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Polysaccharide-based nanoparticles formation by polyeletrolyte complexation of carboxymethylated cashew gum and chitosan

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Abstract Polyelectrolyte complex nanoparticles of chitosan and carboxymethyl cashew gum (CMCG) were prepared with CMCG with two different degrees of substitution (DS = 0.16 and 0.36). The effects of polymer concentration, molar mixing ratio (n^+/n^-) and mixing order of reactants on particle size distribution and zeta potential were investigated. Nanoparticle structure was elucidated by Fourier transform spectroscopy. Particle size of CH/CMCG DS = 0.16 dispersions was smaller than with DS 0.36 for all n^+/n^- ratio investigated. Particle size smaller than 200 nm was obtained when CMCG with DS = 0.16 was used for particle formation. The polydispersity index values were small when CMCG DS 0.36 was used. Increasing the concentration of CMCG led to larger particle size. Zeta potential values for almost all molar mixing ratios were found to be positive (10–32 mV).

Introduction

Polyeletrolyte complexes (PECs) based on chitosan have been proposed for many applications including scaffolds for pulp cell regeneration and matrixes for protein carriers and antibiotics [1, 2] Chitosan, an *N*-deacetylated chitin derivative (Fig. 1), can interact with anionic polysaccharides, such as carboxymethyl Konjac [3], carboxymethyl cashew gum (CMCG) [4, 5], dextran sulfate [2], alginate [6–10] carrageenan [11], chondroitin sulfate [12], and carboxymethyl cellulose [1, 13] to form polyeletrolyte complexes in gels, thin films, micro- and nanoparticles. Chitosan-based nanofilms and nanofibers were also produced for applications such as biosensor [14] and for papain purification [15].

The particle size, swelling and drug delivery behavior of PECs can be affected by the molecular parameters of oppositely charged polyelectrolytes. PEC characteristics depends on many factors such as molar mixing ratio, acidity/basicity, chain flexibility of polyelectrolytes, order of addition and also mixing rate [10, 16–21]. Lee et al. [22] investigated the effect of the deacetylation degree of chitosan on the composition of chitosan/alginate PECs. As the degree of deacetylation increased, PECs with lower alginate content were formed. Becherán-Máron et al. [23] showed that the composition of a chitosan–alginate polyelectrolyte complex was independent of alginate chemical composition as well as of the chitosan molar mass of chitosan. The stoichiometry and particle size of polyelectrolyte complexes formed by chitosan and dextran sulfate have been investigated by Schatz et al. [19, 20] and Drogoz et al. [21]. Drogoz et al. [21] reported that the nature of the polymer in excess strongly affected the mechanism in which colloid particles were formed. The existence of various complexation mechanisms depending on the kind of polyelectrolyte in excess was attributed to the differences in chemical reactivity of the ion in excess and to the conformation and flexibility of the macromolecular chains. When the chitosan was in excess, the molar mass of this

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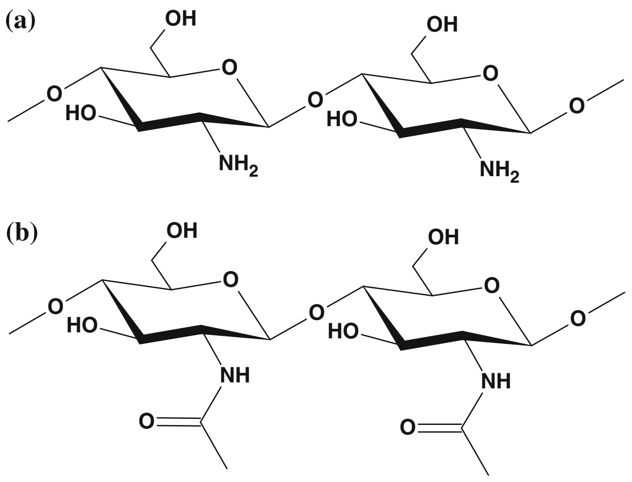

Fig. 1 Structural fragment of fully deacetylated chitosan (**a**) and chitin (**b**)

polysaccharide had a great influence on the submicrometer particle size. The increase in chitosan molar mass resulted in an increase in the particle diameter, whatever the size of the dextran sulfate and degree of acetylation of the polycation [19].

