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Investigation of chemical modification on tosyl-activated polystyrene microsphere magnetic particle surface by infrared microscopy

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토실 활성화된 폴리스티렌 마이크로구체 자성 입자 표면의 화학적 변형에 대한 적외선 연구

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Abstract: The chemical engrafting of polymers on particle surface, plays an important role on selective partitioning of micro/nano-particles in the separated layers of liquid media, such as aqueous two phase systems (ATPSs). Three polymers, dextran, poly (ethylene glycol) and albumin were chosen and chemically conjugated to the polystyrene (PS) magnetic microparticle surface. The attachment of long-chained polymer chains which may switch the partition behavior, can be simply performed by S_N2 substitution of various polymers having primary amine functional groups, with *p*-toluenesulfonyl (tosyl)-activated polystyrene magnetic micro-particles. The surface modification of micro-particle was probed by infrared microscopy. The distinctive peak represents N-H stretching vibration mode for micro-particles after the reaction and it is common for all three polymers examined. The locations of main peaks are similar for all micro-particles but different and distinguishable in fingerprint region.

요 약: 입자 표면에 고분자를 화학적으로 접목하는 것은 수용성 액체 이상 시스템(ATPS)과 같이 분리된 액체 층에서 마이크로/나노입자를 선택적으로 분배시키는데 중요한 구실을 한다. 본 연구에서는 덱스트란, 폴리에틸렌 글리콜, 알부민을 화학적으로 폴리스티렌 자성 마이크로입자 표면에 화학적으로 부착시켰다. 분배 현상을 역전시킬 수 있는 긴 고분자 사슬의 부착은 일차 아민 작용기를 가진 다양한 고분자를 *p*-톨루엔술폰산(토실)기를 부착시킨 폴리스티렌 자성 마이크로입자와의 S_N2 치환에 의해 간단히 수행될 수 있었다. 이 후 적외선 현미경을 사용하여 마이크로입자의 표면 변화의 유무를 검사하였다. 반응 후 입자들은 세 가지 폴리머 모두 N-H 신축 진동 피크를 보였으며 대부분의 주요 피크들의 위치는 반응 전후에 유사하였으나 지문 영역에서 구별 가능한 차이를 보였다.

Key words: tosyl, micro-particle, infrared, spectra, surface modification

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1. Introduction

Polystyrene (PS) magnetic microparticles, commercially called Dynabeads, are monodisperse and uniform compared to other irregularly sized magnetic particles.[1] They are superparamagnetic so they move to the magnet when magnetic field is applied. This enables the easy handling and resuspension of microparticles by magnetic capture and release. Tosylactivated Dynabeads are mainly useful for separating peptides, proteins, cells, and bacteria, which can be conjugated chemically to the particle surface. Also by coupling with antibody proteins, the microparticle acts as a solid support and it is suitable for immunoassay.[2] Over 300 papers can be searched which are related to Dynabeads from Web of Science, and among them about 50 articles are of immunology.

It is well known that the surface property of micro/nanoparticles determines their optical, electrical, and other physicochemical behaviors and applications.[3] Among them, especially partition phenomenon is of importance for separational and analytical purposes.[4] For example, in order to investigate live sender-receiver bacteria microcolony's interactions, expected as a bio-sensing tool by dragging bacteria-containing micro-droplets, the strategy of aqueous two-phase system (ATPS) and partitioning polystyrene (PS)-coated magnetic microparticles into dextran (DEX), was introduced and accomplished by surface modification of microparticles with dextran polymer of similar molecular weight (MW) to DEX solute in ATPS.[5] It followed one of the well-known rules of chemistry, "like dissolves like". However, more thorough theoretical and experimental investigation plus expansion of this phenomenon, have not been independently made after. More concretely it was not clear that the phenomenon is really due to dextran conjugation to microparticles or due to other possible reasons. The use of different conjugated polymers on the microparticle surface may induce dramatic changes of partition behavior in ATPS. Therefore, the establishment of proper and convenient method to probe these chemical changes (*Fig.* 1) on the surface of microparticles are important for further investigation of microparticle partition behaviors in ATPS.

Infrared spectral investigation of materials is greatly beneficial for its non-destructive way of sample analysis, user convenience, fast analysis speed, etc.[6,7] Wide and various applications on IR spectroscopy cover even the confirmation of bacterial attachment and changes on particles[3,8,9] and IR spectroscopy is still considered as one of the most widely used and reliable methods for chemical changes at the surface of materials.[3,6,7,10-15,17,18]

Here the infrared (IR) microscopic investigation results to prove the surface modification of polystyrene (PS)-coated magnetic microparticles, are reported.

