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FULL PAPER

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Cu Nano particles immobilized on silk-fibroin as a green and biodegradable catalyst for copper catalyzed azide-terminal, internal alkynes cycloaddition

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Copper immobilized on silk fibroin was successfully prepared and fully characterized using powder X-ray diffraction, scanning electron microscopy–energy-dispersive X-ray spectroscopy, Fourier transform-infrared, CHN elemental analysis, and inductively coupled plasma-atomic emission spectroscopy. Catalytic activity of this catalyst was examined in the azide-alkyne cycloaddition reaction with internal and terminal alkynes at room temperature under mild conditions. The reusability of the heterogeneous supported Cu catalyst was examined four times without significant loss of catalytic activity.

KEYWORDS
azide-alkyne cycloaddition reaction, click reaction, copper-fibroin catalyst, ligand free

1 | INTRODUCTION

Over the past years, a variety of scientific and methodological development have been achieved, that encourage chemists to use these tools to more easily perform synthesis and related purification/separation processes.

Huisgen 1, 3-dipolar cycloaddition between organic azides and alkynes is one among many synthetic tools that has become quite well-known over the recent decade, mainly due to its key improvement in term of rate and regioselectivity.[1]

The prodigious synthetic potential of primary protocols for Huisgen 1, 3-dipolar cycloaddition reaction between organic azides and alkynes was limited by the significant disadvantages such as heating requirement, prolonged reaction time, and formation of structural isomers due to the lack of selectivity.[2] Wonderful Cu(I) catalyst modification introduced at the dawn of last decade, allowed the cycloaddition to occur at room temperature or with moderate heating leading to the exclusive formation of 1,4-disubstituted triazole with shorter workup and purification steps.[3,4] Now, this method has engulfed almost every part of chemistry and applied sciences.[5,6]

Until now, various catalytic systems, including homogeneous and heterogeneous, have been proposed and employed for copper(I) catalyzed azide-alkyne cycloaddition reactions with terminal and internal alkynes.[7–9] Some of these catalytic systems are Cu(I) salt; $CuSO_4$-ascorbate system[10]; immobilized Cu(I) onto: polymers,[11,12] zeolite,[13] and hydrotalcite,[7] nanomagnetic supported thiourea–copper(I),[14] two magnetic nanoparticle-supported copper(I)[15]; copper nanoparticles[16]; and nanostructured copper oxide.[17]

In catalytic systems, it has always been important to recover the catalyst from the reaction mixture and reuse it. Especially in the case of CuACC reactions which are commonly used for synthesis of substances that are related to biological application,[18] the complete separation of the catalyst from the reaction mixture is a matter of concern due to the toxicity of copper ions to cells and organisms.[19] This illustrates the need to use heterogeneous catalytic systems in which the catalyst is immobilized on a support. However, for homogeneous catalytic systems trace amounts of copper ions can be removed from the reaction mixture and isolated products, by washing with EDTA solution[20] but this procedure is not always convenient, especially in parallel or

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combinatorial systems. Immobilizing the copper catalyst on a heterogeneous support is one of the main strategies to circumvent the aforementioned problems. According to the principles of green chemistry and the need for minimal use of chemicals and the production of Eco-friendly catalysts, many researchers have focused studies on applying natural biopolymers, such as silk,[21,22] starch,[23] cellulose,[24] and chitosan,[8,25] as activated supports for metal particles. Many of the electron-rich functional groups in the protein bind with the metal surface easily, leading to the bioconjugation. The main characteristic of these biopolymers is their high amine group content, leading to interesting chelating properties for metal cations.[25–27] This situation eliminates the need to use surface activator ligands. Sometimes, the sensitivity of these ligands to temperature, moisture and the presence of oxygen, the tedious multistep synthesis, hence the high cost of the ligands, the use of various additives, and the need for an inert atmosphere during the synthesis of the catalyst as well as during the reaction, limits the use of these catalysts.[28–30] Also, uniform dispersion of amino groups leads to uniform dispersion of the synthesized nanoparticles immobilized on these supporting materials. In this paper, for the first time a copper nanoparticle immobilized on silk fibroin was prepared. The catalytic activity of this new heterogeneous catalyst investigated in one-pot synthesis of 1,2,3-triazoles by reaction of terminal and internal alkynes and azides at room temperature under mild conditions.

