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Original Article

FORMULATION OF NANOSTRUCTURED LIPID CARRIERS OF HALOPERIDOL PREPARED BY USING CLARIFIED BUTTER

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ABSTRACT

Objective: To develop the nanostructured lipid carriers (NLC's) using clarified butter.

Methods: Haloperidol-loaded microemulsion templates were prepared by using Smix of Tween 80 and propylene glycol in the ratio 1:2. The selection of the mixture of surfactant and co-surfactant (S_{mix}) and their appropriate proportion were decided by the traditional way of construction of pseudo ternary phase diagrams. 2^2 factorial design was used to check the amenability of the formulation for its successful scale-up. Sonication time and the amount of S_{mix} were selected as independent variables and their influence on the globular size (Y1) of the microemulsions formed was evaluated by using statistical models. Composition of the optimized microemulsion template was further used to prepare haloperidol-loaded NLC's by 'microemulsion quenching method'.

Results: The microemulsion formulations containing Tween 80 as a surfactant and propylene glycol as a co-surfactant exhibited the smallest globular size and hence this composition was used further to implement factorial design as design of experiments. The statistical analysis of the data suggested that the microemulsion formulation can be scaled up successfully. NLC's were prepared from the optimized microemulsion formulation as template. The globular size of NLC's was confirmed by Transmission Electron Microscopy and was observed to be in the range of 300 to 600 nm.

Conclusion: The present work suggested that the latency of the clarified butter as a natural blend of solid lipid and liquid lipids can be successfully explored to prepare nanostructured lipid carriers.

Keywords: Haloperidol, Haloperidol clarified butter, Nano-structured lipid carriers, Factorial design

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INTRODUCTION

The focus of current pharmacotherapy of neuropsychiatric disorders is on maximizing the efficacy of the treatment and reducing the development of adverse effects. This can be achieved not only through the traditional manner of clinical screening of new chemical entities but also through the development of new administrative strategies for established antipsychotics. The first-line antipsychotic medications are usually associated with several systemic side effects leading to poor adherence to the therapy [1]. Brain targeting via intranasal delivery has the potential to minimize cardiovascular, renal, and metabolic side effects of antipsychotic agents that are partly mediated by the blocking of peripheral dopaminergic receptors. Such targeting is also expected to help in alleviating peripheral anticholinergic effects and in minimizing the production of neurotoxic hepatic metabolites of drugs like haloperidol [2, 3]. There are many strategies reported in the literature for the improvement in the delivery of drugs through the olfactory epithelium of the nasal cavity to the brain directly [4]. Nanoparticles and nanocarriers have been widely used in nose-to-brain delivery. Lipid-based nanocarriers are very well-investigated and it is an established technology in the field of pharmaceutics nowadays.

Solid lipid nanoparticles (SLN) and Nanostructured lipid carriers (NLC) are popular nanocarriers for a variety of drug molecules. Nanostructured lipid carriers are preferred to solid lipid nanoparticles to overcome certain limitations of solid lipid nanoparticles [5]. NLCs offer many advantages, such as high encapsulation efficiency for hydrophobic molecules, high efficiency for the nose-to-brain transport, and high long-term stability related to less/absence of polymeric transition during storage [6]. Natural lipids are successfully used in the formulation of NLC due to their biocompatibility and low cost. Natural lipids are plentiful and diverse in composition and thus provide enormous applications if compared with semi-synthetic compounds. Considering the versatile applications of natural lipids, it was decided to develop NLC of haloperidol by using a natural lipid [7]. A thorough literature survey revealed that the butter oil when used along with an intra-nasal formulation of quetiapine fumarate, improved its bioavailability in CNS. Long-chain triglycerides present in the butter oil instigated greater drug accumulation in the brain [8, 9].

