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Releasing property from surface polyion complex gel

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ABSTRACT: Surface polyion complex (sPIC) gels were prepared with a nonionic hydrogel interior core, composed of poly(*N*-vinylformamide and poly(*N*-vinylacetamide), and a chemically bounded polyion complex layer on the outer surface, composed of poly(vinylamine) and poly(acrylic acid). The gels were investigated as controlled drug release models based on electrostatic interactions depending on pH. Methylene blue and allura red were employed as cationic and anionic drug models, respectively, and resulted in the selective adsorption depending on pH conditions. Monovalent and multivalent anionic drug models, allura red and 1,3,6-naphthalenetrisulfonate were observed for their releasing behavior from the sPIC gel. The results indicated that the multivalent anionic drug effectively controlled release depending on pH conditions. We further investigated sPIC gels regarding their ability to control the release of ionic molecules as a function of pH-driven changes in electrostatic interactions. © 2015 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2015**, *132*, 42081.

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INTRODUCTION

Gradient structures on hydrogels have attracted much attention. The simple preparation of an interpenetrating polymer network[1] and the concentration of the crosslinker[2,3] have been investigated for hydrogels with a gradient density of networks. The surface condition of the hydrogel preparation also has a great influence on the physical characteristics.[4] Recently, the unique structural characteristics of gradient hydrogels have also been applied in materials science. For example, the gradient hardness of hydrogels due to their crosslinking densities resulted in different cell adhesion morphologies,[5,6] and even induced migration direction.[7] Proteins[8] and growth factors[9] have also been used in gradients in hydrogels to control cell proliferation. In addition to cell adhesion control, the gradient structure of hydrogels has been applied to actuator[10,11] and drug release[12,13] materials.

A great number of controlled drug release systems have been investigated, taking advantage of their unique chemical characteristics, such as electrostatic interactions,[14,15] solubility differences,[16] hydrogel capsule,[17] and hydrogel mesh size.[18,19] By designing the chemical structures of these hydrogels, intelligent releasing hydrogels have been developed, such as dual stimuli responsive nanocontainers[20] and hydrogels,[21] and insulin molecular responsive hydrogels.[22] Therefore, the design of a gradient network at the molecular level is an important topic.

N-Vinylacetamide (NVA) and *N*-vinylformamide (NVF) are vinyl monomers, and contain nonconjugated vinyl group which is directly connected to a nitrogen atom.[23] A series of polymerization and materials applications using *N*-vinylamides have been reported.[24–28] The unique structure provides a partly cationic poly(vinylamine) (PVAm) after polymerization and the subsequent hydrolysis.[29] It is noteworthy that nonionic poly(*N*-vinylacetamide) (PNVA) and poly(*N*-vinylformamide) (PNVF) have a stable swelling ratio (S.R.) against various pH and temperature conditions, whereas the hydrolyzed part changes to a cationic PVAm (Figure 1). Recently, we applied an interpenetrating polymer network (IPN) to poly(*N*-vinylamide) hydrogel,[30–34] to overcome their low radical polymerizability and limited copolymerization. In these experiments, we developed a novel gradient hydrogel: a surface polyion complex (sPIC) gel,[34] which was composed of a nonionic hydrogel interior core and a chemically bounded polyion complex layer on the outer surface (Figure 1).

Additional Supporting Information may be found in the online version of this article.

