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Formulation and Evaluation of Lornoxicam Loaded Solid Lipid Nanoparticles

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A B S T R A C T

Inflammation is a common phenomenon involving interrelationships of humoral and cellular reactions through a number of inflammatory mediators. Lornoxicam, a new non-steroidal anti-inflammatory drug with analgesic, anti-inflammatory and antipyretic properties, is also effective in relieving symptoms of osteoarthritis, rheumatoid arthritis, ankylosing and low back pain. SLNs were prepared by o/w Microemulsion technique and characterized by particle size analysis, FTIR spectroscopy, drug entrapment efficiency, SEM, *in vitro* evaluation studies. *In vitro* release studies were performed in dissolution cell in phosphate buffer solution of pH 6.8. The kinetics of release was determined and fitted to an empirical equation. The influence of experimental factors such as surfactant concentration, lipid carrier concentration and stirring speed on the nanoparticles size and distribution were investigated to optimize the formulations. At highest speed the resultant SLNs were smaller in size and their size increased with increase in lipid concentration. Smaller size SLNs were obtained with 1 % (1:1 w/v) of lecithin/ tween-80. The *in-vitro* release was found to follow Non- Fickian Diffusion mechanism. Lornoxicam loaded SLNs can be prepared by microemulsion method with narrow size range, high entrapment efficiency.

Keywords: Solid lipid nanoparticles, Lornoxicam, Triglycerides, particle size, SEM, stability, *in-vitro* evaluation studies.

A R T I C L E I N F O

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1. Introduction

Nanotechnology is positively impacting a part of nearly every industry including healthcare[1]. The application of nanotechnology to healthcare also called nanomedicine are requires the intersection of many disciplines including biology, chemistry, physics, chemical and mechanical engineering, material science and clinical medicine. The application of nanotechnology to medicine appears to be a relatively recent trend, the basic nanotechnology approaches for medical application date back several decades. The first example of lipid vesicles which later became known as liposomes were described sin 1965, the first controlled release polymer system of macromolecules was described in 1976, the first long circulating stealth polymeric nanoparticles was described in 1994, the first quantum dot bioconjugate was described in 1998, and the first nanowire nanosenser dates back to 2001.

Polymeric nanoparticles made from non-biodegradable and biodegradable polymers are yet another innovative parenteral carrier system[2]. Advantages of these particles are site-specific targeting and controlled release of the incorporated drugs. However, the cytotoxicity of the polymers after internalization into cells is a crucial and often discussed aspect. Also, large scale production of polymeric nanoparticles is problematic[3]. In the middle of the 1990s, the attention of different research groups has focussed on alternative nanoparticles made from solid lipids, the so-called solid lipid nanoparticles (SLNs or lipospheres or nanospheres). The SLNs combine the advantages of other innovative carrier systems while at the same time minimizing the associated problems. SLN are particles made from solid lipids and stabilized by surfactant(s). By definition, the lipids can be highly purified triglycerides, complex glyceride mixtures or even waxes.

Advantages of solid-lipid nanoparticles [4]

- Small size & relatively narrow size distribution which provide biological opportunities for site-specific drug delivery by solid lipid nanoparticles.
- Controlled release of active drug over a long period can be achieved
- Protection of incorporated drug against chemical degradation.
- Possible sterilization by autoclaving or gama irradiation.
- Solid lipid nanoparticles can be *lyophilized* as well as spray dried.
- Ease of industrial scale production by hot dispersion technique.
- Incorporation of drug can reduce distinct side effects of drug e.g. Thrombophlebit is that is associated with i.v. injection of diazepam or etomidete.

Disadvantages of solid lipid nanoparticles[4]:

However, there are also some potential limitations. Critically highlights potential limitations which might occur:

1. Limitation in drug loading capacity.
2. Drug expulsion during storage.
3. High water content of aqueous SLN dispersions (70–95%).

Lornoxicam is a non-steroidal anti-inflammatory drug of the oxicam class with analgesic, anti-inflammatory and antipyretic properties[5]. It is available in oral and parenteral formulations. Like other NSAIDS, lornoxicam's anti-inflammatory and analgesic activity is related to its inhibitory action on prostaglandin and thromboxane synthesis through the inhibition of both COX-1 and COX-2. This leads to the reduction of inflammation, pain, fever, and swelling, which are mediated by prostaglandins. However, the exact mechanism of lornoxicam, like that of the other NSAIDs, has not been fully determined.

