UDC 546.57:594.3:615.281]:616-001.17 DOI: 10.58407/bht.1.26.8

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DEVELOPMENT OF SILVER–SNAIL MUCUS NANOSYSTEM AND ITS INCORPORATION INTO A MULTIFUNCTIONAL BURN-HEALING CREAM-GEL

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РОЗРОБКА НАНОСИСТЕМИ СРІБЛА НА СЛИЗУ РАВЛИКА ТА ЇЇ ВКЛЮЧЕННЯ ДО МУЛЬТИФУНКЦІОНАЛЬНОГО КРЕМ-ГЕЛЮ ДЛЯ ЛІКУВАННЯ ОПІКІВ

ABSTRACT

In recent years, nanotechnology, nanomaterials have been increasingly integrated into dermatological and cosmetic formulations.

Purpose of the work. To develop a silver nanosystem through an eco-friendly one-step reduction of silver nitrate using lyophilized *Cornu aspersum* mucus as a natural reducing and stabilizing agent, and to incorporate the obtained nanosystem into a multifunctional burn-healing cream-gel.

Methodology. The AgNPs–mucus nanosystem was synthesized by mixing aqueous solutions of silver nitrate and mucus reconstituted in water under stirring at 37–40 °C for 1.5 h, followed by incubation at room temperature under natural light for 48 h. The formation of metal nanoparticles was confirmed by the visual color shift and spectroscopically with the detection of SPR peak at 450 nm. Antimicrobial activity was assessed by the agar disk diffusion method against test strains *E. coli*, *B. subtilis*, and *C. albicans*. The obtained nanosystem was incorporated into a cream-gel base formulated with dexpanthenol, allantoin, lidocaine, and vitamin E, and a detailed technological flow scheme for the manufacturing process was developed

Scientific novelty*.* *C. aspersum* mucus was employed as a dual-function biogenic agent, enabling green synthesis and stabilization of silver nanoparticles, which were further utilized as an active component of a burn-healing cream-gel. The resulting cream-gel combines the antimicrobial, antioxidant, and regenerative effects of snail mucus with the well-known antibacterial activity of nanosilver.

Conclusions. The developed AgNPs-mucilage system demonstrated high antimicrobial efficacy, especially against Gram-positive bacteria and pathogenic dredges, and the gel composition has stability and skin-protective properties, making it suitable for the treatment of burns.

Key words: AgNPs, *Cornu aspersum* snail mucus, eco-friendly biogenic synthesis, burn-healing cream-gel, antimicrobial activity

АНОТАЦІЯ

В останні роки нанотехнології, наноматеріали все частіше інтегруються в дерматологічні та косметичні рецептури.

Мета роботи. Розробити наносистему срібла шляхом екологічно безпечного одностадійного відновлення нітрату срібла з використанням ліофілізованого слизу равлика *Cornu aspersum* як природного відновника та стабілізатора, а також включити отриману наносистему до складу багатофункціонального крем-гелю для лікування опіків.

Методологія. Наносистема AgNPs–слиз равлика була синтезована шляхом змішування водних розчинів нітрату срібла та слизу, ресуспендованого у воді за температури 37–40 °С та перемішування протягом 1,5 годин, з подальшим витримуванням за кімнатної температури і природного освітлення протягом 48 годин. Утворення металевих наночастинок було підтверджено візуально зміною кольору та спектрометрично з фіксацією піку ППР за 450 нм. Антимікробну активність оцінювали методом диско-дифузійного методу на агарі на тест-культурах *E. coli*, *B. subtilis* та *C. albicans*. Отриману наносистему включили до складу крем-гелю, що містив декспантенол, алантоїн, лідокаїн та вітамін Е, розробили технологічну схему його виробництва.

Наукова новизна. Слиз *C. aspersum* був застосований в якості біологічного агенту подвійної дії, що дозволило здійснити екологічний синтез і стабілізацію наночастинок срібла, які були далі використані як активний компонент крем-гелю для лікування опіків. Отриманий крем-гель поєднує антимікробну, антиоксидантну та регенеративну дію слизу равлика з добре відомою антибактеріальною активністю наносрібла.

