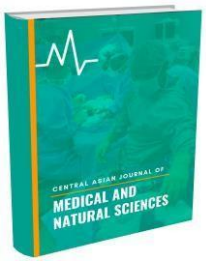

CENTRAL ASIAN JOURNAL OF MEDICAL AND NATURAL SCIENCES
https://cajmns.casjournal.org/index.php/CAJMNS
Volume: 07 Issue: 04 |October 2026 ISSN: 2660-4159

Article

Preservation of Hirdin Antithrombin Activity in a Low-Water-Activity Solid Premix: Formulation Performance and the Human Pharmacodynamic Context of Direct Thrombin Inhibition

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Abstract: Hirudin is a specific direct thrombin inhibitor, and its use depends on retaining conformational integrity and antithrombin activity. We characterized a dry solid premix developed to stabilize hirudin during powder processing and unit-dose filling. Formulation results were considered alongside published human pharmacodynamic studies of recombinant hirudin. The BCLC® premix used trehalose, mannitol and colloidal silicon dioxide for preadsorptive dispersion; citric acid-sodium citrate for local pH control; microcrystalline cellulose and pregelatinized starch as a low-hygroscopicity matrix; and geometric dilution under low humidity. Water activity was 0.26-0.34 in the three optimized formulations. Activity retention was 90.6-92.1%, activity-distribution RSD was 3.8-4.7%, and capsule fill-weight RSD was 2.9-3.4%. Retained activity fell to 76.4% without protective dispersion and staged dilution, 72.9% during high-humidity processing, and 80.1% without the buffer. Intravenous and subcutaneous studies of recombinant hirudin report dose-related prolongation of thrombin time and activated partial thromboplastin time. These pharmacodynamic findings define biomarkers for later studies of stabilized hirudin systems.

Keywords: Hirudin, Direct Thrombin Inhibitor, Antithrombin Activity, Water Activity, Peptide Stabilization, Solid Premix, Anticoagulation

Citation: Ziraoui, A., Mokoena, T., Hassan, H. Preservation of Hirdin Antithrombin Activity in a Low-Water-Activity Solid Premix: Formulation Performance and the Human Pharmacodynamic Context of Direct Thrombin Inhibition. Central Asian Journal of Medical and Natural Science 2026, 7(4), 483-487.

Received: 25th Jul 2026
Revised: 15th Aug 2026
Accepted: 30th Aug 2026
Published: 24th Sept 2026

1. Introduction

Thrombin is the central serine protease that converts fibrinogen to fibrin and reinforces coagulation through several feedback reactions. Direct thrombin inhibitors were developed to block this final enzymatic step more directly than agents whose effect depends on upstream cofactors or several points in the cascade. Hirudin became an important reference compound in this class because of its high affinity and specificity for thrombin, and early clinical work established a clear rationale for studying it as an antithrombotic agent [1], [2].

The clinical history of hirudin also shows why the setting of use matters. Reviews of recombinant hirudins describe a pharmacology that is consistent across intravenous and subcutaneous exposure but not identical across patient groups or indications [3]. Trials of direct thrombin inhibition in acute coronary syndromes broadened that experience beyond laboratory coagulation markers [4]. Recombinant hirudin was compared with intravenous heparin in established lower-limb deep-vein thrombosis [5], desirudin was evaluated against low-molecular-weight heparin after total hip replacement [6], and OASIS-2

compared recombinant hirudin with heparin in acute myocardial ischaemia without ST elevation [7]. These studies demonstrate systemic activity, but they also make clear that dose, route, clinical context and exposure cannot be separated when outcomes are interpreted.

Safety and exposure are similarly linked. Antihirudin antibodies have been documented during lepirudin treatment [8], and anaphylactic or anaphylactoid reactions have also been reported in exposed patients [9]. Those observations do not change the underlying thrombin-inhibitory mechanism, but they place tighter demands on dose control and on knowing how much biologically active material reaches the circulation. More recent delivery research, including self-regulated hirudin systems, reflects the same concern from a formulation perspective: activity must be preserved and exposure must be controlled rather than inferred from nominal drug content alone [10].

