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A study of the antibacterial activity and stability of dyed cotton fabrics modified with different forms of silver

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Abstract: This study compares the effect of colloidal silver nanoparticles and the commercial RUCO-BAC AGP agent with silver chloride as the active component on the antibacterial activity of dyed cotton fabrics. Cotton fabrics were dyed with vat dyes Bezanthren Olive T and Bezanthren Grey FFB. The antibacterial activities of the silver-loaded dyed cotton fabrics were tested against the Gram-positive bacterium *Staphylococcus aureus* and the Gram-negative bacterium *Escherichia coli*. Unlike RUCO-BAC AGP, the synthesized silver nanoparticles deposited onto the dyed cotton fabrics provided maximum bacteria reduction, independent of the applied dye. The stability of the modified cotton fabrics was analyzed in artificial sweat at pH 5.5 and 8.0. Approximately the same amount of silver was released from the differently modified cotton fabrics in artificial sweat. Larger amounts of silver were released in the sweat at pH 8.0.

Keywords: cotton; silver nanoparticles; RUCO-BAC AGP; antibacterial efficiency; artificial sweat.

INTRODUCTION

Approximately 50 % of textiles today are made of cotton (Co) since these fibers are particularly suitable for the manufacture of sport and leisure textiles, medical non-implantables (different bandages, plasters, gauze dressings, lint, wadding, adsorbent pads) and healthcare/hygiene products (surgical gowns and hosiers, sheets, pillowcases, uniforms, blankets).¹ However, Co fibers may act as a nutrient, becoming a suitable medium for microorganism growth and thus frequently antimicrobial properties have to be imparted to cotton products.²

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Various agents have been used for supplying textile materials with antimicrobial properties, but poor efficiency or high toxicity made them unsuitable for long-term use.³ Silver nanoparticles (Ag NPs) are potent and broad-spectrum antibacterial agents.⁴ Ag NPs can be found in room sprays, detergents and wall paints, as well as in washing machines, refrigerators, vacuum cleaners and air conditioners. The concept of Ag NPs application to textile materials relies on the fact that a small amount of deposited nanoparticles can provide the desired antimicrobial activity with long-term durability and stability without the use of highly toxic organic compounds. The antibacterial effect of Ag NPs on Co fabrics has already been studied by several research groups.^{5–12} However, less attention has been paid to the synergism between antibacterial finishing with Ag NPs and dyeing, which is a very important issue from the technological point of view. Hitherto, the influence of reactive dyes and direct dyes on the antibacterial efficiency of Co fabrics treated with Ag NPs was investigated.^{6,10}

In this study, the effect of silver in different forms on the antibacterial properties and color change of Co fabrics dyed with vat dyes were investigated. Comparative examination of colloidal Ag NPs that were synthesized without the employment of any stabilizer and the commercial RUCO-BAC AGP (RB) agent with silver chloride as the active component was performed. Namely, RB consists of micro-structured titanium dioxide that acts as a carrier of silver chloride. In the case of moisture (*e.g.*, perspiration), silver ions with antimicrobial effect are set free from a virtually infinite deposit. The antibacterial activity of Co fabrics was tested against the Gram-negative bacterium *Escherichia coli* and the Gram-positive bacterium *Staphylococcus aureus*. Additionally, the stability of these textile nanocomposite materials was studied in artificial sweat at pH 5.5 and 8.0.

EXPERIMENTAL

Materials and methods

Desized and bleached woven Co fabric (168 g m^{-2}) was cleaned from surface impurities as described in a previous study.¹³ Vat dyes Bezathren Olive T (BO) and Bezathren Grey FFB (BG), both products of Bezema AG, were used for dyeing the Co fabrics.

The schematic procedure of the dyeing of Co fabrics is shown in Fig. 1. Co fabrics were dyed in Polycolor (Werner Mathis AG) laboratory beaker dyer in the bath containing 2 % or 4 % (on weight of fabric – o.w.f.) of a vat dye and reducing agent $\text{Na}_2\text{S}_2\text{O}_4$ (5 g L^{-1}) at a liquor-to-fabric ratio of 10:1. Subsequently, the fabrics were oxidized in a bath containing H_2O_2 (1.5 g L^{-1}), rinsed once with water and treated in a solution of the surfactant Cotoblanc RS (2 g L^{-1}). After rinsing with water, the samples were dried at room temperature.

