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Research Article

FORMULATION AND EVALUATION OF DOUBLE WALLED MICROSPHERES LOADED WITH PANTOPRAZOLE

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ABSTRACT

Pantoprazole is a proton pump inhibitor that has been widely used in the treatment of gastric, duodenal ulcer and also in gastro esophageal reflux disease (GERD), Zollinger-Ellison syndrome. This the most popular drug used in cure and maintenance therapy of peptic ulcer along with antibiotics. It suppresses the acid production by inhibiting the H^+ K^+ ATPase.The present aim of the work was undertaken with one objective to develop gastro resistant drug delivery system for pantoprazole. Pantoprazole is an acid labile drug, which can be degraded in the stomach. Therefore, the drug should be targeted to intestine; to bypass the stomach the gastro resistant double walled microspheric drug delivery system was adopted. The formulations were developed consisting of double wall. The primary wall composed of muco adhesive polymer HPMC and a release controlling polymer sod. Alginate. The second wall coating the primary microspheres was composed of eudragit RS 100. Eudragit RS 100 provides sustained drug release upto 14hrs with the influence of pH 7.4 buffer. The effect of polymer concentration on the particle size, shape drug entrapment efficiency, muco adhesive property, and release study of core microspheres were evaluated.

1. INTRODUCTION TO PPIs[1,2,3]

Proton pump inhibitors (PPIs) decrease gastric acid and gastric secretory volume. PPIs act by blocking the enzyme system responsible for active transport of acid into the gastrointestinal lumen, namely the hydrogen/potassium adenosine triphosphatase (H^+K^+ATPase) of the gastric parietal cell, also known as the "proton pump." Omeprazole, the first drug in this class, was introduced in 1989. Since then, four other PPIs have been introduced: lansoprazole (1995), rabeprazole (1999), pantoprazole (2000) and esomeprazole (2001). In 2003 omeprazole became available over-the-counter in the US. The formulation for the over-the-counter product is omeprazole magnesium, available in other countries as omeprazole multiple unit pellet system (MUPS). Omeprazole is also available in combination with sodium bicarbonate (Zegerid).

PPIs are used to treat peptic ulcers (duodenal and gastric), symptoms of gastroesophageal reflux disease (GERD), healing of erosive esophagitis, and drug-induced ulcers (e.g., non-steroidal anti-inflammatory drugs {NSAIDs}). If H. pylori, the bacterium that causes ulcers, is present, PPIs are given with antibiotics to eradicate H. pylori. The predominant use of PPIs is symptomatic treatment of GERD and gastritis. For gastroesophageal reflux, which causes heartburn and acid regurgitation, the American Gastroenterological Association recommends that patients first try lifestyle modifications and antacids or over-the-

counter histamine-2 receptor antagonists (H2-RAs, commonly called "H2-blockers"). If these steps do not completely control heartburn symptoms, PPIs or high doses of H2-RAs may be prescribed. Many clinicians use H2-RAs as the initial therapy for gastroesophageal reflux.

1.1 Microspheres introduction[16]

Microspheres were introduced long back ago to improve the drug carrying capacity and they are classified to different categories according to their character as follows:

1) Embedded agents, 2) High porosity, 3) Precisely fitting diameters, 4) High precision, coated, high density and activated, doped. The current study deals with the coated Microspheres which they are biodegradable and polymer coated.

1.2 Micro encapsulation methods[15,28]

Air suspension, Coacervation, phase separation, Multiorifice-centrifugal process, Spray drying and congealing, Pan coating, Solvent evaporation techniques, Polymerization.

2. MATERIALS

Pantoprazole sodium was bought from Darvin (P Ltd.)Vijayawada. Eudragit RS 100 was bought from National scientific, Guntur. HPMC, sod. Alginate, liquid paraffin, isopropyl alcohol, sodium hydroxide, acetone and dichloromethane was purchased from National scientific, Guntur. All the chemicals were of analytical grade and double distilled water was used throughout the experiment.

2.1 INSTRUMENTS

U.V double beam Spectrophotometer, GC-MS-SPME, Sonic bath, Rotary solvent evaporator, Magnetic stirrer, Dissolution apparatus.

2.2 .Standard Reaction Conditions

All Microspheres preparation methods carried out at room temperature. And the evaporation process at 60^{o} C.