Висновки. Розроблена система AgNPs–слиз продемонструвала високу антимікробну ефективність, особливо проти грам-позитивних бактерій та патогенних дріждів, композиція гелю має стабільність та шкірозахисні властивості, що робить його придатним для лікування опіків.

Ключові слова: AgNPs, слиз равлика *Cornu aspersum*, екологічно безпечний біогенний синтез, крем-гель для лікування опіків, антимікробна активність

Problem Statement

In recent years, nanotechnology has been increasingly integrated into dermatological and cosmetic formulations (Barel, 2009; Beyth et al., 2015; Rahman, 2022; Jiang et al., 2024). Among nanomaterials, silver nanoparticles (AgNPs) are recognized as advanced antimicrobial agents with remarkable regenerative and wound-healing properties (Kim et al., 2007; Rigo et al., 2013; Kaya et al., 2025). Numerous studies have demonstrated their ability not only to eliminate pathogenic microorganisms but also to promote keratinocyte proliferation, reduce inflammation, and stimulate granulation tissue formation (Vlachou, 2007; Rai et al., 2012, Rowan, 2015). Burn injuries, particularly second degree wounds, involve complex pathophysiological processes such as inflammation, infection risk, and delayed tissue regeneration (El-Kased, 2017; Jeschke, 2020). The other side, вurn injuries cause complex immune-inflammatory responses and severe metabolic disturbances that can lead to multi-organ failure. Burn trauma affects not only physical recovery but also mental health and overall quality of life. Therefore, patients require long-term multidisciplinary care far beyond wound closure to ensure full rehabilitation and well-being. Thus, developing multifunctional topical preparations that combine regenerative, antimicrobial, anti-inflammatory, and analgesic properties is an urgent task for both medicine and cosmetic technology. Analyzing existing formulations, dexpanthenol (Ebner, 2002) and allantoin with (Yazalou, 2024; Ryan, 2017) are recognized as a leading component in burn therapy formulations. AgNPs synthesized from silver nitrate using snail mucus Cornu aspersa show a wide range of antimicrobial effects (Todorova, 2025) and have proven to be quite well established in cosmetic products with therapeutic and preventive effects (Khrokalo, 2025).

Purpose of the work

The purpose of this study is to develop a antimicrobial snail mucus–silver nanosystem (AgNPs-mucus) and incorporate it into a dexpanthenol- and allantoin-based topical cream to enhance second-degree burn healing.

Materials and methods

Synthesis and characterisation of AgNPs-mucus

The lyophilized *Cornu aspersum* snail mucus was obtained directly from the manufacturer EkoStyle snail farm (Kyiv region, Ukraine). As part of a preliminary collaboration with the producer, comprehensive chemical and microbiological analyses were performed (Khrokalo et al., 2022), and an appropriate preservative system was introduced to ensure its suitability for use as an ingredient in medical-grade cosmetic formulations (Khrokalo, 2023). Silver nitrate ($AgNO_3$, ≥99.8 % purity, analytical grade, CAS No. 7761-88-8) was used as a water-soluble crystalline precursor of silver. Lyophilized mucus was reconstituted in distilled water at 36 °C to prepare 0.1 % aqueous solutions. These were mixed with 10 mM $AgNO_3$ solution in a 1:1 ratio. The reaction mixture was stirred at 37–40 °C for 1.5 hours until a color change occurred, then left at room temperature and under natural light for a total of 48 hours. AgNPs formation was monitored visually by color change and confirmed spectrophotometrically by recording the surface plasmon resonance (SPR) band using Hitachi U-2900 UV–visible spectrophotometer at a spectrum range of 200–800 nm.

Antimicrobial activity was assessed by the agar disk diffusion method on nutrient agar plates (Yadav & Tiwari, 2023). Test cultures included Gram-negative *Escherichia coli* UKM B-906, Gram-positive spore-forming *Bacillus subtilis* UKM B-5006 T, and yeast *Candida albicans* D-6. Before conducting the antimicrobial assay, one-day growth inocula of the test cultures were prepared in meat-peptone broth. Immediately before testing, the inoculum density was adjusted to 0.5 McFarland by dilution with nutrient medium and sterile water. The surface of agar plates in Petri dishes was uniformly inoculated using a sterile swab by the broad-streak method. Pre-sterilized filter paper discs

Ø 5 mm were immersed for several seconds in the AgNPs–mucus solution and then placed on the inoculated agar surface. The plates were incubated at 37 °C, and visual assessment was performed after 1 day and 5 days of incubation, recording the diameters of inhibition zones around the discs.

