Swelling Behavior of Thermoreversible Poly(*N*-isopropylacrylamide-*co*-*N*-vinylimidazole) Hydrogels

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ABSTRACT: Thermoresponsive hydrogels based on *N*-isopropylacrylamide and *N*-vinylimidazole were synthesized, and their swelling–deswelling behavior was studied as a function of the total monomer concentration. For copolymeric structures with better thermoresponsive properties with respect to poly(*N*-isopropylacrylamide-*co*-*N*-vinylimidazole) hydrogels, these hydrogels were protonated with HCl and HNO_3, and the copolymer behaviors were compared with those of the unprotonated hydrogels. The temperature was changed from 4 to 70°C at fixed pHs and total ionic strengths. The equilibrium swelling ratio, dynamic swelling ratio, and dynamic deswelling ratio were evaluated for all the hydrogels. © 2004 Wiley Periodicals, Inc. J Appl Polym Sci 94: 1619–1624, 2004

Key words: hydrogels; swelling

INTRODUCTION

Hydrogels can be defined as crosslinked hydrophilic polymers and copolymers that can swell in contact with water or aqueous solutions. Hydrogels are used in many areas, such as biotechnology, bioengineering, pharmacology, medicine, agriculture, and the food industry. According to the type of functional group, the gels undergo reversible and discontinuous volume changes in response to changes in the environmental conditions, including the solvent composition, temperature, salt concentration, and pH.[1–4] Thermally reversible hydrogels have recently been attracting increasing interest in biomedicine and biotechnology. Many scientific works in recent years have dealt with hydrogels incorporating *N*-isopropylacrylamide (NIPAM). The reason is the lower critical solution temperature (LCST) in water of approximately 32°C, which is close to body temperature. The thermoresponsive behavior of poly(*N*-isopropylacrylamide) [poly(NIPAM)] gels has been extensively investigated and modeled by different researchers.[5–14] Poly(NIPAM)-based hydrogels are being used as drug-delivery systems[10] because the critical temperature is close to the temperature of the human body. Copolymers of NIPAM have also been used as carrier matrices for the immobilization of enzymes and cells and for the dewatering of protein solutions.[15–19]

Poly(*N*-vinylimidazole) (PNVI) hydrogels are quite interesting systems that are able to regulate the pH of an aqueous solution[20] and to uptake cations of heavy metals.[21,22] The binding properties of *N*-vinylimidazole (NVI) are due to the electron-donor nitrogen atom at position 3 of the imidazole rings. PNVI hydrogels are neutral, but in acidic solutions, the imidazole groups behave as weak bases[23] and become protonated. Thus, PNVI hydrogels undergo volume changes in response to variations in the pH of the swelling solution. Considering these properties and the complexing and protonation ability of PNVI, we selected NVI as a proper comonomer for NIPAM to produce a thermoresponsive hydrogel with binding abilities with various ligands. The aim of this work was to combine the two comonomers in one structure and to characterize it in terms of the swelling–deswelling degree and swelling–deswelling kinetics of the copolymeric hydrogels and their protonated forms comparatively.

EXPERIMENTAL

Materials

NIPAM and NVI were used as monomers. The former was obtained from Aldrich Chemical Co., Inc., and the second was acquired from Fluka Chemie AG; they were used without further purification. The other chemicals were *N*,*N*′-methylenebisacrylamide (BDH), which was used as a crosslinking agent; sodium persulfate (NaPS; BDH), which was used as an initiator; and *N*,*N*,*N*′,*N*′-tetramethylenediamine (Merck), which was used as an accelerator. $Na_2HPO_4 \cdot 2H_2O$ (Merck) and KH_2PO_4 (BDH) were used to prepare phosphate buffer solutions of pH 7.0. Besides these, HCl and HNO_3 were used to prepare protonation solutions.

