ABSTRACT

The present study was carried out to develop a site-specific gel-delivery system of coenzyme Q10 (CoQ10) for periodontal therapy. Poloxamer 407 (P407) was employed to confer a thermoreversible gel forming ability while carbopol 947P (CBP) and sodium alginate (Alg) were also included for their mucoadhesive power giving rise to Gel-A and Gel-B respectively. The gels were evaluated in adult patients with chronic periodontitis and the clinical parameters viz plaque index (PI), gingival index (GI) and probing depth (PD) were recorded over a sufficient period of treatment (1-21 days). Results revealed that the two formulas had gelation temperature (Tg) in the physiological range whereas they differed in their mucoadhesive force being significantly higher for Gel-A. Both gels displayed a shear thinning property suitable for intrapocket periodontal injection; nevertheless, Gel-A had higher thixotropic behaviour. The release profile and kinetics of CoQ10 from the gels indicated zero-order release from Gel-A but Higuchi-type diffusion from Gel-B. Clinical evaluation was performed in ten patients with chronic periodontitis who received a clinical (plaque index, gingival index, probing depth) examination at the baseline and at 1, 7, 14 and 21 days. The results of the clinical study showed that, the group treated with Gel-A demonstrated significantly higher reduction in all clinical recordings compared to the groups treated with Gel-B or CoQ10 suspension. In conclusion, the significant improvement in clinical parameters indicated the potential of site-specific delivery of CoQ10 in an in situ-gel formulation with high mucoadhesive power and optimized rheological properties.

Keywords: Site-specific delivery, Periodontal therapy, Coenzyme Q10, Poloxamer 407, In-situ gel, Mucoadhesion, rheology

INTRODUCTION

Periodontitis is an inflammatory response in which the structural support to the tooth is destroyed. The disease results in resorption of the alveolar bone, detachment of the periodontal ligament supporting the tooth and formation of a periodontal pocket (lesions between teeth and junctional epithelium) 1. The pocket provides an ideal environment for the proliferation of a variety of pathogenic bacteria. Therapeutic approaches for the treatment of periodontitis include mechanical or surgical methods and administration of systemic antibiotics and/or other therapeutic agents. Systemic therapy has a low benefit to risk ratio 2. The drugs must be given in high doses to maintain an effective concentration in gingival crevicular fluid (GCF) which may cause side effects such as gastrointestinal disorders, development of resistant bacteria and super-infection. With advances in understanding of the etiology and pathogenesis of periodontal disease, attention has been focused on local drug delivery systems 3. These include both sustained and controlled release polymeric systems which when inserted into the periodontal pocket, release active agents for a sustained period of time. Thus intrapocket devices have a high benefit to risk ratio 4-5.

