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New biocompatible polypyrrole-based films with good blood compatibility and high electrical conductivity

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ABSTRACT

A novel *O*-butyryl chitosan (OBCS)-grafted polypyrrole (PPy) film was described. The immobilization was accomplished by photocrosslinking the OBCS onto PPy films under ultraviolet light irradiation. The surfaces of OBCS-grafted PPy film were characterized by attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy and electron spectroscopy for chemical analysis (ESCA). The blood compatibility of the OBCS-grafted PPy film was evaluated by platelet-rich plasma (PRP) contacting experiments and protein adsorption experiments in vitro. These results have demonstrated that the surface with immobilized OBCS shows much less platelet adhesive and fibrinogen adsorption compared to the control surface. The bulk conductivity values of PPy films were measured by a modified four-probe method. The composite films have both good blood compatibility and high electrical conductivity that make them suitable for using as potential biomaterials, such as electrically conducting blood vessel and functionally haemocompatible substrate of biosensor used directly in whole blood.

1. Introduction

Current trend in the development of biomaterials is towards bioartificial polymeric materials. Considerable effort has been made to explore the possibility of obtaining new or better properties from a combination of biopolymer and synthetic polymer [1–5]. Conducting polymers have been the subject of much interest, not only from a fundamental scientific interest but also from a practical point of view, such as various functional applications including solar cells, separation membrane, molecular electronic devices, and biosensing devices. Among the class of conducting polymers, polypyrrole (PPy) takes up the running. Various approaches to hybridize PPy with other materials have been attempted to develop new biomaterials that are full of promise for improving biocompatibility and offering good prospects for more widespread use of synthetic biomaterials [6–8]. Zambonin and co-workers demonstrated that PPy films, modified by covalent attachment of a peptide containing an arginine-glycine-aspartic acid sequence, showed a markedly enhanced bioactivity with respect to osseointegration [6]. Serge and Arielle provide preliminary results that illustrate the potential applications of electrogenerated conducting PPy films functionalized by biotin groups for the fabrication of biosensors [7]. Khor et al. employed the chemical polymerization of pyrrole in the presence of chitosan to obtain chitosan–PPy hybrid biomaterials, but the hybrid did not give a detectable electrical signal [8]. Collier et al. reported PPy–hyaluronic acid bilayer biomaterial showing its conductive, noncytotoxic, and antiogenic properties [9], and Wallace and co-workers reported the synthesis of PPy/heparin composites by electrochemical polymerization on a quartz crystal electrode but such composite polymers were still active in binding thrombin [10]. Jakubiec et al. investigated cellular response to PPy-coated woven polyester fabrics in vitro, possibly for its potential benefits of electrical conductivity [11].

Prospect of the artificial blood vessels made of PPy that have been modified by the addition of new surfaces to create good biocompatibility and electrical performance, as they can be used to monitor a wide range of medically important analytes, such as triglyceride in whole blood, and the status of blood flow that are very important for health condition of an organism [12,13]. The mechanism of blood coagulation and thrombus formation in the cardiovascular system have been considered in connection with properties of vascular wall, blood components, and blood flow [14].

Chitosan, a (1→4)-linked 2-amino-2-deoxy-β-D-glucan, has both reactive amino and hydroxyl groups, which can be used to chemically alter its properties under mild reaction conditions.

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From such chemical properties, many interesting chitosan derivatives were reported especially for biomedical applications [15–17]. Although genuine chitosan is also known for its haemostasis property because of the positively charged nature, there are some chitosan derivates including *N*-acyl chitosans, *O*-diacetyl chitosan, and *O*-butyryl chitosan (OBCS) have already been reported as blood compatible materials [18–22]. OBCS can be considered to provide an inert surface against thrombus formation and blood coagulation [20,21]. This simple modification of existing biomaterials to endow newly prepared biomaterials with specific properties has an advantage of improving biocompatibility without altering the bulk properties of the biomaterials [23].

