Characterization of Poly(methyl methacrylate) by Temperature Gradient Interaction Chromatography with On-Line Light Scattering Detection

Wonmok Lee, Hee Cheong Lee, Taihyun Chang,* and Seung Bin Kim

Department of Chemistry, Pohang University of Science and Technology, Pohang, 790-784, Korea

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ABSTRACT: The molecular weight distribution of anionically polymerized poly(methyl methacrylate) (PMMA) was investigated by temperature gradient interaction chromatography (TGIC). PMMA was well separated under an isocratic elution condition in which C4-bonded silica and acetonitrile were employed as the stationary and mobile phases, respectively. Column temperature was varied from 10 to 60 °C, while six PMMA standard samples (molecular weight range: 8 500−1 327 000) of narrow molecular weight distribution were eluted. Despite the temperature change during elution, the absolute molecular weight of each sample could be determined by light scattering detection. The molecular weight distribution of PMMA standards was not as narrow as that of polystyrene standards. Further fractionation of anionically polymerized PMMA can be achieved by exploiting the high resolution of TGIC.

Introduction

Recently we introduced a novel method, temperature gradient interaction chromatography (TGIC), to characterize the molecular weight distribution of polystyrene (PS) standards.[1−3] Unlike size exclusion chromatography (SEC), TGIC utilizes mainly the enthalpic interaction between solutes and the stationary phase, with the strength of interaction controlled by varying the temperature. The TGIC method has a number of advantages over SEC, such as far superior resolution and increased loading capacity. However, a relatively narrow set of separation conditions for the polymer species to be analyzed has to be identified for TGIC, whereas a wider variety of good solvents for the polymer can be used for SEC.[4] In fact, finding an optimum eluent condition is a general requirement for interaction chromatography; however, this requirement appears far more crucial for the interaction chromatography of polymers than small molecules because the thermodynamic behavior of polymer solutions is more complicated.[5] For example, the importance of selecting a right eluent to achieve isocratic elution of high polymers has been already recognized and the elution characteristics of polymers in solvent gradient HPLC cannot be accurately predicted by solvent strength concept.[6,7] We also found that selection of appropriate separation conditions was crucial to maximize the resolution of TGIC.

Recently, Lochmüller et al.[7] reported the retention behavior of poly(methyl methacrylate) (PMMA) in reversed-phase liquid chromatography (LC). They observed isocratic retention with a finite capacity factor by using proper binary solvent mixtures, thereby demonstrating the feasibility of PMMA characterization by interaction chromatography. In this paper we report the TGIC separation of PMMA using pure acetonitrile as an isocratic eluent. We again observed a better resolution of TGIC compared with SEC for PMMA. In addition, despite the temperature change during elution, we were able to measure the absolute molecular weight distribution by light scattering detection, which should greatly enhance the applicability of TGIC.

Table 1. Characterization of Standard PMMA by SEC and IC

	M_w (× 10^3), M_w/M_n				
sample	SEC/ Cal.[a]	SEC/ LS[b]	TGIC/ Cal.[c]	TGIC/ LS[d]	supplier[e]
I	9.4, 1.14	8.4			8.5, 1.14 (PC)
II	31.1, 1.05	31.3	32.0, 1.008	34.0	31.0, 1.03 (PL)
III	68.4, 1.09	66.0	68.6, 1.02	72.3	68.0, 1.07 (PL)
IV	183, 1.10	187		193	131, 1.02 (PC)
V	501, 1.09	506	533, 1.05	513	501, 1.09 (AP)
VI	1309, 1.28	1291			1327, 1.09 (PC)

[a] SEC characterization result by universal calibration method relative to nine polystyrene standards (mol. wt. range, 950−1 800 000; Polymer Labs.). [b] SEC characterization result by LALLS detection. [c] TGIC characterization result by calibration method relative to SEC/Cal values.[1] [d] TGIC characterization result by LALLS detection. [e] Provided by Polymer Lab Inc. (PL), Pressure Chemical Company (PC), and American Polymer Standards (AP).

