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Differences in the coalescence kinetics of fat emulsions in dependence on the amount of fat and age

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The destabilizing effect of calcium ions on emulsions was studied as a function of the age of the emulsions and the degree of emulsion dilution (2%, 0.2% an 0.02% fat). Particle size measurements were performed both in the Coulter counter and a laser diffraction device equipped with PIDS technology. The data of both instruments showed a good correlation. ζ-Potential was determined by laser doppler anemometrie. The physical stability of the emulsions in 6 mmolar Calcium Chloride decreased with increasing dilution – despite the diminished rate of collision in diluted systems. In addition, an increased electrolyte sensitivity was observed with increasing age of the emulsions – despite enhanced electrostatic stabilization by an increased ζ-potential. Both effects were attributed to an increased binding of calcium ions per surface area of the droplets, i.e. increased ratio calcium ions to surface by dilution and increased binding by the increased charge of aged emulsions.

1. Introduction

The coalescence of fat emulsions depends on various factors and forces, such as electrostatic repulsion, van der Vaals attraction forces, Born hydration, statistics of droplet collision and rigidity of the emulsifier layer [1–4]. Therefore a theoretical prediction of long term stability is only possible to a limited extend. The major influence in coalescence of lecithin stabilized fat emulsions is ascribed doubtless to calcium ions, although the mechanism is not known in detail at the moment. Specific calcium binding residues are discussed [5]. A membrane fusioning effect is also attributed to calcium ions [6], a similar effect seems possible with a lecithin layer around the oil droplets (e.g. via reduction of the rigidity, i.e. lower microviscosity). Regimens for total parenteral nutrition (TPN) are commonly a combination of fat emulsions with solutions of electrolytes, carbohydrates, amino acids and trace elements. The proportion of the components is chosen according to the needs of the patient [7]. Particular in TPN-regimens for babies and infants a high calcium concentration up to 10 mmol/l is required, caused by high electrolyte need and a small volume of liquid, which can be infused. In that range of calcium concentration the electrostatic repulsion of the oil droplets in fat emulsions is minimised [8], i.e. the ζ-potential is only a few millivolts or 0 mV (appr. 6 mmol/l calcium ions).

In previous studies [9, 10] obvious differences in the coalescence behavior of calcium containing diluted fat emulsions were found. In one type of the studies, coalescence was followed using a Coulter Multisizer II [9], stability was assessed by drawing samples from emulsion-electrolyte mixtures. In the other investigation a laser diffractometer was employed [10], which allowed to monitor coalescence directly in highly diluted emulsions. However the differences could not solely be explained by the collision rate. Random collision promotes flocculation and coalescence [11], differences in the collision rate (fat concentration) can therefore cause differences in stability.

This study was performed to assess and to qualify the effects that occur under different conditions – degree of dilution and age of the emulsion – and to demonstrate the change in the emulsion system, even though the outward appearance seemed to be unchanged.

2. Investigations, results and discussion

2.1. Correlation between LS230 measurements and PCS

Photon correlation spectroscopy (PCS) is a frequently used technique to characterize the mean diameter of the bulk population in emulsions. The measuring range is approximately 3 nm to 3 μm. Therefore for stable emulsions PCS is a highly suitable technique to characterize the mean diameter and via the polydispersity index the width of the size distribution. As shown by the Coulter counter data a considerable amount of droplets larger 5 μm was formed during the coalescence. This requires ideally a sizing method covering a broader size range. As a new instrument a LS230 was used, which covers the range from 40 nm to 2000 μm by combining conventional laser diffractometry and PIDS technology.

To compare the PCS with the LS230 the emulsions were characterized with both systems (Table 1). The mean PCS diameters were relatively close to the mean diameters obtained with the LS230. Identical values could not be expected, since the calculation is based on different principles of measurement. PCS gives the intensity weighted diameter, the LS230 calculation is based on a volume distribution. However, both diameters are related closely enough. In addition, the LS230 has the advantage that it covers a size range not accessible by the Coulter counter. The bulk population of about 250 nm is below the detection limit of the Coulter counter (app. 0.7 μm) depending on capillary use. The LS230 appears therefore as a suitable instrument to detect simultaneously relatively small droplets of the bulk population and large coalesced droplets in the lower micrometer range.

