Journal of Innovative Technology, Vol. 2, No. 2, pp. 9-13, September 2020
http://doi.org/10.29424/JIT.202009_2(2).0002

Synthesis of Curcumin Derivative for Ethylenediamine Sensing

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ABSTRACT

Amines are an important class of molecules that are ubiquitous in biology, pharmaceuticals, and industry. Because some amines may be harmful to human health, there is a need for sensitive and selective detection of amines in the fields of public health, food safety, and environmental monitoring. This study synthesized bis-cholesteryl appended curcumin (BCC) from curcumin and cholesteryl chloroformate. When BCC was added to various solvents, it was found that it only formed a gel with ethylenediamine (EDA), indicating that BCC is highly selective in the detection of EDA. The gelation properties of BCC with amines was further investigated under ultrasonic stimulation and explored for amines with different chain lengths. SEM analysis showed that the BCC-EDA molecules self-assembled into dense fibers. It was inferred that the secondary forces among the molecules, such as van der Waals force, polar attraction, and π-π stacking, played an important role in gelation. Based on this evidence, a self-assembly mechanism for gel formation was proposed. The color of BCC also changed from bright yellow to deep red depending on the EDA concentrations used. These results indicate that BCC features dual-detection characteristics with regard to EDA.

Keywords: Curcumin derivatives, Cholesteryl, Ethylenediamine, Gel, Sensing.

1. INTRODUCTION

Amines are ubiquitous and found naturally in many forms such as amino acids and neurotransmitters in all organisms. They are easy to escape into the environmental from industrial emission and food spoilage. Industrial wastewater, agriculture waste incineration, and automobile exhausts are main sources of atmospheric amines. Most amines are toxic and can irritate the skin, mucous membranes, and respiratory tracts through inhalation, ingestion or direct contact. It is harmful to human health to inhale amines in excess of 10 ~ 20 mg L^{-1} (Ayad and Torad 2010). Therefore, there is an urgent need for sensitive and selective detection of amines with low concentration due to increasing demands from public health regulation, food safety, environmental monitoring, and other related social fields such as agrochemical, automotive chemical, and cosmetic industries. There are several technical hurdles to overcome when developing a system for sensitive and rapid detection of amines in industrial and food quality control situations. For the detection of amines, liquid chromatography tandem mass spectrometry is preferred because this has high precision (Latorre-Moratalla, *et al*. 2009). However, these methods require expensive instrumentation and a long period of time for a complete analysis.

Manuscript received June 1, 2020; revised August 7, 2020; accepted August 7, 2020.

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Gao *et al*. (Gao, *et al*. 2016) developed a fluorescent sensor of 2- (2-hydroxyphenyl) quinazolin-4 (3H) -one aggregation-caused (HPQ-Ac) that detected amines through a light-up model. The portable HPQ-Ac sensor was easily prepared by depositing HPQ-Ac directly on filter paper and was able to exhibit fluorescence by exposure to amine vapor of various volatile organic compounds. Protecting the phenoxy group with an acetyl group in HPQ-Ac could effectively quench its fluorescence, by destroying the intramolecular hydrogen bonding process. After reacting with the amine vapor to break the acetyl bond of HPQ-Ac, the HPQ displayed fluorescence through its intramolecular hydrogen bonds. Hawker scholars synthesized Meldrum's activated furan (MAF) for colorimetric detection of amines in the forms of solution, solid, and vapor (Diaz, *et al*. 2017). MAF was able to sense primary and secondary amines by virtue of their reaction to lead to ring opening of MAF, which is a fast and a noticeable color change, easily discernible by the naked eye.

Curcumin is a natural product extracted from Curcuma longa plants (Ismail, *et al*. 2016), which have a wide range of biological applications, such as antioxidants, anti-inflammatories, anti-cancer drugs, radio-sensitization, radiation protection, and renal activity protection. These properties are attributed to its dual antioxidant activity, which can clean reactive oxygen species and reactive nitrogen species. Recently, curcumin has received increasing attention in the application of sensors. For example, curcumin was used in electrochemical sensors as an analytical reagent for the determination of boron, protein, amoxicillin, nucleic acid sensing, and β-cyclodextrin, etc. (Ding, *et al*. 2017; Qin, *et al*. 2018). In addition, curcumin has been used as a sensor for fluoride ions, cyanide ions (Venkataraj, *et al*. 2017), cysteine (Pang, *et al*. 2017), and pH (Pávai, *et al*. 2016). However, thus far there is no data or literature showing curcumin as an application for amine sensing.