Lipids employed for the production of nanocarriers include mixtures of triglycerides, waxes and fatty acids. The lipid phase of nanostructured lipid carriers at room temperature contains both solid (fat) and liquid (oil) lipids. Successful production of NLC's depends on the selection of an appropriate lipid blend. Miscibility of the solid and liquid lipids that are to be used to form NLC at the specific concentrations is one of the fundamental formulation requirements in the preparation of NLC's. Ghee or clarified butter is a traditional dairy product available in India. Its composition provides a natural combination of short and high-chain triglycerides and phospholipids i.e. natural combination of solid lipid and oil [10, 11]. Phospholipids present in the clarified butter are also reported to be efficient permeation enhancers [12, 13]. Therefore clarified butter was selected in this study to prepare NLC's being a natural combination of solid and liquid lipids. Haloperidol was selected as a model antipsychotic drug and the present work describes formulation development of NLCs prepared by using stratified butter (ghee) as a suitable natural lipid blend.

MATERIALS AND METHODS

Materials

Haloperidol was gifted by Appcure Labs Pvt. Ltd. Hyderabad. Clarified butter was purchased from the local market India. Other chemicals used in the formulation were of pharmaceutical grade and the chemicals used in the analysis were of analytical grade and were used as received.

Experimental design

The surfactant, and co-surfactant combination and their appropriate proportion necessary to stabilize the microemulsion of clarified butter was selected by constructing pseudo-ternary phase diagrams

[6]. Full factorial 2^2 experimental designs was planned and implemented to confirm the main factors that influenced globular size distribution in microemulsions of clarified butter. The added intent to implement factorial design was to study interaction effect if any; between the factors selected on the basis of previous research outcomes reported in the literature [14]. After regression analysis of the data generated; the microemulsion formulation with least poly dispersity index was further nano-emulsified. The globular size of NLC's was measured by transmission electron microscopy (TEM).

Selection of surfactant and co-surfactant (Smix)

Lecithin and Tween 80 were screened for the capacity to emulsify clarified butter. Emulsifying capacity was assessed on the basis of the stability of emulsion formed and the globular size of lipid phase measured after keeping the microemulsions for 24hr. After this preliminary screening, Tween 80 was selected as a surfactant to form Smix. Smix is a mixture of the Tween 80 as a surfactant with one of the four (ethanol, isopropyl alcohol, polyethylene glycol 400 and propylene glycol) co-surfactants in the ratio1:1.

Blends of clarified butter with each Smix in the ratios of 1:15, 1:20 and 1:25 at a time were prepared. Then all the blends were heated gently and vortexed to attain complete homogenization. Each blend was slowly titrated with aliquots of warm distilled water until the mixture became turbid. After the addition of each increment of warm water, the mixtures were well stirred and finally were kept in the sonicator for 30 min at room temperature. Initial visual appearance of these mixtures was recorded immediately after attainment of equilibrium and after 24 h. The selection of the Smix was made on the basis of ease of emulsification, globular size and stability of the formed emulsion [15]. These observations are reported in table 1.

Table 1: Stability of preliminary microemulsions prepared by using various co-surfactant

Smix (Surfactant and Co-surfactant)	Clarified butter: Smix	Stability of microemulsion after 24 h
Tween 80+Ethanol	1:15	Stable
	1:20	Stable
	1:25	Stable
Tween 80+	1:15	Stable
Isopropyl alchohol	1:20	Stable
	1:25	Stable
Tween 80+PEG-400	1:15	Unstable
	1:20	Unstable
	1:25	Unstable
Tween 80+Propylene glycol Glycol	1:15	Stable
	1:20	Stable
	1:25	Stable

Construction of pseudo-ternary phase diagram

Pseudo-ternary phase diagrams were constructed by adopting the traditional aqueous titration method [6]. The lipid component of all the microemulsions was clarified butter which is the natural mixture of solid and liquid lipids. Smix was the mixture of propylene glycol as co-surfactant along with Tween 80 as a surfactant in three various proportions as 2:1, 3:1, and 1:2. These ratios were selected on the basis of results obtained by conducting a series of trial and error experiments. The aqueous phase was distilled water. For the construction of each phase diagram, the selected lipid phase and the specific Smix ratio were mixed in weight ratios ranging from 1:9 to 9:1 such that 9 combinations were obtained. The lipid phase was gently heated in a test tube just to melt it. The required quantities of Smix were added and mixtures were vortexed to attain homogenization. These mixtures were titrated with warm water until turbidity is seen. The compositions of the mixtures those retained a transparent or translucent appearance after 24 hr were considered for the construction of pseudo-ternary phase diagrams. CHEMIX 3.51™ software was used to construct a pseudo-ternary phase diagram.