Table 1: Mean diameters and standard deviations of emulsion A97, B93 and C87 calculated from 10 single measurements, performed in a ZetaSizer4 (90°) and a LS230 according to their evaluation routines

Emulsion	Diameter			
	PCS		LS230	
	Mean (nm)	SD (nm)	Mean (nm)	SD (nm)
A97	256	±4.7	268	±2.7
B93	245	±4.0	290	±5.1
C87	288	±4.5	290	±4.4

2.2. Coalescence kinetics of a fat emulsion in dependency of the amount of fat

The three dilutions containing 2%, 0.2% and 0.02% fat were investigated simultaneously in the Coulter counter and the LS230. The differences are shown exemplary for emulsion A97 (Fig. 1).

The overall detected volume after one day, that means the volume of droplets larger than 0.75 μm, increased from about 43,000 $\mu m^3/\mu l$ in the 2% fat emulsion and even to 120,000 $\mu m^3/\mu l$ in the 0.02% fat emulsion. This increase revealed that droplets had coalesced reaching a diameter being in the measuring range of the Coulter counter, that means larger than 0.75 μm. There was even a strong increase in the amount of droplets with a diameter larger than 5.0 μm being most pronounced for the highly diluted emulsion with 0.02% fat. The extend of coalescence is a function of the collision rate of the oil droplets and of the stabilizing effect of the emulsifier layer. Random collision of droplets is a necessary condition [12]. In the dilution of the 2% fat the collision rate is supposed to be much higher than in the more diluted system of 0.02% fat. Assuming a similar stabilizing effect of the emulsifier layer – lecithin in both dilutions – a more pronounced coalescence was expected for the more concentrated emulsion. Fig. 1 shows that the opposite effect occurred.

An explanation for the more pronounced coalescence in the diluted system are differences in the properties of the stabilizing lecithin layer. Lecithin stabilizes droplets by electrostatic repulsion due to its negative charge at pH 7.0 (see below). In addition, the fluidity of the emulsifier layer is an important parameter. Less fluid, i.e. more rigid emulsifier layers minimize coalescence. Düzgünes et al. [6] reported that an interaction with calcium ions increases the fluidity of lecithin membranes. A specific binding of calcium to lecithin was assumed.

The total concentration of calcium ions is identical in both fat emulsions (6 mmol/l). However, one needs to consider the ratio of calcium ions per surface of the emulsion droplets, that means per area lecithin. The surface area in the diluted emulsions is by a factor 10 or 100 lower. The observed increased coalescence in the diluted emulsion is therefore attributed to the increased number of calcium ions per surface area and their effect on reducing the emulsifier layer fluidity.

The dependence of the coalescence kinetics on the degree of dilution highlights that it is rather difficult to make conclusions based on measurements in diluted systems for predicting the destabilizing effects in higher concentrated emulsions. For example, turbidity measurements in diluted emulsions are widely used to assess the destabilizing effect of ions [13]. The data obtained can also explain apparent contradictions regarding the effect of e.g. calcium and magnesium ions on emulsion coalescence. Schuhmann reported a more destabilizing effect of calcium ions compared to magnesium in emulsions containing 20% fat [14]. The ions were added to the 20% fat emulsion, samples drawn after different incubation times and analyzed by Coulter counter. That means it was measured in the original system. The same ions were investigated in diluted emulsions using online measurements in a laser diffractometer [10]. The online measurement in the laser diffractometer requires an extremely high dilution of the emulsion. This changes dramatically the ratio of ions to surface area. In this study the damaging effect of calcium ions was reported to occur earlier and to be of larger extend. Based on the data in this study incubation in original fat concentration and analysis of samples drawn after certain incubation times appears most meaningful.

To assess whether differences in ζ-potential can also contribute to the observed differences in the coalescence kinetics, ζ-potential measurements were performed in 6 mmolar $CaCl_2$ solution. Identical ζ-potentials were found for both emulsions. The ζ-potentials of all diluted $CaCl_2$ containing emulsions were in the range between −4 mV (B93) and 0 mV (A97) in contrast to values of −50 mV (C87) and −41 mV (A97) of the original emulsions (Table 2). Therefore the ζ-potential measurements could give no reason for the obtained differential coalescence kinetics. A higher number of droplets in the capillary of the ZetaSizer would cause multiple scattering, thus the obtained value would be incorrect. This leads to the same conditions for the different emulsions whether the amount of fat in the dilution was chosen.