The technology that small molecules self-assemble into a supramolecular structure has become a popular approach in the bottom-up fabrication of new materials (Bishop, *et al*. 2009; Deindörfer, *et al*. 2006; Fialkowski, *et al*. 2006; Shevchenko, *et al*. 2006; van der Laan, *et al*. 2002; Whitesides and Grzybowski 2002). Particularly, cholesterol-based low molecular weight organo-gelators have attracted great attention because of their versatility in gelation and the diversity of their structures. In our previous studies, the self-assembly of mono- and bis-cholesteryl-appended single gel systems based on isosorbide (Balamurugan, *et al*. 2016), azobenzene (Balamurugan, *et al*. 2014; Kuo, *et al*. 2017), and pyridine (Rizkiana, *et al*. 2015) conjugates have been investigated. These gelation molecules feature functions that immobilize organic liquids. In this study, a bis-cholesteryl appended curcumin (BCC) is synthesized for detection of various amines. BCC can only form a gel with ethylenediamine (EDA), revealing its high selectivity. Besides this gelation, the color of BCC changes from bright yellow to deep red when it comes into contact with EDA, which implies that BCC has dual-detection characteristics.

2. EXPERIMENT

2.1 Synthesis of BCC

BCC was synthesized as reported elsewhere (Rathinam, *et al*. 2020). About 1 g of curcumin (95%, Alfa Aesar) was placed in a 250 mL double-neck round bottom flask and dissolved in dichloromethane (50 mL). To this solution, 0.66 g of dimethylaminopyridine (DMAP, 99%, Alfa Aesar) was added and stirred well. To this mixture, a solution of cholesteryl chloroformate (3.65 g, 97%, Acros Organics) dissolved in dichloromethane was added dropwise over 30 min. The solution in the flask was stirred constantly overnight at room temperature. After completion of the reaction, the solution was transferred to a separating funnel and washed with dilute HCl, aqueous sodium bicarbonate solution, and water, respectively. Then dichloromethane was removed by distillation using a rotary evaporator. The obtained crude product was purified by washing with diethyl ether, as well as re-precipitation in ethanol-water.

2.2 Gelation Test

A pre-calculated weight of gelators (3 mg) and a measured volume of various solvents (0.1 mL) were placed in a glass vial, and the system was heated until all solid materials were dissolved. The solution was then slowly cooled to room temperature, and the test tube was inverted to investigate gel formation. Gel formation was regarded as gelation. If gelators could be dissolved in the solution, it was regarded as a soluble system. However, the systems in which the gelators could not be dissolved, even at boiling point of the solvent, were considered to be insoluble systems. These gels were also used to study the effects of external stimuli such heat and ultrasonication.

2.3 Measurements

Nuclear magnetic resonance (NMR) spectra were obtained using a Bruker AMX-400 high-resolution NMR spectrometer, and the chemical shifts were reported in ppm with tetramethylsilane as an internal standard. Fourier transform infrared (FT-IR) spectra were recorded using a Fourier transform infrared spectrophotometer (Spectrum one, PerkinElmer). The phase transition was analysed using a differential scanning calorimeter (DSC 6000, PerkinElmer) operated at a heating rate of 10 °C/min under a N_2 flow. The sample morphologies were characterized using field-emission scanning electron microscopy (SEM, S4800-I, Hitachi). The xerogel for SEM analysis was prepared by vacuum freeze-drying of the gel formed in the solvent at the critical gelation concentration (CGC) for 12-24 h.