Implementation of 2^2 factorial design

A 2^2 full factorial design was used for the optimization of the composition of microemulsions and to know the impact of sonication time as well as the amount of Smix on the globular size. According to the model total four formulations are possible; the composition of different formulations are shown in table 2. The different independent variables were sonication time (X1) and Amount of Smix (X2). The factor levels were chosen on the basis of the results of an evaluation of preliminary formulations. Dependent factor included Globular size and poly-dispersity index was determined for each formulation.

Table 2: Factorial formulations of micro-emulsions

Ingredients formulation code	Sonication time in min (X1)	Amount of Smix (X2)	Clarified butter: Smix ratio	Distilled water (ml)
F1 (+,+)	60	1:15	1:2	5.5
F2 (+,−)	60	1:10	1:2	5.5
F3 (−,+)	30	1:15	1:2	5.5
F4 (−,−)	30	1:10	1:2	5.5

Briefly, the required amount of clarified butter (1.2 gm) was taken and heated to 10 °C above its melting point. In this molten lipid, the drug (30 mg), surfactant (Tween 80), and co-surfactant (Propylene glycol) were added in calculated amounts and vortexed to form a uniform mixture and an oily pre-concentrate was obtained. This pre-concentrate was dispersed into 5.5 ml of distilled water and vortexed again to form a microemulsion. The microemulsions formed were spread on the glass slide and observed under the Motic Digital Microscope and the globular size of each factorial formulation was recorded.

Preparation and characterization of nanostructured lipid carrier of Haloperidol

Nanostructured lipid carriers (NLC's) were prepared from warm microemulsion templates of composition F1 as mentioned in the above section in table 1. The method of preparation adopted was similar to that reported by Joshi and Patravale in 2006 with slight modifications. The formulation was maintained at a temperature above the melting point of the stratified butter (60 °C). This warm microemulsion (2 ml) was added dropwise in 10 ml of cold water (2–3 °C) containing 1.12 % v/v n-butanol as additional co-surfactant under mechanical stirring at 3000 rpm for 10 min.

Globule size and morphology study by transmission electron microscopy

Morphological analysis for the NLC formulation containing Haloperidol was performed by transmission electron microscopy (TEM). Uranyl acetate (2%) was added to the diluted NLC's. Then, sufficient aliquots were deposited onto carbon film-coated copper grids and dried at room temperature. After drying, micrographs of

the samples were appreciated using a JEOL 1200 EXII microscope operated at 80 kV [7].

Differential scanning calorimeter study

The Differential Scanning Calorimeter Study was performed for NLC formulation. Thermograph was obtained by heating 1–10 mg sample in crimped Aluminum pans at a heating rate of 100C/min, from 40 0C to 330 0C, in a nitrogen atmosphere (flow rate 40 ml/min). Data were analyzed using STAR-SW 9.20 software.

RESULTS AND DISCUSSION

Experimental design

Selection of Smix and the proportion of surfactant: co-surfactant in Smix was determined by constructing pseudo-ternary phase diagrams. Construction of pseudo ternary phase diagrams is the scientific approach that saves time in the selection of appropriate Smix for the lipid phase selected for the study; a number of experimental trials required are less. Phase diagrams also help to find the microemulsion region in ternary/pseudo ternary system with the minimum amount of Smix for micro emulsion formation. Factorial design is a powerful tool to generate and analyze the data in determining the relationship between the factors affecting the formulation process and the desired quality attribute of the formulation. The amount of Smix in proportion to the lipid phase and sonication time are selected as factors or independent variables, as they are reported to influence globular size and the stability of microemulsion [16]. The optimized microemulsion formulation was further converted to a nanostructured lipid carrier. The preparation of nanostructured lipid carriers (NLC) was similar to that reported by Joshi and Patravale in 2006 with slight modifications. This method was adopted because of its obvious advantages, such as the elimination of the need for both specialized equipment and less energy required generating nano-carriers.

