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Research paper

A new method for the preparation of pure acamprosate calcium with a micron particle size

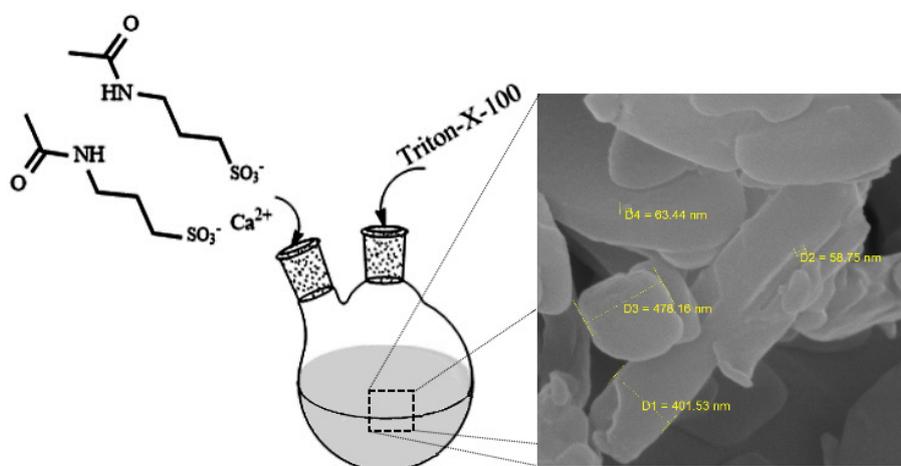
Fatemeh Mobayyen Jarihani, Farajollah Mohanazadeh*

Department of Chemical Technologies, Iranian Research Organization for Science and Technology (IROST), Tehran, Iran

HIGHLIGHTS

- A micron-sized acamprosate calcium sample was synthesized by using Triton-X-100.
- The product has an acceptable purity and is in accordance with international U.S. pharmacopeia.
- The kinetic solubility of acamprosate calcium was improved by size reduction.

GRAPHICAL ABSTRACT



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ABSTRACT

Considering that preparing microparticle drugs enhances their solubility and bioactivity, it is the pharmaceuticals' interest to have more information about the drug's particle size; hence, it is essential to study a drug's particle size and morphology. So far, no work has been done on the particle size of acamprosate calcium. In this work, micronized acamprosate calcium was first prepared, then its water solubility was investigated. To this aim, acamprosate calcium was synthesized from 1,3-propane sultone through two-step reactions. The resulting powder was then micronized using Triton-X-100 using the *in situ* micronization method. The resulting micronized particles were found to be highly pure (99.7 %). The micronized acamprosate calcium's particle size was less than 10 μm . Kinetic solubility studies showed that micronized acamprosate calcium's water solubility had improved compared to bulk particles of acamprosate calcium.

* Corresponding author: Tel.: +9821-56276637 ; Fax: +9821-56276265 ; E-mail address: mohannazadeh@iroست.ir

1. Introduction

Generally, synthesized drugs are bigger than the biological cells, and many are classed Biopharmaceutical Classification System (BCS) class II or IV, which are poor water-soluble compounds [1]. In order to have biological activity, a drug should be less than 5 μm . As a drug becomes more soluble, the medicine particles become smaller, and the drug becomes more active [2,3]. According to the BCS, dissolution is a rate-limiting factor for the drug absorption rate of both class II and IV compounds, resulting in poor bioavailability [4]. Moreover, according to Noyes-Whitney's theories, administering a micron size drug is a primary method to improve the bioavailability of poorly water-soluble drug substances [5].

Various strategies improve the solubility of poorly water-soluble drugs [6-10]. Amongst these methods, increasing surface area by micronization is an effective strategy to overcome these problems [8,11-13]. Micronization is a term used to describe a size reduction technique where the resulting particle size distribution is less than 10 μm . Since the morphology of particles, particle size, and size distribution produced in different industries are usually not appropriate for the subsequent use of such materials, particle design has been gaining importance in manufacturing advanced pharmaceutical compounds [14-16]. Although reducing the particle size has many advantages, such as increased solubility and biological activity, a significant reduction in the particle size also brings disadvantages. For example, nano-size drugs easily pass through the brain membrane and can cause side effects in the brain, or the excessive concentration of nanoparticles in the formulation of tablets can be problematic [17-19]. Also, if the particles are very small, it becomes difficult to formulate tablets using the direct method (C.D. Compaction) due to a decrease in the drop (Flow free).