Even though PPy is widely studied in the medical field, it is still prone to initiate the formation of clots in which platelets and other components of the blood coagulation system are activated in direct contact with blood [22]. By way of a tryout for preventing the formation of clots in this work, we chose OBCS to modify the surface of PPy due to the good blood compatibility of OBCS. Considering the chemical stability of PPy, we employed a novel surface modification technique via the covalent immobilization of OBCS with a photosensitive hetero-bifunctional crosslinking reagent, 4-azidobenzoic acid. This modification method has led us to develop new composite films derived from the crosslinking between the modified chitosan and PPy, and what is more, the films showed high electrical conductivities.

2. Materials and methods

2.1. Materials

Chitosan powder was obtained from Lianyungang Biologicals Inc., China. Its viscosity average molecular weight was 6.7×10^5 g/mol, while the degree of deacetylation was 90%. Pyrrole was obtained from Fluka and doubly distilled prior to use. All other chemicals were of reagent grade and were used without further purification.

2.2. Preparation of 4-azidobenzoate-modified O-butyryl chitosan (Az-OBCS)

A Az-OBCS solution was prepared according to the literature [20]. *O*-Butyryl chitosan (OBCS) (1 g, powder), (1-ethyl-3-(3-dimethylaminopropyl))carbodiimide (EDC) (0.35 g), and 4-azidobenzoic acid (0.2 g) were dissolved in 100 ml methanol. The mixture was stirred at room temperature for 8 h and then concentrated. The product was precipitated by pouring the mixture into acetone (100 ml), and then filtered. The solid was washed for 18 h with methylene chloride to remove the unreacted azide compound completely. After filtration, the solid product was vacuum-dried. The product was dissolved in water to prepare 0.1% Az-OBCS solution.

2.3. Preparation of PPy films

The preparation of PPy films was performed by using a potentiostat (Model 273A, EG & G, Princeton Applied Research, USA), and the electrochemical synthesis of PPy films was performed by galvanostatical electrodeposition on the rough stainless steel substrate in a deionized water solution containing freshly distilled pyrrole (0.1 M) and *para*-toluene sulphonic acid (PTSA, 0.1 M) at the current density of 1 mA/cm^2. The thickness of the prepared PPy films is approximately 0.008 mm.

2.4. Immobilization of Az-OBCS onto PPy films to prepare composite films

The composite films were prepared via a combination of the electrochemical polymerization and photochemical grafting reaction. The 0.1% Az-OBCS solution was cast on a PPy film surface and dried in a brown-colored desiccator. The films were irradiated by a mercury lamp (8 W, 254 nm UV-tube light, Shanghai photoelectric apparatus Inc., China) for 2 min, unbounded Az-OBCS molecules were rinsed with water completely and then dried. Here named it Az-OBCS-g-PPy films (g means grafting). Preparation of another group of PPy films with the procedure is according to the same steps as before with except irradiating, and named it Az-OBCS-c-PPy films (c means coating).

2.5. Characterization

Attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR, Nicolet 170sx, Bruker, Germany) and electron spectroscopy for chemical analysis (ESCA, V.G. ESCALAB MK II, VG, U.K.) were carried out to check if Az-OBCS had been immobilized on the PPy film surface.

2.6. Platelet-rich plasma (PRP) contacting experiments and protein adsorption experiments

There have been several in vitro methods to evaluate blood compatibility of biomaterials [24–26]. We employed platelet-rich plasma (Blood Center of Nanjing Red Cross, China) contacting experiments and protein adsorption experiments: (a) the unmodified PPy, Az-OBCS-c-PPy and Az-OBCS-g-PPy films were immersed, respectively in PBS first and contacted at 37 °C for 1 h with freshly prepared PRP of human blood. The platelet-attached surfaces were coated with gold prior to being observed by SEM (JSM Model 6300 Scanning electronic microscopy, JOEL, Japan). For SEM, six same specimens were observed and that the presented images are representative; (b) in the present work, adsorption of bovine fibrinogen (BFG, F-8630, Sigma, USA) in vitro was employed to evaluate the haemocompatibility of PPy, Az-OBCS-c-PPy and Az-OBCS-g-PPy surfaces. Quantification of protein adsorbed on the polymer surfaces was performed using ^{125}I-labelled protein. The samples were immersed into 1 ml PBS (pH 7.4) at 37 °C, and then 1 ml labelled fibrinogen solution (0.2 mg/ml) was added and mixed. Adsorption tests were carried out at 37 °C for 1 h. After protein adsorption, samples were rinsed three times with 2 ml of butter. The gamma activities were counted with the samples placed in radioimmunoassay tubes by a Gamma Counter (FH-408, Beijing Nucleus Instrument Factory, China). Six replicates were used and evaluated.

