

Potassium ion-recognizable responsive smart materials

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ABSTRACT

Potassium ion (K^+) is the most abundant intracellular metal ion in the body and plays an important role in biological activities. Fabrication of K^+ recognition and response smart materials is of both scientific and technological interests for various applications. Recently, our group have developed a series kinds of K^+ -recognizable responsive smart materials based on 15-crown-5 as K^+ recognizing receptor and thermo-responsive poly(*N*-isopropylacrylamide) (PNIPAM) polymer as actuator. The design, fabrication and performance of these K^+ -recognizable responsive smart materials with various configurations, such as linear polymers, linear-grafted gating membranes, hydrogels and microcapsules, will be describe in this work.

1. INTRODUCTION

Ion recognition and response systems have attracted great interests in recent years due to their versatile applications such as ion detection, ion-responsive controlled-release, and ion-triggered molecular motion, etc. Among physiologically important metal ions, potassium ion (K^+) is the most abundant intracellular metal ion in the body and plays an important role in biological activities. K^+ can not only maintain the intracellular and extracellular osmotic and electrolyte balances with sodium ion (Na^+), but also regulate the biochemical signal transduction throughout the nervous system within the body. The normal serum K^+ level is maintained within a fairly narrow concentration range from 3.5 to 5.5 mM by renal excretion. A K^+ disorder is always associated with certain diseases. In addition, at certain pathological sites in living organisms, serious cytolysis or disabled K^+ - Na^+ pump in cell membrane always results in abnormal increase of extracellular K^+ concentration. Therefore, the fabrication of K^+ recognition and response smart materials is of both scientific and technological interests for various applications such as tissue engineering, targeted drug delivery

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systems, sensors and/or actuators, and so on. Crown ethers are known for the unusual property of forming stable host-guest complexes with specific metal ions. Recently, our group have developed a series kinds of K^+ -recognizable responsive smart materials based on 15-crown-5 as K^+ recognizing receptor and thermo-responsive poly(*N*-isopropylacrylamide) (PNIPAM) polymer as actuator. The design, fabrication and performance of these K^+ -recognizable responsive smart materials with various configurations, such as linear copolymers, linear-grafted gating membranes, and microcapsules, will be describe in this work. These smart materials exhibit remarkably high selectivity and sensitivity towards K^+ due to the formation of 2 : 1 (ligand : ion) “sandwich” host-guest complex between 15-crown-5 and K^+ , which have great potential in the fields of drug delivery systems, diagnostic reagents, artificial internal organs, ion sensors and actuators, and so on.

2. HYPERKALEMIA DIAGNOSIS USING K^+ -RECOGNIZABLE RESPONSIVE LINEAR COPOLYMERS (Yu 2013)

Recently, we present a simple, portable and low-cost method for diagnosis of hyperkalemia by using K^+ -recognizable poly(*N*-isopropylacrylamide-*co*-benzo-15-crown-5-acrylamide) (poly(NIPAM-*co*-B₁₅C₅Am)) linear copolymer as indicator^[1]. The pendent 15-crown-5 units in the linear copolymers can selectively and specifically recognize K^+ to form stable 2:1 “sandwich” host-guest complexes, which cause the copolymer chains to change from the hydrophilic state to the hydrophobic state isothermally. The hyperkalemia can be simply diagnosed by observing the K^+ -induced optical transmittance change of human blood samples with poly(NIPAM-*co*-B₁₅C₅Am) linear copolymer as an indicator. Normal blood samples with low potassium level containing the poly(NIPAM-*co*-B₁₅C₅Am) linear copolymer are almost transparent since the copolymer is hydrophilic and soluble at the operating temperature. However, severe hyperkalemia samples with high potassium level become completely cloudy since the copolymer is hydrophobic and insoluble at this temperature.

2.1 K^+ -Recognizable Abilities of Poly(NIPAM-*co*-B₁₅C₅Am) Linear Copolymers

To systematically investigate the ion-recognizable characteristics of the poly(NIPAM-*co*-B₁₅C₅Am) linear copolymers, the temperature-dependent phase transition behaviors of poly(NIPAM-*co*-B₁₅C₅Am) copolymer and PNIPAM homopolymer solutions containing different metal ions are studied.

