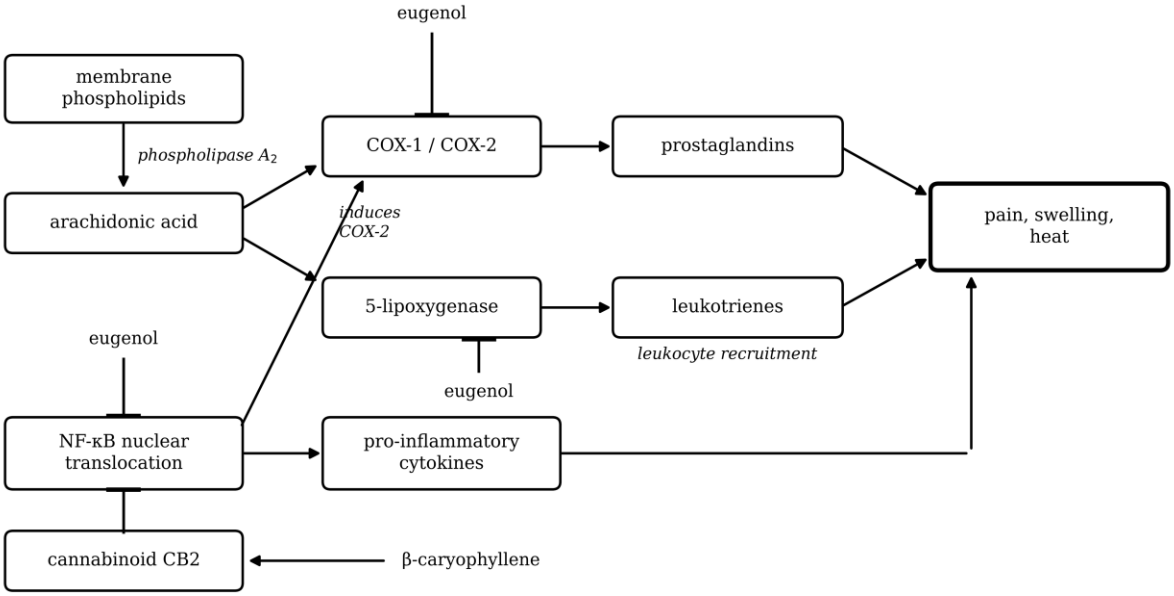


Fig. 1. Points of action of clove oil constituents within the inflammatory cascade. Membrane phospholipids are hydrolysed by phospholipase A_2 to arachidonic acid, which is metabolised by cyclo-oxygenase to prostaglandins and by 5-lipoxygenase to leukotrienes; nuclear factor-kappa B drives transcription of cyclo-oxygenase-2 and of pro-inflammatory cytokines. Blunt-ended lines mark inhibition by eugenol at COX-2, 5-LOX and NF-κB translocation; the arrow marks agonism of the cannabinoid CB2 receptor by β-caryophyllene present in the same oil. Conventional non-steroidal anti-inflammatory drugs act only at the cyclo-oxygenase step. Simplified; not all intermediates are shown.

2.2 Four mechanisms in one oil

The anti-inflammatory basis of clove oil is unusually well mapped, and it is multi-target. Figure 1 locates the points of action within the inflammatory cascade.

Eugenol inhibits cyclo-oxygenase-2 expression and activity, acting on the prostaglandin arm of the arachidonic acid cascade — the same arm targeted by conventional non-steroidal anti-inflammatory drugs, but reached through an effect on expression as well as on catalysis [6,7]. It also suppresses 5-lipoxygenase, and therefore the leukotriene arm, which conventional cyclo-oxygenase inhibitors leave untouched; shunting of arachidonate toward leukotrienes is one recognised limitation of selective cyclo-oxygenase inhibition, and an agent that addresses both arms does not have that problem.

Upstream of both, eugenol prevents the nuclear translocation of nuclear factor-kappa B, the transcription factor that drives expression of cyclo-oxygenase-2 and of the pro-inflammatory cytokines. Clove and eugenol at non-cytotoxic concentrations have been shown to exert immunomodulatory and anti-inflammatory action on cytokine production by murine macrophages [9], and the anti-inflammatory activity of glucosylated eugenol derivatives has been studied directly [11]. Eugenol additionally scavenges reactive oxygen species and blocks voltage-gated sodium channels, the latter conferring the local

anaesthetic action exploited in dentistry for more than a century [7].