3. METHODS [15,28]

3.1 Techniques for Microsphere Production

a)Solvent evaporation[7]**and solvent extraction**:

There are different methods to use microencapsulation by solvent evaporation technique. The choice of the method that will give rise to an efficient drug encapsulation depends on the hydrophilicity or the hydrophobicity of drug. For insoluble or **poorly water-soluble drugs**, the **oil-in-water (o/w) method** is frequently used. This method is the simplest and the other methods derive from this one. It consists of four major steps[17,18] Dissolution of the hydrophobic drug in an organic solvent containing the polymer[19] emulsification of this organic phase, called dispersed phase, in an aqueous phase called continuous phase[20] extraction of the solvent from the dispersed phase by the continuous phase, accompanied by solvent evaporation, transforming droplets of dispersed phase into solid particles[20] recovery and drying of microspheres to eliminate the residual solvent.

3.2. Preparation of double walled microspheres[19,20]

The double walled microspheres were prepared by two step process. In first step the core microspheres of sod. Alginate and HPMC were formulated. The microspheres then dispersed in the organic phase. The organic phase containing polymer in which drug was dissolved then the organic phase was emulsified with liquid paraffin. The solvent was allowed to evaporate and double walled microspheres were collected.

3.3. Formulation of core sodium alginate and HPMC microspheres with drug [47,49,50]

Microspheres were prepared by water in oil emulsification solvent evaporation technique. A 3% polymeric aqueous solution was made in which the drug was dispersed and then the solution poured into 200 ml of light liquid paraffin containing 0.5% span 20 as an emulsifying agent. The aqueous phase was emulsified in oily phase by stirring the system in a 500ml beaker. Constant stirring at 500-1000 rpm was carried out using magnetic stirrer. The beaker and its content were heated at 50^{0}C, stirring and heating were maintained for 4.5 hrs. The aqueous phase was evaporated. The microspheres were washed with n-hexane, separated and dried at room temperature.

3.4. Formulation of double walled microspheres[18,19,20]

The previously formulated microspheres were dispersed in the organic phase (methanol: dichloromethane 1:4). Pantoprazole and the second polymer eudragit RS 100 were dissolved in the same organic phase. The resulting organic phase solution was emulsified in liquid paraffin. 1% span 80

solutions were used as emulsifying agent. Above emulsion was stirred at 500-1000 rpm for 4 hrs for complete evaporation of the organic solution. After complete evaporation of the organic solution the double walled microspheres were collected by vacuum filtration and washed with 3-4 times with n-hexane. The resulted double walled microspheres were freeze dried for 24 hrs.

4. RESULTS AND DISCUSSION

4.1. Morphology and Particle size Determination[10,21]

The size was measured using a microscope with the help of projection microscope, and the mean particle by means of a calibrated stage micrometer with eye piece micrometer.

Procedure

Calibrate the eye piece micrometer using stage micrometer and find out the length of one division of eye piece micrometer. Prepare the slide by using a small quantity of treated microspheres and mount a drop of glycerin and cover with cover slip. Replace the stage micrometer with the prepared slide. Measure the diameter of the microspheres by observing the no. of divisions covered by microspheres.

Calculations

Scale length of stage micrometer = 1mm = 1000µ

1mm = 100 divisions = 1000µ

No. of divisions of stage micrometer = 100 divisions

100 divisions of stage micrometer = 1000µ

Therefore length of each division of stage micrometer

equal to 100 divisions = 1000µ

1 division = 1000 / 100 = 10µ = 0.01mm

Calibration of eye piece micrometer:

Focus stage micrometer and eye piece micrometer and find out the coincidence and do the measurement. 5th division of stage micrometer coincide with 16th division of eye piece micrometer

$$= \frac{\text{No. of divisions of stage micrometer}}{\text{No. of divisions of eye piece micrometer}} \times 10$$

$$= 5/16 \times 10$$

$$= 50/16$$

$$= 3.125\mu m$$

4.2. Surface morphology /Scanning Electron Microscopy (SEM)

The external morphology of the microspheres was studied by scanning electron microscopy using apparatus Philip 505.

