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Research Article

Rheological Assessments on Alginate and Carrageenan as Nanoparticle Carriers for Topical Oral Cancer Drug

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ABSTRACT

Commercially available topical oral drugs in current markets have low efficacy in delivery active load to the infected site due to poor formulation. Delivery of the active ingredients proven to be challenging as compared to skin due the presence of saliva and low shear. The aim of this project to improve formulation and characterised suitable hydrogels which later will be incorporated with nanoparticle drug for oral cancer. The gels are formulated at different pH values (4, 7, 10) and concentrations as such (0.1%, 0.15%, 0.2%, 0.25%, 0.5% and 1.0% for alginate whereas kappa-carrageenan and iota-carrageenan were formulated with 0.25%, 0.5% and 1.0%). The viscosity and zeta potential of the formulated gels are studied using HAAKE™ MARS™ rheometer and Zetasiser Nano-Z respectively. Findings revealed both 1% of kappa-carrageenan and 1% iota-carrageenan of pH 4 and pH 7 are the best candidates for nanoparticle drug delivery as the viscosity and zeta potential for 1% kappa-carrageenan (pH 4), 1% kappa-carrageenan (pH 7), 1% iota-carrageenan (pH 4), and 1% iota-carrageenan (pH 7) amongst the highest as such 70.507±6.190, 61.040±3.199, 59.490±7.799, 67.953±2.034 Pa·s, correspondingly with zeta potential value of -19.4 mV, -20.6 mV, -33.1 mV and -30.4 mV. All hydrogels formulated with different concentration were affected by pH values, by having pH value 4 and 7 appeared to have high viscosity with pseudoplastic behaviour based on the rheological profile, except for alginate due to high density sodium alginate was used in this study.

Keywords: Hydrogels, Nanoparticle, Oral Cancer, Rheology, Topical Oral Drug

Introduction

International Agency for Research on Cancer & Organization [1] reported that there are more than 350,000 new oral cancer cases to date, with countries in Asia recorded the highest number of new cases for more than 200,000 cases. The common factors contributing to the disease are the habit of tobacco chewing, smoking, and alcohol consumption. Early detection of oral cancer symptoms like bleeding soreness that does not heal, formation of lumps or lining in the mouth, loosely fitting denture and tongue pain should be watched out to prevent the adverse effect from the cancer. The current treatments for oral cancer are surgery, radiotherapy, and chemotherapy, but these treatments have some drawbacks to the patient's quality of life. These therapies typically present a variety of side effects for the patients. For example, patients may experience facial disfigurement, speech difficulties, loss of nutrition due to loss of appetite, and risk of cancer cell metastases to other

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parts of the body [2]. Current topical drugs for directly treating oral diseases are lacking as Sankar et al. [3] mentioned that the practices in treating dermatological are adopted to treat oral mucosal disease. Saliva lubrication and mastication may interfere the distribution of drug as it causes the gel matrix to swell, deform and fail to remain at the application site [4,5].

Hydrogels are groups of long chain hydrophilic substance with the ability to retain water. Some commercially available drugs include gelatine, starch, acacia gum and carrageenan used as gelling agent, thickener, emulsifier, and stabilizer [6]. Pharmaceutical application of these hydrogels in forms of microgel dispersions shows to affectively carries active load to targeted site. According Gulrez et al. [7], encapsulating nanoparticle with drugs in the 3-dimensional crosslinked polymers that have swelling capacity, hydrophilic properties and biocompatible makes it an effective carrier. These versatile polymers can be formulated into nanostructures are suitable delivering the biopharmaceuticals or bioactive molecules [8]. Li and Mooney [9] mentioned that hydrogels morphological structure, depending on the variation of the gel porous size can deliver drugs locally in appropriate manner within proper formulation. The wide application hydrogels for medical use are due to its inertness making it nonimmunogenic, sterilizable and biocompatible. Some are currently used as diagnostic, wound dressing and barrier material in bio-adhesion regulation aside from food products like food additives [7,10]. Morphological structure can be tailored by manipulating temperature, pH, ionic strength, and solvent creating diverse physical arrangement of 3-D gel networks [9, 11, 12]. Ahmed [10] further explained swelling action of hydrogel is influenced by physical response like electric field, pressure, light, temperature and chemical response such as pH, ionic strength, solvent composition and molecular species. Rheological properties are the relationship between the shear stress (the force over the surface area) and the shear strain (deformation due to force). Mishra et al. [13] defines rheology as the study of the flow of matter and the deformation. Through the rheological characterization of a material, a general idea of the system's viscoelastic properties behaviour can be obtained. Hydrogels can be classified as the non-Newtonians fluid, of which the relationship of the shear stress and shear rate will not yield a linear relationship [14]. Viscoelastic ability is where the materials can exhibit both liquid and solid characteristics when undergoing deformation. Saha and Bhattacharya [15] further elaborated that there are two types of rheological measurement; small deformation and large deformation of which both are not always correlated to each other. Small deformation typically measures the rheological behaviour according to Young's moduli values as the viscoelastic parameters. The latter test is used to measure shear stress, yield stress, and apparent viscosity. As the mastication activity in the mouth can lead to the deformation of the hydrogels, the rheology properties need to be understood. The hydrogels can then be modified to withstand the minimal shear strain of the teeth and saliva make it the best candidates for the coating of nanoparticles drug delivery in oral [16].

