Carbohydrate Polymers 82 (2010) 648–652

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Lowering the cationic demand caused by PGA in papermaking by solute adsorption and immobilized pectinase on chitosan beads

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ARTICLE INFO

Article history:
Received 21 April 2010
Received in revised form 15 May 2010
Accepted 17 May 2010
Available online 11 June 2010

Keywords:
Chitosan
Adsorption
Immobilized pectinase
Cationic demand
Papermaking

ABSTRACT

Pectins or polygalacturonic acids (PGA) were originated from alkaline peroxide bleaching of mechanical pulps and considered as the dominant troublesome substance, which seriously decreased the runability of the paper machine in process water closure. To find wide application of enzymes in lowering PGA concentration in papermaking industries, cross-linked chitosan beads were prepared. Results showed that the PGA-absorption capability of chitosan beads was greatly affected by its cross-linking degree. The activity of immobilized pectinase on cross-linked chitosan beads were also investigated and the highest activity of binary immobilized pectinase on cross-linked chitosan was achieved using 1.00% of activating reagent or 0.005% of glutaraldehyde. Cationic demand of PGA solutions was obviously lowered by increasing the temperature of enzymatic treatment. GPC analysis showed a sharp decrease in the molecular weight of PGA after enzymatic treatment. Besides, operational stability of the immobilize pectinase was analyzed to evaluate its application potential.

1. Introduction

With the closure of process water stream in papermill, the dissolved and colloid substance (DCS) accumulated in whitewater. Excessive buildup of the DCS in process water stream may decrease the runability of the paper machine and increase corrosion, foaming, pitch, precipitation and scaling (Boegh, Garver, Henry, Yuan, & Hill, 2001; Holmbom, 1997). Acidic polysaccharides were considered as the troublesome dissolved substances, which originated from alkaline peroxide bleaching of mechanical pulps. Prominent among these acidic polysaccharides were pectins, or polygalacturonic acids (PGA) (Boegh et al., 2001). Traditional strategy for reducing the contents of these substances was adding cationic polyelectrolyte for neutralization, which caused extra consumption of hazardous chemicals (Ho & McKay, 2000). However, due to the tremendous increase in the awareness regarding the effects of pollution in pulp and paper industry, it has been highly demanded to replace those traditional processes with “green” processes involving microorganisms and enzymes, which not only provide an economically viable alternative but are also environmental friendly (Holmbom, 1997; Ho & McKay, 2000; Reid & Ricard, 2000; Ricard & Reid, 2004).

Recently enzymes has been tried to solve problems in paper making processes, due to their advantageous properties including high activity, mild reaction conditions, and being environmentally friendly. Among the used enzymes, pectinase was proved to be effective in solving the retention problems and decreasing the cationic demand in whitewater (Reid & Ricard, 2000; Ricard & Reid, 2004; Thornton, 1994). However, short operational lifetime of the free pectinase greatly limited its practical application in papermaking industries. Also, unlike conventional heterogeneous chemical catalysts, free enzymes were dissolved in water in homogeneous catalysis systems, which not only contaminated the product but also cannot be reused in the active form (Krajewska, 2004). Compared to free enzymes, immobilized enzymes were more rigid and resistant to environmental changes. Moreover, the heterogeneity of the immobilized enzyme allowed easy recovery and multiple reuses of enzymes. However, till then no reported was made on the application of immobilized pectinase in treatment of PGA solutions, the main troublesome dissolved substances in process water of papermaking industries.

Among the supports used for immobilization of enzymes, chitosan was well known for its unquestionable advantages. Chitoson had high affinity to proteins, availability of reactive functional groups for direct reactions with enzymes and for chemical modifications, hydrophilicity and mechanical stability (Cetinus & Oztop, 2003; Hung, Giridhar, Chiou, & Wu, 2003; Noda, Furuta, & Suda, 2001; Zeng, Qin, Wang, Chi, & Li, 2008). Several researches had demonstrated the enhancement of both the activity and stability of enzymes immobilized on chitosan (Cetinus & Oztop, 2003; Orrego et al., 2010; Sjoholm, Cooney, & Minteer, 2009). Chitoson was also an absorbent for transition mental ions and organic species because

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0144-8617/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2010.05.028

its amino and/or hydroxyl groups can serve as the coordination and reaction sites. Hence, chitoson was selected as a typical support for pectinase immobilization and its PGA-absorption behavior was also investigated for the first time in this paper. And the effect of immobilized pectinase on degradation of PGA was explored and compared with the absorption effect of chitosan beads on canonic demand of PGA. Additionally, the activity and stability of pectinase immobilized on cross-linked chitosan beads were also investigated.