A common technique for producing micronized drugs is mechanical methods with the milling of larger particles [20,21]. Despite the widespread use of this method, the milling process is not an ideal way to produce fine particles because the properties of the drug and its surface properties change in a mainly uncontrolled manner [14,22]. In addition to milling, other approaches like spray drying and supercritical fluid (SCF) require specialized containment facilities. Although these techniques can produce micronized

particles, they are non-homogenous in particle size distribution when solved in water [23-27]. Hence, a novel technique, called *in situ* micronization, has been developed to overcome the limitations associated with other procedures and produce drugs with homogenous particle size distribution [28,29]. In contrast to other techniques where external processing conditions like mechanical force, temperature, and pressure are required, *in situ* micronization does not require arduous conditions [30-33]. The drug can obtain micron size during a one-step crystal formation without needing further particle size reduction. In this approach, a hydrophilic polymer is used to reduce surface tension. The other factors include changing the type of stirrer [34,35], stirring speed [36], and applying the appropriate temperature [37] during the purification and crystallization of the material [38,39].

Acamprosate calcium, with the chemical name calcium 3- acetamidopropane -1- sulfonate, is a white, odorless powder with the chemical structure $\text{C}_{10}\text{H}_{20}\text{CaN}_2\text{O}_8\text{S}_2$ and a molecular weight of $400.48 \text{ g}\cdot\text{mol}^{-1}$ (Fig. 1). The solubility of acamprosate calcium in water has been reported as 5 and $1.97 \text{ mg}\cdot\text{ml}^{-1}$ in different sources [40,41].

Acamprosate has a chemical structure similar to the amino acids neurotransmitter γ -aminobutyric acid (GABA) and neuromodulator taurine (Fig. 2) [42].

Acamprosate calcium is used in the treatment of alcohol addiction. This drug restores the balance of brain chemicals disturbed by alcohol. Acamprosate calcium normalizes the dysregulation of glutamatergic neurotransmission during chronic alcohol consumption

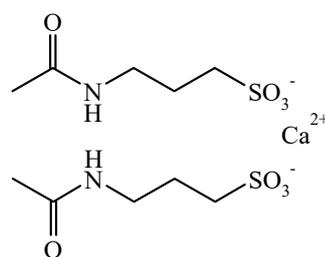


Fig. 1. The chemical structure of acamprosate calcium.

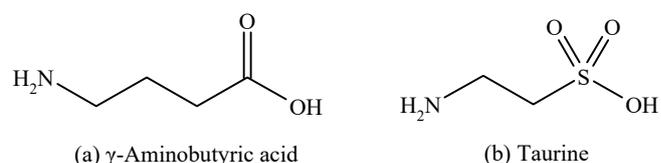


Fig. 2. The chemical structure of amino acids (a) γ -aminobutyric acid and (b) taurine.

and withdrawal [43]. Acamprosate calcium has been used in Europe since 1989 and was approved by the FDA in 2004 [44].

Since acamprosate calcium is poorly soluble in water, the preparation of micronized acamprosate calcium improves its water solubility and may increase the bioavailability of this drug. However, a review of scientific sources shows that no research has been done on the micronization of this compound. In this research, micronized acamprosate calcium was prepared using the *in situ* micronization method. The straightforward procedure and accessibility of the materials make it a preferred industrial method. In this method, the purification and micronization of the sample were done during one process using the precipitation method with anti-solvent. Water was used as a solvent, and acetone as an anti-solvent; both are cheap and commercially available. Non-ionic surfactants, which are commercially available and can be recovered, were used as surface activators. The two other main factors for sample micronization are temperature (the sample dissolution temperature was 60 °C, and the sedimentation temperature was in the room temperature range) and the mixing speed (600 rpm), which are within the acceptable range.

2. Materials and methods

The 1,3-propane sultone was purchased from ALFA AESAR. Ammonia (28% solution), Tween 80, Tween 40, Triton-X-100, PEG 600, PEG 300, PEG 200, sodium hydroxide, acetone, and ethanol were purchased from Sigma-Aldrich. All compounds were used without further purification. The morphology of samples was observed using a Scanning Electron Microscope (JSM-6390 LA SEM, Jeol Co., Tokyo, Japan). The distribution of micronized particles in suspensions was investigated using a Fritsch particle analyzer model ANALYSETTE 22 NeXT Nano.