Selection of surfactant and co-surfactant (Smix)

All the microemulsions formulated with lecithin as a surfactant exhibited larger globular size and signs of phase separation after 24 h. These microemulsions showed creaming of the lipid phase at the top; which was difficult to re-disperse. Whereas microemulsions prepared by using Tween 80, were found stable after 24 h with smaller globular size. The observed differences in the emulsification capacity of Tween 80 and Lecithin might be because of differences in their structure and packing parameters [17]. Thus Tween 80 depicted its better emulsification capacity in the emulsification of the selected lipid component i.e. clarified butter. Also, significant pharmacological effects in CNS are reported in the literature after injecting nanocarriers' formulations coated by Tween 80 when administered intravenously in rats [18, 19].

The use of a single surfactant hardly achieves the negative interfacial tension necessary to cause self-emulsification between the two immiscible phases. Hence, there is always a need to add a co-surfactant along with the surfactant as it reduces the bending stress between the interfaces and provides sufficient flexibility to the interfacial film. Hence, co-surfactants like ethanol, isopropyl alcohol, polyethylene glycol 400, and propylene glycol were screened. As reported in table 1, three of these co-surfactants i.e. ethanol, isopropyl alcohol, propylene glycol showed acceptable emulsification capacity as co-surfactant with Tween 80. However, since the microemulsion formulations containing propylene glycol as a co-surfactant exhibited the smallest globular size amongst all; it was preferred in the design of further formulations.

Construction of pseudo-ternary phase diagram

A self-emulsifying region was identified after constructing pseudo-ternary phase diagrams. This is the first stage of formulation development of nanostructured lipid carriers by microemulsion technique [15, 20]. Amongst the various surfactant/co-surfactant (Smix) ratios that were screened, the maximum microemulsion region was found when formulations were prepared by using Smix of Tween 80 and propylene glycol in the ratio 1:2 (fig. 1). Thus, this ratio of Smix was fixed for the optimization of the stable microemulsion formulation.

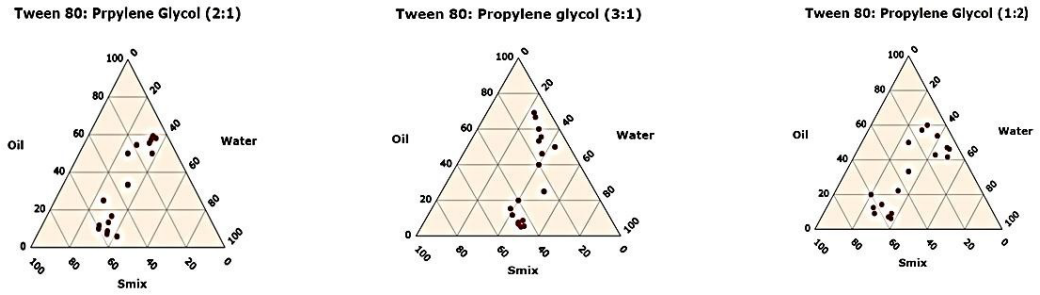

Fig. 1: Pseudo-ternary phase diagrams

Implementation of 2^2 factorial design

Micro emulsions of factorial batches were evaluated for globular size and polydispersity index and the results are reported in table 3. The lower value of the polydispersity index indicates an optimum balance of HLB of Smix at the interface. The formulations F1 and F2 exhibited a smaller polydispersity index and it clearly signified the influence of sonication time on the globular size. The factorial formulations F1 and F2 were prepared after sonication of the pre-mix for 60 min and resulted in the formation of globules of uniform size as evident from the smaller polydispersity indices. It is statistically supported by a useful graphical tool 'Pareto chart' (fig. 2). The Pareto chart is a superior tool to the half-normal plot to check for a more significant effect [21]. The Pareto chart shows the factors/variables in decreasing order of their impact on the response (globular size in the present study). The globular size reduction was highly influenced by sonication time than the amount of Smix added in the formulation in the present study; as indicated by the first position and the height of the bar in the Pareto chart. The design was evaluated by a quadratic model, which bears the form of the equation;

$$\text{Globular size} = 5.12 - 1.27X1 - 0.8317X2 + 0.0813X1X2$$

Table 3: Data for evaluation of factorial batches

Formulation code	Globular size (µm)*	Poly-dispersity index
F1	3.10±0.05	0.108
F2	5.70±1.08	0.183
F3	6.04±2	0.351
F4	5.63±0.7	0.116