Material and Methods

Buffer Solution Preparations

Phosphate buffers were prepared with the addition of PBS tablets and sodium azide (0.02 wt.%), a bactericide agent. The pH was adjusted by adding either sodium hydroxide (1M, NaOH) or hydrochloric acid (1 M, HCl) until the desired pH values were achieved. The pH for the experiments were adjusted to pH 4, 7 and 10 by using pH meter.

Gel Preparations

κ-Carrageenan samples were formulated by adding dry carrageenan powder to a beaker with phosphate buffers prepared previously with the total sample weight of 100 g. The concentrations of the carrageenan samples were set at 1.0 wt.%, 0.5 wt.% and 0.25%. The beakers with respective sample concentrations were covered, stirred at 100 rpm using magnetic stirrer and heated to approximately 70 °C then left to dissolve for 30 minutes [17]. The samples were poured into Falcon 50ml conical centrifuge tubes, covered with parafilm and chilled at 4 °C prior to use. ι -carrageenan samples were prepared with the similar method. Samples were let to set and stored in the chiller prior to next analysis.

Alginate

Sodium alginate gels, were let to set chemically by the addition of Ca^{2+} ions through calcium chloride solution, $CaCl_2$ (1.0 wt.%) using Oyeagu et al. [18] and Rahman et al. [20] methods with a

few modifications. $CaCl_2$ solution was dissolved in Millipore water by magnetic stirrer without any heat treatment due to its highly hygroscopic nature [17]. Sodium alginate solutions with the desired sodium alginate concentrations at 1.0 wt.%, 0.5 wt.%, 0.25 wt.%, 0.2 wt.%, 0.1 wt.% and 0.15 wt.% were formulated respectively with a total of sample weight of 100 g by mixing it with the phosphate buffers. The solutions will be stirred until it fully dissolved for 2 hours and heated at 60 °C. The alginate solutions were mixed slowly, added dropwise into beaker containing 1% $CaCl_2$ solution by using dropper. After the alginate gels set for 15 minutes, the gels were transferred into labelled Falcon 50 ml conical centrifuge tubes and stored in 4 °C chiller prior to next analysis.

Rheology Test

The viscosity of hydrogels was measured with a HAAKE™ MARS™ Rheometer which was equipped with RheoWin 3 software (Thermo Fisher Scientific, Waltham, Massachusetts, USA) to control the probe and to provide measurement and analysis of the results by using method improvised from Rahman et al. [20] The temperature was set at 25 ± 0.05°C with a 5-minute time window to achieve a steady state condition. The plate cartridges (PP35 Ti spindle) were used in every sample. About 1 to 2 ml of microgel suspension was placed on top of the plate. The applied shear rate ranged from 0.1 to 10 s^{-1} for each sample [19].

Zeta Potential

The ζ-potentials of then hydrogel particles were measured using Zetasizer Nano-Z by having modification of methods from Pravinata [19]. The samples were placed in folded capillary electrophoresis cells DTS1070 (Malvern, Worchestershire, UK). Triplicate samples were measured.