2. Materials and methods

2.1. Materials

Chitosan (M_W 9×10^5 Da, Degree of Deacetylation 95%) was provided by Yuhuan Ocean Biochemical Co., Ltd. (Zhejiang, China). Pectin from citrus peel was purchased from Sigma–Aldrich (USA). The Samples of whitewater were gifted by a Southern China Paper Mill producing high-brightness mechanical specialty grades from peroxide-bleached softwood TMP. All the samples used in experiments were brought back on the same day as they were collected and then stored at 4 °C until use. pectinase was purchased from Novozymes (Denmark). 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) was purchased from Aladdin reagent (China) Co., Ltd. All other chemicals were of analytical grade and used without further purification.

2.2. Preparation of cross-linked chitosan beads

The swollen chitosan beads were prepared as follows according to reference (Cetinus & Oztop, 2003; Hung et al., 2003): chitosan solution was prepared by dissolving 2.0 g chitosan flakes into 100 ml of 5% (v/v) acetic acid solution. The resulting solution was dropped into 200 ml of 2.0 M NaOH solution containing 40 ml of ethanol through a needle (diameter, 1.2 mm). The chitosan gelled spheres formed instantaneously. After hardening the chitosan beads were separated and washed with deionized water until the filtrate became neutral. The diameter of the wet beads was approximately 1.8–2.0 mm. Then the swollen beads (5 g) were put into a flask containing 50 ml of glutaraldehyde, and then stirred at 25 °C and 150 rpm for 24 h. After equilibrium, aqueous samples were taken and the concentration of glutaraldehyde was measured by UV/visible spectrophotometer at 234 nm. The amount of cross-linking of glutaraldehyde at equilibrium (g_e) was obtained by

$$g_e(\text{mg/g}) = \frac{(C_{L,0} - C_{L,e})V}{W} \tag{1}$$

where $C_{L,0}$ and $C_{L,e}$ (mg/l) are liquid-phase concentrations of glutaraldehyde initially and at equilibrium, respectively, V is the volume of the solution (l), and W is the weight of chitosan beads used (g).

2.3. Immobilization of pectinase on chitosan beads

Two immobilization methods were tried for pectinase according to references (Cetinus & Oztop, 2003; Hung et al., 2003). Method A: 3 g of chitosan beads were treated by 10 ml 1% (w/v) 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) solution and mixed for 30 min, and then 5 ml of 1% (v/v) pectinase was added into the achieved chitosan–EDC mixture at 25 °C and pH 8.0. After 1 h, the beads were filtered and added into 10 ml 0.005% glutaraldehyde. After 30 min, the supernatant was removed and 5 ml of 1% (v/v) pectinase was added into the beads at 25 °C and pH 8.0. After 3 h, beads were washed thrice in deionized water to remove unbound enzymes.

Method B: 3 g of chitosan beads were mixed with 10 ml 0.005% glutaraldehyde for 30 min, and 5 ml of 1% (v/v) pectinase was added into the cross-linking chitosan beads at 25 °C and pH 8.0. After 1 h, the beads were filtered and added into 10 ml 1% EDC solution for 30 min. And then the supernatant was removed and 5 ml of 1% (v/v) pectinase was added into the beads at 25 °C and pH 8.0. After reaction for 3 h, beads were washed three times in deionized water to remove unbound enzymes. Relative activity (%) of immobilized pectinase was defined as the ratio of the residue enzyme activity to the highest enzyme activity under the examined conditions.

2.4. Cationic demand measurement

Cationic demand was measured using the Mütek Particle Charge Detector (PCD-04) and titrator (PCD-T). The blank and whitewater samples (1 ml) was diluted 10 times with deionized water and then titrated with 0.001 N poly-DADMAC.