The effects of metal ions on the LCST values of poly(NIPAM-*co*-B₁₅C₅Am) copolymer and PNIPAM homopolymer are distinctly different (Fig. 1). As expected, the poly(NIPAM-*co*-B₁₅C₅Am) copolymer with 18.3 mol-% B₁₅C₅Am content presents significantly K^+ -responsive property. As shown in Fig. 1A, the LCST of poly(NIPAM-*co*-B₁₅C₅Am) copolymer exhibits an obvious negative shift in K^+ solution compared with that in deionized water, whereas it has no obvious change in other metal ion solutions. Compared with that in deionized water, the LCST of poly(NIPAM-*co*-B₁₅C₅Am) copolymer in 75 mM K^+ solution decreases about 19 °C (LCST ≈ 13 °C) (Fig. 1A). However, the phase transition behaviors of the PNIPAM homopolymer in various metal ion solutions are almost the same as that in deionized water, showing no ion-

recognition property at all. The LCST values of the PNIPAM homopolymer in deionized water and various metal ion solutions are all around 32 °C (Fig. 1B).

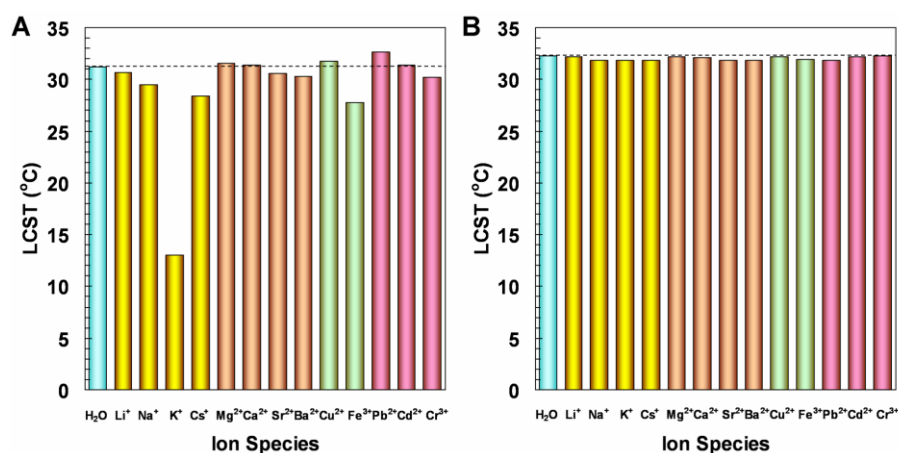


Fig. 1 Effects of metal ions on the LCST values of poly(NIPAM-co-B₁₅C₅Am) copolymer (A) and PNIPAM homopolymer (B). The concentrations of metal ions are all 75 mM.

The distinct negative shift of the phase transition temperature for poly(NIPAM-co-B₁₅C₅Am) linear copolymer in K⁺ solution can be attributed to the formation of stable 2:1 “sandwich” host-guest complexes between adjacent 15-crown-5 units from intra/inter polymeric chains and K⁺, which causes the copolymer chains changing from the hydrophilic state to the hydrophobic state isothermally.

The effects of K⁺ concentration on the LCST of different poly(NIPAM-co-B₁₅C₅Am) copolymers are shown in Fig. 2.

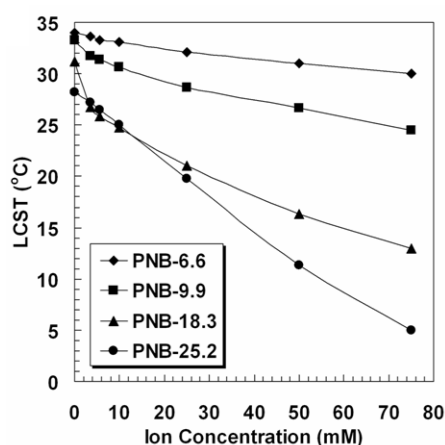


Fig. 2 Effects of K⁺ concentration on the LCST of poly(NIPAM-co-B₁₅C₅Am) copolymers with different molar ratios of B₁₅C₅Am.

With increasing the K⁺ concentration, the negative shift degree of LCST for each copolymer is increased due to the formation of more 2:1 “sandwich” complexes. In addition, the LCST values of the poly(NIPAM-co-B₁₅C₅Am) copolymers in deionized

water decrease slightly as the 15-crown-5 content increases. The B15C5Am units contain hydrophobic phenyl groups, so the hydrophobicity of poly(NIPAM-co-B₁₅C₅Am) copolymer is slightly enhanced with an increase in the content of pendent B15C5Am units in the linear copolymer; as a result, the LCST value of the poly(NIPAM-co-B₁₅C₅Am) copolymer in deionized water shifts to a lower value with increasing the 15-crown-5 content. It is also demonstrated from Fig. 2 that the poly(NIPAM-co-B₁₅C₅Am) copolymers exhibit higher sensitivity to K⁺ with increasing the contents of 15-crown-5 units in the copolymers.