2 | EXPERIMENTAL

2.1 | General

All chemicals were purchased from Merck or Fluka and Aldrich Chemical Companies. Aryl azides **1a-d** were synthesized according to the procedure mentioned in Pokhodylo et al.[31] All yields refer to isolated products. The products were characterized by their spectral data. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded as $CDCl_3$ solutions with tetramethylsilane as an internal standard using a Bruker Avance spectrometer. Fourier transform infrared (FT-IR) spectra were recorded as KBr discs with a Nicolet Avatar 370 FT-IR spectrometer. Elemental compositions were determined through energy-dispersive X-ray spectroscopy (EDS) analysis (model 7353, Oxford Instruments UK), with a resolution of 133 eV. Inductively coupled plasma atomic emission spectroscopy (ICP-AES) analysis was carried out with a Varian VISTAPRO CCD (Australia). Powder X-ray diffraction (PXRD) patterns were obtained using a Bruker D4 X-ray diffractometer with Ni-filtered Cu KR radiation (40 kV, 30 mA).

2.2 | Preparation of support

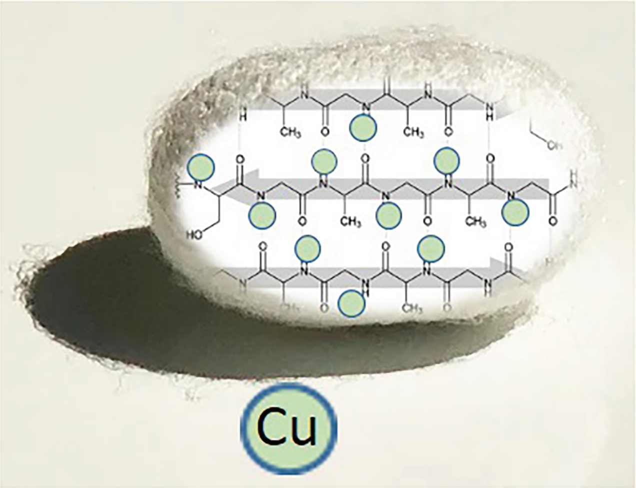
The raw silk fibers were processed three times (30 min each times) in 0.5 wt.% Na_2CO_3 solution at 80°C–90°C to remove sericin (degumming), rinsed with deionized water and dried at room temperature.[32,33]

2.3 | Preparation of supported Cu^0-nanoparticles

The degummed fibroin (0.2 g) dissolved in deionized water (10 ml) and sonicated for 10 min. Then a solution of (0.1 M) copper(Π) acetate (5 ml) was added to mixture and was stirred for 6 h led to the absorption of Cu(Π) on the fibroin surface. Afterwards, copper(Π) supported fibroin was removed from the solution and finally 0.1 M sodium borohydride solution (10 ml) was added dropwise to this and was stirred for 24 h to provide a copper(0)-fibroin catalyst. After completion of the reduction procedure, catalyst was washed four times with water and ethanol (50:50) mixture and dried at room temperature. The Cu/Fib. catalyst structure is schematically shown in Scheme 1.

2.4 | General procedure for AACR reaction

Aryl azides **1a-d** (1 mmol), alkyne **2a-d** (1.2 mmol), tetrabutylammonium bromide, TBAB, (0.1 mmol), and



SCHEME 1 Schematic representation of Cu/Fibroin catalyst

Cu/Fibroin catalyst (0.02 mol%) were added to water (5 ml), and the reaction mixture was stirred at room temperature for appropriation time. After completion of the reaction (monitored by TLC), the reaction mixture was diluted with chloroform (20 ml) and filtered to remove catalyst. The organic layer was washed with water (40 ml × 2), dried and concentrated under reduce pressure to obtain crude residue. The residue was purified by washing with cold methanol to obtained pure products **3a-d** in good to high yields (Scheme 2).

2.5 | Representative spectral data for the selected products

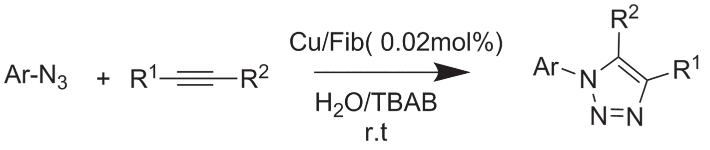
The known products (**3a–h**) were reported previously so these products were identified by ^{1}HNMR and comparison of their physical data with those of authentic samples (Supporting information). But compounds **3i and 3j** are first synthesized so their analytical and spectral data (^{1}H and ^{13}C NMR spectroscopy and mass spectrometry are described below (Supporting information).

2.5.1 | Product 3i

N,N-dimethyl-1-(1-(3-nitrophenyl)-1H-1,2,3-triazol-4-yl) methanamine, mass: m/z = 247 (M^+), ^{1}HNMR ($CDC1_3$, 300 MHz): δ = 2.38 (s, 6H, N-(Me)$_2$), 3.75 (s, 2H, CH_2), 7.78 (t, 1H, J = 8.1, meta-Ph), 8.10 (s,1H, triazole), 8.23 (ddd, 1H, J 1 = 8.1, J 2 = 2.1, J 3 = 0.9, ortho-Ph), 8.33 (ddd, 1H, J 1 = 8.1, J 2 = 2.1, J 3 = 0.9, para-Ph), 8.64 (t, 1H, J = 2.1, ortho-Ph). ^{13}CNMR ($CDC1_3$, 300 MHz): δ = 45.2 (dimethyl amino), 54.2 (CH_2), 115–150, 8C (Ar).

