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**PJBS**

ISSN 1028-8880

**Pakistan  
Journal of Biological Sciences**

**ANSI***net*

Asian Network for Scientific Information  
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

## Determination of Benzidine Using the Analyte Pulse Perturbation Technique in BZ Oscillating Chemical System

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**Abstract:** A simple and sensitive analytical method for the determination of benzidine by the perturbation caused by different amounts of benzidine on the Belousov-Zhabotinskii (BZ) oscillating chemical system involving the Ce (IV)-catalyzed reaction between potassium bromate and malonic acid in an acidic medium is proposed. Results show that a linear relationship between the change in the oscillation amplitude and the concentration of benzidine existed. The calibration curve is linearly proportional to the concentration of benzidine over the range  $3.5 \times 10^{-9}$ - $1 \times 10^{-4}$  M, with the regression coefficient is 0.9975. The optimum condition for the determination has been chosen. Influence of temperature and reaction variables were investigated in detail and the possible mechanism caused by the adding of benzidine on the chemical oscillating system was also discussed.

**Key words:** Oscillating chemical system, benzidine, analyte, determination

### INTRODUCTION

Although there has been much interest in study of mechanism of oscillating chemical reaction, there have been few studies of application to the analytical chemistry<sup>[1,2]</sup>. The introduction of analyte pulse perturbation technique (APP) to the analytical determination by Dolores Perez-Bendito's group<sup>[3]</sup> is a new starting point. Since then, many papers deal with the analytical application, in which organic compounds and BZ reaction are the most part<sup>[4-15]</sup>. For the analytical application, that is, as an analytical method, BZ reaction has simple, sensitive and well repeated feature.

As part of an effort to widen the BZ oscillating chemical reaction in analytical application<sup>[4-7]</sup>, this study reported a new method for the determination of benzidine.

### MATERIALS AND METHODS

**Chemicals:** All chemicals used were of analytical-reagent grade without further purification. Stock solutions of 0.5 M malonic acid, 0.2 M potassium bromate, 0.04 M cerium (IV) were separately prepared with 0.8 M sulfuric acid. Working solutions of low concentration of the benzidine were prepared with bidistilled water and were made freshly just prior to use. Distilled water was used throughout.

**Apparatus:** The instrumental set-up used to implement the oscillating chemical reaction for the determination of benzidine consisted of a glass vessel of 30 mL capacity wrapped in a water recirculation jacket. A CHI 832 electrochemical analytical instrumental (Shanghai Chenhua Instrumental Company) equipped with a computer (LEGEND 586) was used to record the potential change. A Model CS501 thermostat (Chongqing Experimental Instrumental Factory) with an accuracy  $\pm 0.1^\circ\text{C}$  and a Model ML-902 magnetic stirrer (Shanghai Pujiang Analytical Instrumental Factory) were used to maintain the temperature of reaction solutions. The oscillation was monitored by means of two Pt electrodes (Rex, 213, China) and a Type  $\text{K}_2\text{SO}_4$ -1 reference electrode (Shanghai Instrumental Factory). Signals were recorded as a function of time with time step 0.1 s. A micro-injector for injecting sample solution was used.

**Procedure:** To a glass vessel thermostated at  $30^\circ\text{C}$  were added in the following sequence: 6.25 mL of 0.2 M potassium bromate, 6.70 mL of 0.5 M malonic acid, 1.00 mL of 0.04 M cerium (IV) and 6.05 mL of 0.8 M sulfuric acid up to a final volume of 20 mL. The mixture was homogenized by continuous magnetic stirring. Then the indicator and reference electrode were immersed in the mixture and data acquisition by the recorder was started. After the induction period was disappeared, the regular oscillating profile went to a steady state. Then, add variable amounts

of benzidine into the reaction system with a trace injector at the lowest point of the oscillating cycle, respectively to perform the perturbation experiments.

**Cyclic voltammetry:** In order to understand the mechanism of the inhibitory effect of benzidine on the B-Z reaction, cyclic voltammetry was applied to monitor which species reacted with benzidine.

### RESULTS AND DISCUSSION

For a chemical reaction to be the source of auto-oscillating system, it must be far from equilibrium. That is to say, oscillating chemical reactions have their own oscillatory domain. Generally, in the BZ oscillating reaction the concentrations of the potassium bromate and the malonic acid have primary effects on the oscillatory behavior (Fig. 1).