The wonder nutrient: coenzymes Q10 (CoQ10) is a fat-soluble, vitamin-like, benzoquinone compound (Fig. 1) that occurs naturally in the body 6. It functions primarily as an antioxidant, a membrane stabilizer and a cofactor in the oxidative phosphorylation production of adenosine triphosphate (ATP) 7. Its beneficial effects include but are not limited to: delaying the ageing process and increasing longevity, improving heart health, boosting body’s defence system, supporting immune system, minisising free radical damage, fighing gum disease, reducing migraine frequency & length, increasing stamina and endurance, increasing sperm motility leading to enhanced fertility, the symptoms of Parkinson’s disease, coping with the effects of diabetes, improving age-related macular degeneration, and improving exercise tolerance in people with muscular dystrophies 8-10. Research shows that the gums of people with periodontal disease are often significantly deficient in CoQ10 11, 12. Indeed, gingival and leukocytic deficiencies of CoQ10 have been detected in periodontal patients attributed to a systemic nutritional imbalance and not neglected oral hygiene 11, 12. It was suggested that oral intake of CoQ10 may be effective in improving the periodontal disease. Barbieri et al. reported that oral CoQ10 was beneficial in periodontal inflammation by virtue of its intracellular antioxidant activity and its ability to enhance macrophages-phagoecytic activity and to increase granulocyte proliferation 12. However, the oral powders formulations of CoQ10 have poor dissolution in aqueous media, resulting in poor oral bioavailability 13. The market lacks an intrapocket periodontal formulation for CoQ10 and this form is inadequately explored in literature. Therefore, formulations that allow site-specific delivery of CoQ10 i.e. directly in the periodontal pocket are warranted. A recent study reported an intrapocket gel formulations of CoQ10 in chronic periodontitis with promising results both clinically and microbiologically 14. Intrapocket CoQ10 formulations should improve the solubility and stability of this nutraceutical and allow mixing/dilution with body fluids. In this context, water-soluble organic solvents e.g. propylene glycol as well as surfactants e.g. poloxamers were commonly employed as solubilizing excipients 15. Indeed, the poloxamer surfactants at high concentrations (17-25%) were frequently explored not only for their solubilizing power 16 but also for thermoreversible gel formation 17. From a stability viewpoint, the immobilization of drug molecules in a thixotropic gel preparation favours stability due to a complete rest in a solid gel matrix eventually decreasing the chance for chemical degradation 18. Benefits of poloxamer-based systems include: long history of safe and effective use in semi-solid formulations 19, low irritancy and non-sensitizing properties with repeated usage 19, compatibility across a broad pH range and with most acidic, basic, and neutral drugs, consistent and reproducible properties due to their synthetic nature, stability against microbial growth and shear thinning properties that facilitate syringeability or extrusion from product packaging 20. From a periodontist point of view, intrapocket injectable gels are highly convenient since they allow a good filling of the pocket in comparison to solution or solid intrapocket devices and afford improved syringeability with modulated rheological properties 21. Furthermore, inclusion of mucoadhesive polymers to the in-situ gel would offer, beside their high viscosity, site-specific mucoadhesion 22, increased residence time and hence prolonged therapeutic levels in the GCF 23-25. In the present study, a site-specific delivery system of CoQ10 was developed for management of periodontitis. The system was evaluated pharmaceutically as well as clinically to assess its beneficial action to patients with chronic periodontitis.

![Chemical Structure of CoQ10](image.png)

Fig. 1: Chemical Structure of CoQ10
MATERIALS AND METHODS

Materials
CoQ10 was kindly supplied by MepaCo, Egypt; Carbopol® 974P NF (Lubrizol Advanced Materials, Inc. Cleveland, Ohio, USA), poloxamer 407, propylene glycol (ADWIC Co., Egypt).

Equipment
Electric balance (Sartorius, GMBH, Gottingen, Germany); thermostatically controlled magnetic stirrer (Thermolyne Corporation, U.S.A.); Brookfield viscometer (cone and plate; type DVT-2); USP dissolution test apparatus (Pharma test, type PTW, Hainburg, Germany); UV- visible double beam spectrophotometer (Shimadzu, model UV-1601 PC, Kyoto, Japan).

Methodology
Preparation of Coq10 gel formulations
The Poloxamer 407 gel (Polox) was prepared by the cold method as previously described with some modification. Briefly, the mucoadhesive polymers namely: carbopol 974P (CBP) and sodium alginate (Alg) were dispersed in the calculated amount of distilled water at room temperature at a concentration 2% and stirred using a magnetic stirrer. The dispersions were cooled in the fridge; the Polox (18% w/w) was then added slowly and left to hydrate at 4°C with occasional stirring on a vortex mixer.

Before incorporation into the gel, a dispersion of Coq10 was first prepared by mixing the drug (200 mg) and propylene glycol (PG; 5%) in a porcelain dish with stirring till smooth paste formation. The cold CBP/Polox and Alg/Polox solutions were added to the paste and mixed with a glass pestle for 2 min until it sets into a gel giving rise to Gel-A or Gel-B respectively. The gels were then packed into plastic wide mouth container and stored at room temperature (Shah and Donovan 2007). To serve as a control, CBP/Polox (Gel-A) and Alg/Polox (Gel-B) were prepared in a similar manner but without Coq10.