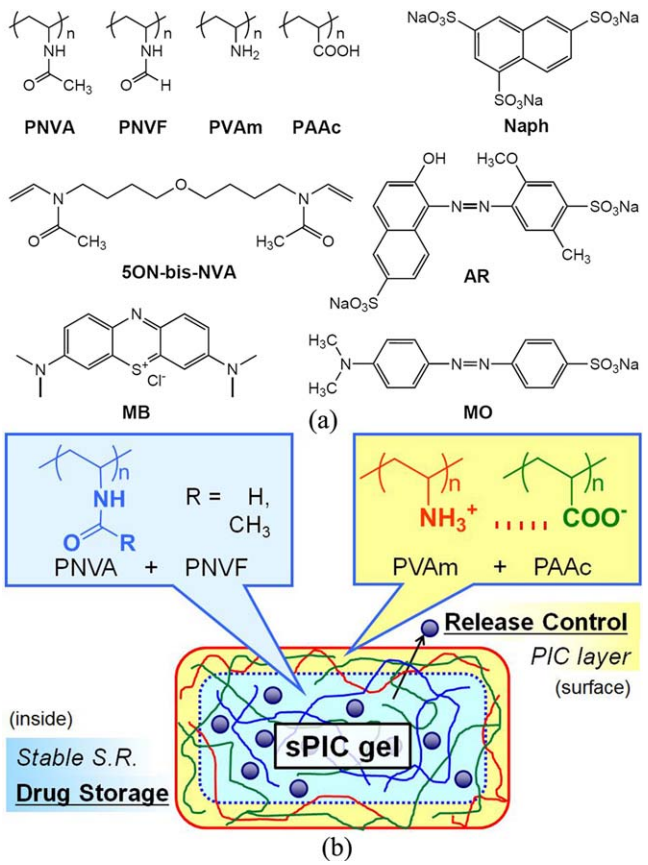

Figure 1. Chemical structures of polymers, crosslinkers, and dyes in this study (a). Schematic illustration of sPIC gel (b). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

The sPIC gel forms a polyion complex layer of PVAm and poly(acrylic acid) (PAAc) at around pH 7, whereas the surface was swollen at pH 2 and pH 12 due to electrostatic repulsion. The layer thickness is about 80 μm for a column-shaped hydrogel (1 cm thickness × 4 mm diameter); thus, the total volume of the sPIC gel is well maintained at any pH because the main interior volume inside is composed of nonionic PNVA and PNVF. In previous studies, we demonstrated a controlled drug release model using fluorescein isothiocyanate (FITC)-dextran ($M_w = 9500$), which was released at pH 2 from a sPIC gel due to the expanded mesh size of the surface, and this release was suppressed at pH 7 by the sPIC gel because a shrunken polyion complex layer was formed on the surface.[34] It was also able to repeatedly turn on and off the control of this releasing behavior. However, in the previous study we could not achieve this releasing control of the low molecular weight FITC-dextran, probably because the shrunken polyion complex layer at pH 7 was not able to prevent drug leakage from the interior core.

Previously, we also tried to use proteins for controlled drug release from sPIC gels. For example, lysozyme ($M_w = 15{,}000$, pI = 11.0), α-lactalbumin ($M_w = 14{,}000$, pI = 5.0), and insulin ($M_w = 5800$, pI = 5.3) have been tested. However, the sPIC gel system was not suitable to control the protein release, because proteins are intricately electrostatically charged polymers. Thus, we searched for a proper drug compound which can be well controlled using the sPIC gel system. So far, electrostatically charged low molecular compounds have not been examined.

In this study, we observed the releasing behavior of electrostatically charged low molecular weight compounds from sPIC gel, in order to understand the properties of sPIC gel. We also suggest a mechanism and a possible application of the sPIC gel as a controlled drug release material, taking advantage of its structurally gradient hydrogel features. Different kinds of drugs controlled by the same hydrogel material, contributes to intelligent material development. Herein, we selected the electrostatically charged low molecular compounds, such as allura red (AR) and 1,3,6-naphthalenetrisulfonate (Naph), as well as FITC-Dex as model compounds.

EXPERIMENTAL

Materials and Measurements

NVA was purchased from Showa Denko (Japan) recrystallized from toluene/cyclohexane (1/3) and dried under a vacuum at room temperature. NVF was purchased from Sigma Aldrich (Japan) and was purified by distillation. Acrylic acid (AAc) was purchased from Wako Pure Chemical Industries (Japan), purified by distillation, and then neutralized with 5NNaOH aq. (27 vol %). *N,N*-methylenebisacrylamide (MBAAm), 2,2′-azobis-(2-methylpropionamidine)dihydrochloride (V-50), 2-propanol, and potassium hydroxide (KOH) were purchased from Wako Pure Chemical Industries (Japan) and were used as received. *N,N*-5-oxanonamethyene-bis-*N*-vinylacetamide (5ON-*bis*-NVA) was used as a cross-linker for poly(NVA-*co*-NVF), and was prepared according to a previously reported method.[30] Ammoniumpersulfate (APS) and *N,N,N′,N′*-tetramethylethylenediamine (TEMED) were purchased from Nakarai Tesque (Japan), and were used without further purification. Methyl orange (MO), allura red (AR), methylene blue (MB), and trisodium naphthalene-1,3,6-trisulfonate (Naph) were purchased from Tokyo Chemical Industry (Japan). The various pH solutions (ionic strength (I.S.) = 0.1*M*) were prepared with 0.01*M* NaOH aqueous solution, 0.01*M* HCl aqueous solution, and NaCl purchased from Wako Pure Chemical Industries (Japan). The pH values were measured using a HORIBA COMPACT pH METER B-212 twinpH.