2.1. Synthesis of 3-aminopropane-1-sulfonic acid

First, a stirred solution of 1,3-propane sultone (6.1 g, 0.5 mmol) in acetone (60 ml) was prepared. Then the solution introduced gaseous ammonia at room temperature at a flow rate of 200 to 250 ml.min⁻¹. The introduction of ammonia continued for 1 h, and the mixture's temperature rose to 45 °C during the

reaction. Next, the reaction mixture was cooled to room temperature and stirred for 2 h. After finishing the reaction, it was diluted with acetone (90 ml) and stirred for 30 min to solidify. Afterward, the solid material was filtered and washed with absolute ethanol (90 %). Finally, the sediments were recrystallized with distilled water and ethanol to afford 3-aminopropane-1-sulfonic acid as a white crystalline powder (6.64 g, 96 %).

2.2. Synthesis of acamprosate calcium

A mixture of acetic acid (1 ml, 17.5 mmol) and acetic anhydride (2 ml, 21.16 mmol) was also prepared. This mixture was then added to a stirred solution of 3-amino-1-propane sulfonic acid (2.5 g, 17.9 mmol) in distilled water (3 ml). After a few seconds, a solution of calcium hydroxide (0.95 g, 12.8 mmol) in distilled water (4 ml) was then added to the reaction mixture. After 1 hour, acetone (25 ml) was added to the reaction mixture and stirred for 30 min. After finishing the reaction, ethanol (50 ml) was added. The resulting compound was solidified, filtered, and dried to give acamprosate calcium (3.3 g, 92 %) for the next step.

2.3. Preparation of micronized acamprosate calcium

A saturated drug solution was prepared by solving 0.5 g of acamprosate calcium in 20 ml of distilled water under heated conditions of 60 °C. The speed of stirring was adjusted to 600 rpm. Then the surfactant solution of the desired compound (1.0 g) in acetone (82 ml) was added to the stirred acamprosate calcium solution dropwise for 40 min. Next, the formed micronized particle was filtered, dried, and weighed. Lastly, the particle size was determined using SEM analysis, and the distribution of micronized particles in suspensions was investigated using DLS analysis.

3. Results and discussion

In this study, acamprosate calcium was first synthesized in two steps. In the first step, the nucleophilic attack of ammonia to 1,3-propane sultone formed 3-aminopropane-1-sulfonic acid. In the second step, 3-aminopropane-1-sulfonic acid was acylated, and calcium salt was formed using calcium hydroxide (Fig. 3). Then, the micronized acamprosate calcium was prepared using a nonionic surfactant. The two factors,

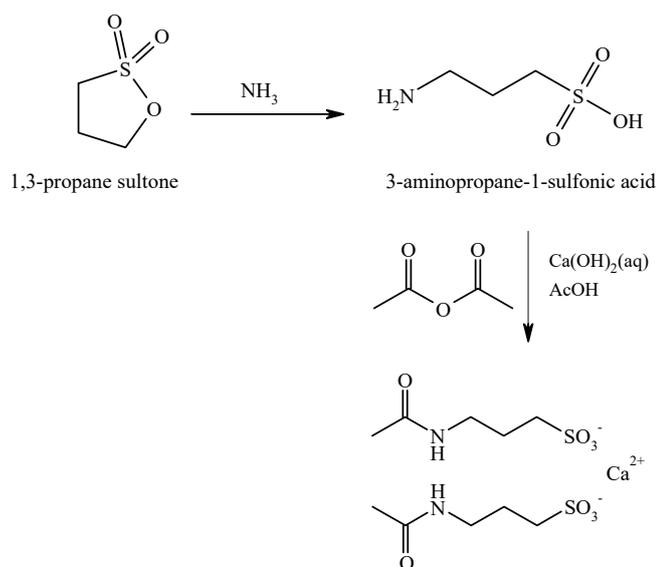


Fig. 3. The route for the synthesis of acamprosate calcium.

surfactant type and stirring speed, were investigated.