2.5.2 | Product 3j

N,N-dimethyl-1-(1-(2-methyl-4-nitrophenyl)-1H-1,2,3-triazol-4-yl)methanamine, m/z = 261 (M^+), ^{1}HNMR ($CDC1_3$, 300 MHz): δ = 2.39 (s, 6H, N-(Me)$_2$), 2.45 (s, 2H,



Ar=3-Nitrophenyl, 2-Nitrophenyl, 2,4,6-trichlorophenyl, 2-Me-4Nitrophenyl
R^1= CO_2Me, H, Ph, -$CH_2N(Me)_2$
R^2= CO_2Me, H

SCHEME 2 General procedure for the synthesis of triazole compounds

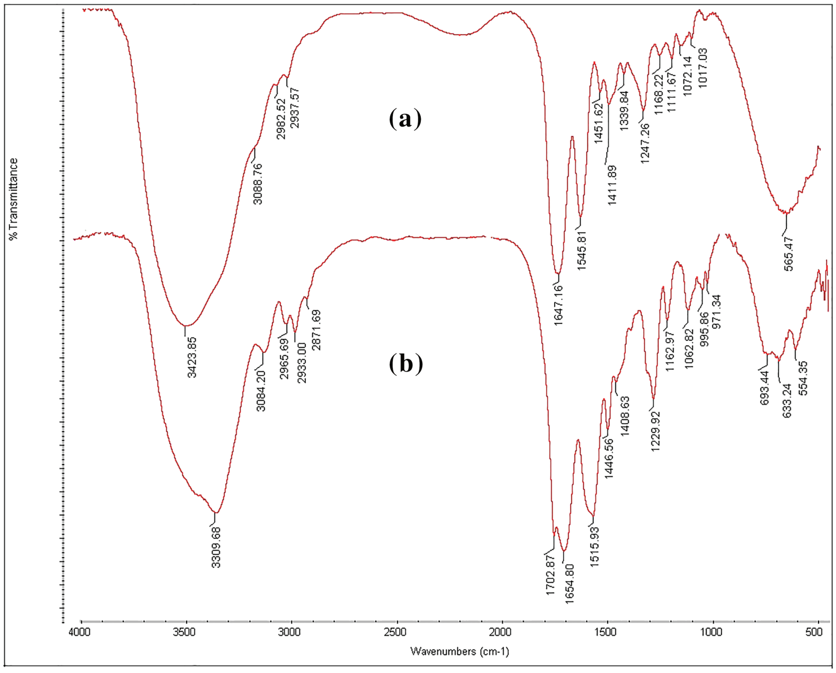


FIGURE 1 Fourier-transform infrared (FT-IR) spectra of fibroin fibers: (a) without copper and (b) with copper NPs

CH3), 3.77 (s, 2H, CH_2), 7.61 (d, 1H, J = 9, ortho-ph), 7.82 (s, 1H, Triazole), 8.25 (dd, 1H, J 1 = 9, J 2 = 3, meta-ph), 8.32 (d, 1H, j = 3, meta-ph). ^{13}CNMR (CDC13, 300 MHz): $\delta = 18.6$ (Me), 45.2 (dimethyl amino), 54.2 (CH_2), 122–147, 8C, (Ar).

2.6 | Recycling and reusing of the catalyst

Due to the fact that the catalyst is insoluble in water, it can be recycled by simple filtration. The separated catalyst was washed with ethanol, dried at room temperature, and reused in the model reaction for four times.

3 | RESULTS AND DISCUSSION

3.1 | Characterization results of the Cu/Fib. catalyst

3.1.1 | Fourier transform-infrared spectra

In the FT-IR spectrum of Pure Silk Fibroin (Figure 1a), the appearance of absorption bands at 1,647 cm and 1,545 cm^{-1}, which are related to the amide—I (C═O stretching) and amide—II (N—H bending), respectively, confirm the Silk II structural conformation (β-sheet). Another absorption band observed at 1,247 cm^{-1} corresponded to amide—III (C—N stretching), which is typically silk I conformation (α-helix or random coil structure).[34] Peaks were observed at 1,411 cm^{-1} is due to symmetric stretching vibrations of carboxylate group and 1,339 cm^{-1} indicate the presence of methylic groups of alanine in the silk chain. The peak at 3,423 cm^{-1} is a symptom of the hydroxyl (—OH) stretching vibration from the phenolic acids of Tyr molecule of SF chain. Infrared (IR) analysis demonstrated that complexation of copper by the fibroin fiber had occurred, with the prominent C—N stretching vibration of the imine (1,247 cm^{-1}) being shifted to a lower frequency (1,230 cm^{-1}) by 17 cm^{-1}, indicative of metal–fibroin interaction.[34]