The fourth mechanism belongs to a different constituent. β-Caryophyllene, a sesquiterpene present in clove oil, is a selective agonist at the cannabinoid CB2 receptor and has been characterised as a dietary cannabinoid [8]. CB2 activation is anti-inflammatory without the central effects of CB1 agonism, and it is a mechanistically independent route.

Anti-inflammatory and antinociceptive activity of eugenol-containing essential oil has been confirmed in experimental animal models [10], and the toxicological profile of eugenol has been examined across human-derived cell lines [12]. The last point deserves emphasis for a formulation scientist: eugenol is not innocuous at high local concentration, and the concentration delivered to the skin is a formulation variable rather than a fixed property of the oil.

2.3 Why essential oils are difficult to formulate

Five liabilities recur across the class and each has a formulation consequence.

Volatility means loss during manufacture and during storage, and it means that the quantity of active in the finished product is not necessarily the quantity weighed into it. Poor aqueous solubility means that a simple aqueous gel cannot carry a therapeutic quantity in dissolved form. Chemical instability toward light, heat and oxygen — eugenol is a phenol and oxidises readily — means that discoloration and loss of assay are the normal failure modes on storage. A strong characteristic odour limits the quantity that is cosmetically acceptable. And irritation on neat application sets an upper limit on concentration that is independent of all of the above.

A nanoemulsion addresses four of these five simultaneously, which is why it is a better match to this problem than to most. Encapsulation within droplets reduces the vapour pressure experienced at the formulation surface and so limits evaporative loss; it shields the phenol from oxygen; it masks odour by reducing the free oil at the interface; and, by raising delivery efficiency, it allows a therapeutic effect from a smaller total quantity of oil and therefore reduces irritation potential. The fifth liability, aqueous insolubility, is not addressed so much as rendered irrelevant, because the oil is dispersed rather than dissolved.

III. NANOEMULSIONS: WHEN THE DRUG IS ITS OWN CARRIER

A nanoemulsion is a kinetically stabilised dispersion of one liquid in another with droplet diameters conventionally below about 200 nm. The terminology deserves care: nanoemulsions are kinetically stable but thermodynamically unstable, whereas microemulsions — despite the name implying larger droplets — are thermodynamically stable and form spontaneously. The distinction has been set out carefully and is not merely semantic, because it determines whether a formulation will eventually separate and what accelerated stability testing can tell you [16,17,19].

Formation and stability have been reviewed in detail [18,20]. Two routes are available. High-energy methods — ultrasonication, high-pressure homogenisation, microfluidisation — supply mechanical energy to rupture droplets and give fine control over size through process parameters. Low-energy methods exploit phase behaviour, with droplet size and stability produced by the temperature phase inversion route depending on composition rather than on shear [21]. For a volatile, heat-sensitive essential oil, the choice is consequential: high-temperature phase inversion risks loss and degradation of the very constituents being formulated, which favours sonication under temperature control, with the caveat that cavitation itself generates local heat and that sonication time is therefore a variable with an upper as well as a lower bound.

The mechanisms by which nanoemulsions enhance skin delivery are reasonably well established. The large interfacial area increases the contact between the oil phase and the stratum corneum and promotes partitioning into it. The surfactants present can fluidise stratum corneum lipids. The formulation can exert an occlusive effect that hydrates the barrier. And in the specific case of clove oil, eugenol is itself a recognised penetration enhancer — a point of real interest here, because it means the vehicle and the active are the same substance and the formulation enhances its own absorption.

The evidence base across actives is consistent. Nanoemulsions have been used as vehicles for transdermal aceclofenac [23] and ketoprofen, the latter with in vitro permeation studies quantifying the gain [24], and a nanoemulsion preparation enhanced

the anti-inflammatory property of aspirin in mice [25].

The directly relevant study is that of Esmaeili and colleagues, who prepared an oil-in-water nanoemulsion of eugenol for topical anti-inflammatory evaluation. The optimum formulation contained 2% eugenol as the oil phase with 14% Tween 20 as surfactant and 14% isopropyl alcohol as co-surfactant in water, giving a polydispersity index of 0.3 and a median droplet diameter of 24.4 nm. In carrageenan-induced paw oedema in rats the nanoemulsions showed significantly improved anti-inflammatory activity after 1.5 h compared with a marketed piroxicam gel. Two further findings are worth noting because they are counter-intuitive: increasing the eugenol concentration did not increase inhibition of inflammation, and the nanoemulsion containing added piroxicam performed less well than the nanoemulsion without it [22].