3. RESULTS AND DISCUSSION

The BCC was synthesized by reacting curcumin with cholesteryl chloroformate in presence of DMAP in dichloromethane. Figure 1 shows the ^{1}H-NMR spectra of curcumin, cholesteryl chloroformate, and BCC, respectively. The spectra exhibited characteristic chemical shifts: 4.6 ppm (s, 2H, O=C–C$\underline{H}_2$–C=O), 7.5-7.7 (m, 2H, C$\underline{H}$=C$\underline{H}$), 7.1-7.4 ppm (m, 3H, Ar–$\underline{H}$), 3.9 ppm (m, 8H, –O–C$\underline{H}_3$ and O–C$\underline{H}$–), 5.4 (t, 2H, C$\underline{H}$–CH$_2$ in cholesteryl), 0.8 ~ 2.1 ppm (m, all methyl protons in cholesteryl unit). Figure 2 presents the FTIR spectra of these chemicals. All spectra revealed an absorption band around 2800 ~ 3000 cm^{-1} representing the C-H stretching vibrations. The strong absorption band at 1755 cm^{-1} in the spectra of cholesteryl chloroformate and BCC corresponded to the COO stretching vibrations. Absorption at 1625 cm^{-1} for the CO stretching vibrations appeared in the spectra of both curcumin and BCC. The band at about 3000 ~ 3400 cm^{-1} for the O-H stretching vibrations was observed in the curcumin spectrum, but not in the BCC spectrum. According to these above-mentioned observations, the BCC was synthesized successfully from curcumin and cholesteryl chloroformate.

BCC was tested to determine whether it could form a gel with various solvents. After heating and cooling, the sample bottles were turned upside down to examine whether the solution in the bottles could flow. If the solution inside could not flow, it was defined as a gel. BCC features an extremely low solubility in alcohols, such as methanol, ethanol, propanol, isopropanol, butanol, hexanol and octanol, and the solutions occur precipitation after the period of heating and cooling. Although BCC has high solubility in THF, DMF, DMSO, chloroform, m-cresol, xylene, and chlorobenzene, these solutions cannot form a gel. BCC was soluble in all amines; however, BCC failed to form a gel in any amine except EDA due to the relatively higher solubility with other amines. The critical gelation concentration of BCC-EDA was found to be 20 g/L. Repeated heating and cooling cycles did not affect the sol-to-gel process. This result indicates that BCC can act as a sensor to detect EDA through selective gelation.

In order to investigate the gelation mechanism, three different diamines with different carbon chain lengths (1,5-Pentanediamine (PeDA), 1,3-Propanediamine (PrDA), and EDA) and various amount of curcumin, cholesteryl chloroformate, and BCC were tested. The experimental results are shown in Table 1. Cholesteryl chloroformate formed a gel in three kinds of amines, shown in Fig. 3. The determined critical gelation concentrations of cholesteryl chloroformate-amine were 20, 150, and 300 mg/L for EDA, PrDA, and PeDA, respectively, increasing with length of amine carbon chain. No matter the amount used, curcumin could not form a gel with the three amines (Fig. 4). Only BCC formed a gel with EDA, but not with the two amines,

