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Development and Evaluation of Mucoadhesive Microspheres of Atenolol

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Abstract

Atenolol is a β-selective adrenergic blocking agent which is prescribed widely in diverse cardiovascular diseases like hypertension, angina pectoris, arrhythmias and myocardial infractions, Atenolol shows maximum absorption in the upper GI regions, also shows bioavailability 40% orally, half life 4-5 hr. Microsphere formulations have advantage over conventional tablet or capsule formulations, since it increases the surface area exposed to the absorption site and thus increasing the absorption of the drug and decreasing the dosing frequency of the drug. For the drugs which show the low absorption in lower part of GIT or having absorption window in the stomach. Thus, Atenolol is a suitable candidate for the development of a gastro retentive drug delivery system. In order to enhance the extent of absorption and to reduce dose, side effect mucoadhesive microspheres were formulated and developed. In this study a bioadhesive microsphere of Atenolol were prepared by emulsion chemical cross linking technique using combination of different polymers (The result of *in vitro* drug release study showed 85 % drug release up to 12 hrs and better mucoadhesion of microsperes

Key words: Mucoadhesive microspheres, Gastro retentive dosage form, Atenolol

Introduction

An ideal controlled delivery system is one which delivers the drug at a predetermined rate, locally or systemically, for a specified period of time. The basic rationale of a controlled drug delivery system is to optimize the biopharmaceutic, pharmacokinetic and pharmacodynamic properties of a drug in such a way that its utility is maximized through reduction in side effects and cure or control of condition in the shortest possible time by using smallest quantity of drug, administered by the most suitable route. [(1).]

The major obstacle in development of controlled release formulation is absorption window in upper GIT and gastric emptying time. Therefore, control of placement of a drug delivery system (DDS) in a specific region of the GIT offers advantages for a variety of important drugs characterized by a narrow absorption window in the GIT or drugs with a stability problem [(2).] These considerations have led to the development of a unique oral controlled release dosage form with gastro retentive properties. Prolonged gastric retention improves bioavailability, reduces drug waste and improves solubility of drugs that are less soluble in a high pH environment.

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It is also suitable for local drug delivery to the stomach and proximal small intestines [(3).] Several approaches have been studied to prolonging the residence time of the dosage at the absorption site and the development of oral controlled release bioadhesive system. Various gastro retentive dosage forms, such as microspheres, and bilayer tablets, have been thoroughly prepared and reported by several research groups [(4).]

Microspheres constitute an important part of these drug delivery systems by virtue of their small size and efficient carrier capacity. However, the success of these microspheres is limited due to their short residence time at site of absorption. It would, therefore, be advantageous to have means for providing an intimate contact of the drug delivery system with the absorbing membranes. This can be achieved by coupling bioadhesion characteristics to microspheres and developing bioadhesive microspheres [(5).]

Atenolol is a β-selective adrenergic blocking agent which is prescribed widely in diverse cardiovascular diseases like hypertension, angina pectoris, arrhythmias and myocardial infractions, Atenolol shows maximum absorption in the upper GI regions, also shows bioavailability 40% orally, half life 4-5 hr[(6)]Administration of conventional dosage form of Atenolol has been reported to exhibit fluctuation in the plasma drug level resulting either in manifestation of side effect or reduction in the drug concentration at the

receptor site. So, the development of oral controlled release dosage form would clearly be advantageous. Moreover the site absorption of Atenolol is in the stomach and proximal portion of the small intestine, the dosage form that is retained in the stomach would increase the absorption, improve drug efficiency and decrease dose requirement[4].

Thus, Atenolol is a candidate for the development of a gastro retentive drug delivery system Atenolol is better absorbed from gastric region, hence it is need to develop gastro retentive drug delivery system i.e. mucoadhesive microspheres.

Material and Methods

Atenolol was gifted by (API) Cipla Ltd., Pune. Chitosan, Gaur Gum, Heavy Liquid paraffin and Glutaraldehyde was gifted by Research lab, Poona. Petroleum ether, Karaya Gum, Xanthan Gum and Acetic acid was gifted by Themis Research lab, Mumbai. Light liquid paraffin was gifted by Sisco research lab, Mumbai. DOSS was provided by Research lab, Poona.

