博士論文

Antioxidant and adhesive properties of bio-inspired gallol polymers

（ガロール基をもつ生体模倣高分子の抗酸化性と接着性評価）

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1. Introduction.

Polyphenols are defined as the chemicals with two or more phenolic groups and most of them contain catechol and gallol groups in their chemical structures.[1] Nowadays, polyphenols enjoy an increasing recognition not only by the scientific community but also by the general public because of their presence and abundance in fruits, tea leaf, vegetables and beverages, whose regular consumption has been claimed to be beneficial for human health.[1,2] Polyphenols also exist in mussel as a form of polyphenolic protein which plays an important role in adhesion to the rocks.[3]

Antioxidants can scavenge reactive oxygen species (ROS) including radical and nonradical oxygen such as $HO^{\bullet}$, $NO^{\bullet}$, O_2, and HOCl derived from biomolecules including low-density lipoproteins, proteins, and oligonucleic acids. All of these species can have deleterious effects on human health.[1,4] Antioxidants such as polyphenols and/or polyphenol-containing compounds prevent or reduce oxidative stress-related chronic diseases and age-related disorders such as cardiovascular disease, carcinogenesis, Alzheimer disease as well as skin deterioration. Therefore, the use of antioxidants capable of scavenging ROS has attracted growing interest.[1]

Adhesives play an outstanding role in our daily life, being widely used in many applications including housing construction, packaging, labeling, and aerospace industry.[4] Specific adhesives that can be used underwater need to be explored because the adhesives used in medical applications are in great demand. However, underwater adhesives present several technical challenges. The adhesives interact with water instead of forming the adhesive bonds to the surfaces or cohesive bonds within the bulk materials when applied to the submerged substrates. Most man-made adhesives do not work well when tasked with such underwater bonding. By contrast, tunicate offers an effective way for the underwater adhesive by using adhesive proteins including the gallol moieties.[5] Therefore, the use of materials containing gallol moieties for the adhesive applications is also interesting.

Although the literature contains a number of studies related to the synthesis and application of catechol polymers, there are only a few about gallol polymers. My research focuses on the antioxidant and adhesive properties of bio-inspired gallol polymers. Throughout the following two sections, I will show that gallol polymers are the potential materials with high antioxidant, adsorption, and adhesive properties, which can be a candidate for the future food packing and bio-adhesive applications.

2. Antioxidant and adsorption properties of gallol polymers.

Catechol polymers become a popular topic in materials science because of their radical-scavenging and adsorption property. Hasegawa and coworkers synthesized the catechol polymers with high H_2O_2 scavenging ability.[6] Detrembleur and coworkers synthesized the poly(ionic liquids) containing catechol moieties which showed redox activity and adsorption

properties.[7] Sutherland and coworkers synthesized catechol-inspired homopolymers which had high adsorption properties on zinc surface.[8] Gallol polymers containing similar chemical structures as catechol polymers also might have good antioxidant and adsorption properties. This inspired me to synthesize the polyvinylgallol (PVGal).

At first, I carried out the reversible addition-fragmentation chain transfer polymerization of 3,4,5-trimethoxystyrene (TMS) and subsequent demethylation to obtain PVGal.[8] The molecular weight of poly(3,4,5-trimethoxystyrene) (PTMS) ranged from 5.4 to 53.4 kg mol^{-1}. Cyanomethyl dodecyl trithiocarbonate was selected as the chain transfer agent. Demethylation of PTMS was performed with boron tribromide to yield PVGal. PVGal with the degree of polymerization of 115 is abbreviated as $PVGal_{115}$. $PVCat_{111}$ and $PVPh_{115}$ were also prepared by the same method using 3,4-dimethoxystyrene and 4-methoxystyrene, respectively, as a monomer.

