Proceedings of ICEGEE 2026 Symposium: AI-Based Medicine and Biological Data Analysis
DOI: 10.54254/2753-8818/2026.AU32190

Construction of Oral Urate Oxidase Delivery System Based on Sodium Alginate/GelMA Composite System and Its Effect on Lowering Uric Acid

Fengrui Hou[1*], Wenfei Chen[1]

[1]*Beijing University of Chemical Technology, Beijing, China*
**Corresponding Author. Email: 2022090050@buct.edu.cn*

Abstract. Hyperuricemia (HUA) is a metabolic disorder caused by purine metabolism dysfunction. Traditional drugs carry risks of liver and kidney damage. Urate oxidase (UOX) directly degrades uric acid, but existing injectable formulations suffer from poor compliance and immunogenicity. This study designed a sodium alginate/gelatin methacryloyl (GelMA) composite oral delivery system: the sodium alginate shell forms a protective barrier in gastric acid, while the GelMA core immobilizes UOX via UV cross-linking and incorporates aprotinin to resist intestinal protease degradation. The grafting rate of GelMA was characterized by FTIR and NMR, and drug loading, acid resistance, and enzyme resistance were optimized. This study provides a new strategy for the development of safe and efficient oral uric acid-lowering drugs. The simulation digestion experiment shows that this system can maintain enzyme activity up to 68%, release UOX in intestinal fluid at the same time, reduce uric acid level by 94% within 30 minutes, and the effect of reducing uric acid is remarkable.

Keywords: Urate oxidase, Oral delivery, Sodium alginate, GelMA, Enzyme immobilization

1. Introduction

1.1. Research background and significance

Hyperuricemia (serum uric acid concentration > 420 μM) is a metabolic disease caused by purine metabolism disorders or reduced uric acid excretion, leading to complications such as gout, hypertension, nephropathy, and cardiovascular diseases. It has become the fourth major health risk factor following hypertension, hyperglycemia, and hyperlipidemia [1]. In China, approximately 182 million people suffer from hyperuricemia, with a prevalence of 13%, including 17 million gout patients. With dietary changes, the incidence of gout has risen continuously over the past 50 years. By 2018, the global market for gout drugs in the U.S., France, Germany, Italy, Spain, and the U.K. is projected to reach $2.28 billion, with an annual growth rate of 18.2% [2].

Lowering serum uric acid levels is critical for treating hyperuricemia. Current drugs fall into three categories [3]:

(1)Xanthine Oxidase Inhibitors:

© 2026 The Authors. This is an open access article distributed under the terms of the Creative Commons Attribution License 4.0 (https://creativecommons.org/licenses/by/4.0/).

DOI: 10.54254/2753-8818/2026.AU32190

Mainly by inhibiting the activity of xanthine oxidase, the last step of uric acid metabolism in the human body, to reduce uric acid production, so as to reduce the level of uric acid in the body, the more common drug on the market at present is feburestat (2-[(3-cyano-4-isobutoxy) phenyl] -4-methyl-5-thiazolcarboxylic acid) [4].

(2)Uricosuric Agents:

It mainly regulates the expression of uric acid transporter in the kidney to promote the transport of uric acid from the blood to the urine, thus excreting uric acid and reducing the level of uric acid in the body. At present, the more common drug on the market is benbromarone tablet ($C_{17}H_{12}O_3Br_2$) [5].

However, the above two drugs will affect liver and kidney metabolism, causing damage to liver and kidney function [6,7].

(3)Recombinant Urate Oxidase (UOX):

Urate oxidase (UOX) is an enzyme that catalyzes the oxidation of uric acid in the purine metabolic pathway, converting it into allantoin, a water-soluble and inert purine metabolite. Since humans lack endogenous UOX, uric acid cannot be efficiently oxidized and removed, making the introduction of exogenous UOX a viable strategy for reducing blood uric acid levels.

In China, the market for anti-gout drugs has been experiencing rapid growth since 2011. The demand for these drugs has been steadily increasing, with annual sales growth rates in key city hospitals consistently exceeding 30%. According to a report by Hengrui Medicine, anti-gout drug sales surpassed 1 billion yuan in 2016, reflecting a rapidly expanding market size. Currently, benzbromarone, colchicine, allopurinol, and febuxostat dominate the market share [8]. As a novel uric acid-lowering agent, urate oxidase holds significant development potential due to its ability to directly decompose uric acid. However, its injectable formulation is associated with immune reactions and poor patient adherence, which limits its widespread clinical application (Table 1.).