*Results are expressed in mean±SD (n=3)

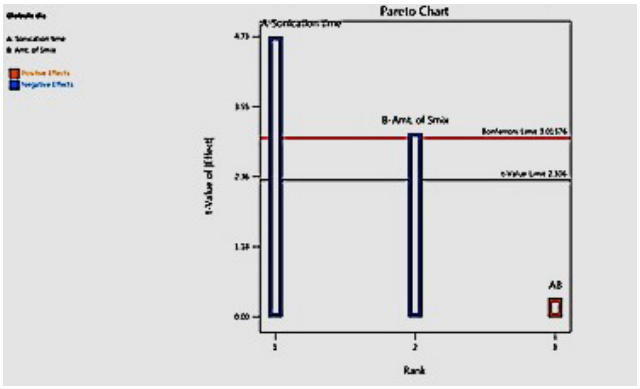

Fig. 2: Pareto chart

The average globular size of all the formulations was 5.12 microns. As sonication time was increased, it reduced the globular size as indicated by the negative coefficient of X1. As the amount of Smix was increased in the formulation, the globular size decreased as indicated by the negative coefficient of X2. But the numeric coefficient of X1 is greater than the coefficient of X2; which indicated that sonication time has more impact on the reduction of globular size than the amount of Smix added in the formulation. The interaction between the factor X1 and X2 was negligible (as the coefficient of X1X2 was small) though both were found to negate the effect of each other.

The contour plot shows the effect of sonication time and amount of Smix added in the formulation on the globular size of the dispersed phase (fig. 3). The globular size was reduced with increasing sonication time and the amount of Smix added in the formulation. With a higher amount of Smix in the formulation, the sonication time had a greater impact on globular size reduction.

The predictability coefficient (R2) value in the 'Fit statistics' is near to one and the difference between the adjusted and predicted R2 values is<0.2 (fig. 4). The analysis of the data suggested that the selected levels of factors (sonication time and amount of Smix added in the formulation) can be used to navigate the design space. This means that the microemulsion of desired globular size with a smaller polydispersity index can be obtained and the preparation process can be successfully scaled up if sonication time as well as the amount of Smix added in the formulation, is properly controlled. This is the point of major concern as the clinical application of nanocarriers system is dependent on globular size and polydispersity index as key parameters [22-24].

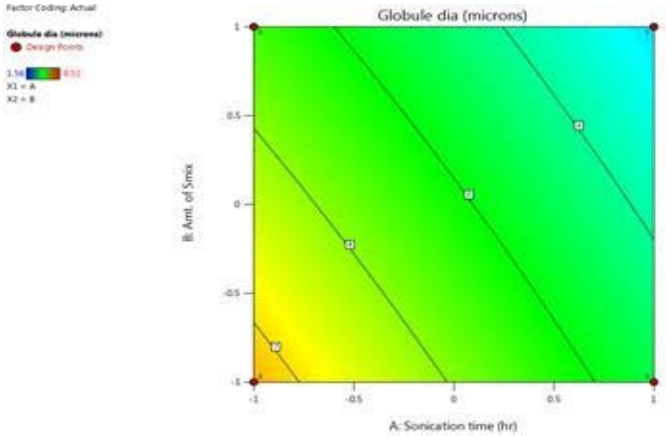

Fig. 3: Contour plot

Fit Statistics

Std. Dev.	0.9305	R^2	0.8002
Mean	5.12	Adjusted R^2	0.7252
C.V. %	18.17	Predicted R^2	0.5504
		Adeq Precision	7.8241

The **Predicted R^2** of 0.5504 is in reasonable agreement with the **Adjusted R^2** of 0.7252; i.e. the difference is less than 0.2.

Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 7.824 indicates an adequate signal. This model can be used to navigate the design space.

Fig. 4: Fit statistics

Preparation and characterization of nanostructured lipid carrier of haloperidol

The method used for the preparation of NLC was similar to that reported by Joshi and Patravale in 2006 with slight modifications. The modified method was adapted because of its obvious advantages, such as the elimination of the need of both specialized equipment and less energy is required to generate Nano-carriers [15, 25]. Hydrophilic co-solvent n-butanol is reported to get distributed rapidly in the aqueous phase of the microemulsion and had a critical role in the formation of lipid nano carriers [26, 27]. A smaller amount of n-butanol was sufficient (1.12 % v/v) to obtain NLC's in the present work. It indicated that the clarified butter is a flexible lipid (less hardness).