That first observation — that more oil did not mean more effect — is the single most useful result in the eugenol nanoemulsion literature for a formulator. It implies that the dose–response relationship saturates within the accessible concentration range, and therefore that the optimisation target should be delivery efficiency and tolerability rather than payload. A design that maximises oil loading is optimising the wrong variable.

3.1 What actually governs delivery from a nanoemulsion

It is widely assumed that smaller droplets mean better skin delivery, and the assumption is often correct but not for the reason usually given. Droplets of 20 to 150 nm are still one to two orders of magnitude larger than the intercellular lipid channels of the stratum corneum, so intact droplets do not squeeze between corneocytes in the way deformable vesicles are proposed to. The size effect is indirect, and it operates through at least four separate mechanisms that a formulator should keep distinct.

The first is interfacial area. For a fixed oil volume, halving the droplet radius doubles the total oil–water interfacial area, and partitioning of a lipophilic constituent out of the droplet and into the stratum corneum is an interfacial process. This is the mechanism most directly tied to size, and it is why size is worth minimising even though the droplets themselves go nowhere.

The second is thermodynamic activity. A constituent dissolved in a very small droplet experiences a different chemical potential from the same constituent in bulk oil, and it is activity rather than concentration that drives partitioning into the barrier.

The third belongs to the surfactant rather than to the droplet. Non-ionic surfactants present at the concentrations typical of these systems interact with stratum corneum lipids and increase their fluidity, lowering the barrier's resistance. This mechanism scales with surfactant concentration, which also correlates with small droplet size — which means that a study varying surfactant level and observing both smaller droplets and higher flux cannot attribute the flux gain to size without holding surfactant constant. Designs that confound the two are common.

The fourth is occlusion. A film of oil and surfactant left on the skin retards transepidermal water loss, hydrates the corneocytes and swells the intercellular route, and this effect increases with oil content rather than decreasing with droplet size — so it can run counter to the first mechanism as oil loading rises.

For clove oil a fifth mechanism applies that is specific to the active. Eugenol is itself a recognised skin penetration enhancer, so the oil phase modifies the barrier it is crossing. This makes the system pleasingly self-consistent and analytically awkward in equal measure: the vehicle, the enhancer and the active are one substance, and their contributions cannot be separated by varying their concentrations independently, because there is only one concentration to vary. A formulator should be explicit that the oil concentration term in any fitted model carries all three effects at once.

IV. FROM NANOEMULSION TO NANOEMULGEL

A nanoemulsion is a mobile liquid, and a mobile liquid applied to a knee or an elbow runs off it. The nanoemulgel — a nanoemulsion dispersed in a hydrogel matrix — is the dosage form that makes the carrier usable, and it has been reviewed extensively as a topical delivery platform [26,27,28].

The gel contributes four things. It provides pseudoplastic, shear-thinning rheology, so that the product is firm in the tube, spreads easily under the finger and stays where it is placed. It extends residence time at the application site, which for a volatile oil also means reduced evaporative loss. It improves cosmetic acceptability — non-greasy, non-staining, easily washed off — which for a chronically applied product is a determinant of adherence rather than a luxury. And it provides a matrix from which release can be modulated, adding a diffusion step that the free nanoemulsion does not have.

That last point is a genuine trade-off and is often glossed over. Adding a gel network interposes a diffusional barrier between the droplets and the skin, and a highly viscous gel can reduce flux relative to the parent nanoemulsion. The gelling agent concentration is therefore not a formulation afterthought but a variable with an interior optimum: enough to give acceptable rheology and residence, not so much as to throttle release. Carbomers, cellulose ethers and poloxamers are the usual choices, with carbomers requiring neutralisation to a pH at which they thicken — a step that must be reconciled with the requirement for a skin-compatible final pH in the region of 5 to 6, and with the ionisation state of a phenolic active.