Cashew gum (CG) (Fig. 2) is an exudate polysaccharide from *Anacardium occidentale* trees and has been previously characterized [24, 25]. The gum is composed of galactose (72%), glucose (14%), arabinose (4.6%), rhamnose (3.2%), and glucuronic acid (4.7%) [24, 25]. The polysaccharide has been modified by carboxymethylation with monochloroacetic as an etherifying agent, resulting in samples with degrees of substitution (DS) between 0.10 and 2.21 [26]. Chitosan/CG gels have been prepared by re-acetylation of chitosan with acetic anhydride, characterized by infrared spectroscopy, thermal analysis, and X-ray diffraction. Addition of CG to the CH gel was found to decrease the pilocarpine release rate in the medium. The release of pilocarpine from CH gel was found to be dependent on pH values [27].

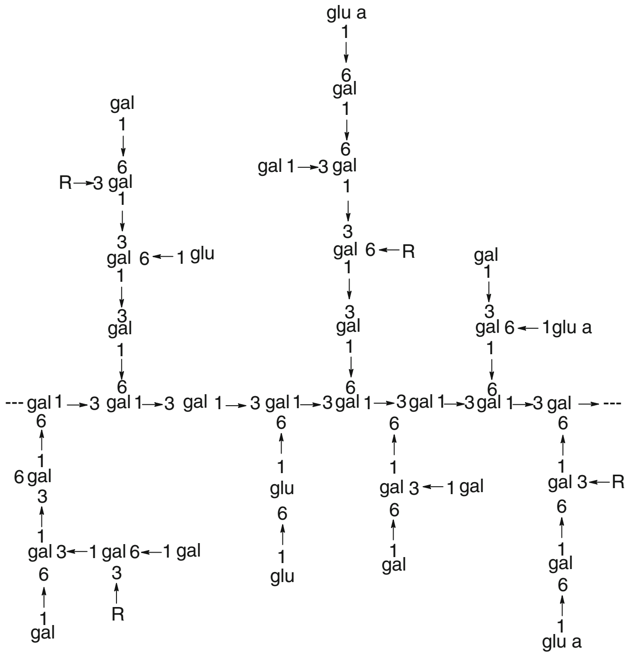

Fig. 2 Structural fragment of cashew nut gum. R1 can be glucose, arabinose, rhamnose, or glucuronic acid and R2 can be glucose chains

In this study, we reported the effect of CMCG DS and solution concentration on the particle size of submicron particle obtained by polyelectrolyte complexation of chitosan and CMCG.

Materials and methods

Materials

Chitosan samples were donated by a local company (Polymar), with degree of deacetylation (DD) of 82% and molar mass of 3.7 × 10^5 g/mol.

Crude samples from CG were collected from native trees in Fortaleza, Ceará, Brazil. They were purified as a sodium salt using a previously described method [23]. Nodules free of bark were selected and dissolved in distilled water at room temperature to give a 5% (w/v) solution. The solution pH was adjusted to approximately 7.0 by addition of diluted aqueous NaOH. The clear solution was successively filtered through sintered glass and the polysaccharide precipitated with ethanol.

Carboxymethylation reaction

The carboxymethylation reaction was performed using the methodology reported by Silva et al. [26] described as follows: purified gum (5 g, 0.0278 mol) was mixed with 5 mL of water until a homogeneous paste was formed. A 10-M NaOH solution was added and the mixture was kneaded for 10 min. After that, monochloroacetic acid (MCA) was mixed thoroughly with the paste. The mixture was maintained at 55 °C for 3 h. The system was neutralized with 1-M HCl and dialyzed against distilled water until all remaining reagents or added salt were eliminated (4–5 days). The solid CMCG samples were recovered by freeze-drying. The conditions applied to obtain the CMCG samples were of CG/MCA/NaOH mol ratios of 1:1:1 and 1:1:2, respectively.