Statistical Analysis

Data for rheological profile was presented as mean ± standard deviation (SD) with the application of one-way analysis of variance (ANOVA) followed by Tukey's test in which was based on the significant value when p value ≤ 0.05 [20].

Results and Discussion

The ideal hydrogels behaviour for oral applications is a non-Newtonian fluid known as pseudoplastic as depicted in Figure 1. The apparent viscosity of pseudoplastic fluid declines through the inclination of shear rate. The characteristic of this fluid is required to ease the flow through of the gels out of tubes or syringe to be applied at the projected site and ability to remain at the applied location. The variation between the velocity and the oral drug tube's diameter is the example of gel's shear rate inside the tube fillings [20]. Thirty-six formulations of hydrogels from three type of polymers namely kappa-carrageenan, iota-carrageenan and alginate with variation in concentrations and pH values tested in this study. The difference of viscosity was measured at the range of 0.1 s^{-1} to 1.0 s^{-1}. Considering the viscosity at shear rate of 1.0 s^{-1} as the initial viscosity, the inclination pattern was denoted in both concentration of hydrogels and the initial viscosity in each hydrogel formulations as displayed in Table 1. Viscosity of the hydrogels formulated in different concentrations as such 0.25%, 0.5% and 1.0% varied significantly at $p < 0.05$ [20,21]. For instance, the viscosities of iota-carrageenan at pH 7 and 1 s^{-1} shear rate were 1.722 ± 0.131 Pas at 0.25%, 15.663 ± 0.575 Pas at 0.5% and 67.953 ± 2.034 Pas at 1.0%. The changes of iota-carrageenan within the stated condition were more prominent as compared to other hydrogel preparations. Based on the results in Table 1, 1% kappa-carrageenan with either pH 4 or 7 and 1% iota-carrageenan formulated using pH 4 and pH 7 phosphate buffer saline were listed as the suitable candidates for oral drug delivery usage. Good hydrogels candidates for oral drug delivery are the gels that had high viscosity value at all three points of shear rates (1 s^{-1}, 5 s^{-1} and 10 s^{-1}

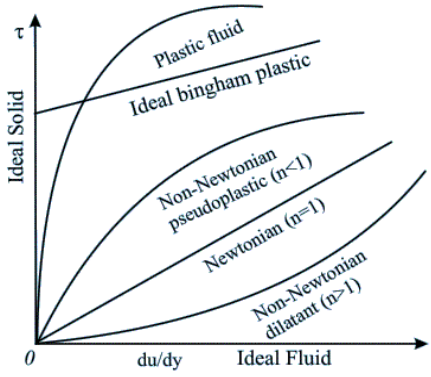

Figure 1. Classification of fluid behaviour with shear stress as a function of shear rate, based on index flow n, termed as non-Newtonian pseudoplastic (Ostwald de-Waele model), Newtonian, non-Newtonian dilatant. Plastic fluid can be found in Herschel Bulkley model [30].

Table 1. Rheological profiles of apparent viscosity from 18 formulated hydrogels that differ in terms of concentration and pH value at specific shear rates

Hydrogels		pH	Apparent Viscosity ± SD (Pa·s) at shear rate		
			1.0 s^{-1}*	5.0 s^{-1}*	10 s^{-1}*
κ-carrageenan	0.25%	4	6.757±2.172^{klmn}	1.531±0.371^{jmn}	0.485±0.058^{jmn}
		7	7.331±0.234 ^{klmn}	1.754±0.136 ^{jlmn}	0.487±0.050^{jkm}
		10	4.849±1.841 ^{klm}	1.094±0.618 ^{jmn}	0.368±0.109^{jmn}
	0.50%	4	20.640±1.961 ^{klmo}	6.757±0.220^{jlmo}	2.821±0.167 ^{jikmo}
		7	22.673±1.620^{iklmo}	6.454±0.139 ^{jlmo}	2.182±0.084^{jkm}
		10	17.927±1.343^{hklm}	5.880±0.580 ^{jlmo}	2.055±0.488^{jkmog}
	1.0%	4	70.507±6.190^{klno}	17.223±1.090^{jnoi}	12.504±1.123 ^{jnoi}
		7	61.040±3.199 ^{klno}	16.060±1.055 ^{jno}	10.920±1.298 ^{jnoi}
		10	52.237±12.116 ^{klno}	14.660±0.874 ^{gjno}	8.027±0.785^{jghno}
ι -carrageenan	0.25%	4	1.673±0.138 ^{ef3}	0.444±0.028^{d3}	0.257±0.019^{d3}
		7	1.722±0.131 ^{ef3}	0.501±0.020^{df3}	0.304±0.006^{de3}
		10	2.224±0.726^{3}	0.592±0.124^{3}	0.327±0.040^{3}
	0.50%	4	16.277±1.219^{2}	4.338±0.289^{df2}	2.236±0.123^{de2}
		7	15.663±0.575^{2}	4.192±0.046^{df2}	2.155±0.026^{def2}
		10	19.607±0.938^{2}	4.613±0.076^{2}	2.327±0.030^{2}
	1.0%	4	59.490±7.799^{ef1}	17.600±0.635^{cd1}	9.733±0.294^{cd1}
		7	67.953±2.034 ^{ef1}	17.560±0.733^{cdf1}	9.469±0.423^{cde1}
		10	55.873±7.320 ^{ef1}	15.2677±0.444^{abd1}	7.950±0.385^{abd1}