The effect of surfactants on the production of fine particles has been thoroughly investigated [45,46]. In this research, non-ionic surfactants were used as surface activators. Ionic surfactants were not used because acamprosate calcium is an ionic compound, and there is a possibility of ion exchange between this compound and the surface activator. Tween 80, Tween 40, Triton-X-100, PEG 600, PEG 300, and PEG 200 were tested as surfactants (Table 1).

In order to evaluate the effect of the first factor, stirring speed, on the particle size of the reaction, different stirring speeds were investigated and are summarized in Table 2.

As seen from Tables 1 and 2, among different surfactants, the best result occurred when using Triton-X-100 at 600 rpm.

The SEM and DSL analyses were used to investigate the particle sizes of the product (Figs. 4 and 5). As

Table 1. The yield of formation of micronized acamprosate calcium.

Acamprosate calcium weight (g)	Surfactant	Micronized particle weight (g)	Yield (%)
0.5	Tween 80	0.23	46
0.5	Tween 40	0.41	82
0.5	Triton-X-100	0.47	94
0.5	PEG 600	0.15	30
0.5	PEG 300	0.30	60
0.5	PEG 200	0.35	70

Table 2. The effect of stirring speed on particle size of acamprosate calcium.

Entry	Rotation speed (r.min ⁻¹)	Particle size (μm)
1	100	7 >
2	200	60 >
3	300	55 >
4	400	25 >
5	500	20 >
6	600	10 >
7	700	10 >
8	800	10 >

shown in Fig. 5, the particle size of all resulting products is less than 10 μm, and 98.1 % of particles were less than 5 μm. The purity of the micronized acamprosate calcium was determined according to international U.S. pharmacopeia using HPLC analysis, which showed that the micronized acamprosate calcium has an acceptable purity (99.7 %, Fig. 6). These microparticles can be used as tablets and capsules after formulation.

3.2. Kinetic solubility studies

In order to evaluate the micronized sample's water solubility compared to the synthesized and bulk samples,

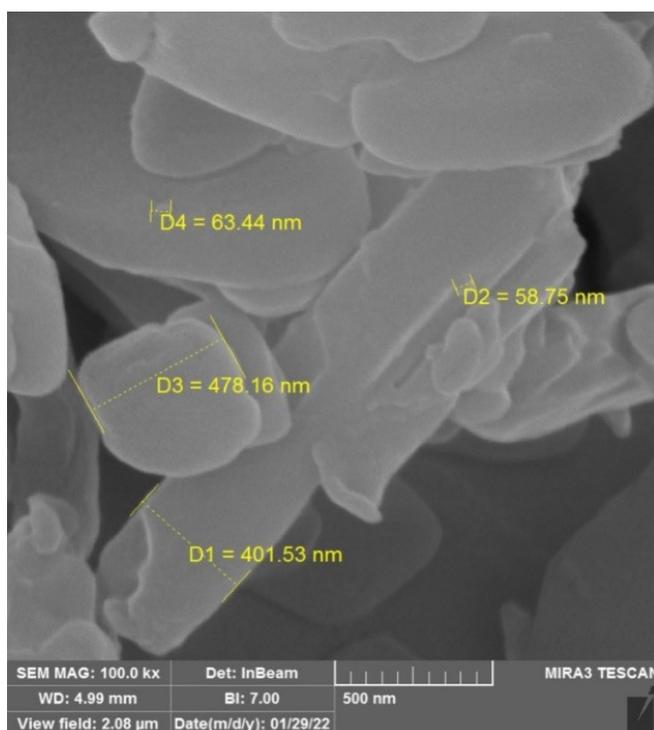


Fig. 4. SEM image of micronized acamprosate calcium.