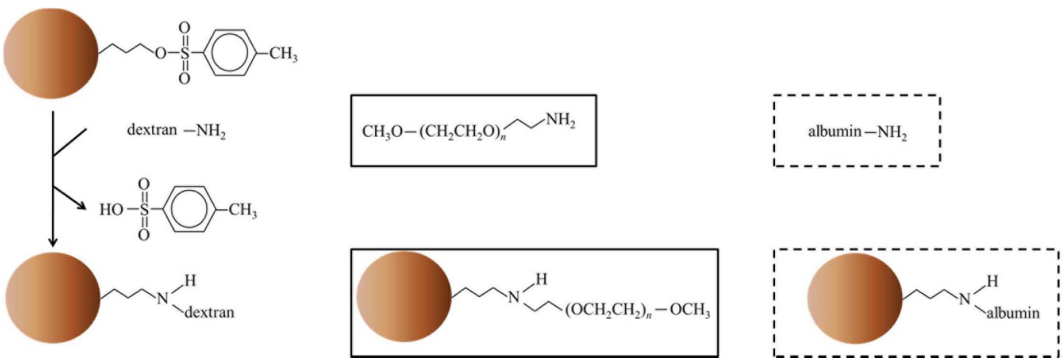

Fig. 1. Reaction scheme of tosylactivated PS microsphere with aDEX, mPEG (boxed in solid line), or BSA (boxed in dashed line). Because the structural complexity of aDEX and BSA, their structures are not drawn in details. Dextran is a branched polysaccharide composed of glucose. aDEX has one or two amine groups per dextran molecule. BSA has primary amine groups at Lys, Gln, Asn, Arg residues and at one end of amino acid chains.

Note that it is the first time for the IR spectra of tosyl-activated PS microsphere conjugated with dextran, poly(ethylene glycol), and albumin to be investigated. The up-to-date results to prove the surface modification of microparticles for switching partition behavior and some further plans on this study will be also discussed. Because the modified microsphere amounts are not enough to perform common IR spectroscopic measurements, FT-IR microscopy was used instead for dealing with small amount (sub-milligram) of microsphere samples in order to obtain their IR spectra.

2. Experimental

2.1. Materials

Bovine serum albumin (BSA, heat shock fraction, protease free, pH 7, ≥98 %) was purchased from Sigma-Aldrich (USA). Methoxy poly(ethylene glycol) amine (mPEG) (MW:~10,000) was purchased from Creative PEGWorks (USA). Amino-dextran (aDEX, MW:~10,000) was purchased from Molecular Probes, Life Technologies (USA). Phosphate buffer saline (PBS) pellets, sodium hydroxide (≥97 %), boric acid (≥99 %) and ammonium sulfate (≥99.5 %) were all purchased from Fisher Scientific (USA). Deionized (DI) water was obtained by Human Power I+ reverse-osmosis (RO) water purification system (Human Corporation, South Korea). Hydrochloric acid (37 %, ACS reagent) was purchased from ACROS Organics, Thermo Fisher Scientific (USA) for pH adjustment. The magnetic micro-particles, 15 mg/mL concentration of tosylactivated Dynabeads® M-280 (diameter 2.8 μm) were purchased from Invitrogen, Life Technologies (Norway). *Fig.* 1 shows the reaction schemes of surface-activated PS magnetic microparticles with three water-soluble polymers described above.

2.2. Surface modification

Conjugation method of amino-dextran to Dynabead M-280 tosylactivated, has been mentioned previously.[5] The same procedures were applied to methoxyl-PEG-amine (mPEG) and BSA (*Fig.* 1 and *Fig.* 2). To summarize, 50 μL of Dynabead suspension was taken after voltexing. After transferring it to 1 mL plastic tube, it was centrifuged shortly and put it on Dynal magnet holder. Then the storage solution was removed and the remaining beads were rinsed by adding 1 mL buffer A (0.3 g boric acid/50 mL water then pH was adjusted to 9.5 by 5 M NaOH and conc. HCl) then the solution was removed after voltexing and attachment to Dynal magnet holder. Then 3~6 mg amino-dextran (aDEX), mPEG or BSA was added with 150 μL of buffer A and 100 μL of buffer C (2 g ammonium sulfate dissolved in 50 mL of buffer A). The mixture was incubated on a roller at 37 °C overnight (12~18 hours). After centrifugation, the dextran-conjugated beads were allowed to attach at the wall of tube by magnet. After removing liquid, 1 mL of buffer D (50 mL PBS pH 7.4 with 0.5 g BSA) was added and the tube was incubated at 37 °C for 1 hour. After removing buffer D, the reacted microspheres were washed with DI water then dried in a vacuum centrifuge (Centrivap Concentrator, LABCONCO, USA) for 3 hrs, then dried more in a vacuum glassware container over 3 hrs in order to remove any water moisture, which may interfere IR spectral detections.

