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Preparation, Physicochemical Characterization and Intestinal Transport Evaluation of CalmGly^{TM}, a Lipid-Encapsulated Magnesium Bisglycinate Delivery System

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Abstract: This study aimed to evaluate a novel lipid-encapsulated magnesium bisglycinate delivery system, CalmGly^{TM}, to overcome the challenges of low bioavailability and poor gastrointestinal tolerability associated with conventional magnesium salts (such as magnesium oxide). The formulation was prepared using high-pressure homogenization combined with spray drying. The system was characterized for encapsulation efficiency, particle size distribution, reconstitution behavior, Caco-2 transepithelial transport capacity, and accelerated stability. Results showed that CalmGly^{TM} exhibited a high encapsulation efficiency of 88.5%, with an average particle size of 168.5 nm and a PDI of 0.18. In the Caco-2 monolayer model, the apparent permeability coefficient (P_{app}) of CalmGly^{TM} reached 1.88×10^{-5} cm/s, which was approximately 10.4 times that of the magnesium oxide control (0.18×10^{-5} cm/s). Furthermore, CalmGly^{TM} maintained a magnesium retention rate of 98.4% after 6 months of accelerated aging. In conclusion, CalmGly^{TM} exhibits high encapsulation efficiency and robust digestion stability, significantly enhancing the absorption rate of magnesium bisglycinate. This delivery system offers a promising new strategy for improving the efficacy of magnesium supplementation and reducing associated side effects.

1. Introduction

Magnesium is one of the most abundant divalent cations in the human body and plays a central role in numerous physiological processes. It is involved in ATP-dependent enzymatic reactions, neuromuscular transmission, protein synthesis, glucose metabolism, cardiovascular function and skeletal health[1,2]. In addition to its metabolic functions, magnesium is also relevant to nervous system regulation through its involvement in neuronal excitability and neuromuscular relaxation[3].

Despite its critical physiological roles, suboptimal magnesium intake remains a significant nutritional challenge across various populations. Driven by factors such as the demineralization of modern agricultural soils, extensive food processing, and excessive caffeine consumption, approximately 31% of the global population is estimated to have insufficient magnesium levels[4,5].

This "Magnesium deficiency" has become an insidious driver of chronic fatigue, sleep disorders, migraines, and cardiovascular diseases[2]. Consequently, magnesium supplementation has established itself as a vital nutritional intervention to support daily mineral requirements, maintain healthy muscle function, facilitate relaxation, and preserve overall metabolic homeostasis.

Commercial magnesium supplements encompass a variety of chemical forms, primarily categorized into inorganic salts (e.g., magnesium oxide and magnesium carbonate), organic salts (e.g., magnesium citrate, lactate, and malate), and chelates (e.g., magnesium bisglycinate)[2]. Magnesium oxide (MgO) is widely utilized due to its high elemental magnesium density and cost-effectiveness. However, its extremely low aqueous solubility leads to poor bioavailability. A human study involving healthy subjects confirmed that the fractional absorption of MgO is approximately 4%; the remaining unabsorbed magnesium ions induce high osmotic pressure within the intestinal lumen, frequently resulting in gastrointestinal distress, such as osmotic diarrhea[6].

In contrast, magnesium bisglycinate offers significant advantages as an organic chelated form. Its ligand, glycine, serves as an inhibitory neurotransmitter that modulates central nervous system excitability, thereby alleviating anxiety and tension while facilitating physiological relaxation[7]. Mechanistically, this chelate is translocated via the peptide transporter 1 (PepT1) pathway in small intestinal epithelial cells, effectively circumventing the competition and interference inherent to ion channels[8]. Nevertheless, the clinical efficacy of conventional magnesium bisglycinate remains constrained by saturable transport kinetics. Due to the limited abundance of PepT1 carriers, elevated single dosages lead to rapid saturation of the transport pathway, causing unabsorbed chelates to be excreted via feces and hindering efficient, high-flux transmembrane delivery. Furthermore, the risk of premature dissociation under fluctuating intestinal pH conditions further compromises the effective concentration reaching the target site.