At the first glance this seems to contradict increased binding of calcium ions to the more diluted 0.02% fat emulsion. However, one needs to consider that for the ζ-potential measurement the emulsion needs to be diluted. Dilution with 6-mmolar $CaCl_2$ leads consequently to the

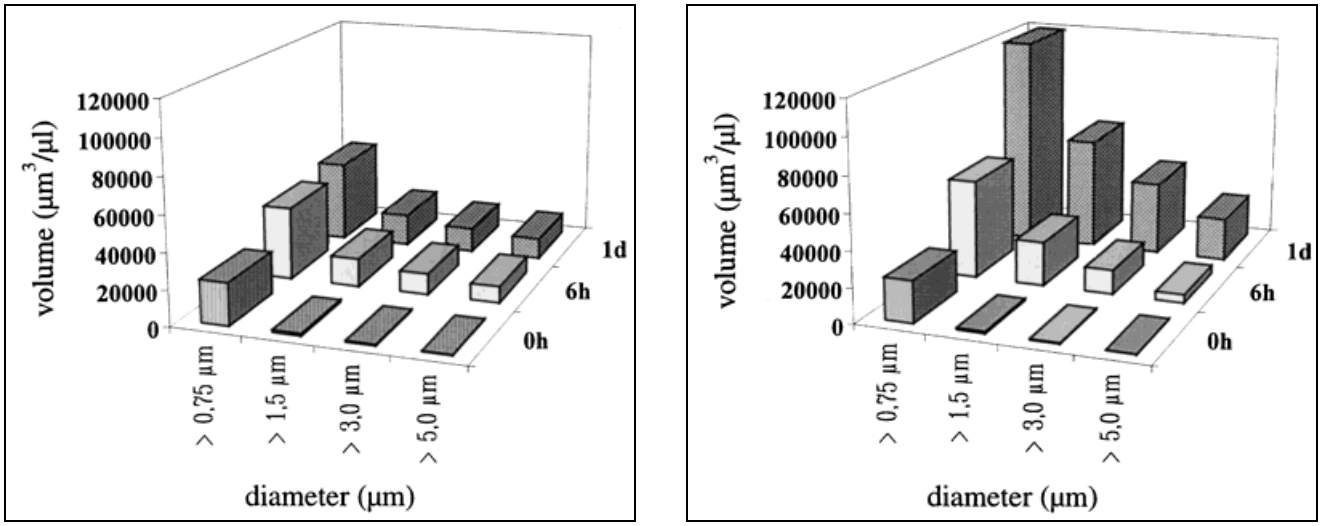

Fig. 1: Increase in the total volume of droplets being larger 0.75 μm, 1.5 μm, 3.0 μm and 5.0 μm per μl fat emulsion. Emulsion batch A97 was diluted with a solution containing 6 mmol/l $CaCl_2$ to 2% fat (left) and to 0.02% fat (right). The increase in the total volumes is a measure for progressing coalescence