BZ oscillating chemical system exhibits periodic change in the concentration of Ce (IV) that reflect in cyclic color or potential change. The color of reaction solution was yellow at the maximum of cycle and it would be disappeared at the minimum of cycle. The Ce (IV)-catalyzed oxidation of malonic acid by bromate ion in sulfuric acid medium involves successive oscillations in the concentrations of oxidized and reduced form of Ce (IV) and Ce (III), which are responsible to the color change observed. Because the analyte can interact with some component in the BZ oscillating system, for example, benzidine can cause the change in the oscillation amplitude to perturb the steady state, the changes are linearly proportional to the analyte concentration.

When a amount of benzidine was added to the BZ system, the first amplitude of oscillation (A) decreases apparently. Figure 2A and B shows typical oscillation profile for the BZ oscillating chemical system in the absence and presence of variable amounts of benzidine perturbation. When the concentration of benzidine was in the range of  $3.5 \times 10^{-9} - 1 \times 10^{-4}$  M,  $\Delta A$  ( $\Delta A$  = the amplitude of the injection cycle - the initial amplitude) is linearly proportional to the benzidine concentration. This is the reason that BZ oscillating chemical reaction can be used to analytical determination.

**Effect of variables on the oscillating system:** Generally speaking, BZ oscillating system is highly vulnerable to external perturbations such as temperature change and the variation of the reaction components in the medium.

The effect of experimental variables on the proposed oscillating reaction was studied in order to establish the optimal working conditions for determining the benzidine. The influence of these variables was carried out with three

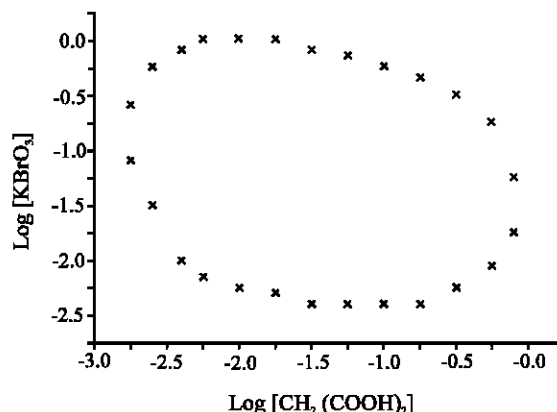


Fig. 1: The oscillatory domain of existence of oscillations for the BZ oscillating chemical reaction in the coordinates of the concentration of potassium bromate and malonic acid. Conditions: 0.04 M Ce (IV) and 0.8 M H<sub>2</sub>SO<sub>4</sub>

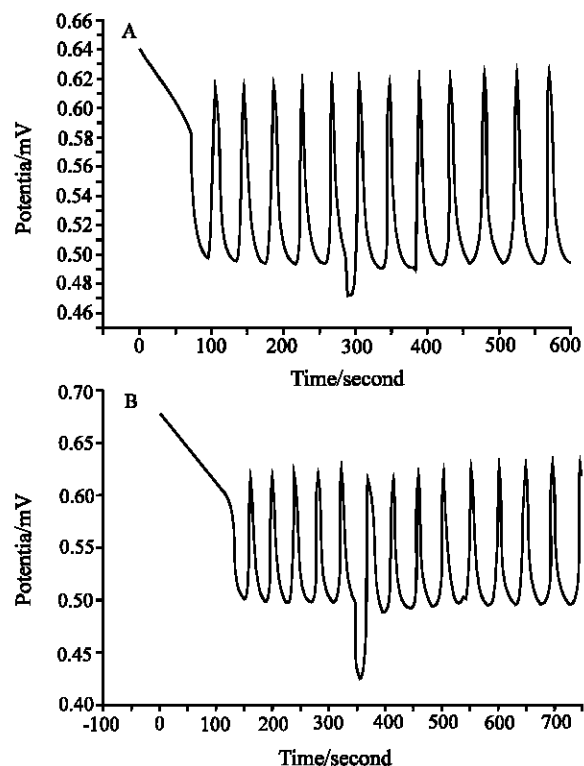


Fig. 2: Typical oscillation profile for the proposed oscillating system obtained in the absence and presence of variable amounts of benzidine perturbation. (A)  $6.5 \times 10^{-7}$  M benzidine; (B)  $1.4 \times 10^{-5}$  M benzidine. Common conditions: 0.04 M Ce (IV), 0.2 M KBrO<sub>3</sub>, 0.5 M CH<sub>2</sub>(COOH)<sub>2</sub> and 0.8 M H<sub>2</sub>SO<sub>4</sub>