Preparation of Mucoadhesive of Atenolol

Preparation: Mucoadhesive microspheres of Atenolol were prepared by emulsification phase separation technique, described by thanoo et al [7] using chitosan and different gums in combinations.

ProcedureAll the ingredients including drug, polymer and excipients were weighed accurately according to the batch formula (Table 1). Chitosan was dissolved in 1% V/V aqueous acetic acid solution. Drug added in polymer solution and the resultant mixture was extruded through a syringe in liquid paraffin (heavy and light 1:1 ratio) containing dioctyl sodium Sulphosuccinate and stirring was

performed using magnetic stirrer, after 10min glutaraldehyde (25% aqueous solution) was added and stirring was continued for 1 h, microspheres thus obtained were filtered washed several times with petroleum ether to remove traces of oil. They were finally washed with water to remove excess of glutarldehyde, dried at room temperature. Formulations B were prepared using same method.

A mixture of chitosan and xanthan gum in aqueous acetic acid solution precipitates hence modified method used for preparation of microspheres of combination of chitosan and xanthan gum (formulation D). In modified method, chitosan added in aqueous acetic acid solution and xanthan gum added in water and both solution added into the oil phase dropwise seperatly but simultaneously. Same modified method is used for formulation C also (7, 8, 9, 10)

Evaluation of formulations:

Particle Size: [7, 8]

The particle size of the microspheres was determined by using Digital electron microscopy method [8]. Approximately 50 microspheres were counted for particle size using a calibrated digital electron microscope.

Drug Entrapment Efficiency: [7, 8]

Microspheres were crushed in a glass mortar and pestle, and the powdered microspheres were suspended in 100 mL of acidic buffer (pH 1.2). After 24 h, the solution was filtered and the filtrate was diluted suitably and absorbance of solution was recorded using UV spectrophotometer at 224 nm and the value obtained used to determine the practical drug content. The drug entrapment efficiency was calculated using the following formula.

Entrapment efficiency = Practical drug content / Theoretical drug content × 100

Drug Release Study: [4, 7]

The drug release study was performed using USP paddle apparatus (Electrolab Model: TDT-08L) at 37°C±0.5°C and at 100 rpm using 900 mL of acidic buffer (pH 1.2) as a dissolution medium. Microspheres were placed in a dissolution vessel. 5mL of sample solution was withdrawn at predetermined time intervals, diluted suitably, and the absorbance of the sample was recorded using UV spectrophotometer at 224 nm [4]. An equal amount of fresh dissolution medium was replaced immediately after withdrawal of the test sample. The % drug dissolved was calculated using disso software (PCP V3).

***In Vitro* Wash-off Test for Microspheres:** [7]

The mucoadhesive properties of the microspheres were evaluated by *in vitro* wash-off test as reported by Lehr et al [7]. A 1-cm by 1-cm piece of rat stomach mucosa was tied onto a glass slide (3 X 1 -inch) using thread. Microspheres were spread (50) onto the wet, rinsed, tissue specimen, and the prepared slide was hung onto one of the groves of a USP tablet disintegrating test apparatus (libinda Mumbai). The disintegrating test apparatus was operated such that the tissue specimen was given regular up and down movements in a beaker containing the acidic buffer pH 1.2. At the end of 3 h, 6 h, 9 h and 12 h, the number of microspheres still adhering onto the tissue was counted.

Equilibrium Swelling Studies of Microspheres: [9, 12]

A preweighed amount (100 mg) of microspheres was placed in acidic buffer pH 1.2 and allowed to swell up to constant weight. The microspheres were removed and blotted with filter paper, and their changes in weight were measured. The degree of swelling (α) was then calculated from the following formula:

$$\alpha = Wg - Wo / Wo$$

Where Wo is the initial weight of the microspheres and Wg is the weight of the microspheres at equilibrium swelling in the medium.

FTIR Spectroscopy:

The interaction between the drug and polymers was studied by using the FTIR spectroscopy, where in IR spectra of atenolol and physical mixture of atenolol with polymers were carried out using the Ker disk method (2 mg sample in 200 mg Ker). The scanning range was 400 to 4000 cm^{-1} and the resolution was $1cm^{-1}$.

