

The study of Au sol synthesized in uncharged and charged star-like copolymers under light irradiation

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Received May, 10, 2019

This work was devoted to the study of the photothermal and photochemical processes occurring under laser irradiation of the nano-systems consisting of gold nanoparticles incorporated into polymer nano-carriers in a non-ionic and anionic form. The UV-vis spectroscopy demonstrated that the intensity of the surface plasmon resonance band is higher for the nano-system synthesized in an anionic polymer; this indicates a slightly larger number of formed nanoparticles. Holographic interferometry in real time didn't register a drastic difference in the behavior of Au sols synthesized in non-ionic and anionic polymer matrices. However, a local heating process took place under the irradiation of both Au sols.

Keywords: nanoscale systems, star-like copolymer, gold nano-particle, holographic interferometry.

Работа посвящена изучению фототермического и фотохимического процессов композитных наноразмерных систем, состоящих из наночастиц золота в полимерном носителе в неионной и анионной форме, происходящих под действием лазерного облучения. Ультрафиолетовая спектроскопия показала, что интенсивность полосы поверхностного плазмонного резонанса выше для наносистем, синтезированных в анионном полимере, что указывает на несколько большее количество образовавшихся наночастиц. Голографическая интерферометрия в реальном времени не показала резкого различия в поведении золей Au, синтезированных в неионной и анионной полимерной матрице. Однако, процесс локального нагрева происходил при облучении обеих золей Au.

Дослідження золів золота, отриманих у незарядженому і в зарядженому зіркоподібному полімері в умовах опромінення світлом Ю.І.Гарагуц, В.А.Павлов, Е.В.Мокринська, Н.Г.Чуприна, Н.А.Давиденко, Н.В.Куцевол, А.П.Науменко, П.А.Вірич

Робота присвячена вивченню фототермічного і фотохімічного процесів композитних нанорозмірних систем, що складаються з наночастинок золота у полімерному наносію у неионній і аніонній формі, що відбуваються під дією лазерного опромінення. Ультрафіолетова спектроскопія показала, що інтенсивність смуги поверхневого плазмонного резонансу вище для наносистем, синтезованих в аніонному полімері, що вказує на більшу кількість наночастинок, що утворилися. Голографічна інтерферометрія в реальному часі не показала різких відмінностей в поведінці золів Au, синтезованих в неионній і аніонній полімерній матриці. Однак, процес локального нагріву відбувався при опроміненні обох золів Au.

1. Introduction

The use of multifunctional polymer nano-carriers which can enhance the target-ori-

ented therapy becomes a modern trend in the anticancer therapy [1]. The main aim is to damage tumors at the cellular level using a selective attack on the tumor cells in order

to reduce significantly the harmful secondary effects on the healthy cells. Recent studies were focused on photo-thermal and photo-dynamic treatments using near-infrared wavelength lasers. A combination of the multifunctional plasmon nano-particles and photodynamic agents could help to achieve a synergistic effect for the tumor therapy [2, 3].

Gold nanoparticles are considered to be photo-thermal agents as well as photo-sensitizer carriers. Their surface plasmon resonance is of high efficiency for light-to-heat conversion, consequently, generating hyperthermia [4]. Also, the gold nano-particles can be easily accumulated in the tumor tissue by the enhanced permeability and retention effect of the tumor [5].

In our recent work it was shown that a nano-composite consisting of gold nanoparticles and photosensitizer chlorine e6 in dextran-graft-polyacrylamide nano-carriers demonstrated a twofold increase of photodynamic efficacy compared to the free photosensitizer for in vitro and in vivo experiments on the malignant cell line MT-4 (human T-cell leukemia) [6]. Singlet oxygen was registered when the nano-composites were exposed to the red (658 nm) laser light (power density 1.1 mW/cm², dose 1 J/cm²).

The present study is focused on understanding the processes occurring under laser exposure in the nano-systems synthesized in non-ionic and anionic polymer nano-carriers.

2. Experimental

Polymer. Copolymers dextran-graft-polyacrylamide (D-g-PAA) with a dextran core ($M_w = 70 \cdot 10^5$ g/mol) and 5 grafted PAA chains in non-ionic and anionic forms were used as a polymer matrix for AuNPs synthesis. Synthesis, molecular parameters and peculiarities of the macromolecular structure of a star-like copolymer D-g-PAA were discussed in details in [7]. The average molecular weight of D-g-PAA (M_w) is equal to $1.57 \cdot 10^6$ g/mol, radius of gyration (R_g) is 67 nm, and polydispersity (M_w/M_n) is 1.81.