4.3. Drug entrapment efficiency or incorporation efficiency

To determine the drug entrapment efficiency or incorporation efficiency the microspheres were crushed in glass mortar and powered, then suspended in 10 ml of methanol, after 24 hrs the solution was filtered and filtrate was analyzed for drug content. The drug incorporation efficiency was calculated by the following

$$Incorporation\ efficiency = \frac{b}{a} \times 100$$

b = calculated amount of drug present in the formulation,

a = theoretical amount of drug present in the formulation.

4.4. Mucoadhesion study

The in vitro mucoadhesive test was carried out using small intestine from chicken. The small intestinal tissue was excised and flushed with saline. Five centimeter segment of jejunum were everted using a glass rod. Ligature was placed at both ends of the segment. 100 microspheres were scattered uniformly on the everted sac from the position of 2 cm above.

Then the sac was suspended in a 10ml tube containing 8 ml of saline by the wire, to immerse in the saline completely. The sac were incubated at 37^{0}C and agitated horizontally. The sac were taken out of the medium after immersion for 0.5, 1, 1.5, 2, and 2.5 hrs, immediately repositioned as before in a similar tube containing 8ml of fresh saline and unbound microspheres were counted. The adhering percent was presented by the following equation.

Mucoadhesion= (no. of microspheres adhered/no. of microspheres Applied) ×100.

4.5. *Invitro* drug release of core microspheres[10]

The prepared formulation was evaluated for *invitro* release by USP dissolution apparatus 1 at 50 rpm and at 37^o C temperatures in order to determine 100% drug release. To evaluate microspheres containing pantoprazole were exposed to 900ml of phosphate buffer (pH 7.4). The samples were collected in pre-determined time intervals from 0 upto 540 min (9 hrs). Pantoprazole concentrations were determined by UV at 289 nm.

4.6. *Invitro* drug release of coated microspheres[21]

The prepared formulation was evaluated for *invitro* release by USP dissolution apparatus 1 at 50 rpm and at 37^{0}C in order to determine 100% drug release. To evaluate gastro resistant microspheres containing pantoprazole were exposed to 300ml of 0.1M HCl. After 1 hr, NaOH (2.6gm) and KH_2PO_4 (6.12gm) aqueous solution (600ml) was added into the medium in order to reach pH 7.4. The samples were collected in pre-determined time intervals from 0 upto 840 min (14 hrs). Pantoprazole concentrations were determined by UV at 289 nm.

4.7. Particle size of the drug loaded microspheres

The particle size and surface morphology was determined with the help of projection microscope Spherical shaped microspheres were observed with stage micrometer and particle size between 30.61µm to 33.5µm

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4.8. Surface morphology

Surface morphology of the core microspheres was examined by scanning electron microscopy (SEM) (PHILIP 505). It was observed that surface of the A1 microspheres were some rough, in comparison to A2, A3, A4 and A5 because it have the higher concentration of sod. Alginate. As the HPMC concentration increased the smoothness in shape of microspheres was observed, as shown in tab 3: A5 showed the least particle size 28.6±0.98µm because it contains higher proportion of HPMC which was due to spherical nature of the microspheres. A1 had the largest proportion of sod. Alginate, showed the largest particle size of 33.0±1.43µm. On increasing the proportion of HPMC the decrease in size of microspheres was observed, that was 33..0±1.43, 32.1±1.54, 30.5±1.65, 29.4±1.23 and 28.6±0.98 for formulation A1, A2, A3, A4 and A5 respectively. This may be due to of increase in availability of the polymer for entrapment of drug particles. A3 shows the particle size in between A4 and A1 because A3 contains the equal proportion of the sod.hpmc and sod. Alginate polymer, the rank order of size A5> A4> < A3>A2>A1.

Drug Entrapment Efficiency

In case of core microspheres, on increasing the concentration of HPMC. Polymer, the amount of drug entrapment will increase as it was observed maximum 74±1.43 in A5 and less 52±1.43 in A1 where the polymer to polymer ratio is 3:1 and 1:3 for HPMC and sod. Alginate, respectively. This was due to the HPMC shows good entrapment efficiency then the polymer sod. Alginate. The rank order of entrapment efficiency A5> A4> A3>A2> A1.

