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Copper-Containing Catalysts for Azide–Alkyne Cycloaddition in Supercritical CO₂

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Abstract: Background: Chemical industry has increased the investment into and innovation capacity to supply chemicals from safe and sustainable sources, which will be essential to offering new solutions and supporting the green transition of the global economy and society. In this sense, the use of green solvents and reusable heterogeneous catalysts has emerged as a promising sustainable process strategy for engineering, chemistry and the environment. In this work, different homogeneous (copper bromide, CuBr and copper(II) acetate, Cu (CH₃COO)₂ · H₂O) and heterogeneous (Cu Wire, Cu Plate, Cu/β-SiC, pre-treated Cu Wire and pre-treated Cu Plate) copper catalysts were tested for the copper(I)-catalyzed alkyne–azide cycloaddition (CuAAC) reaction. In addition, the influence of different reaction media was analyzed, comparing the use of an organic solvent such as toluene and a green solvent such as supercritical CO₂ (scCO₂). Methods: Characterization of the catalysts includes by X-ray diffraction (XRD), Scan Electron Microscopy (SEM), Atomic absorption spectrophotometry (AA) and Temperature Programmed Reduction (TPR). Parameters such as catalyst loading, reaction time, reusability and leaching of the catalysts were studied to obtain more information on the CuAAC reaction in scCO₂. Results: The pre-treated copper plate achieved a 57% increase in reaction yield compared to the non pre-treated copper plate. However, the recovery and reuse of the pre-treated copper plate showed a severe deterioration and a considerable change in its surface. Cu Wire (without pre-treatment) achieved yields of up to 94.2% after reusing it for five cycles. Conclusions: These results suggest the possibility to exploit the combination of heterogeneous catalysts and scCO₂ and justify further research to highlight green solvents and simultaneously address the challenges of reaction, purification and recycling.

Keywords: CuAAC; copper catalysts; scCO₂; green chemistry; heterogeneous catalyst; reusability

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1. Introduction

Cycloaddition using azides and alkynes is an important method for the synthesis of 1,2,3 triazoles, which was firstly reported by Huisgen et al. in 1960 [1]. A particular case of this process is the copper-catalyzed alkyne–azide cycloaddition (CuAAC). The reaction kinetics and mechanism for the CuAAC reaction have been studied in detail since its discovery in 2002 [2,3]. The Cu (+1) catalysis has transformed this cycloaddition into an essentially quantitative and regioselective click reaction, as developed by the Sharpless and Meldal laboratories [2–5]. The CuAAC reaction is generally considered as the most prolific and successful click reaction in drug discovery, bioconjugation applications, and polymer chemistry. CuAAC reaction has found wide application due to its simplicity, applicability and efficiency and is based on the formation of 1,4-disubstituted 1,2,3-triazoles between a terminal alkyne and an aliphatic or aromatic azide in the presence of copper [5–8].

The first example of CuAAC was carried out in water/t-BuOH at room temperature [9]. However, CuAAC reaction is habitually carried out with organic solvents in terms of

polymer and organic chemistry as reaction media, such as N, N dimethylformamide (DMF), toluene or tetrahydrofuran (THF) [10,11]. Traditional organic solvents could potentially cause various health and environmental issues due to their volatility and toxicity, so the reduction in its use is encouraged nowadays by social concern and political regulations. In the past few decades, the use of supercritical fluids (SCFs) as an alternative to the use of traditional organic solvents for chemical synthesis has attracted huge attention [12,13]. Among all SCFs, scCO_2 has received special interest as it is nontoxic, non-flammable, inexpensive and easy to dispose and recycle [14,15].

CuAAC click reactions have been explored and analyzed in many different green solvents and reaction media, including scCO_2 . In 2009, Grignard et al. [16,17] reported the first example of modification of aliphatic polyesters via CuAAC in scCO_2 and the removal of the catalyst (CuI) using scCO_2 extraction. Later, the same authors prepared a polymer with a chain-end functionality through CuAAC in scCO_2 . In 2015, Zhang et al. [18] developed an efficient protocol for the CuAAC catalyzed by $\text{Cu}(\text{CH}_3\text{COO})_2 \cdot \text{H}_2\text{O}$ in supercritical carbon dioxide in the absence of the ligand. A recent work from our group [19] showed the viability of CuAAC with a polyether in scCO_2 using acetate of copper monohydrate as a catalyst. To the best of our knowledge, only four papers can be found in the literature where the CuAAC reaction is studied using scCO_2 as reaction media.

Regarding the catalyst, a wide variety of copper catalysts can be used in CuAAC reaction as long as Cu (+1) ion species are generated [10,20–22]. There are three general methodologies to ensure the presence of Cu (+1) ions in the reaction medium, which can be selected depending on the experimental conditions of the reaction. The first one of these strategies is in situ reduction of copper (II) salts, commonly using copper sulphate pentahydrate and sodium ascorbate as a reducing agent [23,24]. The second strategy is the oxidation of metallic copper and the last one is the addition of copper (I) salts directly. Sometimes, this last strategy is coupled with the addition of certain nitrogen bases or ligands such as N, N-diisopropylethylamine (DIPEA) or N, N, N', N'', N''-Pentamethyl diethylenetriamine (PMDTA), which are able to stabilize the oxidation state +1 of the copper and promote the reaction by reducing the formation of by-products [25,26]. Patricia L. Golas et al. [27] studied different catalyst and ligands, observing that tridentate amine ligands produced faster reaction rates than pyridine-based ligands, the first ones the being most effective ligands in CuAAC.

A new catalysis strategy is currently being developed and studied in the green chemistry context: heterogeneous catalysis in CuAAC. The main benefits attributed to this mode of reaction are the simplicity of processing, recyclability (catalysts preserve their activity throughout several reaction cycles) and minimization of waste, which means a reduction in environmental concerns [21].

There is a great variety of works that have studied different heterogeneous copper catalysts using organic solvents and high temperatures in the CuAAC reaction [11,28–31]. However, there are only two recent papers where a heterogeneous copper catalyst was used in the CuAAC reaction in scCO_2 , and a copper wire was selected as catalyst for both studies [32,33]. Nevertheless, these works did not study in depth the behavior of this catalyst in scCO_2 . Therefore, the CuAAC reaction in scCO_2 is yet far from being fully understood.

In this study, the reaction between methoxy polyethylene glycol (mPEG-alkyne) and coumarin with the azide group was selected as a model of the cycloaddition reaction in order to study the influence on CuAAC of different catalysts and reaction media (Scheme 1). This conjugate can be used in pharmaceutical applications, as it was found to be able to form nanoaggregates to form micelles and exhibits anti-oxidative capacity and fluorescent characteristics for monitoring of disease treatment provided by coumarin [34]. Optimal conditions of pressure and temperature of scCO_2 for the CuAAC reaction were fixed after the previous optimization study [35]. Both homogeneous and heterogeneous catalysts were considered for this work. The homogeneous catalysts selected were CuBr and $\text{Cu}(\text{CH}_3\text{COO})_2 \cdot \text{H}_2\text{O}$ and the heterogeneous catalysts were Cu Wire, a Cu Plate, Cu/ β -SiC, Pre-treated Cu Wire

and a Pre-treated Cu Plate. Silicon carbide pellets (β -SiC) have been widely employed as catalytic support for heterogeneous catalysis, as they exhibit a high thermal conductivity, a high resistance to oxidation, a high mechanical strength, chemical inertness, and average surface area [35–37]. In addition, this work also reports the reusability of the tested catalyst after several cycles in the CuAAC reaction in $scCO_2$.