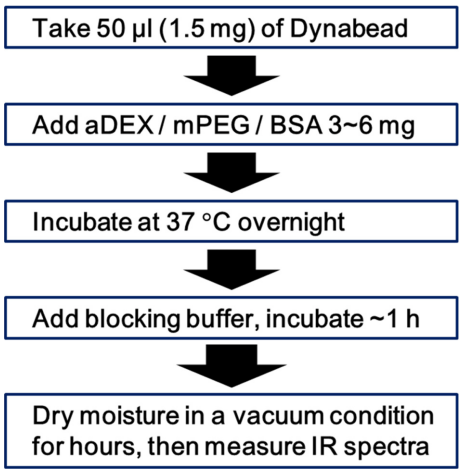

Fig. 2. Preparation of surface-modified PS microparticles for IR measurements.

2.3. Infrared microscope

Infrared spectra were obtained by placing a small amount (~0.5 mg) of surface-modified microspheres

or unmodified microspheres or each substrate powder onto a KBr window then spreading them by pipet tip. Then the window was placed on the xy stage of IR microscope. Infrared spectra were recorded using a Bruker Optics IFS66v/S Fourier transform infrared (FT-IR) microscopic system, operating with a liquid-nitrogen cooled detector, which is located at the National Nanofab Center, Daejeon, Korea (http://www.nnfc.re.kr). Spectra were scanned 64 times with transmission mode, and averaged from 4000 to 600 cm^{-1} with 2 cm^{-1} optical resolution.

2.4. SEM characterization

Scanning electron microscopy (SEM) was performed on a Sirion UHR FEG-SEM (FEI company) at an accelerating voltage of 10 kV and the magnification of 6500X, also located at the National Nanofab Center, Daejeon, Korea (http://www.nnfc.re.kr). The samples were spread on a piece of carbon tape which attached to the SEM mount then coated with Pt for charge dissipation.

2.5. Data analysis

The spectral data files (dpt) were converted to Microsoft Excel-readable files (csv) using OMNIC software (version 7.2a, Thermo, USA). Absorbance to transmittance conversions of spectral data, baseline corrections, and peak findings were all performed with the same software.

3. Results and Discussion

3.1. IR spectral investigation of unmodified PS microsphere

The IR spectrum of tosylactivated polystyrene (PS) magnetic microsphere, commercially available Dynabead, is shown in *Fig.* 3A, with that of PS film (*Fig.* 3B) for comparison. The peaks at 2962 and 2904 cm^{-1} represent $-CH_3$ stretching vibration (methyl group of *p*-toluene from tosyl group). The peaks at 1707 and 1597 cm^{-1} are expected to come from C-C and C=C stretching of benzene ring. The peak at 1513 cm^{-1} seems to be due to styrene C=C stretching of Dynabead. The peak at 1260 cm^{-1} may represent

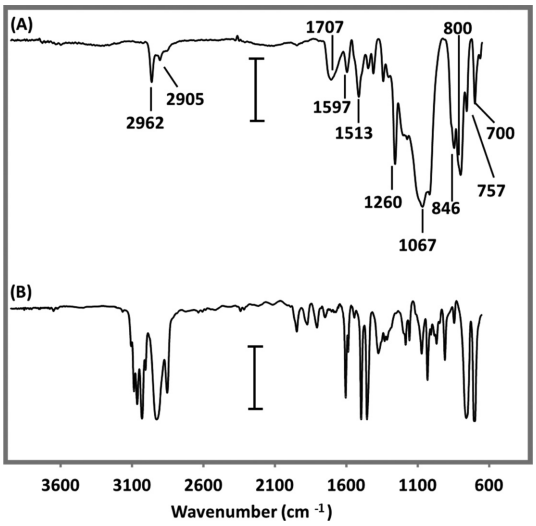

Fig. 3. IR spectrum of (A) Dynabead M-280 tosyl-activated before substitution reaction, (B) PS film. The scale bar represents 5 % transmittance for (A) and 12.5 % transmittance for (B). (B) was introduced from OMNIC library, not by direct detection from IR microscopy. Other conditions are described in Experimental section.