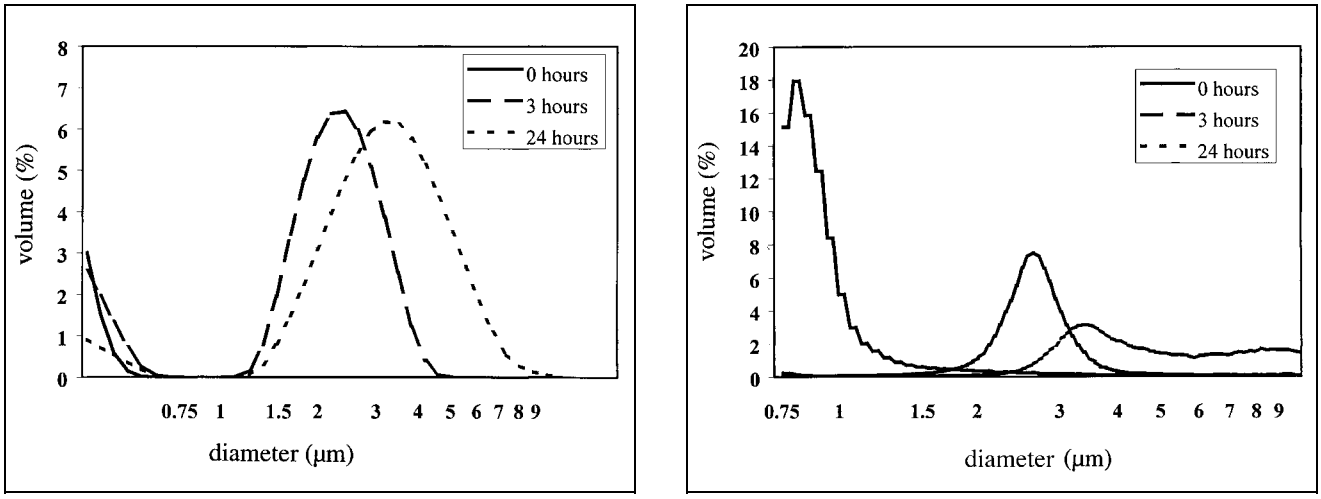

Fig. 2: Emulsion C87 diluted with a solution containing 6 mmol/l $CaCl_2$ to 2% fat. Measurements at 0 h (continuous line), 3 h (broken line) and one day (dotted line) performed with a LS230 (Coulter Electronics) (left) and a Coulter counter (right)

Table 2: ζ-Potentials measured in NaCl containing water (50 μS/cm conductivity) and in a solution containing 6 mmol $CaCl_2$ with a ZetaSizer4

Emulsions	ζ-Potential			
	Distilled water with NaCl (50 μS/cm)		6 mmol/l $CaCl_2$ (415 μS/cm)	
	Mean (mV)	SD (mV)	Mean (mV)	SD (mV)
A97	−41,26	±0,15	−0,42	±0,41
B93	−44,47	±0,64	−4,04	±0,07
C87	−50,54	±0,11	−0,38	±0,07

saturation of the surface and identical ζ-potentials for both systems. To sum up, the ζ-potential being actually present in the more concentrated 2% emulsions is not accessible due to technical procedure of the measurement itself (required dilution).

In addition, it cannot be excluded that the dilution of emulsions leads to the desorption of emulsifier from the interfacial layer between oil and water. For the investigated emulsions this appears unlikely because it could be shown that a surplus of lecithin is present in form of liposomes [15].

2.3. Correlation between data obtained with LS230 and Coulter counter

The time-dependent process of coalescence was followed using a Coulter counter and the LS230 simultaneously. Fig. 2 shows that the data obtained with both systems agree well. With both systems a distinct shift to larger diameters could be detected as function of time. The size distribution curves show a small – but pronounced – second peak on the right after 24 hours. No shoulder was found in the size distribution detected with the LS230. In this case one needs to consider the extremely broad measuring range of the LS230, that means the width of the size classes increases strongly when going to large diameters. The measuring range of the Coulter counter was selected from 0.7 to 30 μm (30 μm capillary) allowing a higher resolution. Fig. 3 shows the shift in size distribution when following the coalescence in the highly diluted 0.02% fat emulsions. It should be noticed that samples were withdrawn from the emulsions and diluted for the laser diffractometer measurement, it was no online measurement. Again both instruments show the more pronounced shift to larger diameters due to increased coalescence in the diluted emulsions.