Silver nitrate (AgNO_3 , Kemika) and the reducing agent sodium borohydride (NaBH_4 , Fluka) of p.a. grade were used for the synthesis of the colloidal Ag NPs.^{14,15} AgNO_3 (8.5 mg) was dissolved in 250 mL of water and purged with argon for 30 min. Under vigorous stirring, NaBH_4 (125 mg) was added to the solution and left for 1 h under an argon atmosphere. The concentration of the Ag colloid was 50 ppm. The synthesized colloid was comprised of nearly spherical nanoparticles with an average diameter of 10 nm.¹³

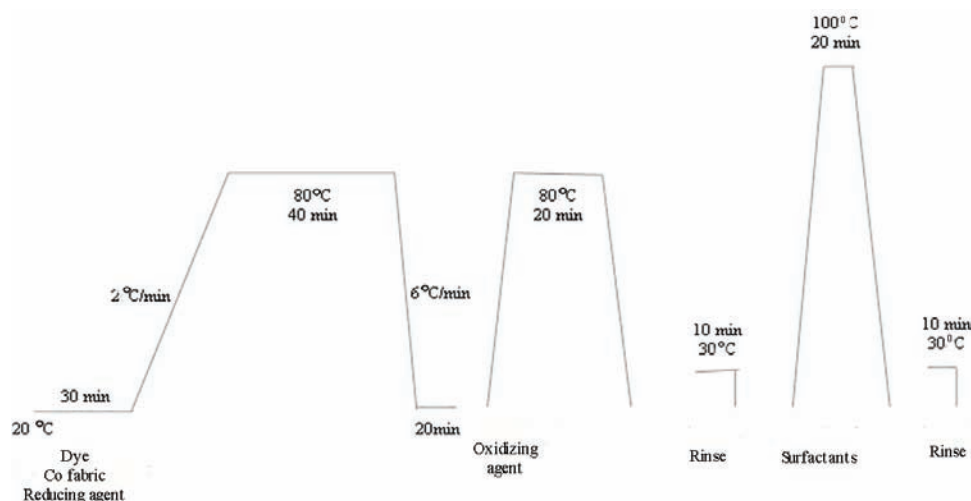


Fig. 1. Dyeing procedure for Co fabrics.

One gram of dyed Co fabric was immersed in 45 mL of the colloidal Ag NPs for 5 min and dried at room temperature. After 5 min of curing at 100 °C, the samples were rinsed twice (5 min) with deionized water and dried at room temperature.

Dyed samples were also treated with RUCO-BAC AGP, a highly concentrated hygiene and freshness system that comply with Öko-Tex Standard 100.¹⁶ The recommended concentration of 0.50 % (o.w.f.) was applied. The samples were treated in a bath (liquor-to-fabric ratio 10:1) for 30 min at 50 °C. The pH value of 5–6 was adjusted using acetic acid. Subsequently, the samples were dried at 130 °C for 4 min.¹⁷

Fiber morphology was followed by scanning electron microscope (SEM, JEOL JSM-6610LV). Gold layer was deposited on the samples before analysis.

The color coordinates of the dyed fabrics (CIE L^* , a^* and b^*) were determined with a Datascolor SF300 spectrophotometer under illuminant D_{65} using the 10° standard observer. Based on the measured CIE color coordinates, the color difference (ΔE^*) was determined as:

$$\Delta E^* = \sqrt{(\Delta a^*)^2 + (\Delta b^*)^2 + (\Delta L^*)^2} \quad (1)$$

where ΔL^* is the color lightness difference between the treated (dyed Co fabric loaded with Ag NPs or RB) and the control (dyed Co fabric without Ag) samples; Δa^* is the red/green difference between the treated and control samples; Δb^* is the yellow/blue difference between treated and control samples.