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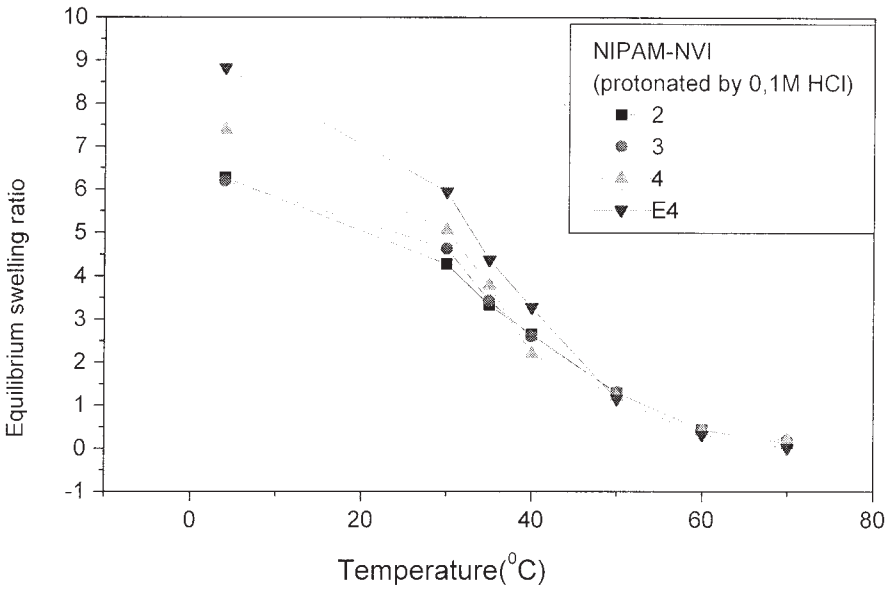
TABLE I
Production Conditions of the Poly(NIPAM-*co*-NVI) Hydrogels

Gel no.	NIPAM/NVI molar ratio	NIPAM (mg)	NVI (μL)	Total monomer (mg/mL)
2	52.43/47.57	50	50	632
3	52.43/47.57	70	70	885
4	52.43/47.57	100	100	1265
E4	52.43/47.57	150	150	1897

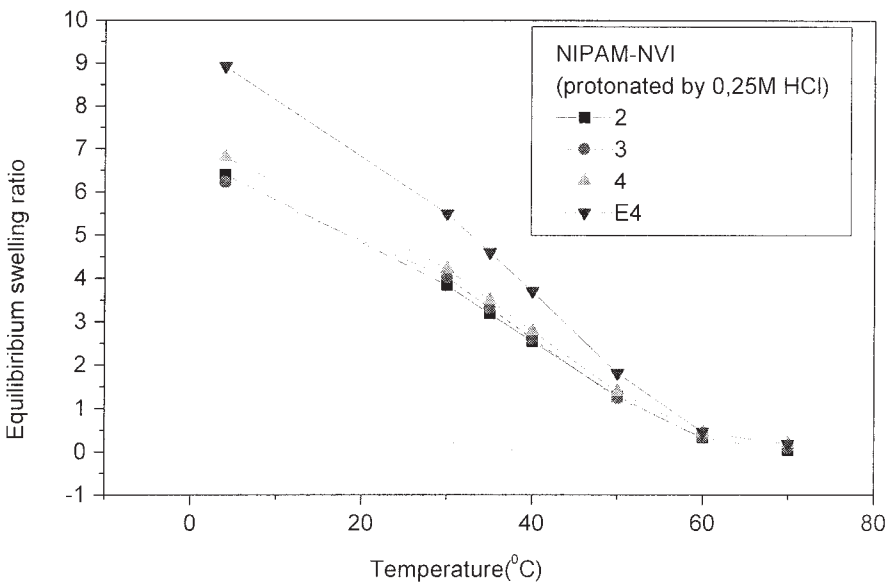
The initiator solution (0.05 mL), NaPS (0.05 g/mL water) accelerator solution (0.05 mL), TEMED (0.1 mL/1.5 mL of water), crosslinking agent (0.05 mL), MBAAm (0.05 g/mL of water), and water (0.02 mL) were included in the copolymerization media of all of the gels listed.

Preparation of the hydrogels

NIPAM and NVI random copolymers with different total monomer concentrations were prepared by radical copolymerization. Copolymeric hydrogels were prepared as described previously.[23] The conditions for the production of the poly(*N*-isopropylacrylamide-*co*-*N*-vinylimidazole) [poly(NIPAM-*co*-NVI)] hydrogels are summarized in Table I. Under these conditions, the monomer conversion was checked by gravimetric determination. As the gelation was almost 100%, the molar ratio percentage of the monomers (i.e., NIPAM/NVI) in the obtained hydrogels was equal to the initial feed compositions.