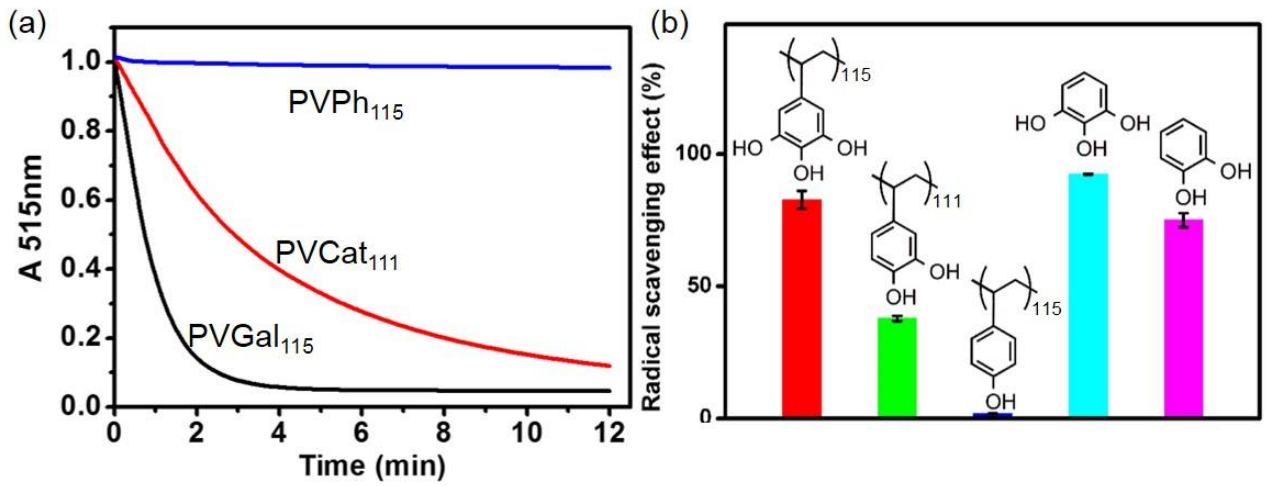


Figure 1 (a) Decoloration kinetic curves of the phenolic polymers, $PVGal_{115}$, $PVCat_{111}$ and $PVPh_{115}$ (250 μM on monomer basis). (b) The antioxidant activity of phenolic polymers (250 μM on monomer basis) and the corresponding small-molecule phenolic compounds (250 μM) at 2 min in the presence of DPPH radicals. All experiments were performed in triplicate. The error bars represent the standard deviation.

Then, I investigated the antioxidant activities of $PVGal_{115}$, $PVCat_{111}$ and $PVPh_{115}$ by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) antioxidant assays. Visual inspection of the color of the DPPH solutions containing the phenolic polymers provided a qualitative measure of antioxidant activity. The addition of PVGal with DPPH radicals resulted in rapid decoloration from purple to yellow, whereas in the case of PVCat, the color was change slowly, and PVPh showed no decoloration, suggesting that the antioxidant activity of PVGal was the highest

among these three phenolic polymers.

I next quantitatively evaluated the decoloration kinetics of DPPH by phenolic polymers on the basis of UV–Vis absorption spectra (Figure 1a). The spectra of the DPPH radicals exhibited an absorbance peak at 515 nm, which progressively disappeared with time. This decrease of absorbance at 515 nm corresponded to the scavenging activity of the tested phenolic polymers. The radical scavenging of $PVGal_{115}$ was faster than those of $PVCat_{111}$ and $PVPh_{115}$ (Figure 1). These results can be explained by the chemical structures of gallol and catechol. Gallol has three OH groups, while catechol just has two OH groups, which indicate that gallol has more possibility to scavenge radical than catechol. Additionally, the average redox potentials of gallol and catechol polymers are 0.48 V and 0.31 V, respectively, which also suggest that gallol polymers are easier to be oxidized than catechol polymers.[7]

Then, I investigated the adsorption of phenolic polymers including $PVGal_{115}$, $PVCat_{111}$ and $PVPh_{115}$ on selected metals and SiO_2 substrates (Figure 2). Adsorption of $PVGal_{115}$ on Au substrates was tested by quartz crystal microbalance (QCM). After 10 min static adsorption on Au substrates, the phenolic polymers adsorbed more than the corresponding small-molecule phenolic compounds (phenol, pyrocatechol and pyrogallol) because polymers have the multivalent effect and advantage on the entropic barrier to adsorption. The adsorbed amounts of $PVGal_{115}$ on Au, Ni, and Al substrates were all higher than those of the $PVCat_{111}$. The reasons of the difference between PVGal and PVCat are twofold. One is the larger number of hydrogen-bonding between gallol and substrates compared to that between catechol and substrates. The other is stronger attractive electrostatic interactions between deprotonated gallol and substrates than those of catechol. Moreover, PVGal showed much more adsorption amount on SiO_2, which is known as a challenging substrate for adsorption, than PVCat and PVPh. The adsorptions on the SiO_2 surface were lower than those on metal surfaces because hydrogen-bonding, coordination and/or van der waals interactions between gallol and the SiO_2 surface was weaker than the interactions between gallol and metal surfaces. These results suggest that the gallol polymers can be a potential building block with better adsorption performance than catechol polymers for coating and adhesives.

