ADVANCED PEPTIDE DELIVERY TECHNOLOGY

Preclinical Technical Dossier

Protixa™ ION System™

Mucosal Transport, Stability, and Safety Characterization

Oral . Intranasal . Sublingual . Topical Delivery Platform

protixa

www.protixa.com

>57×

Barrier Penetration Vs. Saline – Ex Vivo Epithelial Model (Protixa Third-Party Data)

<10 nm

Minimum confirmed nanoparticle size – mucus-penetrating ionic clusters (DLS)

5 ORGANS

Confirmed systemic distribution – in vivo oral mouse study (1 hour post-dose)

NON-CYTOTOXIC-n

ISO 10993-5 – CCK-8 ASSAY (24H & 48H) – 80–100% VIABILITY

4-MONTH STABLE

Room temperature stability – 101.1–101.9% Recovery (hplc) – no cold chain Required

protixa

Protixa ION System in vivo oral data: confirmed multi-organ systemic distribution vs. negligible signal in water control within 1 hour (Section 7). Ex vivo epithelial permeation: >57× enhancement vs. saline control (Protixa third-party data, Section 9). Peer-reviewed ionic liquid class reference: Angsantikul et al., Advanced Functional Materials (DOI: 10.1002/adfm.202002912).

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Protixa ION System - Overview of Findings

The Protixa ION System is a proprietary protic ionic liquid (PIL) excipient platform engineered to solve one of the most persistent problems in modern peptide science: the inability to deliver therapeutic peptides into the body through non-invasive routes (particularly oral ingestion, intranasal administration, and sublingual absorption) without enzymatic degradation, mucosal barrier exclusion, or loss of structural integrity. The system achieves this through a multi-mechanism approach that simultaneously stabilizes the peptide cargo, reduces mucosal viscosity, modulates epithelial tight junctions, and organizes the formulation into nano-scale assemblies with a confirmed sub-10 nm secondary population (5–10 nm, DLS), small enough to penetrate mucus gel networks that trap conventional formulations.

The platform is formed from two naturally occurring, pharmacopoeially accepted components: citric acid (a tricarboxylic acid anion and central Krebs cycle metabolite) and amino acids, specifically lysine and arginine (cationic species). This composition introduces no novel synthetic chemical entities, carries established GRAS and USP/NF regulatory status, and is metabolized through normal biochemical pathways.

The scientific case rests on two complementary bodies of evidence: independent third-party laboratory studies conducted by Zhejiang TianMei Biological Engineering Co., LTD and Zhejiang University of Technology, and a peer-reviewed pharmaceutical study (Angsantikul et al., Advanced Functional Materials, DOI: 10.1002/adfm.202002912) examining a structurally analogous ionic liquid system (CGLY) for oral delivery of monoclonal antibodies. That study demonstrated a 5-fold increase in systemic plasma bioavailability and greater than 4.5-fold increase in intestinal tissue penetration compared to saline controls — providing peer-reviewed class-level validation of the ionic liquid oral delivery mechanism that underpins the Protixa ION System. Note: this 5× figure is from the CGLY reference study; Protixa ION System in vivo oral data demonstrates confirmed multi-organ systemic distribution vs. negligible signal in water control (Section 7).

<10 nm	5 ORGANS	NON-CYTOTOXIC	101.1-101.9%
Sub-10 nm Population	**Systemic Distribution**	**ISO 10993-5 Certified**	**Recovery Rate**
SECONDARY PEAK: 5–10 NM (DLS CONFIRMED)	ORAL FITC STUDY: INTESTINE, LUNG, LIVER, KIDNEY, STOMACH CONFIRMED	CCK-8 ASSAY — 24H & 48H EXPOSURE (80–100% VIABILITY)	ROOM TEMPERATURE STABILITY 1–4 MONTHS (HPLC VALIDATED)

EXECUTIVE SUMMARY

Protixa ION System - Overview of Findings

4-MONTH ROOM TEMPERATURE STABILITY CONFIRMED — NO COLD CHAIN REQUIRED

Independent HPLC-validated stability testing demonstrates that peptides formulated in the Protixa ION System maintain 101.1–101.9% recovery across four consecutive months at room temperature — with zero degradation trend across the entire observation period. This result eliminates cold-chain storage and temperature-controlled shipping requirements, dramatically reducing distribution cost and complexity for oral, sublingual, and intranasal peptide products. All four time points (1, 2, 3, and 4 months) fall well within ICH Q1A(R2) acceptance criteria of 90–110%.

The system was evaluated in an ex vivo epithelial barrier penetration model using full-thickness porcine tissue (0.5 mm) — the most conservative and challenging epithelial model available — and demonstrated greater than 57-fold enhancement in peptide permeation compared to saline controls. This is Protixa's own third-party verified data and represents the headline quantitative performance metric for the platform. Notably, this 57× enhancement was achieved through 0.5 mm thick tissue; oral, sublingual, and nasal mucosal surfaces are approximately 5× thinner (0.1 mm), meaning the 57× figure is a conservative lower bound for mucosal delivery performance. The in vivo oral FITC study (Section 7) confirms this translates to genuine systemic delivery: confirmed fluorescence in five major organs within one hour of oral administration, with negligible signal in the water control.

SCOPE AND REGULATORY NOTICE

All findings presented in this report are based on ex vivo models, in vitro cytotoxicity testing, stability studies, analytical quantification, and in vivo fluorescence distribution studies. These results represent preclinical and laboratory observations and are not clinical claims. The Protixa ION System is an excipient platform composed of established food-grade components. No clinical efficacy claims are made or implied.

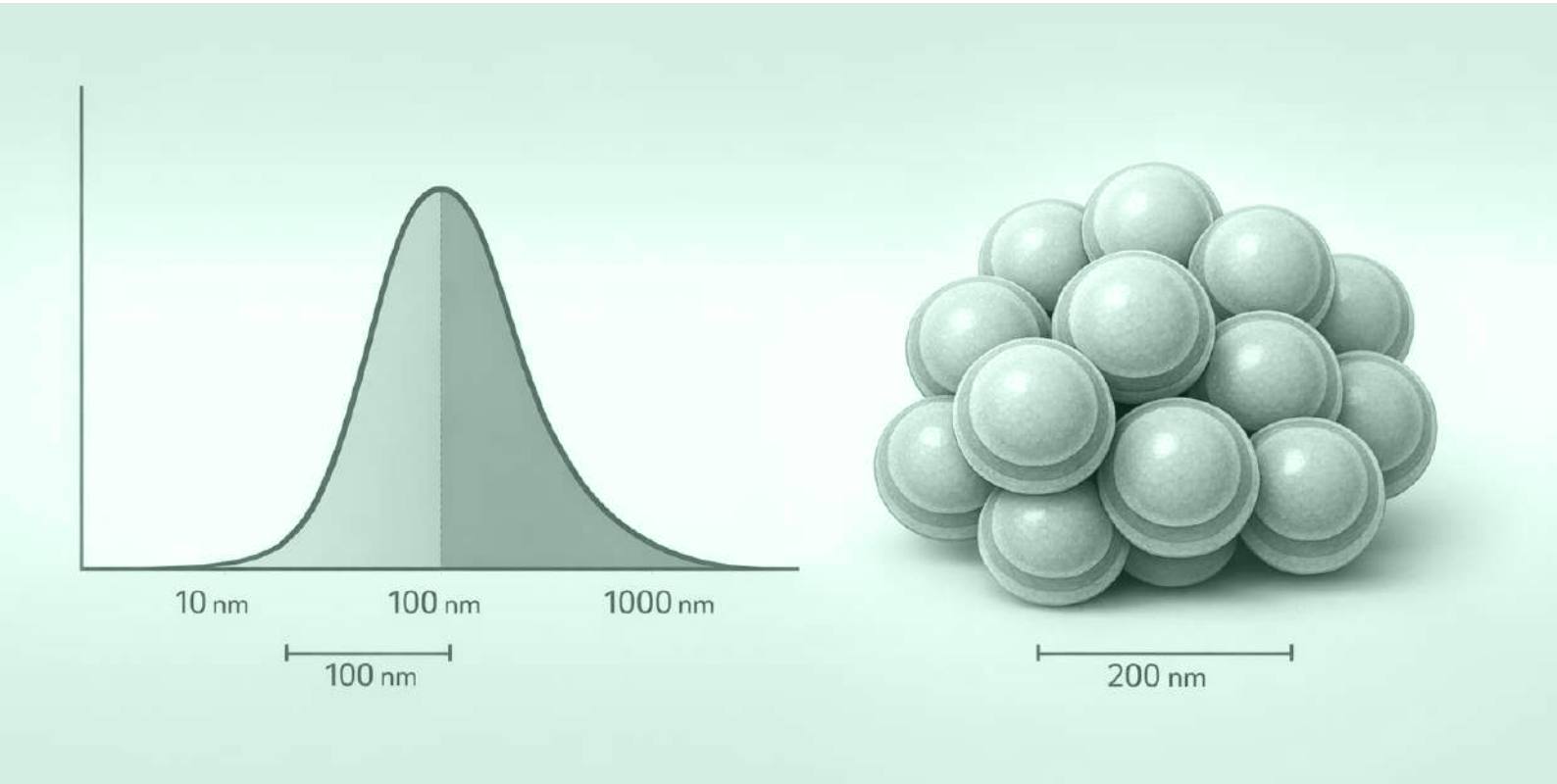
SECTION 1

Introduction:
The Peptide Delivery Challenge

1.1 Why Peptides Fail Without a Delivery System

Therapeutic peptides represent one of the most promising categories in modern medicine and advanced wellness formulation. Their high target specificity, favorable pharmacodynamic profiles, and structural diversity make them uniquely suited for applications ranging from metabolic regulation and tissue repair to immune modulation and neuroprotection. Yet despite these advantages, the clinical translation of peptide therapeutics has been persistently constrained by a single fundamental problem: peptides are extraordinarily difficult to deliver into the body through any route other than injection.

The reasons are rooted in basic biochemistry. Peptides are composed of amino acid chains linked by peptide bonds, which are specifically recognized and cleaved by proteolytic enzymes present throughout the gastrointestinal tract, on mucosal surfaces, and in the bloodstream. When administered orally, a peptide encounters gastric acid (pH 1.5-3.5), which causes direct hydrolysis, followed by pancreatic and brush-border proteases in the small intestine that degrade the peptide before it can reach the epithelial surface. The result is that oral bioavailability of most unformulated peptides is less than 1-2%, and for larger peptides it approaches zero. Sublingual and intranasal routes offer somewhat better prospects due to thinner epithelial layers, but mucociliary clearance and enzymatic activity at these surfaces still severely limit absorption without an effective delivery system.



SECTION 1

Introduction:
The Peptide Delivery Challenge

1.2 The Biological Barriers to Oral, Nasal, and Sublingual Delivery

ENZYMATIC DEGRADATION

Proteases throughout the GI tract, nasal mucosa, and sublingual epithelium cleave peptide bonds. Gastric acid (pH 1.5-3.5) causes direct hydrolysis. Pancreatic enzymes complete degradation in the small intestine before absorption can occur.

MUCUS BARRIER

A viscoelastic hydrogel of cross-linked mucin glycoproteins covers all mucosal surfaces: the GI tract, nasal passages, and sublingual epithelium. Calcium ion cross-links increase viscosity and trap large molecules before they reach epithelial cells.

EPITHELIAL TIGHT JUNCTIONS

Claudins, occludins, and junction adhesion molecules seal intercellular spaces. Molecules above ~1 kDa cannot pass paracellularly. Most therapeutic peptides (1-50 kDa) are effectively excluded without intervention.

FIRST-PASS METABOLISM

Peptides absorbed from the GI tract enter the portal circulation and pass through the liver before reaching systemic circulation. Hepatic enzymes further degrade peptides. Sublingual and nasal routes bypass this barrier entirely.

PEPTIDE AGGREGATION

In aqueous solution, peptides form macro-aggregates. Only the outermost surface interacts with biological tissue. Interior molecules remain inaccessible, dramatically reducing effective concentration at the absorption site.

MUCOCILIARY CLEARANCE

In the nasal cavity and sublingual region, ciliated epithelial cells continuously sweep mucus toward the nasopharynx. This limits the absorption window to minutes for non-mucoadhesive formulations, severely restricting bioavailability.

SECTION 1

Introduction:
The Peptide Delivery Challenge

1.3 Why Ionic Liquid Technology Represents a Paradigm Shift

Conventional approaches to improving oral and mucosal peptide bioavailability have included chemical modification of the peptide itself (PEGylation, cyclization, N-methylation), encapsulation in lipid nanoparticles or polymeric microspheres, co-administration with protease inhibitors, and the use of chemical permeation enhancers such as surfactants, fatty acids, and bile salts. Each of these approaches carries significant limitations: chemical modification can alter pharmacological activity; lipid nanoparticles are complex to manufacture and often unstable; protease inhibitors may disrupt normal digestive function; and chemical permeation enhancers frequently cause membrane disruption, irritation, and cytotoxicity at effective concentrations.

Protic ionic liquids (PILs) formed from naturally occurring organic acids and amino acids offer a fundamentally different approach. Rather than modifying the peptide or disrupting biological membranes, PILs create a unique physicochemical microenvironment around the peptide that simultaneously addresses multiple delivery barriers through non-destructive, reversible mechanisms. When the PIL is formed from citric acid and amino acids (as in the Protixa ION System), both components are endogenous metabolites that the body already recognizes and processes, providing a safety and regulatory profile that is fundamentally superior to synthetic permeation enhancers.

KEY INSIGHT: WORKING WITH BIOLOGY, NOT AGAINST IT

The Protixa ION System does not overcome biological barriers by destroying them. It works with the biology by transiently and reversibly modulating the mucus layer and tight junction architecture through ionic interactions, then allowing the system to return to its baseline state. This reversible, non-destructive approach is the basis of its favorable safety profile and the reason it achieves meaningful oral, sublingual, and mucosal bioavailability without the tissue damage associated with conventional permeation enhancers.