3.1.2 | X-ray diffraction

Powder XRD analysis was applied to identify the crystalline structure of Cu NPs (Cu/Fib.) into the surface of fibroin. As shown in Figure 2, three characteristic diffraction peaks at 2θ values 43.7°, 51. 1°, and 74.6°,

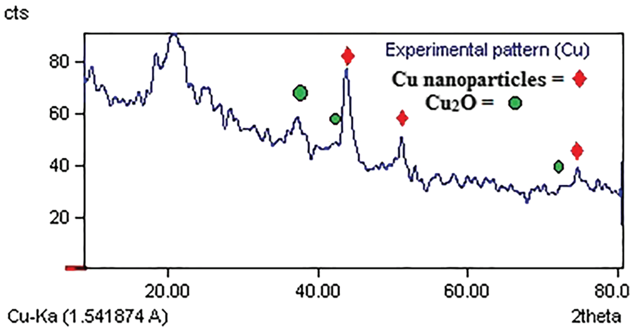


FIGURE 2 X-ray diffraction (XRD) patterns of Cu/Fibroin catalyst

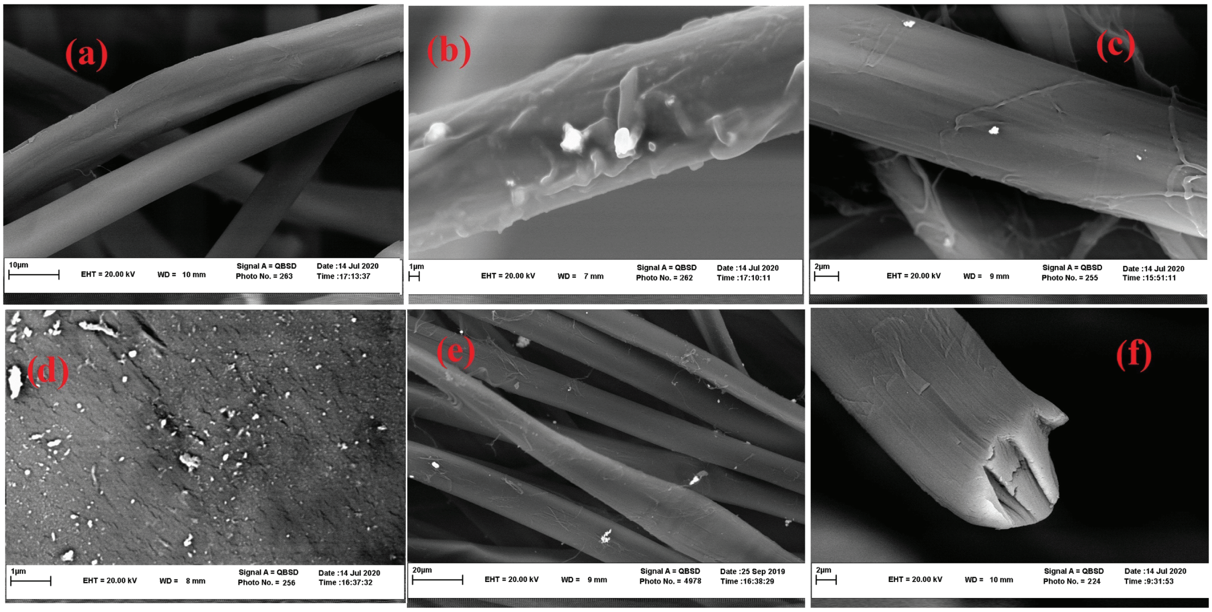


FIGURE 3 Scanning electron microscopy (SEM) images of (a) pure fibroin, (b–e) Cu/Fib.; and (f) cutting section of fibroin fiber

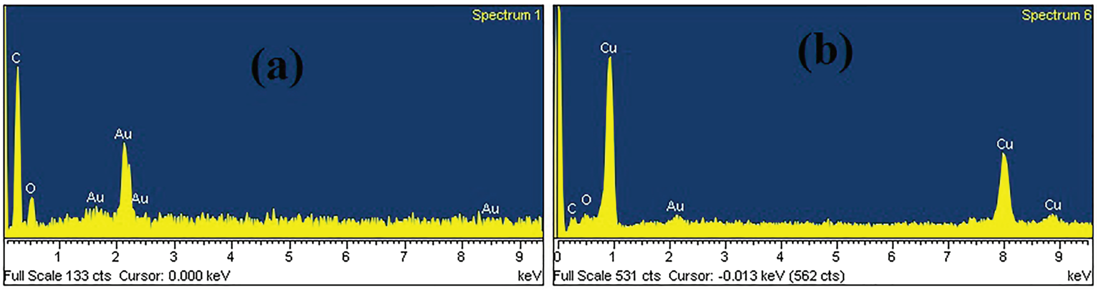


FIGURE 4 Energy-dispersive X-ray spectroscopy (EDS) report of fibroin fibers: (a) before; and (b) after copper loading