An anionic form of the copolymer (further as D-g-PAAan) was obtained via alkaline

hydrolysis of the initial copolymer during 30 min by using sodium hydroxide. The fraction of mers bearing carboxylate groups evaluated by potentiometric titration was equal to approximately 37 %. It was established that in the anionic form, the graft has extremely expanded molecular structure caused the increase of the macromolecule rigidity [7].

Gold nanoparticles synthesis. The AuNPs were synthesized by the chemical reduction of the Au precursor (Tetrachloroauric acid, Aldrich). All synthesized polymers play a role of matrices capable to act as nucleating, capping and stabilizing agents simultaneously.

The 0.012 ml tetrachloroauric acid aqueous solution ($C = 0.1$ M) was added to 0.5 ml of the aqueous polymer solution ($C = 1 \cdot 10^{-3}$ g/cm³) and stirred during 20 min. Then, 0.047 ml of the 0.1 M aqueous solution of sodium borohydride (Aldrich) was added. The final solution was stirred for 30 min. It turned ruby-red in colour, thus the formation of AuNPs was indicated. The reduction process was performed at $T = 25^\circ\text{C}$. The hydrodynamic diameter of the polymer is uncharged and charged for ms as well as the ζ -Potential of the polymer solutions and Au sols are represented in the Table 1.

Dynamic Light Scattering (DLS) and Zeta-Potential Analysis. DLS and Zeta-potential measurements were carried out using a Zetasizer Nano ZS90 (Malvern Instruments Ltd., UK). The apparatus contains a 4 mW He-Ne laser with a wavelength of 632.8 nm; the scattered light is detected at the angle of 173° (backscattering).

UV-visible spectroscopy. UV-visible absorption spectra of the copolymer solutions and Au sols were recorded by a UV-visible spectrophotometer UV1900 from 200 to 800 nm.

Transmission electron microscopy (TEM). For the sample preparation, Cu grids with carbon film were used (Elmo, Cordouan Technologies Bordeaux France). A 5 μl drop was deposited and let adsorbed for 1 min; then the excess of the solution was removed with a piece of filter paper. The observations of the AuNPs were carried out employing two TEMs, Tecnai G2 or CM12

Table 1. The ζ -Potential of the polymer solutions and Au sols

No.	Sample	D_h , nm	ζ -Potential, mV
1	D-g-PAA	49.8±1.3	~ 0
2	Au/D-g-PAA	169.9±3.0	-16.46±0.41
4	D-g-PAA(an)	103.6±9.0	~ 0
5	Au/D-g-PAA(an)	270.4±3.8	-17.55±2.29

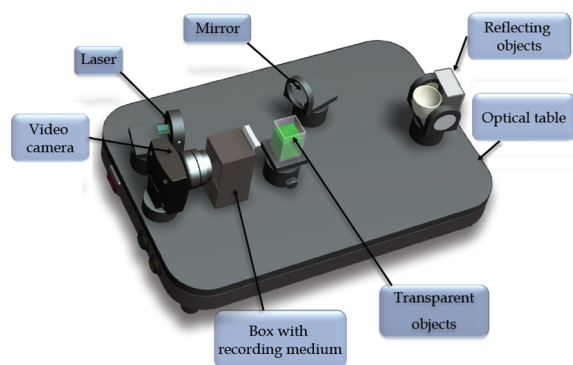


Fig. 1. Double exposure real time Holographic Interferometry setup.

(FEI, Eindhoven Netherlands), and the images were acquired with a CCD Eagle camera on the Tecnai and a Megaview SIS Camera on the CM12.

Holographic Interferometry setup. For study of photothermal and photochemical reactions in the composite nano-scale systems, the method of holographic interferometry in real time was proposed and experimentally applied [8]. The method was tested using the equipment kit [9] to determine the refractive index (n) and its change (Δn) for liquid and gaseous media (Fig. 1). Measurements of Δn on a model object during its illumination includes registration of the hologram of cuvettes with the investigated medium, reconstruction of the hologram, illumination of the medium with an exciting light with a certain wavelength (λ_{irr}) and intensity. Then, using the camcorder, the obtained interference pattern was observed and recorded in the computer for further calculation of Δn by measuring the spatial frequency of the interference bands appearing in the cuvette image. This study was devoted to studying the possibility of hyperthermia process occurring in the nano-system under laser irradiation.

