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Full Length Research Paper

Preparation of ambroxol hydrochloride carboxymethyl chitosan microspheres without burst release

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The aim of this article was to fabricate novel ambroxol hydrochloride carboxymethyl chitosan microspheres without burst release. Firstly the blank carboxymethyl chitosan microspheres were fabricated by the emulsion chemical cross-linking method and the blank microspheres showed a controllable biodegradation *in vitro* in lysozyme solution. Then the ambroxol hydrochloride microspheres were prepared by the column method. The type of bonding between ambroxol hydrochloride and carboxymethyl chitosan microspheres was investigated by X-ray diffraction; the results showed that the drug chemically bonds to the ion exchangeable structure of the microspheres. The evaluation of the microspheres was investigated by dynamic light scattering, scanning electron microscopy (SEM) and UV spectrophotometer. The microspheres were spherical and consistent, and had an average diameter of 7.4 µm, the drug content of the microspheres was 15.3±0.7% (w/w). Finally the *in vitro* drug release was tested in different ionic concentration dissolution mediums. The results showed that the microspheres had a sustained-release profile for 8 h *in vitro* without obvious burst release.

Key words: Ambroxol hydrochloride, carboxymethyl chitosan, lung-targeting; microsphere, *in vitro*.

INTRODUCTION

The development of injectable drug biodegrable microsphere has received considerable attention over the past few years (Langer, 1990; Saito et al., 2001; Ravikumar and Kumar, 2001). This interest has been sparked by the advantages of these delivery systems possess, which include ease of application, localized delivery for a site-specific action, prolonged delivery periods, and improved patient compliance and comfort (Sultana et al., 2009; Hollister, 1989; Levy et al., 1996). Though there are many advantages for the injection biodegradable microspheres, there are still some shortcomings for this dosage form, such as burst, incomplete or uncontrollable drug release (Kumar et al., 2001). Burst release is especially of concern due to its potential to increase side affects which raises a major obstacle for microsphere therapeutic potential. Considerable efforts have been made to moderate this burst release profile (Soriano et al., 1996; Nahata and Saini, 2008), however very few microspheres without burst release have been successfully reported.

Chitosan, a natural linear biopolyaminosaccharide, is obtained by alkaline deacetylation of chitin, which is the second most abundant polysaccharide next to cellulose. Properties such as biodegradability, low toxicity and good biocompatibility make it ideal for use in biomedical and pharmaceutical formulations (Lu et al., 2008). Carboxymethyl chitosan (MCC) is one of the most abundant and familiar kinds of chitosan derivatives, it has been applied in several drug delivery systems (Chen et al., 2004; Ubaidulla et al., 2009).

Recently ion exchange polymers have been used in

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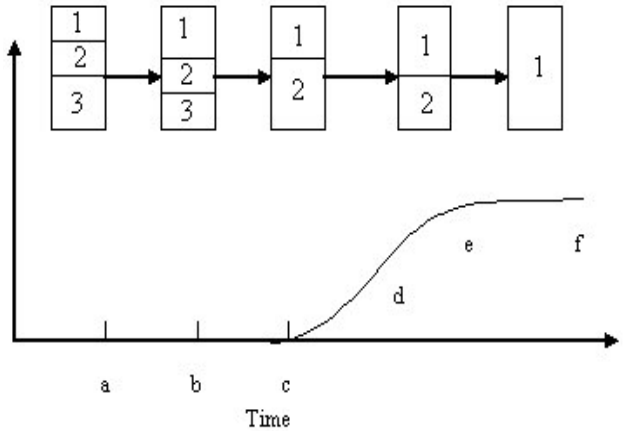

Figure 1. Schemetic diagram of drug loading process in column method. 1, Saturated section; 2, commutative section; 3, uncommutative section.

drug delivery systems (Sriwongjanya and Bodmeier, 1998; Rao et al., 2004). They have a number of attractive properties including increased stability, taste masking, few side effects, and a more uniform absorption and sustained release profile. One of the most impressive advantages of the ion exchange polymers is that theoretically the drug release from the ion exchange polymers is only influenced by the ion concentration in the surrounding medium. Thus when the ion exchange polymers are introduced into the body, the body's natural counter ion concentration stabilizes drug release relieving the concern of a burst release profile. So if we can combine the biodegradable polymer and the ion exchange technology to make the biodegradable and ion exchangeable microspheres that may thoroughly overcome the "burst release" problems. Carboxymethyl chitosan was used in this article as biodegradable polymer because of the carboxymethyl group in it may combine the ionic drug by the ionic bond through the ion exchange process.