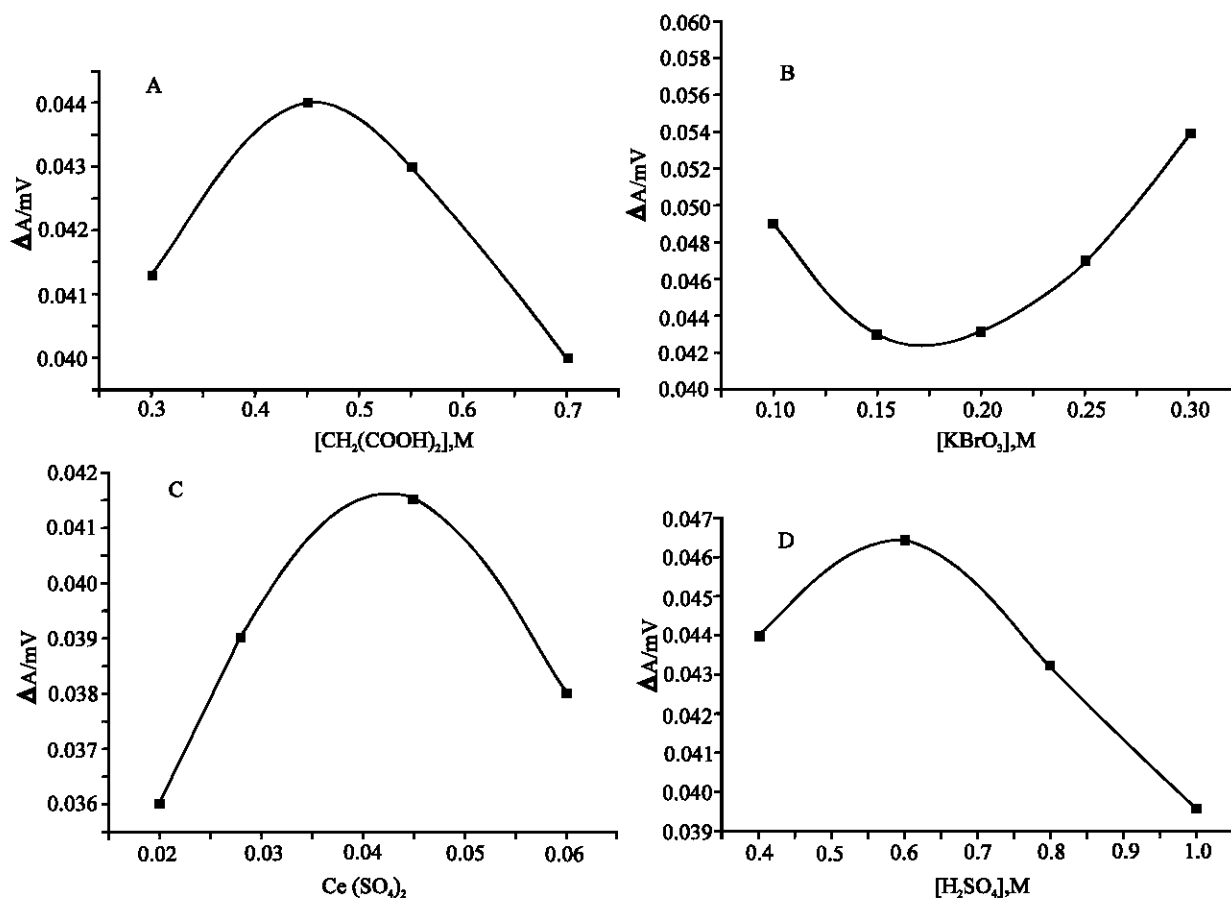


Fig. 3: Influence of the concentration of (A) malonic acid, (B) potassium bromate, (C) cerium(IV) and (D) sulfuric acid on the as benzidine-perturbed oscillating system. Conditions: (A)  $6.5 \times 10^{-7}$  M benzidine, 0.2 M KBrO<sub>3</sub>, 0.04 M Ce (IV), 0.8 M H<sub>2</sub>SO<sub>4</sub>; (B)  $6.5 \times 10^{-7}$  M benzidine, 0.5 M CH<sub>2</sub> (COOH)<sub>2</sub>, 0.04 M Ce (IV), 0.8 M H<sub>2</sub>SO<sub>4</sub>; (C)  $6.5 \times 10^{-7}$  M benzidine, 0.2 M KBrO<sub>3</sub>, 0.5 M CH<sub>2</sub> (COOH)<sub>2</sub>, 0.8 M