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| Complete List of Authors: | Au-Duong, Ai-Nhan; National Taiwan University of Science and Technology, Chemical Engineering<br>Vo, Duc-Thang; National Taiwan University of Science and Technology, Chemical Engineering<br>Lee, Cheng-Kang; National Taiwan University of Science and Technology, Department of Chemical Engineering |
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ARTICLE

# Bactericidal Magnetic Nanoparticles with Iodine Loaded on Surface Grafted Poly(N-vinylpyrrolidone)

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Ai-Nhan Au-Duong, Duc-Thang Vo, Cheng-Kang Lee*

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Bactericidal magnetic nanoparticles was prepared by complexing iodine with poly(N-vinylpyrrolidone) (PVP) grown from the surface of silica coated magnetic nanoparticles (MNPs) via surface-initiated atom transfer radical polymerization (SI-ATRP). Approximately, 10 mg of iodine could be loaded onto one gram of the PVP-grafted MNPs to form bactericidal MNPs@PVP-I. At concentration of 5 g/L, MNPs@PVP-I could achieve 100% bactericidal rate for both Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus* of concentration ~$1x10^{10}$ CFU/mL within 3 min. After used for killing the bacteria in solution, the bactericidal rate of the MNPs@PVP-I decreased to <10% due to the consumption of iodine. The bactericidal rate could be tuned back to 100% when the used MNPs@PVP-I was recharged in a 15 g/L iodine solution for 12 h. The as-prepared bactericidal MNPs@PVP-I could be reused at least 4 times without reducing its 100% bactericidal rate by repeatedly recharging with iodine.

## Introduction

Povidone-iodine (PVP-I), a complex formed between polyvinylpyrrolidone (PVP) and iodine, is a water-soluble and strong antibacterial disinfectant.[1] PVP-I possesses many attractive features, such as broad-spectrum antimicrobial activity against bacteria, molds and certain viruses,[2, 3] high bactericidal efficiency, non-irritation and persistence.[4] Among several commercial forms of PVP-I, such as aqueous solution, film and suppository, the solution form is the most common one in market.[5-8] Some previous studies have taken advantage of the feature property of PVP-I in developing antibacterial materials.[9-12] Iodine complexation with PVP adsorbed on polydopamine modified surface of polypropylene membrane has demonstrated good antimicrobial and antifouling properties.[9] In this recent report, PVP was physically adsorbed onto the polydopamine modified surface mainly via hydrogen bonding. Therefore, the loss of PVP from the polydopamine surface is expected during its applications. Generally, in the application of PVP-I as disinfectant, the iodine molecule will be released from the complex for killing the bacteria, whereas, a great deal of the polymer PVP still remain.[3, 4] PVP although is accepted as a biocompatible polymer but some allergic reaction cases to this polymer have been reported.[13, 14] Thus, the PVP remaining in the disinfection solution is better to be removed by some cleansing processes after application. An iodine-based disinfection solution without free PVP has been prepared by immobilizing PVP on silica gel particles.[12] UV-induced photo grafted process was also used to immobilize PVP on polypropylene film followed by iodine complexation for the preparation of an antimicrobial surface.[10] Antimicrobial fabric prepared by immobilization of PVP-I on non-woven fabric has also been reported can be used for protection wears. [11]

Magnetic $Fe_3O_4$ nanoparticles exhibit good biocompatibility, superparamagnetic property, low toxicity and easy preparation.[15, 16] Various bactericidal magnetic nanoparticles have been prepared based on surface modification with silver nanoparticles, polymeric antimicrobial agents, antibiotics, antimicrobial peptides, and etc. [17,18] The main advantage of using MNPs as bactericidal nanoparticles is that MNPs can be easily retrieved and directed to a specific site by the external magnetic field. However, the bactericidal MNPs with surface grafted antimicrobial agents can only kill the bacteria by contacting. In other words, inhibition zone cannot be observed when its antimicrobial activity is tested in an agar diffusion test. In contrast, MNPs with surface loaded antimicrobial agents such as silver nanoparticles and antibiotics can have stronger bactericidal titer because the antimicrobial agents can be released from the bactericidal MNPs to kill the bacteria even without direct contacting with the MNPs. Usually, the antimicrobial agents once released from MNPs are not replenishable and the retrieved MNPs loses its bactericidal activity significantly. In order to reuse MNPs for antimicrobial application, a facile method for reloading antimicrobial agents to MNPs needs to be developed. Iodine as an antimicrobial agent can be easily replenished to MNPs if PVP could be grafted onto the surface of MNPs due to the strong interaction between iodine and PVP. Therefore, in this work iodine was employed to prepare an effective and rechargeable bactericidal MNPs in using for targeted transport of antibacterial agent and its subsequent removal by an external magnetic field.

SI-ATRP or controlled/‘‘living’’ free radical polymerization has ability to synthesize functionalized polymer grown from the surface in a controlled manner, including grafting density and chain length, that allows the preparation of well-defined dense polymer brushes and hence provides the high capacity of complexing sites for functional molecules.[19-22] In recent years, this method has been widely used to modify a variety of

substrate surfaces, including flat, convex, and concave surfaces such as gold, magnetic nanoparticles, and silica.[23-27] Compared with the some recent studies about grafting PVP onto substrate surfaces,[22, 28] ATRP possesses much milder reaction conditions that can be carried out at room temperature without heating and ultraviolet radiations and in an aqueous solution suitable for immobilizing PVP on the surface of MNPs. Moreover, to the best of our knowledge, there is no report on SI-ATRP of N-vinylpyrrolidone (NVP) from MNPs surface yet. In fact, NVP is a non-conjugated monomer and very reactive propagating radicals will be generated during the polymerization process, as the result, various side reactions could be formed.[19, 22] However, a highly efficient catalyst system CuCl / 5, 7, 7, 12, 14, 14 - hexamethyl-1, 4, 8, 11-tetraazacyclotetradecane ($Me_6$TATD) has been used by Wu et al. to stabilize the propagating radicals and applied successfully to SI-ATRP of NVP on silicon surfaces.[19] In this work, PVP was grafted on the surfaces of MNPs via surface-initiated ATRP (SI-ATRP). To be prepared for SI-ATRP, MNPs was first surface modified with the ATRP initiators [16] and polymerization of NPV was carried out by the help of CuCl/$Me_6$TATD catalyst system.[22, 28] After charging with iodine, the bactericidal activity of the as-prepared MNPs (MNPs@PVP-I) was studied. Besides, the effect of recharging iodine onto PVP grafted MNPs on its bactericidal activity was also evaluated by using Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli* strains.