2.2 Hyperkalemia Diagnosis by Poly(NIPAM-co-B₁₅C₅Am) Linear Copolymers

The above results suggest that when the content of crown ether in the copolymer is large, the poly(NIPAM-co-B₁₅C₅Am) copolymer exhibits more significant sensitivity to K⁺. Therefore, the poly(NIPAM-co-B₁₅C₅Am) copolymer with highest crown ether content (25.2 mol-%) is suitable for diagnosing hyperkalemia. In order to confirm the optimum operating temperature for the diagnosis of hyperkalemia, the temperature-dependent phase transition behaviors of poly(NIPAM-co-B₁₅C₅Am) copolymer in human serum solutions with different K⁺ concentration levels are firstly investigated, and the results are shown in Fig. 3. The serum sample solutions with 4.5 mM, 6.4 mM, and 8.2 mM K⁺ are served as normal potassium, moderate and severe hyperkalemia serum samples, respectively. As expected, the negative LCST shift is enhanced with increasing the K⁺ concentration in the serum samples. That is to say, the thermo-responsive phase transition curve of the poly(NIPAM-co-B₁₅C₅Am) copolymer in hyperkalemia serum is different from that in normal serum sample.

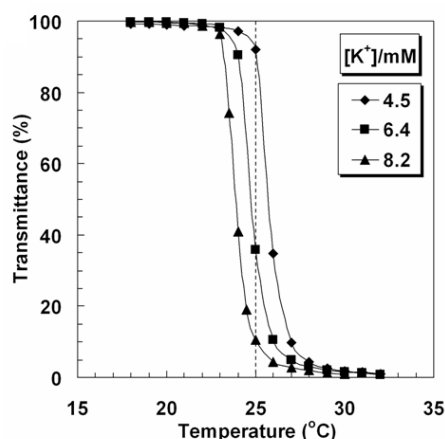


Fig. 3 Temperature-dependent phase transition behaviors of poly(NIPAM-co-B₁₅C₅Am) copolymer in human serum sample solutions with different K⁺ concentration levels.

Therefore, if the operating temperature (T_c) is set between these two LCST values, the poly(NIPAM-co-B₁₅C₅Am) copolymer chains could present different conformational states, which would result in different optical transmittance changes in different serum or plasma samples. In this work, the optimum operating temperatures are selected at 26.5 °C for K⁺ aqueous solutions and 25 °C for human serum samples.

Fig. 4 shows the optical photographs of K^+ aqueous solutions at 26.5 °C (A) and serum sample solutions at 25 °C (B), in which all solutions contain 0.5 wt.-% poly(NIPAM-co-B₁₅C₅Am) copolymer. Obviously, 5.0 mM K^+ aqueous solution containing poly(NIPAM-co-B₁₅C₅Am) copolymer (A1) is transparent. With increasing the K^+ concentration, 6.0 mM K^+ aqueous solution (A2) becomes a little cloudy, whereas 8.0 mM K^+ aqueous solution (A3) becomes completely cloudy. Similarly, serum sample with 4.5 mM K^+ (B1), which is normal serum sample, is almost transparent. Serum samples with 6.4 mM K^+ (B2) and 8.2 mM K^+ (B3), which are moderate hyperkalemia sample and severe hyperkalemia sample, become a little cloudy (B2) and completely cloudy (B3), respectively. That is to say, the hyperkalemia can be simply and clearly diagnosed via K^+ -induced optical transmittance change in sample solution using the poly(NIPAM-co-B₁₅C₅Am) copolymer as an indicator.

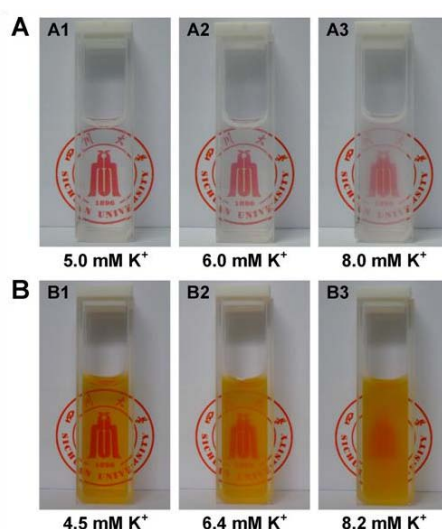


Fig. 4 Optical photographs of K^+ -induced optical transmittance changes in K^+ aqueous solutions at 26.5 °C (A) and serum sample solutions at 25 °C (B).