*Values are Mean ± SD. Statistical analysis were expressed using alphabetical and numerical superscript whereby $p<0.05$ was analysed using ANOVA Tukey statistical test (95% confidence interval) for comparison of: ^{a}iota-carrageenan at pH 4; ^{b}iota-carrageenan at pH 7; ^{c}iota-carrageenan at pH 10; ^{d}iota-carrageenan at 1 s^{-1}; ^{e}iota-carrageenan at 5 s^{-1}; ^{f}iota-carrageenan at 10 s^{-1}; ^{g}kappa-carrageenan at pH 4; ^{h}kappa-carrageenan at pH 7; ^{i}kappa-carrageenan at pH 10; ^{j} kappa-carrageenan at 1 s^{-1}; ^{k} kappa-carrageenan at 5 s^{-1}; ^{l} kappa-carrageenan at 10 s^{-1}; ^{m}1% kappa-carrageenan; ^{n}0.5% kappa-carrageenan; ^{o}0.25% kappa-carrageenan, whilst numerical value of ^{1,2,3} denoted that the significance amongst iota-concentrations. Alginate formulations were omitted from the rheological profiles as the viscosity reading obtained was inconsistent throughout the run. Different letter indicated significant difference.

respectively) [20]. The rheological profiles for 18 alginate formulations were not computed as the viscosity measurements were erratic along the applied shear rate. The formulations of 18 alginate were too rigid to be tested using the parallel spin dle plates attached to the rheometer. The cause of the unreliable viscosity reading of alginate gels may due to the usage of high-density sodium alginate in which produced rigid gels [11].

The determination of the rheological profile of hydrogels aside from alginate in this study can be modelled with Herschel Bulkley or Ostwald de-Waele shown in Table 2. The models were chosen because the contrast of yield stress presence, as Herschel Bulkley made up of consistency factor (K), flow index (n), and yield stress (τ_0) while Ostwald de-Waele (also termed as power-law fluid) only had consistency factor K, and flow index n [21]. The data was selected from each graph via shear stress to the function of shear rate as expressed in the equation $\tau = f(\dot{\gamma})$ and the model was chosen by τ_0. 1% kappa-carrageenan of pH 7 and pH 10 depicted to obey the Herschel Bulkley model whereas the remaining hydrogels followed

Table 2. Rheological profiles of 18 formulated hydrogels that differ in terms of concentration and pH value with Herschel-Bulkley or Ostwald de-Waele model obtained from RheoWin software analysis