Scheme 1. Model CuAAC reaction.

2. Results and Discussion

In this research, the reaction between mPEG—alkyne and coumarin—azide was selected as a model of the CuAAC, in order to study the influence of different copper catalysts and solvents. The results were divided into four main sections. In the first place, the mechanism of heterogeneous catalysts in $scCO_2$ was analyzed at 13 MPa. In the second place, the influence of a catalyst pre-treatment was reported here in order to produce a new alternative to chemical synthesis routes. In the third section, we report a comparison of the yield values obtained for the click chemistry at atmospheric and high pressure with different copper catalyst. Finally, the recyclability and leaching were studied for heterogeneous catalysts.

2.1. Behaviour of Copper Catalyst in $scCO_2$

In this section, the effect of $scCO_2$ on the oxidation state of the catalysts was evaluated with XRD in order to better understand their behavior in the CuAAC reaction. Cu (+1), Cu (+2) oxides and Cu (0) species were observed in the copper catalysts and their intensities compared for the different samples.

Figure 1 shows XRD spectra of acetate of copper monohydrate and copper bromide, before and after being exposed in $scCO_2$ at 13 MPa and 24 h. The peak positions of $Cu(CH_3COO)_2 \cdot H_2O$ at 12.56° , 14.20° , 16.30° , 20.34° , 24.84° , 25.64° , 33.27° , 34.79° , 36.49° , 38.72° , 40.88° , 44.37° , 46.39° , 50.92° , 55.97° , 58.19° , 63.97° , 66.4° and 69.73° are indexed as (110), (002), (11-2), (020), (202), (20-4), (312), (42-3), (13-3), (33-3), (42-5), (22-6), (53-3), (404), (045), (13-7), (060), (641) and (262) hkl planes with space group symmetry of C2/c (15) [37]. The peaks are in good conformity with the pure phase of the Joint committee on powder diffraction standards (JCPDS, PDF-14-0811). When the $Cu(CH_3COO)_2 \cdot H_2O$ was exposed to the supercritical medium, the XRD profile changed (Figure 1a). The exposure of $Cu(CH_3COO)_2 \cdot H_2O$ to $scCO_2$ leads to the formation of several coexisting phases (Cu_2O and CuO). XRD showed the appearance of peaks at 29.46° , 36.29° , 42.15° , 61.13° attributed to Cu_2O and 25.68° , 57.89° attributed to CuO .

This fact indicates that $scCO_2$ at $35^\circ C$ and 13 MPa modified the initial structure of $Cu(CH_3COO)_2 \cdot H_2O$ giving rise to copper species with different oxidation states, which is a very interesting result, in order to achieve excellent yields from the CuAAC reaction. In addition $Cu(CH_3COO)_2 \cdot H_2O$ has been used in $scCO_2$ as catalyst [18,38–40].

Figure 1b shows the XRD spectrum of the $CuBr$, which contains seven peaks that are clearly distinguishable. The prominent XRD peaks at 27.06° , 44.9° , 53.26° , 65.46° , 72.26° , 83.02° and 89.34° are indexed as (111), (220), (311), (400), (331), (422) and (481) hkl planes with space group symmetry of F-43m (216) [41]. The peaks positions are in good agreement with those for $CuBr$ powder obtained from the International Center of Diffraction Data card (ICDD, formerly JCPDS, 06-0292). All of them can be perfectly indexed to crystalline γ - $CuBr$, not only in peak position, but also in their relative intensity [42]. In this case, no

variation in the XRD profile was observed when copper bromide is exposed to scCO_2 at 13 MPa.

Figure 1. XRD spectra of fresh catalysts and after scCO_2 at 13 MPa and 35 °C (a) $\text{Cu}(\text{CH}_3\text{COO})_2 \cdot \text{H}_2\text{O}$ and (b) CuBr.

Figure 2a–c showed XRD spectra of Cu Wire, the Cu Plate and Cu/ β -SiC fresh catalysts and those after being exposed in scCO_2 at 13 MPa, 35 °C and 24 h. The Cu Wire fresh catalyst diffractogram illustrates that the peaks' positions are in good conformity with the pure cubic copper phase from the Joint Committee on Powder Diffraction Standards (JCPDS, PDF-14-0811). No peaks attributable to possible impurities can be observed. The XRD peaks at 43.54°, 50.44°, 74.04°, 90.25° and 95.03° are indexed as (111), (200), (220), (311) and (222) hkl planes with space group symmetry of $\text{Fm}\bar{3}\text{m}$ (225). After exposure to scCO_2 , new peaks appear at 36.76°, 42.15° and 77.05° that correspond to Cu_2O and at 66.13° and 84.82° corresponding to CuO. Since scCO_2 has been used in numerous works as an oxidizing agent for the heterogeneous catalyst [38,42], the formation of Cu_2O and CuO phases on the surface of the copper wire could be explained by the effect of the exposure to this compound.

Figure 2b shows the XRD of the Cu Plate. The peaks at 43.54°, 50.44°, 74.04° and 90.25° are indexed as (111), (200), (220) and (311) hkl planes of Cu (0). No variation in the XRD profile was observed when the copper plate is exposed to scCO_2 at 13 MPa; thus, scCO_2 was not capable of oxidizing the copper plate at these conditions, and therefore did not generate the Cu (+1) ions, which are considered the most active copper species for the CuAAC.

Regarding Cu/ β -SiC, its oxidation stability in scCO_2 was also evaluated (Figure 2c). After exposure to scCO_2 at 13 MPa and 35 °C for 24 h, XRD patterns illustrate the same crystalline structure of the SiC support, with peaks at 35.5°, 59.7°, 71.4°, 75.1° and 89.5°, indexed as (111), (220), (311), (222) and (400) hkl planes with space group symmetry of $\text{F}\bar{4}3\text{m}$ (216). After impregnation of Cu species, the cubic SiC remains as the main peaks. The diffraction peaks related with copper species catalysts were located at 43.3°, 50.4° and 74.1°, coinciding with the (111), (200) and (220) reflections of CuO, respectively, according to JCPDS fiche 04-0836, verifying the presence of this element. Differences in the XRD before and after scCO_2 exposure were not observed (Figure 2c).

Therefore, it can be concluded that XRD of CuBr, Cu/ β -SiC and Cu Plate catalysts did not reveal any variation in their structure after exposure to scCO_2 . However, XRD profiles of $\text{Cu}(\text{CH}_3\text{COO})_2 \cdot \text{H}_2\text{O}$ and Cu Wire changed after contact with scCO_2 at 13 MPa and 35 °C for 24 h, giving rise to Cu_2O in $\text{Cu}(\text{CH}_3\text{COO})_2 \cdot \text{H}_2\text{O}$ and Cu_2O and CuO in Cu Wire, which indicates the ability of scCO_2 to modify the oxidation state of copper in these catalysts.