2.5. Enzyme activity assay

The activity of free and immobilized alkaline pectinase was assayed by measuring the amount of D-galacturonic acid liberated from citrus pectin. One milliliter free pectinase, or 1 g immobilized pectinase was added to the reaction mixture containing 0.5% (w/v) pectin in 5 ml 0.05 M phosphate buffer (pH 8 for free and 8.5 for immobilized pectinase). The reaction mixture was incubated for 30 min at 50 °C for free pectinase and 55 °C for immobilized pectinase. Then the end-product was analyzed using dinitrosalicylic acid (DNSA) reagent. One unit (U) of pectinase was defined as the amount of enzyme required to release 1 μg of D-galacturonic acid per minute per milliliter under the assay conditions.

2.6. The determination of number-average molecular weight of PGA

The number-average molecular weight of PGA was measured by Waters-Wyatt GPC systems equipped with a multi-angle light scattering detector (DAWN HELEOS II). Sodium nitrate aqueous solution (0.2 M, pH 7) was used as mobile phase at a flow rate of 0.5 ml/min. as the standard polymers for calibration

2.7. Treatment of PGA solution with chitosan beads and chitosan-immobilized pectinase

PGA powder (Alfa Aesar) was dissolved into 0.1 mol/l caustic solution for 24 h and then the pH value of the mixture was adjusted to 7.5 by 0.1 mol/l HCl, giving 0.1 or 1 g/l of PGA solutions. 5 g cross-linked chitosan was added into 50 ml of above-mentioned PGA solution (0.1 g/l) and the mixture was stirred at 25 °C for 24 h. Aliquots were withdrawn after equilibrium, diluted 10-fold with distilled H_2O and then titrated with 0.001 N poly-DADMAC. The cationic demand of PGA solution was measured as described in Section 2.4.

3. Results and discussion

3.1. Influence of cross-linking degree on sorption performance of chitosan beads

Generally speaking, the cross-linking degree of chitosan chains with bifunctional groups may greatly affect the sorption performance (Juang et al., 2001; Osifo et al., 2008; Sjoholm et al., 2009). Thus the equilibrium adsorption of PGA on chitosan beads with different degrees of cross-linking was investigated. Considering that the difference sizes of chitosan beads might have potential impact on their absorption performance due to the variation of specific areas, we prepared chitosan beads with the similar size for the

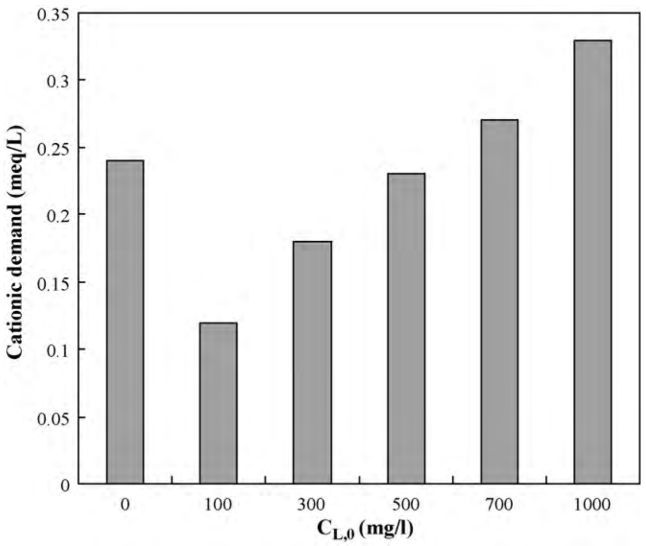

Fig. 1. Effect of chitosan beads with different degrees of cross-linking treatment on cationic demand of PGA solution at 25 °C.

following research. Fig. 1 showed the absorption effects of cross-linking degrees (in terms of different dosages, $C_{L,0}$) of chitosan beads on the cationic demand of PGA at 25 °C. It was found that addition of raw chitosan beads without cross-linking treatment led to obvious decrease of the cationic demand from 0.67 to 0.24 meq/l. And the addition of the chitoson beads with the best $C_{L,0}$ value of 100 resulted in the lowest cationic demand of PGA. Enhanced absorption of chitosan beads by cross-linking might account for this. Further increasing the cross-linking degree of chitoson beads, however, reduced the absorption amount of PGA by chitoson beads.