Lipid-based oral delivery systems have been widely investigated . Lipid-like encapsulation may help protect active ingredients from early gastric exposure, improve aqueous dispersion after reconstitution and facilitate interaction with intestinal epithelial surfaces[9]. Moreover, the biocompatible and biodegradable nature of lipid-based materials minimizes risks associated with in vivo toxicity and accumulation, ensuring their safety for human consumption[10].

CalmGly™ is a lipid-encapsulated magnesium bisglycinate delivery system developed to address these formulation challenges. The system consists of a magnesium bisglycinate core incorporated into a lipid-like encapsulation matrix and converted into a spray-dried powder for practical formulation use. The intended design logic includes nanoscale reconstitution, reduced gastric-intestinal phase release and improved transport across an intestinal epithelial model. A schematic illustration of the distinct intestinal transport mechanisms for magnesium oxide, magnesium bisglycinate, and CalmGly™ across the intestinal epithelium is shown in Fig.1.

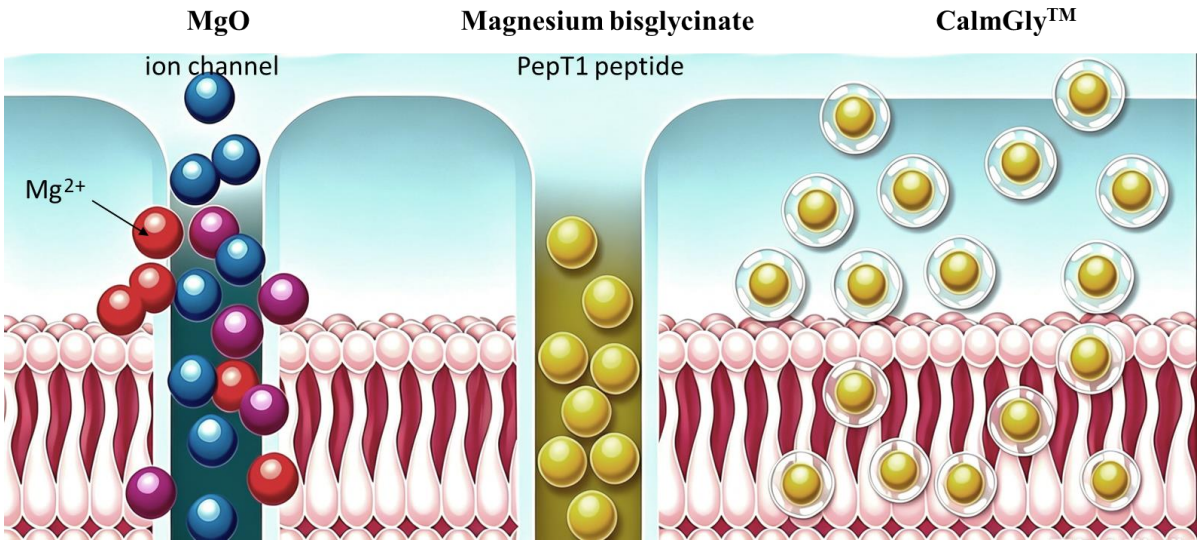

Figure 1. Schematic illustration of the distinct intestinal transport mechanisms for MgO, magnesium bisglycinate, and CalmGly™ across the intestinal epithelium.

2. Materials and Methods.

2.1 Materials

To systematically investigate the efficacy of the lipid-encapsulation technology, three distinct formulations were prepared and categorized into experimental, control groups (Tab.1).

Control formulation: Control groups consisted of unencapsulated magnesium bisglycinate powder or magnesium oxide (MgO).

All magnesium-containing groups were normalized based on elemental magnesium content as determined by inductively coupled plasma optical emission spectrometry (ICP-OES). This normalization was applied in release and transport experiments to ensure that comparisons among formulations were based on equivalent elemental magnesium exposure.

Table 1. Rationale and formulation design of test and control groups.

Group	Sample
Control group 1 (MgO)	Food-grade magnesium oxide
Control group 2 (Magnesium bisglycinate)	Unencapsulated magnesium bisglycinate
Test group (CalmGly™)	Lipid-encapsulated magnesium bisglycinate

2.2 Preparation of CalmGly™

CalmGly™ was fabricated through a sequential three-step process: shear dispersion, high-pressure homogenization (HPH), and spray drying. Initially, magnesium bisglycinate was dissolved in deionized water, followed by the addition of refined phospholipids and lipid-like excipients under continuous stirring. The mixture was processed with a shear mixer (300 rpm, 15 min) to facilitate physical hydration and form a uniform coarse emulsion[11].