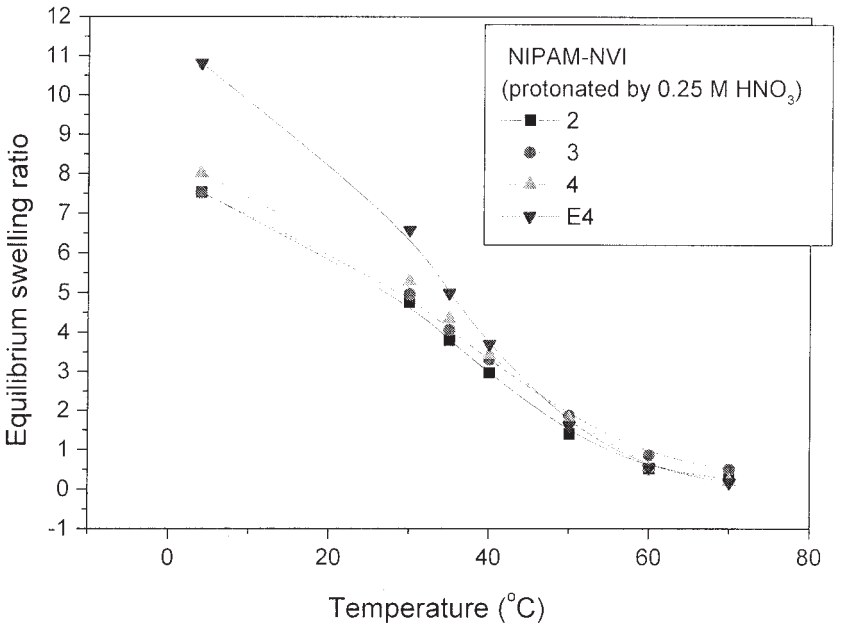


(a)



(b)

Figure 1 Temperature dependence of ESR for the protonated NIPAM–NVI copolymers produced at different total monomer concentrations (NIPAM/NVI = 34.54/65.46 mol/mol): (a) 0.1*M* HCl, (b) 0.25*M* HCl, and (c) 0.25*M* HNO_3.



(c)

Figure 1 (*Continued from the previous page*)

Protonation of the hydrogels

To enhance the swelling capacity of the hydrogels for their use in media of different pHs in biomedical applications, the pendent imidazole groups on the copolymer chains were protonated. For this purpose, the hydrogels were placed in contact with HCL (0.1 or 0.25*M*) and HNO_3 (0.25*M*) for 24 h.[24]

Swelling–deswelling studies

To test the thermoresponsiveness of protonated and unprotonated copolymeric hydrogels, we performed equilibrium swelling and dynamic swelling–deswelling studies in phosphate buffer solutions (pH 7.0, total ionic strength = 0.1) with the gels in the form of short cylinders. These experiments were conducted in a thermostatic water bath.

To obtain the variation of the equilibrium water content of the gels by the medium temperature, we incubated a washed cylindrical copolymer sample prepared in the form of a short cylinder in 75 mL of the buffer solution at a particular temperature for 24 h. At the end of this period, the weight of the gel sample was recorded after the removal of the excess surface water with laboratory tissue. The equilibrium swelling ratio (ESR) was defined as follows:

$$(W_c - W_d)/W_d$$

where W_e is the weight of the gel after the establishment of equilibrium in the buffer solution and W_d is the dry weight of the copolymer sample. The swelling behavior of the hydrogels was investigated by transfer of the gels that were in equilibrium at 70°C into phosphate solutions at 4°C. During the monitoring of the dynamic swelling behavior, the gels were left in the phosphate buffer solution at 4°C. The increase in the water content of the copolymer samples was followed by the determination of the gel weight against time. The dynamic swelling ratio was defined as follows:

$$\phi = (W_t - W_d)/[W_{o(70°C)} - W_d]$$

where ϕ is the swelling ratio, $W_{o(70°C)}$ is the gel weight at 70°C, W_t is the weight of the gel at a particular time, and W_d is the dry weight of the gel. The deswelling kinetics of the copolymer samples were followed by temperature changes in the opposite direction of the swelling behavior investigation. The samples equilibrated in the phosphate buffer solution at 4°C were transferred into another phosphate buffer solution at 70°C. The shrinkage of the hydrogels was followed by the determination of the decrease in the water content of the copolymer samples. The weights of the gels were recorded at particular times. The deswelling ratio was defined as follows:

$$\Theta = (W_t - W_d)/[W_{o(4°C)} - W_d]$$

where Θ is the deswelling ratio, $W_{o(4°C)}$ is the weight of the gel at equilibrium at 4°C, W_t is the weight of the gel at a particular time, and W_d is the dry weight of the gel.