Measurement of gelation temperature of the prepared gels
The gelation temperature ($T_{gel}$) was measured by the simple test tube inverting method exactly as mentioned in previous publications. Two ml aliquot of refrigerated tested formulation was transferred to a test tube and sealed with a parafilm. The tube was maintained in a thermostatically controlled water bath at 25°C. The temperature of the water bath was increased gradually in increments of 1°C in the beginning of the experiment and then 0.1°C increments in the region of sol-gel transition temperature and 0.1°C when it approaches gelation. The tested formulation was left to equilibrate for 5 minutes at each new setting (or for 2 minutes when temperature was increased with an increment 0.1°C). The gelation temperature is taken as the temperature at which the meniscus would no longer move upon tilting through angle 90°. The maximum accepted sol-gel transition temperature tested was 37°C which represents the gingival crevicular fluid (GCF) temperature. The tilting rate, amount of solution and the diameter of the test tube were kept constant throughout the experiment

Measurement of mucoadhesive properties of the prepared gels
The mucoadhesive forces of the prepared gels were determined using mucin tablets and a mucoadhesive force-measuring device as previously mentioned. The detachment force (dyne/cm²) was determined using the following equation

$$\text{Force (dyne/cm²)} = \frac{mg}{A} \quad \text{................. (1)}$$

Where m: the weight of water in grams g: acceleration due to gravity taken as 980 cm/sec².
A: area of the mucin disc (area of contact) and is equal to $\pi r^2$ (r is the radius of the mucin disc).

Measurement of rheological properties of the prepared gels
The rheological properties of the gel formulations were studied using cone and plate (Brookfield) viscometer (cone and plate; type DVT-2 spindle 52) as previously reported. Briefly a sample (0.5 ml) of sol was applied to the lower plate of the viscometer using a spatula. The measurements were made at 25 and 37 $\pm$ 0.1°C using spindle 52 at different shear rates ranging from 5 to 400 rpm. The shear rate (γ) in s⁻¹ and the viscosity ($\eta$) in centipoises (cps) were determined from the instrument reading and fitted to the power law constitutive equation

$$\eta = m \gamma^{n-1} \quad \text{................. (2)}$$

The two dimensionless quantities: the consistency index (m) and the flow index (n) characteristic for each formulation were obtained. If n=1 this indicates Newtonian behavior while if (n) is less than 1, this corresponds to shear thinning flow. The lower the value of (n) the more shear thinning the formulation. The thixotropic behavior of the gels was evaluated by calculating the area of the hysteresis loop formed from the shift of the up and down curves of the rheograms of the 2 intrapocket gel formulations using the trapezoidal rule and calculating the coefficient of variation (C.V.%) at all rates of shear for each formula. Measurements were done in triplicates and statistical tests of significance were done using Student’s t-test at P<0.05.

In vitro drug release study
The in vitro release of CoQ10 from the gel formulations was studied using a modified USP paddle method as previously described. Briefly, a glass cylinder (2.5 cm dia), with a cellulose membrane (Mw cutoff 12,000) tied to one end, was attached to the metallic drive shaft of the dissolution apparatus and immersed in 250 ml of phosphate buffer pH 7.4 maintained at a temperature of 37°C. An accurately measured volume of the formula (5ml), was transferred to the glass tube. The shaft was allowed to rotate at a constant speed (100 rpm). Aliquots of 5 ml were withdrawn at time intervals 0.5, 1, 2, 4, 8, 10, 12 and 14 days and replaced by an equal volume of fresh buffer. The drug content in the withdrawn samples was determined spectrophotometrically at 275 nm and quantified via a standard curve. For comparison, release of CoQ10 from an aqueous suspension prepared with Tween 80 as wetting agent was determined similarly.

Analysis of drug release data
The data obtained from the in vitro release experiments were analysed by the following commonly used exponential equation

$$\frac{M_t}{M} = k t^n \quad \text{............... (3)}$$

$\log \frac{M_t}{M} = \log k + n \log t$

Where $\frac{M_t}{M}$ is the fraction of released drug at time t k: release constant incorporating structural and geometric characteristics of the drug/polymer system.

n: release exponent indicative of the release mechanism. When n is equal to 0.5, the drug is released from the polymer with a Fickian diffusion mechanism (Fick’s model 1). If 0.5 < n <1 this indicates anomalous or non-Fickian release, while if n=1 this indicates zero-order release.