Subsequently, the coarse emulsion was subjected to HPH at 500 bar for three cycles to obtain a

nanoliposomal dispersion with a narrow size distribution [12]. The resulting dispersion was then converted into a free-flowing solid powder using a laboratory-scale spray dryer at an optimized inlet temperature of 175 ℃±5 ℃[13]. The final microencapsulated powder was collected and sealed in light-resistant bags for further characterization.

2.3 Analytical Determination of Mg

The magnesium content was determined using ICP-OES following the methodology described by Bawiec et al[14]. Accurately weighed samples were subjected to acid digestion with concentrated nitric acid (65% HNO_3) at 120 ℃ for 120 min using a DigiPREP MS system. Following cooling and filtration through DigiFILTERs, the samples were diluted to a final volume with ultrapure water. Mg concentrations were quantified using a high-resolution ICP-OES spectrometer based on an external standard calibration curve prepared from certified reference standards.

2.4 Encapsulation Efficiency of Magnesium Bisglycinate

Free and encapsulated magnesium fractions were separated using ultrafiltration centrifugal tubes via high-speed centrifugation at 15,100 × g for 30 minutes [15]. The free magnesium in the resulting filtrate was then quantified using ICP-OES. The encapsulation efficiency (EE) was calculated as follows:

$$EE\ (\%) = [(\text{Total Mg} - \text{Free Mg}) / \text{Total Mg}] \times 100$$

2.5 Particle Size, PDI and Zeta Potential

CalmGly™ powder was reconstituted in deionized water at room temperature. The reconstituted dispersion was analyzed using dynamic light scattering to determine Z-average particle size and polydispersity index (PDI) [12]. Zeta potential was measured to evaluate colloidal stability.

2.6 Reconstitution Behavior

The reconstitution behavior of CalmGly™ and control samples was assessed in deionized water at 25 ° C. The time to complete dispersion and the final state of the solution were recorded.

2.7 In Vitro Gastrointestinal Release

The in vitro release behavior of the samples was assessed using a two-stage simulated gastrointestinal digestion model. Samples (normalized to equivalent elemental magnesium doses) were subjected to gastric digestion in simulated gastric fluid (SGF, pH 2.0) containing 2 mL of 10% pepsin for 2 h at 37 ° C with constant shaking at 100 rpm[14]. Subsequently, the pH of the system was adjusted to 6.5, and 5 mL of 0.4% pancreatin was added to facilitate a further 2-hour intestinal digestion phase under identical conditions[14].

2.8 Caco-2 Cell Culture and Monolayer Formation

Human colon adenocarcinoma (Caco-2) cells were cultured in high-glucose DMEM medium supplemented with 10% fetal bovine serum, 1% non-essential amino acids, and 1% penicillin-streptomycin. The cells were maintained in a humidified incubator at 37 ℃ with 5% CO_2[16]. Upon reaching 80%–90% confluence, the cells were harvested using trypsin and seeded onto the apical

(AP) side of Transwell polycarbonate membrane inserts (0.4 μm, 12 mm diameter) at a density of 2×10^5 cells/cm^2. The culture medium was replaced every two days, and the cells were cultured for 21 days to ensure complete differentiation and the formation of a dense, polarized monolayer[17].

Prior to the transport experiments, the transepithelial electrical resistance (TEER) was monitored using a volt-ohmmeter. Only cell monolayers exhibiting stable TEER values exceeding 500 Ω·cm² were selected for subsequent studies[17].

The cytotoxic effects of CalmGly™ and the control group on Caco-2 cells were assessed using the CCK-8 assay. Cells were exposed to various concentrations of magnesium and incubated for 24 hours. The absorbance at 450 nm was subsequently measured using a microplate reader. The results confirmed that cell viability remained above 90% at the tested concentrations, thereby excluding the possibility of increased permeability resulting from cellular damage.

2.9 Caco-2 Transepithelial Transport Study

Transport experiments were conducted according to the method of Bolan et al[16]. The buffer on the apical side was replaced with 0.5 mL of the diluted digest. To minimize the unstirred water layer, the transport study was performed in a constant temperature shaker at 37 ° C with a shaking frequency of 70 Hz. Sampling of the basolateral solution was selected at 2h.