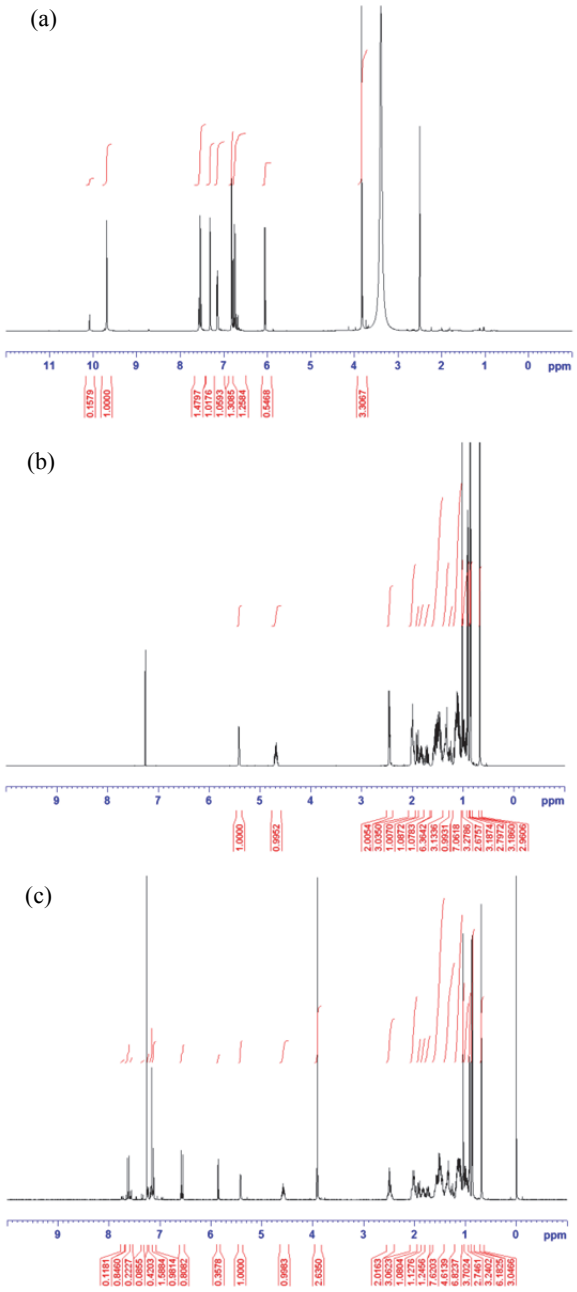

Fig. 1 **^{1}H-NMR spectra of (a) curcumin, (b) cholesteryl chloroformate, and (c) BCC in $CDCl_3$.**

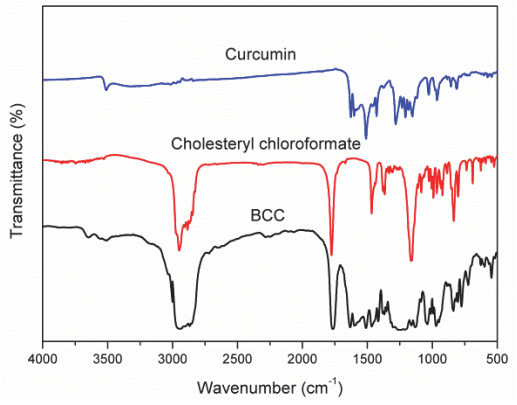

Fig. 2 **FTIR spectra of curcumin, cholesteryl chloroformate, and BCC.**

Table 1 Gelation properties of cholesteryl chloroformate, curcumin, and BCC in various diamines

	EDA (0.1ml)			
	2 (mg)	15 (mg)	30 (mg)	>100 (mg)
Cholesteryl chloroformate	gel	gel	gel	gel
Curcumin	no	no	no	no
BCC	gel	gel	gel	gel

	PrDA (0.1ml)			
	2 (mg)	15 (mg)	30 (mg)	>100 (mg)
Cholesteryl chloroformate	no	gel	gel	gel
Curcumin	no	no	no	no
BCC	no	no	no	no

	PeDA (0.1ml)			
	2 (mg)	15 (mg)	30 (mg)	>100 (mg)
Cholesteryl chloroformate	no	no	gel	gel
Curcumin	no	no	no	no
BCC	no	no	no	no

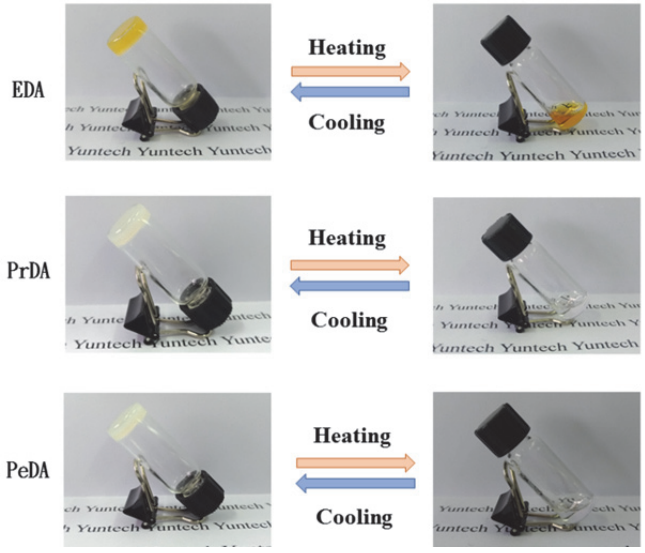

Fig. 3 **Photo images of gelation test for cholesteryl chloroformate (3 mg) in various diamine (0.1 mL).**