Clinical Study
Patient selection
Ten healthy patients with chronic periodontitis, (ages 33-45) were included in this study. All patients attended to Oral Medicine and Periodontology department, College of Dentistry, Al-Azhar University and were given information about the proposed treatment and were asked to sign a formed consent. Each patient had at least three contrafactual periodontal defects with probing depth > 5 mm and/or clinical attachment loss > 5 mm. Root planning and subgingival debridement was performed to thirty periodontal pockets in a split mouth design; which were then divided into three
groups; group I included ten periodontal pockets that received site-specific delivery of Gel-A, group II received site-specific delivery of Gel-B while group III received site-specific delivery of CoQ10 suspension

All participants were given detailed instructions in self performed plaque control measures.

Clinical assessment

Clinical recordings were assessed immediately before treatment (baseline, day 0) then at day 1, 7, 14 and 21 as follows: (1) Plaque index (PI), is an index for estimating the effectiveness of oral hygiene by measuring dental plaque which occurs in areas adjacent to gingival margin and is evaluated on a scale basis as follows: 0 for no plaque, 1 for separate flecks of plaque at the cervical margin of the tooth, 2 for a thin continuous band (up to 1 mm) of plaque at the cervical margin of the tooth, 3 (severe plaque) for a band of plaque wider than 1 mm, at four sites (buccal, lingual, mesial and distal) of each tooth. (2) Gingival index (GI), is a measure of gingival inflammation based on visual inspection of gingiva by periodontist that takes into consideration the color and firmness of gingival tissue along with the presence of blood during probing and recorded on scale basis as follows: score 0 for normal gingiva, 1 for mild inflammation, slight edema, no bleeding on probing, 2 for moderate inflammation, redness, edema, bleeding on probing, 3 for severe inflammation, marked redness, edema, ulcerations and spontaneous bleeding. (3) Probing depth (PD), defined as the distance (in mm) from the gingival margin to the bottom of the pocket and is considered as a measure of the severity of periodontal disease.\[^{21,24,25}\]

Statistical Analysis

All results obtained were statistically analyzed with statistical program SPSS ver 16.0* (Statistical package for scientific studies). Statistical tests of significance were performed using one-way ANOVA followed by LSD method for multiple comparisons. The differences were considered significant when $P < 0.05$.

RESULTS AND DISCUSSION

The use of wonder nutrient, Coenzyme Q10 in an intrapocket gel system necessitates significant formulation efforts prior to clinical studies. In this study, the formulation strategy adopted was: first, to optimize the rheological behaviour of the intrapocket gel to achieve site-specific gelation, high thixotropy and physical stability. Second, mucoadhesive property by incorporation of renowned mucoadhesive polymers and third, hopefully a zero-order release profile of CoQ10 from the intrapocket gel to maintain sufficient amounts of CoQ10 in the GCF.

The gel formulations exhibited a sol-gel transition temperature in the physiological range where Gel-A and Gel-B recorded a $T_{gel}$ of 32°C and 34.5°C respectively (figure 2). These results were in agreement with our previous results.\[^{26}\] The thermo-reversible behaviour is desirable for intrapocket delivery system for good filling of the periodontal pocket and for ease of syringeability.\[^{21}\] Regarding mucoadhesion, results in figure 2 reveals that Gel-A (containing carbopol) exhibited significantly higher mucoadhesive power than Gel-B (containing sodium alginate). These results are in accordance with previous results.\[^{21,25}\] The higher mucoadhesion might result in more prolonged residence time in the pockets of patients allowing higher local concentration of CoQ10 in the GCF.

![Fig. 2: Gelation temperature ($T_{gel}$, °C; green bars) and mucoadhesive force (blue bars) of CoQ10 intrapocket gels.](image)

Fig. 3 depicts a representative viscosity versus shear rate profile showing the viscosity changes that occur by temperature variation. At 25°C the intrapocket gel was in a liquid form and exhibited a Newtonian behaviour (no notable changes in the viscosity over a broad range of shear rates). Conversely at 37°C, a dramatic shear-thinning behaviour was observed in the corresponding profiles of the intrapocket gels indicating temperature-induced gelation.\[^{26}\]

![Fig. 3: Viscosity curves of CoQ10 intrapocket gels at different temperatures](image)

According to equation [3], the values of the flow index ($n$) at 25°C were 0.92 and 0.98 for Gel-A and Gel-B respectively (i.e. close to 1, confirming a Newtonian behavior) while the respective consistency index ($m$) values were 407.5 and 241.2 indicative of lower viscosity at shelf condition (Table 1). However, when the temperature was increased to 37°C, the ($n$) values decreased. The lower the value of ($n$), the more shear thinning the formulation.\[^{28,30}\]
was almost double that for Gel-B (150858 cm²/dyne.s) as shown in table 1. Thixotropic behaviour is a desirable phenomenon where the gel exhibits a shear thinning flow (i.e. becoming less viscous upon agitation/injection from periodontist needle).