Effect on mucoadhesion

To assess the mucoadhesivity of the microspheres *invitro* wash off test was performed for all the formulations. At the end of 4hrs 15 min the percent mucoadhesivity was found 10, 15, 18, 23, 26 for formulation A1, A2, A3, A4 and A5 respectively, shown in table 4. Formulation A5 showed the highest mucoadhesivity due to the presence of higher proportion of HPMC polymer, due to the anionic nature of the polymer, and A1 showed the lowest mucoadhesivity due to higher proportion of sod. Alginate due to the irregular surface was increased.

***Invitro* drug release profile of core microspheres**[15,21]

These studies show the effect of environment of the body on the drug release pattern from the prepared microspheres. The *invitro* release

was observed in phosphate buffer (pH 7.4) for 9 hrs. It was found that the release rate from the all formulation was found to be different for the different polymer proportion used in the formulation 72.0%, 74.0%, 80.0%, 82.0% and 91.0% for formulation A1, A2, A3, and A4 and A5 respectively. shown in table 6, The A5 has highest proportion of polymer HPMC, showed maximum release. While the A1 shows the least drug release after 9 hrs. Due to less swelling action and irregular surface as compared to A5.

5. Evaluation of Double Walled Microspheres [110,21]

5.1. Particles Size and Surface Morphology

The particle size and surface morphology was determined with the help of optical microscope and scanning electron microscope. Smooth spherical shaped microspheres were observed with optical microscope and particle size between 65.952±1.31μm to 82.652±O.82μm. The change in particle size was observed only for some extent.

5.2. *In vitro* drug release profile of double walled microspheres

These studies show the effect of environment of the body on the drug release pattern from the prepared microspheres. The *invitro* release first determined in the pH 1.2 for 2 hrs, all formulation shows no drug release at this pH. Then the pH was increased to 7.4 Phosphate buffer (pH 7.4) for 14hrs. It was found that the release rate from the all formulation was found to be different for the different polymer proportion used in the formulation. 91.352±0.93, 90.452±1.13, 86.252±1.63, 81.152±1.03 and 75.452±1.56% for formulation B1, B2, B3, B4 and B5 respectively. This may be due to of increase in availability of the polymer for entrapment of drug particles. The B1 has lower proportion of polymer eudragit RS 100 showed maximum release, while the B4 shows the least drug release after 14 hrs due to less swelling action and irregular surface as compared to B1.

5.3. Drug release profile of different formulations showing the effect of polymer on drug release from coated microspheres.

After evaluating all the formulation, the formulation A5 which is containing the higher percentage of HPMC showed the goods entrapment efficiency, mucoadhesion, good drug release profile. Therefore it was selected as the best formulation. Then the walled microspheres was formulated by varying concentration of eudragit RS 100, there five formulations was formulated B1, B2, B3 and B4 from B5, on analyzing the all the formulations, B1 was found as best formulation.

6.0. CONCLUSION

Double walled microspheres of pantoprazole were prepared and evaluated. The microspheres thus obtained were subjected to different tests such as particle size, drug entrapment efficiency, mucoadhesive property, release study of core and coated microspheres etc. from this we can conclude that the formulation B1 was considered as the best formulation as the percentage drug release was found to be 91.352% in the presence of phosphate buffer of pH 7.4 after 14hrs which is the greatest among all.

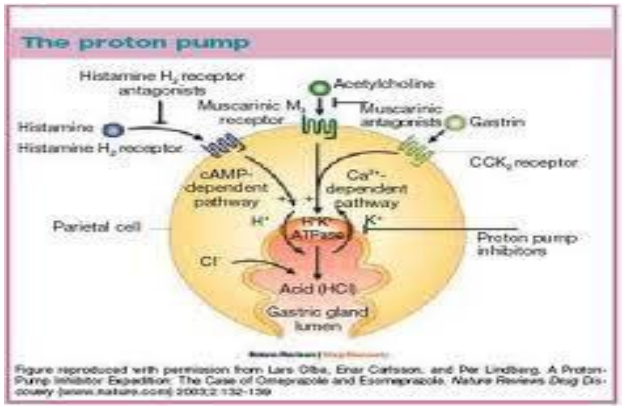


Fig. 1: Mechanism of Action of PPIs

Fig. 2: Basic Steps of Micro Encapsulation by Solvent Evaporation Method

Table 1: Various core formulations using sod. Alginate and HPMC polymer

S. No	formulation	drug (w/w)	hpmc (w/w)	Sod. alginate (w/w)
1	A1	1	1	3
2	A2	1	1.5	2.5
3	A3	1	2	2
4	A4	1	2.5	1.5
5	A5	1	3	1