Experimental Section

The TGIC apparatus was essentially identical to that reported previously.[1−3] A typical isocratic high-performance liquid chromatography (HPLC) apparatus equipped with a C4-bonded silica column (Alltech, Kromasil, 100 Å pore, 250 × 4.5 mm, 5-μm particle size) was used. The mobile phase was acetonitrile, which was used as received from Aldrich (HPLC grade) at a flow rate of 0.5 mL/min. The PMMA samples of narrow molecular weight distribution employed in this study are listed in Table 1. A mixture of six PMMA samples was made in acetonitrile, at a concentration for each polymer sample of 1 mg/mL, and injected through a Rheodyne 7125 injector equipped with a 50-μL sample loop. The column temperature was varied by circulating a fluid from a bath/circulator (Neslab, RTE-111) through a column jacket (Alltech). The chromatogram of PMMA was recorded with a UV/vis detector (LDC Analytical, Spectromonitor 3200) operating at a wavelength of 235 nm, a refractive index (RI) detector (Waters R401), a low angle laser light scattering (LALLS) detector (LDC Analytical, KMX-6), and an evaporative light scattering (ELS) detector (Polymer Lab., PL-EMD950).

For SEC analysis, the same HPLC instrument was used with the following changes. A set of four SEC columns

*To whom correspondence should be addressed.

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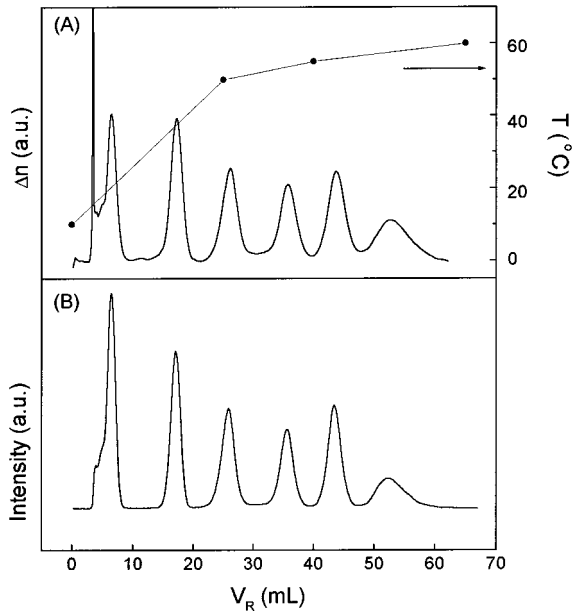

Figure 1. TGIC chromatograms of six PMMA standards listed in Table 1: (A) RI detection; (B) ELS detection. The sharp peak at ~3 mL in the RI chromatogram is attributed to low molecular weight contaminants and is much reduced in ELS chromatogram. The column temperature was varied from 10 to 60 °C in a four-segment linear ramp sequence as shown in the plot.

(Alltech, Jordi DVB; 10^5, 10^3, 10^2 Å and mixed bed; 250 × 10 mm; 5-μm particle size) were connected in series, and the column temperature was maintained at 40 °C with a column oven (Eppendorf, CH-430). Tetrahydrofuran (THF) was used as an eluent at a flow rate of 0.5 mL/min. A mixture of six PMMA samples was made in THF, at a concentration for each polymer of 1 mg/mL, and injected through a Rheodyne 7125 injector with a 100-μL sample loop.

Results and Discussion

As mentioned in the *Introduction*, the right choice of an eluent system is important for successful TGIC separation. We found that other solvent systems, such as a mixture of THF and H_2O (78:22 by volume), work comparably as well as pure acetonitrile. However, because a single solvent system allows us to employ light scattering detection with less complication than a mixed solvent system, acetonitrile was used in this study. Figure 1 shows the TGIC chromatograms of the six PMMA standards recorded by the RI (Figure 1A) and ELS (Figure 1B) detectors. The temperature of the column was raised by a series of four linear ramps from 10 to 60 °C, as shown in the plot. Near complete separation of the six standards was achieved, which is not possible by SEC. For an easy visual comparison, an equivalent SEC chromatogram of the same six PMMA samples is shown in Figure 2, where the lower resolution of SEC is evident. Note that the TGIC chromatogram was recorded by an RI detector, and, even though temperature was varied during the elution, there was no detectable drift in the detector response (we will come back to this result later). For these samples, we always found some contamination by low molecular weight component(s), which appeared as intense peaks in the RI or UV/vis chromatogram at a retention volume (V_R) near 3 mL. A similar observation was reported previously.[7] The intensity of this contribution is much reduced in the ELS chromatogram, as shown in Figure 1(B). This feature is likely due to the high volatility of the low molecular weight component. In addition, we confirmed the removal of these contaminants when we purified the PMMA samples by reprecipitation.

