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Research Article

ANALYTICAL EVALUATION OF FORMULATED OCCULAR NANOASUSPENSION OF PILOCARPINE NITRATE

Kalpana Anumolu*, Vijay Amritharaj R, Suchetha Reddy Aleti and N. Senthil Kumar

Department of Pharmaceutical Analysis, JKK Munirajah Medical Research Foundation College of Pharmacy, Komarapalayam, Namakkal, Tamilnadu, India.

ABSTRACT

Ophthalmic nanosuspension of Pilocarpine nitrate was prepared by quassi emulsion method using Eudragit S 100 and was evaluated for pH, entrapment efficiency, UV, Drug polymer compatibility by FT-IR and DSC. Absorbance of the pure pilocarpine and formulated drug was found to be 207.5nm using phosphate buffer saline as solvent, with molar absorptivity of 0.56×10^{-4} mol^{-1} cm^{-1} and correlation coefficient of 0.9995. The prepared ocular nanosuspension has a pH of 6.4-7.4 and entrapment efficiency was analyzed by UV spectrophotometry at 207.5nm using polymeric solution as reference sample. Drug entrapment studies were done and the percentage drug entrapment efficiency was ranged between 65-80%. DSC studies were carried out for pure drug and physical mixture of pure drug with polymer; data from DSC study conclude that there were no drug polymer interactions between the drug and polymer combinations. Thus the method employed for the preparation of nanosuspension was found to be simple and validated parameters were reproducible.

Keywords: Pilocarpine nitrate, Phosphate buffer saline, Nanosuspension, DSC.

INTRODUCTION

Amongst the various routes of drug delivery, ocular drug delivery is one of the most interesting and challenging endeavours facing the pharmaceutical scientist for past 10-20 years. The need to provide therapy for multifactorial eye diseases such as glaucoma, retinal diseases, and cataract and for surgical complications such as ocular inflammation and infections represents growing opportunities for ophthalmic drug delivery. Among various diseases affecting eye, glaucoma is one of the most prevalent eye disease. Almost 3 million people in the United States and 14 million people worldwide are reported to be suffering from glaucoma. Glaucoma is the third leading cause of blindness worldwide and the second leading cause of blindness in the United States, where it is the leading cause of blindness among blacks and Hispanics.

Efficacy and safety of drugs used for treatment of ocular diseases is matter of concern due to anatomy and physiology of eye function. Normally, the aqueous fluid, which nourishes the eye, is produced by the ciliary body behind the iris (in the posterior chamber) and flows through the pupil to the front of the eye (anterior chamber), where it exits into drainage canals between the iris and cornea (the “angle”). When functioning properly, the system works like a faucet (ciliary body) and sink (drainage canals). Balance between fluid production and drainage between an open faucet and a properly draining sink keeps the fluid flowing freely and prevents pressure in the eye from building up that is Intra Ocular Pressure (IOP) is balanced. Glaucoma occurs when an imbalance in production and drainage of fluid in the eye (aqueous humours) which increases IOP eye pressure to unhealthy levels.

Various drugs are used for treatment of glaucoma. Among them Pilocarpine nitrate is one of the oldest and widely used drug for treatment of glaucoma. Pilocarpine nitrate is an alkaloid obtained from the *Pilocarpus microphyllus*. It is a cholinergic agonist drug. It

has prominent muscarinic action and also stimulates the ganglia-mainly through ganglionic muscarinic receptors. Smaller doses of Pilocarpine nitrate generally causes fall in IOP but higher dose elicit rise in blood pressure and tachycardia which is probably due to ganglionic stimulation. As Pilocarpine nitrate have shorter half-life, the persons who are advised for its medications are recommended for repeated dose administrations. Thus, patients using ophthalmic drops containing pilocarpine nitrate are faced with frequent dosing schedules and difficulty in drop instillation.

Polymers play a vital role in the delivery of adequate concentration of drug/ drugs from a pharmaceutical formulation. Various types of natural, synthetic and semi synthetic polymers are commercialized and still new being carried out synthesize polymers of good acceptability and biocompatibility with various body tissues. In ophthalmic drug delivery, polymers have been employed to increase the viscosity of various eye solutions and also in preparing novel drug delivery system such as nanosuspension, inserts*, in-situ* gels etc. The polymer employed in the preparation of the Pilocarpine nitrate nanosuspension was Eudragit S 100.