Ambroxol hydrochloride (AH) is a new type of expectorant which is widely used clinically in many countries. It is an active metabolite of mucolytic *in vivo*, but currently very few drug delivery systems are available or being investigated which contain AH (Wang and Pei, 2001). Because of the pharmacological actions of the AH, a targeted and sustained release of AH to the lungs would greatly benefit patients.

In this article, we combined biodegadable polymer (MCC) and ion exchange technology to prepare the AH lung targeting microspheres. Firstly the degradability of blank carboxymethyl chitosan microspheres *in vitro* was investigated. The particle size, morphology and drug content of the microspheres were investigated. The type of bonding between AH and carboxymethyl chitosan microspheres was investigated by X-ray diffraction and *in vitro* drug release. Finally, the drug release from the microspheres *in vitro* was tested in different ionic concentration dissolution mediums.

MATERIALS AND METHODS

AH was obtained from the Sigma-Aldrich corporation (St. Louis, USA). Carboxymethyl chitosan, (Mol. wt. 50KD) was obtained from Honghai biotechnology company (Qing Dao, China). Glutaraldehyde was obtained from the Sigma-Aldrich corporation (St. Louis, USA). All reagents were of analytical grade.

Preparation of cross-linked MCC microspheres

Twelve milliliter of MCC aqueous solution (0.25 g/ml) was added to 60 ml liquid paraffin to form a W/O emulsion with 2 g Span80(Weerakody et al., 2008; Sayın et al., 2008). The dispersion was stirred at 800 rpm for 40 min after the addition 2 g glutaraldehyde. The product was filtered and washed with chloroform several times and finally with water and dried at 40℃.

In vitro blank MCC microspheres degradation

The degradability of blank MCC microspheres *in vitro* was investigated in lysozyme solution (Lu et al., 2007). 100 mg blank MCC microspheres (n=3) were immersed in 10 ml lysozyme solution (4 $mg \cdot ml^{-1}$) in PBS (pH 7.4) at 37℃. The degrading solution was replaced with fresh lysozyme solution every 3 days. At 3, 6 and 9 days, the blank microspheres were taken out from the lysozyme solution, rinsed with distilled water, freeze-dried and weighed. The extent of *in vitro* degradation was expressed as the percentage of the weight loss of the blank microspheres after lysozyme treatment.

Preparation of the AH microspheres

The AH microspheres were prepared by a column processes (Jeong and Park, 2008a, b). For the column process, a glass column (size: 1.0 × 20 cm, bed volume: 15 ml) was used. 1 g of MCC microspheres was then slurred with water and transferred to the glass column equipped with a coarse-fritted glass disk at the bottom. To stabilize the packing, the MCC microspheres were backwashed with water using a peristaltic pump and then 8 mg/ml drug solution was pumped upward at a rate of 60 ml/h until there is drug detected in the eluate (time point C in Figure 1). The AH microspheres were then washed off the physical absorption AH with deionized water and dried at 40℃. The diagram of drug loading process is in Figure 1.

Particle size analysis by dynamic light scattering

The mean particle size of the microspheres was measured using a laser light scattering particle size analyzer (LS230, Beckman Coulter, Miami, USA) according to user's manual.

Morphology of the microspheres

The surface of the microspheres was examined by scanning electron microscopy (SEM) (Jeol JSM-6400, Tokyo, Japan). Samples were gold sputter coated (SCD 004 Coater; Bal-Tec, Balzers, Lichtenstein) for 165 s at 15 mA in an atmosphere of argon.

Table 1. The degradation percentage of the blank MCC microspheres *in vitro*.