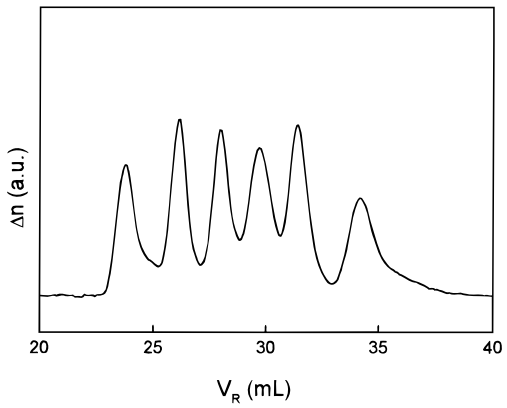

Figure 2. SEC chromatogram of the same PMMA samples as in Figure 1. In comparison with Figure 1, the superior resolution of TGIC to SEC is evident.

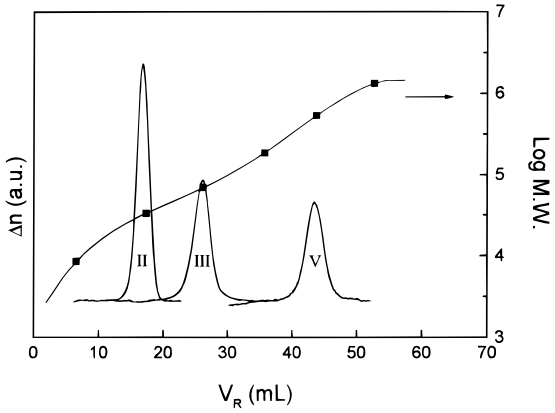

Figure 3. Calibration curve made from the TGIC chromatographic peaks in Figure 1 by fitting to a 4th-order polynomial and the individual chromatograms for Samples II, III, and V. The M_w and M_w/M_n values derived from the calibration curve are listed Table 1.

A calibration curve (log M versus V_R) was constructed from the chromatogram shown in Figure 1(A) by fitting the peak positions to a 4th-order polynomial; the result is displayed in Figure 3. The calibration curve could be changed easily by altering the temperature gradient, and we attempted to optimize the separation efficiency for these six PMMA samples. As a demonstration, two chromatograms taken with different temperature gradients are displayed in Figure 4. It is clear that a slight change in temperature program changes the retention greatly, because retention in TGIC mainly depends on the temperature,[1−3,19] just as it depends on solvent composition in solvent gradient HPLC.[8] Therefore, it is possible in principle to reduce the analysis time by making the temperature gradient steeper. At the moment, however, the long analysis time is required mainly because of two factors: (1) the temperature response of the circulating bath is slow (~1.5 °C/min), and (2) temperature equilibration across the stainless steel column wall is also slow. We have not tried seriously to reduce the analysis time because our focus in this study has been to find a set of conditions for TGIC separation of PMMA that also allows employment of light scattering detection. We believe that this factor

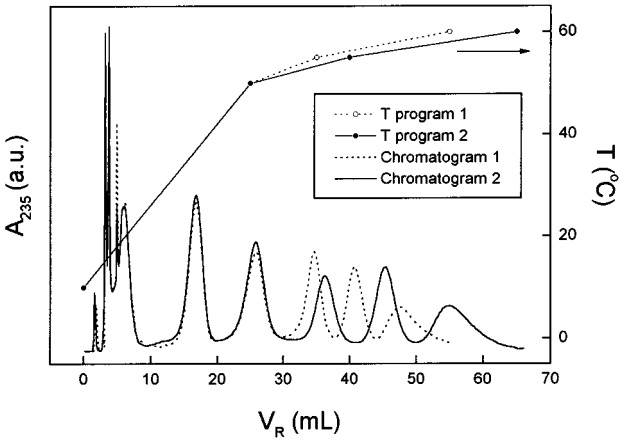

Figure 4. TGIC chromatograms of the same six PMMA samples using two different temperature gradient programs. It is evident that a slight change of the temperature affects the retention significantly.

can be improved by modifying the apparatus, and we are currently working on this practical aspect.