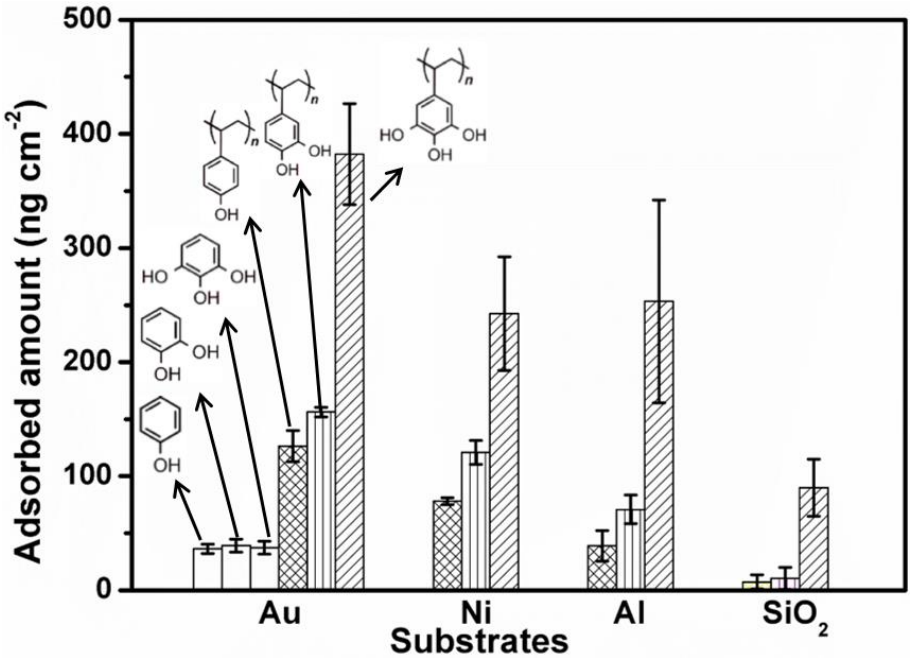
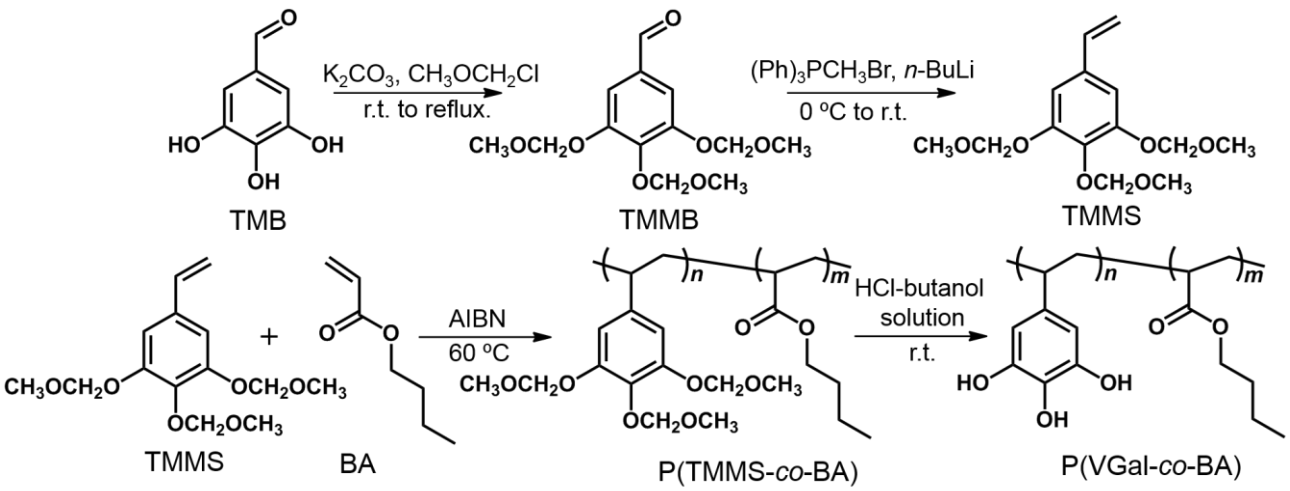


