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Preparation of molecularly imprinted CS membrane for recognizing naringin in aqueous media

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Abstract A molecularly imprinted membrane (MIM) was prepared in aqueous media using chitosan (CS) as a functional polymer, naringin (NG) as a template molecule by phase-inversion technique. The morphologies of the MIM before and after modification with the porogen (PEG) were observed by SEM. The imprinted membrane showed an excellent performance when sulfuric acid was used as a cross-linking agent at the mass ratio of CS:NG = 15:1. The FT–IR spectra confirmed that the formation of hydrogen bond between functional polymer and template molecules. The NG–CS MIM was used to separate NG from neohesperidin/NG in the aqueous media, and the highest permeation percentage was 11.16% for 8 h.

Keywords Chitosan · Naringin · Molecular imprinting technology · Separation · Recognition in aqueous media

Introduction

Due to the dual advantages of the molecular-specific identification and membrane separation, molecularly imprinted membrane (MIM) has been developed for the potential applications, especially in separation, chromatography, and analytics [1–3]. At present, the preparations and applications of molecularly imprinted polymer (MIP) are mainly limited in the organic solvents. However, the researches

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on the hydrophilic compounds and the identifications of natural systems are facing the practical applications of the water-based systems. The molecular identifications in water environment are getting more and more attention [4, 5].

Chitosan (CS) is a biodegradable, biocompatible, and non-toxic amino-polysaccharide obtained by deacetylation of chitin, which is one of the world's most abundant biopolymers. Since CS has been proved to be biologically renewable, biodegradable, biocompatible, non-antigenic, non-toxic, and bio-functional, it is widely used in drug delivery, environmental protection, and membrane separation [6, 7]. CS can be made into an ion exchange, chelating, and adsorption with many substances based on a large number of hydroxyl and amino groups. Unfortunately, these combinations are devoid of selectivity and specificity. The MIP made of CS exhibited a good selectivity [8, 9]. However, the usage of CS as MIM material is rarely reported.

The MIM with specific recognition properties in aqueous media for the naringin (NG, a member of the flavonoid family) was investigated in this study. Flavonoids widely present in fruits, vegetables, and certain beverages, and show biologically interesting properties, such as anti-oxidant, anti-inflammatory, and anti-cancer activities [10, 11]. On the other hand, the content of some flavonoids such as limonin and NG in the food industries (such as, sweeteners, juices) must be controlled because they contribute to their bitter taste, lowering the commercial value of these products. Nowadays, the technologies such as the adsorptive debittering, chemical methods, and ion-exchange resin are used to remove NG from these products. All these methods present some limitations which can alter sensory properties and nutritional value of these products [12].

The preparation of new polymeric imprinted materials possessing highly selective and affinity properties for NG could play a relevant role in solid phase extraction and/or debittering processes. Up to now, the studies of NG MIM have focused only on the organic solvents [13, 14]. The application and preparation of MIM for selecting recognition of NG in aqueous media have not been reported.

In this study, using CS as a functional polymer, NG as the template molecules, and polyethylene glycol (PEG) as porogen, the CS–NG MIM was first prepared in aqueous media by phase-inversion technique. The interactions between NG template molecules and CS functional polymer were investigated by UV and FT–IR. The CS–NG imprinted membrane exhibited selective permeability for the template molecules in aqueous media.

Experimental

Materials

Chitosan with an N-deacetylation degree of 90% was purchased from Guoyao Chemicals Co. Ltd. Naringin (≥98%) was purchased from Nanjing Zelang Medicine Co. Other reagents used for the following investigations were of analytical grade.

Instruments

The characteristic functional groups were investigated by means of NICOLET 380 FT–IR (USA Nicolet Co.). NG absorption in the solutions was studied by CARY 50-type UV–Vis (USA Varian Co.). The contents of NG in the solutions were determined by a high performance liquid chromatography (HPLC) with a Prostar 240 pump (USA Varian Co.). The imprinted membrane morphology was analyzed by means of XL30ESEM/TMP environmental scanning electron microscope (Japan PHILIPS Co.).

