ANTIBACTERIAL PROPERTIES OF MEMBRANES MODIFIED BY ACRYLIC ACID WITH SILVER NANOPARTICLES

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Introduction

Antibacterial properties of different forms of silver have been known for centuries. Also silver nanoparticles have many interesting properties such as: electrical, optical, catalytic oxidation, and antimicrobial [1 – 3]. It is proved that silver nanoparticles in aqueous solutions release silver ions, which are also biologically active [3 – 4]. Silver ions can destroy bacterial cell by interacting with proteins (for example thiol groups form cysteine, forming Ag–S bond), which can result in inactivation of respiratory enzymes. Also they can prevent the DNA replication and may have an impact on the membrane cell structure and permeability [2 – 10]. It is believed that silver nanoparticles can increase permeability of the cell membrane due to their incorporation into the membrane structure, which ultimately leads to release of the cell contents and cell death [3, 8, 9]. In conclusion it is suggested that the antibacterial effect of silver nanoparticles can be described by three main mechanisms:

- release of biocidal silver ions,
- direct impact on the cell membrane by silver nanoparticles,
- silver nanoparticles can generate reactive oxygen species [3].

Also it is postulated that immobilized silver nanoparticles may result in an easy–to–use disinfecting systems, with minimized danger of releasing to the environment silver nanoparticles [9]. Silver nanoparticles exhibit antibacterial properties against broad spectrum of bacteria, such as *Escherichia coli* [2, 4, 11], *Streptococcus sp* [8], *Psuedomonas sp* [8], *Listeria monocytogenes* [11], *Staphylococcus aureus* [4, 11], *Salmonella typhi* [4, 11], *Bacillus subtilis* [4], *Pseudomonas aeruginosa* [4], *Streptococcus mutans* [12].

Colloidal silver has been approved by the U.S. Environmental Protection Agency as a disinfectant agent in hospitals and medical centers [9]. Currently, there is no clear evidence that silver nanoparticles are toxic for humans. The only known negative impact to humans of high concentrations of silver ions in case of long term exposure is skin darkening [3, 6]. Also long term exposure to high concentrations of silver ions can cause a disease called *Argyria* (in case of some genetic determinants), which manifests by the blue–gray staining of skin [2].

Precipitation of silver nanoparticles by reducing agent is one of the most frequently used method for producing silver nanoparticles [4]. In case of this method silver ions derived from silver nitrate can be reduced by sodium borohydride. Also, if modified surface have carboxyl groups it is possible to form bond between silver ions and carboxyl groups. Depending on the concentration of used precipitant, different sizes of nanoparticles may be produced [13]. Also, in case of this modification, nanoparticles will be mostly placed on membrane surface. Another method of producing polymer membranes with nanoparticles, is adding prepared nanoparticles to spinning solution. Then mix matrix membranes are prepared by phase inversion. In case of this modification silver nanoparticles are trapped in the membrane structure. During continuous filtration performed on mix matrix membranes problem of biofouling was not reduced significantly. Most likely this was due to continuous leaching of silver ions from membrane structure. Also in this case most of the silver nanoparticles were trapped in the membrane structure and did not have a direct contact with bacteria. Recent studies postulate that silver nanoparticles should be placed on membrane/contamination boundary. This arrangement of nanoparticles will allow direct contact of biocide with bacteria, which can result in better performance of the process [9, 14].

Materials and method

Polypropylene capillary membranes (PP ACUREL®V8/2HF) produced by MEMBRANA GmbH were used as a substrate during modifications. Basic properties of PP membrane are presented in Table 1.

Table 1. Basic properties of polypropylene membranes produced by MEMBRANA GmbH [15].

Properties	MEMBRANA GmbH
Average pore size	0,2 μm
Burst pressure	> 8 bar
Implosion pressure	> 4 bar
Outer diameter	2,6 mm
Inner diameter	1,8 mm
UFC	2,0 $ml/barcm^2min$
Advancing contact angle	134,9°

Acrylic acid grafting to the membrane surface was performed by Fenton type reaction. This modification was performed to introduce carboxyl groups to the membrane surface. Grafting of acrylic acid by Fenton type reaction is a two-step reaction. In first step a polypropylene membrane is placed for 5 min in a 5 % solution of ethylene glycol dimethacrylate (EGDMA) and 3% solution of cumene hydroperoxide (CHP) in hexane. In the second step membranes were placed for 15 min in a solution containing of 0,1% iron chloride (II), 1% of ascorbic acid and appropriate amounts of acrylic acid (0,1; 0,5; 1; 5; 7; 10; 13; 20%). Ethylene glycol dimethacrylate is used as a crosslinking agent which aims to improve the bond between polypropylene and acrylic acid. Iron ions in the second step is oxidized during Fenton reaction, and then regenerated by ascorbic acid (Fig. 1.) [16].