Table 1. Advantages and disadvantages of existing uric acid-lowering drugs

Drug Class	Drug Name	Advantages	Drawbacks
Xanthine Oxidase Inhibitors	ALLO (allopurinol) [6]	It is effective and has good tolerability, economy and safety. Suitable for patients with renal insufficiency.	Gastrointestinal discomfort, rash, renal toxicity. May cause life-threatening allopurinol hypersensitivity syndrome.
	Febuxostat Tablets [9]	High selectivity for xanthine oxidase and dehydrogenase. Liver metabolism, little kidney damage.	Liver damage, nausea, rash. Cardiovascular safety is controversial.
Uricotropic Drugs	Probenecid	Prevent urate crystallization. Oral absorption is rapid.	More times of medication, poor compliance. May cause renal colic and renal function damage. Many drug interactions.
	Benzbromarone [5]	Less damage to the kidney.	A rare but serious risk of liver damage.
Injectable Urate Oxidase	Fasturtec®(Europe)/ Elitek®(America)	Safe and effective. Rapid uric acid reduction. Easy discharge of metabolites. Less kidney damage.	Low stability, short half-life. Immunogenicity (susceptibility to antibody production). Intravenous injection required [10].
Oral Urate Oxidase	Allena's non-absorbent UOX [11]	Improved stability, half-life and bioavailability. Decreased immunogenicity. It can be delivered to the intestine to degrade uric acid.	Need to solve the problem of gastric acid and intestinal protease decomposition of enzymes (not yet fully realized clinical application).

DOI: 10.54254/2753-8818/2026.AU32190

1.2. Research status at home and abroad

At present, the uric acid oxidase drugs available on the market are mainly injectable drugs. The table (Table 2.) lists the advantages and disadvantages of the currently available UOX drugs. The injectable UOX drug Pegloticase is suitable for the treatment of chronic refractory gout, especially in patients who are ineffective or intolerant to traditional urico-lowering drugs such as allopurinol and febuxoat. Through the oxidation of uric acid to allantoin, the serum uric acid level is rapidly reduced and the tophite is dissolved. In clinical trials, about 40% of patients achieved and maintained target uric acid levels (<6 mg/dL) [12]. However, the drug has a large immunogenicity problem, some patients will produce anti-drug antibodies, resulting in drug efficacy decline or failure. May cause allergic reactions or infusion-related adverse reactions (such as headache, nausea, difficulty breathing, etc.). Due to immunogenicity, some patients cannot maintain efficacy in long-term treatment. In addition, the high cost of treatment limits its wide application [13].

The injectable UOX drug Rasburicase is used to prevent and treat hyperuricemia caused by Tumor Lysis Syndrome (TLS). In patients with TLS, labritase rapidly reduces uric acid levels and prevents acute kidney injury, and the drug acts quickly, usually significantly reducing uric acid levels within 4 hours of administration. But there are also problems with allergic reactions, including anaphylactic shock. There is also a risk of hemolysis, which can induce hemolytic anemia in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency [14]. Labritase is mainly used for acute hyperuricemia and is not suitable for chronic gout or long-term treatment, which is expensive to treat, especially if multiple doses are required [15].

Allena's non-absorbable UOX (ALLN-346) is an orally available urico-lowering drug, and ALLN-346 is a recombinant urico-oxidase (UOX) that has been engineered to be stable and not absorbed in the gastrointestinal tract [11]. The drug acts locally in the intestine and catalyzes the breakdown of uric acid into allantoin, thereby reducing serum uric acid levels. The immunogenicity and infusion reactions of injectable UOX were avoided by oral administration. ALLN-346 is currently in preclinical development and may enter clinical trials in the future where its safety and efficacy need to be further verified, providing a new treatment option for patients with hyperuricemia and gout.

In recent years, oral delivery system made some progress in the field of protein drugs. For example, liposome based carriers can protect drugs from gastric acid destruction, but suffer from low encapsulation rates and poor intestinal stability [16]. Chitosan/pectin composite microspheres release drugs through pH response, but their mechanical strength is poor and difficult to resist intestinal motility [15]. In addition, Allena's non-absorbable UOX (ALLN-346), although genetically engineered to enhance enzyme stability, still relies on complex protein modification technology and is costly6 [11]. These studies indicate that the development of oral delivery systems with high acid and enzymolysis resistance, low cost and simple process is still a difficulty in current research.

DOI: 10.54254/2753-8818/2026.AU32190

Table 2. Advantages and disadvantages of commercially available urate oxidase drugs

Drugs/ Technology	Method of Drug Delivery	Indication	Advantages	Defects/Limitations
Puricase [12]	Intravenous Injection	Refractory gout	Quickly reduce uric acid and dissolve tophite	High immunogenicity, infusion reaction, high rate of treatment failure and high cost.
Rasburicase [14]	Intravenous Injection	Tumor lysis syndrome (TLS)	Quickly reduce uric acid and prevent acute kidney injury	Allergic reaction, hemolysis risk, short-term use only, high cost.
ALLN-346 [11]	Orally Delivery	Chronic hyperuricemia, gout (potential indications)	Avoid injection side effects, improve compliance, intestinal local effects.	It is still in the preclinical stage and needs to be further verified for safety and efficacy.

