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EFFECTS OF -PHENYLALANINE ON THE RADIATION SYNTHESIS OF POLY(, ′-METHYLENEBISACRYLAMIDE- -4-VINYLPYRIDINE) (MICRO)GELS[*]

Qing-de Chen, Xing-hai Shen[**] and Hong-cheng Gao
Department of Applied Chemistry, College of Chemistry and Molecular Engineering,
Peking University, Beijing 100871, China

Abstract The effects of *L*-phenylalanine (*L*-Phe) on the synthesis of poly(*N*,*N′*-methylenebisacrylamide-*co*-4-vinylpyridine) (poly(Bis-*co*-4-VP)) (micro)gels by γ-ray irradiation were studied. The addition of *L*-Phe could not only decrease the gelation dose (D_g) of the synthesis obviously, but also transform the morphology of copolymer from microgel to gel. In addition, the swelling ability of the (micro)gels was also affected in the presence of *L*-Phe. The decrease of D_g was ascribed to the effect of pH, while the transformation of the morphology was ascribed to the effect of *L*-Phe on the stability of the poly(Bis-*co*-4-VP) microgel. Such an effect was confirmed further as compared with the effects of *L*-alanine, *L*-glutamic acid, *L*-arginine, sulfuric acid and aqueous ammonia.

Keywords: *L*-Phenylalanine; γ-Ray irradiation; Gelation dose; Morphology transformation; (Micro)gel.

INTRODUCTION

Microgel and macroscopic gel (gel) are two distinct morphologies of a polymer. Microgels are intramolecularly crosslinked polymer particles of colloidal dimensions (1 nm−1 μm)[1]. They have attracted much attention because of their special rheologic behaviors and potential applications in the fields of biomedicine, paints, coatings, colloid crystals, catalyst carriers, and so forth[1−6]. Gels are two- or multicomponent systems consisting of a three-dimensional network of polymer chains and solvent that fills the space between macromolecules. They have been studied intensively for their characteristics between solid and liquid[7−9]. Both classical polymer chemistry and irradiation chemistry have provided many methods to synthesize (micro)gels. Especially, irradiation techniques have been widely used in the fields of drug delivery, immobilization of biologically active materials and so forth because of their additive-free initiation, easy process control and sterilization along with the formation of (micro)gels[10−12]. Rosiak *et al.*[11] have produced wound dressings on a large scale by irradiation. Drug molecules and biologically active materials are always decomposed or devitalized partly in the irradiation course although monomers can protect them. However, if the absorbed dose is decreased significantly, this problem can be avoided to a great extent.

On the condition that the dose rate was 25.1 Gy/min and the total dose was 1 kGy, a stable microgel dispersion with solid content of about 3.4% and average hydrodynamic radius of 56.2 nm was successfully obtained from the dilute solution of 4-vinylpyridine (4-VP, 0.04 mol/L) and *N*,*N′*-methylenebisacrylamide (Bis, 0.2 mol/L) by surfactant-free emulsion polymerization using γ-ray irradiation[13]. The optimum dose (D_o), *i.e.*, the minimum dose corresponding to the maximum gel ratio, was only about 1 kGy. This low dose is propitious for immobilizing biologically active materials by irradiation and reducing their damage in the process. In addition,

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[**] Corresponding author: Xing-hai Shen (沈兴海), E-mail: xshen@pku.edu.cn

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different additives can affect the stability of the microgel dispersion in different ways[14]. If they are used in the synthesis of the microgel, they can also affect its morphology in different ways. To the microgel dispersion, *L*-Phe has a higher critical flocculation value and cannot make the microgel coagulate completely at the higher concentration of *L*-Phe. Also, the flocculation point of *L*-Phe is less apparent, which is different from other additives[14]. Thus, *L*-Phe may be used to adjust the morphology of copolymer in a relatively wide range of concentration. Recently, easily controlling of polymer morphology transformation from microgel to gel has attracted more attention for both theoretical and practical purposes[15, 16].