Once injected in the pocket, the 3-dimensional structure of the gel reforms and consistency was restored conferring rigidity which results in sustained drug release and stabilization of drug molecules against chemical degradation.

Table 1: Rheological Data of CoQ10 Intrapocket Gels.

<table>
<thead>
<tr>
<th>Mixture</th>
<th>Consistency index (m)</th>
<th>Flow index (n)</th>
<th>Area of Hysteresis loop (cm²/dyne.s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gel-A at 25°C</td>
<td>407.5</td>
<td>0.92</td>
<td>NA</td>
</tr>
<tr>
<td>Gel-A at 37°C</td>
<td>104584</td>
<td>0.209</td>
<td>309115</td>
</tr>
<tr>
<td>Gel-B at 25°C</td>
<td>241.2</td>
<td>0.98</td>
<td>NA</td>
</tr>
<tr>
<td>Gel-B at 37°C</td>
<td>36785</td>
<td>0.204</td>
<td>150858</td>
</tr>
</tbody>
</table>

NA: Not applicable

The rheogram shown in figure 3 reveals the higher thixotropic behaviour of Gel-A compared to Gel-B formulation which is revealed by a larger area of the hysteresis loop (i.e. loop formed by the up and down curve in the rheogram). The value for the area calculated by the trapezoidal rule for Gel-A (309115 cm²/dyne.s) was almost double that for Gel-B (150858 cm²/dyne.s) as shown in table 1. Thixotropic behaviour is a desirable phenomenon where the gel exhibits a shear thinning flow (i.e. becoming less viscous upon agitation/injection from periodontist needle).

Regarding the release of CoQ10, figure 3 depicts the release profile of CoQ10 from the prepared formulations. For the data it could be observed that Gel-A gave almost a linear release profile with 100% drug release in 21 days while the suspension formulation and the Gel-B formulation showed an initial fast release of the drug. Kinetic data in table (1) revealed that the release exponent (n) of equation (3) had value 0.9 which indicates a zero order kinetics for release whereas a Higuchi diffusion mechanism for Gel-B and CoQ10 suspension was demonstrated by n values near 0.5. These results agree with those previously reported by Prabhushankar et al. for levofloxacin periodontal gel.

Table 2: Kinetic Data of CoQ10 Release from different gel formulations

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Release Exponent (n)</th>
<th>Kinetic Constant (k, %/min.x)</th>
<th>Correlation Coefficient (r)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CoQ10 suspension</td>
<td>0.454</td>
<td>1.69112</td>
<td>0.9921</td>
</tr>
<tr>
<td>Gel-A</td>
<td>0.90067</td>
<td>0.91</td>
<td>0.9951</td>
</tr>
<tr>
<td>Gel-B</td>
<td>0.4283</td>
<td>1.491</td>
<td>0.9917</td>
</tr>
</tbody>
</table>

In the clinical study, the gel formulations were compared to CoQ10 suspension. Figures (4-6) summarize the changes in mean plaque index (PI), gingival bleeding index (GI) and probing depth in group I (periodontal pockets that received Gel-A); group II (periodontal pockets that received Gel-B) and group III (periodontal pockets that received CoQ10 suspension).
Fig. 6: Mean reduction in plaque index in group I patients (receiving Gel-A), group II patients (receiving Gel-B) and group III patients (receiving CoQ10 suspensions) at different times of treatment. For every group of pockets, a, b, c and d are significantly different from day 1, 7, 14 and 21 respectively at \(P < 0.05\) using one-way ANOVA followed by LSD test for multiple comparison.

The statistical analysis of the results of the mean reduction in plaque index (PI) showed non-significant changes in PI in the study period between day 1 and day 7. On the other hand, the changes in this parameter were statistically significant at later study periods namely day 14 and day 21 only for group I (receiving Gel-A) but not group II (receiving Gel-B) or group III (receiving CoQ10 suspension). It is noteworthy to note that Group I (receiving Gel-A) demonstrated the highest reduction in PI after 21 days of treatment where it recorded 0.41 ± 0.05 as compared to Group II (receiving Gel-B) and Group III (receiving CoQ10 suspension) with mean reduction in PI 0.28 and 0.24 respectively.

