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Development and evaluation of efavirenz mucoadhesive microspheres

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ABSTRACT

The aim of the present research work was to formulate and evaluate methyl cellulose, HPMC, and HPMC K15 mucoadhesive microspheres for controlled release of Efavirenz. The mucoadhesive microspheres were produced by Ionotropic gelation technique using Methyl cellulose, HPMC, and HPMC K15 in combination with sodium alginate as mucoadhesive polymer in various proportions in combination. Further, the prepared Efavirenz mucoadhesive microspheres were characterized for particle size, morphology, Efavirenz entrapment efficiency, mucoadhesion, *in vitro* drug release, release kinetics and compatibility studies (FTIR &DSC). The Efavirenz Microspheres were discrete and free-flowing. The mean particle size ranged from 707.60 ± 8.32 µm to 921.7 ± 6.03 µm and the entrapment efficiencies ranged from 44.76 to 90.80 %. The Efavirenz entrapment efficiency was found to be dependent on nature and quantity of mucoadhesive polymer used for formulation. Scanning electron microscopy revealed the surface morphology .The FTIR & DSC confirmed stable character of Efavirenz in the drug-loaded mucoadhesive microspheres. The mechanism of Efavirenz release from the mucoadhesive microsphere was found to be anomalous and super case-II transport type. Stability studies were done for the best formulation F12 indicates that there is no change in entrapment efficiency of the formulation. HPMC K15 mucoadhesive microsphere containing Efavirenz was prepared successfully by using an Ionotropic gelation technique.

Keywords: Methyl Cellulose HPMC, HPMC K15M, Efavirenz, mucoadhesive microspheres..

1. INTRODUCTION

The oral route is the most preferred and widely accepted route of administration by the patients and preferred means of delivery of drugs to systemic circulation.[1,2] However oral administration of many of the therapeutic agent in conventional dosage forms has drawbacks such as extensive presystemic elimination, inability to restrain and localize the system at gastro-intestinal tract.[3] In order overcome this draw backs, it has been proposed to coupling the therapeutic agent to mucoadhesive polymeric carrier systems because of their propensity to interact with biological surface for local or systemic drug delivery. [4,5]

Efavirenz is an antiretroviral agent used in treatment of HIV and viral dieses belongs to class II under BCS and exhibit low and variable oral bioavailability due to poor aqueous solubility. [6, 7] Because of its low biological half life and absorbed primary in stomach make it suitable candidate for administration by gastrorentensive dosage form. A novel promising technology for enhancing the bioavailability is a combination of mucoadhesion principle and microsphere technology to result in mucoadhesion microcapsule. [8, 9]

The objective of the present study was to prepare and evaluate mucoadhesive microspheres for enhancing the dissolution rate and bioavailability of efavirenz. The multiunit Microparticulate oral drug delivery systems can be distributed widely throughout the gastrointestinal tract providing a possibility of achieving a longer lasting release of drug at the desired rate thereby reduced dosing frequency and improved patient compliance.

2. MATERIAL AND METHODS

2.1. Materials

Efavirenz, Sodium alginate, Methyl cellulose, HPMC and HPMC K15 polymers were received as gift sample Aurobindo Pharma Ltd, Hyderabad. All other ingredients used were of analytical grade.

2.2. Formulation of Efavirenz mucoadhesive microspheres

The Efavirenz mucoadhesive microspheres were prepared by Ionotropic external gelation technique, the composition of various formulations were mentioned in Table1. Efavirenz and mucoadhesive polymers were individually passed through sieve ≠ 60. The required quantities of mucoadhesive polymers were dissolved in purified water to form a homogenous polymer solution. Efavirenz was added to the polymer solution and mixed thoroughly with stirrer at 400 rpm to form a homogeneous dispersion. The resulting homogeneous dispersion was sonicated for 30 min to remove any air bubbles .For the formation of microspheres the dispersion was then extruded manually drop wise into aluminum sulphate solution (10%) using polyethylene syringe (needle size 24 G). The extruded droplets were retained in the aluminium sulphate solution for 30 minutes to complete the curing reaction and to produce spherical rigid Efavirenz microspheres. [10] The obtained microspheres were collected by decantation, washed repeatedly with distilled water to remove excess aluminum impurity and dried at 45°C for12 hour.