$-SO_3-$ vibration.[6,7] The peak at 1067 cm^{-1} is suspected to be $-CH_3$ rocking vibration or S-O stretching.[10] The peaks at 846 and 800 cm^{-1} also come from C-H bending vibration of benzene rings. The peaks at 757 and 700 cm^{-1} seems to be C-H wagging and twisting peaks of benzene rings of polystyrene. Some peaks which are not assigned in *Fig.* 2A, are subject to be related with sulfonate ($-SO_3-$) group. The overall peaks seem to represent tosyl functional group on the Dynabead surface. Because *p*-toluene sulfonyl (4-methyl benzene sulfonyl, tosyl) functional group covers the surface of the microparticle, the Dynabead spectrum (*Fig.* 3A) is not well matched with that of PS film (*Fig.* 3B). Even though tosyl-activated Dynabead M-280 is known as hydrophobic, it is not clearly mentioned in any references or sources that the surface of Dynabead is additionally coated with other different materials onto polystyrene layer, which covers the iron oxide core of magnetic particle. The spectrum of *Fig.* 3A, has some similarities and differences from that of another kind of Dynabead (Myone® carboxylic acid, which is hydrophilic) of which the IR spectra already has been reported by

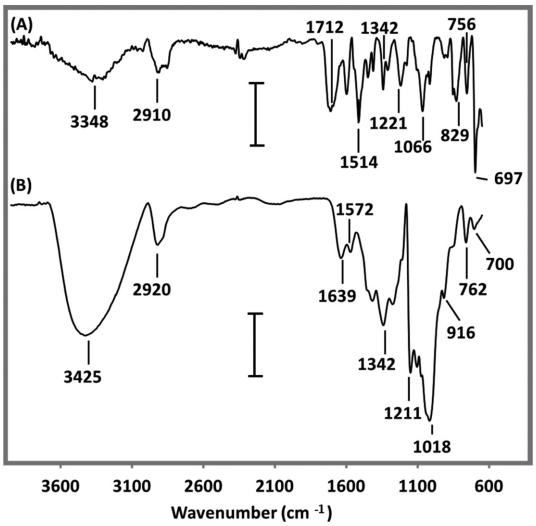

Fig. 4. IR spectrum of (A) Dynabead M-280 tosyl-activated after amino-dextran (aDEX, MW 10,000) substitution reaction, (B) amino-dextran only. The scale bar represents 1 % transmittance for (A) and 5 % transmittance for (B). Other conditions are described in Experimental section.

Kim *et al.*[11] Because the overlaps of peaks from 1500 to 750 cm^{-1} are quite complex, more study such as peak deconvolution analysis, may be needed to make clear of this issue.

3.2. IR spectral investigation of amino-dextran (aDEX) modified microsphere

The IR result of polystyrene (PS) magnetic microsphere, after the surface modification reaction with amino-dextran, is shown in *Fig.* 4A. Also IR spectrum of amino-dextran (MW ~10,000) before reaction is shown in *Fig.* 4B. Various polymer-immobilizations to particle surfaces, have been reported in order to achieve biological goals.[3,11-17] The advantage of attaching amino-dextran to micro/nanoparticles for ATPS is to give the target particles more affinity to dextran-rich phase, which is located at the bottom of two layers (The top layer of ATPS, PEG-rich phase is more hydrophobic. Also the bottom layer, DEX-rich phase is more hydrophilic and dense).[5] Going back to *Fig.* 4A, the peak at 3348 cm^{-1} is probably due to N-H stretching vibration after the substitution reaction with aDEX and the formed secondary amine bond. There may exist some contributions of O-H stretching of dextran also. In *Fig.* 4B, characteristic peaks are at 3425 (N-H stretching), 2920 (C-H stretching), 1639 (C-C stretching), 1572 (N-H bending), 1342 (C-N stretching and C-N-H bending), 1211 (C-O stretching), 1018 (C-H rocking), 916 (C-H twisting), 762 and 700 (C-H wagging and twisting) cm^{-1} and many peaks could be found in similar positions in *Fig.* 4A (3348, 2910, 1342, 1221, 756, 697 cm^{-1}). *Fig.* 4A also share some characteristic peaks of unreacted Dynabead shown in *Fig.* 3A (1712, 1514, 1066, 756, 697 cm^{-1} in *Fig.* 3A). Bhayani *et al.* reported IR spectra of dextran-conjugated inorganic nanoparticles for biomedical applications.[18] The main peaks at 3500, 2950, 2500, 1650 and 1220 cm^{-1} represents O-H, C-H, O-H, C-C and C-O stretching vibration of dextran molecules and were seen in both their dextran and dextran-modified nanoparticles, which are similarly seen in this paper, *Fig.* 4A and 4B.