Preparation of sPIC Gels

sPIC gel was prepared according to the literature.[34] First, poly(NVA-*co*-NVF) hydrogels were prepared by radical copolymerization. Aqueous mixtures of monomer NVA (0.2 M)/NVF (0.8 M), the cross-linker 5ON-*bis*-NVA (3 mol % to monomer), nitrogen bubbled ultrapure water (8 mL), and the initiator V-50 (1 mol % to monomer) were injected into glass tubes of 3.3 mm internal diameter. After polymerization for 4 h at 55°C, the obtained hydrogels were adequately rinsed with ultrapure water. This gel was employed for sPIC gel preparation in the comparison of the released model drugs after 12 h under various pH conditions (Figure 3). The more condensed monomer solution for pre-gel preparation with NVA (0.3 M)/NVF(1.2M) was selected for the initial investigation of model drug release behavior (Figure 4). Then, hydrolysis of the amide groups of poly(NVA-*co*-NVF) hydrogel was performed in order to produce cations. Poly(NVA-*co*-NVF)

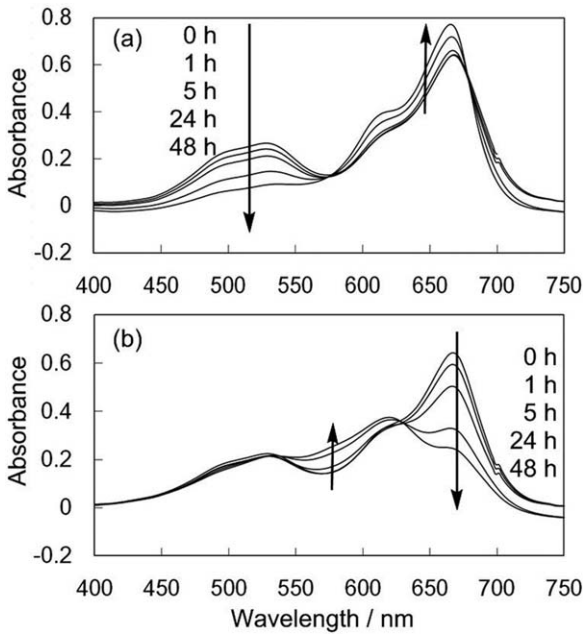

Figure 2. Temporal change of UV spectra for the supernatant of AR and MB mixed solution at pH 2 (a) and pH 12 (b), dissolved in ultrapure water as 1 mg/mL solution, using PAAc/PVAm IPN gel (1 × 2 × 10 mm^3).

hydrogels cut into cylindrical shapes 10 mm in length and 4.4 mm in diameter were immersed into a 2-propanol solution and shrunken. Hydrolysis of the amide groups was then carried out on the shrunken gels in KOH/2-propanol (5 wt %) at 80°C for 3 h. After hydrolysis, the resulting gels were rinsed with aqueous buffer solution (pH 7.4). Finally, anionic AAc was polymerized into the surface cationized hydrogel. Pregel aqueous mixtures of the monomer sodium acrylate solution (0.25 M), the cross-linker MBAAm (5 mol % to monomer), the initiator APS (0.5 mol % to monomer), some hydrolyzed poly(NVA-*co*-NVF) hydrogels and nitrogen bubbled 0.01 MHCl aq. (to 16 mL) were kept at 4°C for 12 h to immerse the hydrogels into the monomer solution (10 mL). TEMED (6.0 or 6.9 μL) was added to the monomer solution 1 h before polymerization, and then the polymerization was carried out for 8 h at 37°C. The obtained hydrogels were rinsed with a large amount of 0.1*M* NaCl aqueous solution.