The antibacterial efficiency of the differently modified Co fabrics was quantitatively assessed using the Gram-negative bacterium *E. coli* ATCC 25922 and the Gram-positive bacterium *S. aureus* ATCC 25923. Bacterial inoculums were prepared in 3 mL of tryptone soy broth (Torlak, Serbia) as the growing medium and left overnight at 37 °C (late exponential stage of growth). 70 mL of sterile potassium hydrogen phosphate buffer solution (pH 7.2) was added to sterile Erlenmeyer flask (300 mL), which was then inoculated with 0.7 mL of a bacterial inoculum. One gram of sterile Co fabric cut into small pieces was placed in the flask and shaken. After 1 h of shaking, 1.0 mL aliquots from the flask were diluted (1:10 and 1:100) with saline solution and 0.10 mL of the solution was placed onto tryptone soy agar (Torlak,

Serbia). After 24 h of incubation at 37 °C, zero time and one-hour counts of the viable bacteria were made. The percentage of bacteria reduction (R , %) was calculated using Eq. (2):

$$R = 100 \frac{C_0 - C}{C_0} \quad (2)$$

where C_0 (CFU – colony forming units) is the number of bacterial colonies on the control Co fabric (dyed Co fabric without Ag) and C (CFU) is the number bacterial colonies on the Co fabric loaded with Ag NPs or RB.^{5,18,19}

Artificial sweat at pH 5.5 and 8.0 was prepared according to ISO 105-E04:1989E.²⁰ One liter of acidic artificial sweat (pH 5.5) contained 0.5 g of *L*-histidine monohydrochloride monohydrate, 5 g of sodium chloride and 2.2 g of sodium dihydrogen orthophosphate dihydrate. One liter of alkaline artificial sweat (pH 8.0) contained 0.5 g of *L*-histidine monohydrochloride monohydrate, 5 g of sodium chloride and 5 g of disodium hydrogen orthophosphate dodecahydrate. The solutions were brought to pH 5.5 and 8.0 with a 0.1 M solution of sodium hydroxide. The Co fabrics (0.300 g) were soaked in artificial sweat at a liquor-to-fabric ratio of 1:50. The samples were incubated in a water bath at 37 °C. After 24 h of incubation, the artificial sweat was collected and the content of released silver was determined using a Perkin-Elmer 403 atomic absorption spectrometer (AAS). The results were calculated as the total amount of silver released into the artificial sweat and normalized with respect to the weight of the fabric sample. In order to determine the total amount of silver on the Co fabric, the samples were burnt and dissolved in nitric acid. The amount of silver was also measured by AAS.

The color fastness of the Co fabrics that had been exposed to artificial sweat was determined spectrophotometrically.

RESULTS AND DISCUSSION

The changes in fiber surface morphology after deposition of Ag NPs onto dyed Co fabric was assessed by SEM. The SEM image of the Co fiber dyed with 2 % BO and loaded with Ag NPs is shown in Fig. 2. It can be noticed that the surface of the Co fiber modified with Ag NPs was mainly covered with NP aggregates of dimensions around 100 nm.

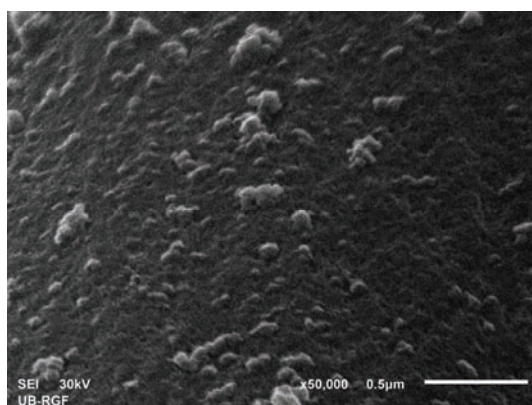


Fig. 2. SEM Image of Co fiber dyed with 2 % BO and loaded with Ag NPs.

The color difference between the Co fabrics loaded with Ag NPs or RB and the control fabric (only dyed Co fabric) was expressed *via* the CIE L^* , a^* and b^* color coordinates. The colorimetric data for the control Co fabrics and the Co fabrics loaded with Ag NPs or RB after dyeing with BG or BO are presented in Table I. The results clearly indicate that treatment of the dyed Co fabrics with RB caused a color change that could not be visually detected since the color difference (ΔE^*) was lower than one. The deposition of Ag NPs onto the Co fabrics dyed with BG also did not significantly affect the color of Co fabric ($\Delta E^* < 1$). However, the deposition of Ag NPs onto Co fabrics dyed with BO led to a considerable increase in color change. This was particularly prominent on the sample that had previously been dyed with 4 % BO.