corresponding to the (1 1 1), (2 0 0), (2 2 0) indices of FCC lattice of metallic Copper nanoparticles (JCPDS card no. 98-410-5041). Because copper nanoparticles are sensitive to oxygen in the air, they can be oxidized to copper(I) oxide (Cuprite). Thus, there are some extra peaks in the XRD pattern attributed to Cu(I) (Cuprite) that are positioned at $2\theta = 36.7$, 42.3, and 73.5, corresponding to the (200) (220) and (311) planes of copper oxide (JCPDS card no. 96-900-7498). The average crystallite sizes of Cu nanoparticles, calculated using the Debye–Scherrer equation ($d = K\lambda/\beta\cos\theta$), is about 30 nm.

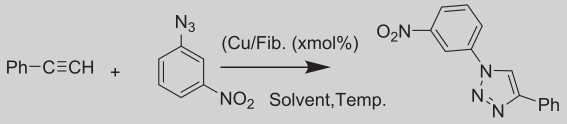
TABLE 1 CHN component of fibroin fibers, before and after Copper loading

Element (% w)	Pure fibroin	Cu/Fibroin
Nitrogen	16.431	15.500
Carbon	45.240	42.830
Hydrogen	5.596	5.288

3.1.3 | Scanning electron microscopy

The morphological features were studied by scanning electron microscopy (SEM) technique. SEM images of

TABLE 2 Synthesis of compound **3e** in the presence of Cu/Fibroin as catalyst under different reaction condition[a]



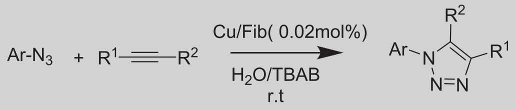
Entry	Catalyst (mol% Cu)	Solvent	Temp. (°C)	Time (h)	Yield (%)[b]
1	0.05	Methanol	50	4	80
2	0.05	H_20	50	4	60
3	0.05	H_20/tButOH (1:2)	50	4	81
4	0.05	H_20/TBAB[c] (10:1)	50	3	83
5	0.05	H_20/TBAB (10:1)	80	3	71
6	0.05	H_20/TBAB (10:1)	r.t	3	90
7	0.02	H_20/TBAB (10:1)	r.t	3	90
8	0.01	H_20/TBAB (10:1)	r.t	3	80
9	-	H_20/TBAB (10:1)	r.t	7	-
10	0.05	n-hexane	50	4	Trace
11	0.05	Toluene	50	4	Trace

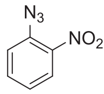
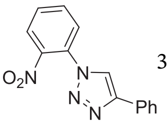
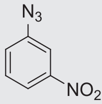
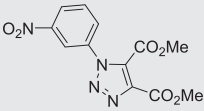
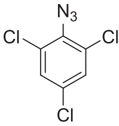
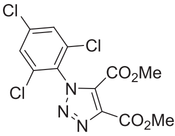
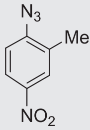
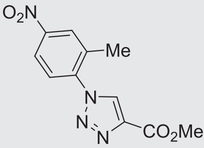
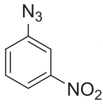
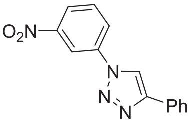
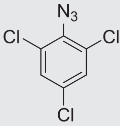
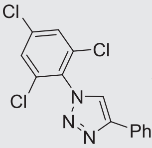
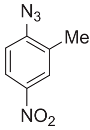
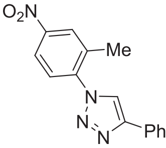
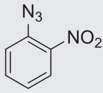
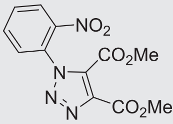
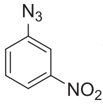
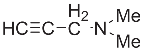
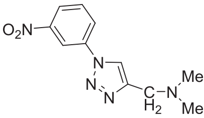
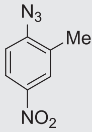
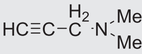
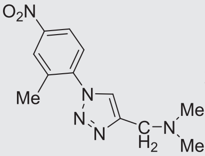
[a] Other conditions: 3-nitroazide (1 mmol), phenylacetylene (1. 5 mmol), H_2O/TBAB (10:1) as a solvent at r.t.

[b] Isolated yield of the pure product based on 3-nitroazide.

[c] Tetra-n-butylammonium bromide.