Preparation of membranes

The imprinted membrane was prepared as follows:

CS and NG (the ratio between NG and CS vary from 1:5 to 1:20) were put in 100 mL 2.0% (v/v) acetic acid with stirring at 60 °C for 4 h. 10 mL of 1.0 wt% PEG 20000 was added into the mixture, and stirred for 2 h, then casted onto a clean glass plate. Subsequently, it was dried overnight in an oven at 60 °C. The membrane was immersed into 0.5 mol/L sulfuric acid solution (or 0.2% glutaraldehyde solution) at the room temperature for 24 h. Then, the membrane was thoroughly washed with distilled water to remove other molecules. NG was removed from the membrane using ethanol as an elate in the ultrasonic bath till no NG was detected by UV measurements (283 nm). The imprinted membrane unused PEG 20000 as a porogen was prepared in a similar manner. And non-imprinted membrane was also prepared by the same method, just without adding the template molecules.

The imprinting effect was evaluated by the permeation amount of NG through membranes in the permeation experiments.

Measurements of the swelling degree of membranes [15]

Before measurement, the membranes were soaked in the different concentration of alcohol–water solution (10, 20, 30, and 40%, respectively) for 24 h at the room temperature to reach the final dilation. Then the surface water was wiped with a filter paper and immediately weighted. The samples were then dried in the oven at 60 °C until a constant weigh was obtained. The swelling degree (S_w) was calculated by the following equation:

$$S_w = \frac{m_s - m_d}{m_d} \times 100\% \quad (1)$$

where m_s and m_d are the wet and dried membranes weight, respectively.

Permeation experiments

Neohesperidin (NHD) is a new generation sweetener with non-toxic, low energy, and high sweetness (950 times more than that of dihydro-chalcone). The sweetness will be decreased due to a small amount of NG contained in NHD. NHD was used as a competitive pollutant to investigate the selective permeation of the CS–NG

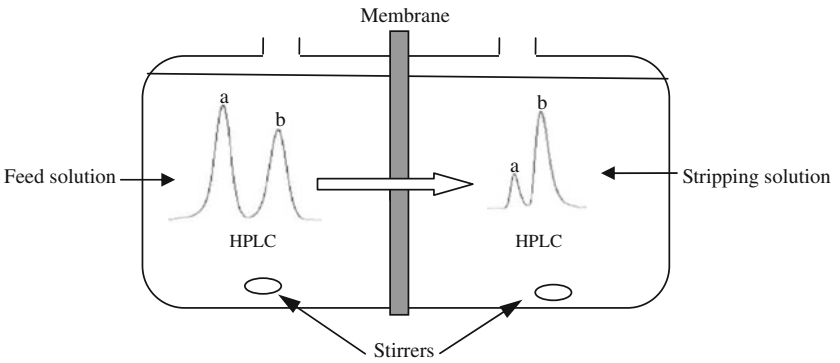


Fig. 1 Schematic diagram of imprinted membrane (*a* NHD, *b* NG)

MIM in this article. As shown in the Fig. 1, the CS–NG imprinted membrane was placed between two rooms (the volume of each room is 25 mL). The feed solution was a blend of 500 μg/mL NG and 500 μg/mL NHD-ethanol aqueous solutions (20%), and the stripping phase is the 20% ethanol aqueous solution with the electromagnetic stirring. 30 μL solutions were taken out from stripping room every 1 h to analyze its composition by HPLC and calculate the permeation amount of NG and NHD.

Chromatography conditions: Microsorb-MV 100-5 C-18 columns (150 × 4.6 mm), UV detection at 283 nm, methanol: 2% HAc (38:62) as mobile phase, flow rate: 1.0 mL/min. The standard curve was obtained by means of external standard method.

Results and discussions

Preparation of NG–CS MIM

The formation of imprinted membrane

The molecular space configuration is controlled by the large benzene ring in NG molecules. The hydrogen bonds between CS and NG improve the combination stability between imprinting molecules and functional polymer, and the selectivity of MIP membrane. The imprinted structure was fixed with the cross-linking agent. After removing the imprinting molecules, the specific cavities were left in the CS network. The formation process of NG–CS MIM was shown in Fig. 2.