Some biologically active materials, such as enzymes, are amphoteric polyelectrolytes. To our knowledge, the studies concerning the effect of amphoteric (poly)electrolytes on the synthesis of (micro)gels are very scarce in the literature. In the present paper, *L*-Phe was selected as model compound to study the effect of amphoteric electrolytes on the synthesis of poly(Bis-*co*-4-VP) (micro)gels. At the same time, the mechanism was suggested by comparing the effects of *L*-alanine, *L*-glutamic acid, *L*-arginine, sulfuric acid and aqueous ammonia with the effect of *L*-Phe.

EXPERIMENTAL

Bis (Beijing Chemical Reagents Company, analytical grade) was purified by recrystallization and dried in vacuum. 4-VP (Merck, > 96%) was purified by vacuum distillation. *L*-Phenylalanine (*L*-Phe), *L*-alanine (*L*-Ala), *L*-glutamic acid (*L*-Glu) and *L*-arginine (*L*-Arg) (Beijing Chemical Reagents Company, biochemical reagents) were used as received. H_2SO_4 and $NH_3 \cdot H_2O$ (A. R. grade) were used without further purification. Water used in the experiments was deionized and redistilled.

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The feed solutions containing Bis, 4-VP, water and *L*-Phe (or other additives, see Table 1) were transferred into test tubes (diameter: 15 mm) to a certain height, bubbled with high-purity N_2 for 20 min to remove O_2, then sealed and irradiated for a period of time at a special location in the ^{60}Co irradiation field whose dose rate was determined by Fricke dosimeter. The dose rate was 23.5 Gy/min except stated otherwise. The samples were numbered according to the absorbed doses. For example, A950 represented the sample whose absorbed dose was 950 Gy in the group A.

After being extracted for 24 h by ethanol and water, respectively, the gel and sol ratio (*g* and *s*) were calculated by the following expressions, respectively:

$$g = W_a/W_b \tag{1}$$

$$s = 1 - g \tag{2}$$

where W_b and W_a are the weights of the (micro)gels before and after extraction, respectively. Before each weighing, all samples were dried under vacuum at 40°C for 24 h.

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The (micro)gel samples dried and exactly weighed were immersed in water at room temperature. Some time later, the swollen samples were taken out and the water on the surface of the samples was blotted up with filter papers, then the samples were weighed again. The swelling degree (*SD*) was calculated as follows:

$$SD\,(\%) = (W_w/W_d) \times 100 \tag{3}$$

where W_w and W_d are the weights of wet and dried samples, respectively.

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The (micro)gels were dried and ground by mortar, then swelled again by water adequately. The swelling gels were collected by filtration and dried in the air at room temperature. After being overgilded, their images were observed by JEOL JSM-5600LV scanning electronic microscope (SEM), operated at 15 kV.

Table 1. Effects of the concentration of *L*-Phe and other additives on the synthesis of poly(Bis-*co*-4-VP) (micro)gels ([Bis] = 0.2 mol/L, [4-VP] = 0.04 mol/L)

Group	Additive	Concentration of additive (mol/L)	pH	Copolymer morphology at maximum gel ratio	D_g (Gy)
A	*L*-Phe	1.0×10^{-2}	8.58	Translucent gel	131.1
B		5.0×10^{-3}	9.06	Translucent gel	148.5
C		1.0×10^{-3}	10.53	Translucent microgel	196.5
D		0	10.80	Translucent microgel	213.2
E	H_2SO_4	5.0×10^{-3}	6.67	Opaque gel	141.1
F	*L*-Ala	1.0×10^{-2}	8.98	Translucent gel	130.0
G	*L*-Glu	5.0×10^{-3}	7.21	Translucent gel	131.5
H	*L*-Arg	1.0×10^{-2}	10.88	Opaque gel	220.3
I	$NH_3 \cdot H_2O$	1.0×10^{-2}	10.98	Opaque gel	228.1

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Gel A950 was taken out integrally from the test tube after irradiation, cut into cylinder pieces with 5 mm height and washed by absolute ethanol until there was no absorbance of the supernatant at 257 nm in order to eliminate the disturbance from a trace amount of soluble substances containing pyridine group. After the residual liquid is being blotted up, the pieces were all immersed in 50 mL water. The absorbance of the supernatant was detected at 257 nm at intervals by using U-3010 Spectrophotometer (Hitachi, Japan) with 1 cm quartz cells until equilibrium. Water was used as the reference in each case. If *L*-Phe in the gel is released completely and there are no other disturbances, the absorbance of *L*-Phe in the supernatant is A_0. For the gel A950, the value of A_0 is measured to be 0.203.