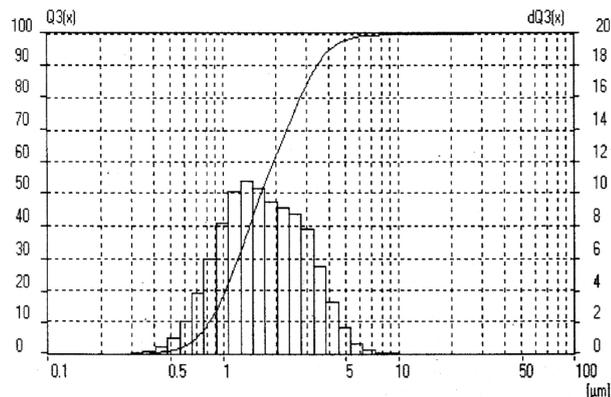
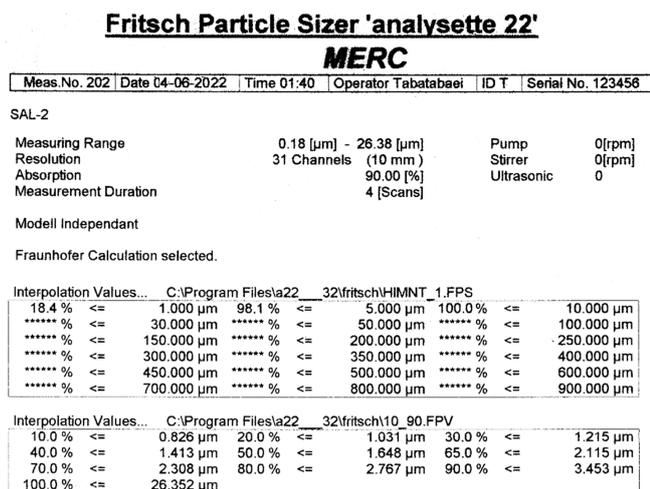


Fig. 5. Distribution of acamprostate calcium particles size.

a certain amount of the two samples was poured into two thimbles. The thimbles were sealed and placed in two flasks containing distilled water at room temperature without stirring. The solutions were sampled at different times, and the amount of acamprostate calcium was determined according to the standard method using an ultraviolet device [47]. As shown in Fig. 7, the solubility rate of the micronized sample is significantly higher than the bulk sample, with particle sizes between 100-70 µm. The micronized sample reached saturation after about 15 min, while the time duration for the bulk samples was about 35 min.

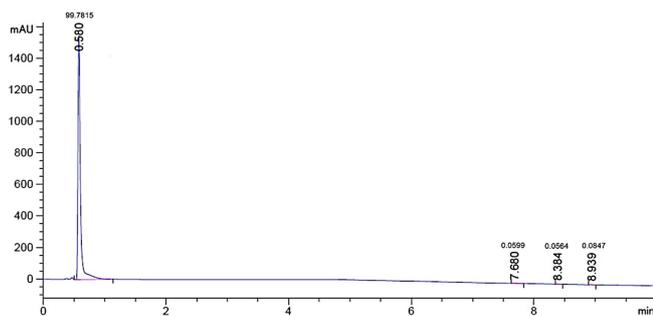


Fig. 6. HPLC analysis of micronized acamprostate calcium according to the international U.S. pharmacopeia.

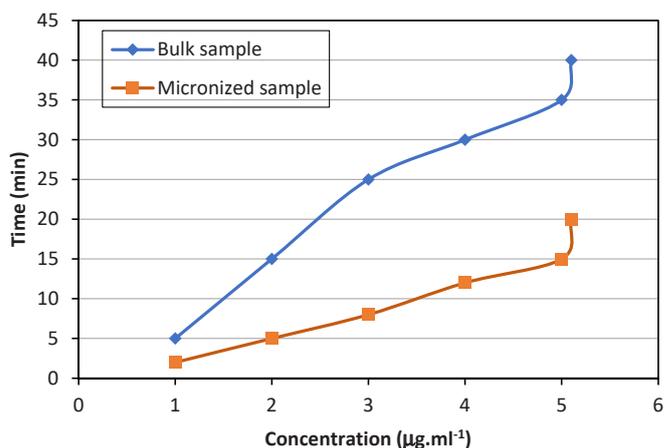


Fig. 7. The effect of particle size on the solubility of acamprostate calcium.

4. Conclusions

Acamprostate calcium was synthesized in two steps from 1,3-propane sultone and ammonia gas. Micronized acamprostate calcium with dimensions of less than 10 µm and high purity (99.7 %) was prepared using Triton-X-100 as a surfactant agent and distilled water and acetone as solvents. The optimal mixing speed was investigated at 600 rpm. The water solubility of the bulk particles of acamprostate calcium and micronized acamprostate calcium was studied. The results showed that the water solubility of the acamprostate calcium had been improved, and the solution of micronized particles became saturated after about 15 min, while this time for the bulk sample was about 35 min.

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