3.3. IR spectral investigation of methoxy-polyethylene glycol amine (mPEG) modified microsphere

The IR spectrum of polystyrene (PS) magnetic microsphere, after the surface modification reaction with mPEG, is shown in *Fig.* 5A. Also the IR spectrum of methoxy-PEG-amine (mPEG, MW ~10,000) before reaction is shown in *Fig.* 5B. PEG-immobilization is one of the widely used chemical treatments for biological and other researches for its non-toxicity and biocompatibility to live cells.[3,5] If the same "like dissolves like" rule for ATPS is applied not only to dextran but also to PEG, PEG-modified particles will prefer partitioning to upper PEG-rich phase, not to the bottom DEX-rich phase, which is the reversal result of the previous study.[5] This partition issue will be dealt more deeply in a separate article. In *Fig.* 5A, the peak at 3416 cm^{-1} is also due to N-H stretching vibration after the substitution reaction with mPEG and the formed secondary amine bond. Some characteristic peaks in *Fig.* 5B are at 2874 (C-H stretching), 1236, 1104 (C-O stretching), and 840 (C-O-C bending or C-C-N bending) cm^{-1}. Similar

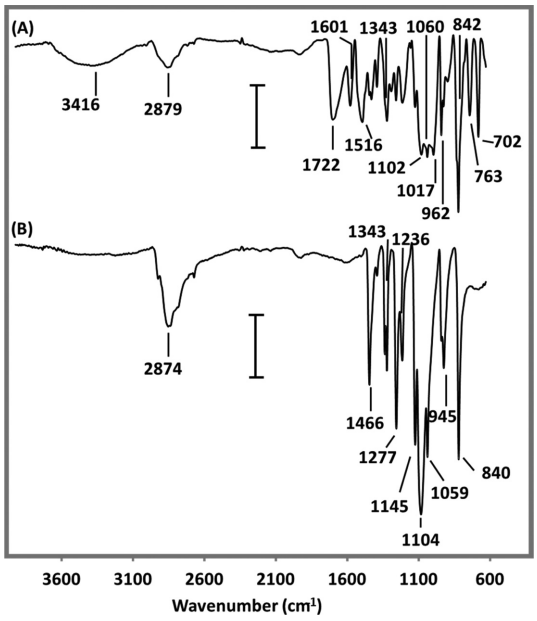

Fig. 5. IR spectrum of (A) Dynabead M-280 tosyl-activated after methoxy-PEG-amine (mPEG, MW 10,000) substitution reaction, (B) mPEG only. The scale bar represents 5 % transmittance for both (A) and (B). Other conditions are described in Experimental section.

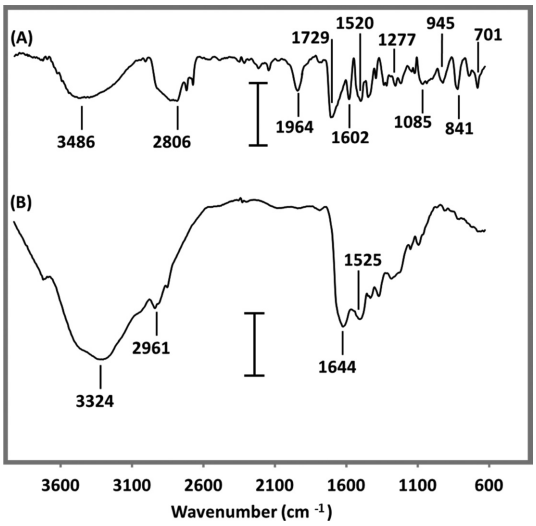

Fig. 6. IR spectrum of (A) Dynabead M-280 tosyl-activated after bovine serum albumin (BSA, MW ~66,500) substitution reaction, (B) BSA only. The scale bar represents 5 % transmittance for both (A) and (B). Other conditions are described in Experimental section.

peaks exist in *Fig.* 5A (2879, 1102, 842 cm^{-1}). *Fig.* 4A also has characteristic peaks of unreacted Dynabead shown in *Fig.* 3A (1601, 1516, 1060, 702 cm^{-1} peaks in *Fig.* 5A) like *Fig.* 4A. Again, the IR spectra could provide the PEG modification information of microsphere with simplicity and rapidity.