Table 2: Showing Various Formulations of Coated Microspheres

S. No	Formulation	Core to coat ratio (w/w)
1	B1	1:0.5
2	B2	1:0.75
3	B3	1:1
4	B4	1:1.5
5	B5	1:1.75

Table 3: Showing Different Particle Sizes of the Core Microspheres

S.No	Formulation	Particle size			Mean	Standard deviation
1	A1	31.57 µm	33.0 µm	34.43 µm	33.0 µm	1.43
2	A2	30.56 µm	32.1 µm	33.64 µm	32.1 µm	1.54
3	A3	28.85 µm	30.5 µm	32.15 µm	30.5 µm	1.65
4	A4	28.17 µm	29.4 µm	30.63 µm	29.4 µm	1.23
5	A5	27.62 µm	28.6 µm	29.58 µm	28.6 µm	0.98

Table 4: Showing Particle Size, Percentage Drug Entrapment and Percentage Mucoadhesion

S. No	Formulation	Particle size (µm)	% of drug entrapment	% of mucoadhesion
1	A1	33..0±1.43	52±1.43	80±2.4
2	A2	32.1±1.54	57±1.43	82±0.98
3	A3	30.5±1.65	66±1.43	84±1.45
4	A4	29.4±1.23	70±1.43	86±0.97
5	A5	28.6±0.98	74±1.43	88±1.20

*Results shown are the mean ±S.D. n=3

Table 5: Determination of Calibration of Pantoprazole

S.No	Concentration	Absorbance
1	2 µm	0.020 nm
2	4 µm	0.033 nm
3	6 µm	0.049 nm
4	8 µm	0.065 nm
5	10 µm	0.076 nm

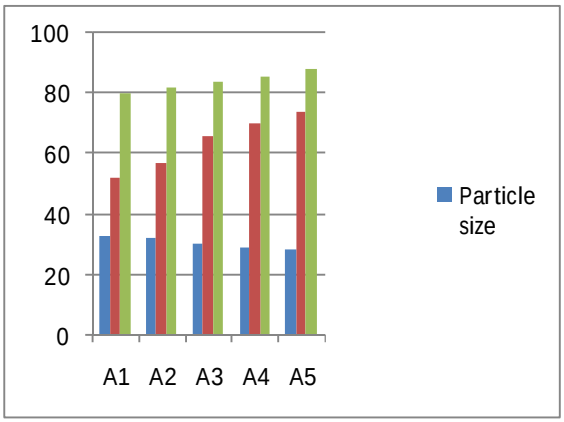
Blank = 0.000 nm, Slope =0.008,
r value = 0.9912

Table 6: Drug Releases of Core Microspheres After 9hrs

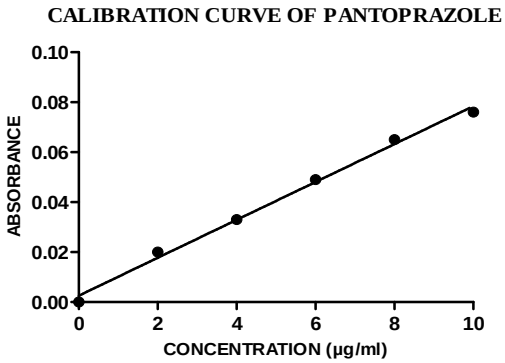
S.no	Formulation	Ratio of hpmc & Sod. alginate	Percentage drug release (9hrs)
1	A1	1:3	72.0
2	A2	1.5:2.5	74.0
3	A3	2:2	80.0
4	A4	2.5:1.5	82.0
5	A5	3:1	91.0