Human pharmacodynamic studies provide a practical bridge between formulation quality and later biological testing. In healthy volunteers, recombinant hirudin produced dose-related changes in activated partial thromboplastin time (aPTT) and other coagulation measures after subcutaneous dosing [11]. A separate study of IK-HIR02 followed thrombin time (TT), aPTT and bleeding time after intravenous and subcutaneous administration [12], while phase I work with PEG-hirudin examined anticoagulant pharmacodynamics and safety across both routes [13]. An angiographic trial in unstable angina extended these observations into a clinical thrombus setting [14]. Together, these reports identify measurable pharmacodynamic endpoints for an active hirudin preparation, without implying that the same exposure is achieved by an untested oral or solid-dose system.

That distinction is especially important for a dry premix intended for later oral evaluation. Recombinant hirudin has been investigated by the oral route in experimental work, where measurable bioavailability and anticoagulant effects were reported in animal models [15]. Before such exposure studies can be interpreted, however, the preadministration material has to meet a more basic requirement: the peptide must remain active during blending and storage, and the low-dose active must be distributed evenly from one unit to the next. Free-water activity, local pH, contact with excipients and the sequence used to dilute a potent peptide can influence these two properties in different ways.

The present study therefore focuses on this upstream formulation problem rather than on clinical efficacy. The BCLC® premix combines preadsorption of hirudin onto colloidal silicon dioxide, trehalose and mannitol as protective solids, geometric dilution with microcrystalline cellulose, citrate buffering and processing under low humidity. We measured water activity, retained antithrombin activity, multipoint activity-distribution RSD, powder flow and capsule fill-weight variability. Published human hirudin studies were used only to define the pharmacodynamic context and the biomarkers that would be relevant if gastrointestinal stability, intact absorption and systemic exposure are demonstrated in subsequent work.

2. Materials and Methods

The premix contained recombinant hirudin dried powder standardized by calibrated antithrombin activity, trehalose, mannitol, pregelatinized starch, colloidal silicon dioxide, citric acid, sodium citrate, magnesium stearate and microcrystalline cellulose. No liquid water was used. Optimized batches were produced at 18-25°C and relative humidity no higher than 35%.

Hirudin was premixed with colloidal silicon dioxide, followed by trehalose and mannitol. A portion of microcrystalline cellulose was added by geometric dilution. Pregelatinized starch, citrate buffer components and the remaining cellulose were then mixed, and magnesium stearate was added briefly for final lubrication. Antithrombin

activity was measured with a chromogenic substrate method. Predefined powder tests measured water content, water activity, angle of repose, multipoint activity RSD and capsule fill-weight RSD.

Human studies were included when they directly measured recombinant hirudin pharmacodynamics, coagulation markers or clinical antithrombotic activity. Parenteral studies described the biology of systemically available hirudin, their outcomes were kept separate from the premix activity measurements.

3. Results

3.1 Formulation performance

All three optimized formulations retained more than 90% activity and had water activity values of 0.26-0.34. Activity-distribution RSD stayed below 5%, and powder flow was compatible with continuous size-0 capsule filling. F1 retained 92.1% activity with an activity RSD of 3.8%.

Without protective preadsorption and staged dilution, activity retention fell to 76.4% and activity RSD increased to 8.9%. High humidity raised water activity to 0.49 and reduced retention to 72.9%.

Removing the citrate buffer produced water activity close to the optimized range but only 80.1% retention. Moisture, local pH and distribution therefore affected different parts of premix performance.

Table 1. Formulation-level physicochemical, activity and capsule-filling performance. F1-F3 are optimized formulations, C1-C3 are mechanistic comparators.

Group	Water %	aw	Activity retention %	Activity RSD %	Angle of repose °	Fill-weight RSD %
F1 optimized	3.2	0.26	92.1	3.8	36.1	2.9
F2 optimized	3.6	0.30	91.4	4.2	37.2	3.1
F3 optimized	4.0	0.34	90.6	4.7	38.4	3.4
C1 no protective preadsorption/staged dilution	5.1	0.43	76.4	8.9	42.8	6.8
C2 high-humidity processing	5.8	0.49	72.9	7.6	44.3	7.2
C3 no citrate buffer	3.5	0.31	80.1	4.5	37.6	3.6