## Experimental

### Materials

Magnetic nanoparticles (Bayoxide E 8706) was purchased from Lanxess, Germany. Sodium metasilicate ($Na_2SiO_3.9H_2O$), hydrochloric acid (HCl, 35–37%), tetraethoxysilane (TEOS, 98%), triethylamine (TEA, 98%), (3-aminopropyl) triethoxysilane (APTES, 98%), and copper (I) chloride (CuCl) were purchased from Acros Organics. N-Vinylpyrrolidone (NVP, 99%), chloroacetyl chloride was obtained from Sigma–Aldrich. CuCl was purified by mixing in acetic acid, washing with methanol, then dried under vacuum at room temperature overnight. The cyclic ligand 5,7,7,12,14,14-hexamethyl-1,4,8,11-tetraazacyclotetradecane ($Me_6$TATD) was prepared based on the procedure reported by Hay et al.[29]. *Staphylococcus aureus* (ATCC6538P) and *Escherichia coli* (BL21) were obtained from TTRI (New Taipei City, Taiwan) and Novagen (Madison, WI, USA), respectively for the antibacterial activity test. All other chemicals were reagent grade.

### Preparation and characterization of PVP-I grafted magnetic nanoparticles

**Surface modification of MNPs with ATRP initiator (MNPs@Cl).** Before MNPs was surface modified with ATRP initiator, silica coating was carried out according to the method of Philipse.[16] MNPs of 2 g was added into 120 mL aqueous solution containing 4.55 g $Na_2SiO_3.9H_2O$ and 0.4 g NaOH. After well-mixed in an ultrasonication bath for 5 min, 2 N HCl was added drop-wise into the MNPs suspension to adjust pH to 6.0. The MNPs was then collected by magnet and washed with deionized water several time. The obtained MNPs (MNPs@$SiO_2$) was further coated with a porous silica layer by the sol–gel process based on the hydrolysis of TEOS in ethanol/ammonia solution. In detail, the MNPs@$SiO_2$ collected were dispersed in a mixture of 50 mL ethanol, 50 mL deionized water and 1 mL concentrated ammonia solution (28 wt %). Subsequently, 200 mL TEOS dissolved in 10 mL ethanol was added to the above dispersion at a rate of 1 mL/min under mechanical stirring and the reaction was carried out at 85°C for 0.5 h. Then, the obtained MNPs@$SiO_2$@TEOS were washed with deionized water several times and dried at 60°C overnight. The obtained MNPs with porous silica coating was further modified with APTES to obtain surface aminated MNPs (MNPs@$NH_2$) by mixing MNPs in a mixture of 60 mL isopropanol and 1.2 mL APTES at 80°C for 2 h under mechanical stirring.[30] After washing with isopropanol followed by deionized water, the amino groups on the obtained MNPs@$NH_2$ were coupled with the initiator for ATRP by dispersing the collected particles in a solution containing 0.9 mL of TEA (6.3 mmol) and 18 mL of dichloromethane. After cooling to 0°C, chloroacetyl chloride (0.45 mL, 5.75 mmol) was added drop-wise to the mixture. The reaction was carried out at 0°C for 0.5 h then at room temperature for 12 h.[22, 28] The obtained initiator-modified MNPs (MNPs@Cl) were washed with ethanol and dried in a vacuum oven and ready to be used for SI-ATRP of NPV.

**PVP grown from surface of MNPs via ATRP (MNPs@PVP).**

ATRP of NVP on the surface of MNPs was carried out first by mixing CuCl (19.8 mg, 0.2 mmol), $Me_6$TATD (56.8 mg, 0.2 mmol), and NVP (2.08 mL, 20 mmol) in a reagent tube. The solution was then sonicated in an ultrasonication bath for approximately 3 min and then degassed by purging nitrogen atmosphere for 30 min. On the same time, the initiator grafted MNPs (MNPs@Cl) was separately dispersed in 4 mL deionized water under nitrogen atmosphere for 30 minutes. ATRP was initiated by adding the NVP containing solution into the MNPs@Cl dispersion and well shaken at room temperature for different time[22, 28] in a rotary shaker. The PVP-grafted MNPs (MNPs@PVP) was collected by a magnet and washed with deionized water, ethanol and dried in a vacuum oven.

**Grafted density and surface characterization.** The amount of PVP-grafted on MNPs was calculated based on the results obtained by thermogravimetric analysing MNPs@PVP (TGA, Model Diamond TG/DTA, Perkin Elmer). The grafted PVP density (mg/mg particles) was defined as eqn (1):

$$\text{PVP} - \text{Grafted density} = \frac{Wt - Wo}{Wo} \quad (1)$$

where Wo, Wt are the masses of the unmodified MNPs and MNPs@PVP, respectively.

Attenuated total reflection–Fourier transform infrared spectra (ATR–FTIR) of MNPs@PVP were obtained by using a Fourier-transform infrared spectrometer (FTS-3500, Bio-Rad). The magnetic property of various preparations of MNPs were measured using Superconducting Quantum Interference Device Magnetometer (SQUID) (Quantum Design, USA, model: MPMS7) at 298 K. Scanning Electronic Microscope (SEM) (JEOL, Japan, JSM-6500F) operated at 15kV was used for observing the size and surface morphology of MNPs dried on a Nuclepore polycarbonate membrane.

**Iodine loading for MNPs@PVP-I preparation.** MNPs@PVP of 0.5 g was dispersed in 30 mL iodine solutions with concentration of 10 ~ 20 g/L prepared in ethanol. The complex reaction between iodine and PVP was carried out at 60°C for 12h. At the end of iodine complexing reaction, the MNPs@PVP-I was collected and soaked in n-heptane for 24 h and washed repeatedly with n-heptane to remove the free iodine.[12] After dried under vacuum at room temperature, the MNPs@PVP-I was ready for bactericidal activity test. The

amount of iodine loaded onto MNPs (g/g particles) was determined by hyposulphite titration. Briefly, 10 mg of MNPs@PVP-I was well-dispersed in 5 mL deionized water containing few drops of 1% starch solution, and then the mixture was titrated with hyposulphite standard solution until the mixture became colorless. The amount of iodine was calculated based on the volume of the hyposulphite standard solution consumed.

**Bactericidal activity of MNPs@PVP-I against *S. aureus* and *E.coli*.** The viable cell counts of bacterial solutions were measured by using the serial dilution and spread plate technique. Activated *S. aureus* and *E. coli* were inoculated into liquid cultures, respectively. The culture was incubated at 37°C for 16 h on a rotary shaker at 200 rpm. Then, 0.1 mL bacterial culture was serially diluted with sterile water and spread on nutrient agar plates. The plates were incubated at 37°C for 16 h and then the number of viable cells (colonies) were manually counted and expressed as mean colony forming units per mL (CFU/mL). The bactericidal activities of MNPs@PVP-I were first measured by the inhibition zone method. A 16 h bacterial culture of 0.1 mL with cell concentration of approximately $10^5$ to $10^6$ CFU/mL was spread on nutrient agar plates. The same amount of MNPs of different surface modifications was first loaded onto 3 mm Whatman filter paper discs then the discs were overlaid on bacteria plated agar surface. The plates were incubated at 37°C for 16 h to check the appearance of clear zone around the paper discs.