The apparent permeability coefficient (P_{app}) was calculated using the following equation:

$$P_{app} = (dQ/dt) / (A*C_0)$$

where dQ/dt is the transport rate of magnesium across the monolayer, A is the surface area of the Transwell membrane and C_0 is the initial magnesium concentration in the apical compartment.

2.10 Accelerated Stability Study

CalmGly™ powder was packaged in sealed aluminum foil bags and stored under accelerated conditions of 40℃ ±2℃ and 75%±5% RH for 6 months. Samples were analyzed at 6 months. Stability parameters included appearance, elemental magnesium content, magnesium retention, encapsulation efficiency, free magnesium ratio, reconstituted particle size, PDI, reconstitution time and signs of powder caking. Elemental magnesium retention was calculated relative to the initial magnesium content.

2.11 Statistical Analysis

All experiments were performed at least in triplicate unless otherwise specified. Results are expressed as mean ± standard deviation. Statistical comparisons between groups were performed using one-way analysis of variance followed by appropriate post hoc tests. $P < 0.05$ was considered statistically significant.

3. Results

3.1 Microstructure Characterization

As shown in Figure 2, the EE of CalmGly™ was 88.5 ± 1.2%, indicating the successful integration of magnesium bisglycinate into the lipid-based delivery matrix. As shown in Fig.2, Upon reconstitution, the system exhibited a Z-average particle size of 168.5±3.6 nm and a PDI of 0.18. A particle size of less than 200 nm is critical for ensuring effective penetration through the intestinal mucus layer[18]. Furthermore, a PDI below 0.2 signifies excellent physical stability,

minimizing the risk of particle aggregation or Ostwald ripening [19].

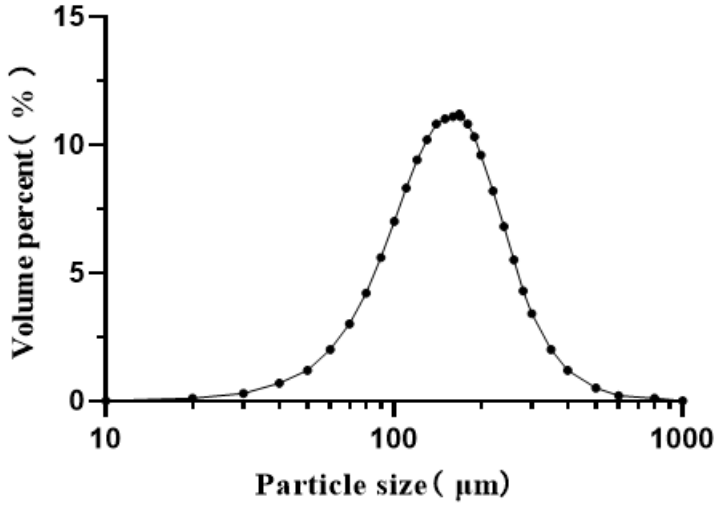

Figure 2. Particle size distribution of CalmGly™ solution by Intensity.

Furthermore, the powder demonstrated rapid reconstitution in water within approximately 30 seconds, yielding a homogeneous and stable dispersion. The combination of high EE, nanoscale particle size, and low PDI confirms the successful development of a structured lipid-encapsulated magnesium bisglycinate delivery system.

3.2 Caco-2 Cell Viability and Monolayer Integrity

Experimental results indicated that Caco-2 cells seeded on Transwell inserts exhibited a significant progressive increase in TEER values throughout the 21-day differentiation period. By Day 21, the mean TEER value of all selected monolayers reached 682±38 Ω· cm^2, which significantly surpassed the pre-defined threshold of 500 Ω· cm2 and the dense barrier criteria reported in the literature [17]. These findings confirm that the cells had formed a continuous, polarized monolayer characterized by high-integrity tight junctions, thereby successfully simulating the human small intestinal epithelial barrier and effectively restricting the paracellular leakage of molecules.

Furthermore, as shown in Tab.2, CCK-8 cytotoxicity assay results demonstrated that at an 80× dilution, the cell viability for both CalmGly™ and all control groups remained above 80% after 24 hours of exposure.