Formulation development and technological design

Based on literature review (Barel et al, 2009) and patent analysis (Lyshchyshyn, 2001; Ryan, 2017; State Higher Educational Institution, 2019; Tobin & Glaze, 2021) a multifunctional burn-healing cream formulation was developed that integrates the obtained AgNPs–mucus nanosystem as one of an active ingredient. The composition was optimized to combine regenerative, antimicrobial, anti-inflammatory, and analgesic effects through the complex together action of AgNPs, dexpanthenol, allantoin, and lidocaine. A technological scheme for the cream manufacturing process was designed, including sequential stages of aqueous and oil phase preparation, emulsification, homogenization under vacuum, cooling, and addition of active components at low temperature to preserve their stability and activity.

Results and discussion

Synthesis and characterisation of AgNPs-mucus

The enhanced technological design resulted in the formation of a stable nanosystem, as evidenced by the color transition from colorless to dark violet. This visual change corresponds to the emergence of SPR associated with the collective oscillations of metal-core nanoparticles. Monitoring this phenomenon over time was important, as it reflected the stability of the formed system and allowed prediction of its key technological properties in the prospective product. The absorption spectrum exhibited a distinct SPR peak at 450 nm both 12 and 48 hours after synthesis (Fig. 1). The narrow and symmetrical peak profile indicated uniform particle distribution and efficient stabilization within the nanosystem.

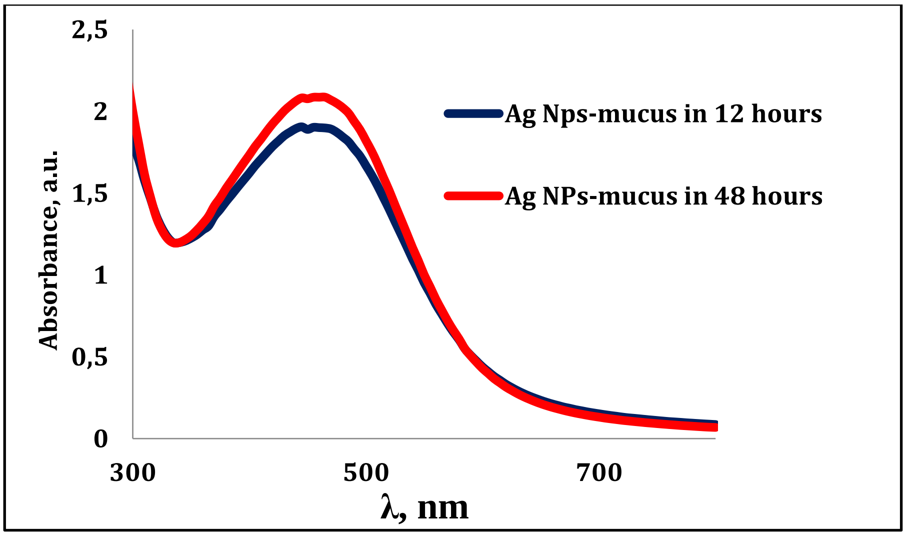

Fig. 1. UV–Vis spectra of AgNPs–mucus at 12 h and 48 h post-synthesis

The evaluation of antimicrobial properties against Gram-positive and Gram-negative bacteria, as well as opportunistic yeasts, represents a crucial biological characteristic of the developed nanosystem. Such assessment is essential, as the nanosystem functions as one of the active components of the after-burn healing cream-gel, contributing to its therapeutic efficacy. Demonstrating broad-spectrum antimicrobial activity confirms not only the biocompatibility and functional stability of the nanosystem but also its potential to prevent microbial contamination and secondary infection in damaged skin tissue, thereby accelerating wound recovery. The Petri dishes were visually inspected after 24 hours and after