Figure 2. XRD spectra of fresh catalysts and after scCO_2 at 13 MPa and 35 °C (a) Cu Wire (b) the Cu Plate and (c) Cu/ β -SiC.

2.2. Pre-Treatment of Cu Wire and the Cu Plate

The pre-treatment was carried out with the main objective of creating copper in an oxidation state (+1) on the surface of these catalysts, in order to accelerate the reaction rate and to maintain the Cu (+1) concentration at a sufficient level during the reaction. In this section, a simple and inexpensive synthesis approach is described for direct growth of Cu_2O and CuO in Cu Wire and Cu Plates.

In the first step, the pre-treatment conditions were decided according to the temperature programmed reduction (TPR) test on Cu metal catalysts, which yielded information about the temperature for the transition from one copper oxide species to another during the reduction process. Figure 3 shows the TPR profiles of Cu Wire and the Cu Plate after oxidation.

For both catalysts, at low temperatures between 200 °C and 400 °C, two reduction peaks can be observed. The first peak corresponds to the reduction of Cu (+2) to Cu (+1) and the second peak corresponds to the reduction of Cu (+1) to Cu (0) [43,44]. The TPR curve of copper wire shows a higher intensity peak at 284.4 °C with a shoulder at 250 °C, whereas, for the copper plate, the reduction profile shows a higher intensity peak at 245 °C with a shoulder at 285 °C. The two reduction peaks overlap in both catalysts, which implies that the transition from one species to another occurs in a short temperature range.

Once the TPR was carried out, the pre-treatment protocol was established as follows. After loading the sample, an initial oxidation of the catalysts was carried out using synthetic air (10 mL/min) and heating of the sample from room temperature up to 400 °C, at a rate of 6.67 °C/min. After reaching 400 °C, the sample was kept at these conditions for 120 min in order to oxidize most of the metal surface and produce CuO [43]. Secondly, with a stream of inert N_2 (50 mL/min), the temperature was reduced to 200 °C with a rate of

10 °C/min. Finally, the reduction of CuO to Cu₂O with H₂ (10 mL/min) took place at 200 °C for 30 min. The aim of this last step was to reduce the copper (+2) oxide species to copper (+1) according to the results of TPR. Figure 4 shows the XRD profile of Cu wire and the Cu plate before and after pre-treatment.

Figure 3. TPR profiles of Cu Wire and the Cu Plate.

Figure 4. XRD spectra of the Cu Plate (a) fresh and (b) after being pre-treated and Cu Wire (c) fresh and (d) after being pre-treated.

XRD profiles of Cu Wire and Cu Plate before pre-treatment show the peaks centered at about at 43.54°, 50.44°, 74.04° and 90.25°, attributed to the (111), (200), (220) and (311) hkl reflection planes, respectively, of pure metallic copper. It can be seen how the intensity corresponding to the pure copper peaks decreases after pre-treatment in both catalysts.

In Figure 4d, showing pre-treated Cu Wire, new peaks were identified at 36.7° , 42.78° , 65.74° and 76.9° assigned to Cu_2O and 44.95° , 81.09° , 82.17° and 92.92° assigned to CuO . Regarding the pre-treated Cu Plate (Figure 4b), it showed new peaks at 36.28° , 42.3° , 65.04° and 77.04° , which are indexed as (111), (200), (220) and (311) hkl planes, which correspond to Cu_2O and 44.35° and 82.62° which are attributed to CuO . Therefore, it is possible to confirm that the pre-treatment process contributes to the formation of Cu_2O and CuO species. Scan Electron Microscopy (SEM) characterization was carried out in order to study the morphology changes (Figure 5).

Figure 5. SEM images of the Cu Plate (a) fresh and (b) after being pre-treated and Cu Wire (c) fresh and (d) after being pre-treated.

In the SEM photographs, it is possible to observe that the surface of the Cu Plate and Cu Wire were oxidized to form appreciable Cu_2O and CuO crystals. However, the effect of the pre-treatment was not uniform over the whole surface of the catalysts. Thus, it can be concluded that both Cu (0), Cu_2O and CuO species coexist within the oxidized copper plate and copper wire, Cu_2O being the dominant oxide after this pre-treatment. However, it is quite clear from the comparison of both (pre-treated Cu Wire and Cu Plate), that in the oxidation–reduction process of Cu Wire many species of CuO were generated compared to the reaction of the copper plate.

2.3. CuAAC Model Reaction with Different Copper Structures

The activity of different copper catalysts in CuAAC with toluene and scCO_2 as solvents was evaluated, as indicated Figure 6, and the results obtained appear in this section.

The coupling of mPEG alkyne with 4-azidomethyl-7-methoxycoumarin (AMMC) using toluene as solvent, different catalysts (CuBr and Cu Wire) and ligands or the absence of ligands was studied in the first place, and a summary of yield results is given in Table 1.

Using toluene as reaction media, the CuAAC reaction with CuBr as a catalyst and no addition of PMDTA did not take place. The addition of PMDTA ligand significantly increased the conversion, reaching a yield of 63.7% after 48 h at the considered conditions. The addition of half of the previous amount of PMDTA produced a slower rate of click product formation with a reduction in the yield values. The use of CuBr as A catalyst could offer the potential to avoid the necessity of reducing agents, as in this salt copper is in the oxidation state +1. However, the Cu (+1) present in this catalyst needs to be

stabilized against moisture and to be efficiently solubilized in the reaction media, requiring in addition avoidance of the disproportionation towards Cu (0) and Cu (+2). For this reason, the reaction was carried out under inert conditions and in the presence of an amine-based ligand such as PMDTA, whose main function is to stabilize the copper complex and increase the reaction rate. CuBr in combination with PMDTA ligand has been commonly used in applications of CuAAC in polymer chemistry [44].

Figure 6. Chart of catalysts used in toluene and scCO_2 .

Table 1. Yield of CuAAC reaction with different catalysts using an organic solvent.

Entry	Copper Source	PMDTA (mmol)	Time (h)	Yield (%)
1	CuBr	-	6	0
2	CuBr	-	12	0
3	CuBr	-	24	0
4	CuBr	-	48	0
5	CuBr	0.05	6	33.6
6	CuBr	0.05	12	34.2
7	CuBr	0.05	24	43.18
8	CuBr	0.05	48	63.7
9	CuBr	0.025	24	11.48
10	Cu Wire	-	6	0
11	Cu Wire	-	12	0
12	Cu Wire	-	24	0
13	Cu Wire	-	48	0
14	Cu Wire	0.05	6	32.55
15	Cu Wire	0.05	12	42.91
16	Cu Wire	0.05	24	78.76
17	Cu Wire	0.05	48	91.92
18	Cu Wire	0.025	24	33.63

Coumarin Azide (0.05 mmol), mPEG-alkyne (0.05 mmol), CuBr (0.05 mmol), Cu Wire (100 mmol) at 80 °C, anhydrous toluene and under inert atmosphere.

Trace copper was removed with heterogeneous adsorbent (silica gel), reaching a final Cu concentration of 2 ppm (detected by AA), which is well below the concentration allowed by the European Medicines Agency (EMA) pharmaceutical industry (15 ppm) [45].