Reported systems support the approach. A nanoemulsion-based gel for transdermal delivery demonstrated the influence of the surfactant–co-surfactant ratio on droplet size and stability [29]; a nanoemulgel improved topical delivery of a natural anti-inflammatory agent with in vivo evaluation [30]; an essential-oil-based nanoemulgel showed topical anti-inflammatory and wound-healing activity in preclinical assessment [32]; and an essential-oil nanoemulsion was developed with antimicrobial and anti-inflammatory evaluation [35]. Reviews specific to phytoconstituent and essential oil nanoemulgels [33] and a Quality-by-Design development of a clove-oil nanoemulgel for topical anti-inflammatory therapy with ex vivo permeation and in vivo evaluation [34] establish that the specific combination proposed here is both feasible and already under active investigation.

V. THE VARIABLES THAT MATTER

Table 2 sets out the formulation and process variables and the quality attributes each governs. Figure 2A shows how the feasible composition region is identified.

Pseudoternary phase diagrams are the standard tool for that identification and should precede any designed experiment. Constructed by aqueous titration of oil–Smix mixtures at fixed surfactant-to-co-surfactant ratios, they delimit the region of composition space within which a clear, low-viscosity nanoemulsion forms rather than a coarse emulsion, a gel or a separated system. Running a Box–Behnken design without first mapping that region risks spending runs on compositions that cannot form the intended product at all, which wastes the design and distorts the fitted surface.

Three variables then dominate. Oil concentration sets the payload and, through the oil-to-surfactant ratio, the achievable droplet size; higher oil content generally increases droplet size and can increase occlusion and therefore penetration, but the eugenol data suggest the effect on efficacy saturates [22]. Smix concentration and ratio control interfacial tension and hence the minimum attainable droplet size; more surfactant gives smaller droplets but raises irritation potential, and for a chronically applied product the surfactant load is a safety variable rather than merely a performance one. Sonication time controls energy input and droplet break-up, with diminishing returns and an eventual reversal as prolonged sonication heats the sample, promotes coalescence and risks degrading thermolabile constituents.

The responses worth modelling follow from this. Globule size and polydispersity index are the conventional pair and are easily measured. Cumulative permeation or steady-state flux through excised skin is the response that actually bears on performance and is the one most often omitted from the design in favour of being measured only on the final batch. Including it as a modelled response is more work but is what converts a particle-size optimisation into a delivery optimisation.

Table 2. Formulation and process variables for a clove oil nanoemulgel, the quality attributes each governs, and the trade-off each embodies.

Variable	Direction of effect	Attributes affected	Trade-off
Essential oil concentration	Higher loading increases droplet size and occlusion; payload rises	Globule size; drug content; occlusion and hydration of the barrier	Efficacy appears to saturate with eugenol concentration, while irritation and odour do not
Smix concentration	More surfactant lowers interfacial tension and reduces attainable droplet size	Globule size; polydispersity; transmittance; physical stability	Smaller droplets against cumulative surfactant exposure on chronic application
Surfactant : co-surfactant ratio	Determines interfacial film packing and the extent of the nanoemulsion region	Size and stability; extent of the feasible region in the phase diagram	Must be fixed before phase-diagram construction; each ratio needs its own diagram
Sonication time and amplitude	Greater energy input reduces droplet size, with diminishing and then reversing returns	Globule size and distribution; constituent integrity	Over-sonication heats the sample, promotes coalescence and degrades thermolabile constituents
Gelling agent type and concentration	Higher concentration increases viscosity and residence but adds a diffusional barrier	Rheology; spreadability; residence time; release rate and flux	Acceptable rheology against throttled release — an interior optimum, not a maximum
Neutralisation and final pH	Carbomer systems thicken only on neutralisation; pH alters phenol ionisation	Viscosity; skin compatibility; partitioning and stability of eugenol	Gel consistency against a skin-compatible pH and the ionisation state of the active
Antioxidant and packaging	Limits oxidation of the phenolic constituents and evaporative loss	Assay on storage; colour; odour	Added excipient burden against shelf life for an inherently oxidisable active