2. Materials and Methods

Lornoxicam is the gift sample of Hetero drugs limited in Hyderabad, Glyceryl mono sterate, Soya bean lecithin, Tween 80 was obtained form K.P Labs hyderabad. Sovents are used analytical grade.

Methodology:

Compatibility studies of drug and polymers:

IR spectral studies lies more in the qualitative identification of substances either in pure form or in combination with polymers and excipients and acts as a tool in establishment of chemical interaction [6]. Since I.R. is related to covalent bonds, the spectra can provide detailed information about the structure of molecular compounds. In order to establish this point, comparisons were made between the spectrum of the substances and the pure compound. FTIR spectra were recorded with a Thermo Nicolet. Japan In the range 450–4000 cm^{-1} using a resolution of $4cm^{-1}$ and 16 scans. Samples were diluted with KBr mixing Powder, and pressed to obtain self-supporting disks. Liquid samples formulations were analyzed to form a thin liquid film between two KBr disks**.**

Preliminary Investigations:

The preliminary studies were carried out by preparing various formulations with different variables in an effort to optimize the formulations for the particle size ranging in nano scale[7]. To select feasible technique, we carried out micro emulsion method using GMS as a lipid carrier. The formulations were categorized in to two sets, 4 formulations in each category namely formulations based on lipid concentration and secondly formulations based on speed of stirrer. Lipid was used in the concentration ranging from 0.5% to 2%. Speed of stirrer was set in the range of 6,500 to 17,500 rpm. The resulted formulations were analyzed for particle size and its distribution. Further, the selected technique was optimized by Surfactent/ Co-surfactent Concentration [Soya Lecthin/Tween-80].

Formulation design:

Procedure for preparation of Lornoxicam loaded SLNs by Microemulsion Technique:

SLNs were prepared from o/w microemulsion technique containing Glycerylmono stearate as a lipid carrier, Soya lecithin as surfactant and tween 80 as co- surfactant. GMS was added drop wise maintained at 70^oC into ice cold water ($2\text{-}3^oC$) with continuous stirring to form SLNs. The samples were sonicated and analyzed for particle size [8].

Microemulsion technique follows good and compatible result over Solvent emulsification diffusion technique. Frothing effect is less pronounced in this technique and negligible amount of lipid carrier stuck to the preparation container. Surfactent/Co-surfactent Concentration (1:1) in the concentration of 1.0% w/v was optimized**.**

3. Results and discussions

Characterization of SLNs

Particle Size Analysis:

Drug loaded nanoparticles were analyzed by CIS-L50 Particle Size Analyzer. The particles were scanned from 0-150µm using lens A. The suspension is taken in a cuvette and diluted with distilled water to give a concentration of 10^{-9} particles with a standard normalizing factor [9]. The cuvettes are made up of polystyrene of 1cm path length. The particles are analysed for its size (length x breadth x volume) by using laser channel beam.

Total content:

Lornoxicam loaded SLNs (1 ml) were diluted to 10 ml of pH 6.8. Final dilution was made with pH 6.8 in its beers range [10]. And total drug content was determined by using UV spectrophotometer at 383nm by taking pH 6.8 as blank

Entrapment Efficiency:

Entrapment efficiency of drug loaded SLNs was determined by centrifugation of samples at 10,000 rpm for 10 min [11]. The amount of free drug was determined in the clear supernatant by UV spectrophotometer at 383 nm using supernatant of non loaded nanoparticles on basic correction

The entrapment efficiency (EE %) could be achieved by the following equation.

$$EE\ (\%) = \frac{W\ \textit{initial drug} - W\ \textit{free drug}}{W\ \textit{initial drug}} \times 100$$

Scanning Electron Microscopy:

Surface morphology of the drug loaded nanoparticles are determined by using a scanning electron microscope (SEM), Model JSM 6330, JEOL, Japan. The samples are dried thoroughly in vaccum desicator before mounting on brass specimen studies, using double sided adhesive tape. Gold-palladium alloy of 120^0A was coated on the sample using sputter coating unit (Model E5 100 Polaron U.K.) in Argon at ambient of 8-10 Pascal with plasma voltage about 20MA [12]. The sputtering was done for nearly 5 minutes to obtain uniform coating on the sample to enable good quality SEM images.