SECTION 2

Platform Architecture and Ionic Chemistry

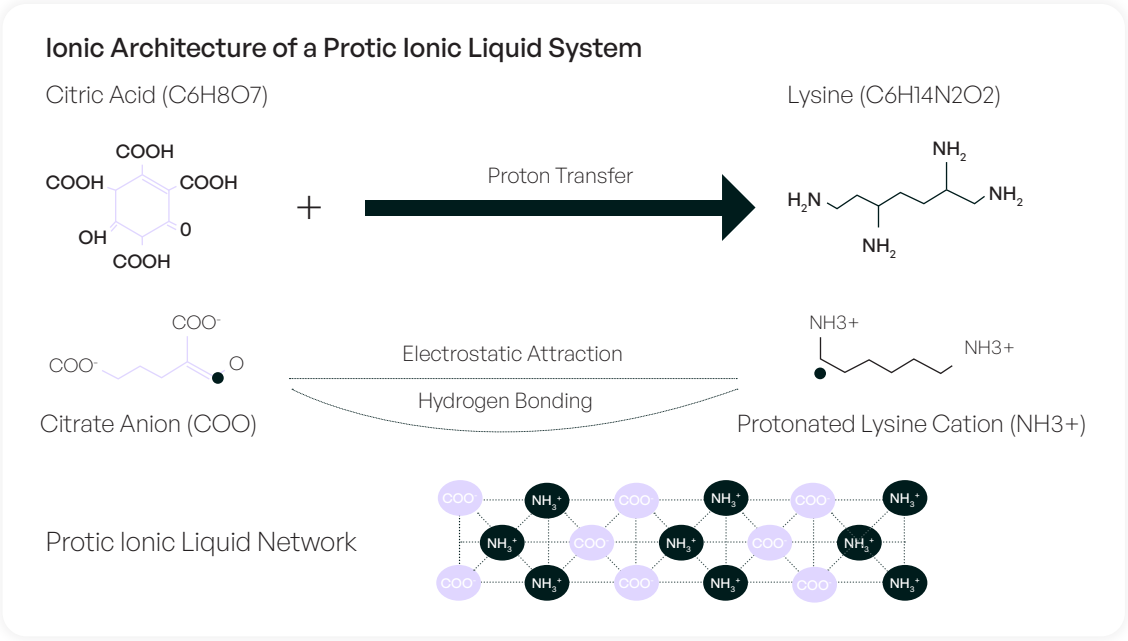
2.1 Ionic Composition and Protic Ionic Liquid Formation

The Protixa ION System is formed through a proton transfer reaction between citric acid (a tricarboxylic organic acid) and amino acids, specifically lysine and/or arginine. In this reaction, a proton is transferred from the carboxylic acid groups of citric acid to the epsilon-amino group of the amino acid, generating a salt pair in which the citrate anion and the protonated amino acid cation are held together by a combination of electrostatic attraction and hydrogen bonding. The resulting material is a protic ionic liquid (PIL): a room-temperature liquid with negligible vapor pressure, high ionic conductivity, and a dense three-dimensional network of non-covalent interactions that creates a uniquely protective and transport-enhancing environment for peptide cargo.

TABLE 2.1 -- PROTIXA ION SYSTEM COMPOSITIONAL OVERVIEW

Component	Role in System	Physiological Status	Category
Citric Acid	Anion; proton donor; Ca2+ chelating agent; pH buffer; mucus fluidizer; protease inhibitor	Endogenous Krebs cycle intermediate	GRAS; USP/NF; E330
Lysine / Arginine	Cation; proton acceptor; mucoadhesive agent; tight junction modulator; nasal/ sublingual retention enhancer	Essential / conditionally essential amino acid	GRAS; USP/ NF; dietary supplement
Peptide Cargo	Active pharmaceutical / bioactive ingredient	Exogenous therapeutic agent	Dependent on specific peptide

2.2 FIGURE 1: IONIC ARCHITECTURE OF THE PROTIXA ION SYSTEM



Proton transfer-driven formation of a hydrogen-bonded ionic network between citric acid (citrate anion, COO-) and amino acid cations (protonated lysine, NH3+) within the Protixa ION System. The resulting protic ionic liquid network provides electrostatic stabilization, hydrogen bonding, and controlled nano-assembly that underpin the platform delivery and stability performance.

SECTION 2

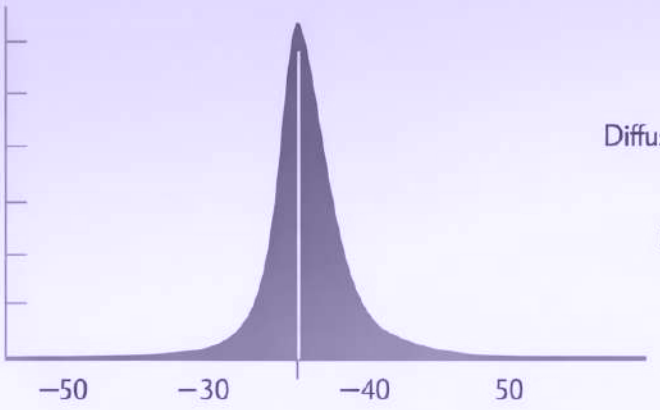
Platform Architecture and Ionic Chemistry

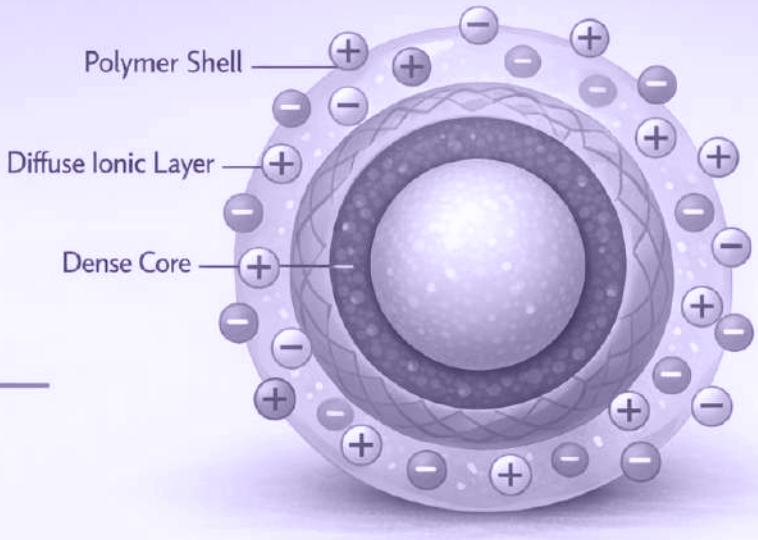
2.3 Molecular Stabilization, Protease Inhibition, and Aggregation Control

Preferential Exclusion: The ionic liquid components organize around the peptide molecule to create a structured hydration shell that thermodynamically favors the native, folded conformation of the peptide. By stabilizing the native conformation, the system reduces the exposure of peptide bond sequences that are recognized by proteolytic enzymes, effectively reducing the rate of enzymatic cleavage. No evidence of irreversible protein denaturation or fragmentation was observed within tested exposure ranges.

Active Site Modulation: The high ionic strength and local pH modulation of the citric acid anion creates a microenvironment less favorable for proteolytic activity. Citric acid-mediated chelation of divalent ions such as calcium (Ca2+) and zinc (Zn2+) is mechanistically consistent with reduced enzyme activity, as many proteases rely on metal ion stabilization for catalytic function. These effects are transient and localized, resolving as the formulation is diluted and dispersed in the GI lumen. Direct protease inhibition assay data is not included in this preclinical package; the protective effect is inferred from the stability and permeation data and supported by the ionic liquid literature.

Aggregation Control: Dynamic Light Scattering analysis demonstrating bimodal nano-scale populations (primary 200-300 nm assemblies with a confirmed sub-10 nm secondary population (5–10 nm range, DLS) supports a stabilized dispersion state rather than uncontrolled macro-aggregation. Dilution-triggered analytical recovery confirms that ionic association is reversible and does not induce irreversible structural alteration or denaturation.





SECTION 2

Platform Architecture and Ionic Chemistry

2.4 Multi-Pathway Mucosal Permeation Enhancement

The Protixa ION System addresses the primary epithelial barriers through three mechanistically distinct but synergistic pathways that together produce the dramatic permeation enhancement observed in laboratory testing across oral, nasal, sublingual, and epithelial barrier models.

MUCUS FLUIDIZATION

Citrate anion chelates Ca2+ ions that cross-link mucin fibers. Reduces mucus viscosity by up to 45% (CGLY study: 0.577 to 0.318 Pa.s). Allows peptide-PIL complex to reach epithelial surface. Active in GI, nasal, and sublingual mucosa. Reversible upon dilution.

TIGHT JUNCTION MODULATION

Amino acid cations interact electrostatically with negatively charged claudin extracellular domains. Triggers transient actin cytoskeleton reorganization. Widens paracellular gaps. 4-5x paracellular transport enhancement confirmed (CGLY Caco-2 study). Fully reversible; TEER returns to baseline.

MUCOADHESION (NASAL/ SUBLINGUAL)

Cationic amino acid groups adhere to negatively charged sialic acid in nasal and sublingual mucosa. Extends residence time on the epithelial surface. Counteracts mucociliary clearance, substantially increasing the absorption window for intranasal and sublingual formulations.

PROTEASE INHIBITION

High ionic strength and local pH modulation reversibly inhibit digestive proteases. Citrate chelates metalloprotease cofactors (Ca2+, Zn2+). Creates a protected microenvironment around the peptide throughout GI and mucosal transit.

PH BUFFERING (ORAL)

Citric acid provides robust buffering capacity. Maintains a protective microenvironment in the acidic gastric environment (pH 1.5-3.5). Prevents acid-induced hydrolysis of peptide bonds during gastric transit.

NANOPARTICLE SELF-ASSEMBLY

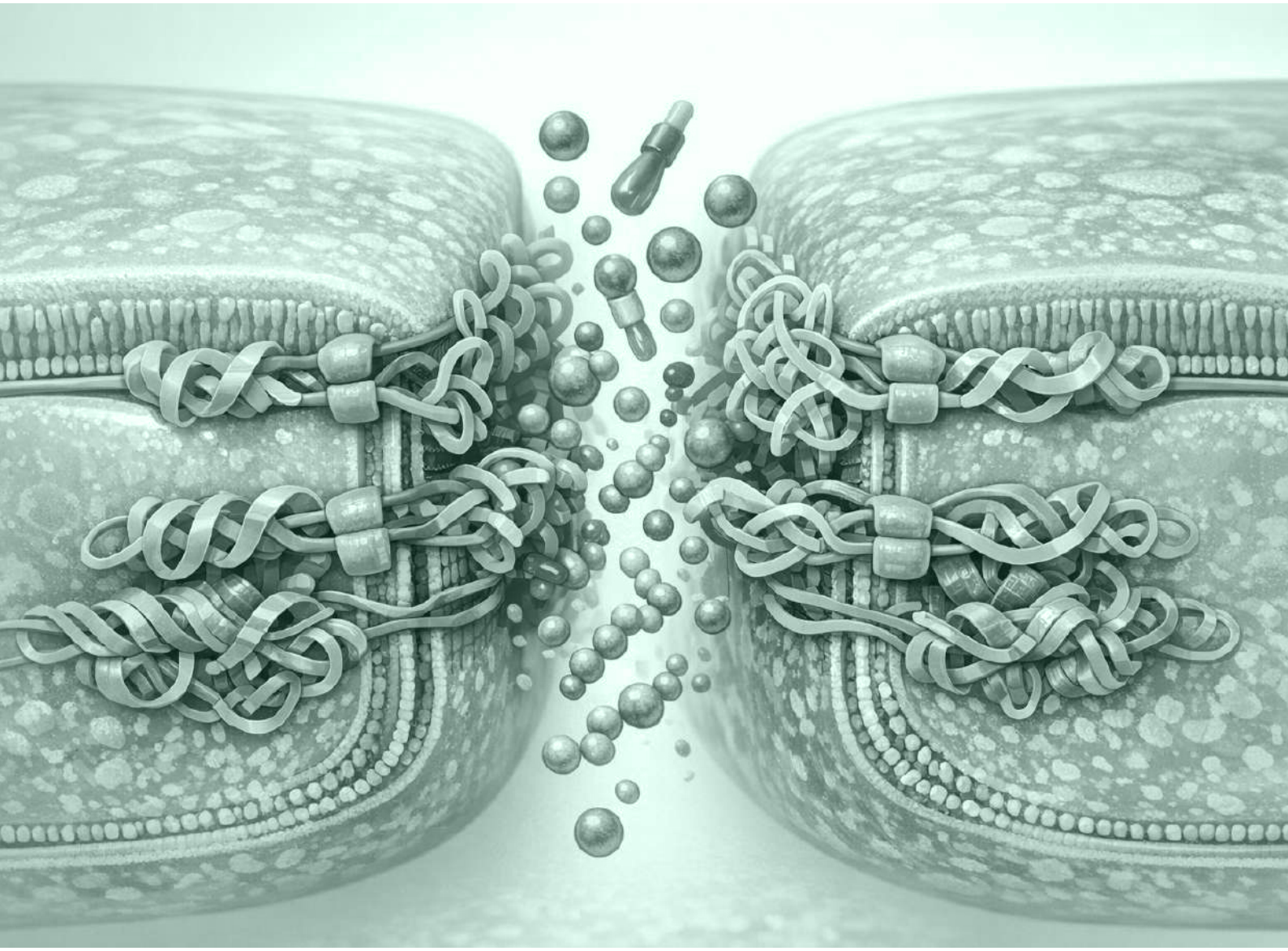
PIL self-assembles into bimodal nanoparticle distribution. Primary: 200-300 nm (peptide-loaded delivery vehicles). Secondary: 5–10 nm (<10 nm confirmed; immediate mucus penetration). Maximizes surface exposure and epithelial interaction efficiency.

SECTION 2

Platform Architecture and Ionic Chemistry

CRITICAL DISTINCTION: IONIC MODULATION VS. MEMBRANE DISRUPTION

The permeation enhancement produced by the Protixa ION System occurs through ionic microenvironment effects and reversible protein-ion interactions, not through membrane disruption, lipid extraction, or cytotoxic mechanisms. Laboratory cytotoxicity testing (CCK-8 assay) confirmed non-cytotoxic classification per ISO 10993-5 at all tested concentrations and timepoints (24h and 48h; 80–100% cell viability), with no evidence of acute inflammatory activation or membrane damage.



SECTION 3

Scientific Precedent:
The CGLY Pharmaceutical Study

IMPORTANT ATTRIBUTION NOTE

This section presents data from a peer-reviewed third-party study on a structurally analogous ionic liquid system (CGLY). This data is included to establish mechanistic precedent and scientific context for the ionic liquid delivery class. It does not constitute direct performance data for the Protixa ION System. Protixa ION System performance data is presented in Sections 4–9.

3.1 Study Overview and Mechanistic Relevance

The direct laboratory data for the Protixa ION System is best understood within the broader scientific context of the ionic liquid delivery class. The core delivery mechanism — the use of a biocompatible ionic liquid to overcome mucosal barriers and enhance oral and mucosal bioavailability — has been independently validated at the pharmaceutical research level by a peer-reviewed study published in Advanced Functional Materials (Angsantikul et al., DOI: 10.1002/adfm.202002912). This study investigated a choline-glycolate ionic liquid system (CGLY) for oral delivery of monoclonal antibodies (~150 kDa) — molecules that face even greater delivery challenges than most therapeutic peptides.

CGLY and the Protixa ION System are distinct formulations: CGLY uses choline and glycolate, while the Protixa ION System uses citric acid and lysine/arginine. The cargo tested in the CGLY study (monoclonal antibodies, ~150 kDa) differs from the peptide cargo relevant to the Protixa ION System. The mechanistic parallels are scientifically meaningful — both are biocompatible ionic liquids operating through mucus viscosity reduction and tight junction modulation — but the CGLY quantitative outcomes (5× bioavailability, >4.5× tissue penetration) represent class-level mechanistic evidence, not direct performance predictions for the Protixa ION System.