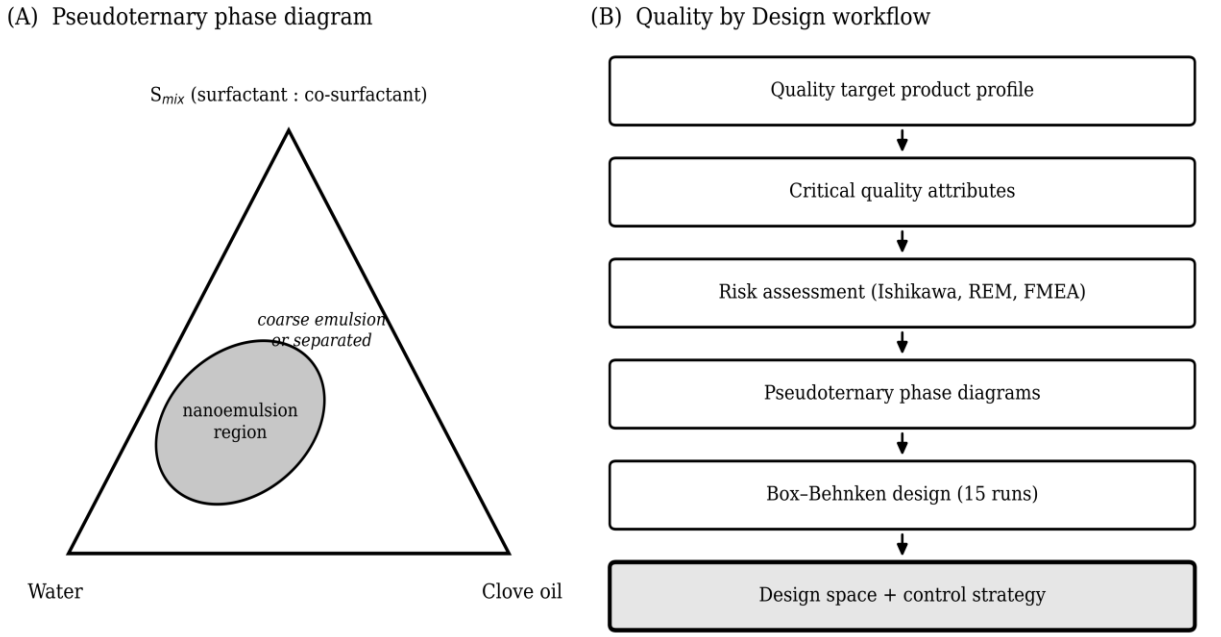


Fig. 2. (A) Pseudoternary phase diagram, shown schematically. Aqueous titration of oil–Smix mixtures at a fixed surfactant-to-co-surfactant ratio delimits the region of composition space within which a clear, low-viscosity nanoemulsion forms; compositions outside it give coarse emulsions, gels or separated systems. Mapping this region precedes the designed experiment, so that runs are not spent on compositions that cannot form the intended product. A separate diagram is required for each Smix ratio. (B) The Quality by Design workflow, in which the documented design space and control strategy — not the single optimised batch — are the deliverable.

VI. QUALITY BY DESIGN: THE DESIGN SPACE IS THE DELIVERABLE

The Quality by Design framework is codified across three ICH guidelines: Q8(R2) on pharmaceutical development, which introduces the quality target product profile, critical quality attributes and the design space; Q9 on quality risk management; and Q10 on the pharmaceutical quality system [36,37,38]. Its principles and their pharmaceutical application have been set out in detail [39,40,41].

Table 3 proposes a quality target product profile and the critical quality attributes derived from it, and Figure 2B shows the workflow.

The sequence matters. A quality target product profile is defined first, from the clinical use: a topical semisolid for localised inflammation, applied two or three times daily, cosmetically acceptable, non-irritant on chronic use, stable for a commercially meaningful shelf life. Critical quality attributes are then derived from that profile rather than from what is convenient to measure — globule size, polydispersity, pH, viscosity and spreadability, drug content, in vitro release and ex vivo permeation. Risk assessment by Ishikawa analysis, an initial risk estimation matrix and failure mode and effects analysis then identifies which material attributes and process parameters actually threaten those attributes, and that assessment is what justifies the choice of factors for the experimental design [37].

A three-factor, three-level Box–Behnken design in oil concentration, Smix concentration and sonication time requires fifteen runs including three centre-point replicates and estimates a full quadratic response surface without the extreme corner combinations of a full factorial — an advantage here, because extreme compositions fall outside the nanoemulsion region established by the phase diagram.

