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# Physicochemical characterization of a reverse micellar solution after loading with different drugs

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Diclofenac sodium (DS), timolol maleate (TM) and pilocarpine hydrochloride (PHCl) were solubilized in a reverse micellar solution (RMS) consisting of lecithin and middle-chain triglycerides (MCT). The influence of these drugs on the physicochemical parameters of the RMS was investigated. Although none of the drugs influenced the size of the associates, the micellar shapes varied considerably. While DS and PHCl increased the anisometry, reverse micelles with TM were spherical. The transformation of the RMS into a lamellar mesophase on contact with water was generally not prevented by any of the drugs but all drugs led to an increased number of defect structures in the liquid crystal. Furthermore the interlayer distance of the lamellar mesophase was reduced by a higher content of DS. In comparison with an RMS containing isopropylmyristate (IPM) instead of MCT, differences in the physicochemical properties of the drug-free RMS and in the influences of solubilized drugs were noticed.

## 1. Introduction

Reverse micellar solutions (RMS) containing lecithin and isopropylmyristate (IPM) transform into a lamellar mesophase on contact with water. This water-contact induced transformation into a semisolid system of liquid crystals enables controlled release of solubilized drugs [1]. Previous studies have shown its applicability for ophthalmic application [2].

Physicochemical parameters of the RMS have been described in previous investigations [3]. The aim of the present contribution is the physicochemical characterization of a modified system using middle-chain triglycerides (MCT) instead of IPM. Not only physicochemical properties of the water-free RMS have been characterized but also the influence of solubilized drugs on the transformation of the RMS into a lamellar mesophase. Additionally, differences in properties of the MCT system and the IPM system are shown.

## 2. Investigations and results

### *2.1. Characterization of water-free RMS systems*

#### *2.1.1. Determination of the intrinsic viscosity*

The intrinsic viscosity [η] reveals information about the particle shape. An intrinsic viscosity of 2.5 goes along with spherical particles whereas anisometric associates cause a deviation from 2.5. Figure 1 shows the standardized specific viscosity in dependence of the lecithin concentration of different RMS systems. In Table 1 the determined intrinsic viscosity [η] and assigned half-axes ratios of the reverse micelles for rod and disc model [4] are shown.

The associates of the drug-free RMS are not isometric but twice as long as wide. While solubilization of DS and PHCl increases the anisometry (the associates are 3–5 times as long as wide), reverse micelles containing TM are spherical.

In comparison to an RMS system containing IPM, the anisometry of the reverse micelles in MCT is less marked (Table 2). It is to be noted that a remarkably high increase in anisometry of PHCl loaded associates does not occur in MCT as it happens in the IPM system.

#### *2.1.2. Particle size measurements*

The diameters of all associates are between 5.0 and 6.5 nm. There is no significant influence of any drug on the particle size of the reverse micelles.

In an IPM system the diameters of drug-free and DS loaded reverse micelles are about 10 nm. While in IPM

**Table 1: Intrinsic viscosity [η] and half-axes ratios a/b of reverse micelles for rod and disc model [4]**

| System | Molar ratio | [η] | a/b (rod) | a/b (disc) |
|---|---|---|---|---|
| RMS | / | 3.13 | 2.0 | 2.0 |
| RMS with DS | 0.441 | 4.85 | 3.8 | 4.9 |
| RMS with PHCl | 0.337 | 4.15 | 3.2 | 3.9 |
| RMS with TM | 0.085 | 2.16 | 1.0 | 1.0 |

For the latter calculation the associate density ϱ was estimated as 1 g/cm³

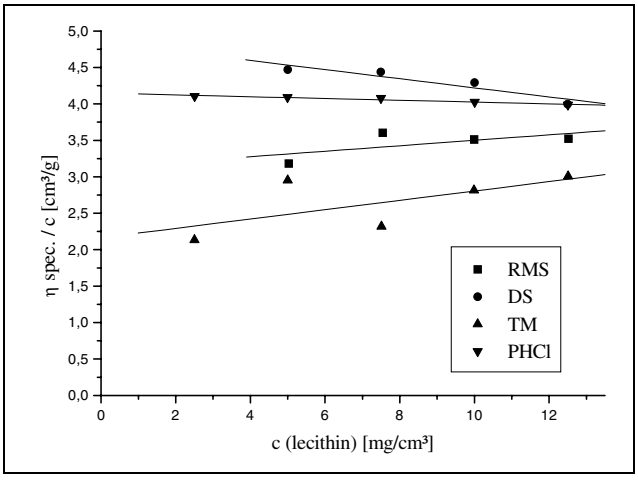


Fig. 1: Standardized specific viscosity versus lecithin concentration. The molar ratios of drug to lecithin are: DS 0.441 (corresponding to 5% (m/m) in the concentrated RMS with 30% (m/m) lecithin); TM 0.085 (1%); PHCl 0.337 (3%).