The bactericidal efficiency of MNPs@PVP-I was evaluated by mixing MNPs@PVP-I solution (in pH 7.4 PBS buffer) with bacteria suspension of concentration about $10^{10}$ CFU/mL at room temperature. These suspensions were shaken for different periods of time before applying an external magnet to collect MNPs@PVP-I. The supernatants were taken for measuring the survival viable cell concentrations. The bactericidal efficiencies of MNPs@PVP-I with different dosage and different contact time were calculated based on following eqn (2):

$$\text{Efficiency rate} = \frac{\text{Number of original cell} - \text{Number of survival cell}}{\text{Number of original cell}} \quad (2)$$

**Reusability of bactericidal MNPs.** Recycling MNPs@PVP for repeated use for bactericide, the PVP grafted MNPs was washed with water followed by ethanol before recharging with iodine. The complex reaction between iodine and PVP was then carried out as described in iodine loading for MNPs@PVP-I preparation. After recharging in each cycle, the MNPs was employed for bactericidal activity measurement. The bactericidal efficiency of the recharged MNPs@PVP-I was evaluated as same as described in bactericidal activity measurement against *S. aureus* and *E.coli*.

## Results and discussion

### Preparation of MNPs@PVP-I

Silica coating was first employed to modify the surface of Bayoxide MNPs to prevent the acidic erosion. In addition to coating using sodium silicate, TEOS coating was also employed to generate an additional porous silica layer as reported by Lin et al.[14] so that surface area available for further functionalization can be significantly increased. APTES modification was carried out on these two surfaces to generated amino groups for the immobilization of the initiator for ATRP. The TEOS coating resulted in a 20% higher surface amino group concentration than that of simple sodium silicate coating (11.4 vs 9.5 mmol/g). The porous silica surface coating was then employed for further MNPs modification. The amount of PVP grafted onto MNPs via ATRP of different reaction time was evaluated by TGA. As shown in Fig. 2, a significant weight loss (~95%) was observed for PVP at ~420 °C. At approximately the same temperature, the weight loss of MNPs@PVP samples increased with ATRP reaction time. A 10% weight loss was observed for MNPs@PVP120 which was obtained after 2 h ATRP reaction. The apparent 1% weight loss at 120°C for MNPs@PVP120 is due to the loss of water bounded to the hydrophilic PVP grafted on MNPs surface. Based on the weight loss percentage observed by TGA, the actual amount of PVP grafted can be calculated based on the weight of samples employed for analysis. As shown in Fig. 3, the amount of PVP grafted on the surface of MNPs increased near linearly with reaction time. Approximately, 90 mg PVP/g particles was obtained for MNPs@PVP120. It is interesting to be noted that the viscosity of the MNPs suspension has a substantial increase when PVP was grafted on MNPs. As illustrated in Fig. S1†, due to its higher viscosity, a much larger amount of MNPs@PVP120 solution remained on the surface of pipette tip as compared with that of MNPs@$SiO_2$ and MNPs@Cl suspension during samples transferring by pipette. Evidently, the significant increase of viscosity is mainly resulted from the presence of PVP on the surface of MNPs.

To further confirm the success of grafting PVP, the surface functional groups of MNPs, MNPs@$SiO_2$, MNPs@Cl, MNPs@PVP were analyzed by FTIR. As shown in Fig. 4, a very strong absorption band appeared at 600 $cm^{-1}$ for every sample which is resulted from the Fe–O bond of magnetic nanoparticles itself. The characteristic bands of Si–O–Si asymmetric/symmetric stretch at 1170 $cm^{-1}$ and 810 $cm^{-1}$ were observed for MNPs@$SiO_2$, MNPs@Cl, and MNPs@PVP that indicates $SiO_2$ was successfully coated onto the surface of MNPs. A weak absorption band at 1665 $cm^{-1}$, corresponding to the imide linkage (O=C–NH–), was observed for MNPs@Cl which indicates that the initiator chloroacetyl chloride was successfully immobilized via the amine groups on MNPs@$NH_2$. When SI-ATRP of NVP was started via the immobilized initiator on MNPs@Cl, a new absorption band at 1670 $cm^{-1}$ which attributes to the stretching vibration of carbonyl groups in the pyrrolidone ring was observed for MNPs@PVP20 and MNPs@PVP120. In comparison with MNPs@PVP20, the MNPs@PVP120 has much stronger absorption intensity which again shows that the amount of PVP grafted on the surface of MNPs increases with the ATRP reaction time. As observed by SEM (Fig. S2†), the size of MNPs@PVP120 (~300 nm) seems slightly larger than that of MNPs@$SiO_2$ (~250 nm) (as estimated by Image J). Besides, MNPs without PVP surface coating (Fig. S2a, c and e†) shows severe particle aggregation in contrast to much well-spread MNPs@PVP120 (Fig. S2e†) on Nucleapore membrane during sample preparation for SEM observation. This again shows that PVP was successfully grafted on the surface of MNPs as a protection coating to prevent the MNPs from aggregating because of the steric repulsion of PVP molecules. The magnetic property of MNPs@PVP120 was also measured by SQUID. As shown in Fig. S3†, the magnetic hysteresis loop obtained shows that both MNPs and MNPs@PVP120 are superparamagnetic particles with saturation magnetism of 74 and 76 emu/g, respectively. This indicates that the PVP surface grafting via SI-ATRP did not affect the magnetic properties of Bayoxide MNPs and the high saturation magnetism maintained results in MNPs@PVP120 very responsive to the external magnet employed for particles collection.

Iodine was loaded onto MNPs@PVP120 by incubating in iodine solution of various concentrations for 12 h. The amount of iodine complexed with the grafted PVP was determined by hyposulphite titration using starch as an indicator (see Fig. S4†). As shown in Fig. 5, the amount of iodine in MNPs@PVP-I increased linearly with the iodine concentration. Approximately, the maximal amount of iodine could be loaded onto MNPs@PVP120 is 10 mg/g at iodine concentration of 15 g/L. In contrast, MNPs@$SiO_2$ which was not surface grafted with PVP shows no iodine content after the same iodine loading process. Since the as-prepared MNPs@PVP-I was thoroughly washed with n-hexane to remove the free iodine, the amount of iodine determined by hyposulphite titration is the one complexed with PVP grafted on MNPs surface.