The effect of cross-linking agents on the swelling degree of membrane

The cross-linking agent is one of the important factors for preparing MIM to improve the cross-linking extent and maintaining a better shape of the "memory" cavity structure. In this study, glutaraldehyde and sulfuric acid were used as cross-linking

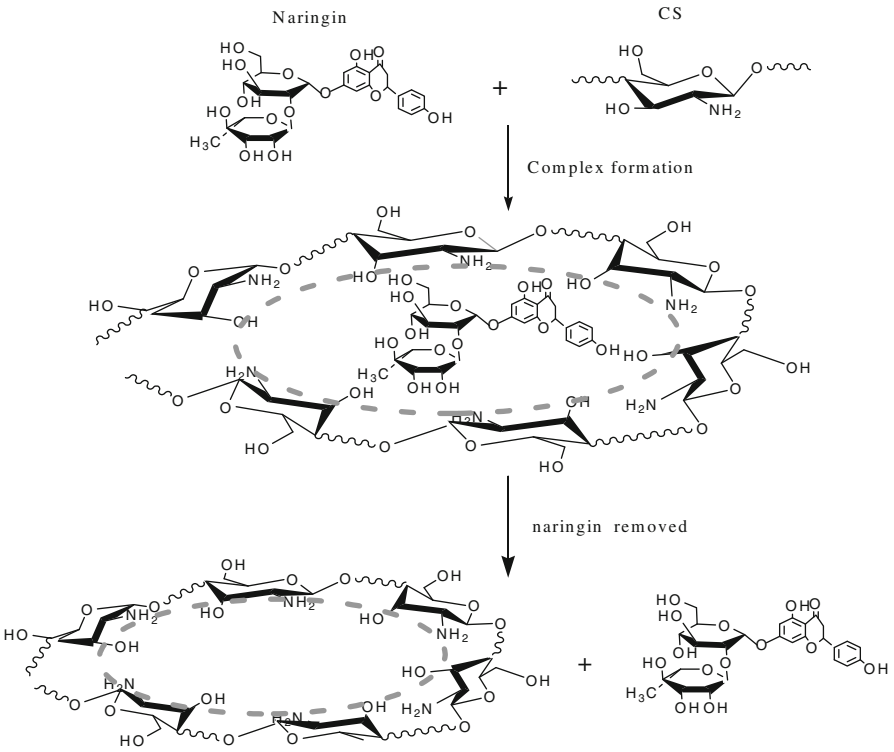


Fig. 2 Schematic diagram of the formation process of NG–CS MIM

agents for preparing MIM, respectively. After cross-linking, the swelling degree of the CS MIM decreased significantly.

CS polymer is a hydrophilic membrane showing a big swelling degree and lower selective property in the water. The CS MIM in a certain concentration of the alcohol–water solution showed good results for recommencing organic solvents such as ethanol. The swelling degrees of the imprinted membranes cross-linked by two cross-linking agents (sulfuric acid -■-, Glutaraldehyde -●-) were reported in Fig. 3. As shown in Fig. 3, the swelling degree of the imprinted membranes cross-linked by sulfuric acid and the glutaraldehyde decreases with the increase of ethanol in the alcohol–water solution.

Using sulfuric acid as a cross-linking agent shown in Eq. 2, $-NH_2$ group in CS membrane was cross-linked with SO_4^{2-} to improve the stability of the membrane structure and the ionic permeability [16], and its recovery rate of NG was 90.8%.

$$CS—NH_2 + H_2SO_4 \rightleftharpoons CS—NH_3^+ \cdots\cdots SO_4^{2-} \cdots\cdots NH_3^+—CS \qquad (2)$$

However, using glutaraldehyde as a cross-linking agent shown in Eq. 3, it is very difficult to elute and recover NG imprinting molecules in the high cross-linking