The point that deserves emphasis is what the exercise produces. A design of experiments used only to locate a single optimised formulation has been used as an expensive optimiser; the regulatory and scientific value lies in the design space — the multidimensional region within which the critical quality attributes remain acceptable — because that is what permits movement within the region without a post-approval change, and that is what documents process understanding rather than process luck. Reporting a design space, a control strategy and the risk assessment that generated them is therefore the deliverable, and the optimised batch is a by-product of it. A precedent exists for exactly this: a topical nanoemulgel formulated by a Quality-by-Design approach with explicit establishment of the design space [31].

6.1 Reading the response surface honestly

A Box–Behnken design produces a fitted quadratic model for each response, and the statistics that accompany it are frequently reported in a way that overstates what has been established.

Three checks separate a usable model from a decorative one. The analysis of variance should show a significant model and, importantly, a non-significant lack of fit: a significant lack of fit means the quadratic form does not describe the data, and no amount of model significance rescues that. The adjusted and predicted coefficients of determination should agree to within about 0.2; a large gap indicates over-fitting, which is common with fifteen runs and nine model terms. And adequate precision, the signal-to-noise measure, should exceed four if the model is to be used for navigation of the design space rather than merely for description.

Centre-point replicates earn their place here. Three of the fifteen runs are replicates at the centre, and their only purpose is to estimate pure experimental error, against which lack of fit is judged. Reporting a design without the replicate variance leaves the central diagnostic uncomputable.

Two interpretive errors recur. The first is treating a non-significant term as evidence of no effect; with fifteen runs the design is modestly powered, and absence of significance for an interaction term is weak evidence that the interaction is absent. The second is extrapolation: a quadratic surface fitted over a defined factor range says nothing reliable outside it, and numerical optimisation routines will happily return an optimum at a boundary that the experiment never visited.

Finally, the confirmation run is not optional. The predicted optimum should be prepared and its

responses compared with the model predictions, with the percentage prediction error reported for each. A design space without a confirmation batch is a model rather than a result, and the confirmation is also the only point at which the whole chain — phase diagram, risk assessment, design, optimisation — is tested against reality.

Table 3. Proposed quality target product profile and the critical quality attributes derived from it for a clove oil nanoemulgel.

QTPP element / CQA	Target	Justification
Dosage form	Topical nanoemulgel, semisolid	Semisolid rheology is required for application to and retention on a localised site
Route and regimen	Topical, two to three applications daily, chronic use	Localised inflammation is managed over months; systemic exposure is to be avoided
Globule size	Below 150 nm	Large interfacial area promotes partitioning into the stratum corneum; sub-200 nm defines the nanoemulsion range
Polydispersity index	Below 0.3	A narrow distribution indicates a uniform, kinetically stable dispersion and more reproducible behaviour
pH	Approximately 5 to 6	Compatibility with the acid mantle of the skin; minimises irritation on chronic application
Rheology	Pseudoplastic, shear-thinning	Firm at rest for retention in the container and on the skin; spreads readily under applied shear
Drug content (as eugenol)	Within specification against a validated assay	Content must be defined against the marker constituent, not against total oil, because oil composition varies
In vitro release	Sustained over 24 h	Matches a two- to three-times-daily regimen and limits peak local concentration
Ex vivo flux	Several-fold that of a plain essential oil gel of equal eugenol content	The comparator that isolates the contribution of nanoemulsification from that of the topical route
Skin irritation	No significant irritation by OECD 404	Eugenol is cytotoxic at high local concentration and the product is applied chronically
Stability	Compliant with ICH Q1A(R2) over the proposed shelf life	Volatile, oxidisable phenolic active in a kinetically stabilised dispersion

VII. HOW SUCH A SYSTEM SHOULD BE EVALUATED

A credible evaluation package has six components.

Oil characterisation. Gas chromatography–mass spectrometry of each batch of clove oil against retention indices, with eugenol, eugenyl acetate and β-caryophyllene quantified [42]. A validated assay for eugenol in the finished nanoemulgel follows from this, and drug content should be expressed against eugenol rather than against total oil.

Nanoemulsion characterisation. Globule size, polydispersity index and zeta potential by dynamic and electrophoretic light scattering; percentage transmittance as a rapid surrogate for dispersion fineness; morphology by transmission electron microscopy; and thermodynamic stability testing by heating–cooling cycles, centrifugation and freeze–thaw, which for a kinetically stabilised system is the test that distinguishes a formulation that will survive from one that will not [17,19].