**Antimicrobial activity of MNPs@PVP-I**

The results of inhibition zone test for qualitatively evaluating the antimicrobial activity of MNPs@PVP-I are shown in Fig. 6. After 16 h incubation, very clear inhibition zones (8.8 mm for *S. aureus* and 9.65 mm for *E. coli*) were observed for MNPs@PVP-I. In contrast, no inhibition zones appeared around MNPs and MNPs@PVP. Iodine solution was also used to treat silica coated MNPs@$SiO_2$, however there is no inhibition zone appeared around MNPs@$SiO_2$-I. This indicates that the iodine can only complex with PVP and indeed will be released to kill both the Gram-positive and negative bacteria. Besides, iodine dissolved ethanol solution was also employed for inhibition test. As shown in Fig. 6, no inhibition zone was observed for the sample of ethanol only, in contrast a significant inhibition zone appeared around iodine dissolved ethanol solution. Evidently, MNPs@PVP-I demonstrated a comparable bactericidal activity as that of iodine-ethanol solution. The effects of dosage and incubation time with bacterial solution on bactericidal efficiency of MNPs@PVP-I against ~1x $10^{10}$ CFU/mL of bacterial suspension were studied by mixing MNPs@PVP-I in *S. aureus* and *E. coli* suspension, respectively. As shown in Fig. 7 and 8 (Table S1† and Table S2†), both *S. aureus* and *E. coli* can be completely killed at MNPs@PVP-I dosage of 5 g/L within 3 min. However, *S. aureus* appeared to be more susceptible to the bactericidal activity of MNPs@PVP-I because after 2 min contact *S. aureus* was completely killed while 3 min was required for *E. coli* at the same dosage. The phenomena of Gram-positive bacteria are more susceptible to the effects of disinfectants than Gram-negative bacteria are already well-reported in the literatures[31, 32] due to the fact that the cell wall of Gram-negative bacteria, consisted of a lipopolysaccharides (LPS) outer membrane and a plasma inner membrane, is more difficult for disinfectants to cross. Very limited bactericidal rates (<10%) were also observed for the iodine solution treated MNPs@$SiO_2$ (MNPs@$SiO_2$-I) at higher dosage and longer contact time. The observed bactericidal rates should not be resulted from the iodine loaded to MNPs@$SiO_2$ because no iodine response was detected for MNPs@$SiO_2$-I. The decreased CFU measured is mainly resulted from the bacteria cells attached to MNPs and/or the natural death of bacteria cells when incubated in PBS buffer for a longer time.

The merit of using PVP grafted on MNPs for complexing iodine as bactericide is also shown in Fig. S5†. As expected, no viable bacterial cells can be detected in a bacterial solution (~1x $10^{10}$ CFU/mL) mixed with 5 g/L MNPs@PVP-I for 3 min. In contrast, a plate full of bacterial colonies was observed for the sample taken from bacterial solution disinfected by employing iodine dissolved in water (~50 mg/L) as disinfection solution. Evidently, the MNPs@PVP-I possesses an enhanced bactericidal activity. Probably, it is because iodine itself is a very volatile molecule, without forming complex with PVP very limited amount of free iodine will exist in aqueous solution for bacteria to be effectively killed.

**Reusability of bactericidal MNPs**

The complex interaction between PVP and iodine is expected to be reversible so that iodine will dissociate from MNPs@PVP-I and becomes free iodine to kill the bacterial cells. As a consequence, the empty complexing sites in the grafted PVP should be able to be recharged with iodine. The rechargeability of used MNPs@PVP-I was evaluated by measuring its bactericidal activity. As shown in Fig. 9 and 10, the effective bactericidal rate of freshly prepared MNPs@PVP-I increased with time. *S. aureus* and *E. coli* was completely killed within 2 min and 3 min, respectively. However, MNPs@PVP-I once has been used to kill the bacteria in solution, it hardly has a significant bactericidal rate for the second use. No appreciable iodine was detected in the spent MNPs@PVP-I. Apparently, the iodine complexed with PVP in the freshly prepared MNPs@PVP-I was near completely consumed during its first use. When the spent MNPs@PVP-I was recharged with iodine for 12 h, its bactericidal rate was returned with similar pattern as that of the fresh one. The iodine recharging could at least repeat 3 times without deteriorating the bactericidal activity of MNPs@PVP-I. As shown in Table 1, the iodine content of MNPs@PVP-I could be maintained at the same level (~10 mg/g particles) as the fresh one and 100% bactericidal rate was also achieved by recharging the MNPs@PVP-I. The high bactericidal rate maintained by recharging iodine to the used bactericidal MNPs also revealed that PVP is stably grafted onto the surface of MNPs@PVP-I via SI-ATRP.

## Conclusion

PVP could be effectively grafted onto the surface of MNPs via SI-ATRP of NVP. After 2 h polymerization, approximately 90 mg PVP/g particles was grafted. The strong complex interaction between PVP and iodine resulted in ~10 mg iodine was loaded in 1 g of MNPs@PVP-I by charging PVP grafted MNPs (MNPs@PVP) with 15 g/L iodine solution for 12 h. MNPs@PVP-I demonstrated a very effective bactericidal activity against *S. aureus* and *E. coli*. Bacteria of ~1x$10^{10}$ CFU/mL could be completely killed within 3 min by mixing with 5 g/L MNPs@PVP-I. Iodine in MNPs@PVP-I was near completely consumed after one bactericidal application. However, the consumed iodine can be recharged by incubating in iodine solution. After 4 repeated bactericidal action and iodine recharging cycles, MNPs@PVP-I showed the same bactericidal level. It reveals the very good rechargeability of MNPs@PVP for the disinfectant iodine. Not only its good iodine rechargeability, MNPs@PVP-I also shows excellent mechanical and chemical stability so that it can be repeatedly applied to kill bacteria and easily retrieved due to its superparamagnetic property. Moreover, the magnetic hysteresis loop obtained shows that MNPs@PVP120 is superparamagnetic particles with saturation magnetism of 76 emu/g. This indicates that the PVP grafted MNPs via SI-ATRP (MNPs@PVP120) can be very easily recycled and used for targeted transport of antibacterial agents by applying an external magnetic field.

## Note and References

Department of Chemical Engineering, National Taiwan University of Science and Technology, Taipei 106, Taiwan, *Email: cklee@mail.ntust.edu.tw

† Electronic Supplementary Information (ESI) available: table of bactericidal efficiency with various time and concentration against *S. aureus* and *E. coli*; images of MNPs@$SiO_2$, MNPs@Cl, MNPs@PVP120 suspension; SEM images of MNPs, MNPs@$SiO_2$ (TEOs), MNPs@ Cl, MNPs@PVP120; the magnetic hysteresis loop of MNPs, MNPs@PVP120 at 298 K; images of MNPs@PVP-I in DI water adding few drops of starch solution. See DOI: 10.1039/b000000x/

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**Table 1** Iodine content and bactericidal efficiency of recharged MNPs@PVP-I

| Recharged MNPs@PVP-I | | Iodine amount (mg/g particles) | Bactericidal efficiency rate (dosage: 5 g/L, 3 minutes, %) | |
|---|---|---|---|---|
| | | | *S. aureus* ($10^{10}$ CFU $mL^{-1}$) | *E. coli* ($1.2 \times 10^{10}$ CFU $mL^{-1}$) |
| Times | 1 | 10.16 | 100 | 100 |
| | 2 | 9.53 | 100 | 100 |
| | 3 | 10.16 | 100 | 100 |