Drug Adsorption Experiment

AR and MB were dissolved in ultrapure water as 1 mg/mL solution. The equivalent volume of AR and MB solution was diluted by NaOH aq. (0.01 N) or HCl aq. (0.01 N) to obtain a 5 × 10^{-3} g/L mixed solution. Into the solution, PAAc/PVAm IPN gel (1 × 2 × 10 mm^3) were immersed and the supernatant was analyzed by UV spectrometry.

Drug Release Under pH 2, pH7, and pH 12

MO, AR, and Naph were used as model drugs. sPIC hydrogels were immersed into a drug solution of high ionic strength (25 g/L in 2*M* NaCl aq.; PIC dissociation conditions) at 4°C for 12 h, and subsequently the sPIC gels transferred to a drug solution of low ionic strength (25 g/L in ultrapure water.; PIC formation conditions) at 4°C for 12 h.

The drug-loaded sPIC gels were immersed into various pH aqueous solutions with ionic strengths equal to 0.1 M. The gel was immersed into 4 mL of phosphate buffered saline (PBS) to start drug release, and each interval of 10 μL was picked up for UV measurement after dilution with 190 mL PBS. The amount of released drug was measured with a UV spectrometer at 490 nm (U-3010, Hitachi High-Technologies Corporation, Japan). The release percentages were determined and compared to the value of cut gels after 12 h at pH 12, with the calibration curve obtained from the UV intensity of the drug aqueous solution with various concentrations.

Pulse Release of AR Under pH 7.4 and pH 2

AR was loaded into sPIC gel with the same procedure as the abovementioned method. The AR-loaded sPIC gels were immersed into pH 2 and pH 7.4 aqueous solutions repeatedly in the required time intervals. The amount of released AR was determined by UV spectrometer (U-3010). The release percentages were determined and compared with the value after 24 h at pH 12 after gel was cut off to release all of the AR inside.

RESULTS AND DISCUSSION

To confirm the electrostatic interactions with the surface layer of the sPIC gel, a separately prepared full IPN of PAAc/PVAm was used for the adsorption of electrostatically charged dyes at first. The PAAc/PVAm was prepared from a hydrolysed PAAc/PNVF IPN, and the amino group introduction was confirmed by FTIR/ATR (Supporting Information, Figure S1). Since PAAc/PVAm, which is a model of the external layer part of the sPIC gel, is positively charged at pH 2 but negative at pH 12, both the cationic methylene blue (MB) and anionic AR were tested as model drugs for adsorption. Figure 2 shows the temporal changes of the UV spectra of the MB and AR mixed solution in the presence of PAAc/PVAm.

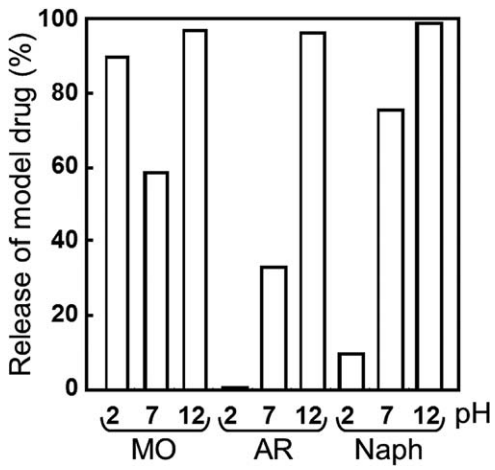

Figure 3. Release of anionic dyes from sPIC gel. Release amount of dye under pH 2, pH 7, and pH 12 conditions, with ionic strengths = 0.1*M*. The release percentages were determined and compared with the value of cut gels after 12 h at pH 12, with the calibration curve obtained from the UV intensity of the drug aqueous solution with various concentrations. Monomer concentration of pre−gel preparation is NVA (0.2*M*)/NVF (0.8*M*).