Fig. 7. Mean reduction in gingival index in group I patients (receiving Gel-A), group II patients (receiving Gel-B) and group III patients (receiving CoQ10 suspensions) at different times of treatment. For every group of pockets treated a, b, c and d are significantly different from day 1, 7, 14 and 21 respectively at \(P < 0.05\) using one-way ANOVA followed by LSD test for multiple comparison.

Regarding the gingival index (GI), the results revealed that the mean reduction in GI was not significant between day 1 and day 7 in the three groups. However, the reduction in this parameter was statistically significant at later study periods i.e. day 4 and day 21 only for group I (receiving Gel-A). For group II (receiving Gel-B) or group III (receiving CoQ10 suspension) the changes were not significant throughout the study period (i.e. from day 1 to day 21).

Similar to PI, the highest reduction in GI after 21 days of treatment was observed in Group I (receiving Gel-A). Concerning the probe depth (PD), the effect of period of treatment was evident and significantly different among the various days of treatment. At any study period, Group I (receiving Gel-A) displayed the uppermost reduction in PD which was significantly different from that displayed in case of Group II and group III.

Fig. 8. Mean reduction in probing depth in group I patients (receiving Gel-A), group II patients (receiving Gel-B) and group III patients (receiving CoQ10 suspensions) at different times of treatment. For every group of pockets, a, b, c and d are significantly different from day 1, 7, 14 and 21 respectively at \(P < 0.05\) using one-way ANOVA followed by LSD test for multiple comparison.
Regional impairment in immune function in the gingiva was demonstrated in patients with periodontitis. An effective way to reduce and/or to control microorganisms in the gingiva of patients with periodontal disease is to increase the efficacy of the immune system of the host. A consensus report by the American Academy of Periodontology stated that 4 to 6 weeks of local delivery seems appropriate to assess the initial response to nonsurgical periodontal therapy, based on evidence of periodontal wound healing. Hence, we evaluated the clinical effect of local delivery of the two gel formulations of coenzyme Q10 as compared to suspension formulation on chronic periodontitis for a study period of 21 days. The results of this study demonstrated the clinical superiority of Gel-A as an adjunctive therapy to scaling and root planing in the three groups of pockets during this the study period where it resulted in significant reduction of PI, GI and PD. These findings were in agreement with those previously reported which suggested that oral administration of CoQ10 may decrease the degree of bleeding tendency in elderly people. Earlier report demonstrated an increase in the mean value of coenzyme Q10 in gingival biopsies during treatment with oral CoQ10 which correlated with extraordinary healing. Moreover, the results of the present study corroborated those reported by Haniola et al. which evaluated topical application of coenzyme Q10 in periodontal pocket with and without subgingival debridement. The study demonstrated a significant improvement in bleeding on probing and probing depth at the experimental sites and suggested that topical application of CoQ10 in adult periodontitis not only as a sole treatment but also in combination with traditional nonsurgical periodontal therapy. Recent studies on local application of CoQ10 in chronic periodontitis revealed the superiority of CoQ10 in combination with root planning and subgingival scaling over treatment based on root planing and subgingival scaling alone. Previous studies have shown that different formulations of the same drug affected the clinical response and/or the pharmacokinetic profile in GCF, saliva and blood. The superior clinical outcomes observed with Gel-A formulation compared to the Gel-B (containing alginate) and CoQ10 suspension could be attributed to its higher mucoadhesive power, optimum release profile, gel-like consistency and the combined solubilizing effect of poloxamer 407 and propylene glycol. For recapitulation, we could be attributed to its higher mucoadhesive power for maximized residence in the pocket as well as a zero order drug release profile. Such formulation proved beneficial in treatment of chronic periodontitis and should be considered as an adjunctive treatment with current dental practice.

ACNOWLEDGEMENT

The author is deeply grateful to Dr. Able Gawish, Co-Ge of Dentistry, Al-Azhar University, Egypt for technical assistance in the clinical work.

REFERENCES


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