Differential Scanning Calorimetric (DSC):

The possibility of any interaction between Atenolol and polymers used in microsphere formulation was assessed by carrying out the thermal analysis of Atenolol and the physical mixture of the polymers along with the Atenolol. The thermal behavior of plane Atenolol and the physical mixtures were studied using differential scanning calorimeter at heating rate of 10^0 C/min. The measurements were performed at a heating range of 30 to 300^0C under nitrogen atmospheres.

Results and Discussion

Table 1: Formulations of Mucoadhesive microspheres of Atenolol

Ingredients	Formulation			
	A	B	C	D
Atenolol (mg)	100	100	100	100
Chitoson (mg)	250	200	200	200
Guar gum(mg)	-	50		
Karaya gum(mg)	-	-	50	
Xanthan gum(mg)	-	-		50

Table 2: Particle size data

Formulation	Particle size (μm)
A	30-40
B	25-45
C	20-40
D	20-40

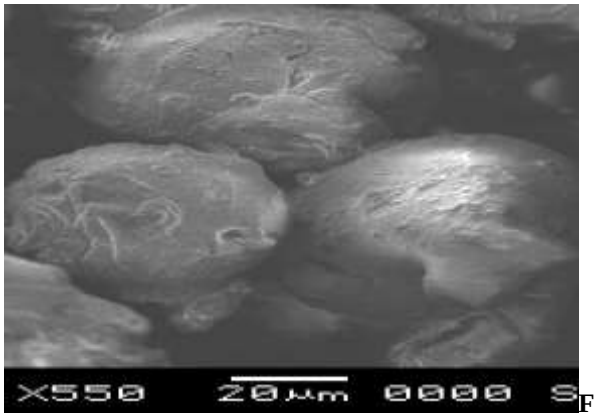


Fig. 1: Shape and size of microspheres

Entrapment efficiency of preliminary formulation:

The drug entrapment efficiency of formulation A in which only chitosan used as polymer was 30%. Formulation B in which combination of chitosan and guar gum used shows 40% entrapment efficiency. Formulation C in which combination of chitosan and karaya gum, formulation D in which combination of chitosan and xanthan gum used shows 42%,44% entrapment efficiency resp. It indicate that combination of chitosan and different natural gums increases entrapment efficiency. combination of chitosan and guar gum shows only slight change in entrapment efficiency and this may be due to high viscosity of guar gum but both being positively charge (14) and hence no significant increase in entrapment efficiency.When combination of chitosan and xanthan gum used it significantly increase entrapment efficiency this may be due to negatively charged xanthan gum which interact with positively charged chitosan.

Table 3: Entrapment efficiency data

Sr.No	Formulation	* % Drug entrapment efficiency
1	A	35±4.032
2	B	40±1.188
3	C	42±2.347
4	D	44±3.031

*Represents mean ± S.D. (n = 3)

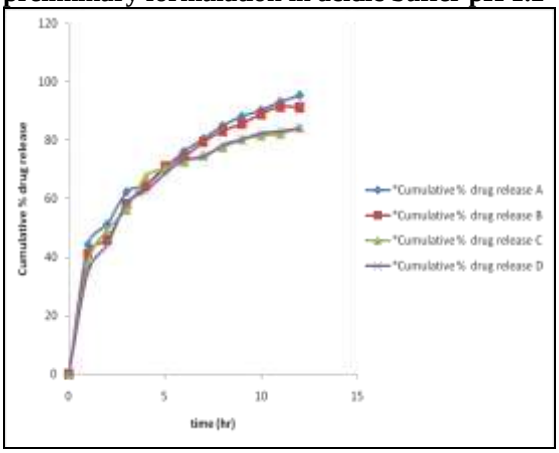
***In vitro* disolution study of formulations:** The preliminary formulations were characterized for their drug release in acidic buffer pH 1.2, the preliminary formulations were prepared for the purpose to do comparative study between chitosan alone and combination of chitosan and different gums. Drug release after 12 h of formulation A was found to be

95%, formulation B was 91%. This indicate that chitosan alone shows fast drug release while combination of chitosan with xanthan gum and karaya gum retard drug release and thus prolong drug release.