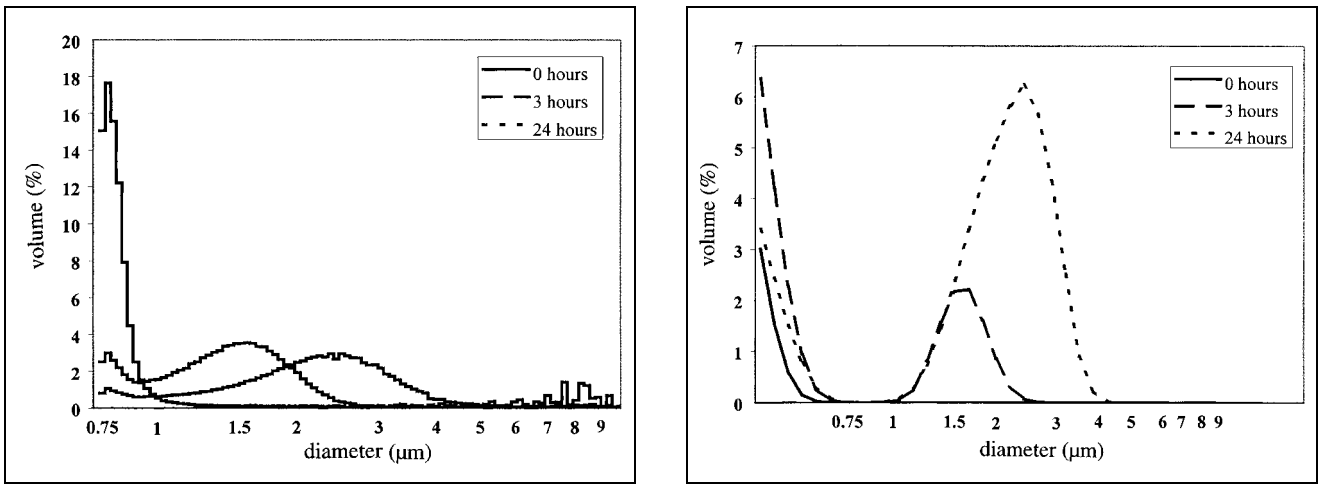

Fig. 3: Emulsion C87 diluted with a solution containing 6 mmol/l $CaCl_2$ to 0.02% fat. Measurements at 0 h (continuous line), 3 h (broken line) and one day (dotted line) performed with a LS230 (Coulter Electronics) (left) and a Coulter counter (right)

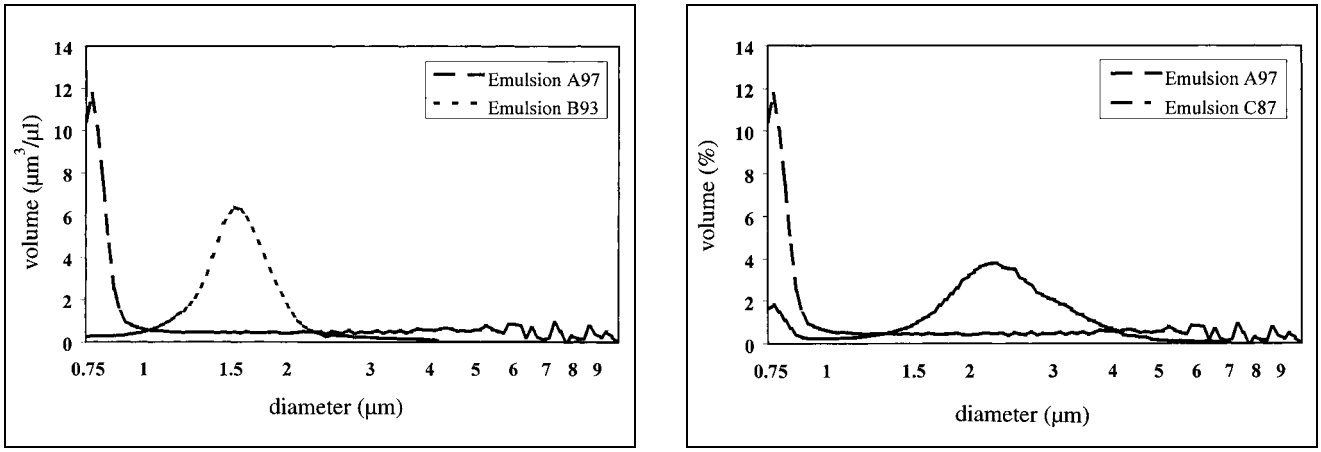

Fig. 4: Coalescence kinetics in dependence on the age. The measurements were performed 3 h after preparation of the emulsion mixtures, containing 6 mmol/l $CaCl_2$ and 0.2% fat, using a Coulter counter. Comparison of emulsions A97 with B93 (left) and A97 with C87 (right)