Days	0	3	6	9
W (mg)	101.7±0.3	85.2±0.6	40.1±0.7	22.6±0.8
Weight loss (%)	0	16.2	60.6	77.8

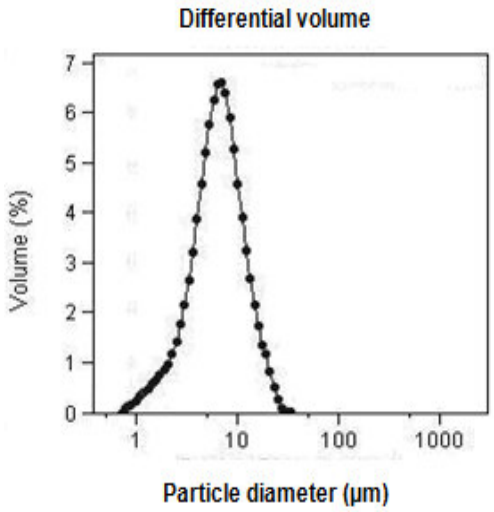

Figure 2. The particle size distribution of AH microspheres.

Determination of drug content in the microspheres

The AH amount in the microspheres was determined by suspending 10 mg AH microspheres in 100 ml NaCl solution (1 mol/L) under magnetic stirring for 10 h at 65℃. The solution was then filtered, and the amount of AH in the filtrate was determined using UV spectrophotometer at 244 nm.

Powder X-ray diffraction properties

Blank microspheres, AH, AH microspheres, physical mixture of AH and microspheres were investigated. Wide-angle-X-ray diffraction was recorded by a X-ray Diffractometer (PANalytical, X'Pert PRO MPD, The Netherlands) using Cu kalpha radiation at 40 KV/40 mA with a secondary nickel beater filter (Jenquin and McGinity, 2008).

In vitro drug release

In vitro drug release investigation was carried out following the USP paddle (apparatus II) method at a paddle speed of 50 rpm (Chuong et al., 2009; Patel et al., 2009). Deionized water, 0.15, 0.5, 1 mol/L NaCl at 37±0.1℃ were used as the dissolution mediums. Microspheres containing 8 mg AH were weighed according to drug content and were placed in the mini dialysis kits (MWCO 6-8 kDa) (GEBA, Gene Bio-Application, Israel), and were immersed in 100 ml dissolution medium. 2 ml dissolution medium was then withdrawn at different intervals (1, 2, 3, 4, 6 and 8 h). The amount of drug released in the filtrate was measured by UV spectrophotometer at 244 nm. And the drug release data in 0.15 mol/L NaCl at 37±0.1℃ was analysis according to the Zero-order, First-order and Higuchi equation.

Statistical analysis

Statistical analysis for the determination of differences in the measured properties between groups was accomplished using one-way analysis of variance and determination of confidence intervals, performed with a computer statistical program (Statistical Analysis System, Version 6.08, SAS Institute, Cary, NC, USA). All data were presented as a mean value with its standard deviation indicated (mean±S.D.).

RESULTS AND DISCUSSION

In vitro blank MCC microspheres degradation

A critical requirement for polymeric matrices in biodegradable injection drug delivery is controllable biodegradation over time. To assess the degradation behavior of blank microspheres, we incubated them in lysozyme solution and monitored change of weight, which is the most relevant characteristic of implanted materials. The results clearly demonstrated that the blank MCC microsphere degraded over time as seen in Table 1 and could be a suitable material for biodegradable injection drug delivery.

Particle size analysis of AH microspheres by dynamic light scattering

The particle size distribution result is in Figure 2. The average particle size of the microspheres was 7.4 μm. Many research groups have demonstrated the micropshere's particle size and the surface properties as the main influential parameters for the microspheres distribution *in vivo* (Huo et al., 2005; Harsha and Rani, 2009), and most microspheres with particle size in the range of 5 to 25 μm would target to the lungs. Because of the pharmacological actions of the AH, a targeted and sustained release of AH to the lungs would greatly benefit patients.

Morphology of the AH microspheres

The scanning electron micrograph of the AH microshpheres is shown in Figure 3. The results showed that AH microspheres were spherical and consistent.