Three intermediate molecular weight PMMA samples were injected separately, and their elution peaks are also displayed in Figure 3. The weight average molecular weight (M_w) and polydispersity (M_w/M_n) value of each polymer were determined according to the calibration curve and are listed in Table 1. The M_w/M_n values are generally smaller than the SEC values, but the difference is not as great as the case of PS in which M_w/M_n from TGIC was much smaller than the values from SEC.[1] This result raises the question of whether the larger M_w/M_n values of PMMA (relative to those for PS) are derived from lower resolution of TGIC for PMMA or attributed to an intrinsically broader molecular weight distribution of PMMA samples. To clarify this question, we used TGIC to fractionate one of the PMMA samples. As shown in Figure 5(A), we divided the TGIC elution peak of Sample III into three fractions. For enhanced resolution, the column temperature was increased slowly at the rate of 1.2 °C/mL (0.6 °C/min) to expand the elution peak, as shown in the top abscissa. Each fraction was then analyzed by TGIC separately, and their chromatograms are displayed in Figure 5(B). The chromatograms of the fractionated samples clearly show a very low dispersion of each fraction, even though the signal-to-noise ratio is poor because of the low concentration of the fractionated samples. This result unambiguously indicates that the larger M_w/M_n values found in PMMA samples are not caused by line broadening associated with TGIC separation but are due to the intrinsically broader molecular weight distribution of PMMA compared with PS. For comparison, SEC chromatograms of the same three fractions are displayed in Figure 5(C). It is clear that SEC cannot resolve the peaks as well as TGIC. This line-broadening effect is well-known to be serious in SEC.[9] It is not surprising for PMMA to show a wider molecular weight distribution than PS because the anionic polymerization of PMMA is known to suffer from a greater extent of side reactions than PS.[10]

Acetonitrile, the eluent used in this TGIC study, is a theta solvent for PMMA at a temperature of 30 °C.[11] Therefore, the eluent is a poor solvent for PMMA, but the elution temperature is maintained well above the phase separation (binodal) temperature. For example, samples I, II, and III do not precipitate at 10 °C, the

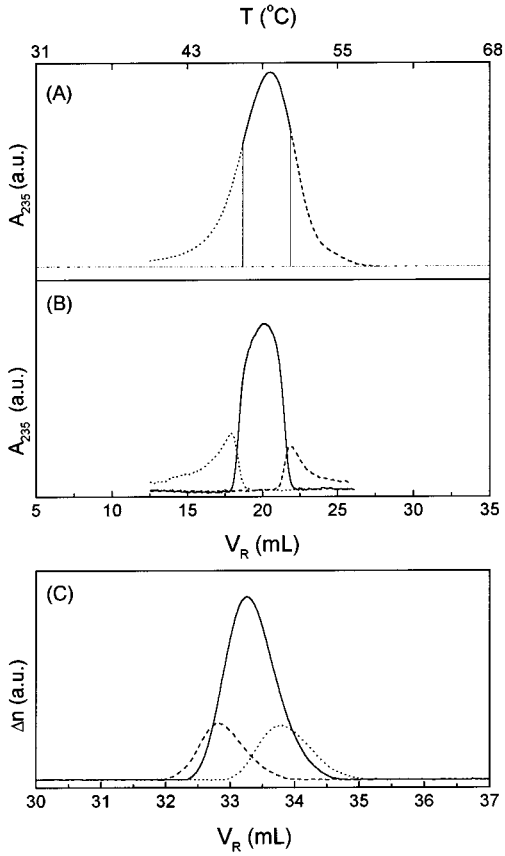

Figure 5. Fractionation of Sample III: (A) TGIC chromatogram of Sample III showing where the fractions were cut; (B) TGIC chromatogram of the resulting fractions; and (C) SEC chromatogram of the resulting fractions.