Table 2. Effect of different dilution ratios of formulations on Caco-2 cell viability after 24 hours of exposure (%).

Group	Undiluted	50×	80×	100×
Control group 1 (MgO)	55.4±4.2	66.2±3.1	83.5±1.8	92.2±1.2
Control group 2 (Magnesium bisglycinate)	62.1±3.8	69.5±2.5	85.8±1.5	95.1±0.8
Test group (CalmGly™)	65.4±3.5	72.1±2.1	87.6±1.2	95.5±0.5

3.3 Caco-2 Transepithelial Transport

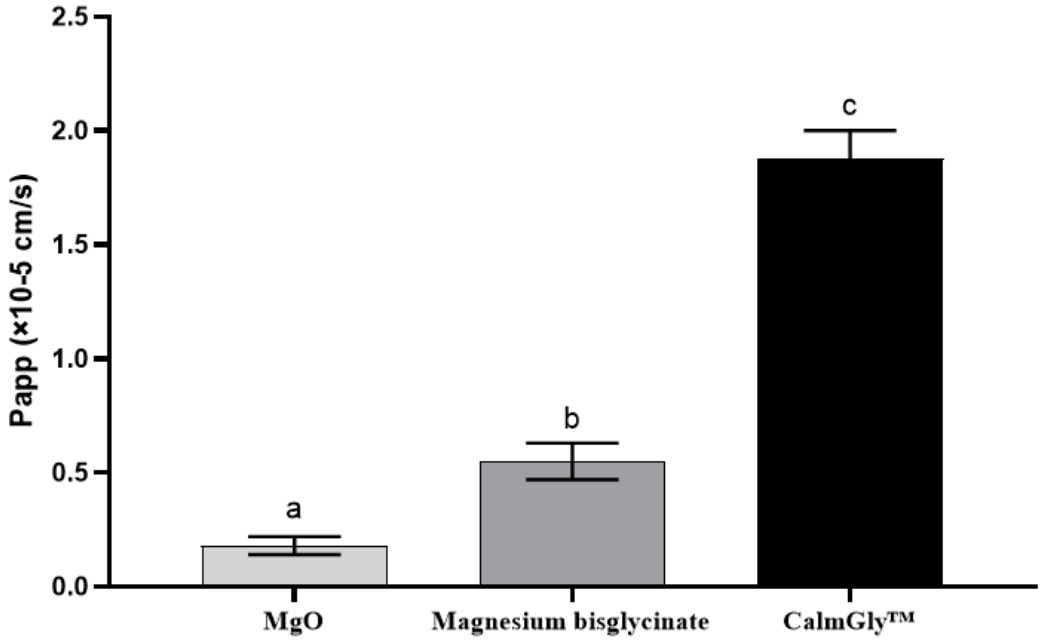

Figure 3. Comparison of P_{app} of CalmGly™, magnesium bisglycinate, and magnesium oxide across Caco-2 cell monolayers.

As shown in Fig.3 and Tab.3, CalmGly™ demonstrated higher transepithelial magnesium transport than magnesium oxide in the Caco-2 model. The P_{app} of CalmGly™ was 1.88×10^{-5} cm/s, compared with 0.18×10^{-5} cm/s for magnesium oxide and 0.55×10^{-5} cm/s for Magnesium bisglycinate. This corresponds to an approximately 10.4 times relative to the magnesium oxide control.

Table 3. Comparison of P_{app} and transport enhancement across Caco-2 cell monolayers for various magnesium formulations.

Sample	**P_{app} ($\times 10^{-5}$ cm/s)**	**Fold vs MgO**
MgO	0.18 ± 0.04	1.0×
Magnesium bisglycinate	0.55 ± 0.08	3.1×
CalmGly™	1.88 ± 0.12	10.4×

3.4 Accelerated Stability

The results presented in Tab.4 demonstrate that CalmGly™ exhibited exceptional physical and structural robustness under accelerated storage conditions. After 6 months of exposure to 40 ℃ ± 2 ℃ and 75% ± 5% relative humidity, the formulation maintained a high magnesium retention rate of 98.4%.The powder maintained acceptable appearance, and no severe caking was observed in the current dataset. Upon reconstitution, the delivery system showed a negligible shift in particle size, measuring 172.3 nm with a low PDI of 0.20. These findings demonstrate that the lipid-encapsulated architecture remained stable and intact throughout the long-term storage period, effectively preventing the aggregation of nanomicelles or the leakage of the encapsulated core.