TABLE I. Colorimetric data for Co fabrics dyed with BG or BO and modified with Ag NPs or RB

Dye	Sample	L^*	a^*	b^*	ΔL^*	Δa^*	Δb^*	ΔE^*
BG 2 %	Control ^a	37.87	-0.31	5.76	-	-	-	-
	Co+Ag NPs	37.56	-0.18	6.49	-0.31	0.13	0.73	0.80
	Co+RB	38.30	-0.30	5.75	0.43	0.01	-0.01	0.44
BG 4 %	Control	29.62	-0.23	5.09	-	-	-	-
	Co+Ag NPs	29.96	-0.26	5.61	0.34	-0.03	0.52	0.62
	Co+RB	30.30	-0.34	5.13	0.68	-0.10	0.04	0.69
BO 2 %	Control	43.38	-44.32	-4.06	-	-	-	-
	Co+Ag NPs	43.31	-42.81	-3.25	-0.07	1.51	0.81	1.71
	Co+RB	43.87	-44.53	-4.16	0.48	-0.22	-0.09	0.54
BO 4 %	Control	34.66	-42.08	-3.21	-	-	-	-
	Co+Ag NPs	34.24	-39.55	-2.00	-0.42	2.53	1.21	2.83
	Co+RB	35.18	-42.42	-3.40	0.52	-0.34	-0.19	0.65

^aDyed fabric without Ag NPs or RB

The antibacterial activity of the Co fabrics was tested against the Gram-negative bacterium *E. coli* and the Gram-positive bacterium *S. aureus*. The Co fabrics dyed with BG and loaded with Ag NPs reached the maximum bacteria reduction (Table II). A similar behavior was exhibited by the Co fabrics modified with RB, although a few bacterial colonies grew in the Petri dishes, leading to a decrease in the reduction of *S. aureus* to 99.8 % in the case of the sample that was dyed with 4 % BG.

The values of bacteria reduction by the Co fabrics dyed with BO and loaded with Ag NPs or RB are given in Table III. Again, the Co fabrics loaded with Ag NPs provided the maximum bacteria reduction, irrespective of the BO dye concentration and bacteria type. The Co fabrics loaded with RB showed good antibacterial efficiency, but the maximum reduction of *S. aureus* bacteria was achieved only in the case of Co fabric dyed with 4 % BO.

The obtained results indicated that both colloidal Ag NPs and RB could be used as efficient antibacterial agents for Co fabrics. They also confirmed that both forms of silver (nanoparticles and ions) exhibited excellent bactericidal pro-

erties. However, the mechanism of bactericidal action of Ag NPs is not yet fully understood. One group of scientists supports the assumption that Ag NPs in an aqueous medium release silver ions which are responsible for killing the bacteria.^{21–24} Another approach relies on the finding of Morones *et al.* who detected Ag NPs attached to the cell membrane and inside the bacteria.²⁵ It is very likely that Ag NPs react with sulfur rich proteins in the bacteria cell membrane and the interior of the cell or with phosphorous-containing compounds such as DNA.^{26,27} Accordingly, the morphological changes in the bacteria cell membrane and possible damage of DNA caused by reaction with Ag NPs disturb the respiratory chain or cell division processes, leading to a cell death.²⁵

TABLE II. Antibacterial efficiency of Co fabrics dyed with BG and loaded with Ag NPs or RB

Sample	Bacterium	Initial number of bacterial colonies, CFU	Number of bacterial colonies on the fabric, CFU	R / %
Control Co (2 % BG)	<i>E. coli</i>	6.4×10^5	2.3×10^5	–
Co+Ag NPs			<10	99.9
Co+RB			45	99.9
Control Co (4 % BG)		2.0×10^5	1.2×10^5	–
Co+Ag NPs			<10	99.9
Co+RB			<10	99.9
Control Co (2 % BG)	<i>S. aureus</i>	2.4×10^4	4.0×10^3	–
Co+Ag NPs			<10	99.9
Co+RB			<10	99.9
Control Co (4 % BG)		3.5×10^4	2.0×10^4	–
Co+Ag NPs			<10	99.9
Co+RB			60	99.8