Table 4:% *In vitro* drug release profile of preliminary formulations in acidic buffer pH 1.2

Time (h)	*Cumulative % drug release			
	A	B	C	D
0	0.000	0.000	0.000	0.000
1	44.251±0.524	41.022±0.245	38.235±0.354	35.102±0.235
2	51.254±0.635	46.058±0.541	49.326±0.362	46.023±0.365
3	62.325±0.421	58.215±0.621	56.224±0.521	58.356±0.654
4	64.652±0.365	64.256±0.841	67.548±0.632	62.634±0.246
5	70.542±0.245	71.025±0.209	70.235±0.523	68.582±0.812
6	76.235±0.654	74.365±0.621	72.543±0.125	73.215±0.294
7	80.541±0.256	79.425±0.501	74.851±0.231	75.024±0.345
8	85.025±0.254	83.254±0.421	77.542±0.356	78.231±0.631
9	88.211±0.245	85.665±0.632	80.214±0.624	80.245±0.601
10	90.341±0.334	89.012±0.521	81.5478±0.701	82.315±0.725
11	93.215±0.654	91.542±0.432	82.12±0.245	83.012±0.812
12	95.421±0.921	91.203±0.632	84.245±0.552	84.023±0.905

Figure 2: % *In vitro* drug release profile of preliminary formulation in acidic buffer pH 1.2



***In Vitro* Wash-off Test for Microspheres** [7] : The bioadhesive strength was determined by the in vitro wash off test as reported by Lehr et al [7].

Table 5: Bioadhesive strength of preliminary formulations

Formulation	Number of microspheres adheres on gastric mucosa after				% Bioadhesive strength
	3h	6h	9h	12h	
A	43	37	31	27	69
B	42	39	33	28	71
C	40	38	30	25	66
D	40	36	31	24	65

Formulation A shows 69% bioadhesive strength, formulation B shows 71% bioadhesive strength formulation C and D shows 66% and 65% bioadhesive strength resp. It indicated that combination of chitosan and guar gum microspheres shows highest bioadhesive strength than other formulations. The G.I.T. mucoadhesive properties of chitosan were in a large part attributed to the electrostatic attraction. chitosan and xanthan gum form cross linking with each other and due to this diffusion of water in polymer network occurs at a lower rate which in turn ,causes an insufficient swelling of polymer complex(12). And in order for strong bonds to develops it is essential that polymer able to establish intimate contact with mucus layer by swelling and absorbing water mucus layer

Swelling study: microspheres formed by combination of chitosan and karaya gum and xanthan gum shows less swellability as the charged group in this polymer react with each other to form complex.

Table 6: Data of swelling study of preliminary formulations.

Sr.No	Formulation	Degree of swelling
1	A	1.56
2	B	1.66
3	C	1.50
4	D	1.54

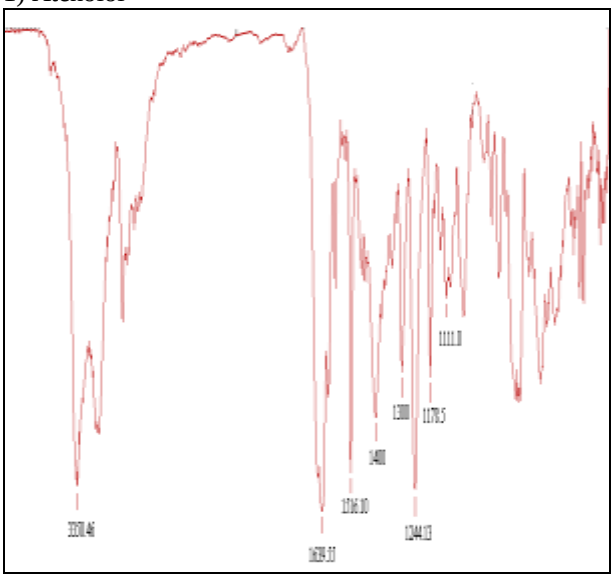
The interaction between drug and polymer was study by FTIR & DSC:

The Drug – Excipients interaction Studies were performed in order to confirm the compatibity of drug in presence of excipients. The Drug – Excipients Compatibility study include FT-IR and DSC studies.