1.3. The research goal and the innovation point

In this study, sodium alginate /GelMA gelatin composite system was constructed, combined with aprotinin to enhance the anti-enzymatic hydrolysis ability, so that uric acid oxidase can resist the erosion of gastric and intestinal fluids respectively, and degrade uric acid in the intestine in a long-term manner. Specific goals are as follows:

a. Natural sodium alginate (anionic polymer) is used as an effective antacid shell to prevent the release of content protein in gastric juice and the damage of protein activity under acidic conditions.

b. The use of double bond crosslinking of drug-loading GelMA skeleton, to firmly fixed uric acid oxidase, prevent sudden release and less contact with intestinal juice.

c. Use add aprotinin enhanced uric acid enzyme digestion resistance, further prolonged uric acid enzyme action.

Innovation point of this experiment is to improve GelMA immobilized enzyme utilization, GelMA gelatin system into the gut, wrapped in uric acid oxidase uric acid oxidase will be fixed within the system and will not release it, this makes the uric acid in the gut can continuously and uric acid oxidase reaction, increase the efficiency of uric acid oxidase. In addition, this study fills a gap in the research on oral UOX delivery. At present, there are few drugs using urate oxidase to treat hyperuricemia at home and abroad, only a few types of injectable drugs developed abroad, and there are few studies on oral delivery system of urate oxidase.

1. Compared with the existing research, this system's innovation is embodied in:

2. (1) Double protection mechanisms: sodium alginate and GelMA synergy, respectively against hydrochloric acid in gastric juice and intestinal protease, without relying on genetically engineered;

3. (2) Immobilization and synergism: UOX is fixed in GelMA network by purple linkage to avoid sudden release and prolong enzyme action time.

4. (3) Low-cost natural materials: Sodium alginate and gelatin are FDA-approved natural polymers, high safety and easy to scale production. The above design solves the key problems such as gastric acid inactivation, high enzymatic hydrolysis rate and complicated process in oral UOX delivery.

2. Materials and methods

2.1. Materials

Reagents: Sodium alginate (Sigma-Aldrich, A2033), gelatin (Gelita, Type A), methacrylic anhydride (Aladdin), UOX (Yuanye Bio, ≥100 U/mg), aprotinin (Beyotime, ≥5,000 U/mg).

Equipment: UV cross-linker (365 nm, 10 mW/cm²), high-speed homogenizer (IKA T25), HPLC (Agilent 1260).

DOI: 10.54254/2753-8818/2026.AU32190

This complex system is composed of a sodium alginate /Ca^{2+} acid-resistant shell and a uric acid oxidase and enzyme inhibitor protein core fixed in it by GelMA. (Figure 1a.)

2.2. Synthesis of GelMA

5g gelatin was dissolved in carbonate buffer at 50℃ (0.1M, pH 9.0), 1 mL of methacrylic anhydride was added drop by drop (molar ratio of gelatin: methacrylic anhydride =1:0.8), followed by nitrogen protection for 3 h, and then purified by dialysis bag with molecular weight of 8-10 kDa for 72 h (change of deionized water every 4 h). Freeze-dried GelMA is obtained (Figure 1c-1d.).

2.3. Preparation of GelMA@ (uricase & aprotinin) microspheres

The mixed oil phase (20mL mineral oil/liquid paraffin, 4mL Spain 80, 1ml Tween 80) was first prepared and stirred to form a clear and transparent light yellow liquid; After that, aqueous solution was added (3mLPBS buffer containing 0.3gGelMA, 15mgLAP, 3mg uricase, 3mg aprotin (over film sterilization)), and then the rotational speed was increased (50% of the maximum rotational speed of magnetic stirring), and a high-speed homogenizer was used to stir at 10,000 rpm for 30 min during the emulsification stage. A relatively stable white emulsion was formed after emulsification for about 30 minutes, and then cured by ultraviolet irradiation. The purple bonding condition was irradiated for 30 minutes at a wavelength of 365 nm and an intensity of 10 mW/cm². After 30 minutes of irradiation, 10mL cyclopentane was added to the emulsion system, and the microcapsules were continuously stirred for 15 minutes to destroy the emulsion system and precipitate and separate. After centrifugation at 8000 RPM for 6 minutes, wash with isopropyl alcohol 2-3 times, collect the microspheres, freeze dry (14-20 hours) or dry under 30 degree vacuum (about 24 hours). Observation under the electron microscope shows that the surface of the microspheres presents a porous structure, and the surface of the microspheres is denser, which is conducive to protecting the internal drugs. (Figure 1b.)

(3) Preparation of sodium alginate /Ca^{2+} gel microspheres: Prepare a 1% (w/v) solution of sodium alginate and a 2% (w/v) solution of calcium chloride. The gel microspheres can be prepared by slowly adding sodium alginate solution to calcium chloride solution with a syringe. After pumping, filtering and collecting the microspheres, freeze-drying can be done.