Cu Wire overcomes some of the limitations of CuBr because it provides a simple way to remove copper from the final product and can be used in subsequent reactions, which has a great importance for biological systems where high levels of copper are not allowed. In addition, the yield results obtained using Cu Wire as catalyst in our experiments, as reported in Table 1, were more promising than the ones obtained for CuBr in toluene,

reaching a yield of 91.92% at 48 h. Díaz et al. [46] reported that the terminal alkynes and internal triazoles could bind to the copper wire surface through σ or π bonding and thereby enhance the CuAAC yield values.

However, as was the case with CuBr, PMDTA was necessary in order to lead the regioselective formation of 1,4-triazol. A possible explanation for the necessity of PMDTA when using the Cu wire catalyst could be that this compound promotes the comproportionation of Cu(+2) and Cu (0) towards the active Cu (I) species in toluene [47]. The residual concentration of copper in the final click product is very low, since the solid catalyst can be removed with simple work-up techniques.

Traditional organic solvents, such as toluene [27,47], could potentially cause various health and environmental problems due to their volatility and toxicity. Significant efforts have been devoted to the development of environmentally benign processes using green solvents. PEGs with MW > 2000 are hard crystalline solids with melting points around 63 °C at atmospheric pressure. Sorption of scCO₂ into a polymer can reduce its melting temperature (T_m) significantly below the one observed at atmospheric pressure. The T_m of the mPEG-alkyne in scCO₂ was between 37 and 43 °C, implying that at 35 °C the CO₂ is starting to act as a lubricant facilitating friction between the polymer chains and softening the polymer, allowing the mPEG-alkyne to start turning into a viscous liquid without the need for organic solvents or elevated temperatures [48]. In this sense, mPEG-alkyne could as main reagent and co-solvent when using scCO₂ as reaction media.

Considering the good physical and toxicological properties of scCO₂, we studied the CuAAC reaction between PEG and coumarin using different catalysts at different times and in the absence of a ligand or base using scCO₂ as reaction media, as shown in Table 2.

Table 2. Yield of CuAAC reaction with different catalysts in supercritical media.

Entry	Copper Source	C/A Molar Ratio ^a	Catalyst Loading (mg)	Time (h)	Yield (%)
1	CuBr	1	7.17	24	0
2	CuBr	1	7.17	48	0
3	Cu(CH ₃ COO) ₂ ·H ₂ O	0.5	5	6	11.74
4	Cu(CH ₃ COO) ₂ ·H ₂ O	0.5	5	12	14.20
5	Cu(CH ₃ COO) ₂ ·H ₂ O	0.5	5	24	82.32
6	Cu(CH ₃ COO) ₂ ·H ₂ O	0.5	5	48	90.12
7	Cu Wire	1	3.177	24	11.60
8	Cu Wire	10	31.77	24	84.54
9	Cu Wire	100	317.73	6	18.26
10	Cu Wire	100	317.73	12	56.78
11	Cu Wire	100	317.73	24	95.96
12	Cu Wire	100	317.73	48	97.80
13	Cu Plate	10	31.77	24	15.70
14	Cu Plate	100	317.73	6	0
15	Cu Plate	100	317.73	12	13.20
16	Cu Plate	100	317.73	24	23.07
17	Cu Plate	100	317.73	48	32.66
18	Cu/ β -SiC	0.1	0.32	24	16.15
19	Cu/ β -SiC	0.5	1.59	6	35.20
20	Cu/ β -SiC	0.5	1.59	12	82.30
21	Cu/ β -SiC	0.5	1.59	24	82.59
22	Cu/ β -SiC	0.5	1.59	48	90.90
23	Cu/ β -SiC	1	3.2	24	91.94
24	Pre-treated Cu Wire	100	317.73	24	97.14
25	Pre-treated Cu Plate	100	317.73	24	89.70

Coumarin Azide (0.05 mmol), mPEG alkyne (0.05 mmol) at 13 MPa, 35 °C. ^a Catalyst/Alkyne molar ratio.

Entries 1-6 correspond to the powder catalysts used in scCO_2 , CuBr and $\text{Cu}(\text{CH}_3\text{COO})_2 \cdot \text{H}_2\text{O}$. CuBr did not show activity in the conditions studied, probably due to the mild reaction conditions and the absence of the ligand. However, $\text{Cu}(\text{CH}_3\text{COO})_2 \cdot \text{H}_2\text{O}$ exhibited an efficient behavior in the same operative reactions conditions and with half the amount of catalyst. The C/A molar ratio of $\text{Cu}(\text{CH}_3\text{COO})_2 \cdot \text{H}_2\text{O}$ was optimized in a previous work [35] and was therefore set at a ratio of 0.5. The molar ratio for CuBr was one. As the reaction was not carried out within 48 h, the amount of catalyst was not further increased due to the difficulty in purification. This fact indicates that scCO_2 at 35 °C and 13 MPa leads to the decomposition of $\text{Cu}(\text{CH}_3\text{COO})_2 \cdot \text{H}_2\text{O}$ to different copper species with different oxidation states, such as Cu, Cu_2O and CuO, including the catalytically active species Cu(I) in CuAAC that arises from Cu_2O .

Subsequently, three heterogeneous catalysts were employed in the CuAAC reaction in scCO_2 , copper wire, the copper plate and copper impregnated in silicon carbide pellets. The proposed mechanism for CuAAC with scCO_2 catalyzed by metallic copper was from Cu(I) as could be seen in Section 2. 2 and the formation of Cu(I) from metal copper is also due to the unfilled valences of the surface atom.

Both heterogeneous catalysts used in the CuAAC reaction, in scCO_2 and in the absence of a ligand showed good catalytic behavior. The entries (7-8-11, 13-16, 18-21-22) were previous experiments used to determine the optimal amount of heterogeneous catalysts based on the yield obtained after 24 h. As can be noticed in Table 2, when the amount of Cu Wire, the Cu Plate and Cu/ β -SiC was increased, the reaction yield value increased too. For Cu Wire and the Cu Plate, the C/A molar ratio used to attain the maximum yield value was 100. However, for Cu/ β -SiC pellets, the reaction could be optimized for a catalyst load of 0.5 C/A molar ratio, as the yield was higher than 90%. As expected, an increase in catalyst loading translates into an increase in the yield.

The results in Table 2 reveal that Cu Wire, the Cu Plate and Cu/ β -SiC were more active than $\text{Cu}(\text{CH}_3\text{COO})_2 \cdot \text{H}_2\text{O}$ and CuBr. The yield obtained in the presence of Cu Wire was higher than those obtained with the Cu Plate and Cu/ β -SiC pellets. However, the amount of copper required to achieve approximately 90% of yield was much less in Cu/ β -SiC pellets than in Cu Wire. The method of purification of the residual copper in the final click product, using the heterogeneous catalyst, was a simple work-up technique (laboratory tweezers) and the residual copper in the sample did not exceed 1 ppm.