3.2. Binary immobilization of pectinase and its catalytic activity in PGA degradation

For better degradation of PGA in whitewater, immobilized pectinase was prepared and the activities of immobilized pectinases prepared by two binary immobilization methods were compared. The immobilization procedures of the two methods were described in Section 2.3. The concentrations of EDC and glutaraldehyde, the key factors influencing the immobilization efficiency, showed obvious effect on activity of chitosan-immobilized pectinase (Table 1), The highest relative activities of immobilized pectinase were achieved with 1.00% of EDC in Method A or 0.0050% of glutaraldehyde in Method B. Under each optimized conditions, the activities of immobilized pectinase prepared by Methods A and B were 52.3 and 72.6 U/g$_{\text{-chitosan}}$, respectively.

Table 1
Effects of EDC/glutaraldehyde concentrations on activity of immobilized pectinase.

EDC or glutaraldehyde concentration (%)		Relative activity (%)
EDC	0.25	75.9
	0.50	80.1
	0.75	86.1
	1.00	100.0
	1.50	98.8
	2.00	97.6
	3.00	88.6
Glutaraldehyde	0.0025	68.3
	0.0050	100.0
	0.0100	93.7
	0.0200	85.0
	0.0500	32.0
	0.1000	4.2

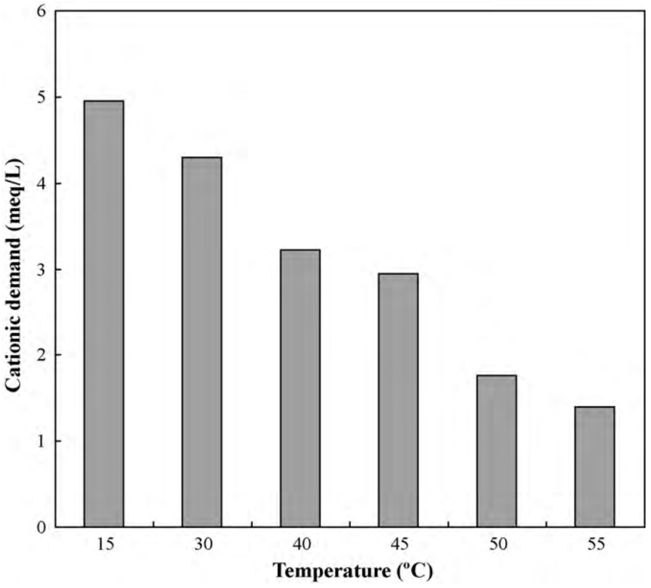

Fig. 2. Effect of temperature on the cationic demand of PGA solutions treated with immobilized pectinase.

It was considered that the binary immobilized pectinase by Method A was first immobilized to the hydroxyl groups of chitosan by activation with EDC, followed by the immobilization of more pectinase molecules through its amino groups by cross-linking with glutaraldehyde. In the second method, pectinase was first immobilized to the amino groups of chitosan by cross-linking with glutaraldehyde followed by the immobilization of more pectinase molecules through its hydroxyl groups which were activated with EDC (Hung et al., 2003; Ting, Tung, Giridhar, & Wu, 2006). The higher binary immobilized pectinase by method B than that by method A might be due to the different deactivation degree of enzymes cross-linked to the chitosan during different immobilization procedures.

Therefore, we decided to use Method B for binary immobilization of pectinase onto chitosan, for further study of the enzymatic treatment of papermaking whitewater.

3.3. Effect of temperature on treatment of PGA solution by immobilized pectinase

Temperature plays an important role in both the activity of enzymes and the thermodynamic equilibrium of reactions. So the effect of temperature on the treatment of PGA solution by immobilized pectinase was firstly investigated. Fig. 2 showed the cationic demand of PGA sharply decreased with the increase of treatment temperature and the lowest cationic demand value was achieved at 55 °C, partly due to the higher degradation rate of PGA at higher temperature. Besides, improved thermo-stability of the pectinase by immobilization might account for the improved catalytic activity of the pectinase at higher temperature (Hung et al., 2003; Chiou & Wu, 2004).