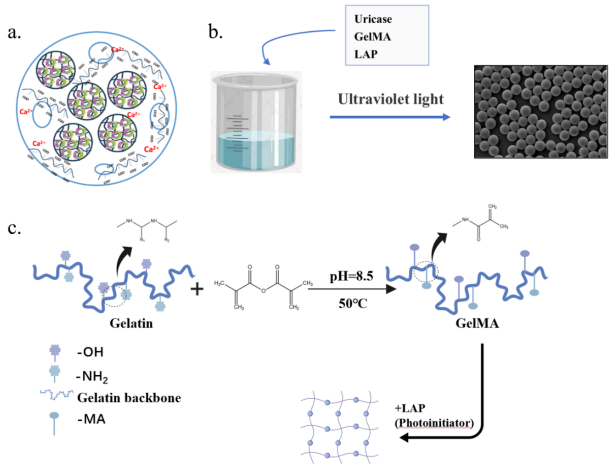

Figure 1. The construction of the oral delivery system

DOI: 10.54254/2753-8818/2026.AU32190

a. Schematic Diagram of Composite Gel System.(Acid-resistant casing: Sodium alginate/Ca^{2+}, Internal drugs: Uric acid oxidase + aprotinin fixed by GelMA.); b Preparation of Microspheres by Emulsion Method.; c. Composite diagram of GelMA.

2.4. Performance test analysis

2.4.1. Anti-gastric acid test

The anti-enzymolysis effect was detected in simulated intestinal fluid (containing trypsin).

2.4.2. Anti-enzymatic hydrolysis test

Take 5 mL pH 8.5(Tris-HC1) 0.3mM UA solution (containing 0.3 mg/mL trypsin), add commercial uricase solution or crosslinked gelatin microspheres into it (as Table 3. shown), incubate at 37 °C for 10min, take 400 uL, dilute with 400 uL deionized water, and add 40 uL 6 M HC1 to terminate the reaction. Test the absorbance at 293 nm.

Table 3. Anti-enzymolysis test experimental group and control group settings

	Commercially Available Uricase Solution (0.025 mg/mL) Additive amount /mL	Crosslinked gelatin microspheres/mg
Control Group 1	0.5	0
Control Group 2	0.5	0
Control Group 3	0.5	0
Inactivated Group 1	0.5 Boiling water treat for 10min	0
Experimental Group 1	0	20(Put in 0.5 mL tris buffer before adding to the test tube)
Experimental Group 2	0	20(Put in 0.5 mL tris buffer before adding to the test tube)
Experimental Group 3	0	20(Put in 0.5 mL tris buffer before adding to the test tube)
Inactivated Group 2		20(Put in 0.5 mLtris buffer. Boiling water treat for 10min)

2.4.3. Enzyme activity release test (protein fixation test in simulated intestinal fluid)

Gelatin microspheres were incubated in pH 6.8 solution, 1mL was extracted at regular intervals, centrifuged, and the supernatant was taken to measure the enzyme activity and observe whether the enzyme activity was improved. Parallel groups 1,2,3 with the same configuration were set up. 20mg gelatin loaded microspheres were added into the buffer solution of 10 mL pH 6.8, incubated in a shaking table at 37℃, and 1mL were removed at 30 min\60 min\120 min\180 min respectively, and centrifuged (10000 rpm,5 min). The supernatant of 80uL was placed in the enzyme reaction system of 1mL of Tris-HCl buffer (pH 8.5), and the reaction was accurately reacted at 37 °C for 5min, and then the reaction was terminated with 5M HCl of 50uL. The supernatant of 200uL was centrifugated (10000 rpm, 5 min) and the change of uric acid concentration was detected with a 96-well plate. The control group was pH6.8 buffer (80uL) without gelatin microspheres. The enzyme activity content in the supernatant was detected by the difference between the control group and the parallel group.

2.4.4. Uric acid degradation efficiency test

The change of uric acid concentration over time was detected by simulated intestinal environment in vitro (HPLC). 10 mg GelMA microspheres containing uricase were placed in the enzyme reaction system of 1 mL Tris-HCl buffer (pH 8.5) and 0.15mM Tris-HCl buffer (pH 8.5) containing uric acid, and the reaction was carried out at 37 degrees Celsius. Remove 1mL at

DOI: 10.54254/2753-8818/2026.AU32190

30min\60min\120min\180min, then terminate the reaction with 5M HCl, centrifuge (10000rpm, 5min) and take 200uL of superserum to detect the change of uric acid concentration with 96-well plate.

2.4.5. GelMA performance characterization- in vitro swelling test

The swelling performance is evaluated by weighing: the GelMA gel sample is immersed in a PBS solution and its weight change at different time points is measured.

2.4.6. GelMA performance characterization - in vitro degradation test

Trypsin was used to accelerate the degradation of GelMA in vitro. Samples containing 10%, 20% and 30% GelMA were immersed in PBS solution containing trypsin, and the weight changes of the samples were measured at different time points.

3. Formatting your paper

3.1. Observation by SEM electron microscope

We observed the microspheres with a SEM electron microscope, and their surfaces presented a porous structure. Moreover, the surface of the microspheres is dense, indicating that a high concentration of microspheres can protect the drugs very well.