TABLE II
ΔESR for the Unprotonated and Protonated NIPAM–NVI Hydrogels

Sample no.	Unprotonated gel	$\Delta ESR_{4-70°C}$ 0.1*M* HCl	0.25*M* HCl	0.25*M* HNO_3
2	6.39	6.50	6.36	7.80
3	6.23	6.0	6.13	7.01
4	5.42	7.19	6.62	7.27
E4	4.54	8.81	8.75	10.64

RESULTS AND DISCUSSION

The biomedical and biological applications of poly(NIPAM) hydrogels may require the derivatization of the gel structure without a loss of thermosensitivity. However, the derivatization of the poly(NIPAM) structure is usually difficult. In our previous study, the influence of the initial NIPAM/NVI molar ratio and total monomer concentration on the swelling properties and thermoresponsiveness was investigated.[23] In that study, we did not observe cracks on the gel surface. The monomer used for the preparation of the hydrogels in this study can bind various molecules such as metal ions and protein structures by forming complexes. Besides, the imidazole groups behave as weak bases in acidic solutions and become protonated. Thus, the hydrogels undergo volume changes in response to variations in the pH of the swelling solution. Therefore, some ligands having interaction abilities with the biological molecules may be incorporated more easily into the gel structure with these properties of NVI groups. Through the addition of an NVI-based structure into a copolymeric gel that is thermoresponsive within the proper temperature range, the binding and pH responsiveness of NVI groups may be used together with the thermoresponsive behavior to control the interactions of various biological molecules with the derivatized gel structure. Therefore, NVI is a good comonomer for the functionalization of NIPAM-based hydrogels.

In our previous studies, poly(NIPAM) gel was produced with different concentrations of the initiator, accelerator, and crosslinking agent. For the variation of the ESR by the medium temperature, the thermoresponsive poly(NIPAM) gel exhibited a transition at 32°C. In our other previous study, the influence of the initial NIPAM/NVI molar ratio and total monomer concentration on the swelling properties was investigated.[23] In that study, a loss of thermoresponsiveness was observed for the NIPAM-based gel structures prepared with higher NVI contents and higher total monomer concentrations. For copolymeric structures with better thermoresponsive properties than those of NIPAM–NVI hydrogels, these gels were protonated, and the copolymer behaviors were compared with those of the unprotonated hydrogels. The effect of protonation on the thermoresponsiveness of the produced gels with different total monomer concentrations was studied. In the polymerization experiments, the total monomer concentration was changed between 632 and 1897 g/mL by the initial NIPAM/NVI molar ratio being fixed at 34.54/65.46. The variation of the ESR of the protonated gels with the temperature is given in Figure 1. The results show that the NIPAM/