***In-vitro* release study:**

The studies were done using USP dissolution apparatus II (Lab India). This test was performed by one tablet from each formulation using 900ml of phosphate C temperature and 50 rpm. Every one buffer 6.8 at 370C hour intervals are taken 5ml sample from each dissolution medium and simultaneously replaced with fresh dissolution medium. Then the samples were analyzed Spectra photo metrically at 383nm [13]. The percentage drug released at time interval was calculated and plotted against time. The cumulative percentage of drug release was calculated.

Mathematical modeling for non linear curve:

The Colloidal systems were reported to follow the zero order release rate by the diffusion mechanism for the release of the drug [14]. To analyse the mechanism for the release and release rate kinetics of the dosage form, the data obtained was fitted in to Zero order, First order, Higuchi matrix and Krosmeyer and Peppas model. Using PCP-DISSO – v2 software. Comparing the r^2-values obtained, the best-fit model was selected.

Zero order kinetics:

Drug dissolution from pharmaceutical dosage forms that do not disaggregate and release the drug slowly, assuming that the area does not change and no equilibrium conditions are obtained can be represented by the following equation [15].

$$Q_t = Q_o + K_o t$$

Where Q_t = amount of drug dissolved in time t, Q_o = initial amount of drug in the solution and K_o = zero order release constant.

First order kinetics: To study the first order release rate kinetics the release rate data were fitted to the following equation.

$$\text{Log } Q_t = \log Q_o + K_1 t / 2.303$$

Where Q_t is the amount of drug released in time t, Q_o is the initial amount of drug in the solution and K_1 is the first order release constant [15].

Higuchi model:

Higuchi developed several theoretical models to study the release of water-soluble and low soluble drugs incorporated in semisolids and or solid matrices [16]. Mathematical expressions were obtained for drug particles dispersed in a uniform matrix behaving as the diffusion media, the equation is

$$Q_t = K_H . t^{1/2}$$

Where Q_t = Amount of drug released in time t, K_H = Higuchi dissolution constant

Hixson- Crowell model:

To study the Hixson – Crowell model the release rate data are fitted to the following equation

$$W_o^{1/3} - W_t^{1/3} = Kst$$

Where W_o is the amount of drug in the dosage form, W_t is the remaining amount of drug in the pharmaceutical dosage form, Ks is a constant incorporating the surface-volume relationship.

Compatibility Study:

The IR spectrum of the pure Lornoxicam sample recorded by FTIR spectrometer is shown in Figure 2 to 5. This was compared with standard functional group frequencies of Lornoxicam as shown in Table. 6.