Degree of substitution

The absolute degree of substitution (DS_{abs}) was determined by potentiometric back-titration. In order to titrate all carboxymethylated groups, either in acid or salt form, 20 mL of the CMCG solutions (2.5% w/v) were passed through an Amberlite IR-120H$^+$ column and freeze-dried. The acid CMCG solution (10 mg/mL) was then titrated with 0.0182-M HCl after addition of a known amount of NaOH. The DS$_{\mathrm{abs}}$ value was calculated through the molar ratio of carboxymethyl acid groups to monosaccharide units, as follows:

$$\mathrm{DS_{abs}} = \text{mol of } -\mathrm{CH_2COOH} \text{ groups/} \\ \text{mol of monosaccharide unit} \tag{1}$$

The molar mass of galactose (180 g/mol), a major constituent of CG [28] was used to calculate the mols of monosaccharide units. An initial value for DS was then determined. The new molar mass of average monosaccharide units was then recalculated taking into account the addition of 58 g/mol (molar mass of $\mathrm{CH_2COOH}$) for each DS increase of 1.0 unit. The method of successive approximation was applied to obtain the final absolute DS. Two samples with DS of 0.16 and 0.36 were obtained and the molar mass for both carboxymethylated samples were similar (1.0×10^4 g/mol).

Preparation of polyelectrolyte complex

Chitosan solution (1 mg/mL) was dissolved in a stoichiometric amount of 0.1-M HCl. Solution pH was then adjusted to 4.0 by addition of 0.1-M NaOH and the amount of acid and alkali were considered for the ionic strength (I) calculation to obtain a final value of $I = 0.05$. CMCG solutions were prepared in deionized water and the pH and ionic strength adjusted to 4.0 and 0.05, respectively. Prior complex formation the solutions (CH and CMCG) were filtered in a 0.22-μm Millipore filter. PECs were prepared by methodology described by Schatz et al. [19, 20] by mixing CH to CMCG solution in appropriated proportion to obtain desired charge ratio (n^+/n^-). The CH solution was added to CMCG solution with low stirring rate and the mixture was kept resting for 24 h before particle size measurement. The particle size of PEC dispersion was monitored up to 21 days of storage time

Characterization of CH/CMCG nanoparticles

Particle size measurement

Particle size and zeta potential measurements of PECs were carried out in a Malvern Zetasizer Nano, model ZS 3600. The hydrodynamic diameter, assuming spherical shape, was measured by dynamic light scattering (DLS) with laser with wavelength of 633 nm and a fixed scattering angle of 90°. The measurements were made in triplicates.

Infrared spectral analysis

The IR spectra of the gum, chitosan, and PEC were recorded in solid stated in KBr pellets using a Shimadzu IR spectrometer (model 8300) operating between 400 and 4000 cm^{-1}.

Optical density

The optical density using a spectrophotometer UV–visible Ultrospec 2000 Pharmacia Biotec at 500 nm using quartz cell with optical pathway of 1 cm.

Scanning electron micrography (SEM)

The scanning electron microscopy was recorded using a Jeol-6360LV field emission. To prepare the SEM sample, a drop of nanoparticles was deposited on carbon stickers on aluminum stubs, dried, and coated with gold.

Results and discussion

Infrared spectroscopy

FT-IR spectrum of CMCG (Fig. 3) in the acidic form showed a broad absorption band at 1743 cm^{-1} assigned to –COOH groups. The peaks at 1140, 1080, and 1030 cm^{-1} are due to stretching vibration of C–O–C from glucosidic bonds and O–H bending of alcohols. The FT-IR spectrum