H<sub>2</sub>SO<sub>4</sub>; (D)  $6.5 \times 10^{-7}$  M benzidine, 0.2 M KBrO<sub>3</sub>, 0.5 M CH<sub>2</sub> (COOH)<sub>2</sub>, 0.04 M Ce (IV)

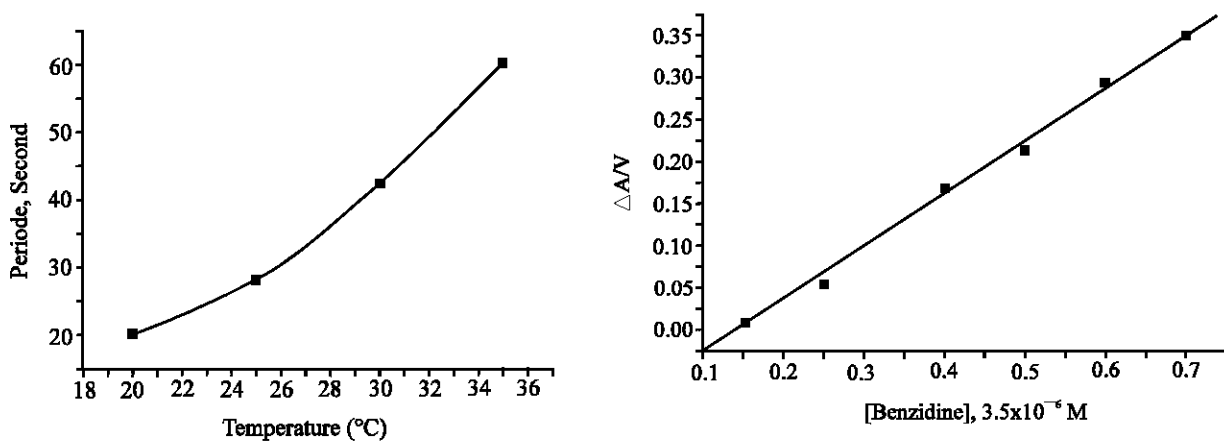


Fig. 4: Effect of temperature on the BZ reaction, Common conditions: 0.04 M Ce (IV), 0.2 M KBrO<sub>3</sub>, 0.5 M CH<sub>2</sub> (COOH)<sub>2</sub> and 0.8 M H<sub>2</sub>SO<sub>4</sub>

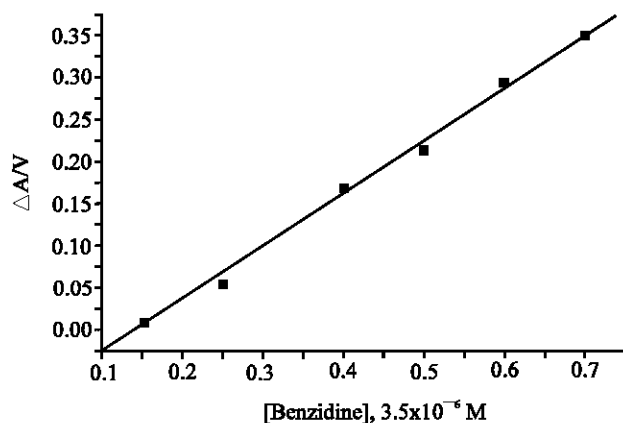


Fig. 5: Calibration curve of the decrease in amplitude vs variable amounts of benzidine. (For other experimental conditions, see under Experimental section)