2.3. Percentage yield

The percentage yield of Efavirenz Mucoadhesive microsphere of various batches was calculated by using the weight of final product after drying. The weight of dried Efavirenz microspheres (W1) was divided by initial total weight of the drug and polymers (W2).

2.4. Particle Size

Particle size and size distribution of the Efavirenz microspheres were measured by sieve analysis method. The Efavirenz microspheres were separated into different size fractions (% mass fraction) by sieving for 5 min using standard sieves having nominal mesh opening of 1.4 mm, 1.2 mm, 1.0 mm, 0.85 mm and 0.71 mm and the mean particle size of the Efavirenz microspheres was determined (Table 2). [11]

2.5. Morphology of Microspheres

The surface morphology and shape of the Efavirenz microspheres was confirmed by scanning electron microscopy using SEM Model – Philips-XL 20. The sample was mounted on to an aluminum stub and sputter-coated with platinum particles in an argon atmosphere.

2.6. Drug Entrapment Efficiency

Entrapment efficiency of prepared Efavirenz microsphere was estimated by method of extraction of drug present in microsphere. The dried efavirenz microspheres (100mg) were taken and extracted in 100 ml of 0.1N HCl for 24 hours in rotary shaker. The solution was filtered through a 0.45 µm filter and the concentration of Efavirenz present in filtrate determined spectrophotometrically at 245 nm (LABINDIA UV-3000+ PC). [12]

Table - 1: Composition of efavirenz mucoadhesive microspheres

Formulation code	Drug: Polymer ratio	Polymer ratio
F1	1:0.5	0.25:0.25 (Sodium alginate: Methyl Cellulose)
F2	1:1	0.5:0.5 (Sodium alginate: Methyl Cellulose)
F3	1:1.5	0.75:0.75 (Sodium alginate: Methyl Cellulose)
F4	1:2	1.0:1.0 (Sodium alginate: Methyl Cellulose)
F5	1:0.5	0.25:0.25 (Sodium alginate: HPMC)
F6	1:1	0.5:0.5 (Sodium alginate: HPMC)
F7	1:1.5	0.75:0.75 (Sodium alginate: HPMC)
F8	1:2	1.0:1.0 (Sodium alginate: HPMC)
F9	1:0.5	0.25:0.25 (Sodium alginate: HPMC K15)
F10	1:1	0.5:0.5 (Sodium alginate: HPMC K15)
F11	1:1.5	0.75:0.75 (Sodium alginate: HPMC K15)
F12	1:2	1.0:1.0 (Sodium alginate: HPMC K15)

2.7. Mucoadhesive test

The mucoadhesive property of Efavirenz microspheres was evaluated by in vitro wash off test. The freshly excised piece of goat intestinal mucosa was mounted on the glass slide using cyanoacrylate glue. About 100 efavirenz microspheres were spread onto each wet rinsed intestinal tissue specimen and immediately afterward the support was hung onto the arm of USP disintegration machine. Now operating the USP disintegration machine, the intestinal tissue specimen was given a slow regular up and down movement in test fluid (0.1N HCL buffer) at 37±0.5^{0}C. At programmed time intervals up to 8 hrs the disintegration machine was stopped and the number of Efavirenz mucoadhesive microspheres still sticking onto the intestinal mucosa was counted and percent mucoadhesion was calculated. [13]

2.8. In Vitro dissolution

The in vitro dissolution studies of prepared Efavirenz microspheres were carried out using USP type II (Electrolab Mumbai, India) dissolution test apparatus. Mucoadhesive Microspheres containing equivalent to 100 mg of efavirenz were introduced into 900 ml dissolution medium of 0.1N HCl for 12 hrs at 37±0.5°C at a rotation speed of 50 rpm. Sample of 5 ml were withdrawn through a filter (0.45 µ) at the programmed interval and replaced with equal volume of fresh 0.1N HCl buffer. The samples were analyzed at 245 nm for Efavirenz content using UV spectrophotometer. The Efavirenz release experiments were carried out in three replicate.[14]

2.9. Release kinetic and mechanism of Efavirenz release

The release rate and mechanism of efavirenz release from the prepared mucoadhesive microspheres were analyzed by fitting the dissolution data into various kinetic models like zero order, first order, higuchi model and korsemeyer peppas. A criterion for selecting the best fit model was based on goodness of fit, high R2 (regression coefficient) value.