Nanosuspension is a submicron colloidal dispersion of drug particles. A pharmaceutical nanosuspension is defined as very finely colloid, biphasic, dispersed, solid drug particles in an aqueous vehicle, size below 1µm ,without any matrix material, stabilized by surfactants and polymers, prepared by suitable methods for drug delivery applications, through various routes of administration like oral, topical, parenteral, ocular and pulmonary route . Nanosuspension not only solves the problem of poor solubility and bioavailability but also alters the pharmacokinetics properties of the drug and thus improves drug safety and efficacy. Nanosuspension differs from nanoparticles, which are polymeric colloidal carriers of drugs (Nanospheres and nanocapsules) and from solid-lipid Nanoparticles (SLN, which are lipidic carriers of drug). Nanosuspension formulation approach is most suitable for the compounds with high log P value, high melting point and high dose. Nanosuspension is reported to enhance absorption and bioavailability of drugs may help to reduce the dose of the conventional oral dosage forms.

The present work is to prepare the nanosuspension of pilocarpine nitrate using Eudragit S 100 polymer by quassi emulsion technique and to validate the prepared occular formulation.

EXPERIMENTAL

Apparatus

UV spectrophotometer (1700PC) of Schimadzu cooperation of Tokyo, Japan was used for recording spectra and absorbance measurements in a quartz cell of 10mm path length. A Toa Electrics model HM-5B pH meter was used for pH measurements, FTIR Jasco cooperation, Japan

Materials

Pilocarpine nitrate and Eudragit S 100 was received as gift-sample from the Yarrow chemicals, Mumbai and all other chemicals used are purchased from SD Fine Chemicals LTD, Mumbai.

Methods

PREPARATION OF OCULAR NANOSUSPENSION

Preparation of Eudragit ocular nanosuspension [41]

Nanosuspension was prepared by the quassi-emulsion solvent diffusion technique. 100mg of drug and 100mg of polymer were co-dissolved at room temperature in 5ml of ethanol and sonicated for 10 minutes. The solution was slowly injected into 50 ml water containing Tween 80 (0.02% w/v) and benzalkonium chloride (0.1% w/v). During injection, the mixture was stirred by mechanical stirrer at various speeds for 1 h. The solution immediately turned into a pseudo-emulsion of the drug and polymer ethanol solution in the external aqueous phase. After completion of stirring, the solution dispersion was subjected to ultra sonication for a period of 10 min. Ethanol residues were left to evaporate off under slow magnetic stirring of the nanosuspension at the room temperature for 8-12 h. The nanosuspension was prepared in triplicate and the following evaluation studies were done.

1. Evaluation of pH

As pH is one of the most important factor which affect the solubility and stability of drugs, pH of all the prepared nanosuspension was determined. The pH of the prepared formulations was checked by immersing digital pH meter in prepared formulations. The prepared ocular nanosuspension have a pH of 6.4-7.4 (Eye have a tolerable capacity of 5-7.4 pH). The pH of the ocular nanosuspension was listed in the table 1.

Table 1: Evaluation of pH

S. No.	Formulation code	pH of the nanosuspension
1	F_1	7.0
2	F_2	6.8
3	F_3	7.2

2. Drug entrapment efficiency

The percentage of incorporated Pilocarpine nitrate (entrapment efficiency) in the prepared formulation was determined spectrophotometrically at 207.5 nm. After centrifugation of the aqueous suspension, amount of the free drug in supernatant liquid was determined by measuring its absorbance at 207.5 nm after proper dilution with phosphate buffer saline of Ph7.4. The amount of incorporated drug that is entrapment efficiency (%EE) was calculated by the following equation:

$$\% \text{ Entrapment efficiency (EE)} = \frac{W_{\text{initial drug}} - W_{\text{free drug}}}{W_{\text{initial drug}}} \times 100$$

The percentage drug entrapment efficiency was ranged between 65-80% as observed and shown in table 6.