REFERENCE

Angsantikul P, et al. “Ionic Liquid-Functionalized Nanoparticles for Oral Drug Delivery.” Advanced Functional Materials. 2020. DOI: 10.1002/adfm.202002912. Conducted on choline-glycolate (CGLY) ionic liquid system for oral monoclonal antibody delivery in Caco-2 cell model and Wistar rat in vivo model.

SECTION 3

Scientific Precedent:
The CGLY Pharmaceutical Study

3.2 Mucus Rheology: Quantified Viscosity Reduction

The mucus layer represents the first physical barrier to mucosal absorption and is structurally stabilized by divalent ion-mediated cross-linking between mucin fibers. Citric acid within the Protixa ION System functions as a calcium chelator, reducing mucin cross-link density and altering mucus rheology. The CGLY study directly quantified this effect using porcine small intestinal mucus (PIM):

TABLE 3.1 -- MUCUS VISCOSITY REDUCTION: QUANTIFIED RHEOLOGICAL DATA

Condition	Mucus Viscosity (Pa.s)	Change	Interpretation
Control (no ionic liquid)	0.577 Pa.s	Baseline	Normal cross-linked mucin gel -- high diffusion resistance
Ionic formulation (12.5% v/v CGLY)	0.318 Pa.s	-45% reduction	Reduced cross-link density -- improved peptide diffusion toward epithelium

This measurable reduction in viscosity (0.577 to 0.318 Pa.s, a 45% decrease) indicates decreased structural resistance and improved diffusion kinetics toward the epithelial surface. From a mechanistic standpoint, lower mucus viscosity increases the probability that intact molecules reach epithelial membranes prior to enzymatic degradation. The Protixa ION System achieves this same effect through citrate-mediated Ca2+ chelation of mucin cross-links.

3.3 Paracellular Pathway Isolation and TEER Reversibility

TEER (Transepithelial Electrical Resistance) analysis demonstrated concentration-dependent reductions in epithelial resistance consistent with transient tight junction modulation. TEER values recovered toward baseline following removal of the ionic liquid, confirming reversibility rather than structural epithelial disruption. Reversible modulation indicates functional permeability enhancement without permanent barrier compromise.

CRITICAL MECHANISTIC FINDING: PARACELLULAR PATHWAY CONFIRMED AS PRIMARY ROUTE

Lucifer yellow transport assays showed a 4-5 fold increase in paracellular marker movement at higher concentrations of the ionic formulation. Inhibition of transcellular pathways did not significantly alter macromolecule transport, definitively isolating the paracellular route as the primary mechanism of enhanced permeability. This means the Protixa ION System works by opening the spaces between cells rather than forcing molecules through cell membranes, which is the mechanistic basis of its favorable safety profile and reversibility.

SECTION 3

Scientific Precedent:
The CGLY Pharmaceutical Study

3.4 Key Findings and Mechanistic Parallels

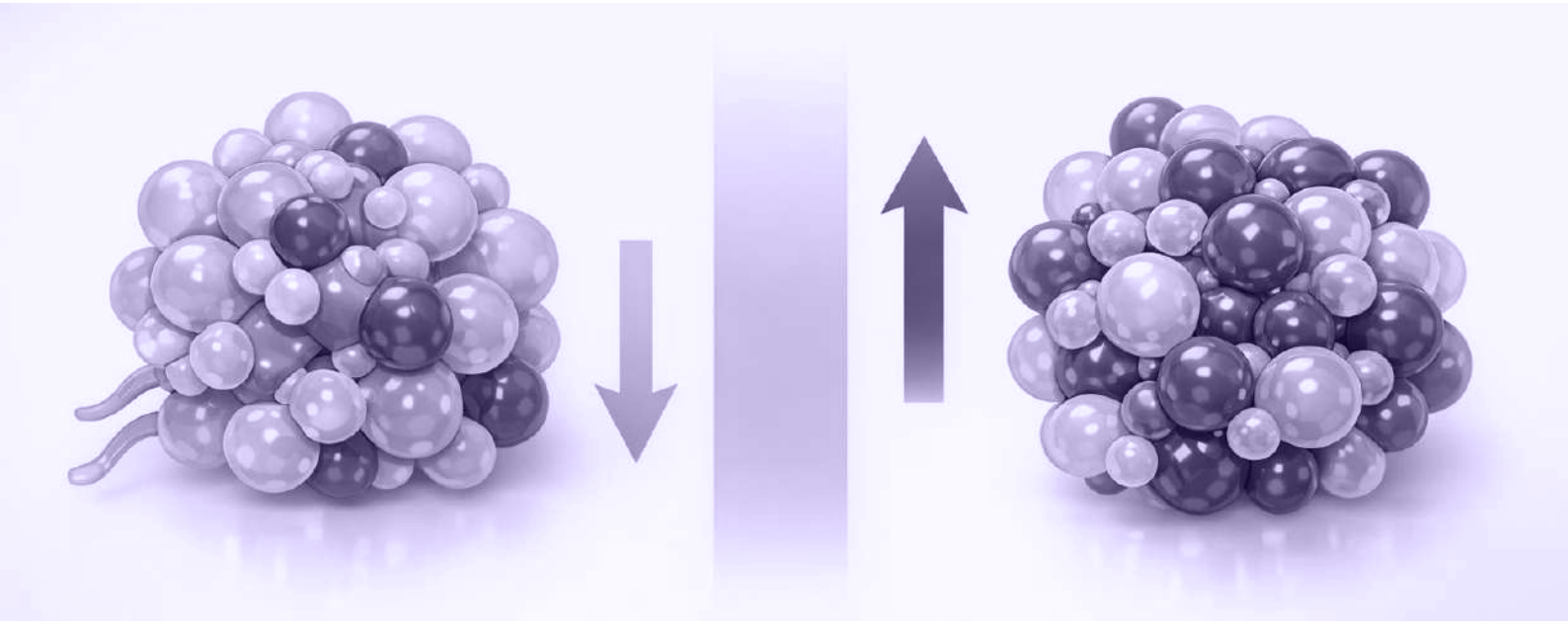
TABLE 3.2 -- CGLY PHARMACEUTICAL STUDY: KEY QUANTITATIVE FINDINGS (ORAL DELIVERY)

Experimental Parameter	CGLY 2:1 Result	Control (Saline)	Enhancement
Antibody structural integrity (CD spectroscopy)	Fully preserved	N/A	--
Antigen-binding function (ELISA)	Fully retained	N/A	--
Caco-2 intestinal cell viability	>80%	100%	Biocompatible
Mucus viscosity (porcine intestinal mucus)	0.318 Pa.s (from 0.577 Pa.s)	0.577 Pa.s	45% reduction
Lucifer yellow paracellular transport	4-5x increase	Baseline	4-5-fold
Transcellular pathway inhibition effect on transport	No significant effect	N/A	Paracellular route confirmed as primary
TEER recovery after ionic liquid removal	Returns to baseline	N/A	Fully reversible
In vivo intestinal tissue penetration (rat jejunum villi)	>4.5x vs. saline	Blocked by mucus	>4.5-fold
Systemic plasma IgG concentration (4h post-oral)	5x higher than control	Minimal absorption	5-fold systemic bioavailability
7-day repeat-dose safety (Wistar rats 625 mg/kg/day)	No toxicity normal GI histopathology normal blood chemistry	N/A	Safe

The side-by-side comparison below highlights the structural and mechanistic parallels between the CGLY system studied in the peer-reviewed literature and the Protixa ION System evaluated in the third-party laboratory studies. Both systems operate through the same dual mechanism of mucus viscosity reduction and tight junction modulation. The key distinction is that the Protixa ION System uses citric acid and amino acids — endogenous metabolites with established GRAS status — rather than choline and glycolate, and has been tested on therapeutic peptides rather than monoclonal antibodies. The mechanistic parallels are direct and scientifically meaningful; the quantitative outcomes from the CGLY study represent class-level evidence for what the ionic liquid delivery mechanism can achieve.

SECTION 3

Scientific Precedent:
The CGLY Pharmaceutical Study



CGLY SYSTEM (ANGSANTIKUL ET AL. -- PEER REVIEWED)

- ✓ Choline + glycolate ionic liquid
- ✓ Tested on monoclonal antibodies (~150 kDa)
- ✓ Caco-2 monolayer + rat jejunum in vivo model
- ✓ 45% mucus viscosity reduction (0.577 to 0.318 Pa.s)
- ✓ 4-5x paracellular transport enhancement
- ✓ Paracellular route confirmed as primary mechanism
- ✓ >4.5x intestinal villi tissue penetration
- ✓ 5x systemic plasma concentration increase
- ✓ Safe at 625 mg/kg/day x 7 days (rat)

PROTIXA ION SYSTEM (TIANMEI / ZUT)

- ✓ Citric acid + lysine/arginine ionic liquid
- ✓ Tested on short-chain therapeutic peptides (short-chain model compound, 2–5 AA)
- ✓ Ex vivo epithelial model + in vivo mouse oral study
- ✓ Mucus fluidization via Ca2+ chelation (same mechanism)
- ✓ Paracellular TJ modulation (same mechanism)
- ✓ Sub-10 nm nanoparticle population confirmed (5–10 nm range, DLS)
- ✓ Multi-organ systemic distribution confirmed (FITC)
- ✓ Non-cytotoxic per ISO 10993-5 (CCK-8, 24h & 48h; 80–100% viability)
- ✓ Conducted: Zhejiang University of Technology

SECTION 4

Analytical Validation and Detection Methodology

Before any quantitative performance data could be generated for the Protixa ION System, a fundamental analytical challenge had to be resolved: the ionic liquid environment that makes the system effective at stabilizing and delivering peptides also interferes with conventional HPLC quantification methods. The PIL forms stable complexes with the peptide cargo, reducing the concentration of free peptide in solution and causing conventional assays to systematically underestimate total peptide content. Addressing this required development of a novel detection methodology specifically validated for ionic liquid-peptide complexes.

4.1 The Analytical Challenge: Complex Formation and Free Peptide Suppression

When a peptide is dissolved in the Protixa ION System, the ionic liquid components form non-covalent complexes with the peptide through electrostatic interactions and hydrogen bonding. These complexes are stable under physiological conditions (which is precisely what makes them effective for delivery), but they also mean that a significant fraction of the total peptide is bound within the complex and is not detectable as free peptide by standard HPLC methods. At 100x dilution, the ionic concentration remains high enough to maintain complex stability, and the measured recovery rate reflects only the free peptide fraction (approximately 80-83%). This is not peptide degradation; it is complex formation, and the distinction is critical for accurate analytical interpretation.

The key insight enabling a reliable detection method was that the complex can be dissociated simply by dilution. When the ionic concentration is reduced sufficiently through 1,000x dilution, the electrostatic and hydrogenbonding interactions that maintain the complex are weakened below the threshold required for stability, and the complex dissociates, releasing the bound peptide as free peptide detectable by HPLC.

TABLE 4.1 -- PEPTIDE DETECTION METHOD DEVELOPMENT: RECOVERY RATE DATA

Condition	Solvent	Dilution	Measured Conc. (mg/mL)	Recovery (%)	Interpretation
Reference (water)	Water	100x	9.35	93.5%	Baseline -- no complex formation
Ionic liquid standard	Protixa ION System	100x	7.99	79.9%	Complex partially intact -- free peptide only
Ionic liquid + NaOH	Protixa ION System + NaOH	100x	8.25	82.5%	NaOH insufficient to disrupt complex
Ionic liquid + NaCl	Protixa ION System + NaCl	100x	8.36	83.6%	NaCl insufficient to disrupt complex
Ionic liquid high dilution	Protixa ION System	1000x	11.23	112.3%	Complete complex dissociation -- validated method

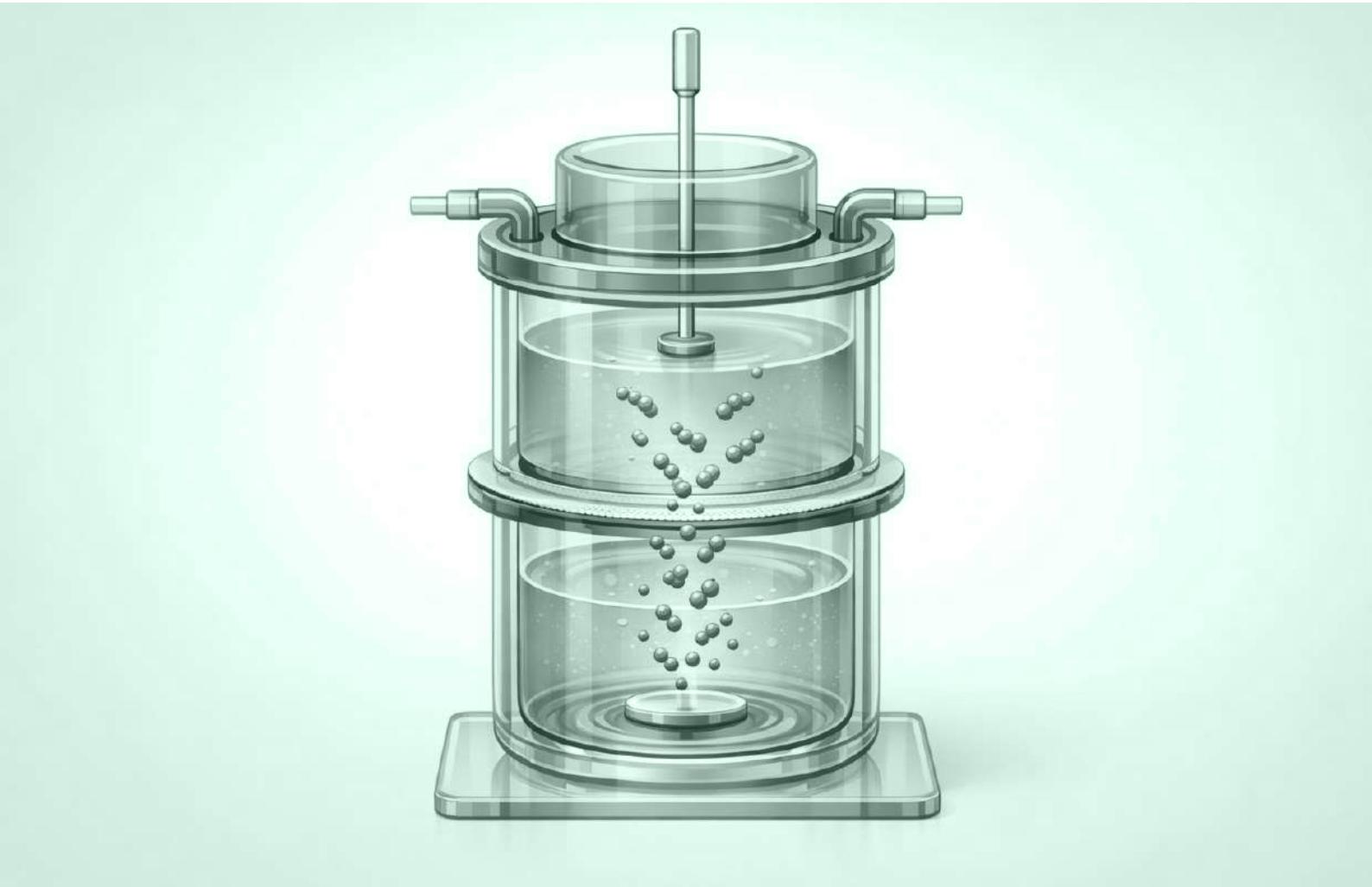
SECTION 4

Analytical Validation and Detection Methodology

The recovery rate of 112.3% at 1,000x dilution is within the acceptable range for HPLC quantification methods (typically 80-120%) and reflects the complete release of all peptide from the complex. This result confirms that the ionic liquid does not degrade or chemically modify the peptide; it forms a reversible complex that can be fully dissociated under appropriate analytical conditions.