3. POSITIVELY K^+ -RECOGNITION RESPONSIVE MEMBRANES (Liu 2012)

A novel positively K^+ -responsive membrane with functional gates driven by host-guest molecular recognition is prepared by grafting poly(NIPAM-co-B₁₅C₅Am) copolymer chains in the pores of porous nylon-6 membranes with a two-step method combining plasma-induced pore-filling grafting polymerization and chemical modification. Due to the cooperative interaction of host-guest complexation and phase transition of the poly(NIPAM-co-B₁₅C₅Am), the grafted gates in the membrane pores could spontaneously switch from “closed” state to “open” state by recognizing K^+ ions in the environment and vice versa; while other ions (e.g., Na^+ , Ca^{2+} or Mg^{2+}) can not trigger such an ion responsive switching function.

3.1 Morphological Analyses of the Grafted Membranes

Fig. 5 shows SEM images of the surfaces and cross sections of the ungrafted nylon-6 membrane substrate and poly(NIPAM-co-B₁₅C₅Am)-grafted membranes with different grafting yields. The ungrafted nylon-6 membrane substrate is constructed with a thin functional porous top layer (Fig. 5A), and honeycombed pores can be clearly seen in the membrane (Fig. 5B). After grafting, it can be seen that the surface pores of the grafted membrane are smaller compared with those of the ungrafted membrane. And, the more the copolymers are grafted, the smaller the pores size (Fig. 5A,C,E). From the cross-sectional SEM images (Fig. 5B,D,F), it can be clearly seen that the grafted copolymers are formed inside the pores throughout the entire membrane thickness. The above results confirm that, the functional copolymers can be grafted onto both the outer surfaces of the membrane and the inner surfaces of the membrane pores.

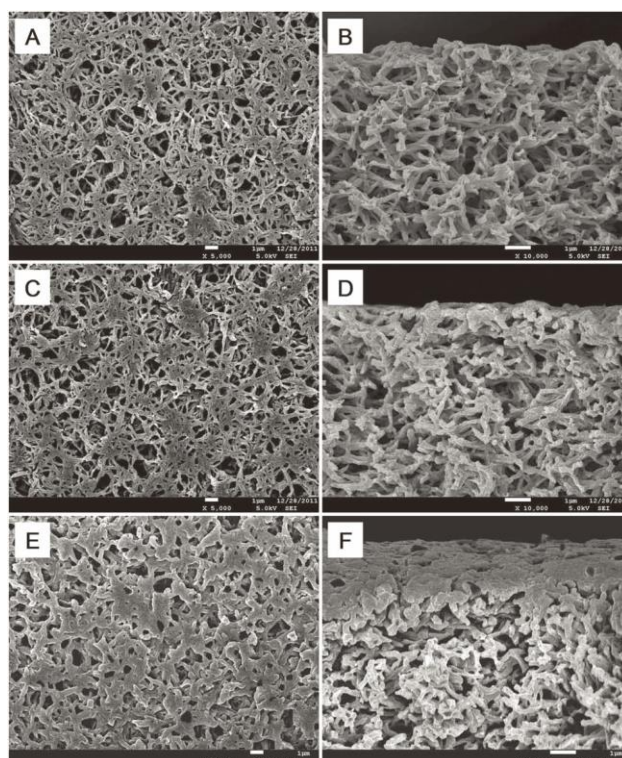


Fig. 5 SEM images of surfaces (A,C,E) and cross sections (B,D,F) of ungrafted nylon-6 membrane (A,B) and poly(NIPAM-co-B₁₅C₅Am)-grafted nylon-6 membranes with grafting yields of $Y_{\text{PNAA}}=2.39\%/Y_{\text{B}_{15}\text{C}_5}=1.22\%$ (C, D) and $Y_{\text{PNAA}}=4.77\%/Y_{\text{B}_{15}\text{C}_5}=2.27\%$ (E, F). Scale bars are 1 μm .