2.4. Coalescence kinetics in dependence on the age of the emulsions

When assessing the effect of electrolyte on emulsion stability it was noticed that there were obvious differences from batch to batch. The batches were, of course, the same in composition and similar in particle size, the only difference was their age. Therefore measurements were performed using emulsions of different age to assess a possible difference in electrolyte sensitivity. The emulsion with expiration date in 1997 (A97) showed no significant change in size distribution after incubation in 6 mmol/l $CaCl_2$ over 24 h (Fig. 4). The emulsion with expiration date 1993 (B93) showed a distinct sift in the size distribution to about 1.5 µm, the emulsion with expiration date 1987 (C87) showed a further sift to the right and additionally a broadening of the distribution (Fig. 4 left and right). Measuring the ζ-potential revealed even the highest potential for the oldest emulsion (C87) being about −50 mV (Table 2). Such an increase is explained by the formation of free fatty acids [16–18] leading to an enhanced physical stability due to increased electrostatic repulsion. A possible explanation is only the different interaction of calcium ions with the emulsifier layer and subsequently a change in its properties, e.g. increased fluidity of the aged emulsions. The increase in negative charge provides more binding sites for calcium, the increased binding of calcium per surface area might cause the increased coalescence seen with the higher diluted emulsion system.

The data demonstrate the limitations to predict long term stability of emulsions and effect of ions on emulsion stability based on assays performed in diluted or even highly diluted emulsion systems. Apart from the probability of droplet collision, the ratio of ions per surface area will change affecting also the physical stability. An increased amount of calcium per surface area caused by a dilution process promotes coalescence. Formation of lysolecithin and free fatty acids increase and the subsequent increase in ζ-potential during the aging of emulsions leads to stabilization of the emulsion in electrolyte-free media. It leads on the other hand to an increased binding of calcium ions and subsequent increasing electrolyte sensitivity with age. It was shown that parenteral emulsions being in principle a metastable system – are physically stable for more than six years [19]. However obviously their physical stability changes when electrolytes are present this rises the question that in TPN regimens the stability might be different when prepared with a fresh emulsion and when prepared with an emulsion shortly before expiration. This appears not critical in low electrolyte systems, but might be a more critical factor in high electrolyte regimens with an a priori stability of only a few days (e.g. high calcium-loaded regimens for infants).

3. Experimental

Parenteral fat emulsions (Lipofundin® MCT 10%; Ch.B.: 6023A81, expiry date 12/97 (A97), Ch.B.: 225681, expiry date 12/93 (B93), Ch.B.: 551281A, expiry date 6/87 (C87)) were provided from B. Braun Melsungen AG, Melsungen, Germany. $CaCl_2$ was purchased from Fluka Chemie AG (Buchs, Switzerland).

The three emulsions were diluted to 2%, 0.2% and 0.02% fat, respectively. The emulsions were put in a syringe and poured through a hose which ended at the surface of the electrolyte solution, only forced by gravitation. The electrolyte concentration of these mixtures was adjusted to 6 mmol/l $CaCl_2$. Batches of 50 ml were made in bottles of high quality glass closed with silicon rubber bungs (a gift from B. Braun, Melsungen, Germany).

The Coulter counter measurements for determination of the volume distribution were done using a Multisizer II (Coulter Electronics GmbH, Krefeld, Germany) equipped with a 30 µm capillary (measuring range 0.75–22.0 µm). To obtain sufficient counting rates different volumes of diluted or native emulsion electrolyte mixture were mixed up with 0.9% NaCl to a total of 100 ml.

For laser diffraction (LD) measurements a LS230 (Coulter Electronics GmbH, Krefeld, Germany) was applied. The decision for the LS230 was made to compare the results of Schuhmann [9] and Diderichs et al. [10], and to show the conformity of both techniques over a wide range of particle size. The sample is put in a volume of 120 ml of a liquid in which the sample is hardly sole.

ζ-potential and photon correlation spectroscopy (PCS) measurements were performed with a ZetaSizer4 (Malvern Instruments, UK) using a 7032 Multi-8-Correlator. The ZetaSizer was equipped with a ZET 5104 capillary, which has a diameter of 4 mm. The ζ-potential measurements took place in distilled water with an adjusted conductivity of 50 µS/cm [8], because in this range a slightly different conductivity causes no change in the obtained ζ-potential.

Every hour a sample was drawn from the gently shaken emulsion mixtures and, if necessary, diluted with 0.9% NaCl solution to get a suitable counting rate when put in the Coulter counter. In this way the nine dilutions (three different emulsions with three different fat contents) were treated over a period of 6 h, additional measurements were performed at day one.

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