3.4. Effect of enzyme dosage and time course of cationic demand of PGA solution

Fig. 3 shows the effect of the dosage of immobilized pectinase on the cationic demand of PGA solution. It was found that the reaction accelerated clearly with the increment in enzyme dosage from 726 to 2904 U/l and then no substantial variation in cationic demand of PGA occurred with further increasing enzyme dosage up to 3780 U/l. To gain a deeper insight into the enzy-

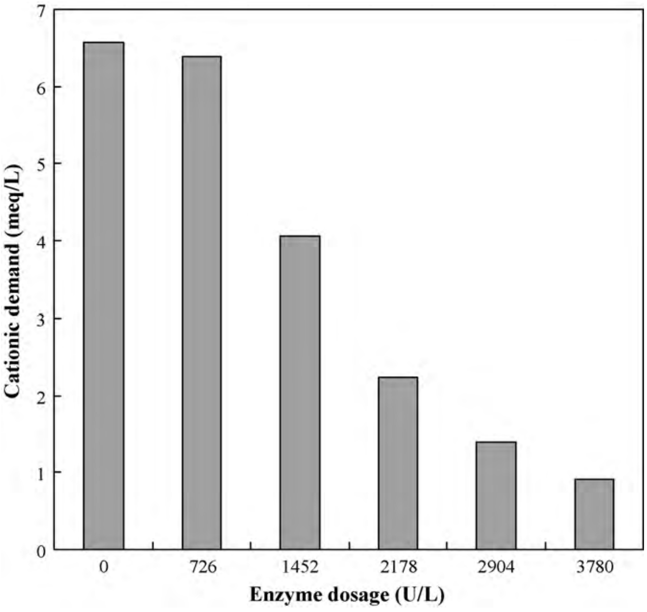

Fig. 3. Effect of immobilized enzyme dosage on the cationic demand of PGA solutions.

matic process, the time course of immobilized pectinase-mediated degradation of PGA was followed (Fig. 4). As can be seen in Fig. 4, the canonic demand of PGA solution underwent a steep drop in about 160 min, and then a smooth decrease, possibly due to the lower concentration of PGA. Compared with enzymatic treatment, much smaller drop of canonic demand of PGA by raw chitosan beads absorption was found in 5 min and then little change in canonic demand was found if further prolonging the treatment time.

3.5. *Number-average molecular weight of PGA treated by pectinase*

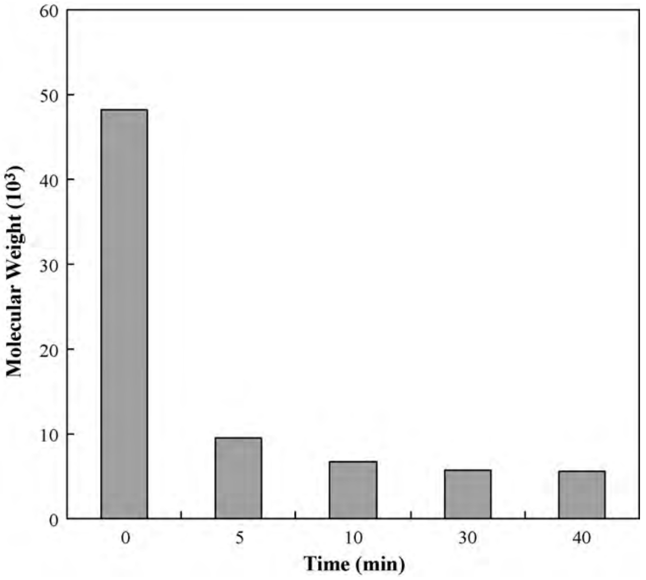

Fig. 5. Molecular weight of PGA treated by immobilized pectinase for different time.

To find out the degradation level of PGA after enzymolysis, number-average molecular weight of PGA solution samples treated by pectinase for different time were analyzed by Waters-Wyatt GPC systems. As shown in Fig. 5, the number-average molecular weight of PGA sharply reduced from 48,150 to 9537 Da after a 5-min enzymatic treatment, and then slowly decreased to 5587 Da if further extending the enzymolysis time to 40 min. This was in account with the observed decrease of cationic demands of PGA solutions (Fig. 4).