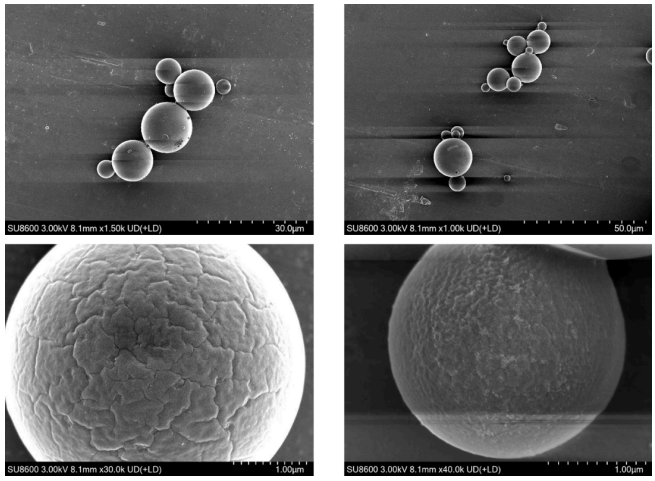

Figure 2. SEM image of GelMA-aprotinin microspheres prepared by emulsification method

3.2. Material characterization

(1) GelMA Performance Characterization - Swelling Test

The quality of 10%, 20% and 30% GelMA gel showed a slight decline and basically maintained a stable state (Figure 2a.), indicating that the swelling rate of the material was low and the material stability was good.

(2) GelMA Performance Characterization - in Vitro Degradation Test

Trypsin was used to accelerate the degradation of GelMA in vitro, and the experimental results showed that GelMA containing 10% had a greater decline in quality and poor enzymatic hydrolysis, while GelMA containing 20% and 30% had almost no change in quality and strong enzymatic hydrolysis resistance (Figure 2b.). So follow-up experiments were done with 20% or 30% gel.

DOI: 10.54254/2753-8818/2026.AU32190

3.3. Delivery system performance: in vitro release effect characterization

3.3.1. Enzyme release test of GelMA microsphere system

In the enzyme activity release test, after gelatin microspheres were incubated in the simulated intestinal fluid at pH 6.8, the activity release rate of urate oxidase (UOX) measured in the supernatant exceeded 100% (Figure 2c.) (for example, the release rate was as high as 250% at 180 min). Theoretically, the enzyme activity release rate should not exceed the theoretical drug load of the microspheres (i.e. 100%), so the result may reflect experimental errors or methodological flaws.

3.3.2. Characterization of uric acid lowering effect of GelMA microspheres

In order to verify the uric acid lowering effect of microspheres, 10mg microspheres were taken in artificial intestinal fluid for uric acid lowering test. First, the uric acid concentration was tested in samples taken at 30min\60min\120min\180min respectively, and it was found that the uric acid concentration at 30min had significantly decreased compared with the initial uric acid concentration (Figure 2d.). Moreover, by comparing the 60min data, it can be seen that the uric acid concentration basically no longer changes after 30min. Therefore, the experiment further shortened the sampling time and tested the samples within 0-30min.

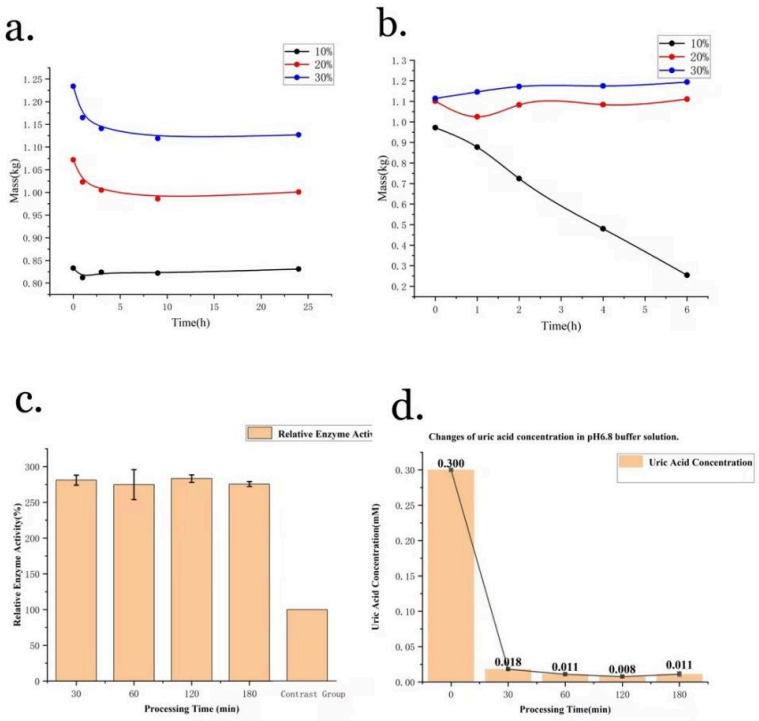

Figure 3. Material characterization delivery system performance

a. GelMA Swelling Test Diagram.
b. Degradation curve of GelMA in vitro.
X-axis: Time (h). Y-axis: GelMA mass.
Group: 10%GelMA microspheres, 20% GelMA microspheres, 30%GelMA microspheres.
c. Enzyme activity release test in pH6.8 solution.