Determination of drug content in AH microspheres and the drug loading efficency

The drug content in AH microspheres was 15.3 ± 0.7% (n=6), as determined by UV absorption at 244 nm. And because the preparation process stopped when the drug

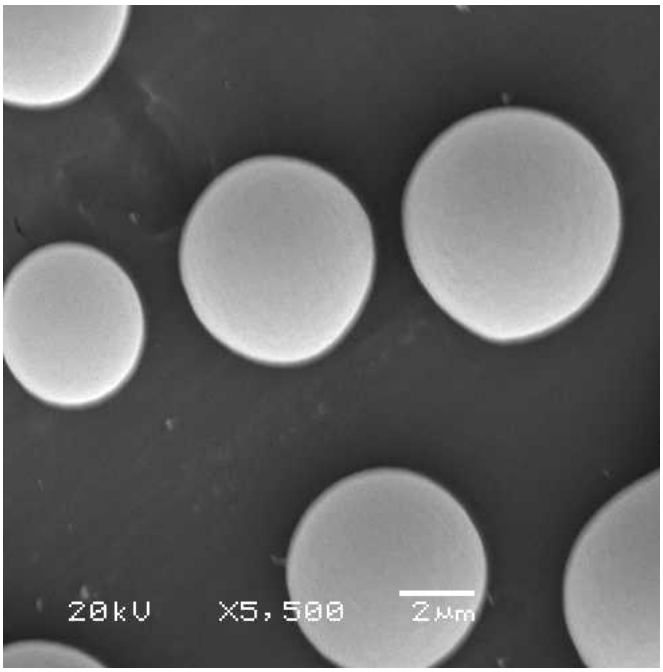

Figure 3. The SEM of AH microspheres.

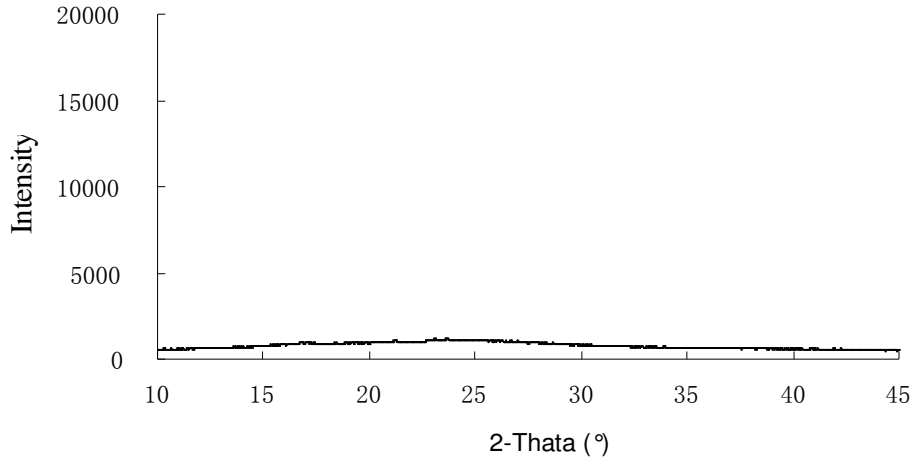

Figure 4. X-ray of MCC microspheres.

was detected in the eluate; the drug loading efficency is 100%. So this microsphere fabrication method would greatly benefit the prospective protein microsphere's fabrication. Most current studies report production of protein microspheres with approximately 30 to 70% drug loading efficiency, leaving the residual protein to go to waste (Hu et al., 2000; Coppi et al., 2001).

Powder X-ray diffraction properties

The type of bonding between AH and carboxymethyl chitosan microspheres was investigated by powder X-ray diffraction analysis. The blank microspheres were amorphous in nature, so were devoid of sharp peaks in Figure 4. The crystallinity of AH was clearly demonstrated by it is unique X-ray diffraction patterns shown in Figure 5, respectively. The diffraction patterns from a physical mixture of 15.3% drug with pure polymer contained sharp diffraction peaks corresponding to the crystalline drug molecules present in the mixture, as displayed in Figure 6. The presence of diffraction peaks in a physical mixture of 15.3% drug with the blank microspheres demonstrated that the presence of undissolved, crystalline drug