Table 4. Accelerated stability endpoints for CalmGly™.

	Initial	6-month accelerated
Appearance	Free-flowing powder	No visible caking reported
EE% of Mg	100%	98.4%
Reconstituted particle size / PDI	168.5 ±3.6 nm / 0.18	172.3 nm / 0.20

4. Discussion

4.1 Structural Integrity and Nano-Architecture: The Benefits of Monodispersity

The physicochemical characterization of CalmGly™ confirms that the integration of high-pressure homogenization and spray drying successfully creates a robustly structured delivery system. The observed Z-average diameter of 168.5 ±3.6 nm is highly significant; particles under 200 nm are known to penetrate the intestinal mucus layer more efficiently than larger aggregates. Furthermore, the PDI of 0.18 indicates a narrow, monodisperse size distribution. Following the logic of solid-state microencapsulation, this uniformity prevents particle growth (Ostwald ripening) and ensures that upon rehydration, the system spontaneously reforms stable nanomicelles. This optimized nano-architecture provides the essential physical foundation for the subsequent leap in magnesium transport efficiency.

4.2 Decoupling Absorption from Protein Carriers: The Membrane-Fusion Pathway

The most critical finding of this study is the 10.4 times in P_{app} compared to the MgO control. Physiologically, magnesium absorption is typically "gated" by the limited abundance of TRPM6/7 and PepT1 protein transporters. By encapsulating the magnesium bisglycinate core within a biomimetic lipid shell, CalmGly™ enables absorption via membrane fusion and endocytosis pathways rather than relying solely on protein-mediated channels, which explains the dramatic enhancement in bioavailability.

4.3 Mechanism of Enhanced Stability

Accelerated stability studies demonstrated the exceptional robustness of the CalmGly™ delivery system. After six months of exposure to stress conditions (40 ℃, 75% RH), the formulation maintained an elemental magnesium retention rate of 98.4%, with its macroscopic powder state and nanostructural integrity remaining virtually unaltered. These findings underscore the superiority of the lipid-encapsulation strategy, where the phospholipid bilayer serves as a hermetic physical barrier, effectively sequestering the magnesium bisglycinate within its protective core. This structural robustness not only ensures a prolonged shelf life without potency loss but also shields the core from premature dissociation in the highly acidic gastric environment. Consequently, the CalmGly™ system remains intact until it reaches the primary absorption sites in the small intestine, facilitating optimal bioavailability.

4.4 Clinical Implications

The ability of CalmGly™ to provide a sustained release profile while maintaining exceptionally high absorption has profound clinical implications. Conventional magnesium supplements, particularly inorganic salts, often cause osmotic diarrhea due to the rapid accumulation of unabsorbed ions in the intestinal lumen. CalmGly™ mitigates this risk by sequestering the

magnesium within micelles, thereby reducing local osmotic pressure and improving gastrointestinal tolerance. Furthermore, the liposomal architecture possesses unique potential for targeted brain delivery. Given that the lipid shell mimics biological membranes, it may more effectively traverse the blood-brain barrier, offering a more reliable nutritional strategy for supporting the central nervous system in conditions such as insomnia, anxiety, and age-related cognitive decline.

5. Conclusion

This study evaluated a lipid-encapsulated nano-microencapsulated delivery system, CalmGly™, for magnesium bisglycinate. CalmGly™ successfully overcame the two major limitations of conventional magnesium supplementation: poor bioavailability and low gastrointestinal tolerability. The findings revealed that, in its solid-state powder form, the system retained 98.4% of its elemental magnesium after six months of accelerated aging, while maintaining a stable nanoscale architecture with a negligible shift in particle size. Furthermore, the transepithelial transport efficiency of the system was 10.4 times that of magnesium oxide, effectively bypassing saturable protein carriers through membrane-fusion and endocytosis pathways. The final assessment indicates that this technology transforms magnesium from a passive, carrier-dependent mineral into an active, high-flux therapeutic agent, promising superior brain-targeting potential and enhanced reliability for neuromuscular and sleep-support applications across diverse populations.

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