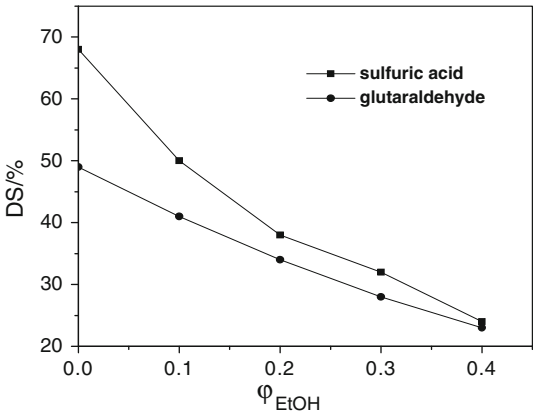
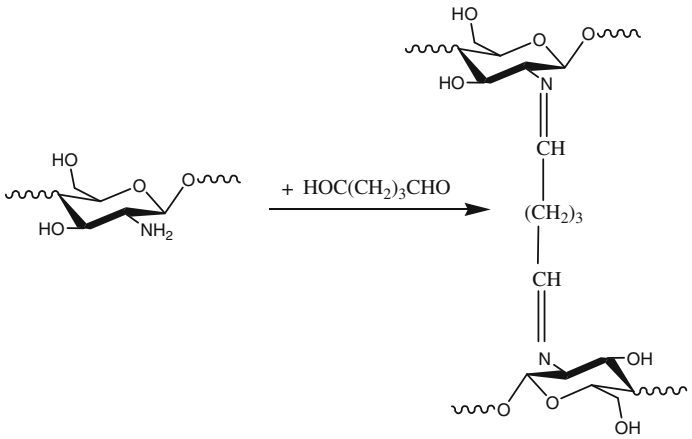


Fig. 3 Swelling degree of membrane in ethanol–water solution

covalent polymer, and the recovery rate of NG was 70.3%. On the other hand, glutaraldehyde will crosslink with more amino groups and reduce the imprinting sites for the template molecules.



(3)

The effect of the mass ratio of CS and NG on the permeation capability of the membrane

The ratio of imprinting molecule and the functional monomer is one of the important separation factors of MIM. The less imprinting molecule and the less imprinted sites are unavailable for the recognition capability of MIM. However, too much imprinting molecule may lead to the formation of membrane defects due to the relative lack of the polymer functional groups. The permeation amount of NG through CS MIM (cross-linked by sulfuric acid) prepared with different mass ratio of CS and NG was investigated. According to the literature [13], the permeation amount of NG can be expressed by the following equation:

$$[S] = \frac{c_e \times V}{m} \quad (4)$$

where $[S]$ is the amount of the NG through the membrane, c_e is the NG equilibrium concentration (after 24 h, determined by HPLC) in the stripping solution (μmol/mL); V is the volume of solution (25 mL); m is the mass of the membrane used (g).

As shown in Fig. 4, the suitable mass ratio was 15:1. The highest permeation amount of the imprinted CS membrane is 21.60 μmol/g_{memb} in the aqueous phase. Compared with the results of Trotta's and Tasselli's study [13, 14], the separation capacity of the CS MIM is much higher.

The effect of porogen on the membrane structure

Mohammad et al. [17] reported that the pore structure and size of polymer would greatly affect the molecular imprinting behaviors. Figure 5 displays the scanning electron microscope photos of the imprinted membranes (cross-linked by sulfuric acid, m_{CS}:m_{NG} = 15:1). The membrane structure is dense (Fig. 5a, the thickness is