2.10. FTIR Studies

Compatibility study of Efavirenz with different mucoadhesive polymers was determined by I.R. Spectroscopy (FTIR) using Shimadzu FT-IR spectrometer model. IR spectra for Efavirenz and powdered Efavirenz microspheres were recorded in a FTIR spectrophotometer with KBr pellets the scanning were done between wave numbers 4000 to 400 cm^{-1} at 4 cm^{-1} resolution.

2.11. DSC studies

Differential scanning calorimetry was carried out on pure drug Efavirenz and Efavirenz loaded microspheres using Shimadzu DSC 60. DSC run were conducted over a temperature range 50-300 °C at a heating rate of 10 °C / min under nitrogen atmospheres.

2.12. Stability Study

Stability studies were carried out for Efavirenz formulation as per ICH guidelines. The best efavirenz microspheres formulation (F12) was sealed in high density polyethylene bottles and stored at 25 ± 2^{0}C/ 60 ± 5 %, 40 ± 2^{0}C/ 75 ± 5 % RH in closed for 90days. The microsphere samples were periodically evaluated for entrapment efficiency and percentage drug release.

3. RESULTS AND DISCUSSION

The prepared Efavirenz microsphere by Ionotropic gelation method was found to be spherical shape and free flowing in nature. The mean particle size of Efavirenz mucoadhesive microspheres ranged from 707.60 ± 8.32 to 921.70 ± 6.03 µm (Table 2). The results revealed that the mean particle size increase with increases in mucoadhesive polymer concentration. It would appear that increasing mucoadhesive polymer concentration produced a significant increase in viscosity of the dispersion, thus leading to an increase of droplet size and finally a higher microsphere size.

Efavirenz microspheres were characterized for size analysis, yield, flow properties and percentage entrapment efficiency. The results are given in Table 2. All the formulations offered good flow property and compressibility.

The percentage entrapment efficiency ranged from 44.76 to 90.80 %. (Table 2). The entrapment efficiency of the microspheres prepared with HPMC K15 was higher than those of microspheres prepared with HPMC and methyl cellulose. From the result it was observed that increase in the concentration of the mucoadhesive polymer increase the entrapment efficacy. This can be attributed to increase in the viscosity of the polymeric solution, which increases the strength matrix formation.

The *in vitro* Efavirenz release profiles for all batches were shown in Figure 2. The Efavirenz release behaviors depend upon the type and amount of mucoadhesive polymers in polymer matrix. Methyl Cellulose -alginate microspheres (F1 and F4) were able to control the Efavirenz release up to 10 hours whereas HPMC & HPMC K15 microspheres were able to control the drug released up to 12 hours. It has been observed that

Methyl cellulose, HPMC, based mucoadhesive microspheres showed comparative ly rapid drug release as compared to HPMC K15 b ased formulations.

The SEM photographs revealed that obtained Efavirenz microspheres were discrete and spherical shape with a rough surface morphology (Figure 1).

Table - 2: Physico chemical properties of Efavirenz mucoadhesive microspheres

Formulation code	Percentage yield	Theoretical drug content (mg)	Entrapment efficiency	Particle size [µm]
F1	79.33	66.6	44.76 ± 0.42	707.6 ± 8.32
F2	85.32	50	54.73 ± 0.87	762.5 ± 13.5
F3	88.46	40	61.65 ± 0.64	815.3 ± 13.1
F4	90.27	33	70.74 ± 0.71	854.6 ± 12.1
F5	81.47	66.6	50.90 ± 0.40	724.3 ± 0.75
F6	90.10	50	62.23 ± 0.55	784.6 ± 13.1
F7	92.16	40	69.56 ± 0.81	833.3 ± 10.0
F8	89.20	33	78.80 ± 0.56	884.6 ± 12.5
F9	80.67	66.6	66.90 ± 0.92	755.3 ± 0.91
F10	86.10	50	73.70 ± 0.85	814.6 ± 12.1
F11	90.32	40	82.60 ± 1.14	858.1 ± 8.18
F12	92.33	33	90.80 ± 0.65	921.7 ± 6.03

Table - 3 : Micromeritics properties of efavirenz mucoadhesive microspheres.