**Table 2: Comparison of the intrinsic viscosity [η] of different RMS systems containing either MCT or IPM**

| System | Molar ratio | [η] (MCT system) | [η] (IPM system) |
|---|---|---|---|
| RMS | / | 3.13 | 6.2 |
| RMS with DS | 0.441 | 4.85 | 6.6 |
| RMS with PHCl | 0.337 | 4.15 | 13.5 |
| RMS with TM | 0.085 | 2.16 | / |

the size of PHCl loaded associates increases up to the triple, in MCT the growth is missing.

*2.1.3. Rheological measurements*

All systems, i.e. both RMS and drug-loaded RMS, exhibit ideal viscous flow properties which indicates minor interactions between the reverse micelles.
Figure 2 shows the influence of the solubilized drug concentration on the dynamic viscosity η of the RMS. Especially after solubilization of DS and PHCl the viscosity increases. This points at an increase of the anisometry of these associates. In contrast, TM has only minor influence. After a slight increase the viscosity remains constant, hence it can be supposed that TM leads to more spherical reverse micelles. The slight increase of the viscosity observed with 0.25% (m/m) TM could be caused by water remaining from the preparation with a highly diluted aqueous solution. The data of the rheological measurements confirm the results of the shape and size determinations.
Although the viscosity of MCT in comparison to IPM is six times higher (30 mPas vs. 5 mPas) the viscosities of the water-free RMS, i.e. of the MCT system and the IPM system, are in the same range (about 150 mPas). In contrast, drug-loaded RMS containing IPM have higher viscosities than equivalent systems containing MCT (Table 3). The more anisometrical associates in the IPM system lead to a higher increase in viscosity.

**Table 3: Comparison of the dynamic viscosity η of RMS systems containing either MCT or IPM**

| System | Drug content (% (m/m)) | η (mPas) (MCT system) | η (mPas) (IPM system) |
|---|---|---|---|
| RMS with DS | 4 | 330 | 750 |
| | 6 | 600 | 2500 |
| | 8 | 1220 | 7000 |
| | 10 | 2460 | 17000 |
| RMS with PHCl | 2 | 170 | 490 |
| | 3 | 220 | 1200 |

### *2.2. Interactions between drugs and lamellar mesophases*

*2.2.1. Polarisation microscopy*

After incorporation of water the RMS transforms into a semisolid system of liquid crystals. By means of polarisation microscopy lamellar mesophases were identified as characteristic textures in all systems, i.e. both RMS and drug-loaded RMS. Typical textures for a lamellar mesophase are maltese crosses, either separate ones or in chains as "oily streaks" [5, 6]. With increasing drug content in the RMS an increase of separate maltese crosses was noticed with all drugs.

*2.2.2. Transmission electron microscopy (TEM)*

After shock-freezing and replication typical colloidal structures of the lamellar liquid crystal such as layered planar surfaces were observed with all drugs investigated. There is no change in the principle structure of the mesophase after solubilization of the drugs. Figure 3 shows a typical micrograph of a lamellar mesophase, of which the layer thickness was estimated to be about 5 nm.