TABLE 3 Synthesis of 1,2,3-triazoles **3a-j** using Cu/Fibroin as catalyst[a]



Entry	Aryl azide	Alkyne	Product[b]	Time (h)	Yield[c] (%)
1	 1a	$Ph-C\equiv CH$ 2a	 3a	2	95
2	 1b	$MeO_2C-C\equiv C-CO_2Me$ 2b	 3b	3	90
3	 1c	$MeO_2C-C\equiv C-CO_2Me$ 2b	 3c	2:40	93
4	 1d	$MeO_2C-C\equiv CH$ 2c	 3d	2	95
5	 1b	$Ph-C\equiv CH$ 2a	 3e	2	90
6	 1c	$Ph-C\equiv CH$ 2a	 3f	3	92
7	 1d	$Ph-C\equiv CH$ 2a	 3g	2	97
8	 1a	$MeO_2C-C\equiv C-CO_2Me$ 2b	 3h	3	93
9	 1b	 2d $HC\equiv C-\overset{H_2}{C}-N\genfrac{}{}{0pt}{}{Me}{Me}$	 3i	2:20	98
10	 1d	 2d $HC\equiv C-\overset{H_2}{C}-N\genfrac{}{}{0pt}{}{Me}{Me}$	 3j	2	94

[a] Reaction conditions: Aryl azide (1 mmol), Alkyne (1.2 mmol), 0.02 mol% Cu/Fibroin and H_2O/TBAB (10:1) as a solvent at r.t. temperature.

[b] All the products were characterized by ^{1}H-NMR.

[c] Isolated yield.

Cu/Fibroin (Figure 3b–e) proved that these copper nanoparticles are almost spherical and narrowly distributed on the smooth surface of the Silk Fibroin (Figure 3a). This homogeneous dispersion was attributed to an interaction between the $Cu(OAc)_2$ and amino acids of the Silk Fibroin, increasing the resistance to the growth of the Cu clusters. The SEM image of cutting section of a fibroin fiber in Figure 3f proves that the sericin was completely removed,[20] and according to Figure 3d, the average particle size is about 50 nanometers.

Comprising between the elemental analysis (EDS) spectrum of pure fibroin and Cu/Fibroin catalyst shows the successful immobilization of Cu nanoparticles on fibroin fibers. As can be seen in Figure 4, no additional peak related to other impurities was appeared in the spectrum.

3.1.4 | Determining of Cu content of Cu/Fibroin catalyst

The Cu content of Cu/Fibroin catalyst was determined using the inductively coupled plasma-atomic emission spectrometry (ICP-AES) technique. This resulted in a value of about 1.45% *w*/w.

Due to confirmation of the copper content resulting from the ICP-AES method, the CHN content was investigated before and after copper loading. The results are shown in Table 1, which shows that the CHN content (% w) decreased from 67.26 to 63.61% w after Copper loading, which is approximately in accordance with the copper content resulting from the ICP-AES method.

3.2 | Catalytic activity of the Cu/Fibroin nanocatalyst in the synthesis of 1,2,3-triazoles

The reaction of 3-nitro-phenylazide (1 mmol), phenylacetylene (1. 5 mmol) for the synthesis of compound **3e** was selected as a model, to optimize the reaction conditions Such as different solvents, amount of catalyst and required optimum temperature of the reaction, and the results are summarized in Table 2.

Initially, we used methanol as solvent in the presence of 0.05 mol% Cu/Fibroin catalyst at 50°C, which resulted 80% yield in 4 h (Table 2, entry 1). The water was then replaced with water as a green solvent in the same condition However, the reaction yield decreased as the solubility of the reactants in the water solvent decreased (Table 2, entry 2). Then *t*-Butanole was added to increase the solubility of the reactants in water (1:2) which increased the reaction yield to 81%.

Tetra-n-butylammonium bromide was also evaluated as a phase transfer catalyst in the water (10:1) and was able to produce product **3e** with high yield (Table 2, entry 4). The highest yield was obtained when the reaction was done in mixture of H_2O and TBAB with the ratio 10:1 at room temperature in the presence of 0.02 mol% Cu/Fibroin (entry 7). Normal hexane and toluene as non-polar solvent were also evaluated, which very small amount of product resulted in 4 h (Table 2, entries 10 and 11). Having these optimized reaction conditions in hand, the scope of reaction was extended to various azides and different terminal and internal alkynes.[8] According to the results shown in Table 3, all phenyl azides carrying either electron-donating or electron-withdrawing groups reacted successfully whit terminal