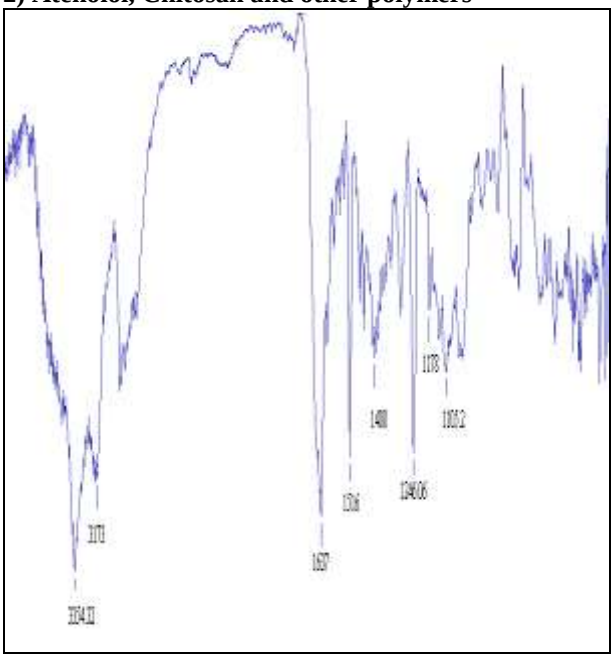
FTIR Spectroscopy: FT-IR spectra of pure, Atenolol physical mixtures of polymers with drug from the figure it was indicated that there is no interaction between drug and polymers because the IR spectra of all physical mixtures retains the main peaks of Atenolol

Figure 3: FTIR Spectra of 1] Atenolol 2] physical mixture of Atenolol, chitosan and other polymers.

1) Atenolol



2) Atenolol, Chitosan and other polymers



Differential Scanning Calorimetry:

The DSC curves of pure atenolol, and physical mixture of atenolol along with the polymers used in formulation shown in Figure 9.5 & 9.6 respectively. Atenolol showed a characteristic sharp endothermic peak at 156.49^{0}C, indicating the melting point of the drug. The obtained DSC curves for the physical mixture of drug with polymers shows the endothermic peak at 153.83^0 C of atenolol and the broad endothermic peak in range 70^0-80^{0}C due to the water associated with polymers. The results of DSC study are revealed that there was no any interaction between atenolol and polymers.

Figure 4: DSC curve of pure Atenolol

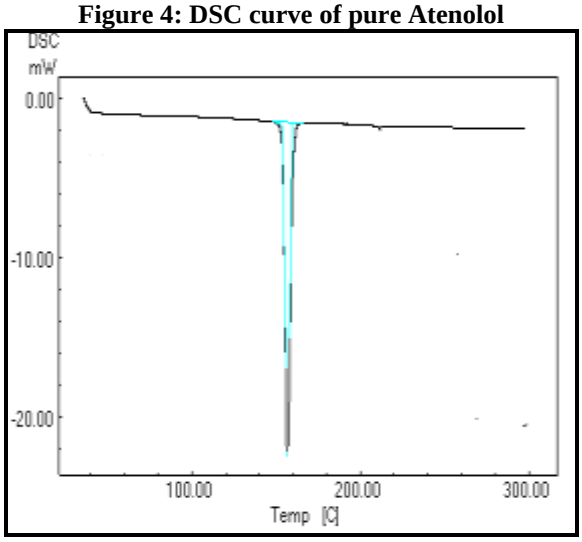
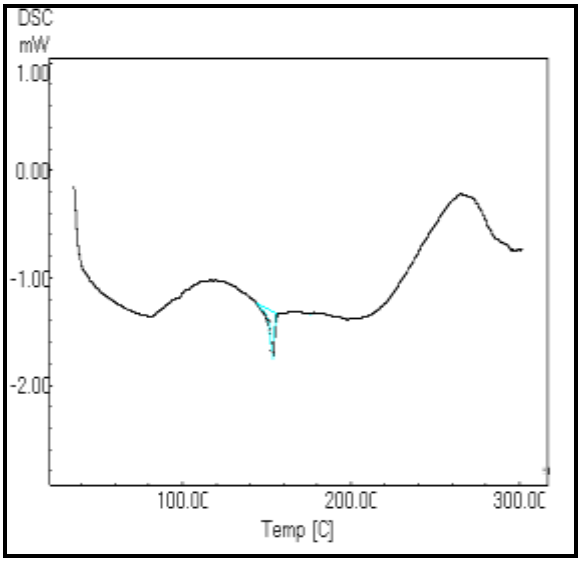


Figure 5:.DSC curve of Atenolol with physical mixture of polymers used in formulation



Stability study: There was no significant change in entrapment efficiency and particle size of drug loaded microspheres stored up to 60 days**.**

***Ex vivo* studies**: % bioadhesive strength shows that large number of microspheres adheres to stomach after

4h while number of microspheres adhering to stomach decreases as the time interval increase from 4 h to 8 h and 12 h.

