

## Research Article

# Towards Boosting Power by Encapsulating Tranexamic Acid into Emulsified Particles

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In aesthetic medicine, during a course of skin whitening treatment, injections must be frequently administered to achieve a strong curative effect. To develop a method to prevent long-term harm due to injections, this study applied a novel technology for the delivery of whitening agents that achieved long-term slow release of agents, thereby reducing the danger of frequent injections. We utilized biodegradable poly(ethylene glycol)-poly(lactide-co- $\epsilon$ -caprolactone) and Span 85 as surfactants and squalene as the core oil to encapsulate and adsorb tranexamic acid in emulsified particles, respectively. The conductivity test determined that the continuous phase of the obtained emulsified particles was aqueous; tranexamic acid did not play a critical role because of its low content. The controlled release experiment demonstrated that the release rate of tranexamic acid from the emulsified matrix was in the sequence of (1) adsorption, (2) encapsulation plus adsorption, and (3) encapsulation. Encapsulating tranexamic acid can efficiently halt the behavior of sudden release and potentially boost the efficacy of whitening.

## 1. Introduction

Tranexamic acid is clinically used as a haemostatic agent because of its antifibrinolytic effect, through which it blocks lysine binding sites on plasminogen molecules [1]. The first publication regarding the use of tranexamic acid to treat melasma was in 1979, and the mechanism for preventing the activation of melanocytes has been highly regarded ever since because of its efficacy [2, 3]. Having minimal side effects and being well tolerated by patients [4], tranexamic acid is promoted as a systemic skin whitening agent in aesthetic medicine [5–7].

Intensive skin whitening treatment usually requires injections two or three times per week for more than 1 month. For example, tranexamic acid is usually applied at a dose of 250 mg twice per day for at least 3 months to effectively treat melasma [8]. To prevent long-term complications due to whitening injections and side effects from the instantaneous high drug concentration of such injections, this study developed a novel delivery technique for whitening drugs with long-term slow release to reduce the occurrence of injury and danger of frequent injections.

Tranexamic acid can be encapsulated in liposomes [9] to achieve a prolonged and sustained release profile. However, this and similar delivery systems require organic solvents and complicated processes, thereby raising the economic impact of further application. In this study, we utilized an emulsion technology to encapsulate or adsorb tranexamic acid through water-in-oil-in-water- (W/O/W-) emulsified particles composed of a biodegradable polymer, poly(ethylene glycol)-poly(lactide-co- $\epsilon$ -caprolactone) (PEG-PLACL), in a water solution, and squalene/Span 85 (sorbitan trioleate, Sigma-Aldrich, Steinheim, Germany) in an oil solution. The kinetic tranexamic acid release behavior of the emulsified particles was analyzed *in vitro* and the release model was described extensively.

## 2. Materials and Methods

**2.1. Emulsion Preparation.** Span 85 and PEG-PLACL were used as surfactants in this study; PEG-PLACL was synthesized according to methods outlined in our previous reports [10, 11]. After purification of the recovered polymer and validation of its composition, the emulsion could be prepared.

We first dissolved 60 mg of PEG-PLACL polymer in 0.39 mL of phosphate-buffered saline (PBS) solution uniformly. During overnight storage at 4°C, the solution got transformed into a white translucent hydrogel. Subsequently, 0.55 mL of the oil phase, composed of squalene and Span 85, was introduced into the PEG-PLACL polymer hydrogel. Finally, we used a homogenizer (polytron PT 2500E, Kinematica AG, Lucerne, Switzerland) at a speed of 6,000 revolutions per minute for 5 minutes to produce an isotropic white emulsion.

Three tranexamic acid-carrying modes of emulsified particles—(a) encapsulation, (b) adsorption, and (c) encapsulation plus adsorption—were prepared through the procedures displayed in Table 1; 3 wt.% tranexamic acid (“TA” hereafter) was introduced in the three tranexamic acid-containing emulsions (“TA<sub>EN</sub>” for encapsulation, “TA<sub>AD</sub>” for adsorption, and “TA<sub>EN+AD</sub>” for encapsulation plus adsorption hereafter). Emulsion without tranexamic acid (“EM” hereafter) served as the control group.

**2.2. Emulsion Properties.** The prepared emulsions were examined for their physicochemical characteristics, including visual appearance, dispersion type, and particle-size distribution. Dispersion properties were determined based on conductivity (ES-51, Horiba, Kyoto, Japan). Particle size at 0, 1, 7, 14, 21, and 28 days was determined using a particle size analyzer (Nano ZS, Malvern Instruments, Worcestershire, UK) by a 1:10,000 dilution in pure water.