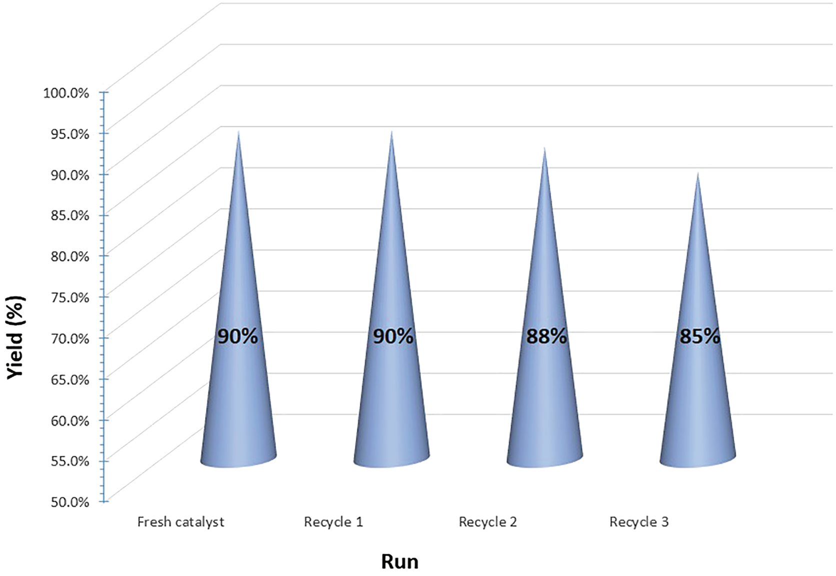
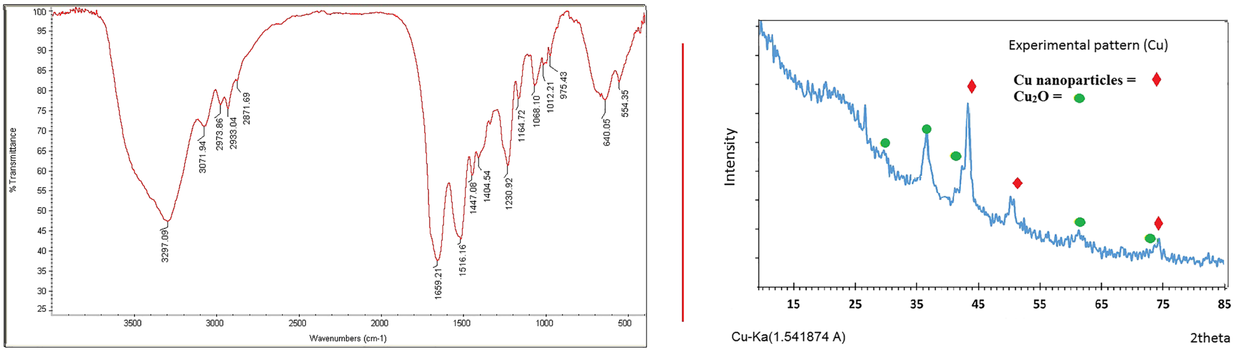


FIGURE 5 Investigation of reusability of the catalyst in the model reaction condition



(a) (b)

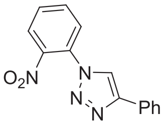
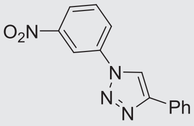
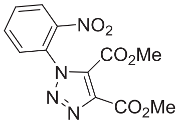
FIGURE 6 Characterization of the Cu/Fibroin catalyst after the third recovery and reuse process. (a) Fourier-transform infrared (FT-IR) spectra; (b) X-ray diffraction (XRD) pattern

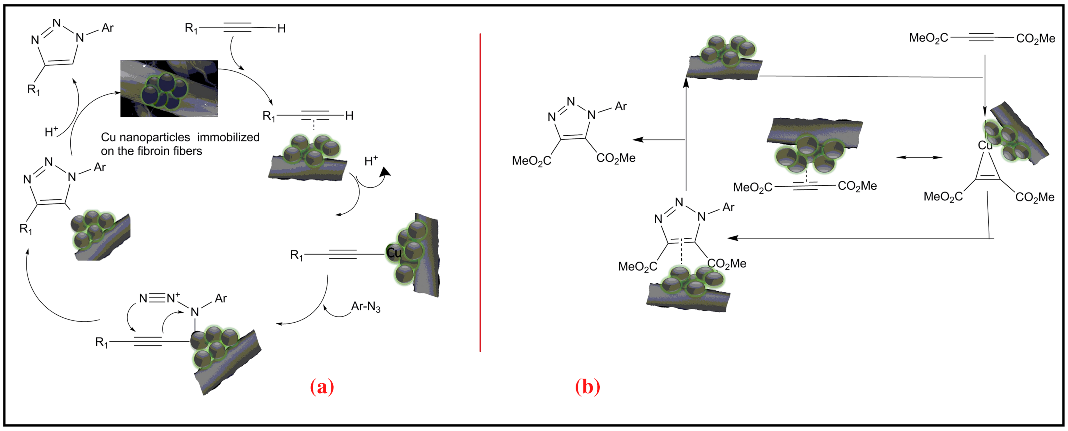
and symmetric internal alkynes and gave the products in high yields within short reaction time. In the case of terminal alkynes, the reaction showed good regioselectivity and produced a high yield of 1,4-disubstituted products.