Figure 2. Adsorption of $PVGal_{115}$, $PVCat_{111}$, and $PVPh_{115}$ on Au, Ni, Al, and SiO_2 substrates monitored by QCM. The small-molecule phenolic compounds (phenol, pyrocatechol, and pyrogallol) were tested only on Au substrates.

3. Underwater adhesive of gallol copolymers.

The man-made glues often fail when trying to stick in water because of hydration-induced softening and dissolution. The wound healing process of tunicate provided an insight to overcome the problems of current adhesives and inspired me to synthesize gallol polymers for an underwater adhesive. However, PVGal were too fragile to be used as adhesive, so I tried to synthesize a soft gallol copolymer (P(VGal-*co*-BA)).

Methoxy groups have been often used as a protecting group in the synthesis of phenolic polymers. However, the deprotection step requires a harsh condition using boron tribromide (BBr_3), whicn can cleave ester group due to its strong reactivity. This harsh deprotection step made the copolymerization with acrylates impossible. Although silyl protecting groups also can be utilized for the synthesis of the catechol polymers, they are not suitable for the synthesis of polymers containing a large amount of vinylgallol units because of the steric hindrance of silyl-protected monomers. I found that the methoxymethyl (MOM) protecting method was suitable for the synthesis of soft gallol copolymer (poly(vinylgallol-*co*-*n*-butyl acrylate), P(VGal-*co*-BA) (Scheme 1)). This method can also be utilized to synthesize poly(vinylcatechol-*co*-*n*-butyl acrylate) P(VCat-*co*-BA), and poly(vinylphenol-*co*-*n*-butyl acrylate) P(VPh-*co*-BA). It can be concluded that the MOM protecting method is a versatile method to synthesize phenolic polymers bearing gallol, catechol, and phenol groups.



Scheme 1. Synthetic route of the P(VGal-*co*-BA) copolymer by MOM protecting method.