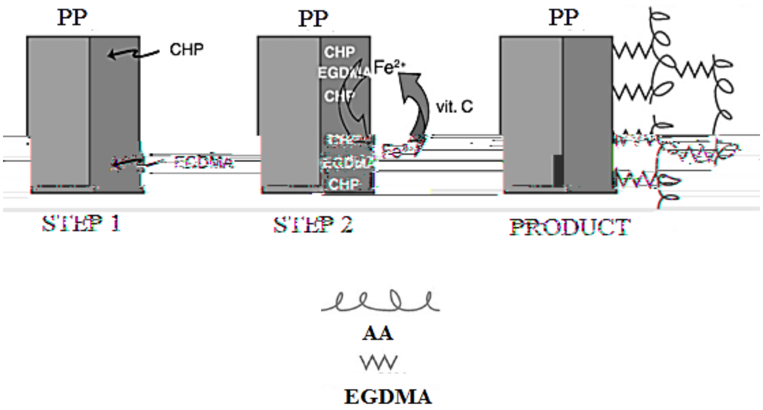

Fig. 1. Scheme of acrylic acid grafting by Fenton type reaction [16].

Synthesis of silver nanoparticles on the membrane surface with carboxyl groups is also a few step process. As a first step pre modified membranes were placed in 0,01 M sodium hydroxide (to convert –COOH groups into –COO$^-$). Then, membranes were placed in a 0,01 M solution of silver nitrate for 15 min. In this step, silver ions were most probably connected with –COO$^-$. Reduction of silver ions to metallic silver occurred thanks to $NaBH_4$. Concentration of sodium borohydride was few times greater than the concentration of silver salt. Modification method was developed on the basis of Mulinger et al. [17]. Scheme of silver nanoparticles synthesis on pre modified membrane surface is presented in Fig.2.

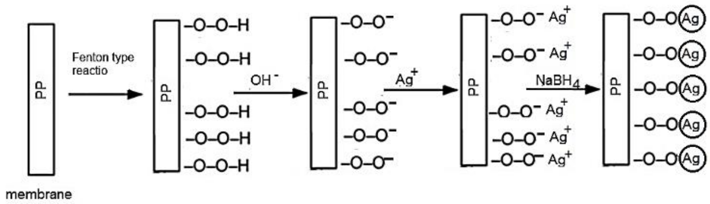

Fig. 2. Scheme of silver nanoparticles synthesis on membrane modified by acrylic acid [based on 18].

FT – IR analysis was performed on a NICOLET 6700 (wave number range from 400 to 4000 cm^{-1}). Thanks to this analysis it was possible to confirm presence of groups characteristic for acrylic acid on the membrane surface.

Plate test for antibacterial and bacteriostatic properties of modified membranes was performed. Membranes were placed vertically in LB (Lysogeny Broth) medium with 1 % of agar, and culture of *Escherichia coli* or *Bacillus subtilis*. Then plates were incubated in 37 °C for 24h. Zone of growth inhibition present near modified material proclaim about it antibacterial properties.

Also test in liquid medium was performed. To asses antibacterial properties of modified membranes, measurement of optical density (OD_{550}) for LB broth after 24 h of incubation with bacteria (*Escherichia coli* and *Bacillus subtilis*) was performed. Adequate number of membranes were placed in 8 ml of inoculated LB broth. Cultures were then incubated in 37 °C for 24 h.