DOI: 10.54254/2753-8818/2026.AU32190

Enzyme activity release rate = E-release(%)= (A-sample−A-control) /A-total × 100%

Where: A-sample: changes in uric acid concentration in the reaction system of the experimental group (calculated by standard curve);

A-control: Uric acid concentration in blank control group (without microspheres).

A-total: Theoretical drug-carrying activity of microspheres (quantification of total enzyme activity by completely dissolving microspheres).

d. Characterization of uric acid lowering effect of 30min\60min\ 120min\180min microspheres.

(3) Anti-Enzymolysis Performance Test of GelMA Microspheres

It can be known from the experimental data that the relative enzymatic activity of gelatin microspheres in trypsin solution is 0.68 (Table 4.), indicating that the microspheres have a good anti-enzymatic hydrolysis effect and retain most of the enzymatic activities.

Table 4. Relative enzyme activity in trypsin solution between control group (commercially available urate oxidase) and experimental group (preparation of gelatin microspheres)

Handling Time(min)	Group	Absorbancy 1	Absorbancy 2	Absorbancy 3	Mean Absorbance	Absorbance Difference	Mean Absorbance Difference	Relative Enzyme Activity
10	Control Group 1	0.135	0.143	0.137	0.138333	0.711		
10	Control Group 2	0.196	0.202	0.191	0.196333	0.653	0.643333	1
10	Control Group 3	0.281	0.285	0.284	0.283333	0.566		
10	Inactivation Group 1	0.847	0.851	0.85	0.849333	0	—	
10	Experimental Group 1	0.401	0.397	0.388	0.395333	0.485		
10	Experimental Group 2	0.52	0.501	0.518	0.513	0.367333	0.434111	0.674784
10	Experimental Group 3	0.451	0.419	0.421	0.430333	0.45		
10	Inactivation Group 2	0.913	0.865	0.863	0.880333	0	—	

4. Discussion

4.1. Advantages of dual protection mechanism

The sodium alginate /GelMA complex system designed in this study achieved efficient oral delivery of urate oxidase (UOX) through a dual protective mechanism. First, the sodium alginate shell formed a dense hydrogel structure through protonation in the gastric acid environment (pH 1.5-3.5), which effectively blocked the destruction of UOX by gastric acid and avoided the premature loss of enzyme activity. After entering the intestine, sodium alginate dissolves in an alkaline environment (pH 6.8-7.4), releasing a UOX kernel fixed by GelMA [17,18] GelMA stably envelops UOX through a three-dimensional network structure formed by optical crosslinking to achieve slow releasing effect, and reduces contact with intestinal protease through steric hindrance [19,20].

The experimental results showed that GelMA microspheres showed significantly better enzyme activity retention rate than commercially available uricase in trypsin solution (relative enzyme activity was 67.5% in the experimental group and 100% inactivation in the control group). This phenomenon can be attributed to the following synergistic mechanisms:

(1) Competitive inhibition of aprotinin:

Aprotinin directly reduces its catalytic efficiency by binding to the active site of trypsin. In this study, aprotinin and UOX were co-loaded on GelMA microspheres, which could form a high

DOI: 10.54254/2753-8818/2026.AU32190

concentration inhibitory environment locally and reduce the degradation of UOX by protease (Figure 3.).

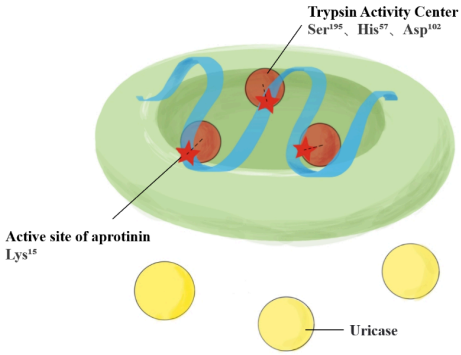

Figure 4. Schematic diagram of competitive inhibition of aprotinin

(2)GelMA immobilization enhances enzyme stability:

The three-dimensional network structure formed by GelMA through purple linkage not only limits the sudden release of UOX, but also reduces the contact probability between trypsin and UOX through steric hindrance effect. Studies have shown that GelMA carriers with high cross-linking density (such as 20%-30% cross-linking degree in this study) can significantly delay the enzymolysis rate through physical barrier action [20].

(3)Enzyme immobilization and active site protection:

Covalent immobilization of UOX may partially mask sites on its surface that are easily recognized by proteases. For example, the methacrylic acid group of GelMA forms a covalent bond with the amino group of UOX through the Michael addition reaction, which may alter the surface charge distribution of the enzyme, thereby reducing its sensitivity to be recognized by the protease [21].