In the experiments, the laser was used with $\lambda_{irr} = 650$ nm which is close to the long-wavelength maximum absorption of Au nanoparticles [5].

3. Results and discussion

The photodynamic *in vitro* testing of the nano-systems synthesized in polymer D-g-PAAan nano-carriers in an anionic form demonstrated higher efficiency in comparison with the nano-composites synthesized in the non-ionic D-g-PAA polymer matrix (Fig. 2). The study was carried out on the malignant cell line MT-4 [6].

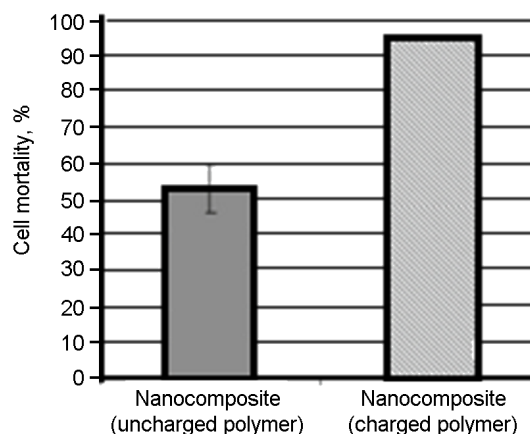


Fig. 2. Photocytotoxicity of nano-composites with Au sols, prepared in non-ionic D-g-PAA and D-g-PAAan polymer matrices.

To understand the reason of such difference in the photodynamic efficiency of the similar nano-composite systems, these systems were studied by TEM, UV-vis and the method of holographic interferometry. We have focused on the impact of AuNPs on the processes that occurring under laser irradiation of both nanocomposites.

It is known that the size and shape of nanoparticles affect the AuNPs biological activity [5]. TEM images of AuNPs, synthesized in the solutions of D70-g-PAA and D70-g-PAAan are represented in Fig. 3.

It is seen that the sols synthesized in the non-charged and anionic polymer matrices differ in size characteristics of AuNPs. The nano-system obtained in the non-ionic polymer matrix contains individual nanoparticles of 1.5–2 nm in size (Fig. 3, c) and some clusters of nanoparticles of 10 to 20 nm. (Fig. 2, a). The AuNPs synthesized in D70-g-PAAan solutions are of 2– nm in size (Fig. 3; b, c).

The reason for the differences in the sols obtained in the solutions of branched non-ionic and anionic polymers may be caused by the chemical nature of the polymer matrices. The interaction of the Au precursor with the anionic polymer matrix takes place with both carbamide (as in non-ionic polymers) and carboxylate groups. Moreover, the interaction has the following different mechanisms: the ion-dipole interaction in the first case, and the electrostatic one in the second case. It is supposed that on the carbamide groups of anionic matrices, the same particles form as in the non-ionic matrices. However, the nanoparticles formed on the carboxylate groups are smaller [7]. The study of the aging process of Au sols prepared in the anionic polymer