Table 2: Drug entrapment efficiency studies

S. No.	Formulation code	Drug entrapment efficiency (%)
1	F_1	77.97
2	F_2	65.87
3	F_3	72.20

3. Determination of λ_{max} of Pilocarpine Nitrate

Preparation of Phosphate buffer saline pH 7.4

2.38g of disodium hydrogen phosphate, 0.19g 0f potassium dihydrogen phosphate and 8g of sodium chloride were dissolved in 1000 ml of distilled water. The pH of the solution was adjusted to 7.4 by using sodium hydroxide solution by pH meter.

Preparation of Pilocarpine nitrate standard solution

Pilocarpine nitrate standard stock solution was prepared by dissolving accurately weighed Pilocarpine nitrate (100mg) in 50ml of phosphate buffer saline pH 7.4. The volume was then made up to 100ml using phosphate buffer saline pH 7.4 to obtain the solution of 1mg/ml(Stock-I). From this standard stock solution, 1ml was transferred to 100ml volumetric flask. The volume was made up to 100ml with phosphate buffer saline pH7.4 (Stock-II, 10µg/ml).

Scanning of Pilocarpine nitrate by UV-Visible spectrophotometer in Phosphate buffer saline pH 7.4

The solution containing 10µg/ml was scanned between 200-400nm and the spectrum was shown in the figure-6. The λ_{max} was found to be 207.5nm and was used as analytical wavelength throughout the study.

Calibration curve of Pilocarpine nitrate

From the Pilocarpine nitrate standard stock solution-II, aliquots of 2, 4, 6,8,10 ml were taken into different volumetric flask and made up to 10ml with the phosphate buffer saline pH 7.4, so as to get drug concentrations from 2-10µg/ml(as Beer-Lamberts range is 2-20 mcg/ml). The absorbance of these drug solutions was determined at 207.5nm. This procedure was performed in triplicate.

The spectrum obtained during determination λ_{max} is shown in the fig no-1. The λ_{max} obtained in accordance with reported values

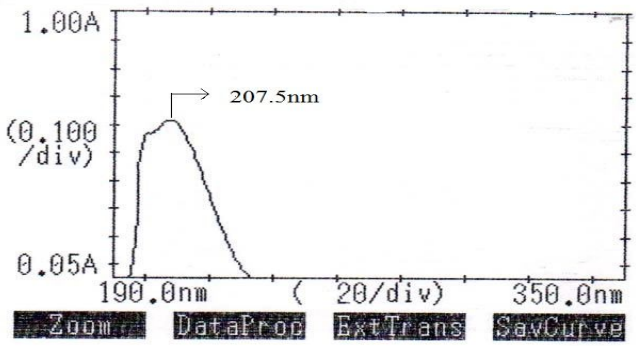

Fig 1: UV spectrum of Pilocarpine nitrate in phosphate buffer saline pH 7.4

Using λ_{max} value of Pilocarpine nitrate, its standard calibration curve was obtained and it is shown in table no-3 and Fig no-2.

Table 3: **Data for calibration curve of Pilocarpine nitrate in Phosphate buffer saline pH 7.4 at 207.5nm**

S. No.	Concentration (µg/ml)	Absorbance at 207.5nm AM±SD
1	0	0.000
2	2	0.112±0.012
3	4	0.0246±0.011
4	6	0.361±0.013
5	8	0.473±0.007
6	10	0.599±0.013

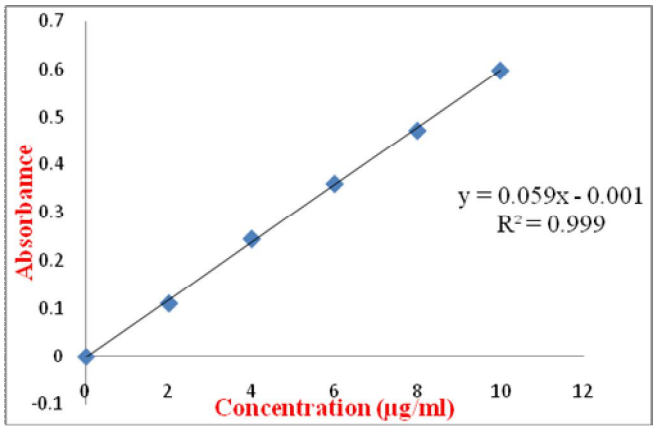

Fig. 2: Calibration curve of Pilocarpine nitrate in Phosphate buffer saline pH7.4

4. Differential scanning Calorimetry (DSC)

Differential scanning calorimetric analysis was performed using Shimadzu DSC-60 system. Sample of Pilocarpine nitrate (10 mg) and physical mixture of Pilocarpine nitrate and Eudragit S100 subjected to DSC studies. Enthalpy changes (ΔH) were calculated from peak areas of samples in order to study the possible polymorphic changes in the formulations.