Hydrogels		pH	Herschel-Bulkley Model*				Ostwald de-Waele Model*		
			r	τ_0 (Pas)	K (Pas^n)	n	r	K (Pas^n)	n
κ-carrageenan	1%	4	-	-	-	-	0.848	68.880	0.174
		7	0.943	2.920	50.700	0.272	-	-	-
		10	0.993	1.508	40.520	0.353	-	-	-
	0.50%	4	-	-	-	-	0.889	23.210	0.204
		7	-	-	-	-	0.621	23.700	0.124
		10	-	-	-	-	0.858	18.680	0.192
	0.25%	4	-	-	-	-	0.227	5.963	0.042
		7	-	-	-	-	0.145	6.894	-0.0303
		10	-	-	-	-	0.0404	3.8710	-0.008
ι -carrageenan	1%	4	-	-	-	-	0.941	65.950	0.204
		7	-	-	-	-	0.908	62.680	0.182
		10	-	-	-	-	0.889	53.610	0.189
	0.50%	4	-	-	-	-	0.905	15.840	0.173
		7	-	-	-	-	0.873	15.520	0.174
		10	-	-	-	-	0.767	18.520	0.129
	0.25%	4	-	-	-	-	0.959	1.4590	0.237
		7	-	-	-	-	0.981	1.573	0.291
		10	-	-	-	-	0.967	1.756	0.252

*The rheological modelling used in this experiment was either Ostwald-de Waele (generalized Newtonian fluid) or Herschel Bulkley (a generalized non-Newtonian fluid) in which differs with the presence of a positive yield stress (τ_0) value.

Ostwald de-Waele model. Similar result of rheological models was obtained by Rahman et al. [20] although the consistency factor and flow index diverge from the finding due to hydrogels preparation method.

Type of fluid could be classified by using flow behaviour index into three categories, for example pseudoplastic if $n < 1$, Newtonian if $n = 1$, and dilatant if $n > 1$ as shown in Figure 6.1 [22,23,30]. All gels displayed pseudoplastic, non-Newtonian behaviour as the value of $n < 1$ based Table 2. Rahman *et al.*, [20] further stated that high consistency factor K value had lower flow index and high gel consistency. It was found that 1% and 0.5% kappa-carrageenan of all pH values, and 1% and 0.5% iota-carrageenan of all pH values portrayed high consistency factor. However, 0.25% kappa-carrageenan showed low flow index value due to the tendency of gel breakage when a stress was applied, as mentioned by Chhabra [22] that smaller n values denoted as the higher degree of shear-thinning aside from the fact that both models did not fit well with the all 0.25% kappa-carrageenan's apparent viscosity.

Hydrogels dispersed in their specific pH buffer for zeta potential measurement used up monomodal analysis to obtain the interfacial property respectively. Figure 2 showed that all hydrogels possessed negative zeta potentials ranging from -0.909 mV, the lowest zeta potential value of 0.5% iota-carrageenan (pH 7) till -33.800 mV, the highest zeta potential value of 0.15% alginate (pH 10) regardless of the type of gels, concentrations and pH values. The negative surface charge of all hydrogels formulated in this study were due to the nature of hydrogels. Patel et al. [24] stated that alginate and carrageenan are anionic polymers because of the presence of anionic such as carboxylic