The Cu Plate was even less active than $\text{Cu}(\text{CH}_3\text{COO})_2 \cdot \text{H}_2\text{O}$, this may be due to the fact that the operating conditions using scCO_2 as the solvent were not selective and were not able to oxidize the copper plate and therefore did not generate the Cu (+1) ions, as could be verified by the XRD in Figure 2b. Nevertheless, the use of the pre-treated Cu Plate improved the reaction yield. The pre-treatment for Cu Wire did not have such a significant effect on the yield of the reaction. Therefore, it can be concluded that the three heterogeneous catalysts considered and scCO_2 as the reaction media offer a very promising result in the CuAAC model reaction.

2.4. Reusability of Heterogeneous Copper Structures in scCO_2

The recyclability of the heterogeneous catalyst was examined in the CuAAC model reaction as shown in Table 3. In addition, the copper leaching calculated from the weight difference was also studied. All the reactions were carried out at 35 °C and 13 MPa for 24 h in order to achieve the highest possible conversion. After each cycle, the surface of the catalysts was cleaned by rinsing with Milli-Q water. The study of the lifetime and level of reusability was considered for the copper plate without pre-treatment due to the low yields obtained in the previous section.

According to Table 3, copper leaching was observed for each cycle, however the leaching did not substantially affect the CuAAC reaction yield. With respect to the Cu/ β -SiC, it was only possible to reuse this catalyst up to a second cycle because the product remained attached to the pellets, blocking the access of fresh reactants to the active centers. In order to wash the catalytic surface, organic solvents (ethyl acetate) and water had to be

used under an ultrasonic bath at room temperature for 30 min. However, from the third cycle onwards, the catalytic activity was negligible.

Table 3. Reusability of the heterogeneous catalyst in scCO_2 .

Cycle		1	2	3	4	5
Copper Wire	Yield (%)	95.9	94.7	95.1	95.4	94.2
	Copper Leaching (%)	-	1.2	6	5	3.4
Cu/ β -SiC	Yield (%)	82.9	79.6	-	-	-
	Copper Leaching (%)	-	2	-	-	-
Pre-treated CW	Yield (%)	97.1	86.6	85.2	25.1	-
	Copper Leaching (%)	-	2.4	5.8	7	-
Pre-treated CP	Yield (%)	89.7	90.1	73.3	13.4	-
	Copper Leaching (%)	-	7.9	12.1	5.6	-

The cleaning of the pre-treated catalysts was conducted with considerable caution because the oxide layer formed in the pre-treatment disappeared after washing, although they showed a high catalytic efficiency up to the third cycle. From this cycle onwards, the structural stability of both pre-treated catalysts was affected. In the third cycle, based on the AA analysis, the amount of copper in the clicked product was 16.7 ppm, so the sample was purified using a chromatographic column of silica gel in order to remove residual copper.

To identify the stability of heterogeneous catalyst, SEM images were also obtained, as shown in Figure 7. Photographs suggest a slight but visible morphological change after five cycles, but despite these changes the overall activity of the Cu Wire still remains relatively high, not showing visible changes in the catalyst structure.

Copper Wire

Cu/ β -SiC

Copper Wire Pre-treated

Copper Plate Pre-treated

Figure 7. SEM images of heterogeneous catalysts in scCO_2 as solvent after five cycles.

The images of Cu/ β -SiC pellets show how the product adheres to the pellet, making it hard to clean the catalyst for another cycle. The pre-treated catalysts exhibited severe weathering and considerable changes in their structures. In fact, the SEM images showed the weathering and fracture of the copper plate from the second cycle onwards and from the third cycle for copper wire.

3. Materials and Methods

3.1. Materials

The following materials were used to carry out the different syntheses: AMMC (synthesis was published in the literature [49–52]) and methoxy mPEG-alkyne 2000 g/mol (Specific Polymers, Castries, France) were used without further purification. Copper wire with a diameter 0.25 mm, plates of copper with a thickness of 2 mm, copper (II) acetate monohydrate and copper (I) bromide were obtained from Sigma Aldrich (Spanish division, Madrid, Spain). Silicon carbide pellets were purchased of SICAT (Strasbourg, France). The solvents used were THF, toluene, and PMDTA which were obtained from Sigma-Aldrich. Carbon dioxide was provided by Air Liquide, Spain, with a purity of 99.8%.

3.2. Synthesis of Click Product in $scCO_2$

Amounts of 0.05 mmol of mPEG-alkyne and 0.05 mmol of AMMC were introduced together with the corresponding amount of catalyst in a high-pressure reactor, in the absence of ligands. Once the reactor was hermetically sealed, $scCO_2$ was pumped out until it reached a pressure of 13 MPa and the reactor was heated until 35 °C. Once the reaction was complete, the heating was switched off and the reactor depressurized with a flow rate of 3 L/min.

3.3. Synthesis of Click Product at Atmospheric Pressure

The procedure for synthesizing the click product at atmospheric pressure started with the introduction of the reagents into a 250 mL flask and then the mixture was stirred at 80 °C. The reagents added were coumarin azide (0.05 mmol), mPEGalkyne (0.05 mmol) and PMDTA (0.05 mmol), using 20 mL of anhydrous toluene as solvent under nitrogen inert atmosphere. The catalyst/alkyne molar ratio was fixed at 1 for the CuBr catalyst and at 100 for the Cu Wire catalyst. Once the reaction finished, a purification of the final product was carried out.

3.4. Click Product Characterization

Gel permeation chromatography (GPC) analyses were performed with a Viscotek chromatograph with two columns (Styragel HR2 and Styragel HR0.5) at 35 °C with a flow of 1 mL/min and THF as eluent. The calibration curves for GPC analysis were obtained with poly (ethylene glycol) standards (from Waters). All reactions were monitored by GPC, from which the rate of loss of starting reagents was determined and yields of the reaction for each particular CuAAC reaction were calculated based on the normalized weight distribution from GPC traces (GPCs are included in the Supplementary Material, Figures S4–S7). In addition, to characterize the final product, nuclear magnetic resonance (NMR), matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI TOF) and Fourier-transform infrared spectroscopy (FTIR) were carried out and the spectra are included in the Supplementary Material (Figures S1–S3) [33]. The amount of copper in the product after reaction and after purification was determined by atomic absorption spectrophotometry (AA), with an error of $\pm 1\%$.

3.5. Synthesis Cu/ β -SiC

Cu/ β -SiC was prepared by the traditional impregnation method. Firstly, the support was placed in a glass vessel and kept under a vacuum at room temperature (~ 25 °C) for 2 h to remove water and other impurities adsorbed on the structure. Secondly, an aqueous solution of $Cu(CH_3COO)_2 \cdot H_2O$ was poured drop by drop over the support, with the

appropriate amount of metal precursor, in order to obtain catalysts with Cu loadings of 6, 3 and 0.6 wt.%. Thirdly, the solvent was removed under a vacuum at 90 °C for 2 h and the pellets were dried at 120 °C overnight. After drying, the samples were calcined under N₂ (10 °C/min) at 600 °C for 4 h.