Results

In order to confirm presence of functional groups characteristic for acrylic acid FT–IR analysis was performed. In Fig. 3. spectra obtained for membranes modified with different concentrations (0,1; 0,5; 1; 5; 7; 10; 13; 20%) of acrylic acid are presented. In case of all modified membranes new peaks appeared. First peak appears in the 3500 – 3000 cm^{-1} region responsible for –O–H bond. Further in the range of 1760 – 1650 cm^{-1} appears peak responsible for carbonyl groups. Also peak in the 1550 – 1610 cm^{-1} area is responsible for the carbonyl group, but most likely it was derived from carboxylates. In addition peak 1100 cm^{-1} is responsible for C–O– bond, which is probably responsible for ester groups.

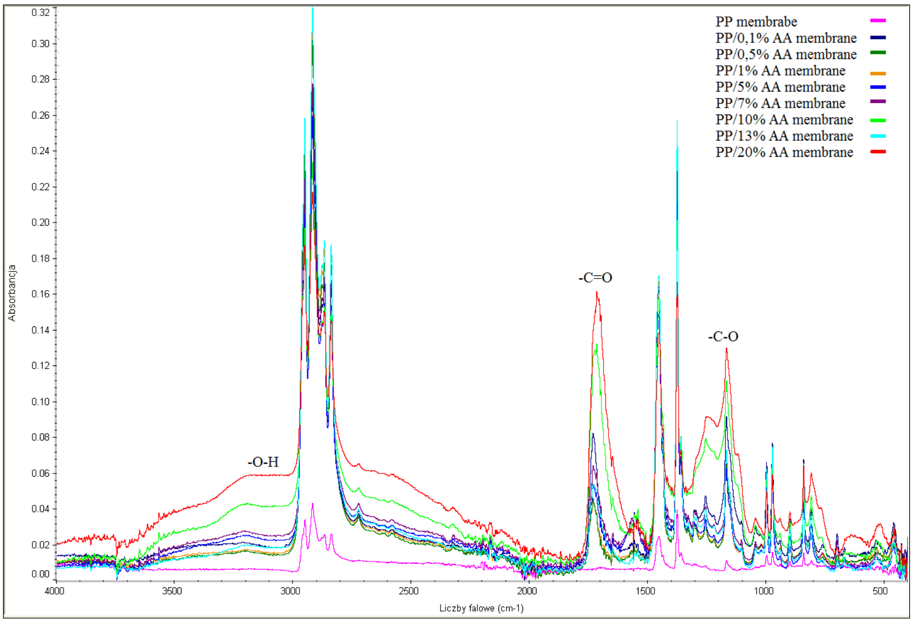

Fig. 3. FT–IR spectra obtained for polypropylene membrane and membranes modified with 0,1; 0,5; 1; 5; 7; 10; 13; 20% acrylic acid.

As it is clearly presented, in case of all modifications presence of carboxyl groups was confirmed.

Membranes modified by acrylic acid with silver nanoparticles were then tested for antibacterial properties. Membranes modified by silver nanoparticles changed color from white to brownish and black.

First antibacterial/bacteriostatic test was performed in solid medium for two kinds of bacteria: *Escherichia coli* and *Bacillus subtilis*. Scan of two plates inoculated with gram positive and negative bacteria and then incubated in 37 °C for 24 h are presented in Fig. 4. Zone of growth inhibition (clear spot) around the membrane confirms antibacterial or bacteriostatic properties of modified material. In Fig. 4. are presented results for PP membrane and 0,1% AA, 0,5% AA, 1% AA, 5% AA, 10% AA and 20% AA membranes modified by silver nanoparticles. In case of plate inoculated by *Escherichia coli*, clear zone of inhibited growth can be observed for membranes modified by nanoAg and 10% of acrylic acid. In case of other modifications, small clear zones were visible, however it was impossible to show it in this picture.

In case of plate inoculated by *Bacillus subtilis*, clear zone of inhibited growth appeared similarly as in the case of *Escherichia coli* for membrane with nanoAg and 10% of acrylic acid. Additionally, in case of membranes modified by 0,1; 1; 5 and 20% of acrylic acid and silver nanoparticles, zone with5 and 20%ilver modificar moal-380(rtiolor c)3(que)7(s