3.6. *Operational stability of the immobilized pectinase in PGA solution*

The operational stability of the immobilized pectinase was examined to evaluate its potential for recycled applications in industrial scales. As described in Fig. 6, after operating repeatedly for 5 batches, the pectinase immobilized on chitoson remained approximately >60% of its original activity. And after used for 8 batches, about 52% residue activity of the immobi-

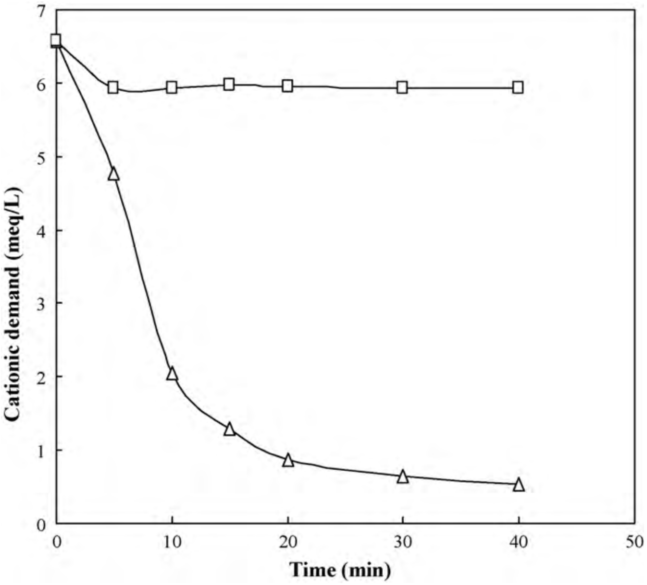

Fig. 4. Time course of cationic demand of PGA solutions treated with immobilized pectinase (△) and cross-linking chitosan beads (□).

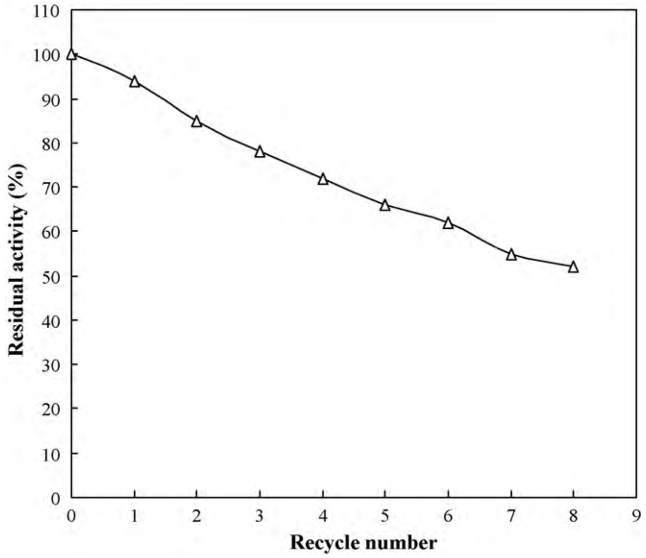

Fig. 6. Operational stability of the immobilized pectinase in PGA solution treatment at 55 °C and pH 8.5.

lized pectinase was found. The enhanced operational stability of chitoson-immobilized pectinase made it an excellent biocatalyst for potential use in water treatment of papermaking industry.

4. Conclusion

In this paper, cross-linked chitosan beads were prepared and its PGA-absorption capability was found to be greatly affected by the degree of cross-linking. Glutaraldehyde-cross-linked chitosan beads also can be served as a suitable carrier for pectinase immobilization. The activity of immobilized pectinase varied with the concentration of EDC or glutraldehyde concentration during immobilization. Treated with the immobilized pectinase prepared under optimized conditions, the molecular weight of PGA sharply reduced from 48,150 to 5587 Da within 40 min. Besides, the immobilized pectinase onto cross-linked chitosan beads showed high activity in treatment of PGA at even much high temperature, indicating an enhanced thermo-stability of the enzyme after immobilization. The immobilized pectinase can be operated repeatedly for 8 batches with nearly half of the original activity remained. The present work revealed the application potentials of cross-linked chitosan beads for lowering canionic demand of PGA by solute adsorption and pectinase immobilization, for potential use in water treatment of papermaking industry.

Acknowledgments

The authors are grateful to the National Natural Science Foundation of China (Grant No. 20906031, No. 20776054), the Science and Technology Project of Guangdong Province (Grant No. 8451064101000807), the Doctoral Program of Higher Education (Grant No. 200805611022, No. 200805611104) and self-determined research funds of SCUT from the college' basic research and operation of MOE (Grant No. 2009ZZ0020) for financial support.

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