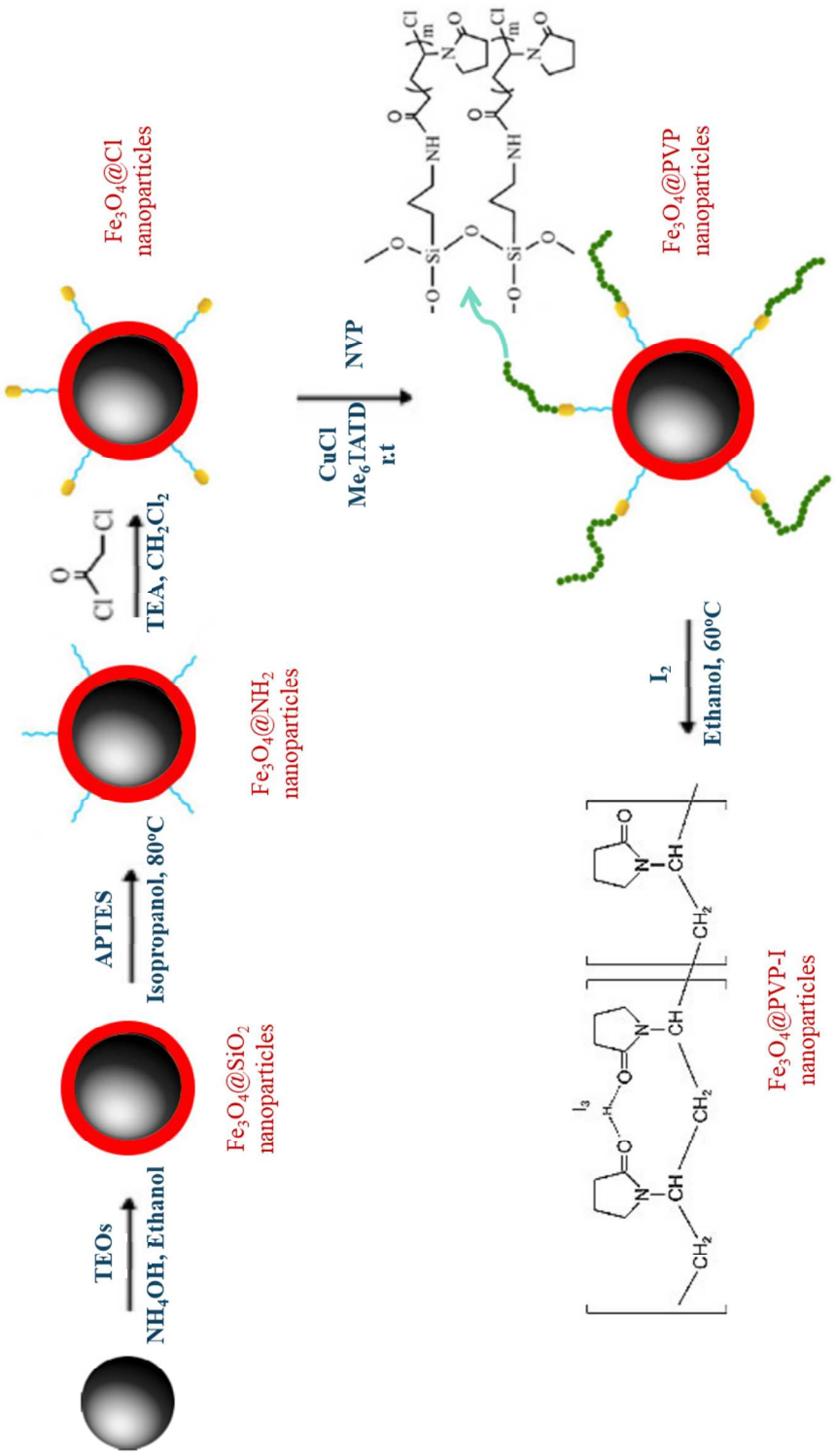


**Fig. 1** Schematic illustration of the preparation of bactericidal magnetic nanoparticles MNPs@PVP-I

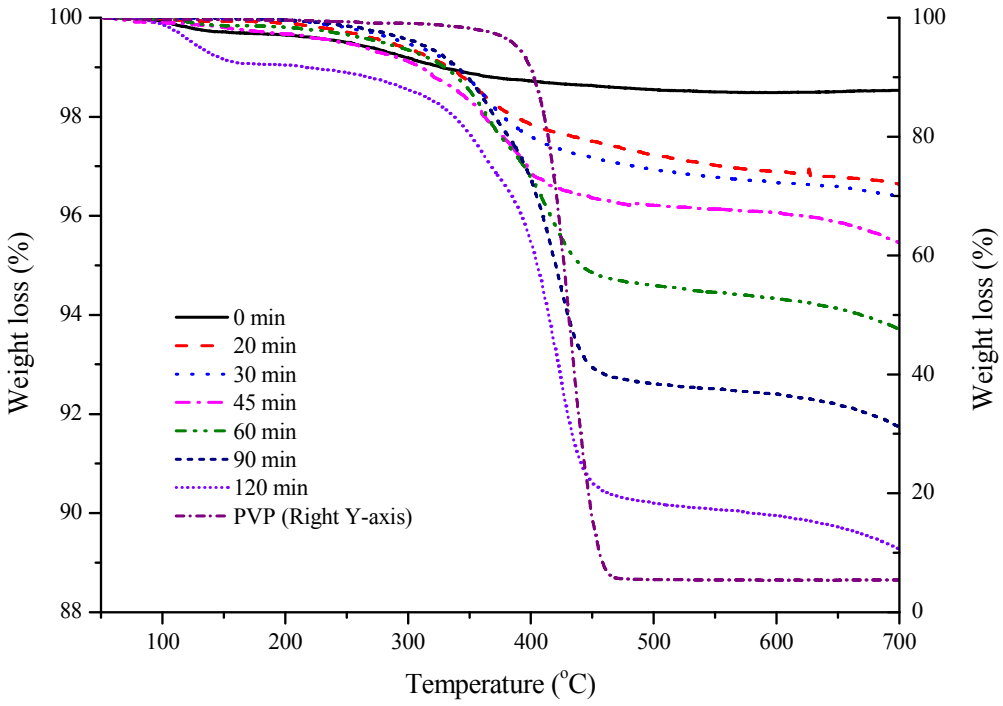


**Fig. 2** Thermogravitric analysis (TGA) of PVP grafted MNPs (MNPs@PVP) prepared at different ATRP reaction time (0-120 minutes) with reference of pure commercial PVP (average M.W. 8, 000, Acros Organics).