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Microgel D950 was treated by absolute ethanol after drying at room temperature, while the treatment of gel A950 was the same as that mentioned in the preceding paragraph. Then all samples were dried at room temperature. FT-IR spectra were recorded as KBr pellets using Thermo Nicolet Nexus FT-IR Spectrometer. The resolution of the samples was 4 cm^{-1} and each spectrum was recorded after 128 scans.

RESULTS AND DISCUSSION

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Effects of L-Phe concentration on D_g and D_o of the synthesis

Fixing the concentrations of Bis and 4-VP and only altering the concentration of *L*-Phe, gel curves are different obviously (Fig. 1a). The figure also shows that gel ratio reaches its maximum at lower dose and D_o decrease obviously with an increase in the *L*-Phe concentration. D_o is about 500 Gy in group A where *L*-Phe concentration is 0.01 mol/L, while in group D where *L*-Phe does not exist, D_o is 950 Gy.

The concentration of Bis is much higher than that of 4-VP in the feed solutions, so the degree of cross-linking of (micro)gels is also much higher. This result does not agree with the fundamental assumption of Charlesby-Pinner equation[10] and other equations[10, 17]. But there is good linear relationship between s and $1/D$ (Fig. 1b) and an empirical expression is therefore brought forward as follows:

$$s = \frac{a}{D} + b \tag{4}$$

where a is the slope of the straight line. When s approaches 1, the corresponding dose is defined as gelation dose (D_g). When s is zero, the corresponding dose is the minimum dose while the gel ratio reaches 1. In practice, this empirical expression is not suitable when the gel ratio nears maximum value. Further work is expected to clarify whether the empirical expression can be applied to other systems. By the empirical expression, we obtained the D_g values of all systems studied. They are close to the values obtained from the crossing points of the extension lines of gel curves with x-axis. The result shows that D_g decreases while the *L*-Phe concentration increases

(Fig. 1b and Table 1). This can help us to explore a method of immobilizing biologically active materials by irradiation and reducing their damage in the process.

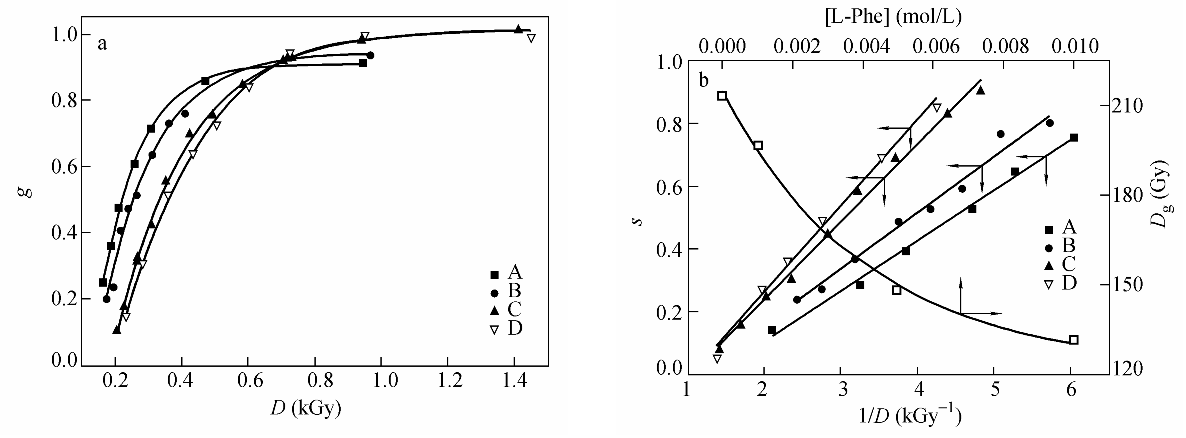

Fig. 1 (a) Effect of *L*-Phe concentration on the gel curve of the synthesis, (b) Gel analysis and relationship between D_g and *L*-Phe concentration

Table 1 also shows that the morphology of copolymer is transformed from microgel to gel while the *L*-Phe concentration increases. In other words, the morphology of copolymer can be adjusted by the *L*-Phe concentration.