CRITICAL QC NOTE FOR LABORATORIES

Any laboratory performing HPLC analysis on Protixa ION System formulations using standard 100x dilution protocols will systematically underestimate peptide content by approximately 15-20%, potentially leading to incorrect dosing calculations or false conclusions about peptide degradation. The correct protocol is 1,000x dilution prior to HPLC injection. This validated method must be specified in all analytical protocols associated with this platform.



SECTION 5

Nanoparticle Characterization

Sub-10 nm Secondary Nanoparticle Population Confirmed

Dynamic Light Scattering (DLS) analysis of the Protixa ION System in aqueous solution reveals a bimodal nanoparticle size distribution with two distinct populations that serve complementary roles in mucosal delivery. The most significant finding is the confirmed sub-10 nm secondary population (5–10 nm range) — a particle size that is physically small enough to penetrate mucus gel pore networks that block conventional formulations. The three key size metrics from DLS characterization are summarized below, followed by a detailed analysis of their mechanistic significance.

<10 nm

Sub-10 nm Secondary Peak

5–10 NM RANGE CONFIRMED (DLS) — BELOW MUCUS PORE THRESHOLD FOR DIRECT PENETRATION

Bimodal

Nanoparticle Distribution

TWO POPULATIONS: SUB-10 NM RAPID-PENETRATING CLUSTERS + 200–300 NM SUSTAINED-RELEASE VEHICLES

200-300 nm

Primary Peak Range

SELF-ASSEMBLED PEPTIDE-LOADED NANOPARTICLES — GI PROTECTION & CONTROLLED RELEASE

One of the most structurally significant properties of the Protixa ION System is its spontaneous self-assembly into nano-scale particles when dissolved in aqueous solution. The system produces a confirmed sub-10 nm secondary nanoparticle population (5–10 nm range, DLS), a scale that is mechanistically critical for mucosal delivery. At sub- 10 nm, these particles are small enough to penetrate the mesh-like structure of the mucus gel network, which has pore sizes typically ranging from 100-500 nm in healthy mucosa. This means the smallest Protixa ION System particles can navigate through mucus pores that would trap conventional formulations, reaching the epithelial surface directly.

SECTION 5

Nanoparticle Characterization
Sub-10 nm Secondary Nanoparticle Population Confirmed

5.1 Dynamic Light Scattering Methodology

Dynamic Light Scattering (DLS) is the gold standard technique for characterizing nanoparticle size distribution in solution. The technique measures time-dependent fluctuations in scattered light intensity from particles undergoing Brownian motion, and uses the autocorrelation function to calculate hydrodynamic diameter. DLS provides two complementary representations: the intensity-weighted distribution (emphasizing larger particles) and the number-weighted distribution (reflecting actual numerical abundance). Both representations are reported for the Protixa ION System. Testing was performed on the peptide/Protixa ION System solution at 10 mg/mL.

5.2 Results: Bimodal Distribution with Confirmed Sub-10 nm Secondary Population

DLS analysis confirmed two distinct nanoparticle populations in the Protixa ION System formulation. The primary peak (200–300 nm, intensity-weighted dominant) represents self-assembled peptide-loaded nanoparticles that serve as sustained-release delivery vehicles during GI transit. The secondary peak (5–10 nm, number-weighted dominant) represents individual ionic clusters and small ionic assemblies — the population responsible for rapid mucus penetration and fast-onset tight junction modulation. Table 5.1 presents the complete characterization data for both populations, including size range, distribution type, physical identity, and functional role in oral and mucosal delivery.

TABLE 5.1 -- DLS NANOPARTICLE SIZE DISTRIBUTION DATA

Peak	Size Range	Center / Minimum	Distribution Type	Physical Identity	Role In Oral/Mucosal Delivery
Secondary Peak	5-10 nm	<10 nm confirmed (5-10 nm range)	Number-weighted dominant	Individual ions / small ionic clusters	Immediate mucus penetration rapid tight junction modulation fast-onset absorption at oral nasal sublingual surfaces
Primary Peak	200-300 nm	~250 nm average	Intensity-weighted dominant	Self-assembled PIL nanoparticles (peptideloaded)	Controlled release GI protection sustained delivery potential endocytic uptake by intestinal epithelial cells

SECTION 5

Nanoparticle Characterization
Sub-10 nm Secondary Nanoparticle Population Confirmed

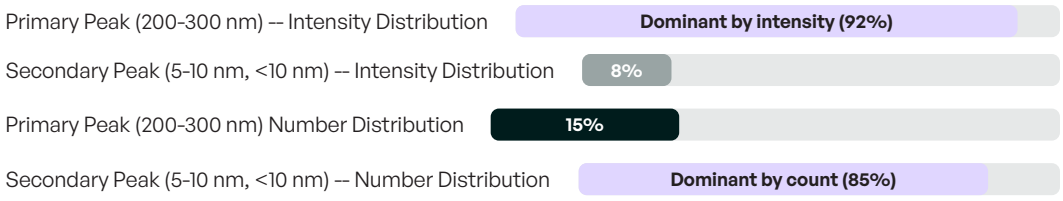
5.3 Why Sub-10 nm Matters for Oral and Mucosal Delivery

The sub-10 nm secondary particle population (5–10 nm, DLS) is not merely a technical measurement. It is a mechanistically meaningful property that directly enables the delivery performance observed in the oral and mucosal studies. Mucus gel networks have pore sizes that vary by location and health status, but typically range from approximately 100-500 nm in the intestinal mucosa and 100-300 nm in the nasal mucosa. Conventional nanoparticle formulations with particle sizes of 200-500 nm are partially or completely excluded from these pores, limiting their access to the epithelial surface.

The sub-10 nm ionic clusters in the Protixa ION System are well below the lower bound of mucus pore sizes, meaning they can navigate freely through the mucus gel network regardless of local pore size variation. This is the physical basis of the rapid-onset permeation enhancement observed in the ex vivo model (28.5x enhancement at just 30 minutes) and the confirmed systemic distribution in the in vivo oral study within one hour of administration.

The larger nanoparticles (200-300 nm primary peak) serve a complementary function: they act as sustainedrelease reservoirs that protect the peptide cargo during GI transit and release it gradually as the nanoparticle structure is disrupted by the biological environment. The combination of sub-10 nm rapid-penetrating clusters and 200-300 nm sustained-release nanoparticles creates a two-phase delivery profile that maximizes both the speed and duration of mucosal permeation enhancement.

FIGURE 5.1 -- NANOPARTICLE SIZE DISTRIBUTION: INTENSITY VS. NUMBER REPRESENTATION



Note: *Relative proportions are representative of DLS bimodal distribution pattern. The sub-10 nm secondary peak (5–10 nm range, DLS) is the headline metric for mucus penetration capability.*

SURFACE AREA AMPLIFICATION

When peptides aggregate into large macro-clusters in aqueous solution, only the outermost surface is exposed to the intestinal environment. By dispersing into sub-10 nm ionic clusters, the Protixa ION System dramatically increases the surface area-to-volume ratio. A sphere of 1 mm diameter dispersed into sub-10 nm particles increases total surface area approximately 125,000-fold. This geometric amplification of surface exposure is a fundamental driver of the enhanced oral and mucosal absorption performance observed with the system.

SECTION 6

System Stability Studies

Peptide stability is one of the most significant practical challenges in formulation and distribution. Therapeutic peptides are susceptible to thermal denaturation, photolytic cleavage, oxidative degradation, and hydrolytic cleavage in aqueous solution. The conventional solution is cold-chain storage at 2-8 degrees C or freezing at -20 degrees C, adding substantial cost and logistical burden to the peptide supply chain.

The Protixa ION System addresses peptide stability through the same ionic microenvironment that drives its delivery performance. The dense network of electrostatic and hydrogen-bonding interactions within the PIL creates a protective cage around the peptide that restricts conformational flexibility required for degradation reactions, excludes water molecules from the immediate vicinity of the peptide backbone, provides a physical barrier against photolytic and oxidative attack, and chelates metal ions that would otherwise catalyze oxidative degradation pathways.

6.1 Long-Term Room Temperature Stability

Long-term stability testing was conducted by storing Protixa ION System-peptide formulations (10 mg/mL initial concentration, using a short-chain therapeutic peptide as the model compound (short-chain peptide, 2–5 amino acids)) at room temperature for periods of one, two, three, and four months. Peptide concentrations were monitored using the validated 1,000x dilution HPLC method. Recovery rates at all four time points fall within 101.1–101.9%, well within ICH Q1A(R2) acceptance criteria of 90–110%, with no evidence of degradation trend across the full four-month observation period.

TABLE 6.1 -- LONG-TERM ROOM TEMPERATURE STABILITY DATA (SHORT-CHAIN THERAPEUTIC PEPTIDE, 10 MG/ML)

Storage Duration	Condition	HPLC AUC	Concentration (mg/mL)	Recovery (%)	Assessment
Initial (T=0)	Room temperature	--	10.00 (nominal)	100%	Reference standard
1 Month	Room temp (~20-25 C)	140.59	10.11	101.1%	Excellent -- no degradation
2 Months	Room temp (~20-25 C)	118.44	10.11	101.1%	Excellent -- no degradation
3 Months	Room temp (~20-25 C)	67.76	10.12	101.2%	Excellent -- no degradation
4 Months	Room temp (~20-25 C)	75.30	10.19	101.9%	Excellent -- no degradation

SECTION 6

System Stability Studies

6.2 Accelerated Thermal and Photostability Testing

Two accelerated stress conditions were evaluated: thermal stress at 37 degrees C for three days and photostress under intense light exposure for ten days. Both conditions represent significantly more aggressive stress than would be encountered under normal storage or handling conditions.

TABLE 6.2 -- ACCELERATED STABILITY TESTING DATA (SHORT-CHAIN THERAPEUTIC PEPTIDE, 10 MG/ML)

Stress Condition	Duration	HPLC AUC	Concentration (mg/mL)	Recovery (%)	Assessment
Thermal Stress	37 degrees C x 3 days	152.69	10.58	105.8%	Excellent thermal stability
Photostress	Intense light x 10 days	150.80	10.46	104.6%	Excellent photostability

The six stability data points — four room temperature time points and two accelerated stress conditions — collectively demonstrate that the Protixa ION System provides robust peptide protection across the full range of storage and handling conditions relevant to commercial distribution. Recovery rates at all six conditions fall within the ICH Q1A(R2) acceptance range of 90–110%, with no evidence of degradation trends over time or under stress. The key recovery values are summarized below.

101.1%

Recovery
1 MONTH ROOM TEMPERATURE

101.1%

Recovery
2 MONTHS ROOM TEMPERATURE

101.2%

Recovery
3 MONTHS ROOM TEMPERATURE

101.9%

Recovery
4 MONTHS ROOM TEMPERATURE

105.8%

Recovery
37 DEGREES C X 3 DAYS

104.6%

Recovery
INTENSE LIGHT X 10 DAYS

SECTION 6

System Stability Studies

PRACTICAL IMPLICATION: COLD-CHAIN ELIMINATION

Peptides formulated in the Protixa ION System do not require refrigeration or cold-chain logistics for storage periods of at least four months, as confirmed by HPLC-validated stability data showing 101.1– 101.9% recovery across all time points. This eliminates the need for temperature-controlled shipping, reduces spoilage risk, and dramatically reduces the cost and complexity of international distribution, particularly relevant for oral, sublingual, and intranasal peptide products intended for broad consumer or clinical distribution.

SECTION 7

In Vivo Systemic Distribution
Oral Administration Study

The in vivo oral distribution study is the most direct and compelling demonstration of the Protixa ION System core value proposition: the ability to deliver peptides orally and achieve genuine systemic distribution throughout the body. This study moves beyond in vitro and ex vivo models to demonstrate, in a living animal, that orally administered peptides formulated in the Protixa ION System cross the gastrointestinal epithelium, enter the systemic circulation, and distribute to multiple organs, a result that is completely absent in the control group receiving the same peptide in water.

7.1 Experimental Design: FITC Fluorescence Tracking

The in vivo distribution study used fluorescence imaging to track the systemic distribution of orally administered peptides. The peptide was labeled with FITC (fluorescein isothiocyanate), a fluorescent dye that emits green fluorescence when excited by blue light. Mice were orally administered 10 mg of FITC-labeled peptide formulated in the Protixa ION System (experimental group) or in water (control group). One hour post-administration, the animals were sacrificed and major organs (intestines, lungs, liver, kidneys, and stomach) were harvested and imaged for FITC fluorescence.

7.2 In Vivo Oral Distribution Results

One hour after oral administration, organs were harvested from both the experimental group (peptide in Protixa ION System) and the control group (peptide in water) and imaged for FITC fluorescence. The results were unambiguous: the experimental group showed confirmed fluorescence signal in all five major organs examined, while the control group showed negligible signal in every organ. This binary outcome (systemic distribution versus no systemic distribution) from the same peptide at the same dose in the same animal model, with the only variable being the presence of the ionic liquid delivery system, represents the most direct possible demonstration of the platform's oral delivery capability. The organ-by-organ results are presented in Table 7.1.