3.6. Pre-Treatment of Cu Wire and Cu Plate

Before the pre-treatment, the sample reducibility was studied by H₂-TPR. Analyses were conducted in a commercial Micromeritics AutoChem 2950 HP analyzer unit with a thermal conductivity detector (TCD). After loading 0.13 g, the sample was reduced by 5 v/v.% H₂ (10 mL/min) at a heating rate of 5 °C/min up to 900 °C. Then, the sample was cooled down and an oxidation step started, using synthetic air (10 mL/min) and a heating rate of 5 °C/min up to 900 °C. After oxidation, the reduction step was repeated to check the formation of the different copper oxides.

Pre-treatment of copper wire and the copper plate was carried out with the main objective to increase the reaction rate by generation of Cu (I) species on the surface of the wire and plate. Samples were pre-treated in a tubular quartz reactor (75 cm length divided at the middle into two parts with two different diameters, 1.5 and 0.7 cm, respectively). The feed systems consist in three lines, for the feeding of O₂/N₂ (synthetic air, 99.99% purity) used for oxidizing the sample, H₂ (99.99% purity) used to reduce the sample and N₂ (99.99% purity) used to keep the sample inert. After loading the sample, the sample was heated from room temperature to 400 °C at 6.67 °C/min in a continuous flow of synthetic air stream. After reaching 400 °C, the sample was kept at these conditions for 120 min with a synthetic air stream (10 mL/min). In the third stage the temperature was decreased to 200 °C while a stream of nitrogen (50 mL/min) was passed through the reactor with a rate of 10 °C/min. Finally, a hydrogen stream (10 mL/min) was passed through for 30 min.

3.7. Catalyst Characterization

The amount of copper in the catalyst was determined by AA, with an error of ±1%, using a SPECTRAA 220FS. All catalysts were also characterized before and after reactions by X-ray diffraction (XRD), with a Philips PW-1710 instrument, using Ni-filtered Cu K α radiation ($\lambda = 1.54056 \text{ \AA}$). The samples were scanned at a rate of $0.02^\circ \cdot \text{step}^{-1}$ over the range $20^\circ \leq 2\theta \leq 100^\circ$ (scan time 4 s per step) and the diffractograms were compared with the JCPDS-ICDD references. In addition, the Cu Wires, the Cu plates and the synthesized catalyst were depicted by means of scanning electron microscopy (SEM) by using an FEI QUANTA 250 with a wolfram filament operating at a working potential of 10 kV (FEI Company, Hillsboro, OR, USA).

3.8. Purification Steps

The purification process of the final product depended on the solvent (toluene or scCO₂) and catalyst (Cu Wire, Cu Plate, CuBr, Cu(CH₃COO)₂·H₂O and Cu/ β -SiC). The purification of the product obtained using toluene as reaction media started with the solvent removal using a rotary evaporator. Once click product were removed from the solvent traces; the next steps focused on the elimination of the catalyst. CuBr and Cu(CH₃COO)₂·H₂O were removed through a chromatographic column, whereas copper wire, the copper plate and Cu/ β -SiC were removed from the final product using laboratory tweezers. When scCO₂ was used as solvent only the catalyst elimination step was needed in the purification process.

4. Conclusions

The CuAAC reaction has been widely used in the synthesis of polymers. In this work, it is demonstrated that the ligand, solvent, copper catalyst and the interaction between catalyst and solvent had a significant effect on the CuAAC reaction. XRD showed the modification of the oxidation state of Cu(CH₃COO)₂·H₂O and Cu Wire in scCO₂, however CuBr, the Cu Plate and Cu/ β -SiC suffer no change. In addition, a pre-treatment procedure

for Cu Wire and the Cu Plate was developed based on TPR results, which increase the amount of Cu (+1) species on the surface of both catalysts, which was confirmed by XRD spectra and SEM images.

Activity in the CuAAC reaction was compared using toluene and scCO_2 as reaction media and different types of catalysts. From the study of this comparison, it can be concluded that, when toluene is used as a solvent, the yield values exhibited significant increase using Cu Wire instead of CuBr. In addition, PMDTA was necessary in order to carry out the reaction. In the case of using scCO_2 as reaction media, CuAAC was carried out properly with $\text{Cu}(\text{CH}_3\text{COO})_2 \cdot \text{H}_2\text{O}$, Cu Wire, pre-treated Cu Wire, a pre-treated Cu Plate and Cu/ β -SiC as catalysts. However, with the Cu Plate the yield value was low and with CuBr there was no conversion at all.

The recovery of the catalysts was studied, and it was found that Cu Wire could be used up to five cycles, pre-treated Cu Wire up to three cycles and the pre-treated Cu Plate and Cu/ β -SiC up to the second cycle without important decrease in the activity. The relationships of various aspects such as the solvents and catalysts proposed here suggest further modifications that can be made to improve the performance of the CuAAC reaction, confirming that the use of eco-friendly scCO_2 as reaction media is the most interesting alternative to organic solvents.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/catal12020194/s1>. Figure S1. (up) FTIR: (a) BMMC, (b) AMMC (down) ^1H -NMR Spectrum of AMMC. Figure S2: FTIR comparative between starting reagents mPEG-alkyne and AMMC (azido-coumarin) and click product, coumarin-PEG (CouPEG). Figure S3: ^1H NMR of coumarin azide and click product. Figure S4: MALDI TOFF MS of (a) starting mPEG-alkyne and (b) click product CouPEG. Figure S5: GPC of CuAAC reaction at 80 °C, inert atmosphere and toluene with Cu Wire. Figure S6: GPC of CuAAC reaction at 80 °C, inert atmosphere and toluene with CuBr. Figure S7: GPC of CuAAC reaction corresponding to the preliminary study in scCO_2 at 13 MPa and 35 °C. (a) Cu Wire at C/A = 1, 10 and 100, (b) the Cu Plate C/A = 10, 100, Cu/ β -SiC, C/A = 0.1, 0.5 and 1. Figure S7: GPC of CuAAC reaction corresponding to the assess of different catalysts in scCO_2 at 35 °C and 13 MPa. (a) Cu Wire (6, 12, 24, 48 h), (b) the Cu Plate (12, 24, 48 h), (c) Cu/ β -SiC (6, 12, 24, 48 h), (d) pre-treated Cu Wire (24 h), (e) the pre-treated Cu Plate (24 h) and (f) $\text{Cu}(\text{OAc})_2 \cdot \text{H}_2\text{O}$ (6, 12, 24 and 48 h).