Formulation code	Bulk density[a]	Tapped density [a]	Compressibility index [a]	Hausner's ratio [a]	Angle of Repose [a]
F1	0.496 ± 0.045	0.546 ± 0.055	9.10 ± 0.83	1.10 ± 0.01	6.40 ± 1.65
F2	0.460 ± 0.016	0.525 ± 0.020	12.20 ± 0.42	1.130 ± 0.005	12.50 ± 1.060
F3	0.462 ± 0.016	0.527 ± 0.010	13.40 ± 0.43	1.15 ± 0.005	21.90 ± 0.010
F4	0.474 ± 0.008	0.560 ± 0.017	14.60 ± 1.03	1.16 ± 0.011	29.10 ± 0.85
F5	0.452 ± 0.017	0.489 ± 0.019	7.40 ± 0.27	1.076 ± 0.005	10.57 ± 0.640
F6	0.435 ± 0.005	0.487 ± 0.013	10.00 ± 0.77	1.110 ± 0.017	18.10 ± 1.40
F7	0.437 ± 0.009	0.504 ± 0.012	13.20 ± 0.28	1.146 ± 0.005	22.81 ± 1.001
F8	0.438 ± 0.007	0.514 ± 0.010	14.70 ± 0.24	1.166 ± 0.005	26.79 ± 0.950
F9	0.497 ± 0.021	0.563 ± 0.020	10.80 ± 0.17	1.080 ± 0.001	10.20 ± 2.08
F10	0.486 ± 0.028	0.559 ± 0.030	11.40 ± 0.32	1.120 ± 0.005	15.40 ± 1.17
F11	0.480 ± 0.010	0.550 ± 0.010	13.40 ± 1.25	1.150 ± 0.015	19.70 ± 0.620
F12	0.470 ± 0.008	0.540 ± 0.01	14.10 ± 1.00	1.160 ± 0.060	29.90 ± 0.250

[a] Mean ± SD, *n* = 3.

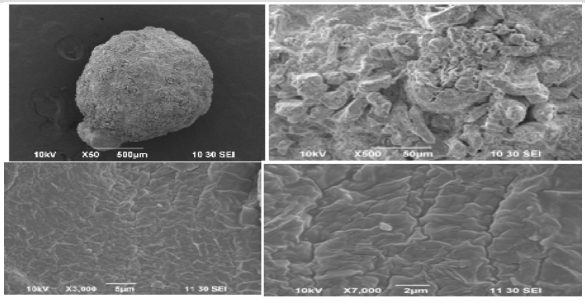

Figure - 1: Scanning electron photomicrographs of formulation F12: A) 50 X, B) 500 X, C) 3000 X, D) 7000 X.

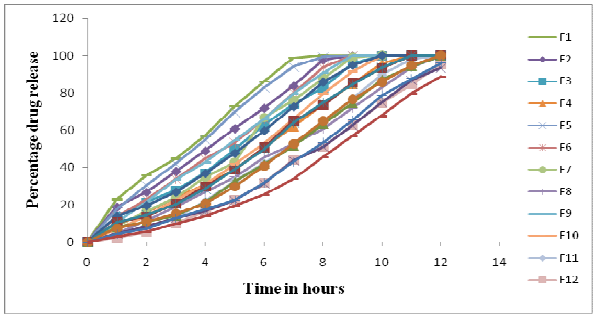

Figure - 2: Comparative release profile of formulation F1 to F12.

Drug release kinetic data for Efavirenz microspheres was shown in Table No. 3. All the formulations (F1 to F12) follow zero order release kinetics with regression values ranging from 0.979 to 0.999. Korsmeyer-Peppas plots, 'n' value ranges from 0.745 to 1.542 indicating that the Efavirenz release mechanism followed anomalous transport and super case-II transport mechanism.

The results of the in-vitro mucoadhesion studies of efavirenz microspheres formulations were shown in table 4. Percentage mucoadhesion of efavirenz formulations increased with the increase in concentration of mucoadhesive polymers. The higher mucoadhesion of HPMC K15 based mucoadhesive microspheres may be attributed to the higher molecular weight of HPMC K15 than HPMC and Methyl cellulose based microspheres.