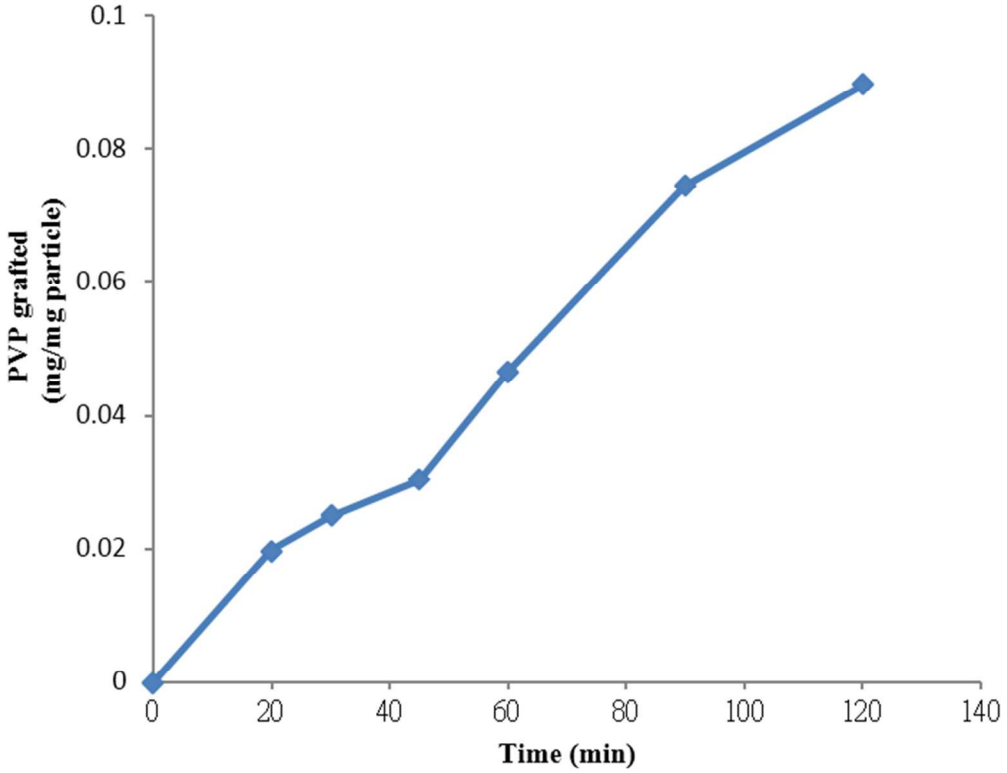


**Fig.3** Effect of ATRP reaction time (0-120 minutes) on the amount of PVP grafted on MNPs@PVP based on the weight loss percentage observed by TGA.

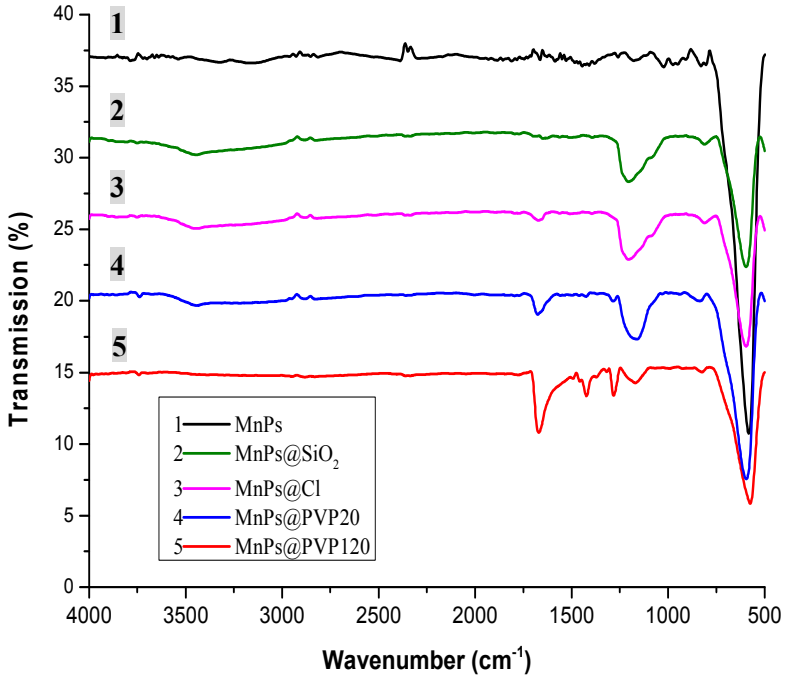


**Fig.4** FT-IR spectra of magnetic nanoparticles (MNPs) with different surface modification of naked MNPs, MNPs@$SiO_2$, MNPs@Cl, MNPs@PVP20 and MNPs@PVP120 (ATRP reaction time 20 and 120 minutes respectively).

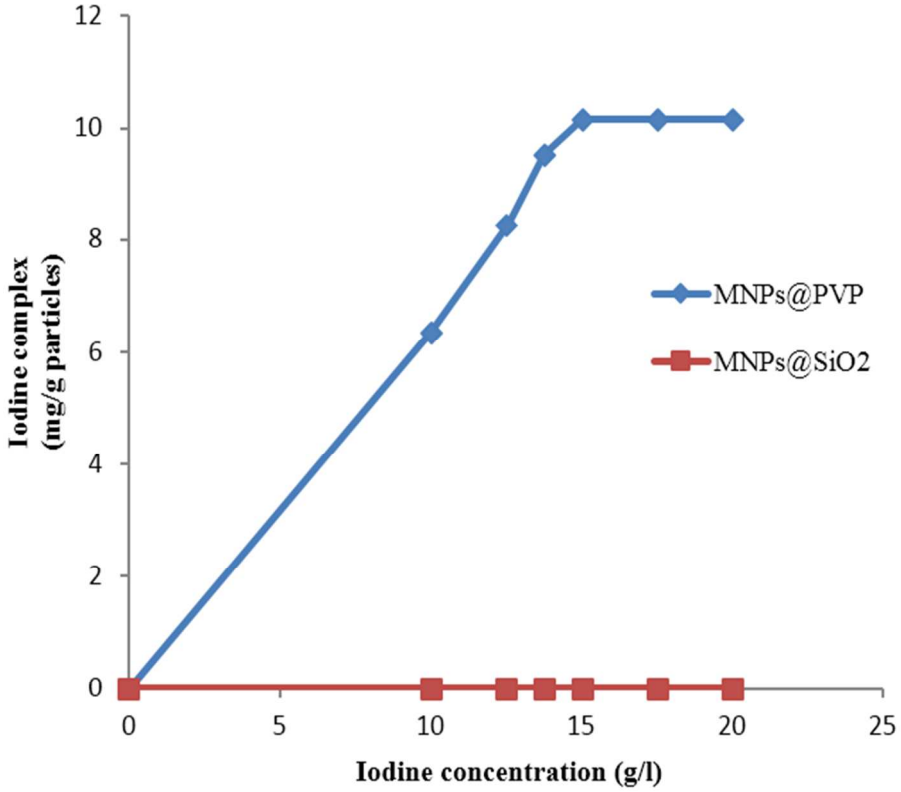


**Fig.5** Amount of iodine complexed with magnetic nanoparticles (MNPs@$SiO_2$ and MNPs@PVP120) calculated from hyposulphite titration method after charging in iodine solution for 12h with MNPs concentration of 16.67g/L