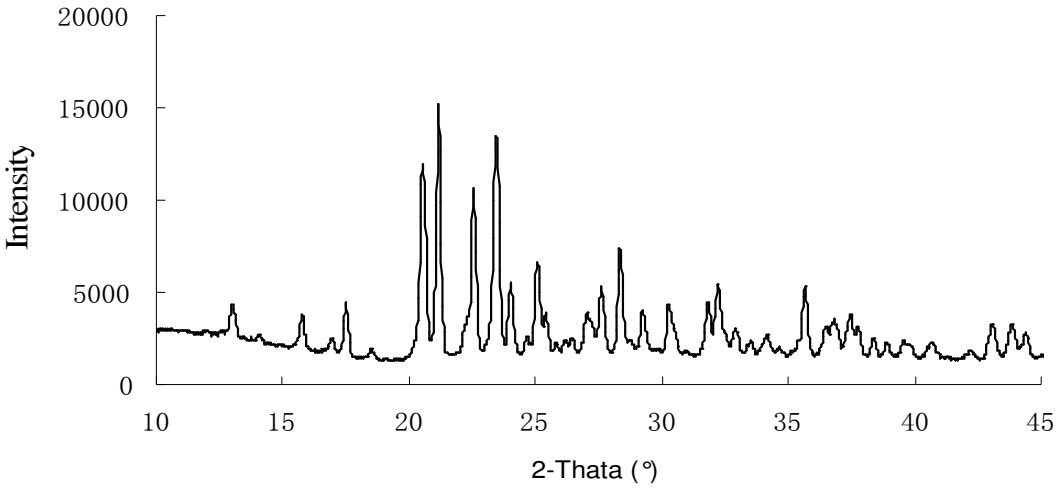

Figure 5. X-ray of the AH.

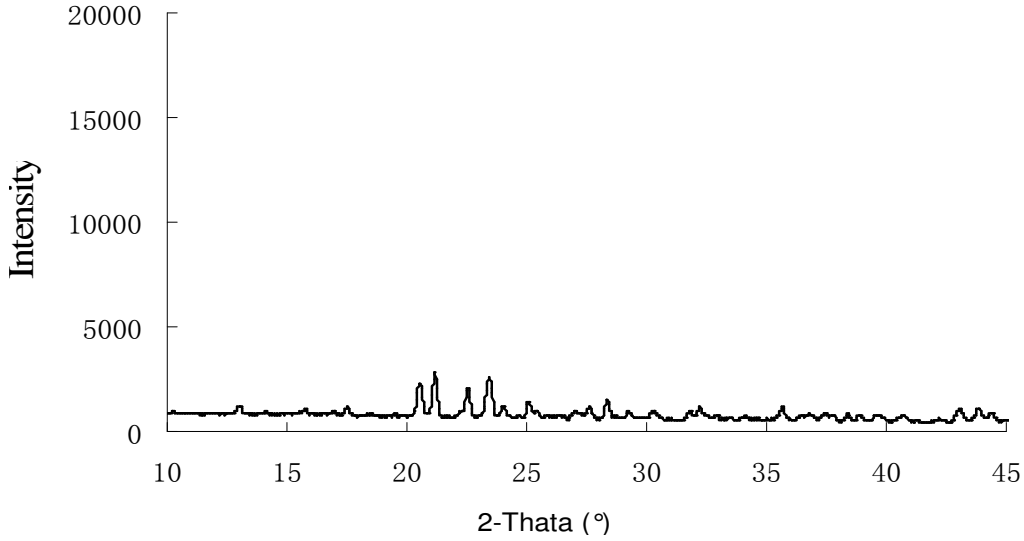

Figure 6. X-ray of the physical mixture.

dispersed in the matrix would exhibit diffraction peaks when exposed to X-rays. The diffraction patterns from AH microspheres containing 15.3% of AH were displayed in Figure 7 respectively, and did not contain any peaks associated with crystalline drug molecules. These diffraction patterns were identical to those of the pure polymer, shown in Figure 5, suggesting the drug presented in an amorphous state within the polymer matrix. It showed that the AH is chemically bonded to MCC microspheres.

In vitro drug release

The *in vitro* drug release results are shown in Figure 8. The results showed that the microspheres released minimum AH in water, and with the increase of ionic strength in the dissolution mediums, the drug released faster. The results showed that the drug release process was mediated by the ion exchange functionality from the microspheres. In addition, the microspheres did not demonstrate the “burst release” at the initial stages. The drug was released in a sustained manner for 8 h in physiological isoosmolar 0.15 mol/L NaCl, and more than 90% of loaded drug was released after 8 h. The results also showed that the AH was combine with the MCC microspheres with chemical bond. And the drug release data’s analysis results are in Table 2. The results showed that the Zero-order equation fit for the drug release process.