Table -3- Release Kinetic parameter of Efavirenz from mucoadhesive microspheres

Formulation code	Zero order	First order	Higuchi	Korsmeyer peppas	n-value
F1	0.985	0.832	0.974	0.987	0.745
F2	0.993	0.810	0.972	0.986	0.810
F3	0.992	0.599	0.960	0.980	0.921
F4	0.992	0.800	0.942	0.991	1.050
F5	0.993	0.635	0.984	0.997	0.863
F6	0.995	0.618	0.965	0.994	0.932
F7	0.993	0.769	0.964	0.996	1.063
F8	0.996	0.912	0.959	0.999	1.228
F9	0.999	0.756	0.976	0.999	1.027
F10	0.992	0.576	0.948	0.996	1.188
F11	0.988	0.778	0.945	0.989	1.386
F12	0.979	0.895	0.906	0.994	1.542

Table - 4: Results of *in vitro* wash off test in 0.1N HCL

Hours	1	2	3	4	5	6	7	8
F1	75	60	51	45	40	27	10	4
F2	80	71	62	56	49	30	21	9
F3	83	75	66	58	50	37	25	11
F4	87	78	68	61	53	41	29	17
F5	89	82	76	70	58	44	30	12
F6	94	88	80	75	61	52	35	15
F7	96	90	81	77	65	55	39	18
F8	97	95	89	83	68	58	41	22
F9	100	93	84	77	63	53	44	31
F10	100	97	90	85	77	69	58	45
F11	100	98	92	86	78	72	62	58
F12	100	100	95	89	82	76	70	69

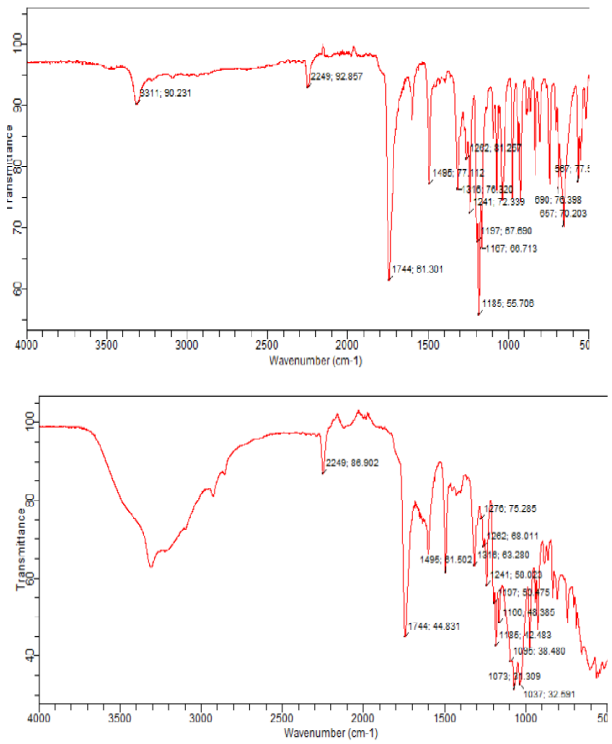

Figure - 3: FTIR spectra of A. Pure Efavirenz B. Formulation containing HPMC K15 (F12)

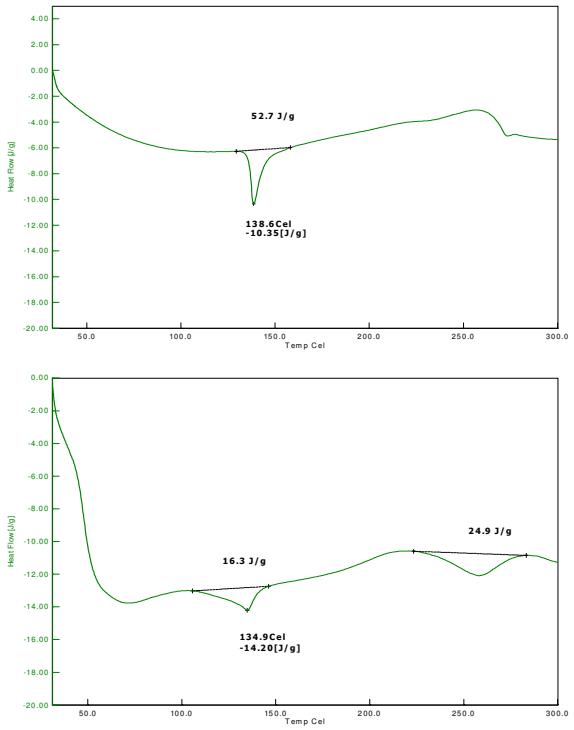

Figure - 4: DSC Thermograms of A. Pure efavirenz B. Formulation containing HPMC K15 (F12)