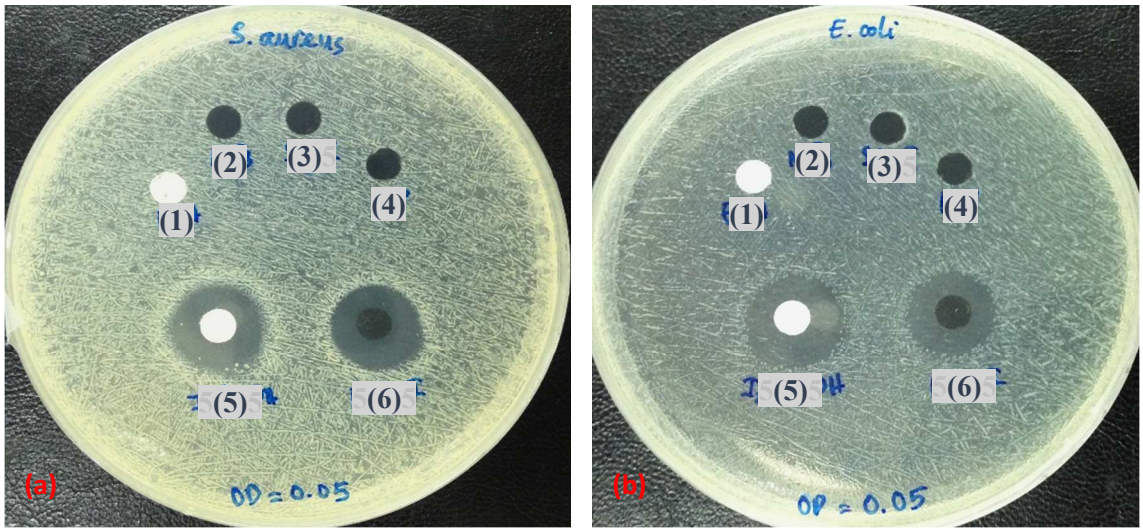


**Fig. 6** Inhibition zones test after incubating at 37°C for 16 h the overlaid discs of ethanol (1); MNPs (2); MNPs@$SiO_2$-I (3); MNPs@PVP (4); iodine in ethanol solution (5); MNPs@PVP-I (6) against *S. aureus* (a) and *E. coli* (b)

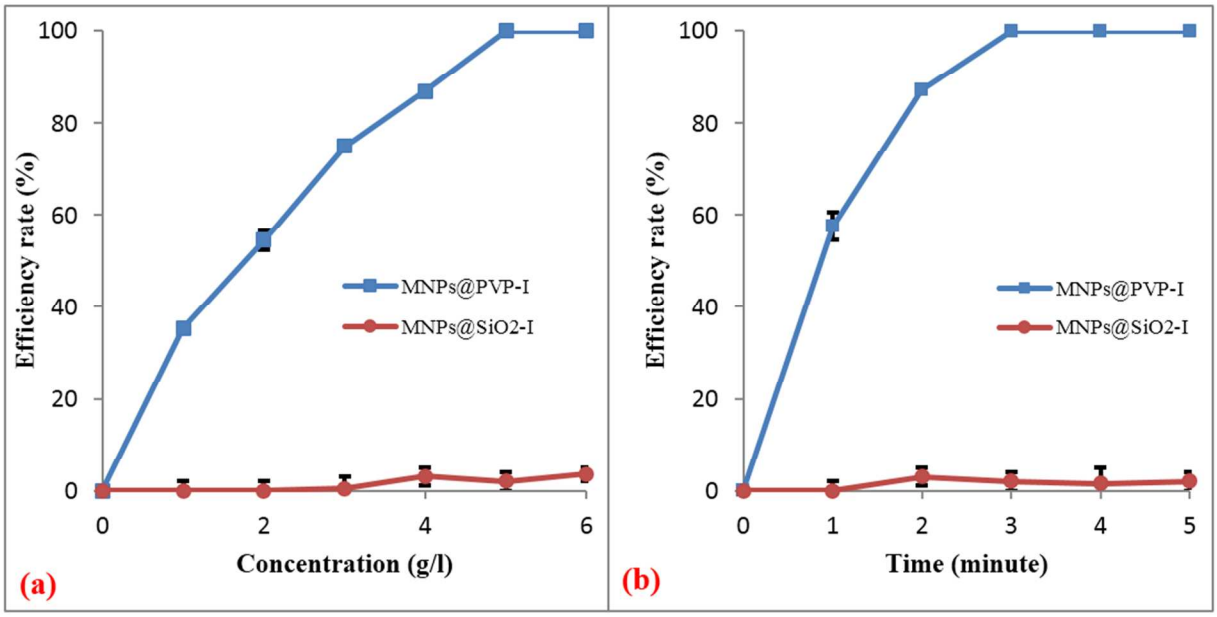


**Fig.7** Effect of bactericidal MNPs concentration (a) and incubation time (b) against *S. aureus* (bacteria suspension of concentration about $10^{10}$ CFU/ml) at room temperature

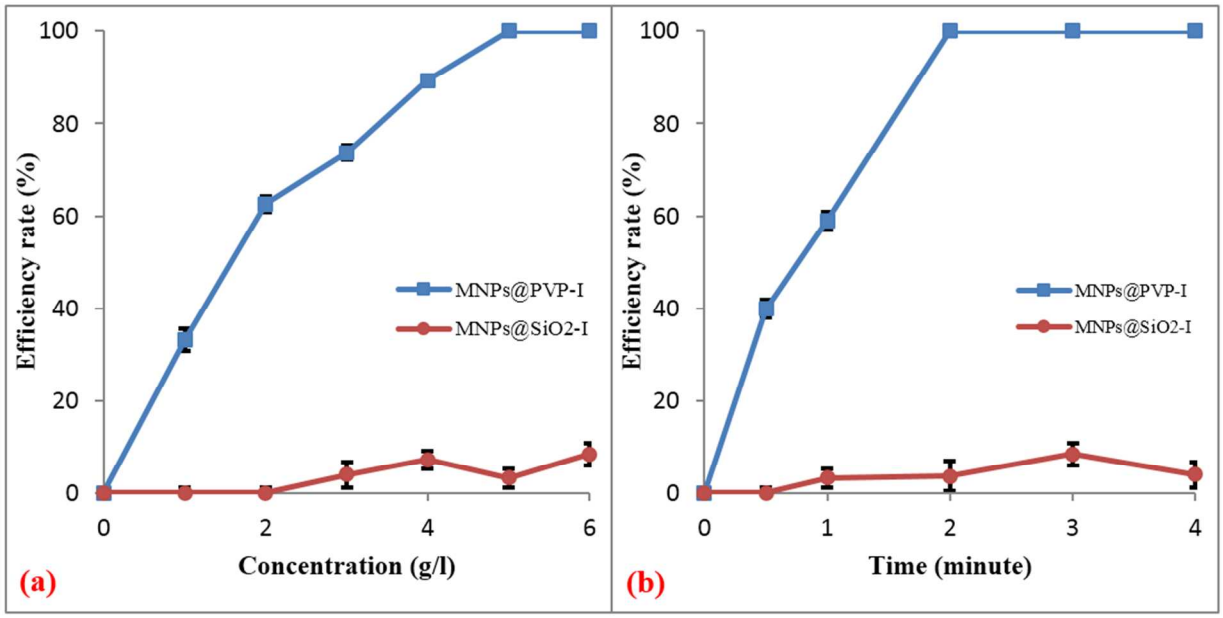


**Fig.8** Effect of bactericidal MNPs concentration (a) and incubation time (b) against *E. coli* (bacteria suspension of concentration about $10^{10}$ CFU/ml) at room temperature