TABLE 7.1 -- IN VIVO ORGAN DISTRIBUTION: FITC FLUORESCENCE SIGNAL (1 HOUR POST-ORAL ADMINISTRATION)

Organ	Experimental Group	Control Group	Interpretation
Intestines	Strong fluorescence -- localized within intestinal villi structures	Negligible signal	Primary absorption site -- peptide reached absorptive epithelial surface of villi not just intestinal lumen
Liver	Significant fluorescence detected	Negligible signal	Hepatic distribution -- confirms portal vein absorption pathway
Lungs	Significant fluorescence detected	Negligible signal	Pulmonary circulation -- confirms entry into systemic bloodstream
Kidneys	Significant fluorescence detected	Negligible signal	Renal filtration -- confirms systemic circulation and whole-body distribution
Stomach	Significant fluorescence detected	Negligible signal	Gastric absorption -- confirms multi-site GI uptake

SECTION 7

In Vivo Systemic Distribution
Oral Administration Study

7.3 Mechanistic Interpretation: Villi Localization and Absorption Pathway

The strong intestinal signal, specifically localized within intestinal villi structures, is particularly significant. The intestinal villi are the finger-like projections of the intestinal epithelium that dramatically increase the absorptive surface area of the small intestine. Fluorescence signal was localized within the villi rather than remaining in the intestinal lumen, confirming that the peptide penetrated the mucus layer, reached the epithelial surface, and was absorbed into the villi tissue. The villi localization is the anatomical correlate of the tight junction modulation and mucus fluidization mechanisms described in Sections 2 and 3.

The hepatic signal is consistent with portal absorption: peptides absorbed from the small intestinal villi enter the portal vein and pass through the liver before reaching systemic circulation. The pulmonary and renal signals confirm genuine systemic circulation.

THE SINGLE MOST IMPORTANT DATA POINT IN THIS REPORT

The difference between the experimental group (multi-organ systemic distribution with villi localization) and the control group (negligible signal in all organs) represents the entire value proposition of the Protixa ION System in a single experiment. The same peptide, the same dose, the same animal model, the same time point. The only variable is the presence of the ionic liquid delivery system. Without it: no systemic delivery. With it: confirmed distribution to intestinal villi, liver, lungs, kidneys, and stomach within one hour of oral administration.

7.4 Mechanistic Context: CGLY In Vivo Tissue Penetration

The CGLY pharmaceutical study (Section 3) provides mechanistic context for the villi localization observed in the Protixa ION System oral FITC study. In the CGLY study, FITC-labeled IgG was injected directly into the jejunum of Wistar rats formulated in either CGLY 2:1 or saline. Without the ionic liquid, antibody transport was blocked by the mucus barrier and signal remained in the intestinal lumen. With CGLY, the antibody permeated the mucus and was absorbed into the villi. This peer-reviewed finding supports the mechanistic plausibility of the villi localization observed in the Protixa ION System FITC study, though the two studies used different ionic liquid formulations, different cargo molecules, and different animal models. The CGLY data is presented as class-level mechanistic precedent, not as a direct quantitative prediction of Protixa ION System performance.

SECTION 8

Safety Profile
Cytotoxicity Assessment (CCK-8 Assay)

The safety profile of any excipient platform intended for oral, sublingual, or mucosal administration must be rigorously characterized before the platform can be responsibly deployed in formulations intended for human use. For the Protixa ION System, safety evaluation focused on the most fundamental question: does the ionic liquid environment cause cellular damage at concentrations relevant to its use as a delivery vehicle? The answer, based on CCK-8 cytotoxicity assay data, is unambiguously negative.

8.1 CCK-8 Assay Methodology

The CCK-8 (Cell Counting Kit-8) assay is a colorimetric method for determining cell viability and cytotoxicity based on the reduction of WST-8 tetrazolium salt to a yellow-colored formazan dye by cellular dehydrogenases in living cells. The CCK-8 assay is accepted by regulatory agencies as a standard in vitro safety assessment tool per ISO 10993-5. Cells were co-cultured with Protixa ION System formulations for 24 hours and 48 hours, representing short-term and extended exposure conditions relevant to oral, sublingual, and mucosal contact.

TABLE 8.1 -- CCK-8 CYTOTOXICITY ASSAY RESULTS

Exposure Duration	Test Article	Cell Viability (%)	ISO 10993-5 Classification	Assessment
24 hours	Protixa ION System	80-100%	Non-cytotoxic per ISO 10993-5 (>70% threshold)	PASS ✓ - Excellent biocompatibility
48 hours	Protixa ION System	80-100%	Non-cytotoxic per ISO 10993-5 (>70% threshold)	PASS ✓ - Excellent biocompatibility

SECTION 8

Safety Profile
Cytotoxicity Assessment (CCK-8 Assay)

The CCK-8 results classify the Protixa ION System as non-cytotoxic per ISO 10993-5 at both 24-hour and 48-hour exposure timepoints. Beyond the primary viability data, the safety profile of the platform is supported by six additional lines of evidence — spanning cellular, mechanistic, compositional, and in vivo dimensions — that together establish a comprehensive biocompatibility case. These are summarized below.

- ✓ **No Cytotoxicity at 24 Hours**

  Classified non-cytotoxic per ISO 10993-5 (threshold: >70%). Cell viability 80–100% across all tested concentrations. No statistically significant reduction in cell number or metabolic activity.
- ✓ **No Cytotoxicity at 48 Hours**

  Viability maintained at 80-100% after extended exposure. No progressive cytotoxicity observed with increased exposure duration.
- ✓ **No Acute Immune Activation**

  No evidence of acute immune activation or inflammatory marker elevation observed under laboratory testing conditions.
- ✓ **Reversible Tight Junction Modulation**

  TEER values return to baseline after ionic liquid removal (CGLY study). No permanent tissue alteration. Paracellular pathway modulation is transient and fully reversible.
- ✓ **Food-Grade Excipients Only**

  Citric acid (E330, GRAS) and amino acids (GRAS, USP/NF). No novel synthetic chemical entities. Both components are endogenous metabolites present in normal human physiology.
- ✓ **Confirmed by Independent In Vivo Study**

  The analogous CGLY ionic liquid system showed no toxicity in a 7-day repeat-dose rat study at 625 mg/kg/day, with normal GI histopathology and blood chemistry throughout.

8.2 Mechanistic Basis of Biocompatibility

The favorable cytotoxicity profile of the Protixa ION System is mechanistically grounded in the nature of its components. Citric acid is a central intermediate in the Krebs cycle and is present in virtually every cell in the human body. It is also a common food additive (E330). Lysine and arginine are essential nutrients. The combination of these two components in a protic ionic liquid configuration does not generate any novel chemical entities or reactive intermediates. The proton transfer reaction that forms the PIL is a simple acid-base reaction that produces a salt, the same type of reaction that occurs when citric acid is consumed in food and encounters the basic amino groups of dietary proteins.

SECTION 9

Mucosal Barrier Penetration
Ex Vivo Epithelial Permeation Model

To quantify the Protixa ION System's ability to drive peptides across epithelial barriers (the same class of barriers that govern oral, sublingual, and mucosal absorption), an ex vivo permeation study was conducted using a porcine tissue model. This study provides the most directly quantifiable measurement of the system barrier-penetration capability, expressing the enhancement as a precise fold-increase relative to a saline control under identical experimental conditions.

9.1 Model Selection and Scientific Rationale

Porcine tissue is the gold standard preclinical model for human epithelial permeation studies. The porcine tissue used in this study had a thickness of 0.5 mm — representing one of the thickest and most challenging epithelial barriers in the body. This is 5× thicker than the mucosal surfaces relevant to oral, sublingual, and intranasal delivery. The respiratory tract mucosa, sublingual epithelium, and intestinal mucosal surfaces have a thickness of only approximately 0.1 mm, and their epithelial cells are structurally thinner, less keratinized, and more permeable than skin. This means that the permeation enhancement measured in this study represents a conservative lower bound for the enhancement achievable in oral, sublingual, and mucosal delivery applications — the actual mucosal enhancement is expected to be substantially higher.

WHY 57× IS A CONSERVATIVE FLOOR — NOT A CEILING

The >57-fold enhancement was measured through 0.5 mm thick porcine skin — the hardest biological barrier tested. This is the same thickness as full-thickness human skin. The scientist who conducted this study explicitly noted:

> *"The thickness of the pig skin used was 0.5 mm, while the thickness of the respiratory tract mucosa was 0.1 mm, and its epithelial cells were relatively thin compared with pig skin, which was easier to achieve transdermal absorption.*

In other words: the test was deliberately run on the most difficult barrier. Oral mucosa, sublingual epithelium, and nasal mucosa are all approximately 5× thinner and structurally more permeable. The 57× figure is therefore a worst-case, conservative lower bound. The in vivo oral FITC study (Section 7) — which confirmed systemic distribution to five organs within one hour — is consistent with substantially higher effective mucosal enhancement in practice.

The experimental setup used a standard Franz diffusion cell configuration. The receptor fluid was sampled at defined time intervals (0.5h, 1h, 2h, 4h, 6h, and 24h), and peptide concentration in each sample was measured by HPLC using the validated 1,000x dilution method. Two formulations were tested in parallel: the experimental group (peptide + Protixa ION System) and the control group (peptide + saline).

SECTION 9

Mucosal Barrier Penetration
Ex Vivo Epithelial Permeation Model

9.2 Epithelial Permeation Data

Cumulative peptide permeation was measured at six time points over 24 hours: 0.5h, 1h, 2h, 4h, 6h, and 24h. At every time point, the Protixa ION System formulation substantially outperformed the saline control, with enhancement ratios ranging from 28.5-fold at 30 minutes to greater than 57-fold at the 24-hour endpoint. The rapid onset at 30 minutes — already 28.5-fold above control — is particularly significant: it demonstrates that the mucus fluidization and tight junction modulation effects are not slow-acting phenomena but begin within minutes of application, consistent with the immediate availability of the sub-10 nm ionic clusters identified in the DLS characterization. The complete time-course data is presented in Table 9.1.

TABLE 9.1 -- EX VIVO EPITHELIAL BARRIER PERMEATION DATA: CUMULATIVE PERMEATION OVER TIME

Time Point	Experimental Group (mg)	Control Group (mg)	Enhancement Ratio	Cumulative Rate
0.5 hours	0.114 mg	0.004 mg	28.5x	1.14%
1 hour	0.163 mg	0.005 mg	32.6x	1.63%
2 hours	0.152 mg	0.005 mg	30.4x	1.52%
4 hours	0.186 mg	0.006 mg	31.0x	1.86%
6 hours	0.217 mg	0.006 mg	36.2x	2.17%
24 hours (Final)	0.459 mg	0.008 mg	>57x	4.59%

9.3 Analysis and Interpretation

The permeation data reveals several important features of the Protixa ION System delivery kinetics. First, the enhancement effect is rapid in onset: at the earliest time point measured (30 minutes), the experimental group had already achieved 28.5x greater cumulative permeation than the control, indicating that the mucus fluidization and tight junction modulation effects begin within minutes of application, consistent with the rapid-acting sub-10 nm ionic clusters (5–10 nm, DLS) identified in the particle characterization analysis. Second, the permeation rate is sustained over the full 24-hour measurement period.

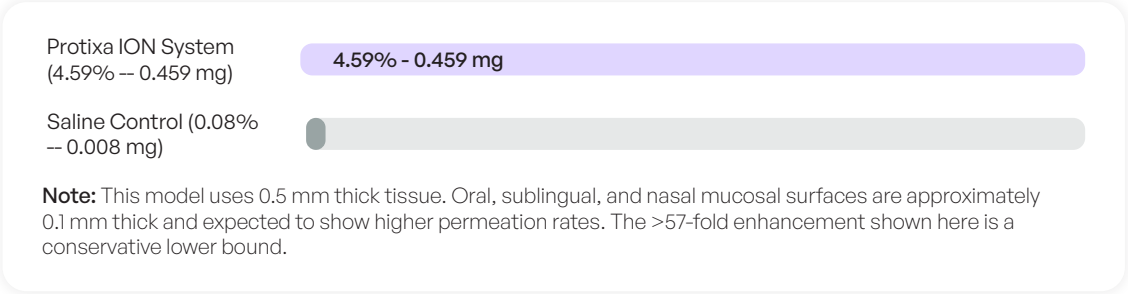
Third, and critically for the interpretation of oral and mucosal delivery potential: the tissue model used in this study (0.5 mm thickness) is significantly thicker and less permeable than the mucosal surfaces relevant to oral, sublingual, and intranasal delivery. The greater than 57-fold enhancement demonstrated in this conservative model should therefore be understood as a lower bound for the permeation enhancement achievable in oral, sublingual, and mucosal delivery applications, a conclusion directly supported by the multi-organ systemic distribution observed in the in vivo oral study (Section 7).

SECTION 9

Mucosal Barrier Penetration

Ex Vivo Epithelial Permeation Model

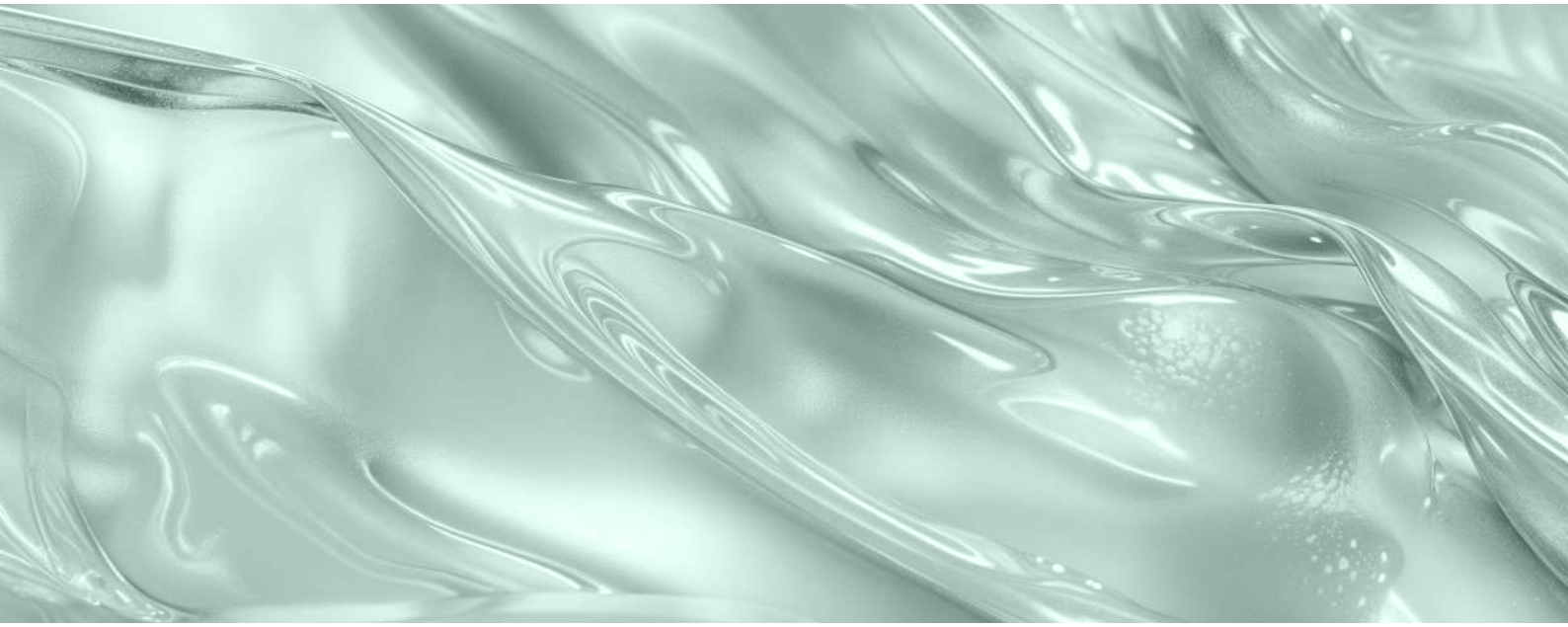
FIGURE 9.1 -- 24-HOUR CUMULATIVE EPITHELIAL PERMEATION: PROTIXA ION SYSTEM VS. SALINE CONTROL



Note: This model uses 0.5 mm thick tissue. Oral, sublingual, and nasal mucosal surfaces are approximately 0.1 mm thick and expected to show higher permeation rates. The >57-fold enhancement shown here is a conservative lower bound.