3.2 2.4. Positively K^+ -Responsive Gating Characteristics of the Grafted Membranes

The dynamic changes in solution fluxes across the poly(NIPAM-co-B₁₅C₅Am)-grafted membrane with grafting yields of $Y_{\text{PNAA}}=4.77\%/Y_{\text{B}_{15}\text{C}_5}=2.27\%$ in pure water and aqueous solutions containing different metal ions at 25 °C are shown in Fig. 6. When pure water is used, the grafted copolymer chains swell and the membrane pores are “closed” at 25 °C, as a result that water flux is as low as 4.5 kg m⁻² h⁻¹. In contrast, the presence of K^+ in the environment at 25 °C induces an isothermal shrinkage of the copolymer chains that causes the “opening” of membrane pores, and thus the flux

suddenly increases to as large as $245.4 \text{ kg m}^{-2} \text{ h}^{-1}$. As expected, Na^+ , Ca^{2+} and Mg^{2+} ions nearly do not cause any obvious change of the solution flux across the membrane. The very slight differences between the flux in pure water and those in Na^+ , Ca^{2+} and Mg^{2+} solutions are the results of the salting-out effects on the grafted copolymers. Repeatability of K^+ -responsive switching function is also investigated by repeatedly changing the environmental solution between pure water and 0.1 M K^+ solution. Once the environmental solution is changed from 0.1 M K^+ solution into pure water at 25 °C, the grafted poly(NIPAM-co-B₁₅C₅Am) copolymers exhibit a rapid shrinking response behavior to “close” the pores; as the result, the flux decreases suddenly. The results show that, the fabricated membrane with grafted poly(NIPAM-co-B₁₅C₅Am) gates satisfactorily exhibits positively K^+ -responsive characteristics. The response behavior, which is driven by host-guest molecular recognition, is reversible and reproducible.

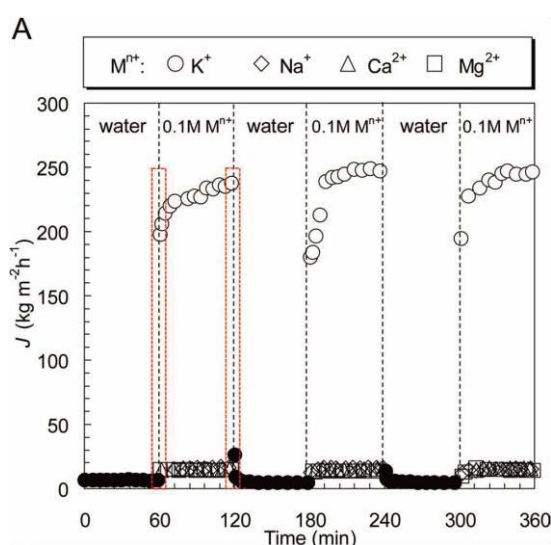


Fig. 6 Isothermally dynamic change in solution flux through the poly(NIPAM-co-B₁₅C₅Am)-grafted membrane with grafting yields of $Y_{\text{PNAA}}=4.77\%/Y_{\text{B}_{15}\text{C}_5}=2.27\%$ in pure water and aqueous solutions containing different metal ions. The concentration of metal ion is 0.1 M.

4. K^+ -RECOGNITION RESPONSIVE MICROCAPSULES WITH SQUIRTING RELEASE MECHANISMS (Liu 2011)

K^+ -recognition capsules are developed to translate K^+ -recognition into a squirting release function. Upon recognition of K^+ , the capsules shrink rapidly and squirt out encapsulated oil cores due to the cooperative interaction of host-guest complexation and phase transition in capsule membranes. The microcapsule membrane is composed of 15-crown-5 units as K^+ sensors, NIPAM units as actuators, and acrylamide (AAm) units as hydrophilicity adjusters. The poly(NIPAM-co-AAm-co-B₁₅C₅Am) core-shell microcapsules are prepared with oil-in-water-in-oil (O/W/O) double emulsions as templates, which are generated with a microfluidic approach.

4.1 Volume Phase Transition Behaviors of the Microcapsule

As shown in Fig. 7, the LCST of poly(NIPAM-co-AAm-co-B₁₅C₅Am) microcapsule containing 10% B₁₅C₅Am and 10% AAm increases to 46 °C in deionized water (DI water) due to the introduction of AAm units. When the environmental DI water is replaced by 0.1 M K⁺ solution, the LCST of poly(NIPAM-co-AAm-co-B₁₅C₅Am) microcapsule shifts negatively to 35 °C. Therefore, the isothermal K⁺-recognition of poly(NIPAM-co-AAm-co-B₁₅C₅Am) microcapsule can be operated at 37 °C, which is near the body temperature. When the K⁺ concentration is 0.1 M, the poly(NIPAM-co-AAm-co-B₁₅C₅Am) microcapsule shows K⁺-recognition-triggered isothermal volume shrinkages as large as 80–85% at 37 °C.