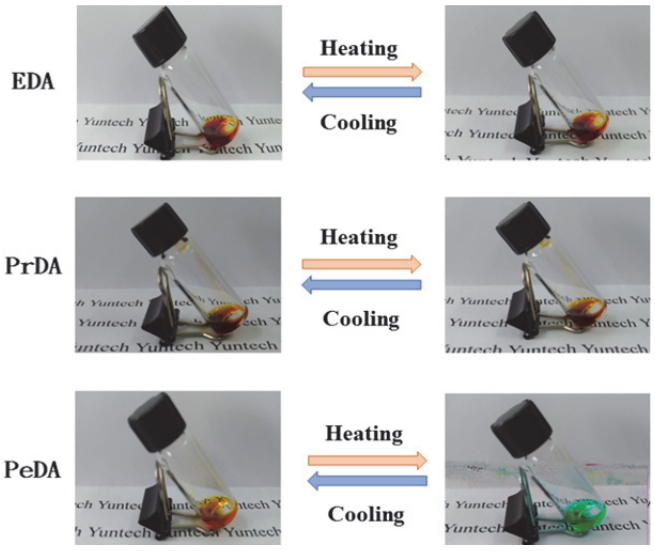

Fig. 4 **Photo images of gelation test for curcumin (3 mg) in various diamine (0.1 mL).**

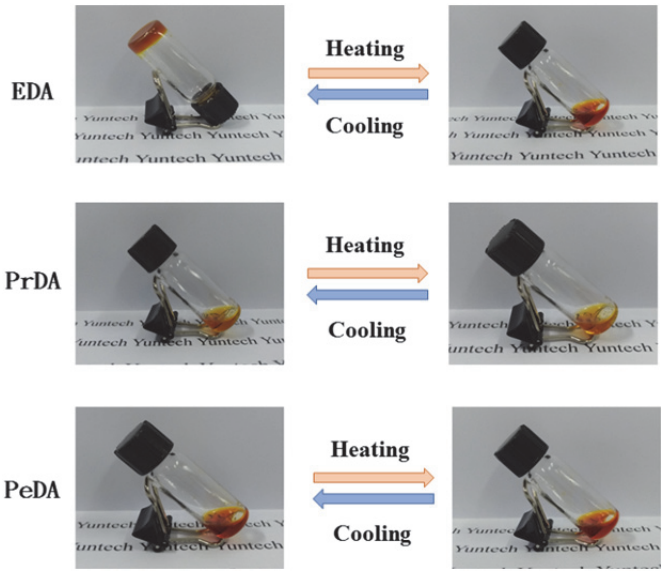

Fig. 5 Photo images of gelation test for BCC (3 mg) in various diamine (0.1 mL).

even if 100 mg of BCC was added (Fig. 5). As a result, BCC exhibits selective detection of EDA through curcumin modification to cholesteryl chloroformate. The mixture of BCC and EDA was also tested by ultrasonication. Even after a long time, BCC would not dissolve in EDA using ultrasonication at room temperature. The color of the solution turned to black and red, but no gel formed. Figure 6 shows the DSC curves of the BCC-EDA. A weak endothermic peak at 42°C can be observed in the heating curve, whereas a weak exothermic peak around 51 °C appears in the cooling curve. These peaks are related to the melting and gelation processes of the BCC-ED. The hysteresis behavior (ΔT = 9°C) displays the existence of molecular relaxation during the phase transition between liquid and gel.

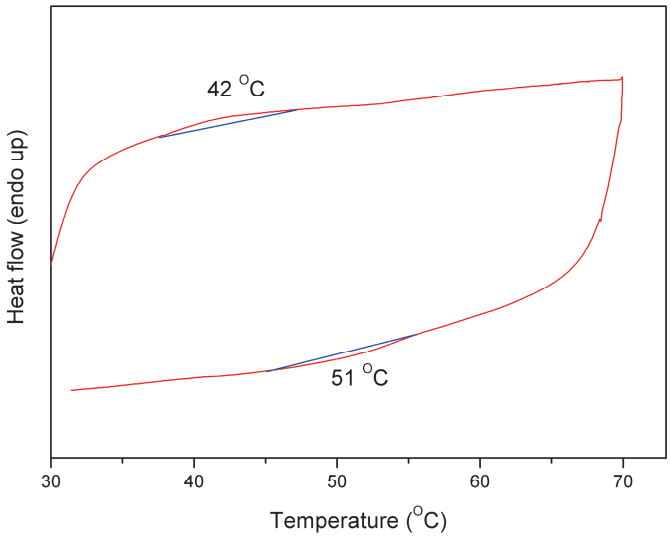

Fig. 6 DSC curves of BCC-EDA gel.