lowest temperature employed. The higher molecular weight samples (IV, V, and VI) precipitate at 10 °C, but dissolve completely before the system reaches the theta temperature, which is significantly below the final elution temperature. These measurements were carried out at concentration of 1 mg/mL. In addition, we found that the polymer solutions are easily supersaturated so that each takes 1−2 h to precipitate in a stirred vial at 10 °C. Thus it is possible that even high molecular weight PMMA remains dissolved for the entire separation process. The TGIC separation was also found to be sensitive to the choice of stationary phase, indicating that the stationary phase is not a passive support material but plays an important role in the separation. For example, if a C18-bonded phase is used instead of the C4-bonded phase, PMMA is more strongly retained at the same elution condition, as shown in Figure 6, where two chromatograms obtained with C4-bonded phase (top) and C18-bonded phase (bottom) columns are displayed. For a direct comparison, the two chromatograms were obtained using the same temperature program and columns of identical dimension, particle size, and porosity. Although this behavior requires further study to elucidate the detailed thermodynamic nature of the interaction between PMMA and the stationary phase, longer alkyl chains appear to interact with PMMA more efficiently. In addition, both bare silica and CN-bonded phase columns behave quite differently from the alkyl-bonded columns, and we were not able to find a proper separation condition with them. Combining these observation, we conclude that the TGIC separation mechanism of PMMA is not a simple precipitation−redissolution process.[12,13]

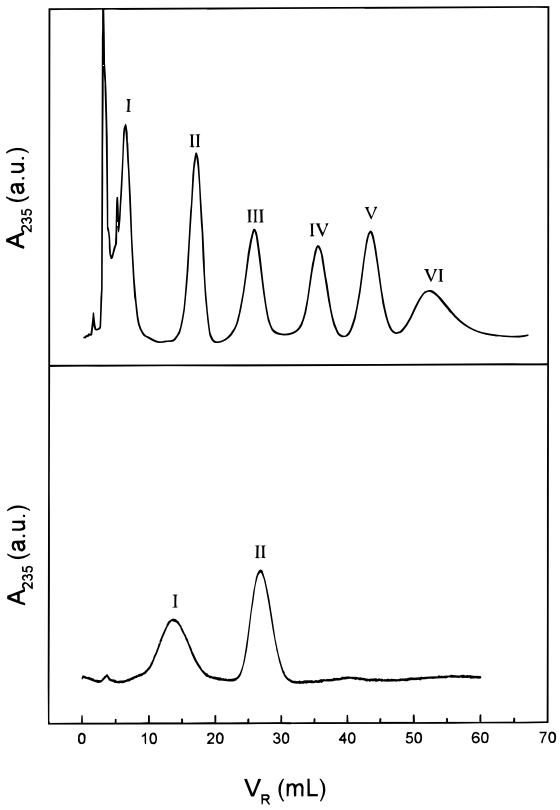

Figure 6. TGIC chromatograms of six PMMA samples obtained with C4- (top, Alltech, Kromasil) and C18- (bottom, Alltech, Nucleosil) bonded stationary phase columns. For a direct comparison, the chromatograms were obtained under the same temperature program and columns of identical dimension, particle size, and porosity (100 Å pore, 250 × 4.5 mm, 5-μm particle size). The C18-bonded phase retains PMMA more strongly than the C4-bonded phase.

It is interesting to note, however, that the eluents used for TGIC separation of both PS and PMMA are poor solvents for the respective polymers, because the interactions between the stationary phase (hydrocarbon in the reversed-phase HPLC column used in this study) and the polymer chain segments are not quite favorable. Because aliphatic hydrocarbons are not good solvents for PS or PMMA, such a poor solvent condition would provide an environment where temperature could play a decisive role in the molecular weight-dependent separation. If solvent quality is good, interaction with the stationary phase becomes relatively weak and size exclusion effect dominates; as a result, the polymers are eluted before the injection solvent. We previously demonstrated this behavior by simultaneous characterization of a mixture of PS and PMMA, where PS was separated by an interactive mechanism and PMMA was separated by a size-exclusion mechanism.[3] In that case, the temperature change did not affect the retention characteristics in the SEC regime significantly. If the solvent is too poor, then polymer chains adhere to the stationary phase too strongly to be eluted out in a reasonable time period. Therefore, it is best to carry out the separation near the interaction-to-size exclusion transition point, where the size exclusion effect compensates the interaction effect.[14−16] We are currently working on a description of the thermodynamics involved in the separation process to elucidate the separation mechanism more quantitatively.