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References

- Huisgen, R.; Mloston, G.; Langhals, E. The first two-step 1,3-dipolar cycloadditions: Non-stereospecificity. *J. Am. Chem. Soc.* **1986**, *108*, 6401–6402. [[CrossRef](#)]
- Rostovtsev, V.V.; Green, L.G.; Fokin, V.V.; Sharpless, K.B. A stepwise huisgen cycloaddition process: Copper(I)-catalyzed regioselective “ligation” of azides and terminal alkynes. *Angew. Chem.* **2002**, *41*, 2596–2599. [[CrossRef](#)]
- Kolb, H.C.; Finn, M.G.; Sharpless, K.B. Click Chemistry: Diverse Chemical Function from a Few Good Reactions. *Angew. Chem. Int. Ed.* **2001**, *40*, 2004–2021. [[CrossRef](#)]
- Meng, G.; Guo, T.; Ma, T.; Zhang, J.; Shen, Y.; Sharpless, K.B.; Dong, J. Modular click chemistry libraries for functional screens using a diazotizing reagent. *Nature* **2019**, *574*, 86–89. [[CrossRef](#)] [[PubMed](#)]
- Kolb, H.C.; Sharpless, K.B. The growing impact of click chemistry on drug discovery. *Drug Discov. Today* **2003**, *8*, 1128–1137. [[CrossRef](#)]
- Van Dijk, M.; Rijkers, D.T.S.; Liskamp, R.M.J.; van Nostrum, C.F.; Hennink, W.E. Synthesis and Applications of Biomedical and Pharmaceutical Polymers via Click Chemistry Methodologies. *Bioconjug. Chem.* **2009**, *20*, 2001–2016. [[CrossRef](#)]

7. Crescenzi, V.; Cornelio, L.; di Meo, C.; Nardecchia, S.; Lamanna, R. Novel Hydrogels via Click Chemistry: Synthesis and Potential Biomedical Applications. *Biomacromolecules* **2007**, *8*, 1844–1850. [\[CrossRef\]](#)
8. Nwe, K.; Brechbiel, M. Growing Applications of “Click Chemistry” for Bioconjugation in Contemporary Biomedical Research. *Cancer Biother. Radiopharm.* **2009**, *24*, 289–302. [\[CrossRef\]](#)
9. Sokolova, N.V.; Nenajdenko, V.G. Recent advances in the Cu (I)-catalyzed azide–alkyne cycloaddition: Focus on functionally substituted azides and alkynes. *RSC Adv.* **2013**, *3*, 16212–16242. [\[CrossRef\]](#)
10. Binder, W.H.; Sachsenhofer, R. ‘Click’ Chemistry in Polymer and Material Science: An Update. *Macromol. Rapid Commun.* **2008**, *29*, 952–981. [\[CrossRef\]](#)
11. Ellanki, A.R.; Islam, A.; Rama, V.S.; Pulipati, R.P.; Rambabu, D.; Krishna, G.R.; Reddy, C.M.; Mukkanti, K.; Vanaja, G.R.; Kalle, A.M.; et al. Solvent effect on copper-catalyzed azide-alkyne cycloaddition (CuAAC): Synthesis of novel triazolyl substituted quinolines as potential anticancer agents. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 3455–3459. [\[CrossRef\]](#) [\[PubMed\]](#)
12. Kazarian, S. Polymer Processing with Supercritical Fluids. *Polym. Sci.* **2000**, *42*, 78–101. [\[CrossRef\]](#)
13. Shieh, Y.-T.; Su, J.-H.; Manivannan, G.; Lee, P.; Sawan, S.; Spall, W. Interaction of supercritical carbon dioxide with polymers. I. Crystalline polymers. *J. Appl. Polym. Sci.* **1996**, *59*, 695–705. [\[CrossRef\]](#)
14. Prajapati, D.; Gohain, M. Recent Advances in the Application of Supercritical Fluids for Carbon–Carbon Bond Formation in Organic Synthesis. *Tetrahedron* **2004**, *60*, 815–833. [\[CrossRef\]](#)
15. Oakes, R.S.; Clifford, A.A.; Rayner, C.M. The use of supercritical fluids in synthetic organic chemistry. *J. Chem. Soc. Perkin Trans. 1* **2001**, *9*, 917–941. [\[CrossRef\]](#)
16. Grignard, B.; Schmeits, S.; Riva, R.; Detrembleur, C.; Lecomte, P.; Jérôme, C. First example of “click” copper(I) catalyzed azide-alkyne cycloaddition in supercritical carbon dioxide: Application to the functionalization of aliphatic polyesters. *Green Chem.* **2009**, *11*, 1525–1529. [\[CrossRef\]](#)
17. Grignard, B.; Calberg, C.; Jerome, C.; Detrembleur, C. “One-pot” dispersion ATRP and alkyne-azide Huisgen’s 1,3-dipolar cycloaddition in supercritical carbon dioxide: Towards the formation of functional microspheres. *J. Supercrit. Fluids* **2010**, *53*, 151–155. [\[CrossRef\]](#)
18. Zhang, W.; He, X.; Ren, B.; Jiang, Y.; Hu, Z. Cu(OAc)₂·H₂O—An efficient catalyst for Huisgen-click reaction in supercritical carbon dioxide. *Tetrahedron Lett.* **2015**, *56*, 2472–2475. [\[CrossRef\]](#)
19. Gracia, E.; García, M.T.; Borreguero, A.M.; de Lucas, A.; Gracia, I.; Rodríguez, J.F. Functionalization and optimization of PLA with coumarin via click chemistry in supercritical CO₂. *J. CO₂ Util.* **2017**, *20*, 20–26. [\[CrossRef\]](#)
20. Haldón, E.; Nicasio, M.C.; Pérez, P.J. Copper-catalysed azide-alkyne cycloadditions (CuAAC): An update. *Org. Biomol. Chem.* **2015**, *13*, 9528–9550. [\[CrossRef\]](#)
21. Chassaing, S.; Bénateau, V.; Pale, P. When CuAAC ‘Click Chemistry’ goes heterogeneous. *Catal. Sci. Technol.* **2016**, *6*, 923–957. [\[CrossRef\]](#)
22. Neumann, S.; Biewend, M.; Rana, S.; Binder, W.H. The CuAAC: Principles, Homogeneous and Heterogeneous Catalysts, and Novel Developments and Applications. *Macromol. Rapid Commun.* **2020**, *41*, 190–359. [\[CrossRef\]](#) [\[PubMed\]](#)
23. Hu, G.; Chen, J.; Fan, Y.; Zhou, H.; Guo, K.; Fang, Z.; Xie, L.; Wang, L.; Wang, Y. A promoted copper-catalysed Azide-alkyne cycloaddition (CuAAC) for broad spectrum peptide-engineered implants. *Chem. Eng. J.* **2022**, *427*, 130918. [\[CrossRef\]](#)
24. Meldal, M.; Diness, F. Recent Fascinating Aspects of the CuAAC Click Reaction. *Trends Chem.* **2020**, *2*, 569–584. [\[CrossRef\]](#)
25. Wang, Q.; Chan, T.R.; Hilgraf, R.; Fokin, V.V.; Sharpless, K.B.; Finn, M.G. Bioconjugation by Copper(I)-Catalyzed Azide-Alkyne [3 + 2] Cycloaddition. *J. Am. Chem. Soc.* **2003**, *125*, 3192–3193. [\[CrossRef\]](#) [\[PubMed\]](#)
26. Chan, T.R.; Hilgraf, R.; Sharpless, K.B.; Fokin, V.V. Polytriazoles as Copper(I)-Stabilizing Ligands in Catalysis. *Org. Lett.* **2004**, *6*, 2853–2855. [\[CrossRef\]](#)
27. Golas, P.L.; Tsarevsky, N.V.; Sumerlin, B.S.; Matyjaszewski, K. Catalyst Performance in “Click” Coupling Reactions of Polymers Prepared by ATRP: Ligand and Metal Effects. *Macromolecules* **2006**, *39*, 6451–6457. [\[CrossRef\]](#)
28. Fauché, K.; Cisnetti, F. Magnetic Fe₃O₄ nanoparticles bearing CuI-NHC complexes by an “auto-click” strategy. *Inorg. Chim. Acta* **2021**, *520*, 120312. [\[CrossRef\]](#)
29. Chassaing, S.; Kumarraja, M.; Sido, A.S.S.; Pale, P.; Sommer, J. Click Chemistry in CuI-zeolites: The Huisgen [3 + 2]-Cycloaddition. *Org. Lett.* **2007**, *9*, 883–886. [\[CrossRef\]](#)
30. Parveen, S.; Gurjaspreet, G.K.; Jandeep, S.; Harminder, S. Robust and Versatile Cu(I) metal frameworks as potential catalysts for azide-alkyne cycloaddition reactions. *Rev. Mol. Catal.* **2021**, *504*, 111432. [\[CrossRef\]](#)
31. Gurjaspreet, S.; Jasbhinder, S.; Akshpreet, S.; Jandeep, S.; Manoj, K.; Kshitiz, G.; Sanjay, C. Synthesis, characterization and antibacterial studies of schiff based 1,2,3-triazole bridged silatranes. *J. Organomet. Chem.* **2018**, *871*, 21–27. [\[CrossRef\]](#)
32. Jiang, Y.; He, X.; Zhang, W.; Li, X.; Guo, N.; Zhao, Y.; Xu, G.; Li, W. Metallic copper wire: A simple, clear and reusable catalyst for the CuAAC reaction in supercritical carbon dioxide. *RSC Adv.* **2015**, *5*, 73340–73345. [\[CrossRef\]](#)
33. Gracia, E.; García, M.; De Lucas, A.; Rodríguez, J.; Gracia, I. Copper wire as a clean and efficient catalyst for click chemistry in supercritical CO₂. *Catal. Today* **2018**, *346*, 65–68. [\[CrossRef\]](#)
34. Behl, G.; Sikka, M.; Chhikara, A.; Chopra, M. PEG-coumarin based biocompatible self-assembled fluorescent nanoaggregates synthesized via click reactions and studies of aggregation behavior. *J. Colloid Interface Sci.* **2014**, *416*, 151–160. [\[CrossRef\]](#)
35. López, S.; Gracia, I.; García, M.T.; Rodríguez, J.F.; Ramos, M.J. Synthesis and Operating Optimization of the PEG Conjugate via CuAAC in scCO₂. *ACS Omega* **2021**, *6*, 6163–6171. [\[CrossRef\]](#)