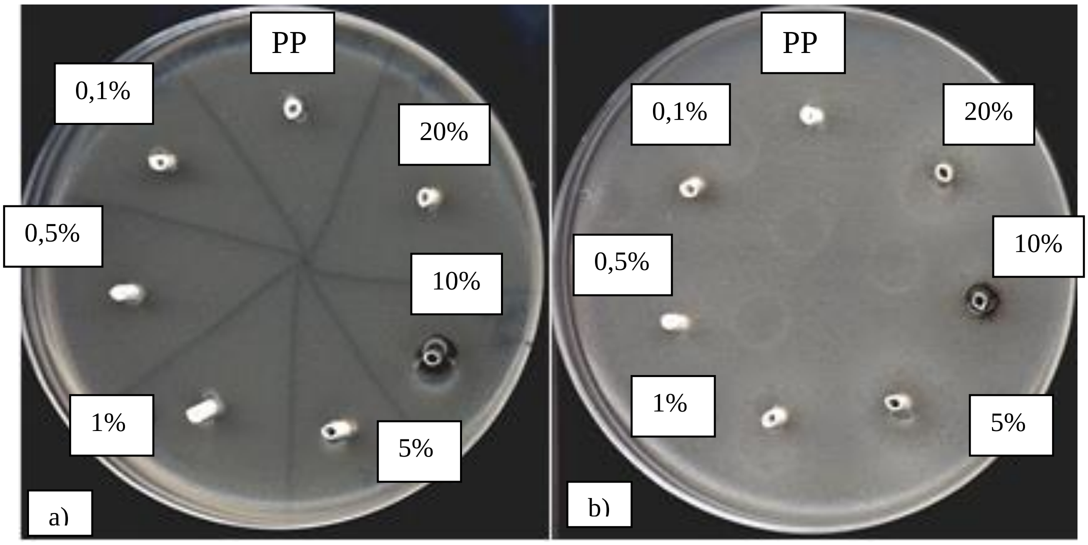

Fig. 4. Plates inoculated by a) *Escherichia coli*; b) *Bacillus subtilis* with PP and modified membranes then incubated for 24 h at 37 °C.

Scan of two plates inoculated with *Escherichia coli* (on differentiating medium) and *Bacillus subtilis* and incubated in 37 °C for 24 h are presented in Fig. 5. Presented results were obtained for PP membranes and 5% AA, 7% AA, 10% AA and 13% AA membranes modified by silver nanoparticles. In case of *Escherichia coli* growth inhibited zones can be observed for membranes modified by 10 and 13 % of acrylic acid and nanoAg. Also minor clear zones are visible for membranes modified by 5 and 7% AA and silver nanoparticles.

In case of plates inoculated by *Bacillus subtilis* clear zones of inhibited growth are visible for membranes modified by 10 and 13 % of acrylic acid and nanoAg.

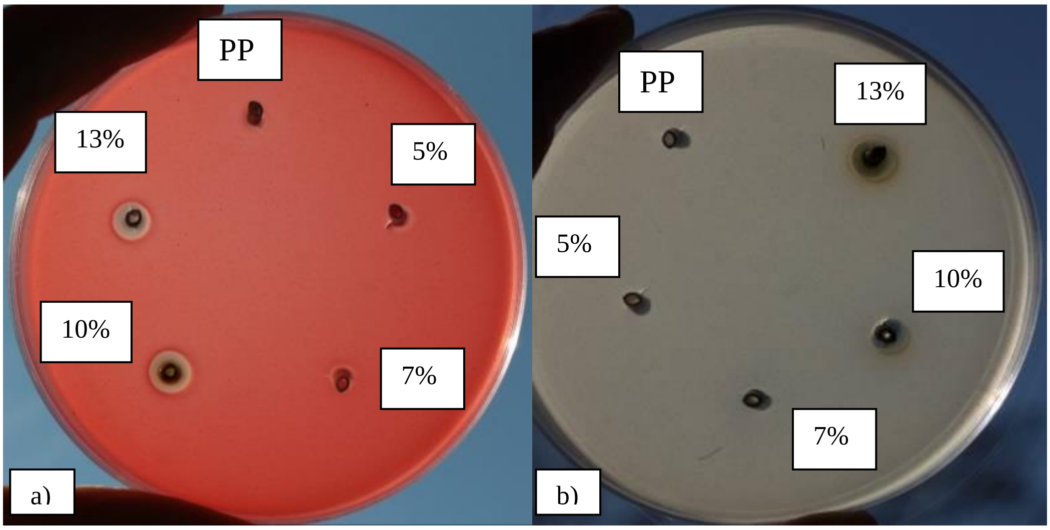

Fig. 5. Plates inoculated with a) *Escherichia coli*; b) *Bacillus subtilis* and PP and modified membranes then incubated for 24 h at 37 °C.