However, this study did not independently evaluate the protective effect of aprotin-free GelMA microspheres on UOX, so it is not possible to fully distinguish the independent contribution of aprotinin inhibition from GelMA immobilization. In the future, the synergistic effect of the two can be further quantified by controlled experiments, such as the preparation of microspheres containing only GelMA-UOX. In addition, the crosslinking density of GelMA may affect the enzymolysis rate, and the relationship between the crosslinking degree (such as 10%, 20%, 30%) and the enzyme activity retention rate needs to be systematically studied to optimize the carrier design.

4.2. Comparison with existing studies

Compared with traditional uric acid lowering drugs (such as allopurinol), the system can degrade uric acid by enzyme, avoid the interference of xanthine oxidase inhibitors on liver and kidney metabolic pathways, and thus avoid the risk of renal toxicity. Compared with the genetically engineered UOX oral drug developed by Allena (which enhances anti-enzymolysis through protein modification), this system uses a natural polymer carrier design, which significantly reduces the development cost while ensuring acid-resistant and anti-enzymolysis properties. Oral delivery avoids the immunogenicity problems associated with repeated injections, and patient compliance is significantly improved (oral administration can be reduced to 1-2 times per week) compared to injectable forms of UOX drugs such as Pushcaril. This advantage is particularly important in the

DOI: 10.54254/2753-8818/2026.AU32190

long-term management of hyperuricemia, especially in patients with gout who require lifelong medication.

5. Results and prospects

The sodium alginate /GelMA composite oral delivery system constructed in this study showed significant advantages in acid-resistant and enzymolysis resistance, enzyme stability and safety. Compared to prior art:

1. Compared with the traditional injectable urate oxidase (such as Pushikari, Labritase) :

(1) This system avoids the immunogenicity problems of injections and the inconvenience of frequent administration through oral delivery, and the patient compliance is significantly improved.

(2) The dual protective mechanism of sodium alginate shell and GelMA immobilization technology made the activity retention rate of UOX in the environment of gastric acid and intestinal protease (67.5%) significantly better than that of commercially available enzyme solution (complete inactivation), and there was no need to rely on costly genetic engineering modification.

2. Compared with other oral delivery technologies (e.g. liposomes, chitosan microspheres) :

(1) This system uses natural polymer materials (sodium alginate, gelatin), which has both FDA approved safety and the feasibility of large-scale production, and solves the problems such as low encapsulation rate (liposome) and insufficient mechanical strength (chitosan/pectin microspheres) of existing carriers.

(2) GelMA's purple immobilization technology achieved slow release and long-term effects of UOX, avoiding the sudden release effect of traditional carriers, and further prolonged enzyme activity by synergizing with aprotinin to inhibit protease degradation.

(3) Compared with oral drugs under development (such as ALLN-346), this system enhances enzyme stability through physical immobilization rather than protein modification, reduces development cost and production complexity, and avoids the possible intestinal local action limitations of non-absorbent UOX.

(4) The oral delivery system of urate oxidase established in this study has significant uric acid lowering effect, which is much higher than that of other oral drugs. For example, the Military Medical Research Institute and other teams used the food-grade probiotic Lactococcus lactis lactate NZ9000 to express the urate oxidase derived from aflatoxus, and the efficiency of inhibiting the rise of uric acid in mice after oral administration was only half of that of this study system [22]. The renal safety of engineered bacteria needs to be further verified.

In this study, sodium alginate /GelMA composite oral delivery system was successfully constructed, which has significant anti-acid and anti-enzymatic properties, high efficiency and safety, and can effectively deliver UOX to the intestine and degrade uric acid, providing a new strategy for the treatment of hyperuricemia.

Although in vitro experiments have verified the anti-acid and anti-enzymatic properties of the composite system, its in vivo metabolism and long-term safety need to be further evaluated by animal experiments. For example, the biological distribution of sodium alginate shell after dissolution in the intestine, the match between the degradation rate of GelMA and the release kinetics of UOX need further study.

Future research could extend the system to oral delivery of other protein drugs (such as insulin or antibody drugs). By adjusting the crosslinking density of GelMA or introducing targeted modifications, site-specific release of the drug is expected to be achieved. In addition, the development of multistage response vectors (such as enzyme response or REDOX response) can

DOI: 10.54254/2753-8818/2026.AU32190

further improve delivery efficiency and provide a better solution for the treatment of metabolic diseases.

References

[1] DEHLIN M, JACOBSSON L, RODDY E. Global epidemiology of gout: prevalence, incidence, treatment patterns and risk factors [J]. Nat Rev Rheumatol, 2020, 16(7): 380-90.

[2] LIU R, HAN C, WU D, et al.Prevalence of hyperuricemia and gout in mainland China from 2000 to 2014: a systematic review and meta-analysis [J].Biomed Res Int, 2015, 2015: 1-12.