5 days of incubation to assess the development of inhibition zones around the discs. The formation of a clear, transparent inhibition zone around the discs was indicative of bactericidal activity, while the appearance of a turbid yet distinct zone denoted bacteriostatic action, reflecting temporary inhibition of microbial growth with subsequent recovery. The results of the antibacterial assay are shown in Figure 2. The growth of the *E. coli* was inhibited by the AgNPs–mucus complex (zones of inhibition being 3 mm), its effect on the gram-positive bacterium *B. subtilis* was via zones of inhibition being 6 mm) and yeast *C. albicans* – 5 mm. Thus, AgNPs–mucus system exhibited the greatest inhibitory effect against the Gram-positive bacterium *B. subtilis* and yeast *C. albicans*, indicating that the nanosystem is also effective against eukaryotic fungal cells, likely through membrane disruption and oxidative stress induction. In contrast, the Gram-negative bacterium *E. coli* showed the lowest sensitivity to the formulation.

A B C

Fig. 2. Antimicrobial effect of AgNPs–mucus against *E. coli* (A), *B. subtilis* (B), and *C. albicans* (C) as evidenced by inhibition zone diameters

Formulation development and technological design

The developed formulation represents a balanced oil-in-water emulsion. The aqueous phase, containing demineralized water, glycerin, and carbomer, provides hydration, viscosity, and structural stability of the cream-gel matrix. The oil phase, composed of vaseline oil, almond oil, and shea butter, ensures emollient and nourishing properties, forming a protective lipid film that prevents transepidermal water loss. The inclusion of dexpanthenol and allantoin contributes to epithelial repair and anti-inflammatory effect. Lidocaine hydrochloride provides local analgesic action, which is crucial in burn therapy. The novel ingredient AgNPs–mucus exhibits multifunctional activity with strong antimicrobial and regenerative potential. Tocopherol serves as an antioxidant stabilizer of both the formulation and biological membranes, while phenoxyethanol and benzyl alcohol maintain microbiological purity and product safety during storage. The full formulation of the developed cream-gel is summarized in Table 1.

The manufacturing process involved sequential preparation of the aqueous and oil phases, their emulsification followed by cooling, and finally incorporation of active ingredients (Fig. 3). At the initial stage, purified demineralized water and disodium EDTA were loaded into reactor No. 1 equipped with a propeller stirrer and a thermostated jacket. Under continuous agitation, carbomer was slowly dispersed, followed by the addition of glycerin and allantoin at 70 °C until a uniform gel-like mass was obtained. Dexpanthenol and lidocaine hydrochloride were introduced last into the aqueous phase, and the mixture was stirred for several additional minutes before heating was switched off. The prepared aqueous phase was then transferred by pump 2 into collector 3 for preliminary cooling to approximately 25 °C.

In parallel, the oil phase was prepared in melting boiler No. 4 by introducing vaseline oil, shea butter, almond oil, and the emulsifiers cetyl alcohol and polysorbate 80. The components were heated to 50 °C, after which the pre-cooled aqueous phase was gradually introduced into the main reactor via pump 5.

Table 1

Composition of the burn-healing cream-gel formulation

No	Component	INCI name	% of mass	Action in product
Phase A (water phase)				
1	Demineralized water	Aqua	59-66 (up to 100)	Main solvent, hydratation
2	Glycerin	Glycerin	4.0-4.5	Humectant, moisture retention
3	Carbomer	Carbomer	0.5-0.8	Thickener, stabilizer
4	Lidocaine	Lidocaine Hydrochloride	1.5-2.0	Local anesthetic
5	Allantoin	Allantoin	0.5-1.0	Soothing, anti-inflammatory
6	Disodium EDTA	Disodium EDTA	0.03-0.05	Chelating agent, improves carbomer stability, enhances preservative efficacy
Phase B (oil phase)				
7	Vaseline oil	Paraffinum Liquidum	5.5-6.0	Emollient, skin protection
8	Almond oil	Prunus Amygdalus Dulcis Oil	5.5-6.0	Nourishing and softening
9	Shea butter	Butyrospermum Parkii Butter	3-3.5	Moisturizing, nourishing
10	Cetyl alcohol	Cetyl alcohol	3.5-4.0	Co-emulsifier and consistency enhancer
11	Polysorbate 80	Polysorbate 80	2.5-3.0	Emulsifier
Phase C (active phase)				
12	Dexpanthenol	Panthenol	3.5-4.5	Regeneration, epithelial repair
13	AgNPs-mucus	Silver (and) Snail Secretion Filtrate	0.5-1.0	Antibacterial, regenerative
14	Vitamin E	Tocopherol	0.2-0.3	Antioxidant
15	Phenoxyethanol	Phenoxyethanol	0.5-0.6	Preservative
16	Benzyl alcohol	Benzyl alcohol	0.2-0.3	Preservative