*2.2.3. Small angle X-ray diffractometry (SAXD)*

To determine the influence of solubilized drugs on the interlayer spacing of the lamellar mesophase (Fig. 4), different RMS with a constant water content of 10% (m/m) were investigated with SAXD. In all cases the ratio of the

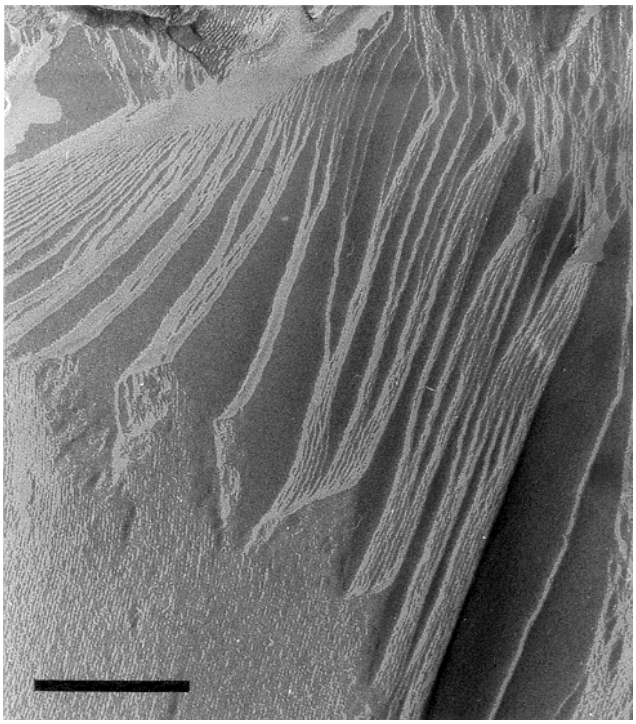

Fig. 3: Typical structure of the lamellar mesophase with 2% (m/m) PHCl observed by transmission electron microscopy (bar = 238 nm).

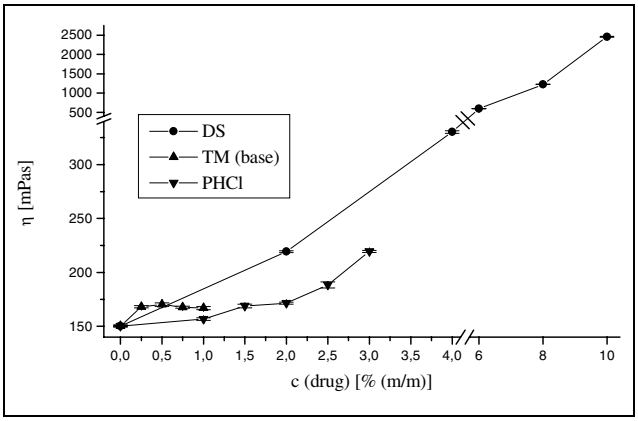


Fig. 2: Influence of drug concentration on the dynamic viscosity η of the RMS (n=3).

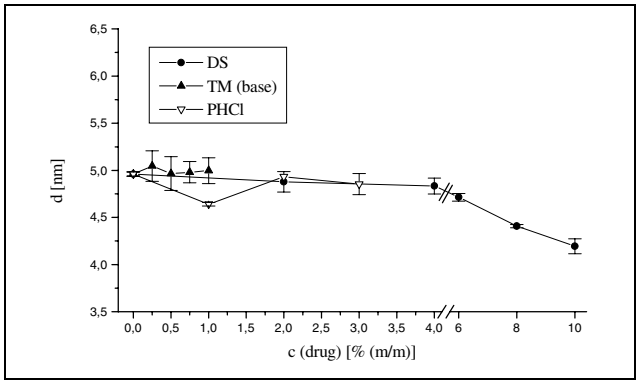


Fig. 4: Interlayer spacing d of the lamellar mesophase versus drug concentration (n = 2). The water content of all systems is 10% (m/m).