[3] LIN P, CHEN Z, LIN J. Therapeutic drugs of gout: The progress in target selection [J]. International Journal of Rheumatic Diseases, 2024, 27(1): e15022.

[4] HUANG X, DU H, GU J, et al. An allopurinol-controlled, multicenter, randomized, double-blind, parallel between-group, comparative study of febuxostat in Chinese patients with gout and hyperuricemia [J]. International Journal of Rheumatic Diseases, 2014, 17(6): 679-86.

[5] STAMP L K, HASLETT J, FRAMPTON C, et al. The safety and efficacy of benzbromarone in gout in Aotearoa New Zealand [J]. Internal Medicine Journal, 2016, 46(9): 1075-80.

[6] WRIGHT D F B, DUFFULL S B, MERRIMAN T R, et al. Predicting allopurinol response in patients with gout [J]. British Journal of Clinical Pharmacology, 2016, 81(2): 277-89.

[7] RICHETTE P, BRIèRE C, HOENEN-CLAVERT V, et al. Rasburicase for tophaceous gout not treatable with allopurinol: an exploratory study [J]. J Rheumatol, 2007, 34(10): 2093-8.

[8] LV W, CHEN H, ZHOU P, et al. Mechanisms Associated With Renal Injury in Hyperuricemia and Strategies for the Development of Natural Active Substances [J]. International Journal of Rheumatic Diseases, 2025, 28(2): e70096.

[9] XU S, LIU X, MING J, et al. A phase 3, multicenter, randomized, allopurinol-controlled study assessing the safety and efficacy of oral febuxostat in Chinese gout patients with hyperuricemia [J]. International Journal of Rheumatic Diseases, 2015, 18(6): 669-78.

[10] BOSLY A, SONET A, PINKERTON C R, et al. Rasburicase (recombinant urate oxidase) for the management of hyperuricemia in patients with cancer [J]. Cancer, 2003, 98(5): 1048-54.

[11] PIERZYNOWSKA K, DESHPANDE A, MOSIICHUK N, et al. Oral Treatment With an Engineered Uricase, ALLN-346, Reduces Hyperuricemia, and Uricosuria in Urate Oxidase-Deficient Mice [J]. Front Med (Lausanne), 2020, 7: 569215.

[12] SUNDY J S, BARAF H S B, YOOD R A, et al. Efficacy and Tolerability of Pegloticase for the Treatment of Chronic Gout in Patients Refractory to Conventional Treatment Two Randomized Controlled Trials [J]. Jama-Journal of the American Medical Association, 2011, 306(7): 711-20.

[13] GEORGE R L, SUNDY J S. PEGLOTICASE FOR TREATING REFRACTORY CHRONIC GOUT [J]. Drugs of Today, 2012, 48(7): 441-9.

[14] SHIN H Y, KANG H J, PARK E S, et al. Recombinant urate oxidase (Rasburicase) for the treatment of hyperuricemia in pediatric patients with hematologic malignancies: Results of a compassionate prospective multicenter study in Korea [J]. Pediatric Blood & Cancer, 2006, 46(4): 439-45.

[15] LI C, HEIN S, WANG K. Chitosan-Carrageenan Polyelectrolyte Complex for the Delivery of Protein Drugs [J]. International Scholarly Research Notices, 2013, 2013(1): 629807.

[16] XIE C, LUO Q, ZHANG Y, et al. Micro/Nanomotors for Oral Delivery of Drugs: From Design to Application [J]. Advanced NanoBiomed Research, 2024, 4(2): 2300057.

[17] SUN X, LIU C, OMER A M, et al. Dual-layered pH-sensitive alginate/chitosan/kappa-carrageenan microbeads for colon-targeted release of 5-fluorouracil [J]. Int J Biol Macromol, 2019, 132: 487-94.

[18] SINGH J, NAYAK P. pH-responsive polymers for drug delivery: Trends and opportunities [J]. Journal of Polymer Science, 2023, 61(22): 2828-50.

[19] KAMATA H, LI X, CHUNG U-I, et al. Design of Hydrogels for Biomedical Applications [J]. Advanced Healthcare Materials, 2015, 4(16): 2360-74.

[20] ABASALIZADEH F, MOGHADDAM S V, ALIZADEH E, et al. Alginate-based hydrogels as drug delivery vehicles in cancer treatment and their applications in wound dressing and 3D bioprinting [J]. J Biol Eng, 2020, 14: 8.

[21] ESIMBEKOVA E N, TORGASHINA I G, NEMTSEVA E V, et al. Enzymes Immobilized into Starch- and Gelatin-Based Hydrogels: Properties and Application in Inhibition Assay [J]. Micromachines (Basel), 2023, 14(12).

[22] CHENG H, XIONG GQ, CUI JZ, et al. Oral administration of Lactococcus lactis expressing uric acid oxidase regulates the blood uric acid level in mice [J]. Journal of Biotechnology, 2024, 40(11): 4111-9 (in Chinese).