**2.3. Controlled Release.** Based on the previous reports [10, 11], the in vitro release system was operated using a dialysis tube method. An emulsion solution of 300  $\mu$ L was placed in the dialysis chamber, and a cutoff at the 200 nm membrane pore size was set. Subsequently, the dialysis chamber was sunk in the 50 mL centrifuge tube, which contained 5 mL of PBS in a constant 37°C circulation holder. At predetermined time points, a 60  $\mu$ L sample solution in the centrifuge tube was withdrawn for quantitative analysis of tranexamic acid release, and a fresh 60  $\mu$ L of PBS was added to maintain a stable sample volume of 5 mL.

The spectrofluorimetric method was adopted to determine the concentration of tranexamic acid [12]. In brief, the 60  $\mu$ L sample solution was mixed with 30  $\mu$ L of formaldehyde (20%, v/v) and 15  $\mu$ L of acetylacetone (8.4%, v/v) and heated at 95°C for 10 minutes. After cooling to room temperature for 20 minutes, the 415 nm excitation and 480 nm emission were applied to the sample solution and the fluorescence intensities were measured using a microplate reader (SpectraMax M2, Molecular Devices, Sunnyvale, CA, USA). A calibration curve using aliquots of standard solutions was used to calculate the concentration of tranexamic acid in the sample solution.

### 3. Results and Discussion

**3.1. Emulsion Properties.** The appearance of each of the three prepared emulsions—(a) encapsulation, (b) adsorption, and (c) encapsulation plus adsorption—was homogeneous and white. Almost no difference was observed among the three

emulsions in terms of appearance. Table 2 presents the conductivity of the ingredients and formulations used in this study. The conductivity of the pure oil—squalene—could not be measured with a conductivity meter and was categorized as nondetectable (“N.D.” in Table 2). The distilled deionized water (DDH<sub>2</sub>O) lacked ions and thus had no conductivity. The aqueous solutions, namely, PBS, PEG-PLACL in PBS, TA, TA<sub>EN</sub>, TA<sub>AD</sub>, TA<sub>EN+AD</sub>, and EM, had a higher level of electrolytic conductivity than the oil sample. The four emulsion formulations, namely, TA<sub>EN</sub>, TA<sub>AD</sub>, TA<sub>EN+AD</sub>, and EM, demonstrated conductivity values between those of squalene and PBS; this implied that the continuous phase of the emulsion was the aqueous phase. The four emulsions had similar values, thereby revealing that the small amount of tranexamic acid was not a key factor for conductivity.

Figure 1 presents the particle sizes of the four emulsion formulations at different time points during a 28-day storage period. The particle sizes ranged from large to small in the order of EM, TA<sub>AD</sub>, TA<sub>EN+AD</sub>, and TA<sub>EN</sub>. Particle size was affected by the presence of tranexamic acid, which caused the particles to shrink. The results also indicated that the particle sizes of all four emulsion formulations decreased over time. The particles ranged in size between 300 and 1,300 nm.

**3.2. Controlled Release.** Figure 2 presents the 28-day release behavior of tranexamic acid in three emulsions compared with that in the aqueous solution. The release of tranexamic acid in the TA group was rapid and exhausted within 3 days, thereby achieving equilibrium tranexamic acid concentration. TA<sub>AD</sub> exhibited slow controlled release only in the early stage and subsequently tended toward nonformulated tranexamic acid beyond 100 hours. The equilibrium tranexamic acid concentration was similar to the condition of tranexamic acid without formulation. Slow release behaviors of tranexamic acid were observed in the TA<sub>EN+AD</sub> and TA<sub>EN</sub> emulsions. For TA<sub>EN+AD</sub>, two stages were observed in the release curve, and transition occurred at 26 hours. A two-stage release behavior was also noted for TA<sub>EN</sub>, for which transition also occurred at approximately 26 hours. These findings indicated that not all tranexamic acids were encapsulated during the encapsulation process. Before 26 hours, desorption of tranexamic acid on the surfaces of particles dominated the release, whereas diffusion of tranexamic acid encapsulated in the emulsified particles dominated the later stage of release. Throughout the entire 700-hour period, TA<sub>EN</sub> encapsulation was highly efficacious, and the equilibrium concentration of tranexamic acid was lowest in this emulsion formulation.

Studies have demonstrated that the W/O/W emulsion can provide protection against degradation for water-soluble compounds and exhibit prolonged and sustained release [13–15]. In the present study, the controlled release data (Figure 2) revealed that tranexamic acid, which is water soluble, was released from water-containing particles. In addition, the conductivity results (Table 2) indicated that the emulsion had a high affinity to water and was an O/W-

TABLE 1: Ingredients of emulsion formulation and preparation sequence.