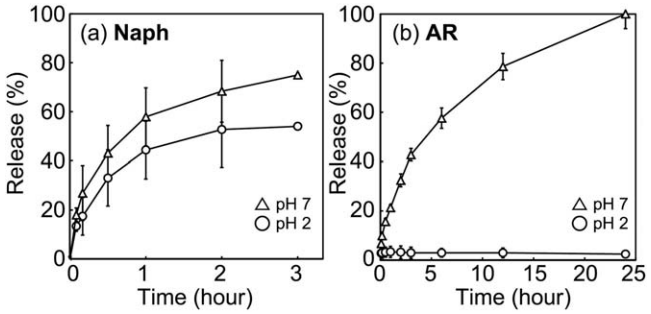

Figure 4. Release behaviors of Naph (a) and AR (b) from sPIC gel under pH 2 and pH 7 conditions. The release percentages were determined and compared with the value of cut gels after 12 h at pH 12, with the calibration curve obtained from the UV intensity of the drug aqueous solution with various concentrations. Monomer concentration of pre-gel preparation is NVA (0.3*M*)/NVF (1.2*M*).

The AR peak around 529 nm gradually decreased at pH 2, which was due to adsorption by the PAAc/PVAm gel because of the electrostatic interactions with the positively charged hydrogel and the negatively charged AR, whereas the intensity around 665 nm of MB did not change very much [Figure 2(a)]. These results implied that the cationic MB barely adsorbed to the positively charged PAAc/PVAm at pH 2. On the other hand, the PAAc/PVAm gel was negatively charged for the carboxyl group of PAAc at pH 12; thus, the cationic MB could interact with the PAAc/PVAm gel, resulting in a gradual decrease of the peak around 665 nm. During the quenching process, the peak around 529 nm due to AR was unchanged [Figure 2(b)]. The PAAc/PVAm gels after each process became red and blue in color (Supporting Information, Figure S2), which suggested that selective adsorption had occurred due to the electrostatically charged PAAc/PVAm IPN.

Next, we tried the controlled release of electrostatically charged compounds from the sPIC gel, selecting a monovalent anion (MO), a divalent anion (AR), and a trivalent anion (Naph) as drug models (Figure 3). Each dye was dissolved in 2*M* NaCl solution at 0.5 g/L at pH 7.4, and the sPIC gel was dipped into the dye solution for 12 h.[34] The pH values of AR, Naph, and MO solutions were 6.0, 7.3, 6.4 at 0.5 mg/mL in 2*M* NaClaq. The sPIC gel was then transferred into the same dye solution in 0.1*M* NaCl at 0.5 g/L in order to keep the compound inside the sPIC gel. After encapsulation of the drug model, the sPIC gel was immersed into three solutions of varying pH (pH 2, pH 7, and pH 12), and the amounts of dye released over 12 h were compared by the intensities of their UV spectra, diluted in fivefold volume of phosphate buffered saline solutions. The assumed total amount of drug was calculated after the sPIC gel was cut into two parts to expose the interior core part for 12 h at pH 12.

Slightly smaller amounts of MO release were observed at pH 7, as compared to pH 2 and pH 12, probably due to the condensed layer of the sPIC gel on the outer surface, but almost no electrostatic effect was observed between pH 2 and pH 12, suggesting that the monovalent MO turned nonionic at pH 2. However, the divalent AR was not released at pH 2, probably because of the strong interaction of the divalent anion with the ammonium ion of the PVAm in the sPIC gel. The amount of AR released increased at pH 12 with the electrostatically negative repulsion. This tendency was also recognized in the trivalent anion of Naph. The electrostatically positively charged sPIC gel on the surface at pH 2 would trap the anionic low molecular compound, whereas it was released under pH 7 and pH 12 conditions.

Releasing behaviors of AR and Naph from sPIC gels against time were monitored in Figure 4. The difference of releasing amount between pH7 and pH2 was recognized in both cases of Naph and AR, but AR release at pH 2 was significantly prevented [Figure 4(b)]. The photos of each solution with sPIC gels clearly showed drug adsorption at pH 2 (Supporting Information Figure S3). The various substituents in the AR structure contribute more effectively to stay inside the sPIC gel under pH2 condition, such as hydrogen bonding and $\pi-\pi$ stacking interaction.