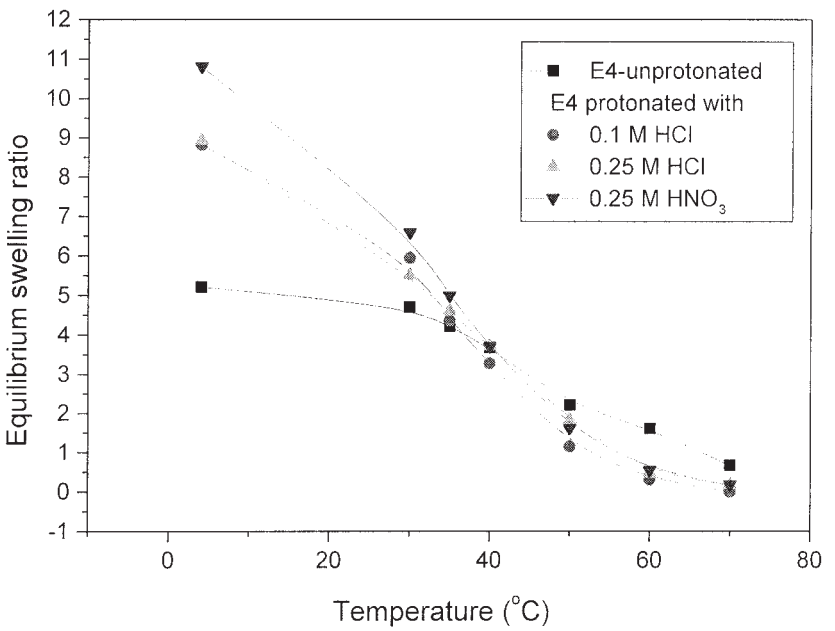


Figure 2 Temperature dependence of ESR for the E4 sample. The total monomer concentration was 1897 mg/mL.

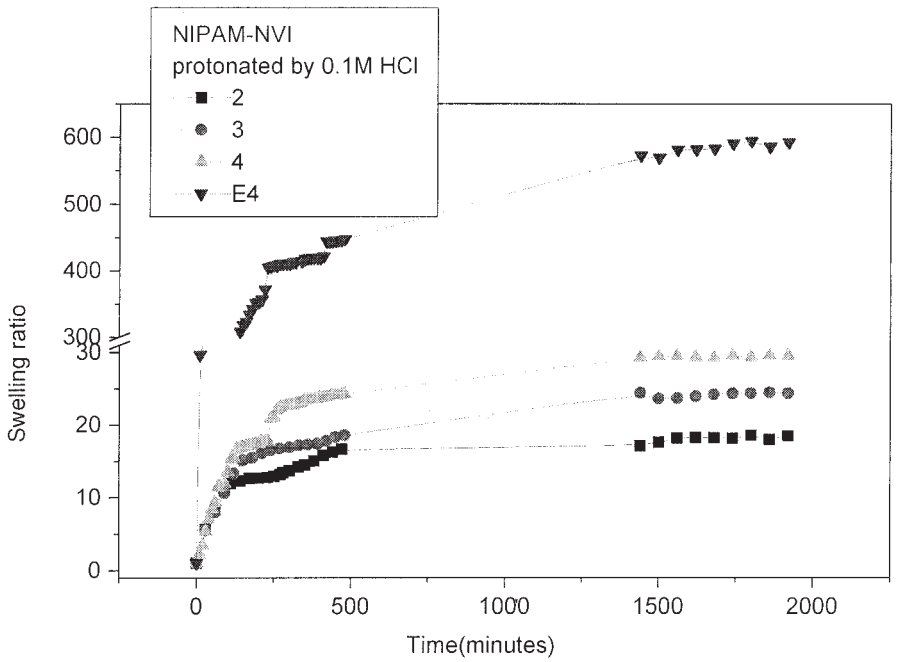


Figure 3 Swelling kinetics of protonated NIPAM–NVI copolymers produced at different total monomer concentrations. The magnitude of the step input for the medium temperature was 66°C (from 70 to 4°C).

NVI gels exhibited deswelling behavior at higher temperatures. The poly(NIPAM) gels swelled when cooled below the LCST and collapsed when heated above the LCST. This was because the amido group of NIPAM in the polymeric structure had an intermolecular hydrogen bond with the surrounding water at a low temperature, which turned into an intramolecular hydrogen bond over its transition temperature. This caused the hydrogel hydration capacity to decrease and the hydrophobicity of the isopropyl groups of the poly(NIPAM) gel to increase. These two effects caused the bond water in the hydrogel to change into free water, and the gel exhibited a volume-phase transition around its LCST. As shown in Figure 1, the temperature range at which the volume changed (the transition region) was shifted to higher temperatures and