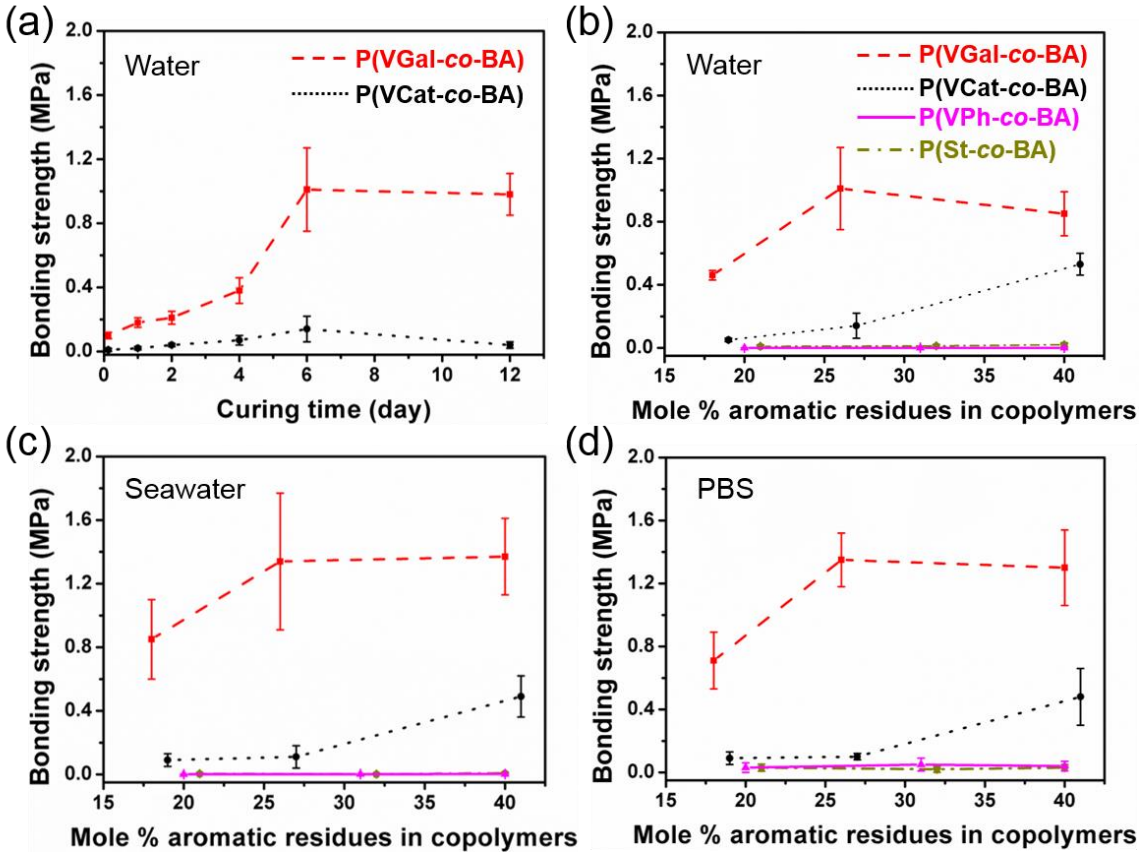


Figure 3. (a) Effect of curing time on bonding strength with P(VGal$_{26\%}$-*co*-BA$_{74\%}$) and P(VCat$_{27\%}$-*co*-BA$_{73\%}$) under water condition. Bonding strengths of P(VGal-*co*-BA), P(VCat-*co*-BA), P(VPh-*co*-BA) and poly(styrene-*co*-*n*-butyl acrylate) P(St-*co*-BA) after 6 days under water (b), seawater (c), PBS (d) conditions. Error bars indicates standard deviation, n ≥ 5.

In order to investigate the gallol copolymers used for adhesive applications, I studied the adhesive properties of P(VGal-*co*-BA), P(VCat-*co*-BA), P(VPh-*co*-BA) and P(St-*co*-BA) on polished aluminum substrates under water, seawater, and phosphate-buffered saline (PBS) by lap shear adhesion test (Figure 3).

First, I investigated the effect of curing time on bonding strength (Figure 3a). Bonding strength of P($VGal_{26\%}$-*co*-$BA_{74\%}$) in an underwater condition increased as the curing time proceeded and reached saturation (1.01 ± 0.26 MPa) at approximately 6 days (Figure 3a), while bonding strength of P($VCat_{27\%}$-*co*-$BA_{73\%}$) was relatively low and reached 0.14 ± 0.08 MPa even after 6 days curing. 6 days were selected as the optimized curing time for the later bonding strength test in wet conditions.

P(VGal-*co*-BA) copolymers have higher bonding strength than P(VCat-*co*-BA) in water, seawater, and PBS conditions (Figure 3b, 3c, and 3d). In general, bonding strength is the outcome of interfacial and cohesive interactions. In my case, the stronger bonding strength of P(VGal-*co*-BA) is due to slightly higher interfacial interactions between P(VGal-*co*-BA) and substrates, and stronger cohesive interactions inside P(VGal-*co*-BA).

4. Conclusion

In summary, I synthesized polyphenol-inspired polymers containing gallol groups. The antioxidant activity of this synthesized PVGal was higher than that of PVCat. The adsorption of PVGal on Au, Ni, Al, and SiO_2 substrates was higher than that of PVCat and PVPh. Moreover, the P(VGal-*co*-BA) copolymer, which was synthesized using the MOM protecting method, showed greater bonding strength than P(VCat-*co*-BA) and P(VPh-*co*-BA) under all conditions tested. These results help attest to the value of using blueprints inspired from the tunicate when designing new materials. Such a kind of gallol polymers may aid the development of the antioxidant and underwater adhesive needed for industrial or biomedical applications.

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Publications

(1) Kan Zhan, Hirotaka Ejima, Naoko Yoshie, *ACS. Sustainable. Chem. Eng.* **2016**, *4*, 3857–3863.
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