FT-IR spectra of pure Efavirenz and Efavirenz loaded microspheres were compared and shown in figure 3. The FT-IR spectra of Efavirenz loaded Microspheres showed the characteristic peaks of the pure drug indicating that there was no interaction between the Efavirenz and mucoadhesive polymers. The thermogram of Efavirenz exhibited a sharp endothermic peak at 138.6°C shown in (Figure 4), which corresponds to its melting point. The characteristic peak of Efavirenz was well recognized in the drug-loaded mucoadhesive microspheres. Thus, there was no interaction between Efavirenz and mucoadhesive polymers.

The X-ray diffractograms of Efavirenz and formulation F12 are shown in figure 5. Pure Efavirenz has shown characteristic intense peaks due to its crystalline nature. Whereas, in case of formulation F12 showed less intense peak of low intensity, revealing amorphous dispersion of the drug after entrapment into mucoadhesive microspheres.

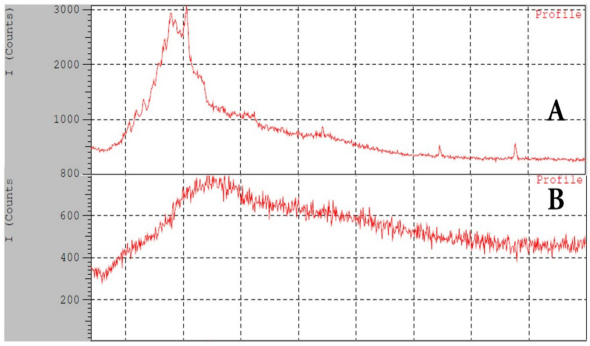

Figure - 5: XRD pattern of A. Pure Efavirenz B and Best formulation F12.

Table - 4: Percentage entrapment efficiency and mucoadhesion of the F12 formulation

Stability condition	Sampling Day	Percentage Entrapment efficiency [a]
4 ^{0}C / Ambient	0	82.60 ± 1.137
	30	82.17 ± 0.557
	60	81.92 ± 0. 631
	90	81.29 ± 0.347
25°C/ 60 RH	0	82.60 ± 1.137
	30	82.42 ± 0.539
	60	82.07 ± 0.482
	90	81.75 ± 0.457
40°C/ 75RH	0	82.60 ± 1.137
	30	82.03 ± 0.656
	60	81.47 ± 0.573
	90	81.11 ± 0.495

[a] Mean ± SD, n = 3

3.1. Stability Study

After 3 months storage of F12 formulation at $4^0C \pm 1^0C$ / Ambient ,25 ± 2^0C/ 60 ± 5 % RH, 40 ± 2^0C/ 75 ± 5 % RH, percentage entrapment efficiency were checked and found to be almost similar to the initial values. There was no significant change in any value and also the physical appearance. So it can be said that Efavirenz mucoadhesive microspheres prepared with HPMC K15 is stable.

4. CONCLUSION

The HPMC K15 mucoadhesive microspheres containing Efavirenz can be successfully prepared by Ionotropic technique. The present method was quite simple, rapid and does not imply the use of toxic organic solvent. The method also achieves good micrometric properties and better encapsulation efficiency. The prepared mucoadhesive microspheres were spherical and free flowing. The entrapment efficiencies ranged from 44.76 to 90.80 % and mean size was in the range of 707.60 ± 8.32 µm to 921.70 ± 6.03 µm. The Efavirenz release depends upon the mucoadhesive polymer type and concentration in the polymer matrix. Thus the results demonstrate the potential use of HPMC K15 polymer for preparation of controlled delivery Efavirenz mucoadhesive microspheres and prolonged residence at the absorption site. Further in-vivo activities are required to confirm the claim of beneficial effect of Efavirenz in the form of HPMC K15 mucoadhesive microspheres.

Conflicts of interest

All authors have none to declare

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