Gel characterisation. pH, which should sit in the skin-compatible range; viscosity and flow behaviour, with pseudoplasticity demonstrated rather than assumed; spreadability; and — the step most often omitted — re-measurement of globule size in the gelled product, since the shear and the pH shift of gelation can alter

the droplets that were so carefully optimised in the parent dispersion.

Release and permeation. In vitro release with the data fitted to zero-order, first-order, Higuchi and Korsmeyer–Peppas models to characterise the mechanism [43,44], and ex vivo permeation through excised skin in Franz diffusion cells with steady-state flux and permeability coefficient reported. The comparator set should include a plain clove oil gel at equal eugenol content, not only a marketed product: without the plain gel arm the study demonstrates the benefit of a topical vehicle rather than the benefit of nanoemulsification.

In vivo activity and tolerability. The carrageenan-induced rat paw oedema model remains the standard assay and has been in continuous use since its description [45]. Skin irritation should be assessed by the Draize approach as modernised in OECD Test Guideline 404 [46,47]; for a product containing a phenolic constituent with known cytotoxic potential at high concentration [12], and applied chronically, this is a substantive endpoint rather than a formality.

Stability. Accelerated testing to ICH Q1A(R2) [48], monitoring not only eugenol assay but also globule size, phase separation, pH, viscosity and colour. For a volatile phenolic oil the informative measurements are the ones most likely to be omitted: loss of total oil by evaporation from the container, and appearance of oxidation products by chromatography.

VIII. GAPS AND OUTLOOK

Several gaps are worth naming directly.

The standardisation gap. Essential oil composition varies with origin, season and distillation, yet many formulation studies characterise the oil once or not at all. Batch-wise chromatographic characterisation is inexpensive and is the precondition for the quantitative relationships a designed study is meant to establish.

The gelled-product gap. Droplet size, polydispersity and zeta potential are routinely reported for the parent nanoemulsion and rarely re-measured after incorporation into the gel, even though gelation involves shear and, for carbomers, a pH shift. A nanoemulgel characterised only through its parent nanoemulsion has not been characterised.

The comparator gap. Studies commonly compare a nanoemulgel with a marketed synthetic product and conclude that the nanoemulgel is effective. The comparison that isolates the contribution of nanoemulsification is against a plain gel containing the same quantity of the same oil, and it is reported far less often.

The saturation gap. The finding that increasing eugenol concentration did not increase anti-inflammatory effect [22] has not been systematically followed up. If the dose–response saturates at accessible concentrations, then the correct optimisation objective is minimum effective oil content, and much of the field is optimising for the opposite.

The surfactant gap. Reported essential oil nanoemulsions frequently use surfactant concentrations of the order of ten per cent or more — in the eugenol study, 14% Tween 20 with 14% isopropyl alcohol [22]. For a product applied over a large area several times daily for months, the cumulative surfactant exposure is a safety consideration in its own right, and minimising it deserves to be a formal design objective rather than a constraint noted in passing.

IX. CONCLUSION

Clove oil is an unusually well-matched active for a nanoemulsion-based topical product. Its pharmacology is multi-target and well characterised: inhibition of cyclo-oxygenase-2 and 5-lipoxygenase, suppression of nuclear factor-kappa B translocation, antioxidant activity and sodium channel blockade from eugenol, with cannabinoid CB2 agonism contributed by β-caryophyllene in the same oil. Its liabilities are physical rather than pharmacological, and a nanoemulsion addresses most of them at once — with the particular elegance that the active serves as its own oil phase and, being a recognised penetration enhancer, promotes its own absorption.

Converting that dispersion into a nanoemulgel supplies the rheology, residence and acceptability a chronically applied topical product needs, at the cost of an additional diffusional barrier that makes gelling agent concentration a real optimisation variable rather than a formulation detail.

The Quality by Design framework is the right way to explore that space, but its value is easily mislaid. A Box–Behnken design used only to find one good batch has been used as an expensive optimiser. Used

properly — preceded by phase-diagram mapping and formal risk assessment, including permeation as a modelled response rather than a final check, and concluding with a documented design space and control strategy — it produces process understanding that outlives the particular batch. Add to that batch-wise standardisation of the oil, re-characterisation of the droplets after gelation, a plain-gel comparator that isolates what nanoemulsification actually contributes, and an explicit objective of minimising both oil and surfactant load, and the result is a study whose conclusions would survive scrutiny. Each of those elements is standard somewhere in the literature; the contribution lies in assembling them.

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