THE 57× NUMBER IN CONTEXT: A CONSERVATIVE FLOOR, NOT A CEILING

The >57-fold epithelial permeation enhancement is Protixa's own third-party verified data, measured through 0.5 mm thick porcine tissue — the most challenging epithelial barrier available. Oral, sublingual, and nasal mucosal surfaces are approximately 5× thinner (0.1 mm) and structurally more permeable. The 57× figure is therefore a conservative lower bound for mucosal delivery performance — not a ceiling. The in vivo oral FITC study (Section 7) independently confirms that this permeation enhancement translates to genuine systemic distribution in a living animal model — the same peptide, the same dose, the same time point, with and without the ionic liquid delivery system.



SECTION 10

Delivery Route Applications

The Protixa ION System is a multi-route platform capable of enhancing peptide delivery across oral, intranasal, sublingual, and topical application routes. The same core ionic liquid composition addresses the fundamental biological barriers present at all mucosal and epithelial surfaces. The four delivery routes are presented here in order of their primary scientific and commercial significance, with oral, intranasal, and sublingual delivery representing the platform most strategically important applications due to their systemic delivery potential and the compelling in vivo and pharmaceutical study data supporting them.

01

Oral / Gastrointestinal

Primary route. Protects peptide from gastric acid and proteases. Fluidizes intestinal mucus. Modulates tight junctions. Confirmed multi-organ systemic distribution in mouse FITC study. Supported by CGLY pharma study (5x bioavailability). Villi localization confirmed.

02

Intranasal / Mucosal

High-priority route. Mucoadhesive amino acids extend nasal residence time. Bypasses first-pass hepatic metabolism. Highly vascularized nasal epithelium enables rapid systemic absorption. Thinner mucosa (0.1mm) than tissue model; enhanced permeation expected. Mucoadhesive retention extends absorption window.

03

Sublingual / Buccal

High-priority route. Thin sublingual epithelium (0.1mm). Bypasses first-pass metabolism entirely. Rapid systemic absorption. Same ionic mechanism (mucus fluidization + TJ modulation) applies. Mucoadhesive amino acids extend sublingual residence time.

04

Topical / External Skin

Supporting route. >57x enhancement demonstrated in ex vivo model. Suitable for skin-targeted peptides (tissue-repair peptides, metabolic peptides, and other bioactive compounds). Primary differentiation of platform lies in oral, nasal, and sublingual delivery.

SECTION 10

Delivery Route Applications

10.1 Oral and Gastrointestinal Delivery

The oral delivery route presents the greatest challenge for peptide bioavailability but also offers the greatest potential benefit in terms of patient convenience, compliance, and cost. The Protixa ION System addresses the oral delivery challenge through a comprehensive multi-mechanism approach that targets each of the major GI barriers in sequence. In the stomach, citric acid provides robust pH buffering that maintains a protective microenvironment around the peptide, shielding it from the highly acidic gastric environment (pH 1.5-3.5). As the formulation passes into the small intestine, the citrate anion begins to fluidize the intestinal mucus layer by chelating Ca^{2+} ions that maintain mucin cross-links, while the amino acid cations interact with claudin proteins in the tight junctions to create a transient paracellular transport window. The in vivo FITC distribution study (Section 7) confirmed that this multi-mechanism approach achieves genuine systemic absorption, with fluorescence detected in intestinal villi, liver, lungs, kidneys, and stomach within one hour of oral administration.

10.2 Intranasal and Mucosal Delivery

The intranasal route offers several unique advantages for peptide delivery. First, the nasal epithelium is significantly thinner than the tissue model used in the ex vivo permeation study (approximately 0.1 mm versus 0.5 mm), suggesting that the permeation enhancement achievable through the nasal route would be substantially greater than the already impressive results observed in the ex vivo model. Second, the nasal route bypasses firstpass hepatic metabolism entirely — peptides absorbed through the nasal epithelium enter the systemic circulation directly without passing through the liver, a significant pharmacokinetic advantage for peptides that are extensively metabolized by hepatic enzymes. Third, the intranasal route provides direct access to the lymphatic and vascular networks of the nasal submucosa, enabling rapid systemic distribution without the absorption delays associated with GI transit.

The Protixa ION System is particularly well-suited for intranasal delivery because of the mucoadhesive properties of its amino acid cation component. The protonated lysine and arginine cations carry a strong positive charge that adheres to the negatively charged sialic acid residues in the nasal mucosa, extending the residence time of the formulation on the nasal epithelium and counteracting mucociliary clearance.

SECTION 10

Delivery Route Applications

10.3 Sublingual Delivery

The sublingual route (delivery of formulations beneath the tongue) represents one of the most strategically important and underutilized routes for peptide delivery. The sublingual epithelium is thin (approximately 0.1 mm), highly vascularized, and relatively permeable compared to the intestinal epithelium. Peptides absorbed sublingually enter the systemic circulation directly through the sublingual veins, bypassing first-pass hepatic metabolism entirely, a significant advantage for peptides that are extensively metabolized by hepatic enzymes following oral absorption.

The Protixa ION System is mechanistically well-suited for sublingual delivery through the same pathways that drive its oral and nasal performance. The citrate anion fluidizes the sublingual mucus layer through Ca2+ chelation, the amino acid cations modulate tight junctions in the sublingual epithelium through electrostatic claudin interaction, and the mucoadhesive properties of the cationic amino acids extend residence time beneath the tongue, counteracting the natural tendency of sublingual formulations to be swallowed before adequate absorption can occur. The sub-10 nm ionic clusters (5–10 nm, DLS) are particularly relevant for sublingual delivery, as their small size enables rapid penetration of the thin sublingual mucus layer and direct access to the epithelial surface.

SUBLINGUAL + ORAL COMBINED STRATEGY

For maximum systemic bioavailability, the Protixa ION System may be used in formulations designed for both sublingual absorption (rapid onset, bypasses first-pass metabolism) and subsequent oral/GI absorption (sustained delivery as the formulation is swallowed). This dual-phase absorption strategy could provide both rapid peak concentrations and extended duration of action from a single administration.

10.4 Topical and External Skin Application

The topical application route is supported by the ex vivo epithelial permeation data presented in Section 9, which demonstrated greater than 57-fold enhancement in peptide transport across full-thickness tissue compared to saline controls. For topical applications where the target is the skin itself rather than systemic circulation, the Protixa ION System enables peptides to penetrate beyond the surface layers and reach the viable epidermis and upper dermis, where they can interact with keratinocytes, fibroblasts, and other skin cells. This is particularly relevant for peptides with established roles in skin biology, such as collagen-stimulating peptides, wound-healing peptides, and anti-inflammatory peptides. While topical application represents a well-supported and commercially relevant use case, the primary scientific and commercial differentiation of this platform lies in its oral, intranasal, and sublingual delivery capabilities.

SECTION 11

Study Limitations and Preclinical Scope

Scientific integrity requires explicit acknowledgment of the scope and limitations of the preclinical data presented in this dossier. The following limitations should be considered by any scientist, physician, formulator, or regulatory reviewer evaluating this data package.

11.1 Model and Species Limitations

All permeation data (Section 9) was generated using an ex vivo porcine tissue model. While porcine tissue is the accepted gold standard for human epithelial permeation studies, ex vivo models do not fully replicate the dynamic biological environment of a living organism, including active transport mechanisms, blood flow, lymphatic drainage, and immune responses. The in vivo data (Section 7) was generated in mice; interspecies differences in GI physiology, mucus composition, and metabolic activity mean that direct extrapolation to human pharmacokinetics requires clinical validation.

11.2 No Human Clinical Data

The Protixa ION System has not been evaluated in human clinical trials. No human pharmacokinetic, pharmacodynamic, or safety data exists for this platform at the time of this report. All bioavailability and absorption claims are based on preclinical models and peer-reviewed class-level literature. Clinical translation will require IND-enabling studies, Phase I safety evaluation, and route-specific pharmacokinetic studies in human subjects.

11.3 Qualitative vs. Quantitative In Vivo Data

The in vivo oral distribution study (Section 7) provides qualitative fluorescence imaging data confirming multiorgan systemic distribution. It does not provide quantitative plasma concentration data, absolute bioavailability percentages, or pharmacokinetic parameters (Cmax, Tmax, AUC, t½) for the Protixa ION System specifically. The 5× systemic bioavailability figure cited in this document is from the peer-reviewed CGLY study (Angsantikul et al.) and is not a direct measurement of Protixa ION System performance.

11.4 Single Peptide Model

Stability and permeation studies were conducted using a short-chain therapeutic peptide (short-chain peptide, 2– 5 amino acids) as the model compound. The Protixa ION System stabilization mechanism operates through ionic microenvironment effects on the peptide backbone — electrostatic shielding, hydrogen bonding, and water exclusion — rather than through sequence-specific interactions, making the findings broadly applicable across peptides in the range of approximately 2–200 amino acids. Performance data for the specific model compound used in these studies is available upon request. Formulators working with larger peptides (>10 kDa) or peptides with unusual secondary structure are encouraged to request peptide-specific evaluation data.

SECTION 11

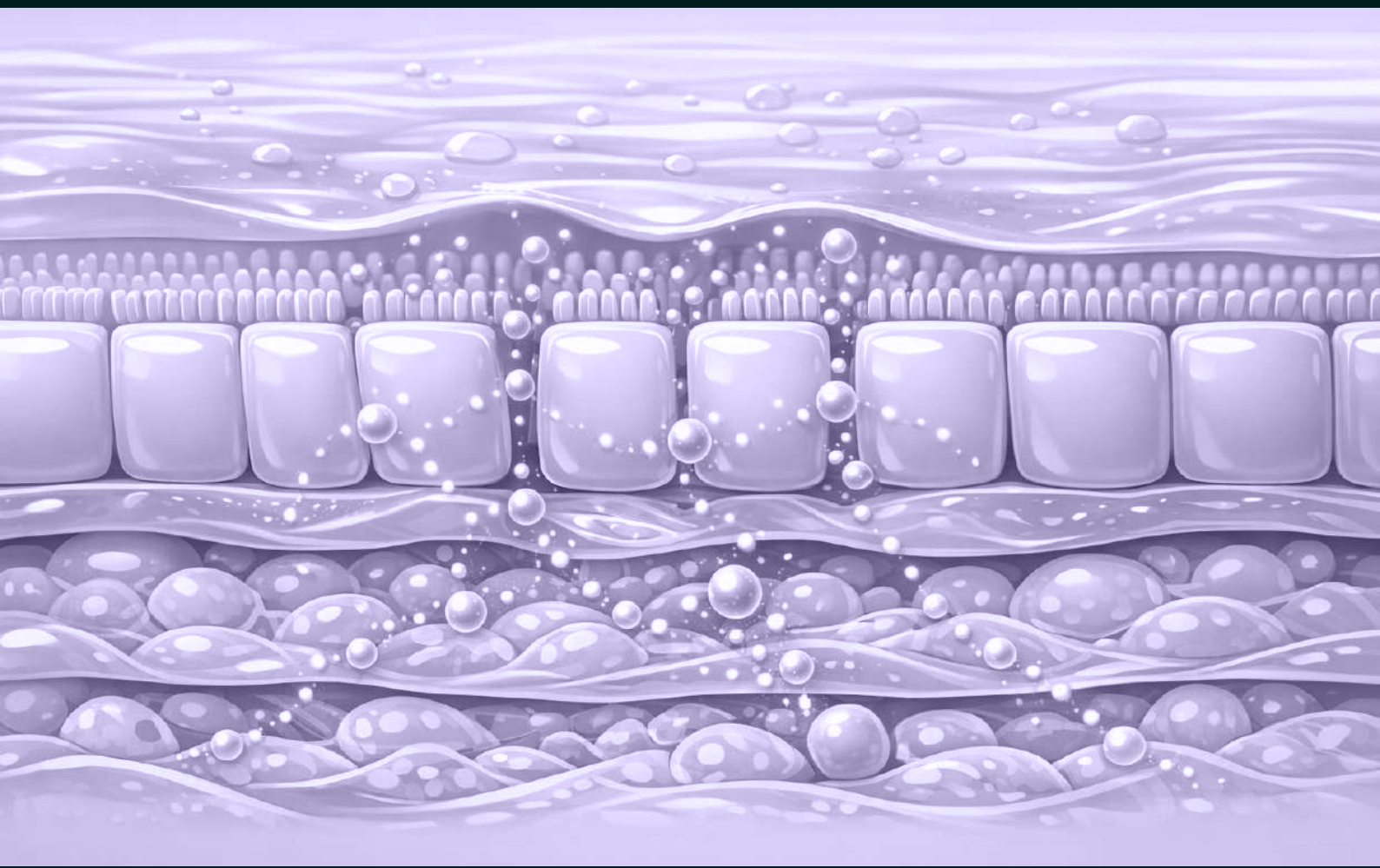
Study Limitations and Preclinical Scope

11.5 Statistical Reporting

The data presented in this dossier reflects the results of the third-party test report as conducted by Zhejiang TianMei Biological Engineering Co., LTD and Zhejiang University of Technology. Full statistical analysis including n-values, standard deviations, and p-values is available in the source test report (Appendix A). The quantitative data presented in the tables throughout this document represents the reported experimental measurements from that report.

PRECLINICAL DATA PACKAGE — INTENDED USE

This dossier is intended to support formulation development decisions, investor scientific due diligence, and preliminary regulatory strategy discussions. It is not intended as a substitute for IND-enabling studies, clinical trial data, or regulatory submissions. All claims are preclinical and should be interpreted within that context.



SECTION 12

Regulatory Positioning and Excipient Safety Profile

The Protixa ION System is composed entirely of excipients with established regulatory acceptance across multiple jurisdictions and application categories. This means that the regulatory pathway for products incorporating the Protixa ION System is substantially shorter and less uncertain than for products based on novel synthetic delivery systems.