Effects of L-Phe and dose rate on the gel ratio

Fixing total absorbed dose and only altering dose rate, the result of gel analysis shows that the gel ratios of group A are not affected significantly by dose rate in the selected dose rate range, but those of group D decrease when the dose rate becomes higher. This is ascribed to the fact that microgel dispersion which can flow is obtained from the feed solution D by γ-ray irradiation, while gel which cannot flow is obtained from the feed solution A (Table 1). When the dose rate is higher, the free radicals generated during unit interval will have higher density and become easier to lose the ability of initiation in the microgel dispersion because of their recombination. This makes the energy usage efficiency at higher dose rate smaller than that at lower dose rate.

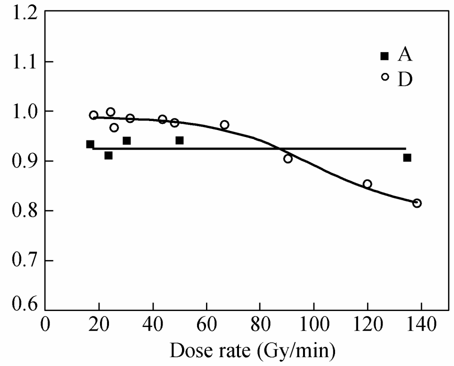

Fig. 2 Effect of dose rate on the gel ratio of groups A and D Total doses: about 950 Gy

Effects of different additives on the synthesis

During the course of irradiation, water absorbs most of the irradiation energy and mainly generates ·OH, H and e_{aq}^-. The former plays a main role in polymerization and the crosslinking process[18]. In weakly basic feed solution of group D at pH 10.80, ·OH can react with OH^- as follows:

$$\cdot OH + OH^- \rightleftharpoons \cdot O^- + H_2O \tag{5}$$

whose reaction rate constant is 1.2×10^{10} L · mol^{-1} · s^{-1}[19]. The reaction rate constants of acrylamide with ·OH and ·O$^-$ are 6.6×10^9 and 6.5×10^8 L · mol^{-1} · s^{-1}[20], respectively, so the initiation ability of ·O$^-$ is weaker than that of ·OH. This makes the efficiency of initiation decrease. L-Phe, a weakly acidic amphoteric electrolyte with an isoelectric point (pI) at 5.48[21], can react with 4-VP to a certain extent and decreases the pH value of the solution. So reaction 5 is restrained, which makes the concentration of ·OH and the rate of polymerization and crosslinking increase. Accordingly, the value of D_g and D_o decrease in the order of groups D, C, B and A (Table 1 and Fig. 1).

Then, *L*-Ala, *L*-Glu, *L*-Arg, sulfuric acid and aqueous ammonia were used to study the mechanism. All of the results are shown in Fig. 3 and also compiled in Table 1.

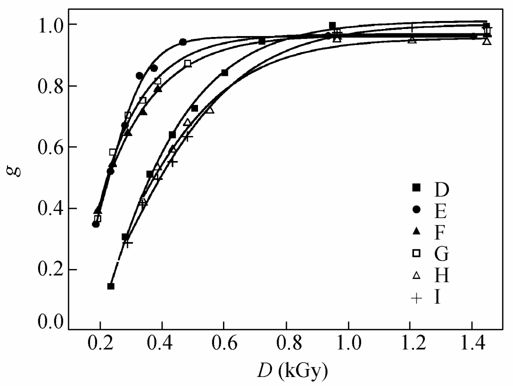

Fig. 3 Effects of different additives on the gel curve of the synthesis

The sulfuric acid and the weakly acidic amphoteric electrolytes *L*-Ala (pI = 6.02[21]) and L-Glu (pI = 3.22[21]) can work in a similar way. Table 1 and Fig. 3 show that D_g and D_o of groups E, F and G are all lower than that of group D. Because the yield of ·OH is kept constant in the pH range from 5 to 9[22], the value of D_g of groups E, F and G are nearly identical, although their pH values are different obviously (Table 1).