Furthermore, pulse release was achieved using AR between pH 2 and pH 7 (Figure 5). The AR release was repeatedly triggered and suppressed, although the amount gradually decreased. These results showed that it is possible for electrostatic compounds to undergo controlled release with an on-off action depending on the pH. The ionic strength condition has showed a clear difference for drug release (Supporting Information Figure S4). Using the property, the selective drug release from sPIC gel has been devised, although the clear selectivity is not achieved at present, probably due to the interaction among drugs in sPIC gel (Supporting Information Figure S5).

The mechanism for the releasing control by the sPIC gel is proposed in Figure 6. The sPIC gel possesses a polyion complex layer, which is shrunken at pH 7. When the drug is electrostatically neutral and at a high molecular weight ($M_w \sim$ 10,000), it is released at pH 2 or pH 12 due to the mesh size being larger than at pH 7, and therefore the release is

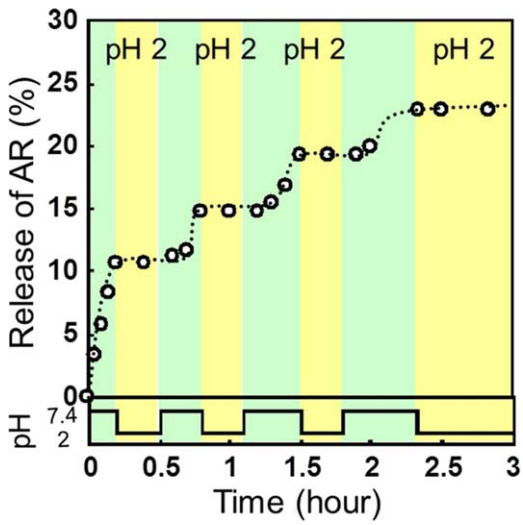

Figure 5. Release of AR from sPIC gel. pH-responsive stepwise release of AR at pH7.4 and 2. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

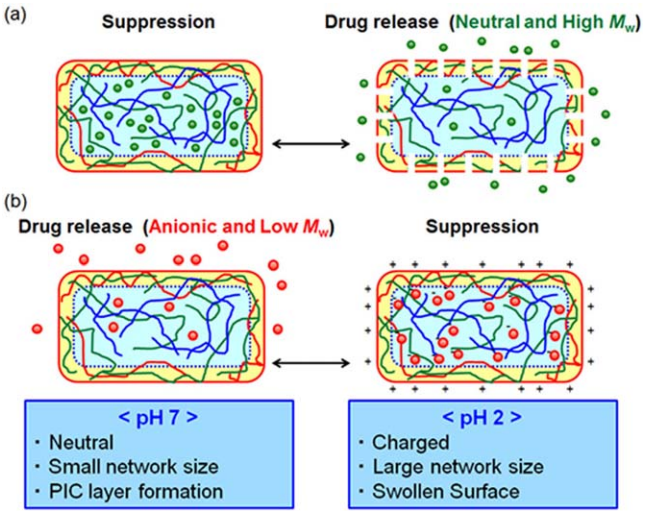

Figure 6. Proposed mechanism of the controlled drug release using sPIC gel. The drug release was suppressed at pH 7, and then released at pH 2 when the drug is electrostatically neutral and high molecular weight (a). The drug was released at pH 7, and then suppressed at pH 2 when the drug is electrostatically negative charged and low molecular weight (b). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

suppressed at pH 7 [Figure 6(a)].[34] On the other hand, the drug is released at pH 7 when the drug is electrostatically negatively charged and at a low molecular weight, because the mesh size is not sufficient to trap the diffusing drugs, but the release is suppressed at pH 2 due to the electrostatic interactions [Figure 6(b)].

CONCLUSIONS

The sPIC gel was employed as a controlled drug release system under various drug models. The polyion complex layer of PAAc/PVAm showed the selective adsorption of electrostatically charged model compounds at pH 2 and pH 12, respectively. Taking advantage of these electrostatic interactions, a low molecular weight drug model showed controlled release, depending on the pH conditions. Conversely, an electrostatically charged polymer as a drug model did not display controlled release, and differed from the previously reported drug release control, in which a neutral, high molecular weight compound was used. This study revealed that the proper chemical structure is necessary for controlled release with a sPIC gel system. We expect sPIC gels will be applied for controlled drug release material in the future.

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