TABLE 12.1 -- REGULATORY STATUS OF PROTIXA ION SYSTEM COMPONENTS

Component	FDA GRAS Status	USP/NF Monograph	EU Food Additive	Application Categories
Citric Acid	GRAS (21 CFR 184.1033)	USP/NF monograph	E330	Food pharmaceutical cosmetic nutraceutical
L-Lysine	GRAS (dietary supplement)	USP/NF monograph	Permitted amino acid	Food pharmaceutical dietary supplement cosmetic
L-Arginine	GRAS (dietary supplement)	USP/NF monograph	Permitted amino acid	Food pharmaceutical dietary supplement cosmetic

The regulatory acceptance of all three components across food, pharmaceutical, cosmetic, and nutraceutical categories means that the Protixa ION System can be incorporated into products across a wide range of application categories without introducing novel regulatory complexity. Table 12.2 maps the primary application categories to their relevant regulatory frameworks and identifies the specific pathway through which the Protixa ION System is compatible with each.

TABLE 12.2 -- APPLICATION CATEGORY FRAMEWORK

Application Category	Regulatory Framework	Key Requirements	Protixa ION System Status
Nutraceutical / Dietary Supplement	FDA DSHEA (US); EFSA (EU)	GRAS excipients; structure/function claims only	Fully compatible -- all components GRAS
Cosmetic / Cosmeceutical	FDA 21 CFR 700 (US); EU Cosmetics Regulation	Safety assessment; no drug claims; INCI listing	Fully compatible -- established cosmetic excipients
Pharmaceutical (OTC/Rx)	FDA NDA/ANDA/505(b)(2); ICH guidelines	IND/NDA filing; clinical trials; GMP manufacturing	Compatible -- pharmacopoeial excipients; 505(b)(2) pathway available
Research Use Only (RUO)	No specific regulatory framework	Clearly labeled RUO; no human use claims	Fully compatible

REGULATORY NOTICE

All findings presented in this report are based on ex vivo models, in vitro cytotoxicity testing, stability studies, analytical quantification, and in vivo fluorescence distribution studies conducted in animal models. These results represent preclinical and laboratory observations and are not clinical claims. The Protixa ION System has not been evaluated in human clinical trials. No claims of therapeutic efficacy, disease treatment, or clinical bioavailability are made or implied. All product applications must comply with applicable regulatory frameworks. Protixa does not manufacture drugs. The Protixa ION System is excipient technology.

SECTION 13

Practical Implications for Formulators and Compounders

For peptide formulators and compounders, the Protixa ION System represents a platform-level change in what is achievable with non-injectable peptide products. The data presented in this dossier translates directly into practical formulation advantages across every dimension that matters in product development: delivery efficacy, stability, safety, regulatory positioning, and manufacturing simplicity. The comparison below places the Protixa ION System's laboratory-measured performance directly alongside the baseline performance of conventional aqueous peptide formulations, making the practical magnitude of each advantage explicit.

TABLE 13.1 -- PROTIXA ION SYSTEM VS. CONVENTIONAL PEPTIDE FORMULATION: KEY PARAMETERS

Parameter	Conventional Aqueous Formulation	Protixa ION System	Advantage
Oral systemic delivery	Negligible (FITC control: no organ signal)	Multi-organ distribution confirmed in vivo (villi localization)	Qualitative transformation -- from zero to systemic
Oral bioavailability (peer-reviewed ionic liquid class reference)	Near zero for most peptides	5x Increase -- CGLY pharma study in vivo rat (Angsantikul et al.); Protixa in vivo data: confirmed multi-organ distribution vs. negligible control	5x systemic bioavailability (class reference); qualitative systemic delivery confirmed (Protixa data)
Sublingual absorption	Rapidly swallowed; minimal absorption	Mucoadhesive retention + TJ modulation + mucus fluidization	Extended residence + enhanced epithelial access
Nasal mucosal residence time	Minutes (mucociliary clearance)	Extended via mucoadhesive amino acid cations	Substantially increased absorption window
Minimum nanoparticle size	Macro-aggregates (variable uncontrolled)	<10 nm confirmed (5-10 nm range DLS secondary peak)	Mucus pore penetration capability

SECTION 13

Practical Implications for Formulators and Compounders

Parameter	Conventional Aqueous Formulation	Protixa ION System	Advantage
Epithelial barrier penetration	~0.08% (24h ex vivo model)	4.59% (24h ex vivo model)	>57-fold Improvement
Room temperature stability (2 months)	Significant degradation expected	101.1% recovery (no degradation)	Cold-chain elimination
Thermal stability (37 C 3 days)	Degradation likely	105.8% recovery	Tropical storage compatible
Cytotoxicity profile	Peptide-dependent	Non-cytotoxic per ISO 10993-5 (CCK-8; 80–100% viability at 24h & 48h)	Excellent biocompatibility
Regulatory excipient status	N/A (water/saline)	GRAS; USP/NF; E330	Established regulatory acceptance

CRITICAL QC NOTE FOR LABORATORIES

When performing HPLC quantification of peptide content in Protixa ION System formulations, always use 1,000x dilution (not 100x) prior to injection. Using 100x dilution will yield recovery rates of approximately 80-83%, which may be incorrectly interpreted as peptide degradation. The 1,000x dilution method yields recovery rates of 112.3%, confirming complete peptide integrity and full complex dissociation.

SECTION 14

Technical FAQ
Questions from Scientists and Physicians

Q1 Why not simply dissolve peptides in saline or water for oral or sublingual administration?

Simple aqueous dissolution fails to address three fundamental barriers. First, peptides in aqueous solution form macro-aggregates, exposing only the outermost surface to biological interaction while interior molecules remain inaccessible. Second, oral, sublingual, and nasal mucosa contain viscous mucus designed to trap and exclude large molecules; saline does not meaningfully reduce mucus viscosity. Third, large molecules do not readily pass between epithelial cells, and saline does not alter paracellular permeability. The result is that peptide + saline formulations showed negligible systemic distribution in the in vivo oral FITC study, while the Protixa ION System formulation produced confirmed fluorescence in five major organs within one hour.

Q2 What makes the Protixa ION System different from common oral or mucosal permeation enhancers?

Most conventional oral permeation enhancers (including surfactants, bile salts, fatty acids, and synthetic polymers) work by disrupting the lipid bilayer structure of intestinal cell membranes. While effective at increasing permeability, these mechanisms are inherently damaging to the epithelium and produce cytotoxicity, irritation, and inflammation at effective concentrations. The Protixa ION System works through ionic structuring rather than membrane disruption: it reduces mucus viscosity through Ca2+ chelation, modulates tight junctions through electrostatic protein interactions, and organizes the formulation into nano-scale assemblies with a confirmed sub- 10 nm secondary population (5–10 nm, DLS), all without damaging cell membranes. The CCK-8 cytotoxicity data (non-cytotoxic per ISO 10993-5; 80–100% cell viability) confirms that this mechanism-based approach achieves permeation enhancement without the cytotoxic trade-off associated with conventional enhancers.

Q3 How does the system protect peptides from gastric acid and digestive enzymes?

The Protixa ION System protects peptides from GI degradation through two complementary mechanisms. First, citric acid provides robust pH buffering that maintains a protective microenvironment around the peptide in the acidic gastric environment, preventing acid-induced hydrolysis. Second, the high ionic strength of the PIL creates a local microenvironment that reversibly inhibits proteolytic enzymes through active site blockade and metal cofactor sequestration (citrate chelates Ca2+ and Zn2+ required by many proteases). These protective effects are transient and localized, resolving as the formulation is diluted in the GI lumen, but they provide a critical window of protection during the absorption phase.

SECTION 14

Technical FAQ
Questions from Scientists and Physicians

Q4 What is the evidence for oral systemic delivery?

Two independent bodies of evidence support oral systemic delivery. First, the in vivo FITC fluorescence study (Section 7) demonstrated confirmed fluorescence in five major organs (with signal specifically localized within intestinal villi structures) within one hour of oral administration in mice, with negligible signal in the water control group. Second, the CGLY pharmaceutical study (Section 3) demonstrated a 5-fold increase in systemic plasma antibody concentration following oral ionic liquid administration in rats, with the absorbed antibody confirmed to be structurally intact and fully functional by ELISA. Together, these two studies provide both qualitative (organ distribution with villi localization) and quantitative (plasma concentration) evidence of oral systemic delivery.

Q5 How does intranasal delivery with this system differ from conventional nasal sprays?

Conventional nasal sprays deliver peptides in aqueous solution that is rapidly cleared by mucociliary action within minutes, severely limiting the absorption window. The Protixa ION System addresses this limitation through mucoadhesion: the protonated amino acid cations (lysine, arginine) adhere to the negatively charged sialic acid residues in the nasal mucosa, extending residence time substantially. Additionally, the system fluidizes the nasal mucus layer (enabling access to the epithelial surface) and modulates tight junctions (enabling paracellular transport). For peptides requiring rapid systemic onset, the intranasal route offers a significant pharmacokinetic advantage: absorption directly into the systemic circulation without hepatic first-pass metabolism, with the mucoadhesive properties of the system substantially extending the nasal residence time beyond what conventional aqueous nasal sprays can achieve.

Q6 What are the advantages of sublingual delivery with the Protixa ION System?

Sublingual delivery offers three key advantages over oral delivery: it bypasses first-pass hepatic metabolism entirely (peptides absorbed sublingually enter systemic circulation directly through sublingual veins), it provides faster onset of action (the sublingual epithelium is thin and highly vascularized), and it avoids the harsh proteolytic environment of the GI tract. The Protixa ION System enhances sublingual delivery through the same mechanisms that drive its oral and nasal performance (mucus fluidization, tight junction modulation, and mucoadhesive retention) while the sub-10 nm ionic clusters (5–10 nm, DLS) provide rapid penetration of the thin sublingual mucus layer. A combined sublingual + oral strategy may provide both rapid peak concentrations and extended duration of action from a single administration.

SECTION 14

Technical FAQ
Questions from Scientists and Physicians

Q7 Does the system damage the intestinal, sublingual, or nasal epithelium?

No. Laboratory testing confirmed non-cytotoxic classification per ISO 10993-5 in the CCK-8 assay (80–100% cell viability), with no significant cytotoxicity and no acute inflammatory indicators under tested conditions. The CGLY pharmaceutical study confirmed this finding in vivo, showing normal GI histopathology and unaltered mucosal structure after 7 days of repeat oral dosing at 625 mg/kg/day in rats. Permeation enhancement occurs through ionic microenvironment effects and reversible protein-ion interactions, not chemical irritation or membrane destruction. The tight junction modulation is transient and reversible, with TEER values returning to baseline after removal of the ionic liquid.

Q8 Does the system protect peptides from degradation during storage?

Yes, comprehensively. Stability studies demonstrated 101.1–101.9% recovery after 1–4 months at room temperature, 105.8% recovery after 3 days at 37 degrees C, and 104.6% recovery after 10 days of intense light exposure. The protective mechanism operates through multiple pathways: the dense hydrogen-bonding network restricts peptide conformational mobility (preventing thermal denaturation), Ca2+ chelation by citrate prevents metal-catalyzed oxidative degradation, exclusion of bulk water reduces hydrolytic cleavage, and the overall ionic microenvironment inhibits protease activity. This stability profile eliminates cold-chain requirements for at least four months of confirmed room temperature storage.

Q9 How does this compare to the CGLY system studied in the pharmaceutical literature?

The CGLY system (choline + glycolate) and the Protixa ION System (citric acid + lysine/arginine) are both biocompatible protic ionic liquids that operate through the same dual mechanism of mucus viscosity reduction and tight junction modulation. The Protixa ION System uses amino acid cations (lysine/arginine) rather than choline, which provides additional mucoadhesive properties (through interaction with sialic acid in nasal and sublingual mucosa), additional protease inhibitory activity (through active site blockade), and a more direct physiological relevance (amino acids are endogenous metabolites). The citric acid anion provides stronger chelating activity than glycolate, potentially producing more effective mucus fluidization. The CGLY study demonstrated a 5-fold increase in systemic antibody bioavailability in vivo, a benchmark that the Protixa ION System is designed to meet or exceed for peptide cargo.

SECTION 15

Conclusion

The Protixa ION System represents a scientifically rigorous, experimentally validated, and practically deployable solution to one of the most persistent challenges in modern peptide science: the inability to deliver therapeutic peptides effectively through oral, sublingual, and mucosal routes without enzymatic degradation, barrier exclusion, or loss of structural integrity. The data presented in this report, generated by independent third-party laboratories and supported by peer-reviewed pharmaceutical research, demonstrates that the system achieves its core objectives across all three dimensions of evaluation (efficacy, safety, and stability) with results that are quantitatively compelling and mechanistically well-understood.

TABLE 15.1 -- CONSOLIDATED SUMMARY OF ALL KEY EXPERIMENTAL FINDINGS

Domain	Key Finding	Quantitative Result	Scientific Significance
Oral Systemic Delivery (In Vivo)	FITC fluorescence -- mouse oral study (1h)	Multi-organ distribution: intestinal villi liver lungs kidneys stomach	Confirms genuine systemic absorption via oral route in living animal villi localization confirms epithelial uptake
Oral Bioavailability (Pharma Precedent)	CGLY study -- systemic plasma IgG (rat 4h)	5x increase vs. saline control	Peer-reviewed quantification of oral ionic liquid bioavailability enhancement
Intestinal Tissue Penetration (Pharma Precedent)	CGLY study -- rat jejunum in vivo	>4.5x vs. saline control	Direct measurement of mucosal villi penetration in living tissue
Minimum Nanoparticle Size	DLS secondary peak (number-weighted)	<10 nm confirmed (5-10 nm range DLS)	Below mucus pore size threshold -- enables direct mucus penetration
Mucus Viscosity Reduction	Porcine intestinal mucus (CGLY study)	0.577 to 0.318 Pa.s (45% reduction)	Quantified rheological evidence of mucus fluidization mechanism
Paracellular Pathway Confirmation	Transcellular inhibition study (CGLY)	No effect on transport -- paracellular route confirmed primary	Mechanistic isolation of paracellular pathway as primary transport route
Epithelial Barrier Penetration (Ex Vivo)	Ex vivo porcine tissue model (24h)	>57x enhancement 4.59% vs 0.08%	Quantitative barrier penetration -- conservative model (0.5mm tissue)
Long-Term Stability	Room temperature storage (1–4 months)	101.1–101.9% recovery (HPLC)	Eliminates cold-chain requirement for at least 4 months
Thermal Stability	37 degrees C accelerated aging (3 days)	105.8% recovery	Compatible with tropical storage and body temperature exposure
Photostability	Intense light exposure (10 days)	104.6% recovery	No light protection required robust against photodegradation
Cytotoxicity	CCK-8 assay (24h and 48h)	Non-cytotoxic per ISO 10993-5 (80-100% viability)	Classified non-cytotoxic at all tested concentrations and timepoints
Analytical Validation	HPLC recovery (1000x dilution)	112.3% recovery	Validated QC method for ionic liquid-peptide formulations

SECTION 15

Conclusion

15.1 The Three Core Conclusions

IT WORKS -- ORAL, SUBLINGUAL, AND MUCOSAL DELIVERY CONFIRMED

The Protixa ION System enables oral, sublingual, and mucosal delivery of therapeutic peptides through six measurable, mechanistically understood pathways: mucus fluidization via Ca2+ chelation (45% viscosity reduction quantified), paracellular tight junction modulation via electrostatic claudin interaction (4-5x paracellular transport, confirmed as primary mechanism), mucoadhesion for extended nasal and sublingual residence, protease inhibition via active site blockade and metal cofactor sequestration, pH buffering for gastric protection, and nano-scale particle self-assembly to a confirmed sub-10 nm secondary population (5– 10 nm, DLS) for mucus pore penetration. The combined effect produces confirmed multi-organ systemic distribution with intestinal villi localization following oral administration in vivo, greater than 57-fold enhancement in epithelial barrier penetration, and independent pharmaceutical-grade validation of a 5-fold systemic bioavailability increase.