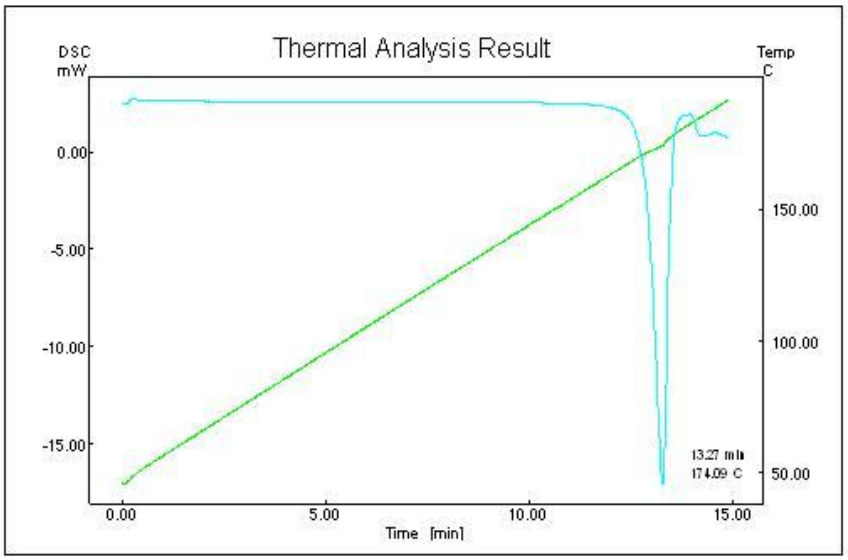

Fig. 3: DSC of Pilocarpine nitrate

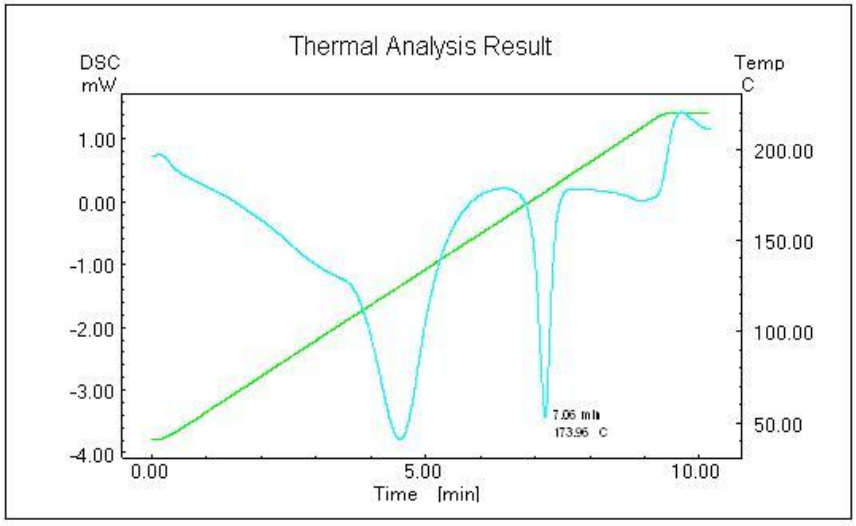

Fig. 4: DSC of Pilocarpine nitrate + Eudragit S100

Data from DSC study conclude that there were no drug polymer interactions between the drug and polymer combinations (Pilocarpine nitrate and Eudragit S100).

5. Drug polymer compatibility (FT-IR)

Drug polymer compatibility studies were carried out using FTIR spectrophotometer using KBr discs. Infrared spectra of pure Pilocarpine nitrate and physical mixture of drug- polymer were taken between 4000-400 cm^{-1}.