2.7. Measurement of electronic conductivity of films

To avoid that the probes in standard four-probe method may well penetrate thin chitosan coating and direct contact with underneath PPy, the bulk conductivity values of PPy films were measured by a modified four-probe method (Department of Physics, Nanjing University, China).

3. Results and discussion

In the present study, a hybrid material was prepared by covalent bondings between OBCS and PPy. Azide compounds containing $-N_3$ groups are known to release N_2 molecules under UV irradiation and

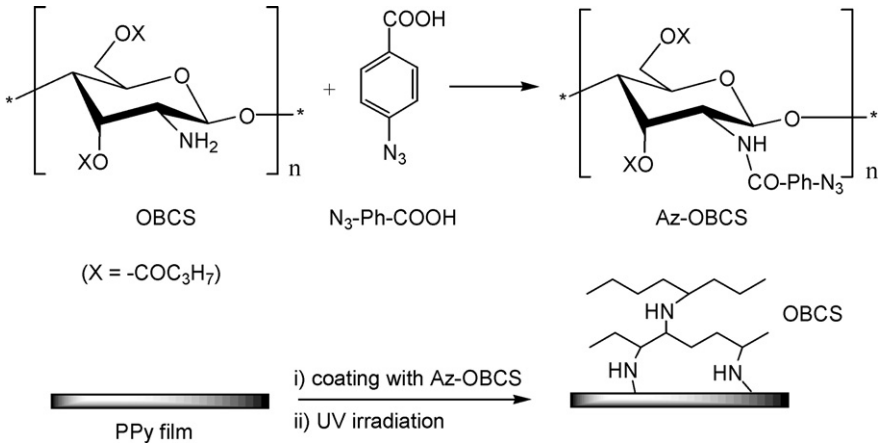

Fig. 1. Reaction scheme for the preparation of Az-OBCS and immobilization scheme of OBCS on a PPy film.

to be converted into a highly reactive nitrene group. The nitrene group can abstract the hydrogen atom from C–H bonds of substrates and then combined with the carbon radicals of the substrates. A derivative of *O*-butyryl chitosan bearing azide groups (Az-OBCS) was prepared by the reaction of OBCS with *para*-azidobenzoic acid. Then, the Az-OBCS after coated on a PPy film was cross-linked upon UV irradiation (Fig. 1) [27].

Fig. 2(a) shows FTIR spectrum of Az-OBCS, from which it could be seen that there was obvious absorption peak at 2126.2 cm^{-1}, which is the characteristic of –N3 [28]. This result confirmed the above conclusion that the azide group had been bonded on the OBCS molecules. The sharp peak at 1741.81 cm^{-1}, characteristic of the –C=O stretching vibration frequency of the amide group was also presented here. From Fig. 2(b), so many characteristic peaks of PPy were obtained. After crosslinked with Az-OBCS, the IR spectrum of the Az-OBCS-g-PPy film showed a sharp band at 1735 cm^{-1}, characteristic of the –C=O stretching vibration frequency of the amide group in the Az-OBCS-grafted PPy film (OBCS-g-PPy) (see Fig. 2(c)). With, those characteristic peaks of PPy with a little shift were also presented in the spectrum. All results indicate that Az-OBCS was photografted to the PPy film as expected.

The change in the chemical composition of the PPy film after modification was also investigated by ESCA analysis. The C 1s and N 1s peaks in the ESCA spectra for the unmodified PPy and modified OBCS-g-PPy films were shown in Fig. 3. The new peaks (C 1s and N 1s) appeared at the ESCA spectra of Az-OBCS-g-PPy in contrast with that of blank PPy surface (Fig. 3(b-1, b-2)). The C 1s peak for unmodified PPy could be resolved into two component peaks due to hydrocarbon (–C–C–C–) peak at 284.60 eV and conjugated carbon (–C–C=C–) peak at 286.79 eV (see Fig. 3(a-1)). After modified by Az-OBCS, the C 1s peak could be resolved into four component peaks due to hydrocarbon (–C–C–C–) peak at 284.60 eV, ester carbon (–COO–) peak at 285.75 eV, ether carbon (–C–O–C–) peak at 286.52 eV and amide (–NH–CO–) peak at 288.12 eV (see Fig. 3(b-1)). These new peaks presented all result from grafting of OBCS onto the surface of PPy film.