Also antibacterial test in liquid medium was performed. In case of this experiment LB broth was inoculated by *Escherichia coli*. Initial OD_{550} of culture was 0,1, then culture was incubated with membranes for 24 h at 37 °C. After 24 h of incubation OD_{550} was measured once again. Increase of this parameter indicated microorganism growth. Also it is imported to stress out that this test do not differentiate life and dead bacteria. Only in case of cell lysis the reduction of OD_{550} will be observed. Graph presenting antibacterial effect of modified membranes is presented in Fig. 6. The biggest bactericidal effect was obtained for membranes modified by 10% of acrylic acid with silver nanoparticles. Then, in the order of antibacterial properties will be membrane modified by 0,1 %; 0,5%; 20%;1% and 5% acrylic acid with silver nanoparticles. In case of this method of modification growth of AA concentration does not go in pair with growth of number of free carboxyl groups. Also antibacterial effect is strictly connected with amount of silver nanoparticles. Which is connected with number of free carboxyl groups present on the membrane surface.

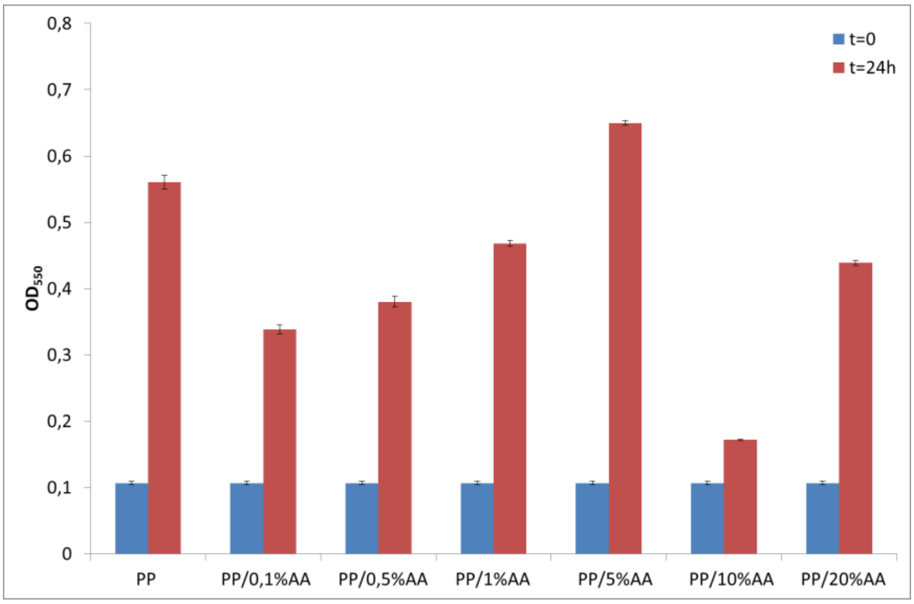

Fig. 6. Effect of acrylic acid concentration on antibacterial properties of membranes with immobilized silver nanoparticles on *Escherichia coli*.

The best result were obtained for membrane modified by 10% of acrylic acid and silver nanoparticles. However, that the test does not distinguish dead cells from alive. Only in case of membranes modified by 10% AA/nanoAg cfu test was performed. For modified membranes there was ~10^{-2} cfu/ml, but for polypropylene membrane ~$3\cdot10^{-8}$ cfu/ml.

In case of next experiment in liquid media, LB broth was inoculated by *Bacillus subtilis*. Initial OD_{550} of culture was 0,1, then culture was incubated with membranes for 24 h at 37 °C. After 24 h of incubation OD_{550} was measured once again. However, in the case of this experiment, bacterial cultures were infected, most likely it was fungal infection. Nevertheless, it was decided to run this experiment and see how modified membranes cope with multicultural culture. Graph presenting antibacterial effect of modified membranes on infected *Bacillus subtilis* culture is presented in Fig. 7. Antibacterial properties were observed only in case of membrane modified with 10% acrylic acid with nanosilver. In case of other modifications no antibacterial effect was observed.