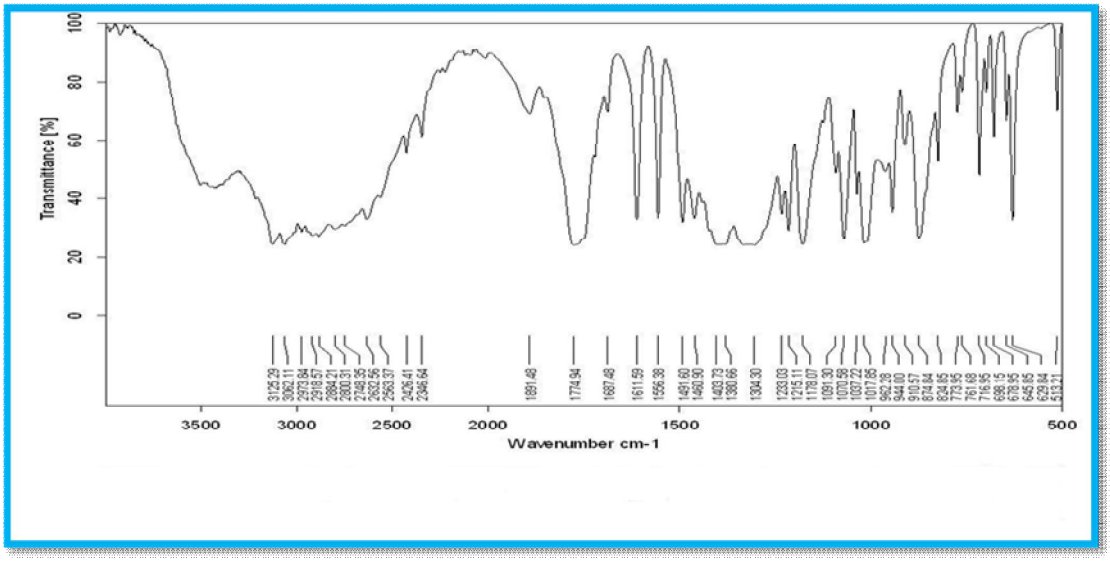

Fig. 5: IR Spectra of pure pilocarpine nitrate

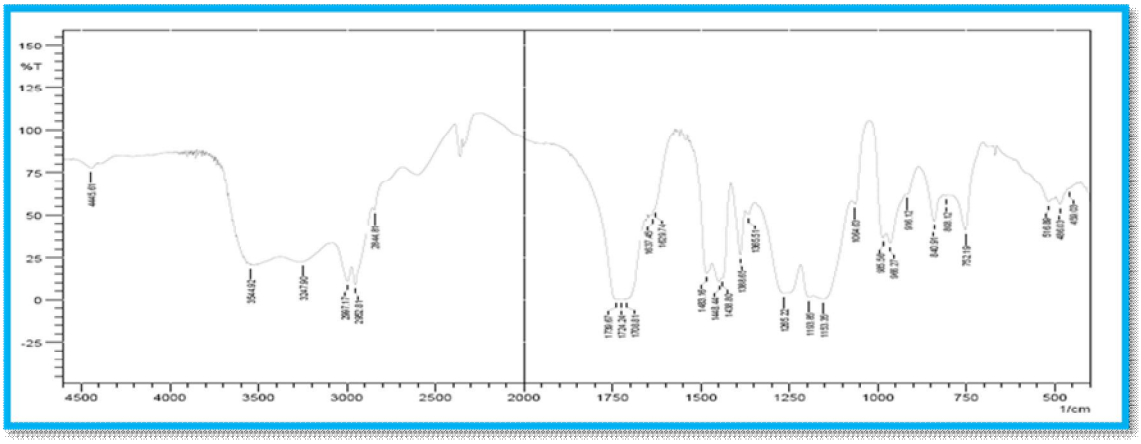

Fig. 6: IR spectra of Eudragit S 100

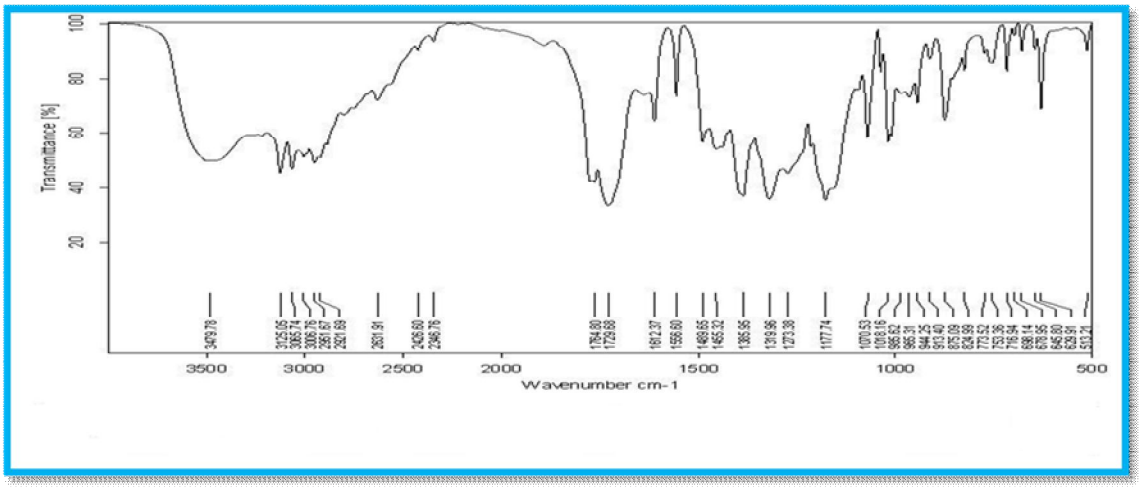

Fig. 7: IR spectrum of pilocarpine nitrate+Eudragit s 100

Table 4: IR interpretations of drug and polymer

S. No.	I.R spectrum	Groups	Peaks (cm^{-1})	Stretching /deformation
1.	Pilocarpine nitrate	C=N	1687.48	Stretching
		C-O	1070.58	Stretching
		C=O	1774.94	Stretching
		C-N	1233.03	Stretching
		C-H	2918.57	Stretching
2.	Physical mixture of Pilocarpine nitrate and Eudragit S100	C=N	1689.64	Stretching
		C-O	1070.53	Stretching
		C=O	1764.80	Stretching
		C-N	1273.38	Stretching
		C-H	292.69	Stretching

The drug polymer interactions was ruled out, as there was no major shifts in the absorption bands (peaks) of Pilocarpine nitrate in presence of polymeric combination viz.. Eudragit S 100.

RESULTS AND DISCUSSION

Absorption spectra

Pilocarpine nitrate drug solution of 2-10 mcg/ml were prepared using phosphate buffer saline of pH 7.4 and spectra was obtained at 207.5nm. Calibration curve was plotted and correlation coefficient was found to be 0.9995.

As pH is one of the most important factor which affect the solubility and stability of drugs, pH of all the prepared nanosuspension was determined. The pH of the prepared formulations was checked by immersing digital pH meter in prepared formulations. The prepared ocular nanosuspension have a pH of 6.4-7.4 (Eye have a tolerable capacity of 5-7.4 pH) results were discussed in table no1. Drug entrapment studies were carried out and the percentage drug entrapment efficiency was ranged between 65-80% as observed and shown in table no-2.DSC study conclude that there were no drug polymer interactions between the drug and polymer combinations (Pilocarpine nitrate and Eudragit S100) results were discussed in fig 3 and 4.