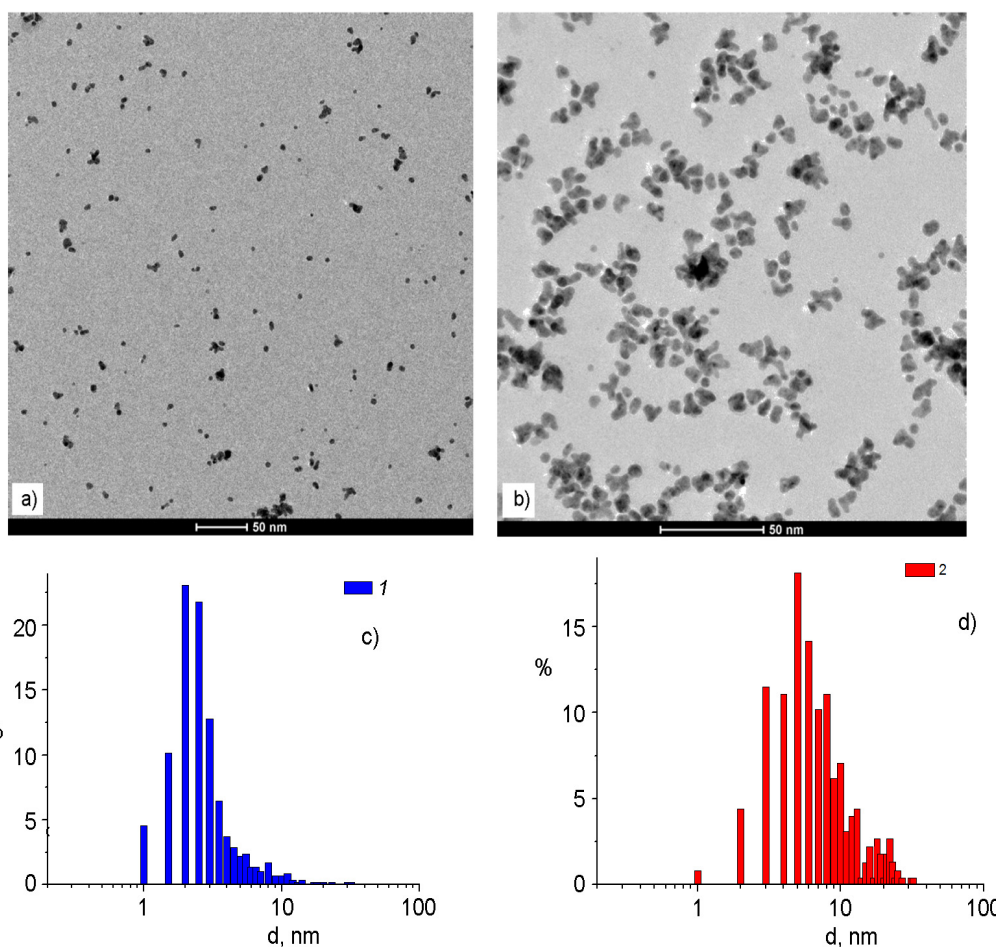


Fig. 3. TEM images (a,b) and size distribution of nanoparticles (c,d) in Au sols synthesized in D70-g-PAA (a, c) and D70-g-PAA(an) solutions (b, d).

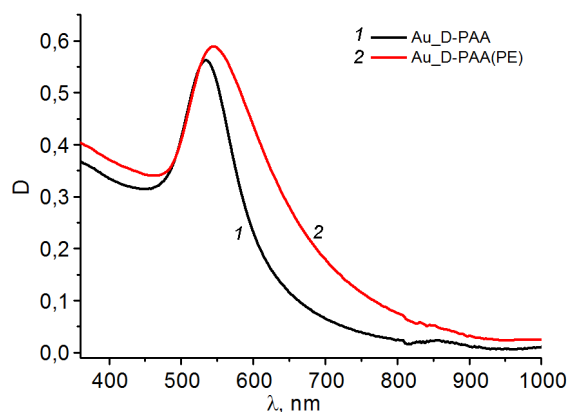


Fig. 4. Absorption spectra of nano-systems: D70-g-PAA/AuNPs (curve 1); D70-g-PAA(PE)/AgNPs (curve 2).

matrix revealed the disappearance of small nano-particles and increase in size of nanoparticles formed on the amide functional group of the polymer chains [10].

UV-Vis spectra of the AuNPs synthesized in the solutions of D70-g-PAA and D70-g-

PAAan polymers are shown in Fig. 4. A well defined surface plasmon resonance (SPR) for the gold nano-particles was observed for all the prepared sols. The difference in absorption curves for the sols synthesized in the non-ionic and anionic star-like polymer matrices is evident. The spectrum of the D70-g-PAAan nano-system is red-shifted and has greater full width at half maximum. It is known that for metallic nano-particles, such important characteristics as the frequency and spectral line half-width (the decay of the surface plasmon resonance) depend essentially on their size, namely, when the size of the nano-particles increases, the resonant wavelength increases too (red shift), and an increase in the spectral half-width (expansion) resonance is observed. That is, the spectral line position and shape in the spectral band indicate an increase in the size of the nano-particles for the nano-system on the base of a charged star-like copolymer.