3.4 IR spectral investigation of bovine serum albumin (BSA) modified microsphere

The IR spectrum of BSA (MW ~66.5 kDa) before reaction is shown is *Fig.* 6A. Also polystyrene (PS) magnetic microsphere, after the surface modification reaction with BSA, is shown in *Fig.* 6B. Protein-immobilization to particle surfaces, has been routinely used for immunoassay and other biological analyses.[3,14] Moreover, BSA often used to complete the modification or conjugation reaction for Dynabead by dissolving and adding them in the blocking buffer, as in the vendor's manual, in order to remove any residual tosyl groups which possibly remain after the reaction with target substrates (such as aDEX or mPEG, in this case) because BSA has amine groups from amino acid chains and it is not harmful to cells. However, it may affect partitioning in ATPS because it is hydrophilic, indeed. On the other hand, unreacted or raw PS particles are expected to be hydrophobic because of benzene rings of polystyrene. 4-methyl benzene sulfonyl or tosyl group, is also expected to work similarly to be hydrophobic, which may prefer hydrophobic PEG-rich upper layer in ATPS, rather than hydrophilic DEX-rich phase. From *Fig.* 6A, the peak at 3486 cm^{-1} is thought to be partially due to N-H stretching vibration after the substitution reaction and the formed secondary amine bond, at least, since albumin also has some other hydroxyl and amine groups in its structure. In *Fig.* 6B, characteristic peaks at 3324, 2961, 1644 and 1525 cm^{-1} seem to represent -C(=O)-O- stretching, C-C (with C=O) stretching, C-H bending and O-H or N-H bending vibration and could find some similarly positioned peaks in *Fig.* 6A (2806, 1520 cm^{-1}). *Fig.* 6A also share some characteristic peaks of unreacted Dynabead shown in *Fig.* 3A (1602, 1520, 1085, 701 cm^{-1} in *Fig.* 5A). From Bhayani *et al.*, IR spectra of BSA-

Table 1. Peak positions and assignments from IR spectra of tosylactivated PS microsphere, before and after substitution reactions with aDEX, mPEG and BSA

Wavenumber (cm^{-1})				Functional group
Before reaction (Fig. 3A)	Reacted with aDEX (Fig. 4A)	Reacted with mPEG (Fig. 5A)	Reacted with BSA (Fig. 6A)	
-	3348	3416	3486	N-H amide stretching; O-H stretching
2962, 2905	2910	2879	2806	C-H stretching; C-C stretching
1707	1712	1722	1729	Aromatic ring stretching
1597	1599*	1601	1602	Aromatic ring stretching; N-H bending
1513	1514	1516	1520	C=C stretching
1260	1221	1235*	1277	SO_3 stretching; C-O stretching
1067	1066	1060	1085	C-H rocking; S-O stretching; C-O-C stetching
800	829	842	841	Aromatic C-H bending
757	756	763	761*	Aromatic C-H wagging
700	697	702	701	Aromatic C-H twisting

*peaks are not assigned in *Fig.* 4A, 5A and 6A.

conjugated nanoparticles were also included.[18] But because of the complexity of BSA structure and many functional groups, they did not provide clear peak assignment of the BSA IR spectrum, although the broad and overlapped spectral pattern of BSA, was the same as *Fig.* 6B.

The peak energy (distinguished by different wavenumbers) and possible vibrational modes of surface-activated PS microparticles, before and after the substitution reaction with three different water-soluble polymers (aDEX, mPEG and BSA) are summarized in *Table* 1. N-H stretching vibration around 3400 cm^{-1} (broad peaks) seems obvious after the reactions are completed. (Note that no peaks were found for unreacted tosylactivated microbeads at 3300-3500 cm^{-1}). It may be due to the O-H stretching vibration from the attached dextran or albumin since they have hydroxyl groups in their structures. However, for poly(ethylene glycol) it does not have any hydroxyl groups so the secondary amine group formed by S_N2 substitution reaction (*Fig.* 1), is the only source for the peak at 3416 cm^{-1} (*Fig.* 4A). Because different vibrational peak energies share similar positions in *Table* 1 (e.g. around 1600, 1250 and 1050 cm^{-1}), it would be a better approach to recognize immobilization of different polymers by comparing fingerprint region (2000~650 cm^{-1}) of IR spectra and *Fig*. 3A, 4A, and 5A are distinguishable one another in this aspect. Overall, the peaks of microparticle after the reaction are much similar to those of microparticle before substitution reaction, rather than raw polymers (aDEX, mPEG, and BSA) so that a lot of main peaks are located at the same positions (*Table* 1) and peak shapes around 1700 and 1600 cm^{-1} are alike. However, characteristic heights and distributions of peaks build up different fingerprints for each polymer-modified micro-particles, compared to raw tosylactivated microparticles. For example, Peaks at ~1060 cm^{-1} exist for all micro-particles but the relative peak heights are quite different among them, which peak component analysis would help to explain better about these spectral differences in the future.