Table 7: For Particle Size and Percentage Drug Release After 14 hrs

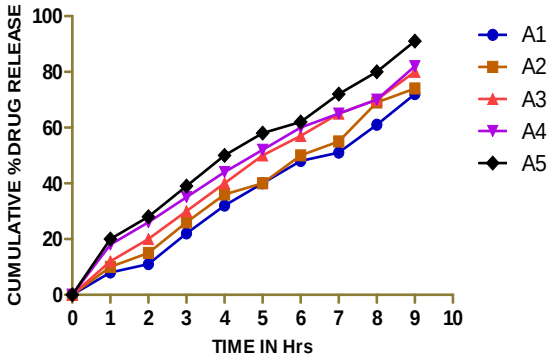
S. No	Formulation	Core : Coat	Particle Size (um)	Percentage Drug Release (14 Hrs)
1	B1	1;0.5	65.952±1.31	91.352±0.93
2	B2	1:0.75	70.552±0.97	90.452±1.13
3	B3	1:1	75.252±0.79	86.252±1.63
4	B4	1.1.5	78.452±1.25	81.152±1.03
5	B5	1.1.75	82.652±O.82	75.452±1.56



Graph 1: Showing Entrapment Efficiency, Particle Size and Percentage Mucoadhesion



Graph 2: Calibration Curve of Pantoprazole



Graph 3: Invitro Drug Release Profile of Different Formulations Showing the Effect of Drug and Polymer on Drug Release from Core Microspheres of Sod. Alginate and HPMC

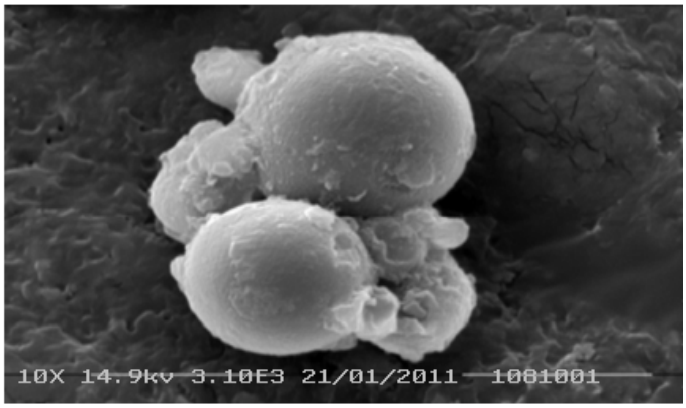


Fig. 3: SEM photograph of core microspheres (Formulation A1)

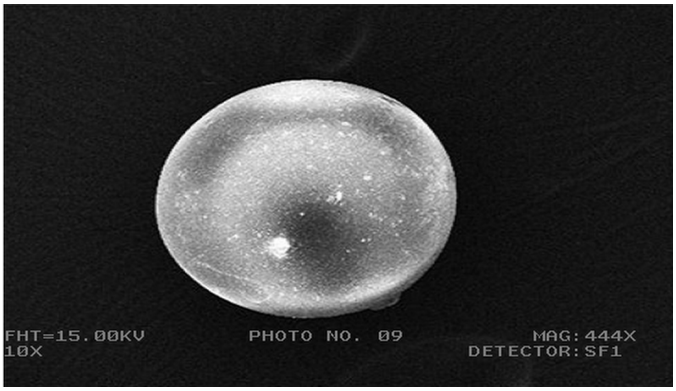
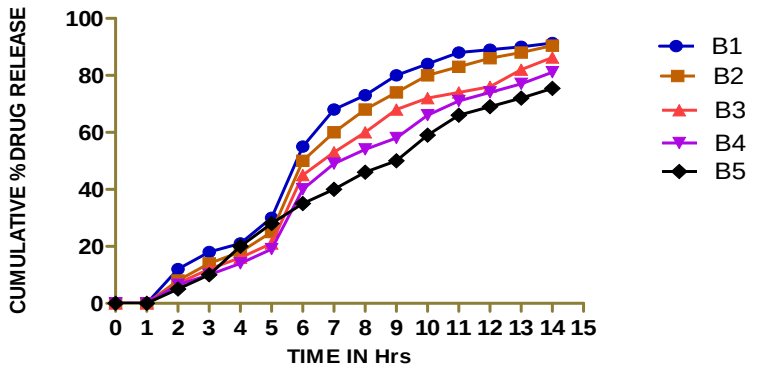


Fig. 4: SEM Photograph of Coated Microspheres (Formulation B1)



Graph 4: Invitro drug release profile of different formulations showing the effect of polymer on drug release from coated microspheres.

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