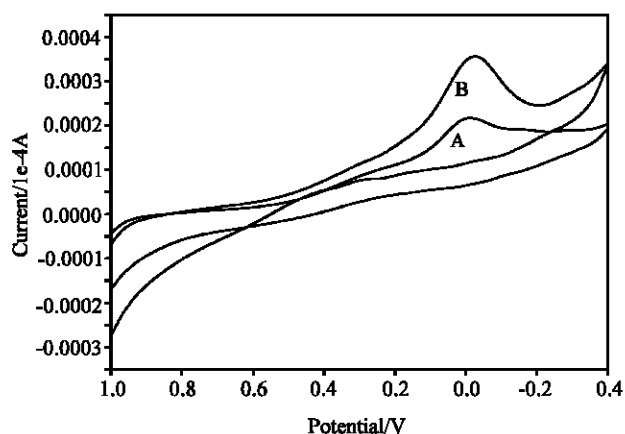


Fig. 6: Cyclic voltammograms of Ce (IV) at concentrations of benzidine of (A) 0, (B)  $6.5 \times 10^{-7}$  M. Common conditions: Temperature, 30°C; Scan rate 0.05 V/s; Sensitivity 1E-5; 0.04 M Ce( $\phi$ ) plus 0.8 M H<sub>2</sub>SO<sub>4</sub>

Table 1: Influence of foreign ions and species on the determination of benzidine

| Ions and species   | Tolerated ratio |
|--|-----------------|
| NO <sub>3</sub> <sup>-</sup> , PO <sub>4</sub> <sup>3-</sup> , NO <sub>2</sub> <sup>-</sup> , CO <sub>3</sub> <sup>2-</sup> , S <sup>2-</sup> , SCN <sup>-</sup> , SO <sub>4</sub> <sup>2-</sup> , Br <sup>-</sup> | 800             |
| Ba <sup>2+</sup> , Al <sup>3+</sup> , Co <sup>2+</sup> , Cu <sup>2+</sup> , Zn <sup>2+</sup> , Ni <sup>2+</sup>  | 500             |
| Vitamin B <sub>6</sub> , Fe <sup>2+</sup> , Fe <sup>3+</sup>   | 100             |
| F <sup>-</sup> , Cl <sup>-</sup> , I <sup>-</sup>  | 80              |

criteria<sup>[16]</sup>. To obtain a constant and maximum value of  $\Delta A$  in the determination of benzidine, the effect of the concentrations of sulfuric acid, potassium bromate, malonic acid and cerium (IV) were checked.

The effect of malonic acid concentration was studied over the range from 0.3 to 0.7 M. When the concentration of malonic acid was 0.50 M, the maximum response of the benzidine perturbation was obtained (Fig. 3A). The influence of the potassium bromate concentration was investigated over the range 0.1 to 0.3 M. The results show that a higher concentration of potassium bromate caused a larger change of amplitude (Fig. 3B). Because of a compromise between maximum sensitivity (amplitude) and minimum analysis time (period of the oscillating system), the concentration of potassium bromate chosen was 0.2 M. In contrast, the increase in cerium (IV) concentration from 0.02 to 0.06 M, a higher cerium (IV) concentration will cause a shorter induction period before the oscillation begin. A concentration of 0.04 M, which was finally adopted as optimal, was chosen in order to maximize the system response to the perturbation (Fig. 3C). Changes in the sulfuric acid concentration over the range from 0.4 to 1.0 M also had a significant effect on the oscillation amplitude and period. The effect of sulfuric acid concentration in the presence of benzidine was

illustrated in Fig. 3D. A concentration of 0.8 M was finally adopted.

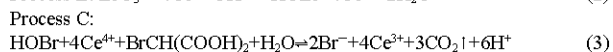
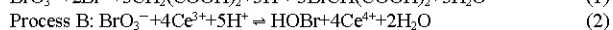
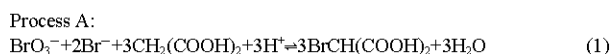
The periodicity in the rate of heat evolution during oscillation in some autocatalytic systems has been described by Körös *et al.*<sup>[17]</sup>. Thus, the influence of temperature was investigated over the range 20-40°C (Fig. 4). Raising the temperature from 20 to 40°C, the oscillation period dramatically decreased whereas the oscillation amplitude remained almost constant. However, the system response to the benzidine perturbation was not altered by the temperature change. A temperature of 30°C was therefore chosen as optimal as it ensured correct, reproducible oscillations.