3.5. Investigation of unreacted and modified microspheres by field emission scanning electron microscope (FE-SEM)

The SEM images of microspheres before and after the reaction with three polymers, are shown is *Fig.* 7. The changes of size and shape of microparticles before and after surface-modification seem negligible, which means the covalent bondings are achieved rather than physical adsorption of unreacted polymers onto the surface of microparticles. The triplicated measured diameters for *Fig.* 7A, 7B, 7C and 7D were within 2.50~2.87 μm (data not shown).

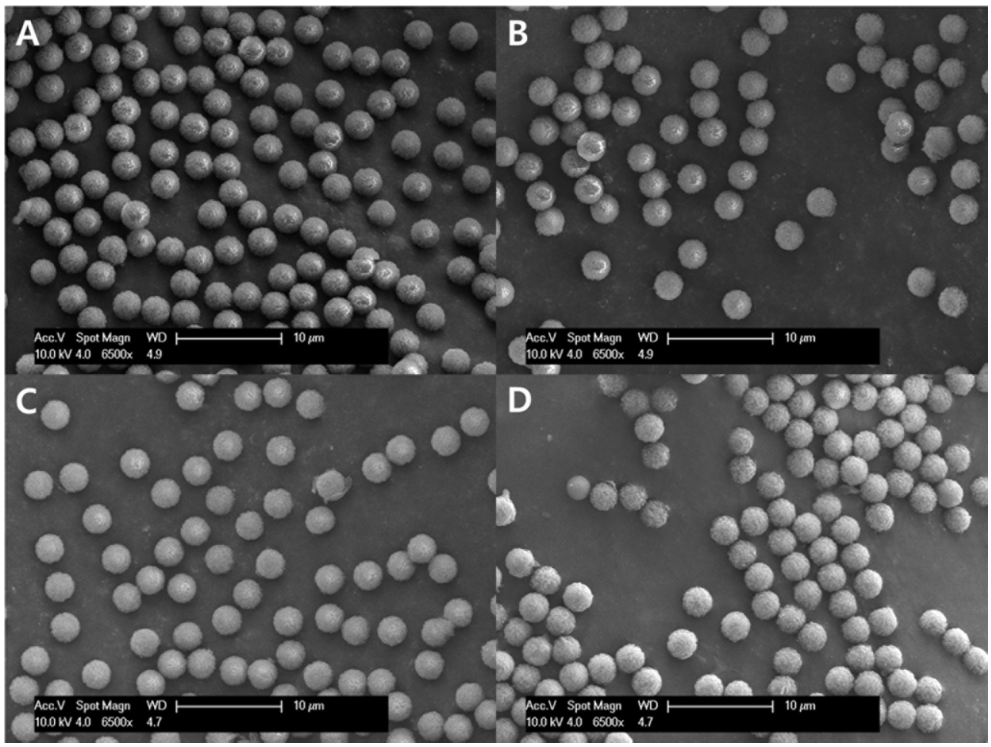

Fig. 7. SEM images of PS magnetic microspheres: (A) Tosylactivated, before reaction, (B) after the reaction with aDEX, (C) after the reaction with mPEG, (D) after the reaction with BSA. All scale bars are 10 mm. Other conditions are described in Experimental section.