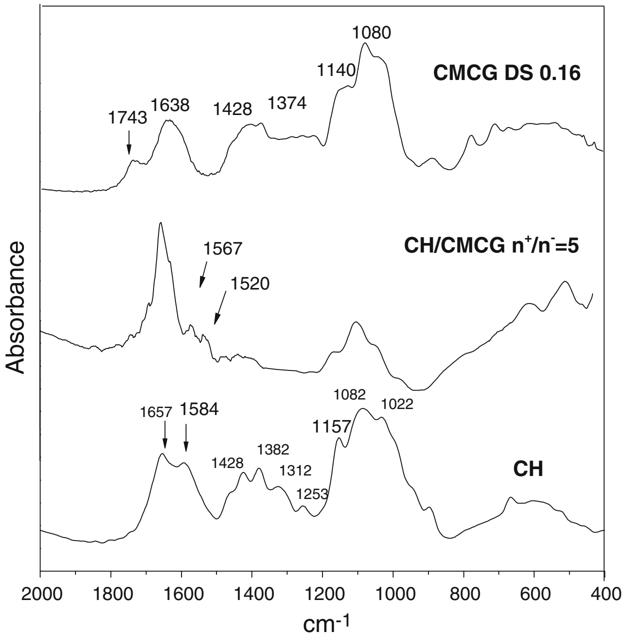

Fig. 3 FTIR spectra of CMCG DS 0.16, CH/CMCG DS 0.16 and CH

of chitosan showed absorption band at 1657 and 1570 cm^{-1} assigned to amide I and the –NH$_3$$^+$ deformation, respectively. Signals from CMCG and CH are observed for CH/CMCG DS 0.16 sample. The appearance of a new absorption band at 1560–1520 cm^{-1} assigned to –NH$_3$$^+$ groups is a strong evidence the formation of polyelectrolyte complexes [28, 29].

Optical density

The formation of PEC colloidal particles was observed using optical density measurement at 500 nm. Chitosan and CMCG do not absorb light at this wavelength and the increase of optical density when the two polyelectrolytes were mixed is an indication of the presence of insoluble particles [19]. Optical densities of nanoparticle (NP) formed by the addition of chitosan solution (1 mg/mL) in a CMCG solution (1 mg/mL), denoted as CH/CMCG, using a desired amount of each solution in order to form polyelectrolyte complexes with molar charge ratio (n^+/n^-) ranging from 0.1 to 20, are shown in Fig. 4.

For CH/CMCG DS 0.16 sample the optical density is almost constant for particles with n^+/n^- ratio between 0.2 and 0.8. A slight increase in OD is then observed followed by a OD decrease when an excess of positive charge is present (n^+/n^- ratio higher then 5). Flocculation was not observed for PECs formed with CMCG DS 0.16. Higher values of OD were observed when the CMCG DS was increased for 0.36. Colloidal particles formed for CH/CMCG DS 0.36 samples increase with increasing of n^+/n^- ratio, between 0.1 and 0.8. At n^+/n^- ratio = 1 flocculation was observed. For n^+/n^- ratio between 2 and 20 a decrease of OD was observed. Similar behavior was observed for dextran sulfate and chitosan polyelectrolyte complexes [20] when an excess of charge (positive or negative) is present. Authors suggested that a stabilizing shell is formed around the particle preventing further aggregation and so a decrease in OD values is obtained.

Effect of DS of CMCG on particle size

Figure 5 shows the effect of DS of CMCG on particle size as a function of charge ratio. For both DS particle size decreases slightly with increasing molar charge ratio. As expected a high particle size is observed when a high CMCG DS is used. For NP made with CMCG DS 0.16, two behaviors were observed: for $1 < n^+/n^- < 5$, particle size was smaller than 200 nm, while for $n^+/n^- < 1$ values ranging from 400 to 200 nm were obtained. Nanoparticles formed with CMCG DS 0.36 have particle size ranging from 400 to 250 nm. The polydispersity index values were small when nanoparticles were formed using CMCG with DS 0.36 (PI ranging from 0.1 to 0.3) than with DS 0.16 (PI ranging from 0.2 to 0.5).

Effect of CMCG solution concentration on particle size and zeta potential

The effects of CMCG solution concentration on zeta potential and particle size (Fig. 6) were investigated for CH/CMCG NP with charge molar ratio (n^+/n^-) of 20 and 0.2. Increasing CMCG concentration from 1 to 2 mg/mL resulted in an increase of particle size, this behavior being observed for both CMCG DS investigated. Similar behavior was also observed by Sun et al. [30] who reported as it being due to an increase in the number of nanocomplexes formed leading to particle aggregation. At concentration of 1 mg/mL the zeta potential values ranging from 15 to 32 mV (Fig. 6b) for both DS investigated. High positive value was obtained for CMCG sample with lower