Figure 7 shows the SEM image of the BCC-EDA xerogel, which presented one-dimensional fibrous aggregates with a length of a few microns and a diameter of 100 ~ 200 nm. Fibrous aggregates were entangled forming a network. Observing the BCC molecular structure, the –OCOO- group showed a polar attraction with $–NH_2$. Moreover, the benzene groups of the curcumin unit and the cycloalkanes of the cholesteryl unit contributed a π-π stacking force and van der Waals force, respectively. The attraction forces can induce regular self-assembly of both BCC and amines. Too long chain in the amine structure may hinder the self-assembly process of BCC molecules. As a result, only EDA can form a gel with BCC. Besides gelation, the color also changes when BCC is mixed with various EDA. Figure 8 shows how the color of the mixture changes from light yellow to dark yellow, then continues to deepen into a dark red as the EDA concentration increases from 10^{-1} to 10 M in dichloromethane. This means that BCC can also detect EDA by spectral analysis.

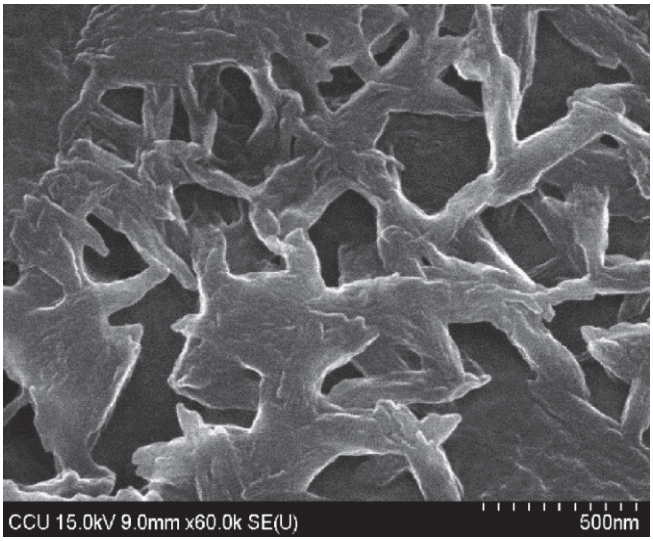

Fig. 7 SEM image of BCC-EDA xerogel.

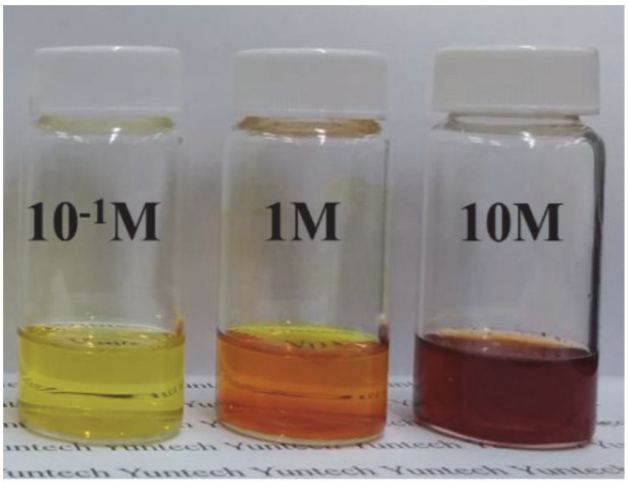

Fig. 8 Photo images of various EDA (0.5 mL, in dichloromethane) concentrations in BCC (5 mL, 1 mM, in dichloromethane).

4. CONCLUSIONS

In summary, BCC was successfully synthesized for use in detection of amines. This was confirmed by H-NMR and FTIR. The BCC formed a gel with EDA but not with other amines, revealing high selectivity. The sol-gel transition of BCC-EDA can be repeated for several cycles through a heating and cooling process. The formation of a gel was attributed to the secondary forces among the molecules, such as van der Waals force, polar attraction, and π-π stacking in the mixture. Different concentrations of EDA caused color changes in the BCC, ranging from bright yellow to deep red. BCC successfully detected EDA by both gelation and colorimetric methods.

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