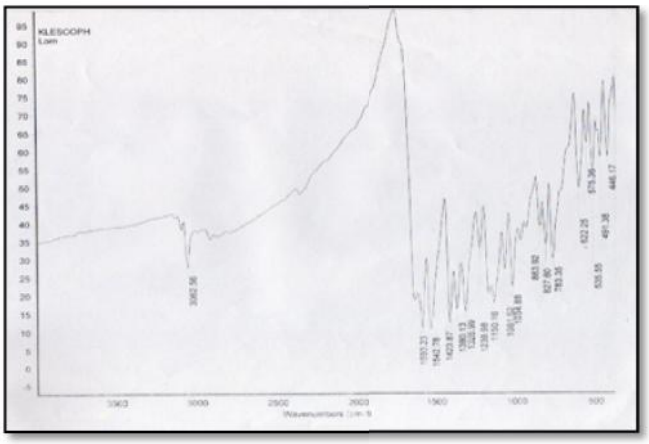


Figure 1: FTIR spectra of Lornoxicam

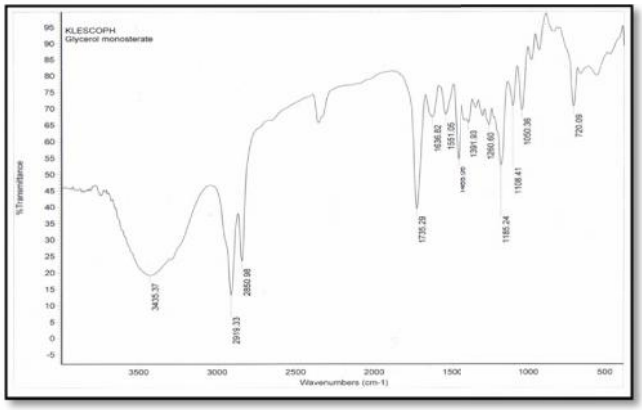


Figure 2: FTIR spectra of Glycerylmono Stearate

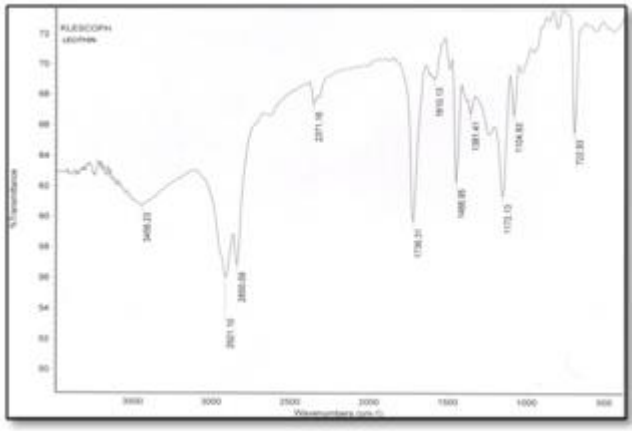


Figure 3: FTIR spectra of Lecithin

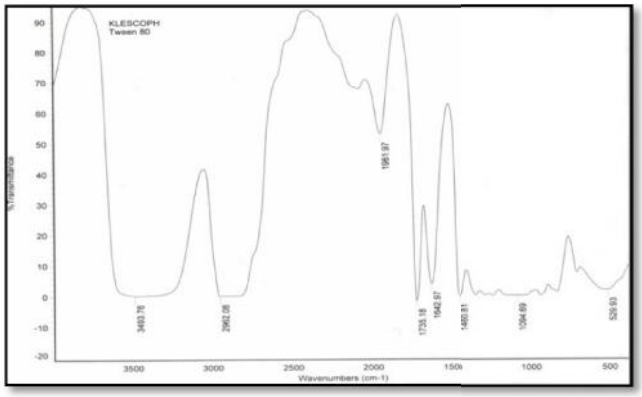
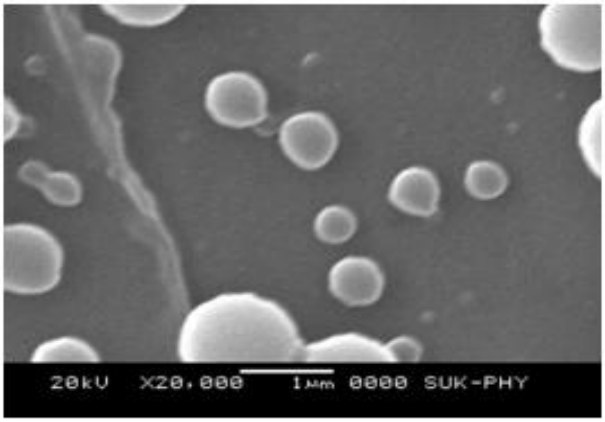


Figure 4: FTIR spectra of Tween-80



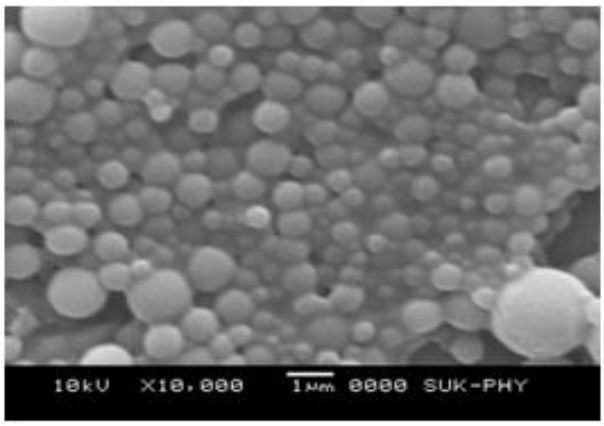


Figure 5: SEM Pictures of Lornoxicam loaded solid lipid nanoparticles

SEM reports of Lornoxicam loaded SLNs:

The developed micro emulsion of LN-II were freeze-dried both in the presence and in the absence of a cryoprotectant (5%, w/w mannitol), simply by gentle hand agitation without evident size of the resulting micro emulsion. Morphology and structure of SLNs were determined using scanning electron microscopy (SEM) are shown in the figure 5.