As the weak base $NH_3 \cdot H_2O$ and the weakly basic amphoteric electrolyte *L*-Arg (pI = 10.76[21]) can increase the pH values of the solution and the yield of ·OH increases slowly in the pH range from 11 to 9[22], the value of D_g of groups H and I are higher than that of group D (Table 1).

The additives can affect the D_g and D_o of the synthesis of poly(Bis-*co*-4-VP) (micro)gels and their morphology correspondingly. During the irradiation of the solution of group A, transparent solution, translucent microgel dispersion and translucent gel appear in succession, so the formation of gel comes through an intermediate stage of microgel.

In groups A and B, *L*-Phe can make the generated microgel flocculate and coagulate in succession, but microgel cannot be coagulated completely. A slight phase separation is favorable for the polymerization of double bonds on the surface of the microgel[13] with the residual Bis, and the formation of translucent gel. But the concentration of *L*-Phe in the feed solution C is so low that it cannot make the microgel to flocculate and therefore microgel is reserved. Because *L*-Ala and *L*-Glu also belong to weakly acidic amphoteric electrolytes like *L*-Phe, translucent gel is obtained when their concentrations are 1.0×10^{-2} and 5.0×10^{-3} mol/L, respectively (Table 1).

Because the microgel is electropositive, the ability to coagulate by SO_4^{2-} is stronger than that by Cl$^-$. The ability to coagulate the microgel by HCl is stronger than that by *L*-Phe obviously[14], so the ability to coagulate the microgel by sulfuric acid is stronger than that by *L*-Phe. Because of serious phase separation, opaque gel is obtained when the concentration of sulfuric acid is 5.0×10^{-3} mol/L and when the gel ratio reaches maximum (Table 1).

Because the ability to coagulate the microgel by $NH_3 \cdot H_2O$ is stronger than that by *L*-Phe[14], opaque gel is obtained when the concentration of $NH_3 \cdot H_2O$ is 1.0×10^{-2} mol/L and the gel ratio reaches maximum. The action on the microgel by *L*-Arg, a weakly basic amphoteric electrolyte, is similar to that by $NH_3 \cdot H_2O$ (Table 1).

The above results show that translucent microgel dispersion and gel can be obtained respectively only when weakly acidic amphoteric electrolytes are used and their concentrations are appropriate. If other additives are used, their concentration range for adjusting the morphology of copolymer is narrower than that of the system of weakly acidic amphoteric electrolytes and therefore opaque gel is likely to be obtained. Weakly acidic amphoteric electrolytes are suitable in a relatively wide concentration range for adjusting the morphology of copolymer. This is one more effective method in addition to that of controlling absorbed dose, dose rate, concentration of monomers and so forth.

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In the swelling studies, A950 and D950 were selected because the absorbed doses in their syntheses were identical and their gel ratios had reached the maximum. Figure 4 shows that the saturated swelling degree of A950 is lower than that of D950. Combining with their SEM images (Fig. 5), it can be concluded that the degree of crosslinking increases, the voidage becomes smaller and its swelling degree decreases because of the effect of *L*-Phe. The obtained gel is so rigid that it can keep its appearance during the process of swelling and drying. The obtained microgels without the effect of *L*-Phe agglomerate irreversibly in the course of drying. In the agglomerated microgel, the degree of crosslinking is much higher in the microgel, but the voidage among the microgels is bigger. So the agglomerated microgel has a higher saturated swelling degree and the characteristics of chapped gel is generated after being swelled and dried. In other words, the swelling ability of the (micro)gel can also be adjusted by *L*-Phe.