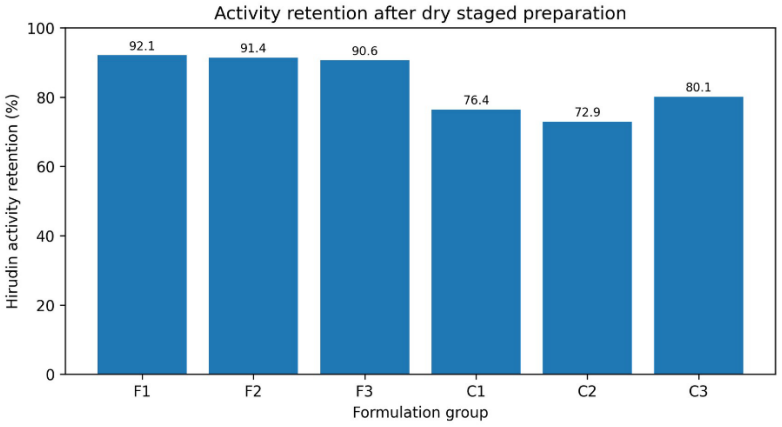

Figure 1. Hirudin activity retention across optimized and comparator powder formulations.

3.2 Published human evidence

Verstraete et al. studied 231 healthy volunteers and found dose-proportional aPTT prolongation within 30 minutes after subcutaneous recombinant desulfatohirudin [11]. In 24 healthy volunteers, intravenous and subcutaneous IK-HIR02 produced dose-dependent increases in TT and aPTT [12]. Randomized phase I studies of PEG-hirudin in 75 healthy volunteers also measured anticoagulant pharmacodynamics after intravenous and subcutaneous dosing [13].

Table 2. Representative human pharmacodynamic studies of recombinant hirudin.

Study	Population	Exposure	Key observation
Verstraete et al., 1993	231 healthy volunteers	Subcutaneous recombinant hirudin	Dose-proportional aPTT prolongation
Schenk et al., 1996	24 healthy volunteers	IV/SC recombinant hirudin	Dose-related TT and aPTT prolongation
Esslinger et al., 1997	75 healthy volunteers	IV/SC PEG-hirudin	Randomized phase I anticoagulant pharmacodynamics

4. Discussion

The comparator formulations separate three manufacturing problems. Water activity reflects free water available for peptide degradation, while citrate buffering controls local acid-base variation.

Preadsorption and geometric dilution address the small mass fraction of hirudin relative to the excipients. No single excipient explains the full pattern of retained activity and uniformity.

Direct thrombin inhibition depends on active hirudin reaching the circulation. Parenteral human studies produced rapid, dose-related anticoagulant responses [11]-[13], and an angiographic unstable-angina trial related hirudin dosing to coronary thrombus activity [14]. These studies identify coagulation measures that can be used when exposure from a stabilized system is examined.

Oral delivery adds questions of gastrointestinal stability and intact peptide absorption. Recombinant hirudin variant rHV2-K47 showed measurable oral bioavailability and anticoagulant effects in animal models [15]. The premix data address the earlier manufacturing step by preserving activity before administration. Later work can measure intact hirudin exposure and relate it to aPTT, TT, ecarin-based assays or other thrombin-sensitive biomarkers.

Research Scope and Future Perspectives

The next experimental stage can first measure gastrointestinal stability and intact peptide absorption. Defined doses could then be related to systemic exposure and coagulation pharmacodynamics. These experiments would connect premix activity and uniformity with the biological response after administration.

5. Conclusion

The low-water-activity, buffered premix preserved hirudin activity while maintaining acceptable distribution and capsule-filling consistency: the three optimized formulations retained 90.6-92.1% antithrombin activity, with activity-distribution RSDs of 3.8-4.7% and fill-weight RSDs of 2.9-3.4%, whereas removal of protective dispersion, high-humidity processing, or omission of citrate buffering reduced performance in distinct ways. These findings indicate that moisture control, local pH management and staged dilution act as complementary manufacturing controls rather than interchangeable formulation choices, and they provide a better-defined starting material for subsequent

exposure studies. The present work is limited to premix stability, powder uniformity and comparison with published parenteral pharmacodynamic evidence, it does not establish gastrointestinal stability, intact oral absorption, systemic bioavailability or clinical anticoagulant efficacy for the BCLC® formulation. The next stage should therefore measure peptide survival through gastrointestinal conditions, quantify intact hirudin exposure, and relate achieved concentrations to aPTT, TT, ecarin-based assays or other thrombin-sensitive endpoints under defined dosing conditions.

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