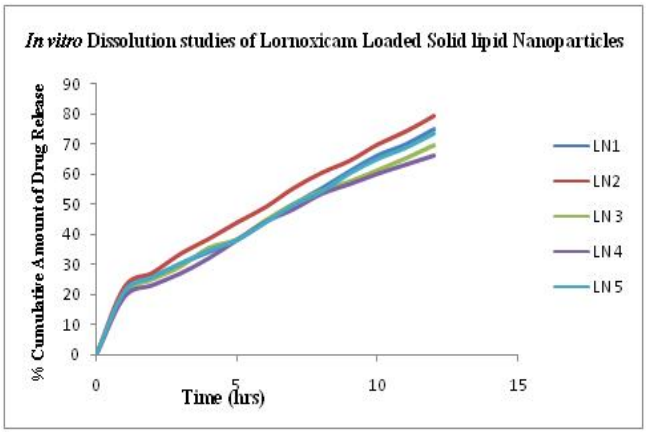


Figure 6: *In-vitro* Drug Release Profile of Lornoxicam loaded solid lipid nanoparticles

Discussion:

In the present investigation an attempt has been made to formulate Lornoxicam loaded solid lipid nanoparticles using lipids as an carrier and prepared Lornoxicam loaded SLNs are subjected for characterization and evaluation studies.

Compatibility Studies:

Drug polymer compatibility studies were carried out using IR-200 (FTIR) to establish the possible interaction in the SLNs formulations. It was found that there was no possible interaction in between drug and lipid carrier in their individual form and in formulations too. With different surfactants such as lecithin and tween-80 when kept for one month in different conditions. Results are tabulated in Table no. 6. Compatibility studies were also carried by using DSC, which is a qualitative analytical tool for assessing the interactions. The pure form and the formulations are studied

after one month storage at different conditions. It was found that the thermal peaks of drug are identical in formulations with those entire lipid carriers. This indicates that, there is no interaction in the formulations. Results are tabulated in table no.2

Characterization & Evaluation of SLNs:

Particle size Analysis: The formulations with GMS such as LN showed wide distribution in particle size ranging from 780nm to 920nm; the particle size analysis reveals that the size reduction was with varying speeds and size increment with varying lipid carrier concentrations. As the lipid concentration was increased, more particles were aggregated resulting in a increased particle size. When the concentration of the lipid exceeded 1.0% with a fixed concentration of surfactants, there were insufficient surfactants available to coat the surface of all the lipid droplets, resulting in particle aggregation and an increase in particle size**.**

Scanning electron microscopy (SEM):

This was performed to study the surface morphology of the particles, although the particles were abundantly found and they were spherical in their shape. Thus, both types of surfactants produced better surface characteristics. The smooth surface of SLNs is because of presence of Lecithin and it could be attributed to its proteinous nature. The surface morphology of the SLNs had not been altered by the type of lipid carrier, concentration of lipid carrier and speed.

Total Drug content and Entrapment Efficiency:

The experimental results indicate that the concentration of lipid, speed had critical effects on the Lornoxicam incorporation efficacy. The entrapment efficiencies of SLNs made from different concentrations of lipid carrier was come up in the ascending order the reported reasons for that the entrapment efficiency is lower for the sample with lower lipid concentration. The cooling process leads to super saturation of the drug and subsequently to drug crystallization prior to lipid crystallization. The little bit reduction in entrapment efficiencies was observed with the varying speed. Among the one lipid carriers formulations prepared with GMS (44.23% to 55.14%) .

***In-vitro* Dissolution Studies:**

In-vitro drug release data from the SLNs were carried out for 12hrs and graphically represented as % CDR v/s time profile. The Cumulative Percent drug released after 12hrs for LN was 19.37 to 79.02% respectively.