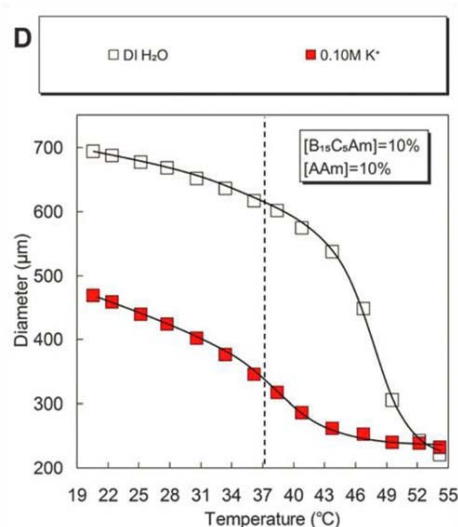


Fig. 7 Volume phase transition behaviors of poly(NIPAM-co-AAm-co-B₁₅C₅Am) microcapsule.

4.2 Volume Phase Transition Behaviors

Because the density of the oil phase is slightly lighter than that of the aqueous phase in the O/W/O emulsions, the membrane thicknesses of polymerized microcapsules are usually not so even (Fig. 8). Such uneven membrane thickness is beneficial to the squirting release mechanism of the capsule, because the thinnest point in the membrane is usually the point for pressure-induced breakage.

As shown in Fig. 8, the poly(NIPAM-co-AAm-co-B₁₅C₅Am) microcapsules are stable in DI water at 37 °C. When the environmental DI water is replaced by 0.2 M K⁺ solution at this temperature, the poly(NIPAM-co-AAm-co-B₁₅C₅Am) microcapsules exhibit very fast shrinkage. Because the oil core is incompressible and cannot pass through the membrane via diffusion, the liquid pressure inside the microcapsule increases rapidly due to the membrane shrinkage. When the internal pressure increases to a critical value, the membrane ruptures suddenly and the encapsulated oil core squirts out as a result.

Given the K⁺-recognition-responsive squirting release mechanism, the proposed microcapsules are expected to be used in various applications such as K⁺-responsive smart drug delivery systems, K⁺ sensors and actuators, and K⁺-triggered microdevices.

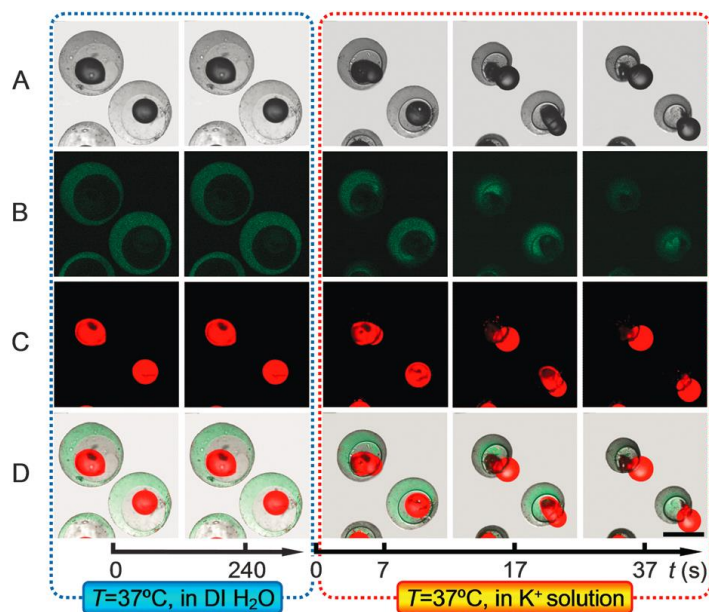


Fig. 8 CLSM images of K^+ -recognition squirting release behaviors of poly(NIPAM-co-AAm-co-B₁₅C₅Am) microcapsules when the environmental solution is changed from DI water to 0.2 M K^+ solution at 37 °C. The scale bar is 500 nm.

5. CONCLUSIONS

A series kinds of K^+ -recognizable responsive smart materials with various configurations, such as linear copolymers, linear-grafted gating membranes, and microcapsules are introduced in this work. These smart materials exhibit remarkably high selectivity and sensitivity towards K^+ due to the formation of 2 : 1 (ligand : ion) “sandwich” host-guest complex between 15-crown-5 and K^+ , which have great potential in the fields of drug delivery systems, diagnostic reagents, artificial internal organs, ion sensors and actuators, and so on.

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