For the unmodified PPy film, the N 1s chart could reflect a combination of the contribution from two peaks at 399.70 eV and 401.19 eV that belong to the one- and two-dimensional structure of the nitrogenous carbon (–NH–C–C–), respectively [29] (see Fig. 3(a-2)). However, there is a new peak appeared at the N 1s spectrum of OBCS-g-PPy. The N 1s chart could reflect a combination of the contribution from the nitrogenous carbon (–NH–C–C–) at 399.17, 401.91 eV and amide peak (–NH–CO–) peak at 400.35 eV (see Fig. 3(b-2)). The latter peak at 400.35 eV is just attributed to

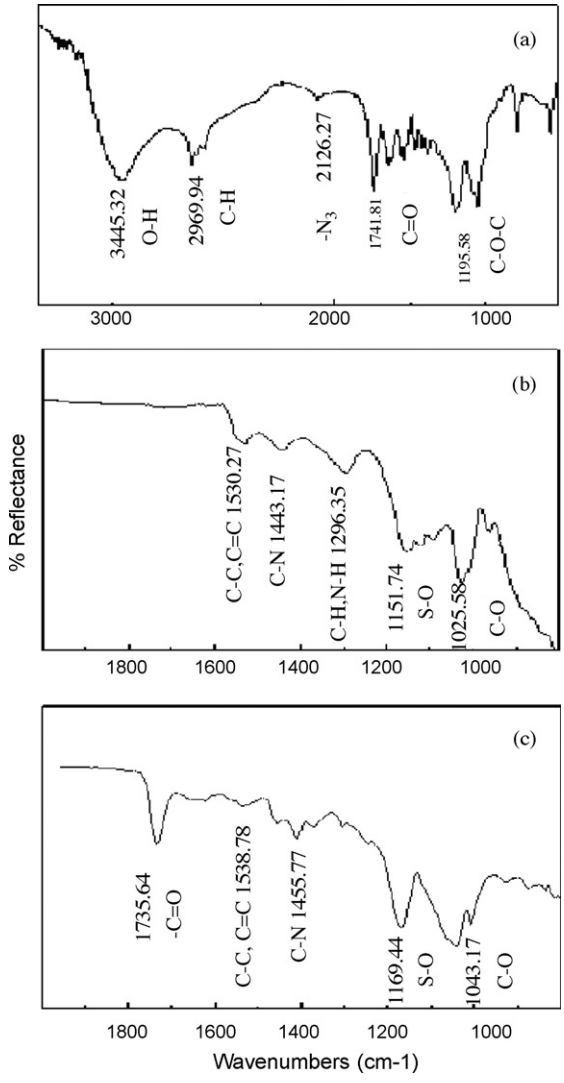

Fig. 2. FT-IR spectra of (a) Az-OBCS; (b) PPy and (c) Az-OBCS-g-PPy.