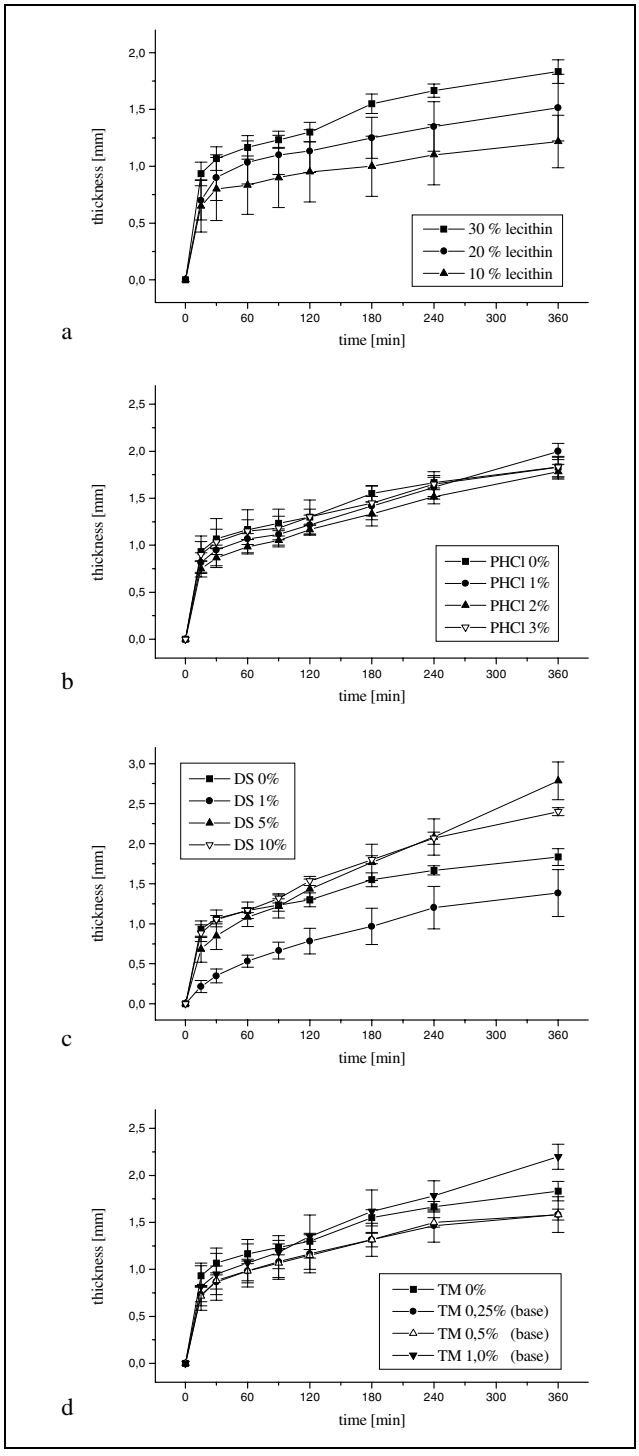


Fig. 5: Liquid crystal growth versus time (n = 3). a: without drug b: with PHCl c: with DS d: with TM

measured interferences corresponds to 1 : 1/2 : 1/3 which is characteristic for lamellar mesophases [7]. While solubilized TM has no influence on the interlayer spacing d, 1% (m/m) PHCl reduces d. Higher contents of PHCl have again no influence on d. Furthermore the layer thickness was reduced by solubilized DS in higher amounts. Also in RMS containing IPM, DS leads to a reduced interlayer distance whereas PHCl has no significant influence.

### *2.2.4. Liquid crystal growth on hydrogel*

Prior to controlled release of the solubilized drug a transformation of the RMS into a lamellar mesophase on contact with water has to occur. In order to determine the drug influence on the liquid crystal formation, the growth of the mesophase was investigated in contact with a hydrogel as water donator (Fig. 5). Growth rate and maximum thickness of the liquid crystal depend on the lecithin concentration of the RMS. The more material is available for liquid crystal formation the higher is the thickness of the mesophase.

PHCl has no significant influence on the liquid crystal growth. Low contents of DS and TM seem to reduce the maximum thickness whereas high contents of both lead to an increase in maximum thickness. Whether differences observed are significant or not has to be investigated in further studies.

## 3. Discussion

Although none of the investigated drugs influence the size of the associates, the micellar shapes vary considerably. DS and PHCl increase the anisometry whereas reverse micelles with TM are spherical. This finding may be caused by a different incorporation of each drug into the reverse micelles (Fig. 6). DS and TM, both amphiphilic, seem to be localized between the lecithin molecules in the reverse micelles. Due to the bigger lipophilic area of TM, the associates probably are forced into a spherical shape. PHCl is likely to be solubilized in the hydrophilic core of the reverse micelles and thus causes also an increase in anisometry.

In contrast to an RMS containing IPM, the associates in an RMS with MCT are smaller (6 nm vs. 10 nm). Furthermore the significant increase in size with PHCl up to the triple, noticed in IPM, does not occur as well as the anisometry of the reverse micelles is generally less marked in MCT systems. Also, PHCl could be solubilized to a smaller extent in the MCT system than in the IPM system (3% vs. 5% (m/m)). The physicochemical properties of the lipophilic solvent such as polarity and viscosity seem to influence size and shape of the reverse micelles.