To determine the applicability of catalyst recovery, the same model reaction was again studied under the optimized conditions. The catalyst was recovered according to the procedure mentioned in Section 2 and reused in a similar reaction, for three more times. The catalyst showed high stability after it was reused four times with only a slight reduction of its activity. Results have been shown in Figure 5. FT-IR and powder XRD, were carried out to characterize the catalyst after the third recovery and reuse process, which does not show any considerable change in the morphology. However, an increase in the intensity of diffraction peaks related to Cu_2O (cuprite) was observed in the recovered catalyst (Figure 6) compared to the fresh catalyst (Figure 2). This is due to the oxidation of copper nanoparticles by air during catalyst recovery.

In order to compare the efficiency of the synthesized catalyst and other previous catalytic systems used in the CuACC reactions, studies were performed, and the results are given in Table 4. As can be seen in Table 4, these comparative results establish the advantage of the Cu/Fib. catalyst over the previously reported methods (based on catalyst loading and reaction times and yield).

TABLE 4 Comparative results between our study and other previously reported catalytic systems in the synthesis of some triazole compounds

Entry	Product	Catalyst/condition	Yield (%)	Ref.
1	 (3a)	Cu/Fibroin (0.02 mol%), H_2O/TBAB, rt, **2 h**	95	This work
		Dialkyldithiocarbamic acid copper complexes, (0.005 mmol), acetonitrile, rt, **11 h**	89	Velasco et al.[37]
		Cu $(PPh_3)_2NO_3$ (0.5 mol%), rt, solvent free, **24 h**	45	Wang et al.[38]
2	 (3e)	Cu/Fibroin (0.02 mol%), H_2O/TBAB, rt, **2 h**	90	This work
		Cu $(PPh_3)_2NO_3$ (0.5 mol%), rt, solvent free, **5 h**	81	Wang et al.[38]
		Aryl halide, NaN_3, CuI/D-lucosamine KOH, 1:1(DMF + H_2O), 80°C–90°C, ethanol, phenylacetylene, Sod.ascorbate, rt, **16 h**	92	Murthy et al.[39]
		$C_{32}H_{32}Cu_2N_{10}{}^{(2+)} * 2F_6P^{(1-)}$ (0.3 mol%), sodium L-ascorbate (3 mol%), methanol, rt, **N_2, 16 h**	99	Han et al.[40]
3	 (3h)	Cu/Fibroin (0.02 mol%), H_2O/TBAB, rt, **3 h**	93	This work
		Catalyst free, chloroform, 30°C–70°C, **15 days**	44	Kauer et al.[41]



SCHEME 3 Proposed CuAAC reaction mechanism for (a) terminal and (b) internal alkynes

3.3 | Proposed reaction mechanism

Under our conditions, the presence of CuNPs on the surface of fibroin fibers probably acts as the catalyst. A tentative reaction mechanism for the synthesis of 1H-1,2,3-triazoles catalyzed by CuNPs under heterogeneous conditions is proposed in Scheme 3. It is proposed that, in the first step, a terminal alkyne is coordinated to CuNPs supported on fibroin, which activates the C—H bond. As a result, the corresponding copper–alkylidine complex is formed on the surface of the fibroin fibers. This is a very favorable step because Cu metal is well known to exhibit high alkynophilicity for terminal alkynes. In the second step, synthesized aryl azides attack the copper-alkylidine complex followed by intramolecular cyclization, finally leading to formation of a five-membered ring of triazole as a product. In the case of internal alkynes, π-coordination between alkyne and copper(I) spices leading to activation of alkyne and can acts as driving force for this reaction.[35,36]

4 | CONCLUSION

In conclusion, novel copper nanoparticles immobilized on silk fibroin (Cu/Fibroin) were successfully prepared and fully characterized using FT-IR, SEM, EDS, XRD, ICP, and CHN techniques. We have developed a simple new catalytic method for the synthesis of 1,2,3-triazoles from aryl azides and terminal and internal alkynes in the presence of Cu/Fibroin NPs as ligand-free, efficient, reusable, and heterogeneous catalysts. Some attractive features of this protocol are high yields, simple procedure to produce catalyst, easy work-up, high catalytic activity, good regioselectivity with terminal alkynes, reactivity toward internal alkynes and reusability of the catalyst. The catalyst can be used at least four times.

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AUTHOR CONTRIBUTIONS

Hakimeh Sadat Mirzaei Hesari: Conceptualization; data curation; formal analysis; investigation; methodology; project administration; validation; visualization. **Hossein Eshghi:** Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; resources; supervision; validation; visualization. **Seyed Mohammad Seyedi:** Conceptualization; methodology; project administration; supervision.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

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