IT IS SAFE -- BIOCOMPATIBLE BY DESIGN

The Protixa ION System is composed of food-grade excipients (citric acid and amino acids) that are endogenous metabolites with established pharmacopoeial acceptance. CCK-8 cytotoxicity testing confirmed non-cytotoxic classification per ISO 10993-5 at all tested concentrations and timepoints (24h and 48h; 80–100% cell viability), with no evidence of acute immune activation or membrane disruption. The independent CGLY pharmaceutical study confirmed safety in a 7-day repeat-dose in vivo study at 625 mg/kg/day, with normal GI histopathology and blood chemistry. The permeation enhancement mechanism is reversible and non-destructive; TEER values return to baseline after ionic liquid removal.

IT IS STABLE -- COLD-CHAIN INDEPENDENT

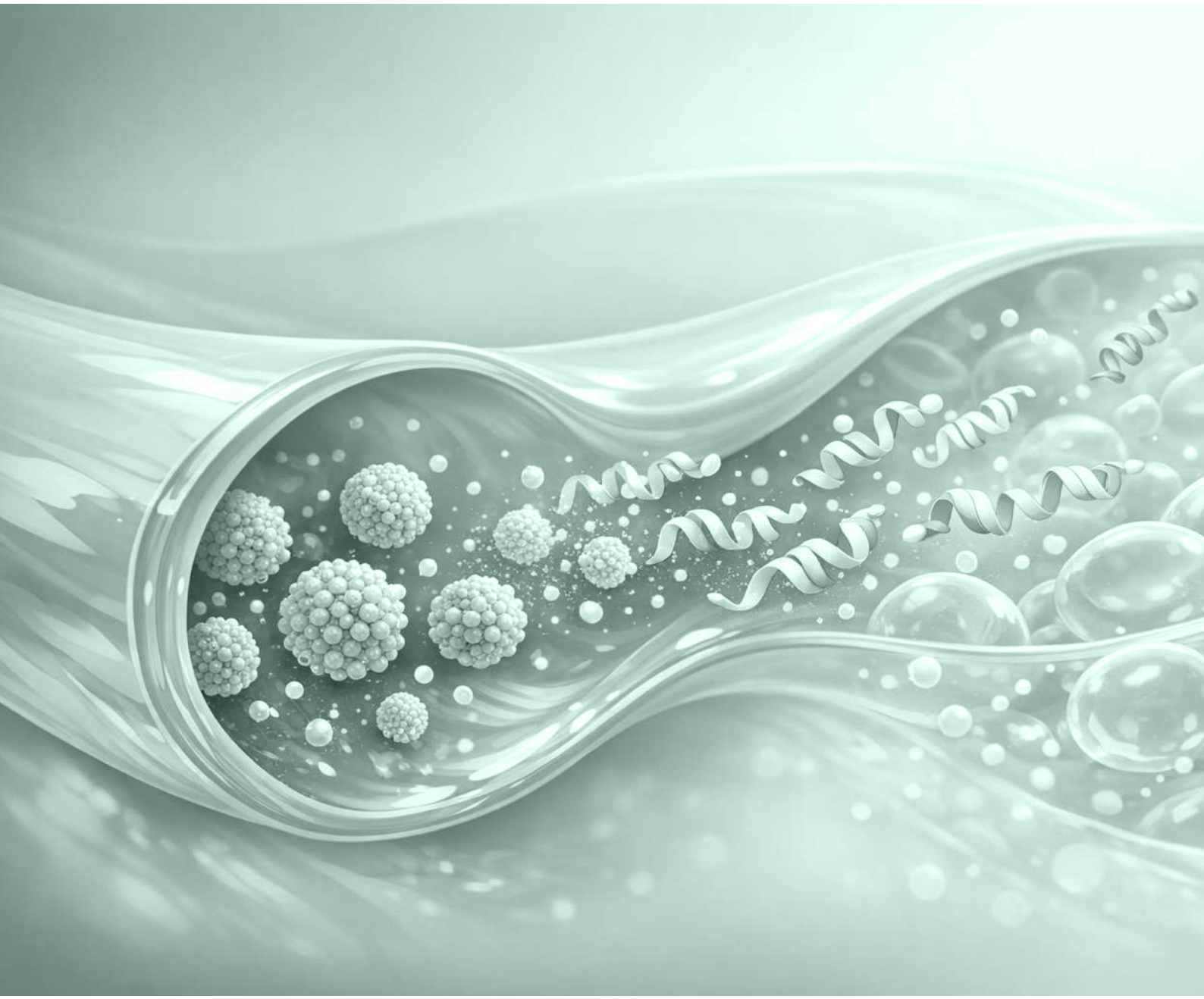
Peptides formulated in the Protixa ION System maintain 101.1–101.9% recovery after four months at room temperature, 105.8% recovery after three days at 37 degrees C, and 104.6% recovery after ten days of intense light exposure. These results demonstrate comprehensive protection against thermal, photolytic, and hydrolytic degradation pathways, enabling room-temperature storage and distribution without coldchain requirements.

SECTION 15

Conclusion

15.2 Path Forward

The logical next steps for platform development include: controlled pharmacokinetic studies measuring plasma peptide concentrations following oral, sublingual, and intranasal administration to quantify absolute bioavailability; route-specific optimization studies for sublingual delivery and intranasal delivery; peptide-specific formulation development for high-priority therapeutic targets; and preparation of regulatory dossiers for specific product applications in the nutraceutical, cosmetic, and pharmaceutical categories.



Appendix A

Third-Party Test Report -- Zhejiang TianMei Biological Engineering Co., LTD

The following section reproduces the complete findings from the independent third-party test report issued by Zhejiang TianMei Biological Engineering Co., LTD, with cellular and animal studies conducted at Zhejiang University of Technology. The report was issued on January 28, 2026, under the supervision of investigator Zihan Zhang.

APPENDIX TABLE A.1 -- TESTING LABORATORY IDENTIFICATION AND STUDY CREDENTIALS

Parameter	Detail
Testing Laboratory	Zhejiang TianMei Biological Engineering Co. LTD (TIANMEI CHEMICALS)
Cellular and Animal Study Laboratory	Zhejiang University of Technology
Principal Investigator	Zihan Zhang
Study Objective	Feasibility Test of Rapid-ion technology in peptide delivery
Report Date	January 28 2026
Test Article Concentration	10 mg/mL (peptide in Rapid ion / Protixa ION System)

A.1 Test Item 1: Particle Size (Dynamic Light Scattering)

Testing Method: Dynamic Light Scattering (DLS) test of the peptide/Rapid Ion solution (10 mg/mL).

Results: Bimodal distribution. Primary peak: 200-300 nm (self-assembled nanoparticle system). Secondary peak: 5–10 nm (<10 nm confirmed).

Conclusion: The bimodal distribution originates from the self-assembly properties of RI in solution. The sub-10 nm secondary population (5–10 nm range) enables penetration of mucus gel pore networks. These nanoparticles are hypothesized to enhance peptide stability and facilitate controlled release.

A.2 Test Item 2: Ex Vivo Porcine Tissue Penetration Testing

Testing Method: Delivery efficiency assessed using an ex vivo porcine tissue model (0.5 mm thickness). Peptide solution (10 mL) mixed with Rapid Ionic system (1 mL) administered to tissue surface; cumulative permeation measured as a function of time.

Appendix A

Third-Party Test Report -- Zhejiang TianMei Biological Engineering Co., LTD

APPENDIX TABLE A.2 -- EX VIVO PERMEATION RAW DATA

Time Point	Experimental Group (mg)	Control Group (mg)	Enhancement
0.5 hours	0.114	0.004	28.5x
1 hour	0.163	0.005	32.6x
2 hours	0.152	0.005	30.4x
4 hours	0.186	0.006	31.0x
6 hours	0.217	0.006	36.2x
24 hours	0.459	0.008	>57x

Conclusion: The Rapid Ion system enhanced delivery by over 57-fold compared to the control group. The 24-hour permeation rate was 4.59%. Note: Porcine tissue thickness was 0.5 mm; respiratory tract mucosa, sublingual epithelium, and intestinal mucosa are approximately 0.1 mm and more permeable -- the 57-fold enhancement is a conservative lower bound for mucosal delivery applications.

A.3 Test Item 3: Peptide Stability

APPENDIX TABLE A.3 -- STABILITY RAW DATA (SHORT-CHAIN THERAPEUTIC PEPTIDE, 10 MG/ML)

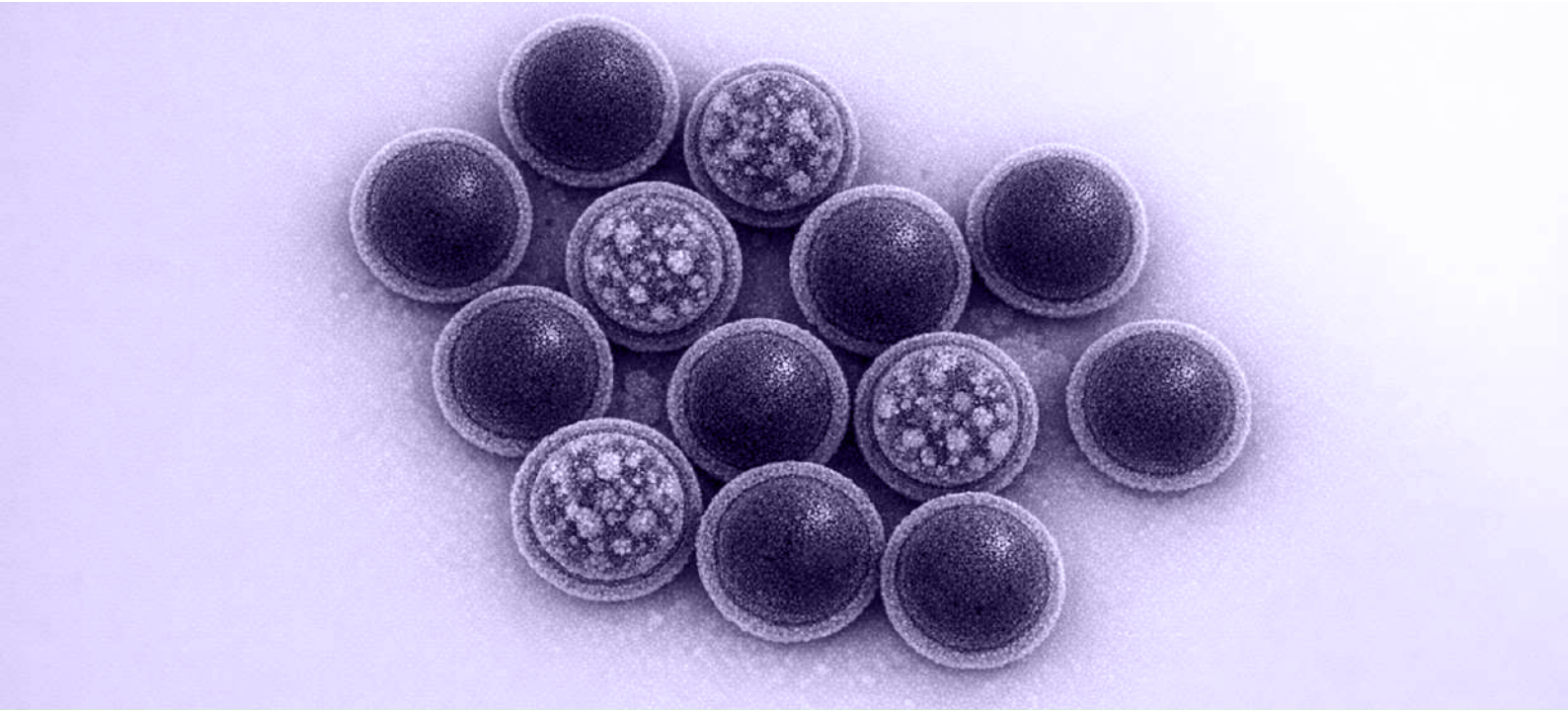
Condition	Dilution	HPLC AUC	Concentration (mg/mL)	Recovery (%)
1 Month RT	1000x	140.59	10.11	101.1%
2 Months RT	1000x	118.44	10.11	101.1%
37 degrees C x 3 days	1000x	152.69	10.58	105.8%
Sunlight x 10 days	1000x	150.8	10.46	104.6%

A.4 Test Item 4: Cellular Toxicity (CCK-8 Assay)

Conclusion: After co-culturing cells with ionic liquids for 24h and 48h, cell viability remained between 80% and 100%. No significant impact on cell vitality was observed, demonstrating excellent biocompatibility.

Appendix A

Third-Party Test Report -- Zhejiang TianMei Biological Engineering Co., LTD



A.5 Test Item 5: In Vivo Distribution (Oral)

Testing Method: Peptides labeled with FITC. Mice orally administered 10 mg of FITC-labeled peptide/Rapid Ion formulation. One hour post-administration, systemic fluorescence signals detected throughout the body. Control: FITC + water. Experimental: FITC + ionic liquid.

Conclusion: Significant fluorescence signals detected in intestine (localized within villi structures), lungs, liver, kidneys, and stomach of the IL group. Control group exhibited only negligible signals, confirming that the Rapid Ion system effectively promotes systemic delivery in vivo.

A.6 Detection Methodology Validation

APPENDIX TABLE A.4 -- PEPTIDE DETECTION METHOD DEVELOPMENT RAW DATA

Condition	Solvent	Dilution	Result (mg/mL)	Recovery (%)
Water reference	Water	100x	9.35	93.5%
Ionic liquid standard	Rapid Ion	100x	7.99	79.9%
Ionic liquid + NaOH	Rapid Ion + NaOH	100x	8.25	82.5%
Ionic liquid + NaCl	Rapid Ion + NaCl	100x	8.36	83.6%
Ionic liquid high dilution	Rapid Ion	1000x	11.23	112.3%

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All findings presented in this document are based on preclinical and laboratory studies and are not clinical claims. The Protixa ION System is an excipient platform. All product applications must comply with applicable regulatory frameworks.