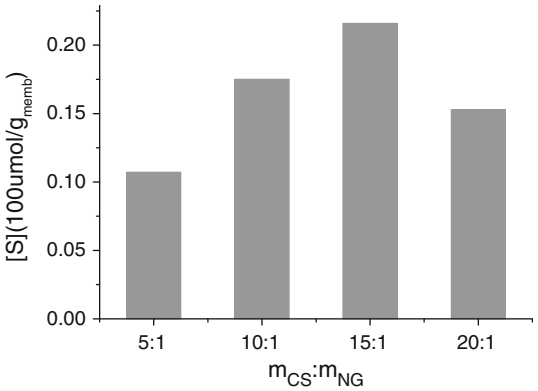


Fig. 4 Effect of the mass ratio of CS and NG on the permeation amount

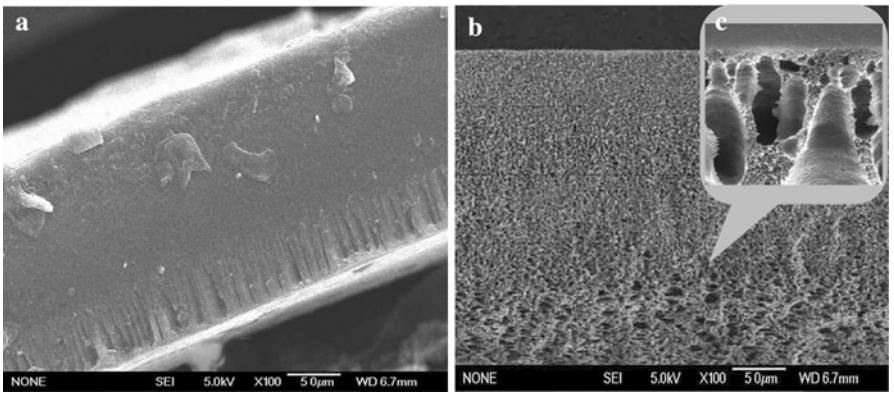


Fig. 5 SEM of imprinted membranes [**a** without PEG (×100), **b** including PEG (×100), **c** including PEG (×10,000)]

about 120 μm), when PEG was not added in. A loose and gully-like membrane structure was observed in Fig. 5b, when PEG was added in. The interactions in CS molecular chains are weakened, and lead to increasing the free volume and flux of the CS membrane due to the PEG having a higher flexibility and non-side groups. However, the higher porogen content will result in increasing the viscosity and reducing the speed of proliferation [18].

The interactions between imprinting molecules and functional polymer

UV analysis

Figure 6 displays UV absorption spectra of NG, CS, and elate of imprinted membrane (cross-linked by sulfuric acid, $m_{CS}:m_{NG} = 15:1$). The absorption peak at about 283 nm is corresponding to the benzene ring in NG molecules (curve a), while the CS functional polymer without any absorption peaks (curve b) was observed. The absorption peak in Fig. 6c is over lap to the Fig. 6a. It confirms that the elate molecules are NG. After eluting, cavities were left in the imprinted membrane perfectly matching with NG molecules [19].

FT–IR spectra

Figure 7 displays FT–IR spectra of non-imprinted membrane, NG and imprinted membrane (cross-linked by sulfuric acid, $m_{CS}:m_{NG} = 15:1$, before NG was removed). The spectrum of non-imprinted membrane shows the absorption peaks at about 3435 cm^{-1} for the $–NH_2$ and –OH groups in CS, at about 2920 and 2881 cm^{-1} for the aliphatic C–H stretching vibration, 1630 cm^{-1} for the absorption peaks of the rest NH_2CO in CS, and 1031 cm^{-1} for the C–O group. The spectrum of NG exhibits the characteristic peaks of –OH at 3422 cm^{-1}, C=O at 1643 cm^{-1}, C–O–C at 1063 cm^{-1}, and the benzene ring at about 1579, 1518, and 1450 cm^{-1}. In the Fig. 7c, the broaden absorption peak at about 3500–3000 cm^{-1} is corresponding to the hydrogen bond strength. In comparison with that of Fig. 7c, the C=O absorption peaks in Fig. 7b moves from 1643 to 1628 cm^{-1} and the C–O–C

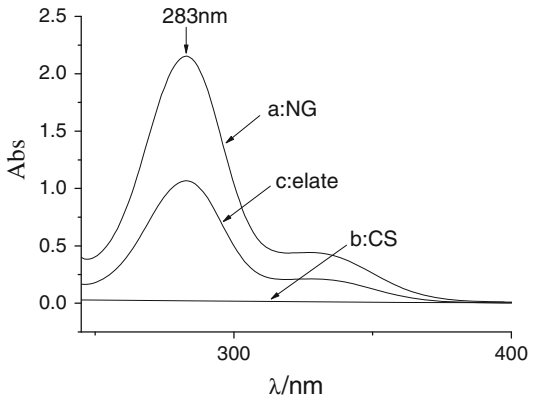


Fig. 6 The UV spectra of NG (*a*), CS (*b*) and the elate of imprinted membrane (*c*)

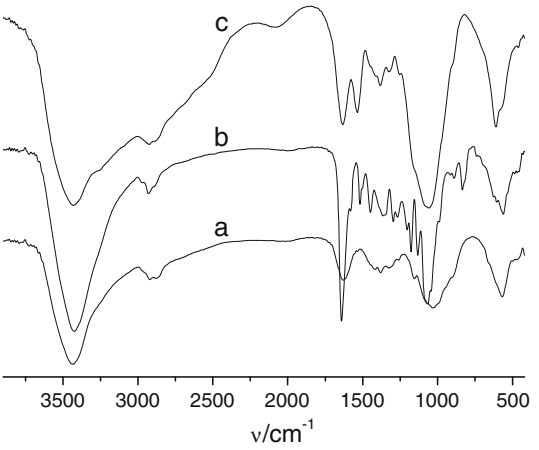


Fig. 7 FT–IR spectra of non-imprinted membrane (*a*), NG (*b*), and imprinted membrane (*c*)

absorption peaks moves from 1063 to 1058 cm^{-1}. These changes are due to the formation of hydrogen bonds between $–NH_2$ and –OH in the functional polymer CS and OH, C=O, C–O–C in the template molecule NG.