A single component eluent is used in this system, so light scattering detection can be easily employed for

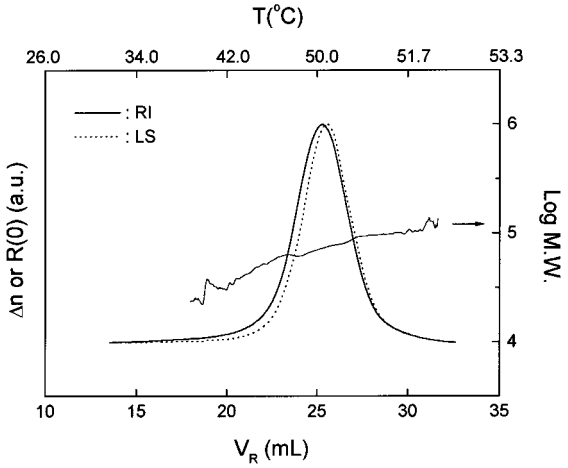

Figure 7. TGIC chromatograms of Sample III recorded by the RI and LALLS detectors. The log (MW) versus V_R relationship determined from the two chromatograms is also displayed. The temperature change during elution is shown on the upper abscissa.

absolute molecular weight determination if the temperature is controlled back to a stable value when the eluted samples reach an RI or LALLS detector, either of which is sensitive to fluctuation in temperature. To reduce the baseline drift induced by temperature variation, the eluted solution was passed through a 1-m length tubing prior to a UV detector, followed by a LALLS detector, and then finally through an RI detector. We found no detectable drift of the RI and LALLS detector response, as shown in Figures 1 and 7. The magnitude of dn/dT for acetonitrile (measured with an Abbe refractometer to be −0.00056/K) is not large, but enough to cause a noticeable drift of the RI detector. Therefore, our moderate effort of postcolumn temperature control must have been good enough to reequilibrate the temperature to room temperature and thereby avoid noticeable drift in the baseline.

In addition to the elimination of background refractive index drift, dn/dc must be kept constant during a chromatographic run for accurate molecular weight determination by light scattering detection. In a separate measurement of dn/dc with a differential refractometer (LDC Analytical; KMX-16) over the temperature range 10−60 °C, we found that d(dn/dc)/dT of the PMMA/acetonitile system was 7.5×10^{-5} mL/g/K, which translates into a change in dn/dc of the system from 0.128 at 10 °C to 0.132 at 60 °C. This variation, ∼3%, would cause a maximum 6−7% deviation in the molecular weight determination because the scattered light intensity is proportional to (dn/dc)^2. In fact, the actual deviation should be much less than the bounds because the temperature of the eluted solution is largely reequilibrated not to show any drift in the RI detector response curve. Consequently, the extent of uncertainty due to temperature change in molecular weight determination by on-line light scattering detection in TGIC should be insignificant considering the precision of the light scattering technique itself. Figure 7 shows two chromatograms of Sample III monitored by RI (solid line) and LALLS (dotted line) detectors. The molecular weight distribution of the elution peak determined from the detector responses, also plotted in the figure, shows a fairly stable response of the LALLS detector. The determined absolute weight average molecular weights of PMMA samples are listed in Table 1. In calculating these molecular weights, a fixed dn/dc value at 25 °C

(0.129 mL/g) was used. As shown in Table 1, molecular weights determined by light scattering are in good agreement with those obtained by the calibration method. This result emphasizes the distinct advantages of the temperature gradient method over the solvent gradient method[6−8,12,13,17−19] in that only the former allows use of an RI or a light scattering detector.

In conclusion, we found that PMMA can be separated with very high resolution by TGIC employing acetonitrile as the eluent and a C4-bonded column as the stationary phase. Moreover, the level of resolution is sufficient that TGIC can be easily used to further fractionate the anionically polymerized PMMA. The elution temperature is generally much higher than the precipitation temperature and the retention depends on the choice of stationary phase, so the separation mechanism does not appear to involve precipitation−redissolution but rather sorption of polymer chains to the stationary phase. The molecular weight distribution of anionically polymerized PMMA was somewhat broader than anionically polymerized PS. Light scattering detection, as well as RI detection, can be easily employed in TGIC; no detectable drift was observed in the response of either detector when the eluted solution is made to pass through a moderate length of tubing and a UV/vis detector to reequilibrate the temperature.

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