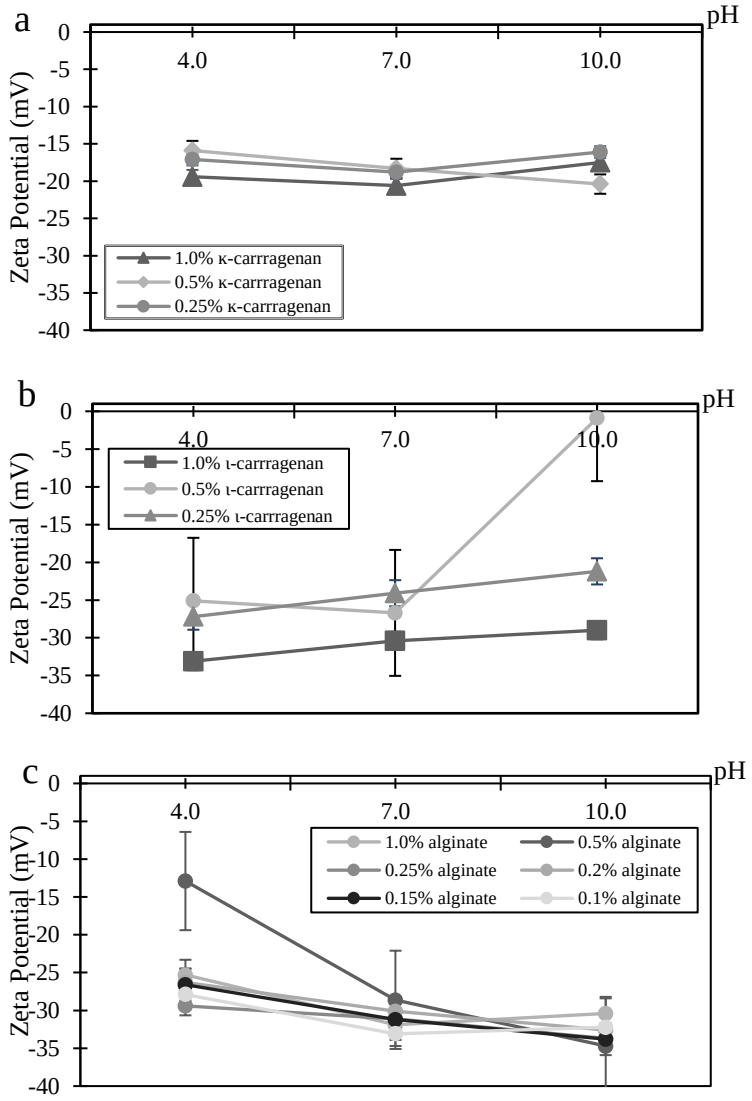

Figure 2. Graph of average zeta potential for (a) kappa-carrageenan, (b) iota-carrageenan, and (c) alginate formulations at different pH.

group in alginate whereas sulphur group for both iota-carrageenan and kappa-carrageenan. According to Al-Zebari et al. [25] the stability of dispersion system generally determined by electrostatic repulsion of any zeta potential, either positive or negative value, larger than 30 mV. Zeta potential value smaller than 10 mV as such for 0.5% iota-carrageenan (pH 7) was example of unstable reading due to the presence of aggregates [24,26]. Kappa-carrageenan had the lowest overall zeta potential value by the range of -15.9 mV (at 0.5%, pH 4) to -20.6 mV (at 0.5%, pH 10) compared to the other hydrogels' formulations. The result satisfied with the Senthil et al. [27] findings although the value slightly varies due to different concentration and preparation method were used. The average zeta potential for iota-carrageenan increased (with the declination of negative zeta potential) as the alkalinity of the hydrogels increased whilst the zeta potential value for both kappa-carrageenan and alginate in overall concentration decreased (with the increment of negative zeta potential value) across pH 4 to pH 7. Honary and Zahir [28] described that the cancer cell surface has negative charges due to the presence of anionic inner layer cell membrane like phospholipids and pH values ranges from 5.7 until 7.8, causing the extracellular cancer cells to be slightly acidic than the normal, healthy cells. High pH hydrogels could be used to comply with acidic or neutral drugs while low pH could be incorporated with basic drug to increase the stability and efficacy of

the drug. Negative zeta potential could be adjusted to a lower value by adding a surface modifier to increase the nanoparticles stability causing the changes in steric effects and hydration force [8]. In this study, 1% iota-carrageenan of pH 4 and 7, 0.5% iota-carrageenan of pH 7 and 4, 1% kappa-carrageenan of pH 7 and 4, 0.1%, 0.15%, 0.2%, 0.25%, 0.5% and 1.0% alginates of pH 7 and pH 10 were the suitable hydrogel candidates to be used as delivery system for oral drug. Negative surface charge of hydrogel nanoparticles with specific pH could be applied as drug delivery system due to several advantages such as automated trigger of drug release actions at specific organs or cell site like endosomes that favour pH 5.5 as described by Gonçalves *et al.* [8], controlled release and degradation of nanoparticle drug without harming healthy cells and drug activity [24]. Besides, hydrogels owned swelling controlled system in which able to swell in the presence of water or any fluids before delivering the loaded nanoparticle drug to diffuse into the targeted site [29].

Conclusion

Based on the rheological profiles and the zeta potential values, 1% of either kappa-carrageenan or iota-carrageenan formulated using phosphate buffer at pH 4 and pH 7 were the suitable candidates to be used in oral nanoparticle drug delivery system in the future. It was found that pH affected the hydrogels formulation whereby 1.0% iota-carrageenan at pH 4 depicted the highest value in viscosity and stability in term of average zeta potential value. Alginate formulations were among the most stable hydrogel yet, the formulation with a low density of alginate is recommended to overcome the rigid hydrogel formation for a better rheological profile result.

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