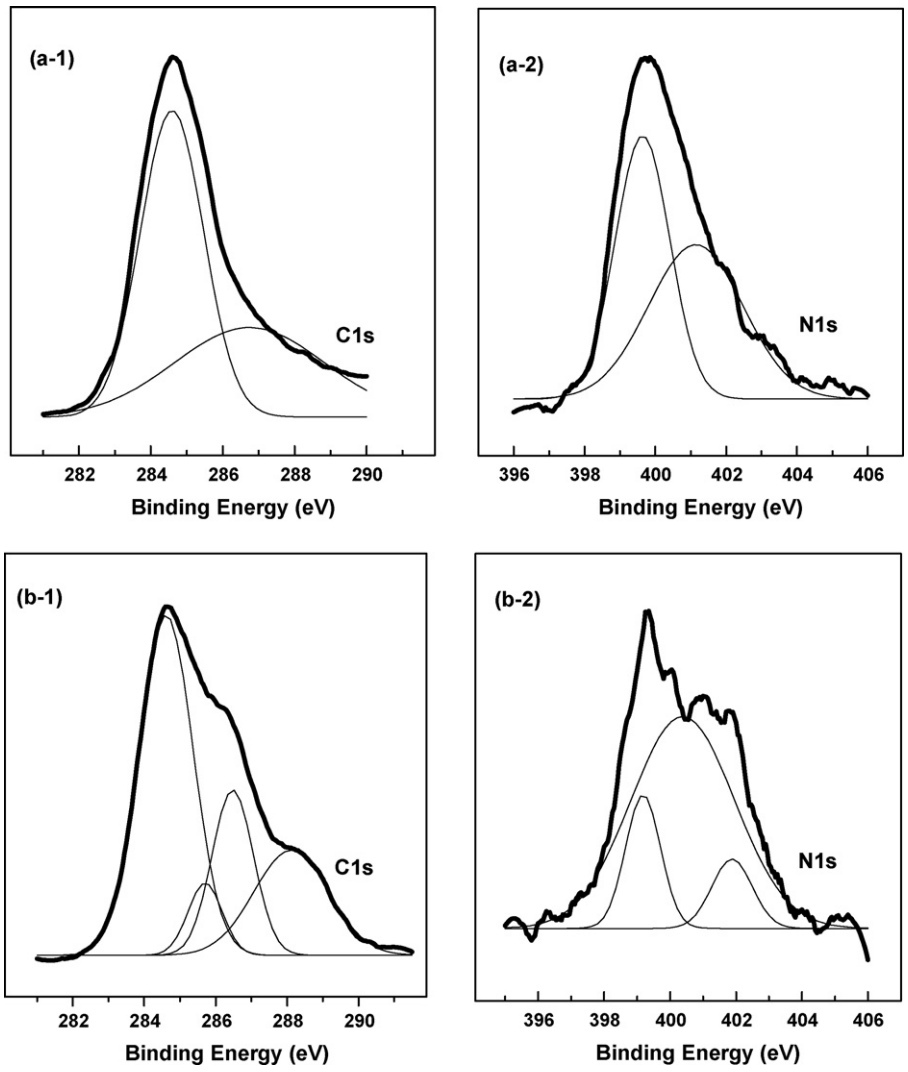

Fig. 3. Carbon 1s (C 1s) and Nitrogen 1s (N 1s) XPS spectra of (a-1 and a-2) PPy and (b-1 and b-2) Az-OBCS-g-PPy films.

OBCS. The ESCA results also indicate that OBCS has been successfully grafted onto the PPy surface.

The ESCA atomic composition of the PPy film was analyzed as follows: C 1s, 64.78%; O 1s, 21.63%; N 1s, 9.64%; and S 2p, 3.95%. However, the element content of the OBCS-g-PPy film was dramatically changed to C, 64.18%; O, 29.16%; N, 4.64%; and S, 2.02%, which is ascribed to the modified surface of the PPy film overlaid with grafted Az-OBCS chains. Taken together, the data indicated that Az-OBCS was well grafted to the surface of the PPy film.

In order to check biocompatibility of the modified film, the adhesion of human blood platelet was tested to the surfaces of PPy, Az-OBCS-c-PPy and Az-OBCS-g-PPy films (Fig. 4). The surface of the PPy film and Az-OBCS-c-PPy film showed high platelet adhesion, and most of the adhered platelets were distorted with pseudopodia. However, the surface of the Az-OBCS-g-PPy film had nearly no adhered platelet. PPy surface is rough and microporous, which may help the tight physical coating of chitosan as well. However, the results of PRP contacting experiments mean that there is poor adhesion strength of OBCS to PPy without covalent bonding. The platelet adhesion test revealed that the Az-OBCS-g-PPy film showed the superior bonding of OBCS to PPy and excellent anti-platelet adhesion. It is also clear that the improved antithrombogenicity should be attributed to OBCS [20,21].