**Application to the determination of benzidine:** It was known that perturbing the oscillating system by injecting a trace of sample containing a given amount of benzidine caused a change in the oscillation amplitude that was quantitatively related to the analyte concentration in the injected sample. Under the optimal experimental conditions described above, the calibration curve (Fig. 5) over the concentration range from  $3.5 \times 10^{-9}$  to  $1 \times 10^{-4}$  M was obeyed the following linear regression equation:

$$\Delta A = -0.0919 + 0.6293 [\text{benzidine}] \quad r = 0.9977$$

Oscillating systems are very easily altered by the presence of foreign species or ions in the reaction medium. Consequently, the effect of some possible interferences from species and ions were investigated. For this purpose, the oscillating system was perturbed with a sample containing a fixed amount of benzidine and variable amounts of interferences. Results show that the proposed method is acceptably selective. Commonly, the reducing character of the species and ions often had a strong influence on the extent to which they were tolerated the more reductant, the more interfering<sup>[18]</sup>.

**The possible mechanism:** The mechanism for the BZ reaction has been investigated extensively. The famous FKN mechanism<sup>[19]</sup> proposed by three authors was generally accepted. It describes two major processes (Process A and Process B), which alternatively control the BZ reaction and result in oscillations in the concentration of the intermediate species. The third process C is a link between Process A and Process B. The overall reactions are simplified below,



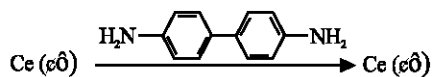
The key of mechanism is autocatalytic and a single-electron oxidation carried out by  $\text{BrO}_2^-$  in Process B. The autocatalytic species is  $\text{HBrO}_2$ . The process can be inhibited by any species that are able to compete  $\text{HBrO}_2$  with the chain-branching sequence. The concentration of  $\text{Br}^-$  in the BZ system plays an important role<sup>[20]</sup> and it determines whether Process A or Process B was in control at a particular time. Process B is suppressed when  $[\text{Br}^-]$  is too high as the following reaction will be starting,



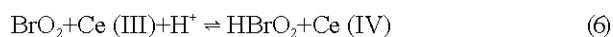
This reaction removes  $\text{HBrO}_2$  before it can enter the chain-branching sequence. The net effect of Process A, however, is to remove  $\text{Br}^-$  from the system. Autocatalytic oxidation by Process B begins when Process A has drives  $[\text{Br}^-]$  to such a sufficiently low value that Reaction (4) can no longer compete successfully for  $\text{HBrO}_2$ . The onset of Process B is accompanied by a rapid production of Ce (IV), which becomes a reactant in Process C to regenerate  $\text{Br}^-$ . Beyond supplying  $\text{Br}^-$  to suppress Process B, Process C also resets Process B by reducing Ce (IV) back to Ce (III). The reaction continues until the concentration of one of the reactants falls below the level necessary to sustain the cycle.

To clarify which species in the BZ system reacted with benzidine, the cyclic voltammograms of benzidine were recorded in the following media: I:  $\text{H}_2\text{SO}_4 + \text{Ce(IV)} + \text{benzidine}$ ; II:  $\text{H}_2\text{SO}_4 + \text{KBrO}_3 + \text{benzidine}$ ; III:  $\text{H}_2\text{SO}_4 + \text{CH}_2(\text{COOH})_2 + \text{benzidine}$ . The results indicate that only Ce (IV) can react with benzidine (Fig. 6).

The possible mechanism is that when benzidine is introduced into the system, it can be oxidized by Ce (IV) immediately, so that the concentration of Ce (IV) in the system decreases and that of Ce (III) increases, so the value of in  $([\text{Ce(IV)}] / [\text{Ce(III)}])$  decreases sharply and the potentiometric oscillation decreases apparently. The reaction scheme can be described below,



As the concentration of Ce(III) increases, it can react with free radical  $\text{BrO}_2^\bullet$ ,



The Reaction 6 is performed very quickly and the Ce (IV) regenerates in no time. So the system can reconvert regular oscillation state as initial. This is consistent with variation of  $[\text{Br}^-]$  over time.

## ACKNOWLEDGMENTS

This work was sponsored by the Project of Excellent Teachers Foundation of Education Ministry, China (3076); the Project of International Cooperation between China and Ukraine (043-05) and the Project of KJCXGC-01, NWNNU, China.

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