The TEM image (fig. 5) revealed typically spherical, intact NLC's with globular size in the range of 300 to 600 nm. The DSC thermograph (fig. 6) of the NLC formulation exhibited broad endothermic peak

around 102 °C due to the evaporation of water present in the formulation. The melting endotherm of the haloperidol completely disappeared in it. This clearly indicated that the haloperidol is in completely solubilized form in the formulation.

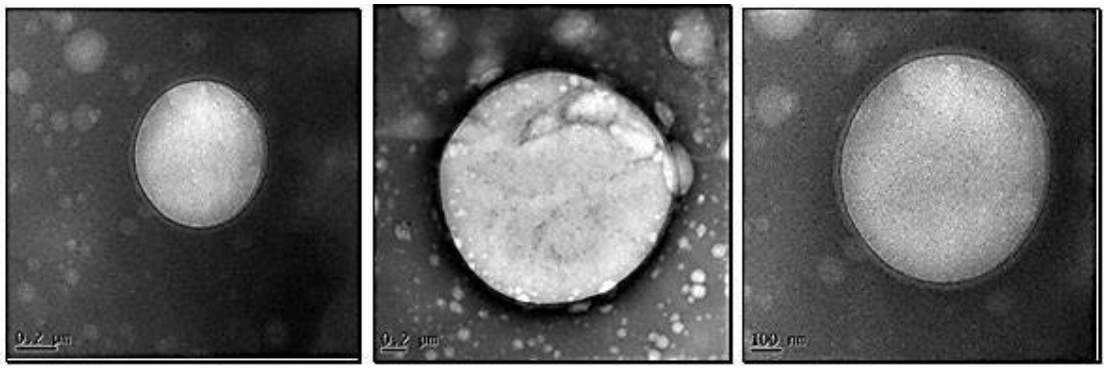

Fig. 5: TEM images of haloperidol loaded NLC's

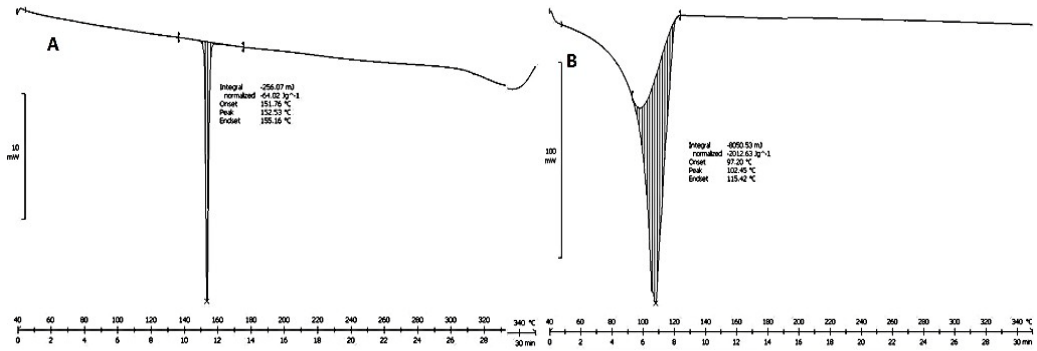

Fig. 6: DSC thermograph of plain haloperidol (A) and haloperidol loaded NLC's (B)

CONCLUSION

Selection of an appropriate blend of solid lipid and liquid lipid is the most challenging part in the development of NLC's. The aim of the present study was to formulate a new NLC with the naturally available combination of solid lipid and liquid lipid composition of food grade, i.e. clarified butter. The haloperidol-loaded NLC's were successfully prepared by the energy-saving 'microemulsion quenching method,' and the scalability of the process was evaluated by implementing the factorial design as the design of experiments. Thus, this technique can be explored further, as there are high chances of regulatory approval to clarified butter as a novel natural lipid component in NLC formulation.

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Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

The authors declare no conflict of interest, financial or otherwise.

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