A hydration of the polar lecithin heads leads to a transformation of the reverse micelles into a mesophase which was identified as a lamellar liquid crystal. None of the investigated drugs prevent the transformation but an increase of separate maltese crosses was noticed upon increasing drug content. This increase could be caused by an increase in defect structures within the planar lamellar mesophase according to a change in molecular geometry of both lecithin and the solubilized drug. Moreover, a drug influence on the interlayer distance of the liquid crystal was noticed. Supposing an equivalent incorporation

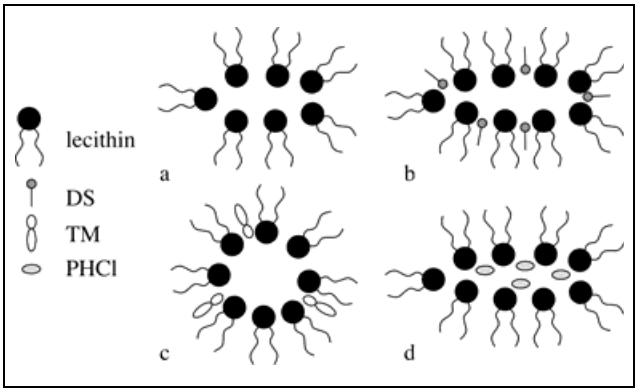


Fig. 6: Schematic structure of the reverse micelles. a: without drug b: with DS c: with TM d: with PHCl

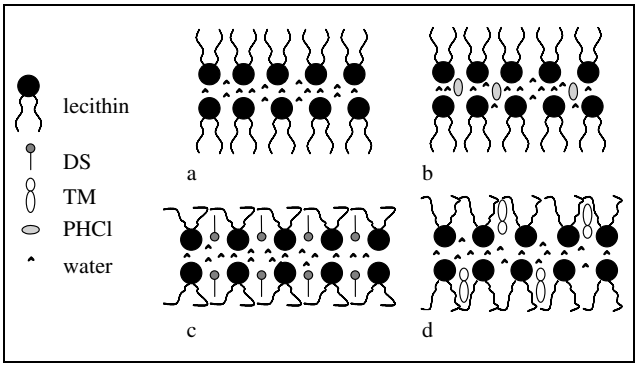


Fig. 7: Schematic structure of a single bilayer of the lamellar liquid crystal. a: without drug b: with PHCl c: with DS d: with TM

of the drugs as in the reverse micelles (Fig. 7); i.e. DS and TM between the lecithin molecules and PHCl in the hydrophilic regions of the liquid crystal; DS could reduce the layer thickness due to the fact that DS is a shorter molecule than lecithin. On account of the flexible lipophilic region of lecithin, chain folding can occur together with a decrease in the layer thickness. TM is a larger molecule than DS; therefore chain folding cannot occur. PHCl, being localized in the hydrophilic region, has no influence on the layer thickness.

## 4. Experimental

### *4.1. Materials*

Diclofenac sodium (DS) was purchased from Synopharm (D-Barsbüttel); timolol maleate (TM) and pilocarpine hydrochloride (PHCl) were supplied by Dr. Winzer Pharma (D-Olching). Phosal® 53 MCT is a solution of purified soybean lecithin Phospholipon® 90 (54.6% (m/m)) in a mixture containing mainly middle-chain triglycerides (about 30% (m/m)). Other ingredients are mono- and diglycerides, oleic acid and ethanol in minor concentrations. The product was supplied by Rhône-Poulenc-Rorer (D-Köln). Miglyol® 812, a mixture of middle-chain triglycerides, was purchased from Hüls (D-Witten). Water was used in bidistilled quality. Pilocarpine hydrochloride (PHCl) used for RMS systems containing IPM was supplied by Dr. Mann Pharma (D-Berlin). Other materials used for these systems are in accordance with [3].

### *4.2. Methods*

The preparation of RMS systems containing IPM was performed as reported earlier [3].