Kinetic Study:

The release study was further investigated for the kinetic studies. Various kinetic models were applied and their values were noted. LN I to LN 5 was found to follow the zero order models. From the n values obtained it can be said that the diffusion followed Non-Fickian mechanism.

Table 1: Formulation design of Lornoxicam loaded SLNs by microemulsion Technique

Formulation codes	Lipid %W/V	Drug (mg)	Conc. of surfactant/ co- surfactant W/V (1:1)	Speed (rpm)
LN -I	0.5	8	1.0	6,500
LN -II	0.5	8	1.0	9,500
LN -III	1.0	8	1.0	9,500
LN - IV	1.5	8	1.0	13,500
LN -V	1.0	8	1.0	13,500

Table 2: Compatibility study of drug, Lipid and formulations by FTIR spectroscopy

Lipid/Drug/ Formulation.	Important IR Spectral peaks of different groups, wave length in cm^{-1}				
	C-H Stretch (Aromatic)	C=O Stretch	C-H Stretch (Aliphatic)	C-O-C Stretch	S=O
Lornoxicam	3062.56	1593.23	------	-----	1326.99
G.M.S	------	1735.29	2919.33	1185.24	-----
Lecithin	------	1736.31	------	1466.95	-----
Tween-80	------	1735.18	2962.08	-----	-----

Table 3: Mean Particle Size of Lornoxicam Loaded SLNs using G.M.S as a lipid carrier by ME Techniques

Formulation code	LN -I	LN -II	LN -III	LN -IV	LN -V
Mean Particle Size (nm)	790nm	780nm	800nm	920nm	780nm

Table 4: Drug Entrapment Efficiency with GMS as a lipid carrier

Formulation code	LN -I	LN -II	LN –III	LN -IV	LN -V
Practically total drug content	88.42%	87.58%	89.40%	86.74%	87.30%
Drug entrapment efficiency	46.12%	45.23%	48.80%	51.14%	47.42%

Table 5: *In-vitro* Dissolution studies of Lornoxicam loaded SLNs

S.No	Time (hrs)	% Cumulative Amount of Drug Release				
		LN1	LN2	LN 3	LN 4	LN 5
1	1	21.4	22.5	20.3	19.3	21.1
2	2	26.04	27.2	25.1	23.2	25.8
3	3	30.4	33.5	29.3	27.08	30.3
4	4	34.3	38.4	35.5	32.1	34.6
5	5	38.3	43.8	38.3	38.1	38.1
6	6	44.2	48.8	44.5	44.09	43.8
7	7	50.1	55.1	50.01	48.3	49.7
8	8	55.4	60.2	54.3	53.4	54.1
9	9	61.1	64.2	57.4	56.6	60.08
10	10	66.4	69.6	61.2	60.08	64.9
11	11	70.1	73.9	65.1	63.1	68.6
12	12	75.1	79.02	69.4	66.1	73.3

Table 6: Kinetic data of various models for release Lornorxicam loaded solid ipid nanoparticles

Formulation code	Zero order		First order		Matrix		Hixon Crowell		Best fitting model
	R	k	R	k	R	k	R	K	
LN 1	0.9962	0.0105	0.9962	-0.0001	0.9143	0.0295	0.9962	0.0000	Zero order
LN 2	0.9984	0.0780	0.9984	-0.0008	0.9280	0.2211	0.9984	0.0003	Zero order
LN 3	0.9987	0.0676	0.9987	-0.0007	0.9283	0.1915	0.9987	0.0002	Zero order
LN 4	0.9988	0.0648	0.9988	-0.0007	0.9223	0.1832	0.9988	0.0002	Zero order
LN 5	0.9969	0.0700	0.9968	-0.0007	0.9179	0.1977	0.9968	0.0002	Zero order

4. Conclusion

Lornoxicam loaded SLNs can be successfully formulated from Micro emulsion Technique to enhance the efficacy of drug at the target area by reducing the side effects from the dose. The formulated SLNs of Lornoxicam showed particle sizes in the range of 740nm-920nm, with good surface characters. These SLNs were prepared using different lipid carriers & mixture of surfactants, by varying concentrations of lipid carriers along with the speed. Increment in particle size was observed with varying concentrations of lipid carriers. Smaller size is obtained with varying speed. the particle size had influence on the *in vitro* drug release was observed.

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