Upon combination of the two phases, a high-shear homogenizer operating at 3 000 rpm and a vacuum pump 8 were activated to promote emulsification and prevent air entrainment. After complete emulsification, both the vacuum and homogenizer were turned off, and cooling water was circulated through the reactor jacket to reduce the product temperature below 30 °C.

During the final stage of manufacturing (at temperatures below 30 °C), the active components were added sequentially: the antioxidant vitamin E, the AgNPs–mucus nanosystem, and the preservatives phenoxyethanol and benzyl alcohol. To ensure uniform dispersion of the actives throughout the formulation, the homogenizer was restarted at a reduced speed of approximately 500 rpm. After final mixing, the homogenizer and stirrer were stopped, and a representative sample of the cream-gel was sent for quality control. Following a positive evaluation, the finished product was transferred to collection tank 9 and subsequently to the packaging line.

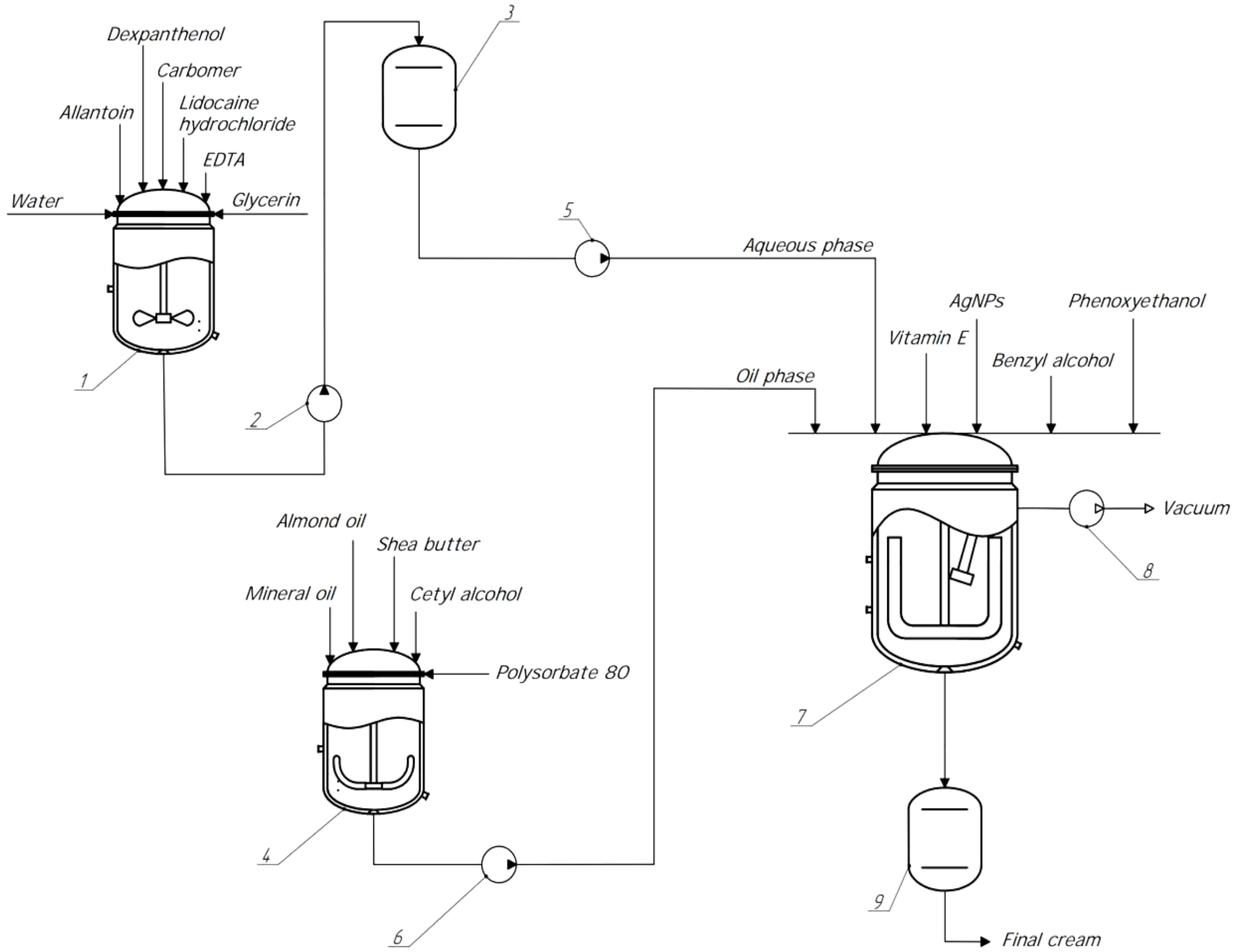

Fig. 3. Technological flow diagram illustrating the manufacturing process of the burn-healing cream-gel

Conclutions

AgNPs-mucus synthesized and stabilized by *C. aspersum* snail mucus was successfully incorporated into a multifunctional burn-healing cream-gel. The nanosystem exhibited stable optical characteristics, confirming the formation of well-dispersed, uniformly sized nanoparticles suitable for topical application. The developed formulation represents a synergistic integration of nanotechnology and natural bioactive ingredients, combining regenerative, antimicrobial, and antioxidant effects essential for effective burn treatment. The novelty of this study lies in the utilization of *C. aspersum* mucus as both a natural reducing and stabilizing agent, ensuring an environmentally friendly and biocompatible synthesis route. The resulting AgNPs–mucus nanosystem merges the biological activity of snail mucus with the broad-spectrum antibacterial efficacy of silver, enhancing the therapeutic potential of the cream-gel matrix. Overall, the optimized composition provides a stable and homogeneous emulsion with moisturizing, protective, and antimicrobial properties that support rapid skin regeneration and protection against secondary infection. The