#### *4.2.1. Preparation of the reverse micellar solutions*

Phosal® 53 MCT was adjusted to a lecithin concentration of 30% (m/m) with Miglyol® 812. The mixture was stirred with a teflon coated magnet for 1 h. DS was dispersed as a solid in the RMS whereas TM and PHCl had to be added as aqueous solutions (PHCl : water 1 : 2; TM : water 1 : 16 (m/m)). The hereby incorporated water which intermediately transformed the RMS into a lamellar mesophase was evaporated together with the ethanol of the Phosal® 53 MCT while stirring the RMS at a temperature of 323 K for 24 h in order to solubilize the drugs. The drug-free RMS was prepared in the same way to evaporate the small amount of ethanol. The influence of DS on physicochemical properties of the RMS was determined in concentrations up to 10% (m/m). TM was investigated in concentrations up to 1% (m/m) of the timolol base and PHCl up to 3% (m/m), both concentrations are known to be commonly used in ophthalmic formulations [8].

#### *4.2.2. Capillary viscometry*

The kinematic viscosity ν of low concentrated RMS was measured with an Ubbelohde capillary viscometer (Schott, D-Hofheim) at 293 K. By means of the different RMS densities, the dynamic viscosity η was determined from the kinematic viscosity ν. Then the standardized specific viscosities $\eta_{spec.}/c$ (lecithin) shown in Fig. 1 were calculated. To determine the intrinsic viscosity [η] the graphs were extrapolated to the zero concentration of lecithin.
The density ϱ of the low concentrated RMS was measured with a DMA 46 instrument (Anton Paar, A-Graz) at 293 K.

#### *4.2.3. Rheological measurements*

Rheological measurements were performed at 293 K on a rheometer CVO/CS (Bohlin Instruments, GB-Cirencester) with a cone and plate measuring geometry CP 1/20. The shear stress was 0.3–100 Pa. The dynamic viscosity η was determined from 24 points in a shear stress range of 40–80 Pa.

#### *4.2.4. Photon correlation spectroscopy (PCS)*

PCS measurements were performed with a Zetasizer 3 (Malvern, D-Herrenberg) modified with a He/Ne laser model 127 (Spectra Physics, USA-Mt. View, Cal.). After dilution with MCT to a lecithin concentration of 5% (m/m) the different RMS were investigated under an angle of 90° in a measuring cell AZ 10 tempered at 293 K. The solutions were filtered through a sterile filter (0.22 μm) prior to the measurement.

#### *4.2.5. Polarisation microscopy*

RMS systems with a water content of 7% (m/m) were observed with a photo microscope type III (Zeiss, D-Oberkochen) using crossed polarizers and a λ-plate to generate a path difference of 550 nm.

#### *4.2.6. Transmission electron microscopy (TEM) of freeze-fractured specimen*

RMS systems with a water content of 5% (m/m) were shock-frozen in melting nitrogen at 63 K between two flat gold holders. The frozen samples were fractured at 173 K in a BAF 400 instrument (Balzers, D-Wiesbaden). Samples were shadowed with platinum/carbon (2 nm) at 45° and with pure carbon (20 nm) at 90° for replica preparation. A chloroform-methanol mixture (1 : 1 (v/v)) was used for cleaning the replicas. The replicas on uncoated grids were viewed with a transmission electron microscope EM 300 (Philips, D-Kassel).

#### *4.2.7. Small angle X-ray diffractometry (SAXD)*

SAXD studies were performed in a compact small angle system connected to a PW 1710 generator including a PW 2213-25 X-ray tube with a copper anode (Philips, D-Kassel). A nickel foil served as $K_\beta$ filter. The tube voltage was 40 kV and the anode current 25 mA. An OED PSD-50M position sensitive detector (Braun, D-München) was linked to a ASA-SAX card (Braun, D-München) for interference analyzing. RMS systems with a constant water content of 10% (m/m) were investigated for 800 s between kapton foils in vacuum to reduce scattering effects.

#### *4.2.8. Liquid crystal growth on hydrogel*

A hydrogel consisting of 3.5% (m/m) hydroxyethylcellulose (Hercules Powders, USA-Wilmington) and water was filled in limulus test tubes (2 g). After centrifugation of the gel for 10 min at 2000 rpm, 1 g of the RMS was layered on the hydrogel. The thickness of growing liquid crystal was measured by using a caliber.

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