4. Conclusions

The surface modification of tosyl-activated polystyrene magnetic microparticles with dextran, poly (ethylene glycol) and albumin, was performed and examined with infrared microscope for the first time. This paper shows that 1) the formation of N-H stretching peak happened and is obvious of existing N-H amine bond on the microparticle surfaces, especially when poly (ethylene glycol) is attached. 2) Different surface modifications by three polymers produce their distinctive IR spectral patterns in fingerprint region (2000~650 cm^{-1}). Surface-modified micro-particles share the IR spectral characteristic peaks of each substrate polymer and the micro-bead, both (but share more on micro-particle side and less on polymer side). Although cross-checking using different analytical methods such as Raman spectroscopy, might be more persuasive to prove chemical change, IR microscope could provide evidence for surface modification of polystyrene magnetic microsphere with different substrates such as polyethylene glycol (PEG), dextran (DEX), and bovine serum albumin (BSA). 3) SEM investigation did not show considerable changes of surface, size and shape of microspheres before and after the reaction, which means the IR spectral changes are not by the physical adsorption but by the chemical modification of microparticle surface. Finally, the IR spectral results of microparticles for three polymers are expected to be useful to explain different partition behaviors of these microparticles when they are added to PEG-DEX aqueous two-phase systems (ATPSs), in the future[19]. More peak analyses about the IR spectra on this paper, are possible and could be one of the next subjects on the further study.

Acknowledgements

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References

1. I. Šafarík and M. Šafaríková, *J. Chromatogr. B*, **722**(1-2), 33-53 (1999).
2. J. Yan, D. Horák, J. Renfeld, M. Hammond and M. Kamali-Moghaddam, *J. Biotechnol.*, **167**(3), 235-240 (2013).
3. L. Wang, W. Zhao and W. Tan, *Nano Res.*, **1**(2), 99-115 (2008).
4. P-A. Albertsson, Chapter 3 and Chapter 4, In 'Partition of Cell Particles and Macromolecules'. 2nd Ed., John Wiley & Sons, NY, USA, 1986.
5. C. K. Byun, I. Hwang, W. S. Choi, T. Yaguchi, J. Park, D. Kim, R. J. Mitchell, T. Kim, Y-K. Cho and S. Takayama, *J. Am. Chem. Soc*., **135**(6), 2242-2247 (2013).
6. C. K. Byun, T. Parker, C. Liang, I. Kendrick, N. Dimakis, E. S. Smotkin, L-J. Jin. D. Zhuang, D. D. DesMarteau, S. E. Creager and C. Korzeniewski, *Anal. Chem.*, **84**(19), 8127-8132 (2012)
7. C. Korzeniewski and C. K. Byun, *ECS Trans,* **64**(3), 719-727 (2014).
8. H. M. Al-Qadiri, M. Lin, A. G. Cavinato and B. A. Rasco, *Int. J. Food Microbiol.*, **111**(1), 73-80 (2006).
9. R. Davis, A. Deering, Y. Burgula, L. J. Mauer and B. L. Reuhs, *J. Appl. Microbiol.*, **112**(4), 743-751 (2012).
10. C. K. Byun, I. Sharif, D. D. DesMarteau, S. E. Creager and C. Korzeniewski, *J. Phys. Chem. B*, **113**(18), 6299-6304 (2009).
11. Y. H. Kim, G. Y. Kim and H. B. Lim, *Bull. Korean Chem. Soc.*, **31**(4) 905-909 (2010).
12. H.-S. Jung, D.-S. Moon and J.-K. Lee, *J. Nanomater*, Article ID 593471 (2012).
13. F. Reymond, C. Vollet, Z. Plichta and D. Horák, *Biotechnol. Prog*, **29**(2), 532-542 (2013).
14. F. N. Mutua, P. Lin, J. K. Koech and Y. Wang, *Mater. Sci. Appl*, **3**(12), 856-860 (2012).
15. D. V. Quy, N. M. Hieu, P.T. Tra, N. H. Nam, N. H. Hai, N. T. Son, P. T. Nghia, N. T. V. Anh, T. T. Hong and N. H. Luong, *J. Nanomater*, Article ID 603940 (2013).
16. R. Wilson, D. G. Spiller, A. Beckett, I. A. Prior and V. Sée, *Chem. Mater.*, **22**(23), 6361-6369 (2010).
17. S. Lan, X. Wu, L. Li, M. Li, F. Guo and S. Gan, *Coloids Surf. A: Physicochem. Eng. Aspects,* **425**, 42-50 (2013).
18. K. R. Bhayani, S. N. Kale, S. Arora, R. Rajagopal, H. Mamgain, R. Kaul-Ghanckar, D. C. Kundaliya, S. D. Kulkarni, R. Parsricha, S. D. Dhole, S. B. Ogale and K. M. Paknikar, *Nanotechnol.*, **18**, 345101 (2007).
19. C. K. Byun, M. Kim, D. Kim, *J. Liq. Chromatogr. & Related Technol.* To be submitted.