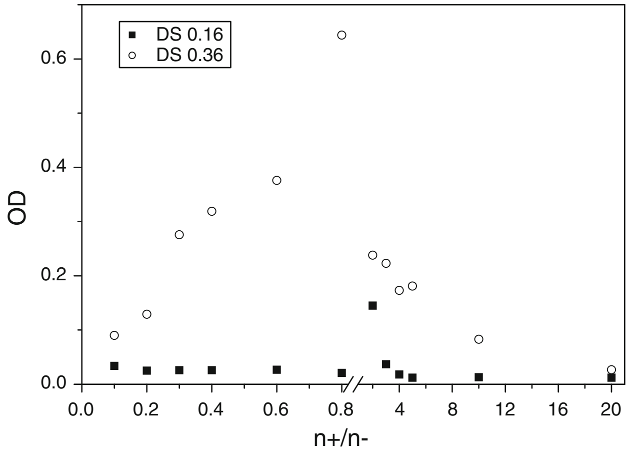

Fig. 4 Effect of n^+/n^- molar ratio on optical density

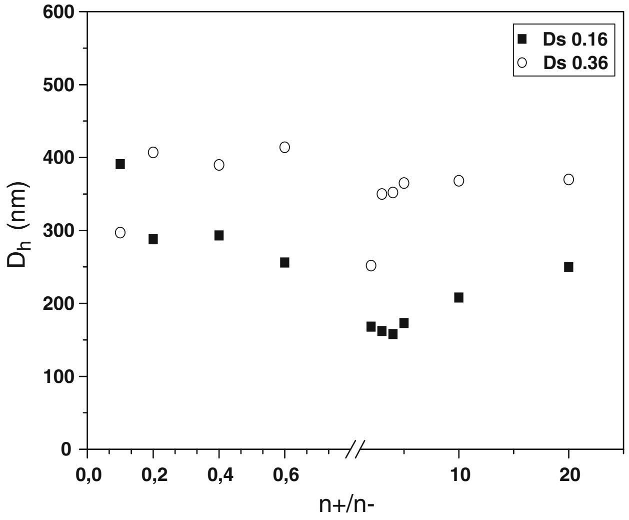

Fig. 5 Effect of degree of substitution of CMCG DS on particle size

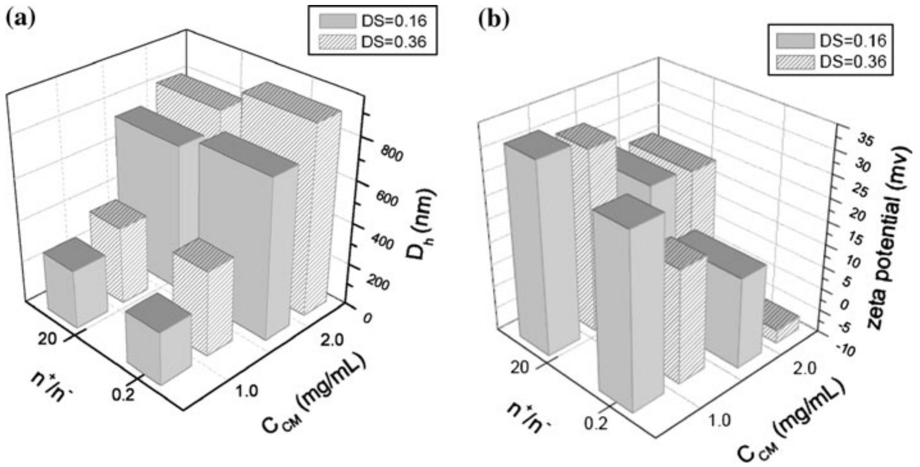

Fig. 6 Effect of concentration of CMCG on particle size (**a**) and zeta potential (**b**)

DS (0.16), higher n^+/n^- ratio (20) and lower CMCG concentration. As CMCG concentration increases a decrease in zeta potential values is observed and negative value (−7.2 mV) was found for CH/CMCG DS 0.36 at 0.2 charge ratio.