Sequence	Ingredients	TA	Formulation			EM
			TA <sub>EN</sub>	TA <sub>AD</sub>	TA <sub>EN+AD</sub>	
1	PEG-PLACL	—	60 mg	60 mg	60 mg	60 mg
2	PBS	—	390 $\mu$ L	390 $\mu$ L	390 $\mu$ L	390 $\mu$ L
3	Tranexamic acid	—	60 mg	-	30 mg	—
4	Squalene/span 85	—	550 $\mu$ L	550 $\mu$ L	550 $\mu$ L	550 $\mu$ L
5	—			Homogenizing		
6	Tranexamic acid	60 mg	—	60 mg	30 mg	—
7	PBS	1940 $\mu$ L	940 $\mu$ L	940 $\mu$ L	940 $\mu$ L	1000 $\mu$ L

TABLE 2: Conductivity of ingredients and formulations used in this study (mean  $\pm$  standard deviation,  $n = 3$ ).

Ingredients or formulations	Conductivity (mS/cm)
TA	0.811 $\pm$ 0.001
TA <sub>EN</sub>	0.266 $\pm$ 0.002
TA <sub>AD</sub>	0.246 $\pm$ 0.001
TA <sub>EN+AD</sub>	0.244 $\pm$ 0.005
EM	0.279 $\pm$ 0.001
PEG-PLACL in PBS	1.615 $\pm$ 0.001
PBS	6.680 $\pm$ 0.010
DDH <sub>2</sub> O	N.D.
Squalene	N.D.

N.D.: nondetectable.

emulsified system. Therefore, the conclusion based on these two results is that the emulsified particles containing tranexamic acid exhibited W/O/W characteristics; this agrees with our findings obtained by using ovalbumin and inactivated viruses as models [11, 13].

**3.3. Kinetic Model.** A simple empirical equation known as a power law, expressed as follows, can extensively and successfully describe the first 60% of the release curves [16–18]:

$$\frac{C}{C_{\infty}} = kt^n, \quad (1)$$

where  $C/C_{\infty}$  is the fractional solute concentration,  $C_{\infty}$  is the equilibrium concentration,  $t$  is the release time,  $n$  is the diffusion exponent, and  $k$  is the release constant. Figure 3 shows the curve fitting of the first 60% of the release data in Figure 2 obtained using Equation (1), and Table 3 presents a summary of the curve fitting result of a best-fit power law equation and correlation coefficient denoted as  $R^2$ . The results indicated that the goodness of fit, or  $R^2$ , was greater than 0.95 in all cases. The power law release model adequately predicted the first 60% of release behaviors, even though this study had two transport barriers, namely, the emulsion particle and dialysis membrane. The power law release model, used to describe the release mechanism in a system with multiple transport barriers, is deemed applicable in the literature for applications such as release from drug-loading porous polymeric scaffolds [18]. In the present study, the values of  $n$  for the groups of TA, TA<sub>AD</sub>, TA<sub>EN+AD</sub>, and TA<sub>EN</sub> were 0.3696, 0.4926, 0.5873, and 0.7398, respectively. TA<sub>EN+AD</sub> and TA<sub>EN</sub> had values higher than 0.5,

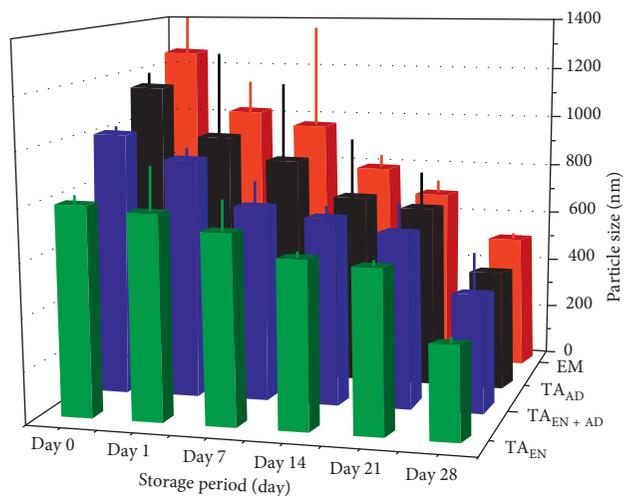


FIGURE 1: Particle sizes of the four emulsion formulations at various time points in the storage period (mean  $\pm$  standard deviation,  $n = 3$ ).