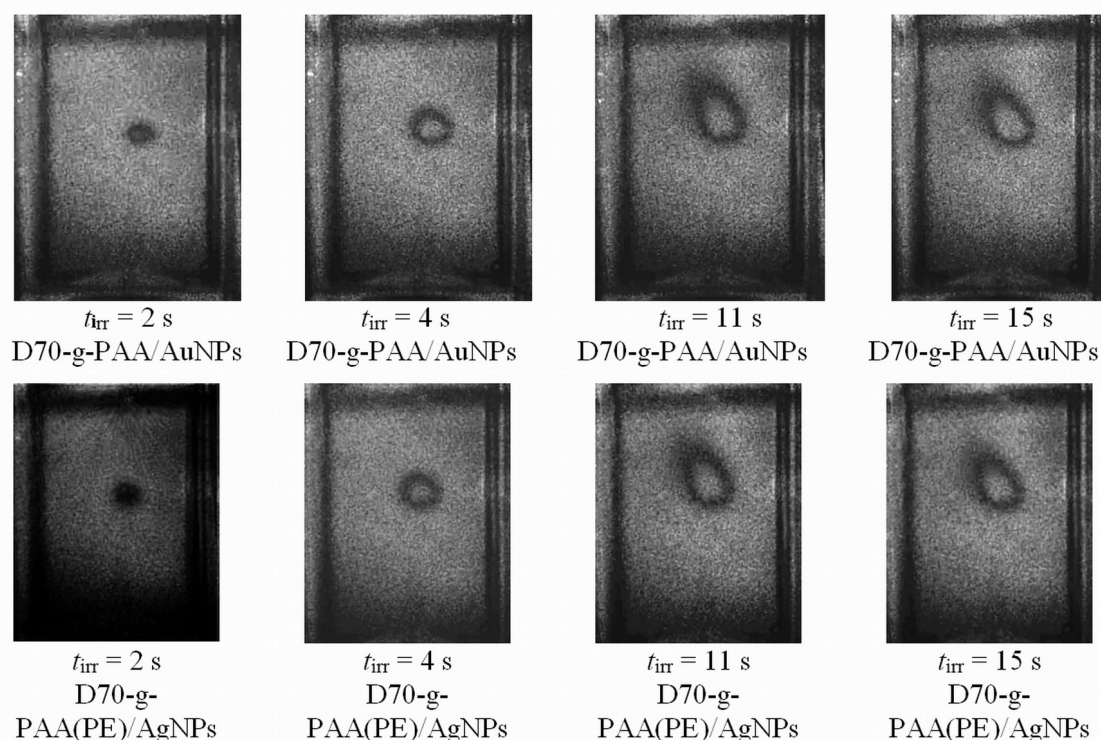


Fig. 5. Photographs of cuvettes with the solution during laser light illumination with $\lambda_{irr} = 650$ nm and power of 10 mW.

The method of holographic interferometry allows registering the change in the refractive index in a local volume. Fig. 5 demonstrates the behavior of both Au sols synthesized in the non-ionic and anionic polymer solutions under red laser ($\lambda_{irr} = 650$ nm) illumination. It should be noted that the wave length and power density of the laser was the same as for *in vitro* experiments [6]. It is evident that over time (t_{irr}) after the illumination begins, the number of interference bands increases testifying an increase of Δn . After ceasing the light, the interference pattern relaxes and the bands disappear. The interference bands are localized only in the volume limited by the laser beam and there is no observed diffusion of the heated solution into other parts of the cuvette. However, the drastic difference in the behavior of Au sols synthesized in the non-ionic and anionic polymer matrices was not registered.

Fig. 6 shows that the Δn linearly depends on the laser radiation power within the range of 0.1 to 10 mW. It is evident that the relaxation time $\tau_r = 3 \pm 0.2$ s does not depend on the laser radiation power. To explain the experimental results, a phenomenological model is proposed which takes into account the features of the molecular structure of the star-like Dextran-graft-Polyacrylamide copolymer with incorporated Au nano-particles. Since