The haemocompatibility of materials is generally considered to have relation to a protein adsorption process, because adsorbed proteins may trigger the coagulation sequence [30]. The adsorption of bovine fibrinogen onto the surfaces of PPy, Az-OBCS-c-PPy and Az-OBCS-g-PPy films in the BFG protein solution (0.1mg/ml) was measured as $2.827 \pm 0.083\ \mu g/cm^2$, $2.815 \pm 0.080\ \mu g/cm^2$ and $1.673 \pm 0.076\ \mu g/cm^2$, respectively. These results demonstrate that just the grafting of OBCS onto PPy decreases the fibrinogen adsorption to the film. From these data, the Az-OBCS-g-PPy film was further confirmed to offer the improved blood compatibility.

We also devoted our attention to the electronic conductivity of Az-OBCS-g-PPy films. The bulk conductivity values of unmodified PPy films were measured about 28.3 ± 0.3 S/cm by a standard four-probe method. Surprisingly, the conductivity of Az-OBCS-g-PPy films was measured about 27.5 ± 0.3 S/cm, which did not decrease significantly compared to that of the unmodified PPy films. Attack to pyrrole such grafting methods applied to other polymers were also not to affect the bulk properties and very thin layers prepared with the grafted polymers on substrate surfaces [31,32].

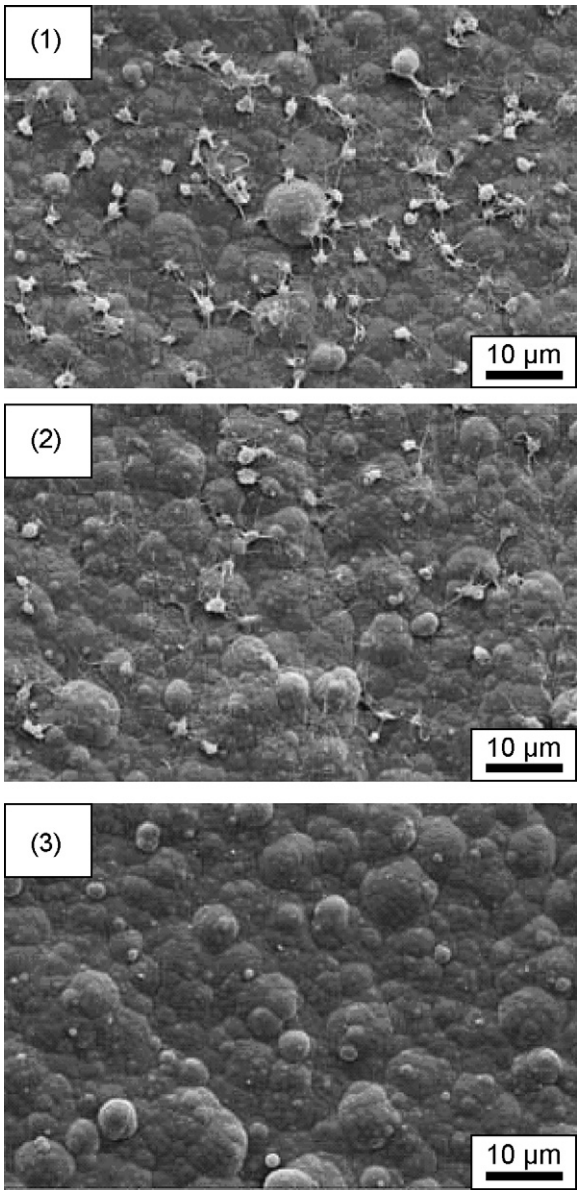

Fig. 4. SEM images of (1) unmodified PPy; (2) Az-OBCS-c-PPy; (3) Az-OBCS-g-PPy surfaces exposed to a PRP solution for 1 h.

4. Conclusions

In conclusion, the hybrid film of Az-OBCS-g-PPy prepared by photo-crosslinking shows good blood compatibility and high electrical conductivity. Such an approach to combine the respective advantages of OBCS and PPy and endow new features to hybrid materials could open a way to increase the scope and versatility of biopolymer-conducting polymer hybrid systems that provides new and interesting properties in future. For example, such materials have a realistic potential to solve the problems related to blood compatibility still placing major restrictions on the successful clinical application of intravascular sensors for long-term real-time monitoring [33]. Moreover, it can also be applied to a field to research the influence of a low potential on blood components in vitro. Utilizing the electrical property and haemocompatibility of the hybrid film of Az-OBCS-g-PPy developed in this study will be the focus of our future work to obtain more bio-applications.

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