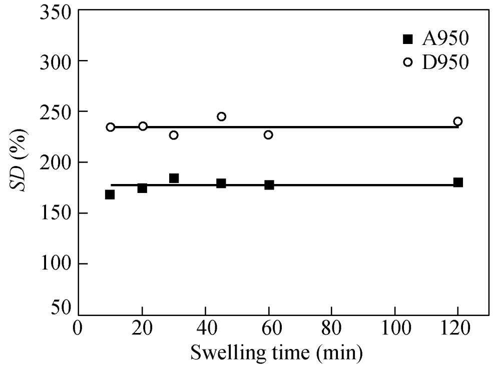

Fig. 4 A plot of swelling degree versus swelling time of A950 and D950

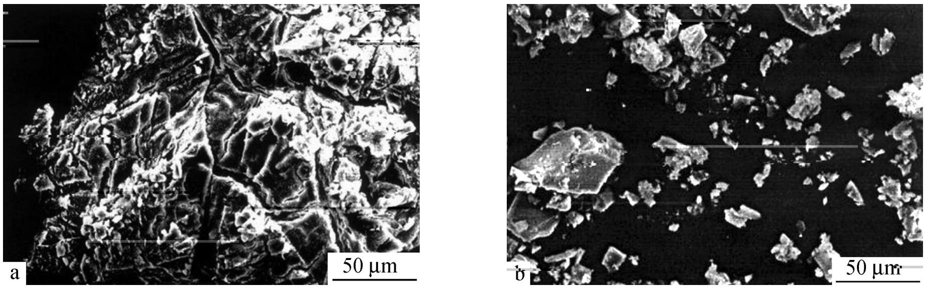

Fig. 5 SEM images of microgel D950 (a) and gel A950 (b) after being swelled and dried

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After the first release equilibrium, the absorbance of the supernatant is measured to be 0.152. In other words, about 25% of *L*-Phe is still remained in the gel, which is similar to the situation in high crosslinking systems[23–24].

As the molar percentage of *L*-Phe is much lower than those of other solutes in the feed solution A, the IR spectra of A950 and D950 are alike in their appearances (Fig. 6). According to our previous study[13] and the spectra of *L*-Phe and the mixture of D950 with *L*-Phe, it is considered that, in the IR spectrum of A950, the characteristic peaks at 1655 cm^{-1} (stretching vibration of C=O) and 1530 cm^{-1} (scissoring vibration of N-H) come from Bis, the shoulder peak at 1603 cm^{-1} is composed of stretching vibration of C=C from benzene ring of *L*-Phe and stretching vibration of C=N from pyridine ring of 4-VP unit, and the absorption at 1560 cm^{-1} is partly contributed by the characteristic absorption of scissoring vibration of N-H from *L*-Phe which is overlapped by the broad peak of scissoring vibration of N-H from Bis.

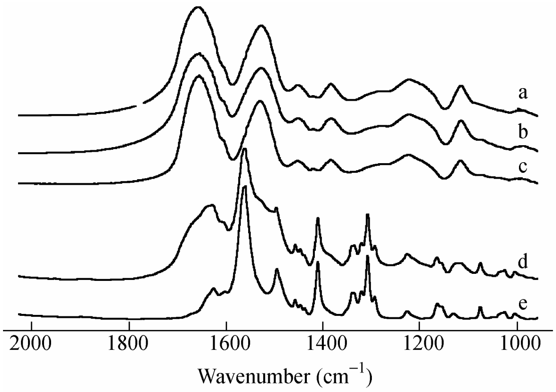

Fig. 6 FT-IR spectra of (a) A950 (after release), (b) A950 (before release), (c) D950, (d) D950 + *L*-Phe and (e) *L*-Phe

Because the molar concentration of Bis in the feed solutions A and D is equal and the absorbance of 1655 cm^{-1} (A_{1655}) is free from the interference of the absorbance of *L*-Phe, it is suitable to select A_{1655} as standard. Whether *L*-Phe exists in the gel or not can be ascertained by the ratios of A_{1603}, A_{1560} to A_{1655}. The average ratios measured in three parallel experiments are listed in Table 2.

Table 2 shows that the two ratios of A950 (after release) all intervene between those of D950 and A950 (before release), respectively. According to estimation, about 27% of *L*-Phe is remained in the gel, which is close to the result of UV. Further work is expected to elucidate the existence of *L*-Phe remained in the gel in detail.

Table 2. Results of FT-IR analysis of A950 and D950

Sample	A_{1603}/A_{1655}	A_{1560}/A_{1655}
A950 (before release)	0.48	0.61
A950 (after release)	0.40	0.51
D950	0.37	0.47

CONCLUSIONS

The addition of *L*-Phe could not only decrease D_g

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