findings highlight the potential of AgNPs–mucus biogenic nanosystems as innovative active components for advanced dermal formulations in burn care and regenerative. The AgNPs–mucus nanosystem exhibited superior antimicrobial activity against Gram-positive bacteria and yeast-like fungi, a finding of particular clinical relevance given that these microbial groups constitute the predominant pathogenic flora colonizing compromised skin barriers, including burn wounds.

Funding / Фінансування
This study did not receive external funding / Це дослідження не отримало зовнішнього фінансування.

Data Availability Statement / Заява про доступність даних
Dataset available on request from the authors / Набір даних доступний за запитом до авторів.

Institutional Review Board Statement / Заява інституційної ревізійної ради
Not applicable / Не застосовується.

Заява про інформовану згоду / Informed Consent Statement
Not applicable / Не застосовується.

Conflict of interest / Конфлікт інтересів
The authors declare no conflict of interest / Автори заявляють про відсутність конфлікту інтересів

Declaration on Generative Artificial Intelligence and AI-enabled Technologies in the Writing Process / Декларація про генеративний штучний інтелект і технології на основі штучного інтелекту в процесі написання
This study did not use generative artificial intelligence or AI-enabled technologies to collect, analyze, or interpret data / У цьому дослідженні не використовувався генеративний штучний інтелект або технології штучного інтелекту для збору, аналізу чи інтерпретації даних.

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Received: 20.11.2025. **Accepted:** 26.12.2025. **Published:** 06.04.2026.

Ви можете цитувати цю статтю так:

Khrokalo L., Volova U., Skiba M. Development of silver-snail mucus nanosystem and its incorporation into a multifunctional burn-healing cream-gel. *Biota. Human. Technology.* 2026. № 1. P. 91-99. DOI: https://doi.org/10.58407/bht.1.26.8

Cite this article in APA style as:

Khrokalo, L., Volova, U., & Skiba, M. (2026). Development of silver-snail mucus nanosystem and its incorporation into a multifunctional burn-healing cream-gel. *Biota. Human. Technology,* (1), 91-99. https://doi.org/10.58407/bht.1.26.8

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