36. Alterovitz, S.A.; Woollam, J.A. Cubic Silicon Carbide (β -SiC). In *Handbook of Optical Constants of Solids*; Palik, E.D., Ed.; Academic Press: Burlington, MA, USA, 1997; pp. 705–707. [CrossRef]
37. García-Vargas, J.M.; Valverde, J.L.; Díez, J.; Sánchez, P.; Dorado, F. Preparation of Ni-Mg/ β -SiC catalysts for the methane tri-reforming: Effect of the order of metal impregnation. *Appl. Catal. B Environ.* **2015**, *164*, 316–323. [CrossRef]
38. Nguyen, D.L.; Leroi, P.; Ledoux, M.J.; Pham-Huu, C. Influence of the oxygen pretreatment on the CO₂ reforming of methane on Ni/ β -SiC catalyst. *Catal. Today* **2009**, *141*, 393–396. [CrossRef]
39. de Meester, P.; Fletcher, S.R.; Skapski, A.C. Refined crystal structure of tetra- μ -acetato-bis-aquodicopper(II). *J. Chem. Soc. Dalton Trans.* **1973**, 1973, 2575–2578. [CrossRef]
40. Herbert, M.; Montilla, F.; Galindo, A. Supercritical carbon dioxide, a new medium for aerobic alcohol oxidations catalysed by copper-TEMPO. *Dalton Trans.* **2010**, 39, 900–907. [CrossRef]
41. Mathew, K.; Zheng, C.; Winston, D.; Chen, C.; Dozier, A.; Rehr, J.J.; Ong, S.P.; Persson, K.A. High-throughput computational X-ray absorption spectroscopy. *Sci. Data* **2018**, *5*, 180151. [CrossRef]
42. Yang, M.; Zhu, J.J.; Li, J.J. Cubic assembly composed of CuBr nanoparticles. *J. Cryst. Growth* **2004**, *267*, 283–287. [CrossRef]
43. Sahle-Demessie, E.; Gonzalez, M.A.; Enriquez, J.; Zhao, Q. Selective Oxidation in Supercritical Carbon Dioxide Using Clean Oxidants. *Ind. Eng. Chem. Res.* **2000**, *39*, 4858–4864. [CrossRef]
44. Meldal, M. Polymer “Clicking” by CuAAC Reactions. *Macromol. Rapid Commun.* **2008**, *29*, 1016–1051. [CrossRef]
45. ICH Q3D Elemental Impurities | European Medicines Agency. Available online: <https://www.ema.europa.eu/en/ich-q3d-elemental-impurities> (accessed on 18 November 2021).
46. Díaz, D.; Punna, S.; Holzer, P.; McPherson, A.K.; Sharpless, K.B.; Fokin, V.V.; Finn, M.G. Click chemistry in materials synthesis. 1. Adhesive polymers from copper-catalyzed azide-alkyne cycloaddition. *J. Polym. Sci. Part A Polym. Chem.* **2004**, *42*, 4392–4403. [CrossRef]
47. Bell, C.A.; Jia, Z.; Perrier, S.; Monteiro, M.J. Modulating catalytic activity of polymer-based CuAAC “click” reactions. *J. Polym. Sci. Part A Polym. Chem.* **2011**, *49*, 4539–4548. [CrossRef]
48. López, S.; Ramos, M.J.; García-Vargas, J.M.; García, M.T.; Rodríguez, J.F.; Gracia, I. Carbon dioxide sorption and melting behaviour of mPEG-alkyne. *J. Supercrit. Fluids* **2021**, *171*, 105182. [CrossRef]
49. Oh, C.; Yi, I.; Park, K.P. Nucleophilic vinylic substitution of halocoumarins and halo-1,4-naphthoquinones with morpholine. *J. Heterocycl. Chem.* **1994**, *31*, 841–844. [CrossRef]
50. Sivakumar, K.; Xie, F.; Cash, B.M.; Long, S.; Barnhill, H.N.; Wang, Q. A Fluorogenic 1,3-Dipolar Cycloaddition Reaction of 3-Azidocoumarins and Acetylenes. *Org. Lett.* **2004**, *6*, 4603–4606. [CrossRef]
51. Kusanur, R.A.; Kulkarni, M.V.; Kulkarni, G.M.; Nayak, S.K.; Row, T.N.G.; Ganesan, K.; Sun, C.-M. Unusual anisotropic effects from 1,3-dipolar cycloadducts of 4-azidomethyl coumarins. *J. Heterocycl. Chem.* **2010**, *47*, 91–97. [CrossRef]
52. Chaurasia, C.S.; Kauffman, J.M. Synthesis and fluorescent properties of a new photostable thiol reagent “BACM”. *J. Heterocycl. Chem.* **1990**, *27*, 727–733. [CrossRef]