TABLE III. Antibacterial efficiency of Co fabrics dyed with BO and loaded with Ag NPs or RB

Sample	Bacterium	Initial number of bacterial colonies, CFU	Number of bacterial colonies on the fabric, CFU	R / %
Control Co (2 % BO)	<i>E. coli</i>	2.3×10^5	2.2×10^5	–
Co+Ag NPs			<10	99.9
Co+RB			2.8×10^2	99.8
Control Co (4 % BO)		8.0×10^5	2.5×10^5	–
Co+Ag NPs			<10	99.9
Co+RB			1.4×10^2	99.9
Control Co (2 % BO)	<i>S. aureus</i>	2.5×10^5	7.4×10^4	–
Co+Ag NPs			<10	99.9
Co+RB			30	99.9
Control Co (4 % BO)		2.3×10^5	6.7×10^4	–
Co+Ag NPs			<10	99.9
Co+RB			<10	99.9

The slightly better antibacterial activity of the Co fabrics loaded with Ag NPs can be attributed to the larger amount of silver present, which was detected

by AAS (Table IV). The larger amount of silver that was found in the samples modified with Ag NPs can provide longer antibacterial activity during the exploitation of textile products. It is also clear that the silver content in the Co fabrics modified with RB was almost constant being independent of the applied dye and the dye concentration. On the contrary, the total amount of silver in the Co fabrics loaded with Ag NPs varied with the concentration of the dyes and was larger in the Co fabrics that were dyed with the higher concentration of the dyes (4 %), indicating the existence of some interaction between the dye molecules and Ag NPs.

TABLE IV. Initial silver content and total silver released into artificial sweat

Sample	Initial silver content $\mu\text{g g}^{-1}$	Silver released into	Silver released into
		artificial sweat, pH 5.5 $\mu\text{g g}^{-1}$	artificial sweat pH 8.0 $\mu\text{g g}^{-1}$
Co+BG 2 %+Ag NPs	31.97	13.15	16.35
Co+BG 4 %+Ag NPs	52.41	12.83	18.63
Co+BO 2 %+Ag NPs	32.15	12.40	16.00
Co+BO 4 %+Ag NPs	63.67	12.93	17.35
Co+BG 2 %+RB	18.84	12.48	17.78
Co+BG 4 %+RB	17.36	12.20	16.33
Co+BO 2 %+RB	18.20	12.10	17.93
Co+BO 4 %+RB	18.75	12.13	18.58

The stability of the investigated systems is of major interest for the exploitation and maintenance of textile products. Therefore, the stabilities of the differently modified Co fabrics were examined in artificial sweat. Namely, the composition of sweat varies between individuals. However it also depends on the body region, age, season, diet, degree of acclimation, infection status and level of activity.²⁸ Hence, the stability of modified Co fabrics was tested in alkaline and acidic artificial sweat. AAS measurements of sweat solutions after 24 hours of contact with the Co fabrics modified with Ag NPs or RB revealed that silver was released. The amounts of silver released from the Co fabrics into artificial sweat are shown in Table IV. Obviously, all the investigated samples released almost the same amount of silver into sweat, independent of the dye type and concentration, as well as of the silver form applied. However, approximately 39 % more silver was released into alkaline sweat (pH 8.0) compared to acidic sweat (pH 5.5). Although the Co fabrics modified with Ag NPs and RB showed equivalent trends of silver release, a larger amount of Ag was retained in the Co fabrics loaded with Ag NPs.

Additionally, the color fastness of Co fabrics in artificial sweat was studied. The data on color change and color fastness of the Co fabrics loaded with Ag NPs and RB after exposure to acidic and alkaline sweat for 24 h are given in Table V. The color fastness was evaluated in accordance with the ISO 105-A05

standard and the AATCC standard.^{29,30} The color changes after 24 h of rinsing in artificial sweat could not be visually detected as the color difference (ΔE^*) was lower than one. The only exception was the Co fabric dyed with 4 % BO and loaded with Ag NPs that was exposed to artificial sweat at pH 8.0. All samples exhibited good color fastness since the color fastness grades ranged from 4–5 according to both standards.