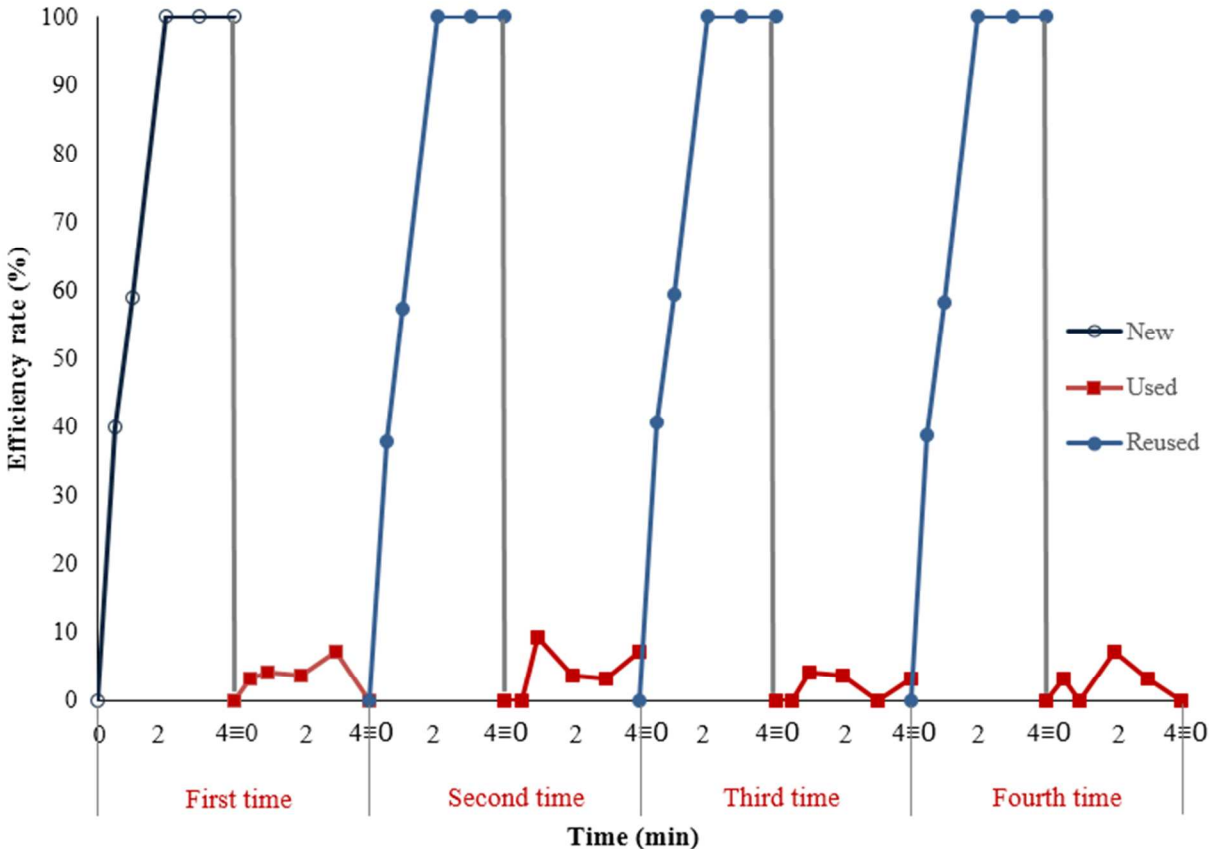


**Fig 9** Effect of bactericidal MNPs@PVP-I (fresh, used and reused) against *S. aureus* (dosage: 5 g/l, 4 minutes) (bacteria suspension of concentration about $10^{10}$ CFU/ml) at room temperature

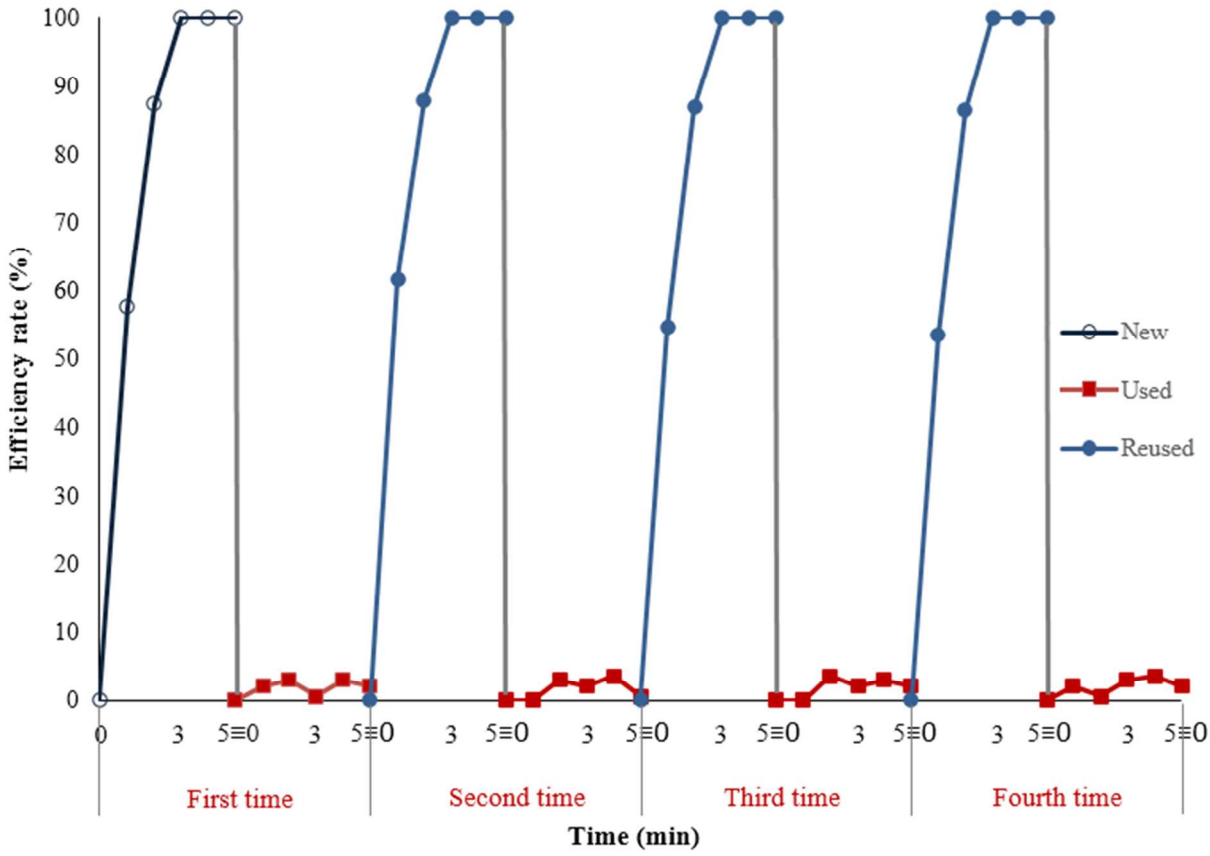


**Fig 10** Effect of bactericidal MNPs@PVP-I (fresh, used and reused) against *E. coli* (dosage: 5 g/l, 5 minutes) (bacteria suspension of concentration about $10^{10}$ CFU/ml) at room temperature

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