Selectivity permeation experiments

Using NG and NHD as separation mixtures, as shown in Fig. 1, the NHD intensity in the left room is obviously larger than that of in the right room after the permeation. NG was selectively separated from the mixture of NG and NHD by using of the imprinted membrane.

The curves of the permeation percentage (P.P.) changed with permeation time (P.T.) were determined. As shown in Fig. 8, the P.P. of NG through imprinted membrane (IM, cross-linked by sulfuric acid, $m_{CS}:m_{NG} = 15:1$) is much larger than

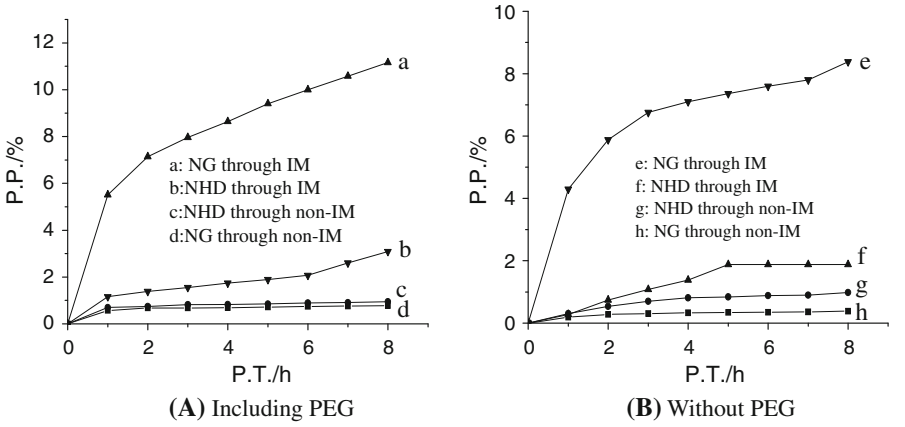


Fig. 8 Permeation curves of the mixture solutes through IM and non-IM. **a** Including PEG. **b** Without PEG

non-imprinted membrane (non-IM). The P.P. of NG is 11.16% (curve a), 8.38% (curve e) through IM and 0.78% (curve d), 0.38% (curve h) through non-IM, respectively for 8 h. The permeation percentage of the NHD molecules through IM (curve b and f) and non-IM (curve c and g) are almost the same.

The P.P. was calculated by the following equation:

$$\text{P.P.} = \frac{c_t}{c_0} \times 100\% \tag{5}$$

where c_0 is the NG initial concentration (μg/mL); c_t is the NG concentration in the stripping solution at the time t (μg/mL).

Imprinted channels can recognize template, but they also act as template specific gates between large pores [19]. During the imprinting process, channels can be generated by template molecules increasing the fraction of microspores in the polymer and producing structures complementary to that of the template. Imprints in general represent cavities that can bind template molecules with strength, corresponding to the number and nature of the functional groups, located in these cavities. Permeates with different structure will possess different diffusion paths and this phenomenon can be responsible for molecule separation.

Conclusions

The CS–NG MIM was prepared in aqueous media using NG as imprinting molecules, PEG as porogen, CS as a functional polymer based on its good biocompatibility, biodegradability, non-toxicity, and low cost. The CS–NG imprinted membrane was used to separate NG from NHD/NG in an aqueous media. The effects of the different mass ratio of CS and NG, linkage reagent and porogen on membranes structure and properties were investigated. The MIM morphologies before and after modification of the porogen were observed by SEM. The imprinted membrane showed an excellent performance when sulfuric acid was used as the cross-linking agent and the mass ratio of CS and NG was 15:1. The highest permeation percentage of NG was 11.16%.

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