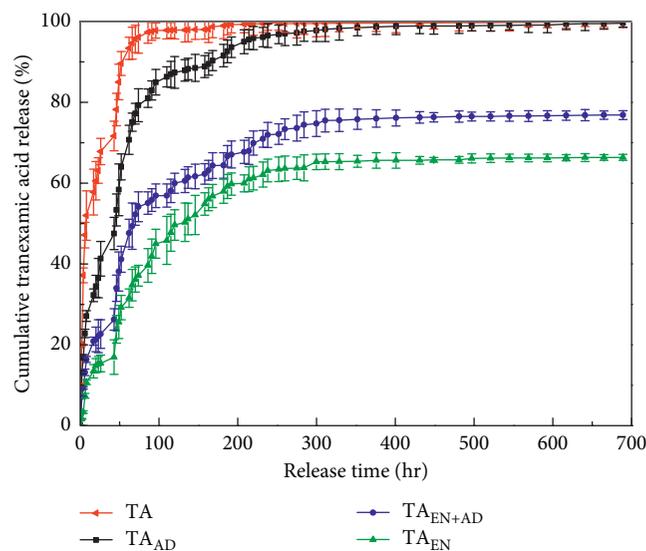


FIGURE 2: Kinetic release behaviors of tranexamic acid in various emulsified particles.

indicating that their release behaviors were characterized by anomalous (non-Fickian) transport between Fickian diffusion and zero-order release [18].

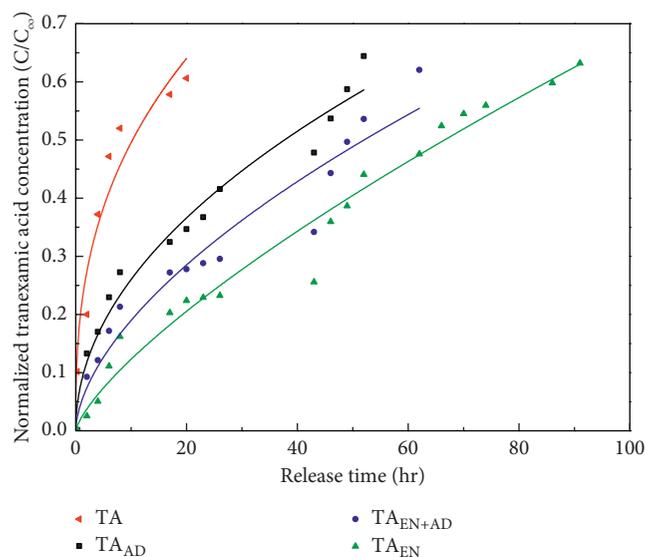


FIGURE 3: Curve fitting of tranexamic acid release in various emulsified particles.

TABLE 3: Curve fitting for fractional solute concentration over time.

Formulation	Best-fit equation	$R^2$
TA	$C/C_{\infty} = 0.2116 t^{0.3696}$	0.95
TA <sub>AD</sub>	$C/C_{\infty} = 0.0837 t^{0.4926}$	0.97
TA <sub>EN+AD</sub>	$C/C_{\infty} = 0.0491 t^{0.5873}$	0.95
TA <sub>EN</sub>	$C/C_{\infty} = 0.0224 t^{0.7398}$	0.98

## 4. Conclusions

To provide a method for preventing harm to the human body due to frequent whitening injections, this study prepared three tranexamic acid-containing emulsions, namely, TA<sub>EN</sub>, TA<sub>AD</sub>, and TA<sub>EN+AD</sub>, for slow controlled release. The release data demonstrated that all three emulsions had controlled release functions. The release rates, from rapid to slow, were adsorption, encapsulation plus adsorption, and encapsulation. The release of tranexamic acid from these three emulsions can last for at least 15 days and potentially boost whitening efficacy. Finally but critically, from the perspective of safety, W/O/W-emulsified particles can be easily injected using a small-gauge needle to conceptually diminish local reactions to W/O-dispersion colloidal clusters. Such application requires in vivo investigation of single-dose whitening injections.

## Abbreviations

DDH <sub>2</sub> O:	Distilled deionized water
EM:	Emulsion without tranexamic acid
PBS:	Phosphate-buffered saline
PEG-	Poly(ethylene glycol)-poly(lactide-co-
PLACL:	$\epsilon$ -caprolactone)
TA:	3 wt.% tranexamic acid
TA <sub>AD</sub> :	3 wt.% tranexamic acid-containing emulsions by adsorption

TA<sub>EN</sub>: 3 wt.% tranexamic acid-containing emulsions by encapsulation

TA<sub>EN+AD</sub>: 3 wt.% tranexamic acid-containing emulsions by encapsulation plus adsorption.

## Data Availability

Previously reported PEG-PLACL synthesis data were used to support this study and are available at doi:10.1002/jbm.b.31352 and <https://doi.org/10.1007/s11095-009-9898-y>. These prior studies are cited at the relevant places within the text as references [10, 11].

## Disclosure

The founding sponsors had no role in the study design; collection, analysis, or interpretation of data; composition of the manuscript; or decision to publish the results.

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

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