FTIR studies were carried out for pure drug polymer and physical mixture of pure drug with polymer (Eudragit S 100). IR spectra were shown in fig no-5, 6, and 7. The drug polymer interactions was ruled out, as there was no major shifts in the absorption bands (peaks) of Pilocarpine nitrate in presence of polymeric combination viz.. Eudragit S 100.

From the results, it was concluded that the formulations which contain drug: polymer ratios of 1:1 result the nanosuspension with good prolong release of medicament. However, we fail to get any conclusion on effect of concentration of ethanol on pharmacokinetic properties of formulations.

The prepared nanosuspension have shown good physical parameter (pH) in which the eye which have the tolerable capacity limit of 5-7.4.

CONCLUSION

Method of preparation employed for preparing nanosuspension was found to be simple and reproducible. The polymer used was non-toxic, relatively less expensive. Pilocarpine nitrate was prepared by quassi emulsion method using Eudragit S 100 and validated for pH, λ_{max} determination, entrapment efficiency, DSC, FTIR. Results obtained were within the limits indicating the proposed method for the formulation is good for single dosing and validation results were accurate and precise. As the present process validation study was carried out with limited number of process and formulation variables, increasing the number of this variable and further *in-vivo* study may result in optimization of ideal formulation with well established safety and efficacy profile.

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