Conclusion

The results of the present study indicate that mucoadhesive microspheres of Atenolol with controlled drug release can be successfully prepared by emulsification phase separation technique using combinations of different polymers. It exhibited well controlled and delayed release pattern. This study concludes that, all the four formulations have good drug release up to 12 hrs. Combination of chitosan and anionic polymers like xanthan gum and karaya gum were retard release. Combination of chitosan and other polymers does not affect mucoadhesive strength much more. Chitosan microspheres showed 35% entrapment efficiency while microspheres containing combination of chitosan and xanthan gum showed highest entrapment efficiency (44%).

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References

1. Brahmankar DM, Jaiswal SB. Biopharmaceutics and Pharmacokinetics, A Tretise, 1st Edn., Vallabh Prakashan, Delhi.1995; 337-41.
2. Singh BN, Kim KH. Floating drug delivery systems: an approach to oral controlled drug delivery via gastric retention. J Control Release. 2000; 63: 235-9.
3. Ali J, Arora S, Khar RK. Floating drug delivery System: A Review. AAPS Pharm Sci Tech.
4. R. natarajan*, G .Elishaba Raj, M.Rangapriya and N N.Rajendran,,' optimization and evaluation of mucoadhesive microspheres of atenolol ',International journal of research in pharmacy and chemistry 1(3);722-729
5. Rishi RK. Gastroretentive Drug Delivery System. The Pharm Review. 2007;
6. Indian Pharmacopoeia 1996, Government of India, ministry of health and family welfare's, Vol-I, published by the controller of publications, The Indian pharmacopoeia commission Ghaziabad,:587-588.
7. Patel JK, Patel MM. Stomach specific Anti Helicobacter Pylori Therapy: Preparation and Evaluation of Amoxicillin-Loaded Chitosan Mucoadhesive Microspheres. Current Drug Del. 2007; 4: 41-50.
8. Patel J.K, Patel R.P, Amin A.F, Patel M.M. 2005. Formulation and Evaluation of Mucoadhesive Glipizide Microspheres. AAPS PharmSciTech. 06(01): E 49-E55. DOI: 10.1208/pt0601109
9. Chaurasia M, Chourasia M.K., Jain N.K. 2006. Cross-Linked Guar Gum Microspheres: A Viable Approach for Improved Delivery of Anticancer Drugs for the Treatment of Colorectal Cancer. AAPS PharmSciTech. 7(3): Article 74. DOI: 10.1208/pt070374
10. Brown MB, Lim ST, Martin GP, Berry DJ. Preparation and Evaluation of the *in vitro* Drug Release properties and Mucoadhesion of Novel Microspheres of Hyaluronic acid and Chitosan J Controlled Rel. 2000; 66: 281–92
11. Kazuhiro M, Jian W, Yasuhiko T, Dianzhou B. Evaluation of Gastric Mucoadhesive properties of Aminated gelatin Microspheres. J Controlled Rel. 2000; 73: 223–31.
12. Martin Loa, Sanem Argin-Soysala, Peter Konasb Y. Effect of Complexation conditions on Xanthan–chitosan Polyelectrolyte Complex Gels. Food Hydrocolloids. 2009; 23: 202-9
13. Guang-Hui Maa, Lian-Yan Wang a, Yong-Hong Gub, Zhi-Guo Sua. Preparation and Improvement of Release behavior of Chitosan Microspheres containing Insulin Int J Pharmaceutics. 2009; 311: 187–195.
14. Thakkar HP, Murthy RR. Effect of Cross-linking agent on the characteristics of Celecoxib loaded Chitosan Microspheres. Asian J pharmaceutics. 2008; 2: 246-51.

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