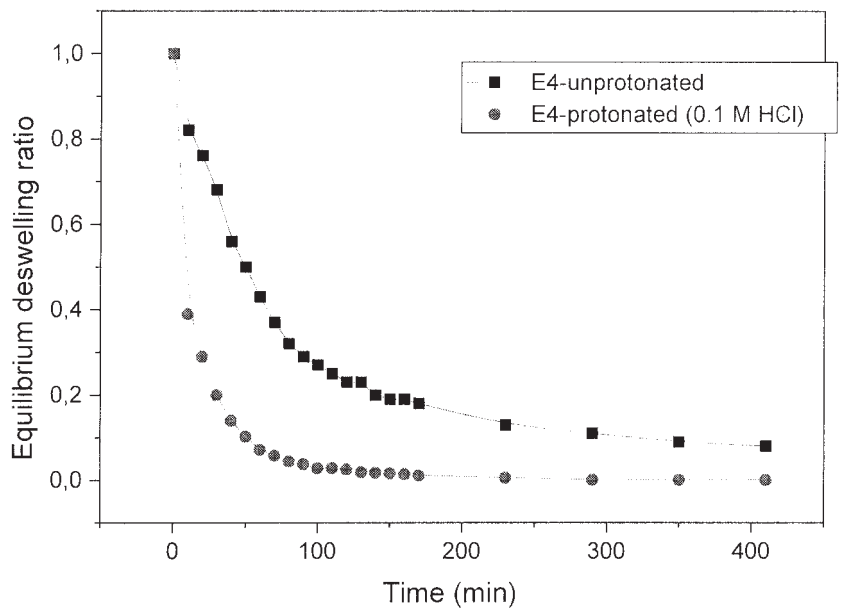


Figure 4 Deswelling kinetics of the unprotonated and protonated E4 sample. The magnitude of the step input for the medium temperature was −66°C (from 4 to 70°C).

broadened with the unprotonated NIPAM/NVI gel and all protonated gels with respect to the poly(NIPAM) gel. The LCST was regarded as the hydrophilic/hydrophobic balance of the copolymeric structure. When the hydrophilic moiety was incorporated into the network, the hydrophilic/hydrophobic balance was shifted toward a more hydrophilic nature, and its LCST shifted to a higher temperature. In the protonated gels, with an increase in the number of positive charge groups, the volume change at the transition increased because of increasing electrostatic interactions between the same charged groups, and the transition temperature rose. As shown in Figure 1, the ESR of the protonated gels increased with increasing total monomer concentration. The protonated gels had higher thermoresponsiveness because the equilibrium water content of the gel at 4°C and the ESR difference (ΔESR) between 4 and 70°C increased with the protonation of the gel. The ΔESR values are shown in Table II. The results show that the ΔESR values of the unprotonated gels between 4 and 70°C decreased with increasing total monomer concentration, but the ΔESR values of the protonated gels in the same temperature interval increased with increasing total monomer concentration. Representative curves of the temperature dependence of the ESR are shown in Figure 2 for the E4 sample. The protonated samples exhibited higher thermoresponsiveness because the equilibrium water content of the gel at 4°C and ΔESR between 4 and 70°C increased with protonation. This was due to the coulombic repulsion between newly formed $^{+}$N—H groups from the protonation of the imidazole groups. Charged groups on the polymer chains gave rise to a strong repulsive interaction over short ranges but caused attraction over long ranges.[25]

The variation of the dynamic swelling ratio of the protonated gels was investigated. An increase in the swelling rate and the fastest shrinkage were observed with the protonated gels (Figs. 3 and 4). The initial rates of swelling of the unprotonated and protonated gels are presented in Table III. This table indicates that the initial swelling rates decreased with increasing total monomer concentration in the unprotonated gels. The increasing total monomer concentration made the hydrogel more compact, and this caused decreasing initial swelling rates. If the free volume in the hydrogel was too low, water might be unable to penetrate the polymer matrix to initiate the swelling process. The results showed that the initial swelling rates of the protonated hydrogels were greater than those of the unprotonated hydrogels because of the repulsive interactions between neighboring $^{+}$N—H groups. The number of vinylimidazole groups in the polymer matrix increased with an increase in the total monomer concentration, and the coulombic (electrostatic) repulsion of the newly formed $^{+}$N—H groups from protonation increased.

TABLE III
Initial Swelling Rates [(g of Water/g of Gel)/min] for the Unprotonated and Protonated NIPAM–NVI Hydrogels

Sample no.	Unprotonated gel	$\Delta ESR_{4-70°C}$	
		0.1*M* HCl	0.25*M* HCl
2	1.34×10^{-2}	1.17×10^{-1}	2.83×10^{-1}
3	1.32×10^{-2}	1.21×10^{-1}	2.47×10^{-1}
4	1.25×10^{-2}	1.53×10^{-1}	1.77×10^{-1}
E4	1.21×10^{-2}	3.32	3.34

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