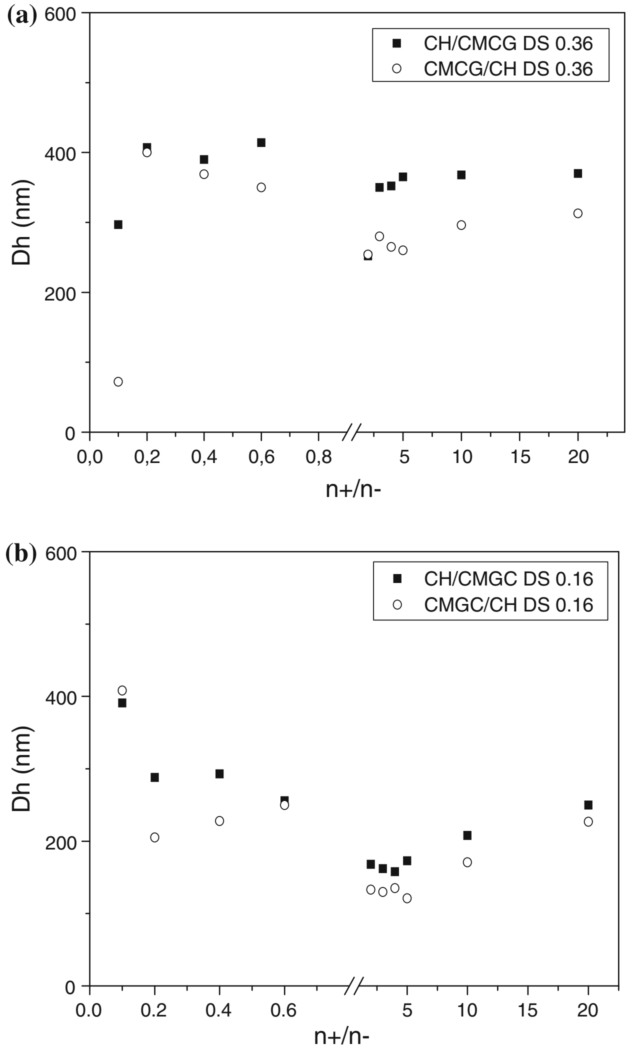

Fig. 7 Effect of mixing order of polyelectrolyte on particle size in CMCG concentration 1 mg/ml

Effect of polyelectrolyte mixing order on particle size

Figure 7 shows the effect of mixing order as a function of charge ratio and CMCG DS on particle size. Adding chitosan to CMCG DS 0.36 (CH/CMCG) results in particle size higher then when CMCG DS0.36 is added to CH solution (CMCG/CH) (Fig. 7a). This behavior is more pronounced for charge ratio higher then 5. The same behavior is observed when CMCG DS 0.16 sample is used; however, the increase in particle size is smaller then when the PEC was formed with CMCG DS 0.36.

SEM image of CMCG DS 0.36/CH nanoparticle in a molar charge ratio (n^+/n^-) equal to 5 is shown in Fig. 8. The image shows aggregated spherical particles with size ranging from 200 to 350 nm. Similar values were also found for the same sample using DLS (Fig. 7).

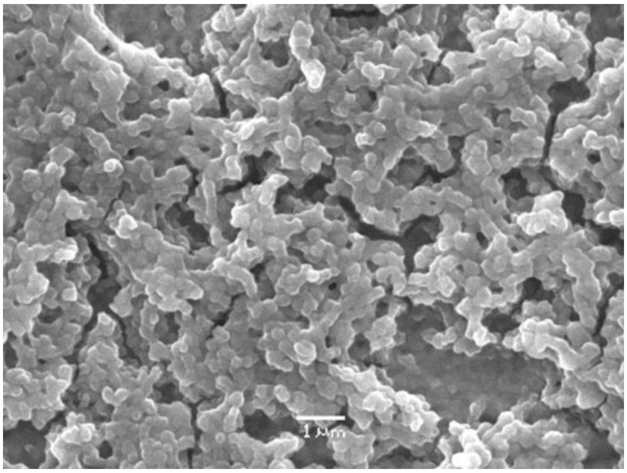

Fig. 8 SEM image of CMCG DS 36/CH in n^+/n^- ratio = 5

Conclusion

Polyelectrolyte complex nanoparticles were successfully prepared using polysaccharides (chitosan and a CG derivative) as starting materials. Particle size was found to be dependent on molar ratio, mixing order of reactants, polymer concentrations and CG derivative DS, but zeta potential values for almost all samples were positive and showed little variation (from 10 to 32 mV). Using different CMCG DS, data obtained revealed that the small the CMCG DS, the small (>200 nm) were the resulting particle sizes. Increasing CMCG concentration resulted in an increase of particle size, yielding particle as big as 800 nm, a behavior observed for both CMCG DS. It is noteworthy to mention that negative particle was obtained for high polymer concentration, high DS and low charge ratio.

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