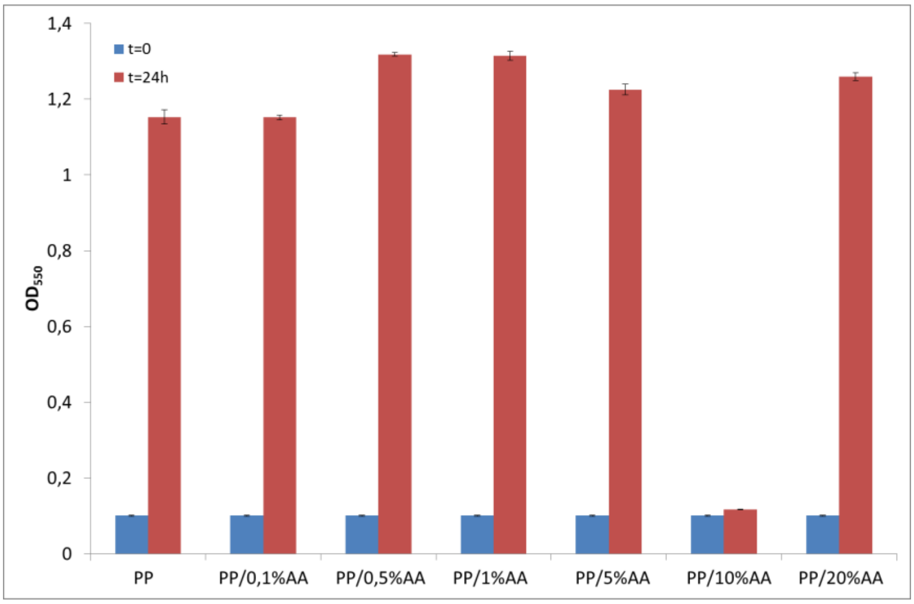

Fig. 6. Effect of acrylic acid concentration on antibacterial properties of membranes with immobilized silver nanoparticles on *Bacillus subtilis*.

Based on obtained data, it can be concluded that membranes modified with 10% of acrylic acid and silver nanoparticles have strong antibacterial properties. Also membranes modified by 10% acrylic acid with silver nanoparticles exhibited antibacterial properties against gram positive and negative bacteria both in solid and liquid medium.

Conclusions

It is possible to modify membrane by acrylic acid by Fenton type reaction. Thanks to this modification free carboxyl groups were introduced to the membrane surface. Also presence of free carboxyl groups allowed to form bond between silver nanoparticle and membrane surface.

In the next stage the membrane modified work were subjected directly modifying the silver nanoparticles . Such modified membranes were tested in a bactericidal manner analogous to the previous modification. The best results , as in the case of the acrylic acid grafting was obtained for 10 % of the membrane modified with acrylic acid nanoAg . This membrane had a strong bacteriostatic / bactericidal against both solid-phase Escherichia coli and Bacillus subtilis . Similarly, in a liquid medium displayed a significant decrease in the content of micro-organisms after 24 h of culture in the case of both types of bacteria. It is also worth noting that in the case of Bacillus subtilis cultures infected membrane modified with 10 % of acrylic acid nanoAg was able to reduce the number of microorganisms , including those derived from infections , fungal likely .
As the results of this study it has been possible to modify the surface so as to give it additional properties reactive . The membranes have become not only a physical barrier , but also the reactive barrier which in recent years has become a new paradigm membranes. Based on the obtained results it can be concluded that examined issues require further study and evaluation mainly filtration processes , as well as scale-up in case of modification processes . In the case of plasma activation is an area seldom used for the modification of polymers , in particular membranes and still requires a lot of research , however, presented results allow to look optimistically to such modifications . At the same time , zinc oxide modifications seem to be very promising for both photocatalytic properties and bactericidal . Would be worth developing further research in this area , so as to understand the capabilities and limitations of

this type of membranes. According to the author not worth continuing research on the modification of the manganese oxide nanoparticles and commercial silver nanoparticles , at least in the field of microfiltration membrane . Very interesting proved to opportunities offered by the membrane grafted with acrylic acid and, depending on the concentration used during the modification they can find different applications . As in the previous modifications , and such membranes, acrylic acid modified acrylic acid and silver nanoparticles , it is necessary to continue research in order to discover the capabilities and limitations of such modified membranes.

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