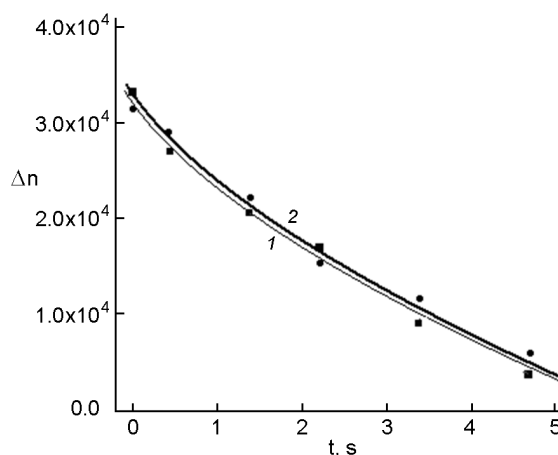


Fig. 6. Relaxation of Δn during time t after switching off laser light with $\lambda_{irr} = 650$ nm and a power of 10 mW after illumination.

under illumination with $\lambda_{irr} = 650$ nm light the variation of n is observed mainly in the small volume of the cell near the laser beam transmission region (Fig. 5), the following model consideration can be proposed.

Absorption of laser light with $\lambda_{irr} = 650$ nm occurs in Au nanoparticles. Since the fluorescence in the investigated samples is not observed and there are no reasons for photochemical transformations, it can be assumed that the dissipation of all absorbed light en-

ergy happens by excitation of oscillations. Namely, all absorbed light energy is transformed into thermal energy.

Since Au nanoparticles have a high thermal conductivity and they are placed close to fragments of polymeric molecules, it can be supposed that thermal vibrations from the Au nano-particles are transferred to the nearest similar fragments and from them to other Au nanoparticles within a single large polymeric molecule. Such a stepped mechanism of transferring heat energy within a single large polymer molecule must also take into account the energy dissipation to excite vibrations of H₂O molecules located inside the free space of the polymer molecule.

After thermal excitation of an individual polymer molecule with Au nanoparticles, the excitation of vibrations of neighbor H₂O molecules and further excitation of neighboring polymer molecules occur. Therefore, as a result of this cascade process, the aqueous medium in the cell is not immediately heated but only after the local heating of the polymer molecules with Au nanoparticles in the areas where the light was absorbed.

In our experiments, the change of n is observed mainly from the change of n of the aqueous medium. Therefore, this change is seen only near the volume in the cell where the laser light with $\lambda_{irr} = 650$ nm is absorbed. The surface plasmon involves laser heating of AuNPs in the water-based sols.

Thus, the process of local heating has been registered under laser irradiation of nano-composites containing AuNPs. These results are in good agreement with our recent study of Au colloids prepared in the D-g-PAA solution [12]. The temperature of the AuNPs was determined via the broadening of the SPR absorption band. The heating showed a resonant character. Namely, it was demonstrated that the temperature of the AuNPs depends strongly on the laser frequency detuning from the SPR in the AuNPs. The sharp increase of AuNPs temperature to about 316 K was obtained at moderate laser intensity of $5\text{Å} \sim 10^3$ W/cm².

The present study gives an impetus to understand the importance of the AuNPs in the nano-composites for a photo-dynamic therapy (PDT). However, the reason of higher efficiency for the PDT with the nano-systems synthesized in the anionic polymer matrix in comparison with non-ionic ones is not clear. The interaction of non-ionic and anionic polymer nano-carriers with a cell membrane should be the next stage to understanding the mechanism of action of hybrid nano-composites for photo-dynamic anticancer therapy [13].

4. Conclusions

Thus, the difference in absorption curves for the Au sols synthesized in non-ionic and anionic star-like polymer matrices was found. The intensity of the SPR band was a bit higher for the AuNPs/D70-g-PAAan nano-system. The absorption of laser light ($\lambda_{irr} = 650$ nm) in Au nanoparticles was registered by the method of real-time holographic interferometry. It was established that all absorbed light energy was transformed into thermal energy as the interference bands were localized only in the volume limited by the laser beam. No drastic difference in the behavior of the Au sols synthesized in the non-ionic and anionic polymer matrices was registered. The effect of the local heating can enhance the efficiency of the nano-composite with incorporated AuNPs for photodynamic therapy, because of the hyperthermia effect.

Acknowledgements. This publication is supported in part by the Ministry of the Education and Science of Ukraine: joint Ukrainian-Belarusian research and development projects "Design and physico-chemical properties of novel multicomponent nano-systems for the treatment and diagnostics of solid tumors" (2019-2020).

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