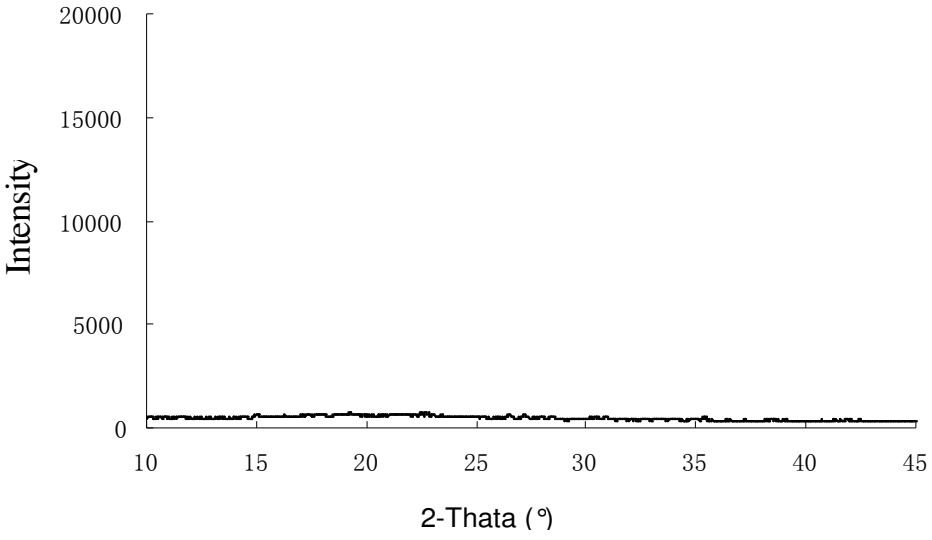

Figure 7. X-ray of AH microspheres.

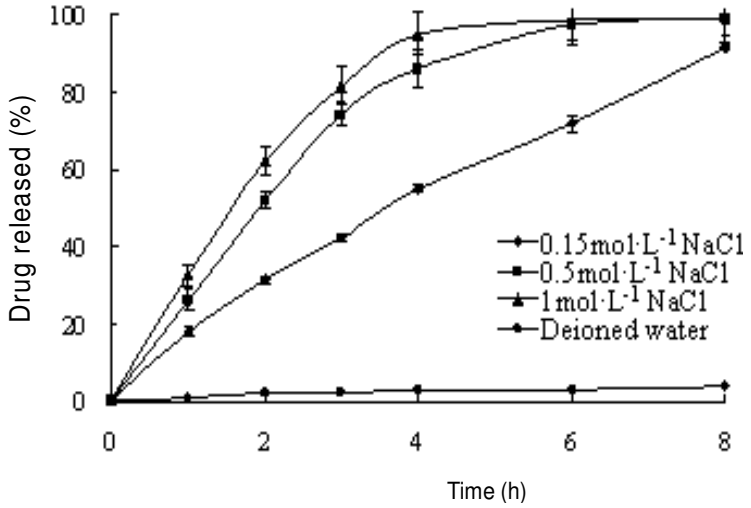

Figure 8. Effects of ion concentration on drug release from the AH microsphere.

Table 2. Results of mathematical study for drug release from the microsphere.

Functions	Expression	Equation	r
Zero-order	Q=Bt+A	Q=15.33t+0.82	0.994
First-order	ln(100-Q)=Bt+A	ln(100-Q)=-0.46t+4.17	0.815
Higuchi	$Q=Bt^{1/2}+A$	$Q=37.93t^{1/2}-11.13$	0.903

Conclusion

Novel lung-targeting sustained-release AH microspheres were prepared and characterized. The results clearly demonstrated that the blank MCC microspheres had a good degradation character *in vitro*. The AH microsphere's particle size was appropriate for a lung-targeting purpose. The X-ray diffraction and *in vitro* drug

dissolution results indicated the drug chemically bonded to the ion exchangeable structure of the microspheres. The microspheres had a sustained-release profile *in-vitro* without the burst release phenomenon. These microspheres containing AH thus demonstrated a great potential therapy option with appropriate biocompatility, sustained release profiles and appropriate particle size for lung targeting.

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