TABLE V. Color change of Co fabrics loaded with Ag NPs and RB after 24-h exposure to acidic and alkaline sweat

Sample	ΔL^*	ΔE^*	Color fastness, ISO A05	Color fastness, AATCC
pH 5.5				
Co+BG 2 %+Ag NPs	0.30	0.71	4–5	4–5
Co+BG 4 %+Ag NPs	0.06	0.43	4–5	4–5
Co+BO 2 %+Ag NPs	0.30	0.98	4–5	4–5
Co+BO 4 %+Ag NPs	0.55	0.88	4–5	4–5
Co+BG 2 %+RB	0.52	0.55	4–5	4–5
Co+BG 4 %+RB	0.26	0.34	5	5
Co+BO 2 %+RB	0.59	0.94	4–5	4–5
Co+BO 4 %+RB	0.49	0.70	4–5	4–5
pH 8.0				
Co+BG 2 %+Ag NPs	0.25	0.62	4–5	4–5
Co+BG 4 %+Ag NPs	–0.30	0.43	4–5	4–5
Co+BO 2 %+Ag NPs	0.09	0.69	4–5	4–5
Co+BO 4 %+Ag NPs	0.79	1.64	4	4
Co+BG 2 %+RB	–0.41	0.44	4–5	4–5
Co+BG 4 %+RB	–0.04	0.10	5	5
Co+BO 2 %+RB	0.22	0.73	4–5	4–5
Co+BO 4 %+RB	0.28	0.87	4–5	4–5

CONCLUSIONS

Cotton fabrics dyed with the vat dyes Bezanthren Olive T and Bezanthren Grey FFB and subsequently loaded with synthesized colloidal silver nanoparticles exhibited excellent antibacterial activity, reaching the maximum possible bacterial reduction (Gram-negative bacterium *E. coli* and Gram-positive bacterium *S. aureus*). Although the dyed Co fabrics modified with commercial RUCO-BAC AGP agent with silver chloride as an active component also provided good antibacterial properties, the maximum bacteria reduction was not always achieved. The better antibacterial properties of the Co fabrics modified with the silver nanoparticles could be due to the larger amount of silver in these samples, which was confirmed by atomic absorption spectroscopy. The larger amount of silver in these Co fabrics could provide longer antibacterial activity during the exploitation. The treatment with RUCO-BAC AGP did not significantly affect the color of the cotton fabrics. However, the cotton fabrics dyed with Bezanthren Olive T

underwent considerable color change after the treatment with colloidal silver nanoparticles.

The results also indicated that silver was released from the cotton fabrics during 24-h long exposure to sweat at pH 5.5 and 8.0. All the studied samples released almost the same amount of silver into the sweat, independent of the dye type and concentration, as well as of the silver form applied. However, about 39 % more silver was released into the alkaline sweat compared to the acidic sweat. The color fastness of the cotton fabrics exposed to sweat was satisfactory.

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ИЗВОД

ИСПИТИВАЊЕ АНТИБАКТЕРИЈСКЕ АКТИВНОСТИ И СТАБИЛНОСТИ ОБОЈЕНЕ ПАМУЧНЕ ТКАНИНЕ МОДИФИКОВАНЕ РАЗЛИЧИТИМ ФОРМАМА СРЕБРА

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У овом раду се пореде ефекти колоидних наночестица сребра и комерцијалног анти-микробног агенса RUCO-BAC AGP са сребро-хлоридом као активном компонентом на анти-бактеријску активност бојене памучне тканине. Памучне тканине су бојене редуционим бојама *Bezanthren olive T* и *Bezanthren green FFB*. Антибактеријска активност сребра је тестирана према Грам-позитивној бактерији *Staphylococcus aureus* и Грам-негативној бактерији *Escherichia coli*. За разлику од RUCO-BAC AGP, синтетисане наночестице сребра депоноване на обојену памучну тканину су обезбедиле максимум бактеријске редуkcије независно од примењене боје. Стабилност модификованих памучних тканина је анализирана у вештачком зноју при рН 5,5 и 8,0. Готово иста количина сребра се ослобађа са различито модификованих памучних тканина у вештачком зноју. Већа количина сребра се ослобађа у зноју при рН 8,0.

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