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# MICROCALORIMETRIC INVESTIGATION OF THE GROWTH OF THE *ESCHERICHIA COLI* DH5α IN DIFFERENT ANTIBIOTICS

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The effects of *Amoxicillin Sodium* and *Cefuroxime Sodium* on the growth of *E. coli* DH5α were investigated by microcalorimetry. The metabolic power–time curves of *E. coli* DH5α growth were determined by using a TAM air isothermal microcalorimeter at 37°C. By evaluation of the obtained parameters, such as growth rate constants ($k$), inhibitory ratio ($I$), the maximum heat power ($P_m$) and the time of the maximum heat power ( $t_m$), one found that the inhibitory activity of *Amoxicillin Sodium vs*. *E. coli* DH5α is enhanced with the increasing of the *Amoxicillin Sodium* concentration, and the *Cefuroxime Sodium* has a stimulatory effect on the *E. coli* DH5α growth when the concentration is about 1 μg $mL^{-1}$. The $IC_{50}$ for the *Amoxicillin Sodium* and the *Cefuroxime Sodium* are 1.6 and 2.0 μg $mL^{-1}$, respectively, it implicates that the *E. coli* DH5α is more sensitive to *Amoxicillin Sodium* than *Cefuroxime Sodium*.

***Keywords:*** *Amoxicillin Sodium, Cefuroxime Sodium, E. coli DH5α, inhibition, microcalorimetry*

## Introduction

Antibiotics are the most important bioactive and chemotherapeutic compounds made by microbiological synthesis. It has been proved significance of antibiotics in various fields like medicinal chemistry, agriculture and food industry. The antibiotics have been classified into many groups such as penicillins and cephalosporins according to their structures. Each group of antibiotics has different antimicrobial activities [1].

*Amoxicillin Sodium* is a semi-synthetic penicillin widely used in clinical chemotherapy because of its broad spectrum of antimicrobial activity and low toxicity. It has activity against both gram-positive and gram-negative bacteria [2, 3]. *Cefuroxime Sodium* is the second-generation cephalosporins which has broader spectrums of activity *vs.* gram negative coverage. It can eliminate bacteria that cause many kinds of infections, including lung, skin, bone, joint, stomach, blood and urinary tract infections. Both drugs have been listed in the current Pharmacopoeias such as European Pharmacopoeia and British Pharmacopoeia.

Microcalorimetry is a simple and straight-forward method for the study of microorganisms, as it permits the online tests of bioactivity screening and can obtain a lot of important information which can not be observed by other methods [4–11]. The study on microbial growth can provide a continuous measurement of heat production and supply the power–time curves which can describe the growth process without disturb the normal activity of the bio-system [12–14]. Therefore, it has been extensively used in the study of the interactions between drugs and cells based on the abundant thermodynamic and kinetic information [15–21].

In the present study the effect of *Amoxicillin Sodium* and *Cefuroxime Sodium* on the growth of *E. coli* DH5α has been explored. The relationship between antibiotic activity and microbe has been studied by microcalorimetry. Results can provide useful information for the application of antibiotics in clinical treatment.

## Experimental

### *Materials*

The *E. coli* DH5α used for the present study was supplied by Biomass Conversion Technology Group, Dalian Institute of Chemical Physics, CAS, Dalian 116023, P.R. China. The strain of *E. coli* DH5α was stored in 10% glycerol solution at –20°C and cultivated at 37°C with Luria–Bertani (LB) culture medium.

The LB culture medium (pH=7.0–7.2) consists of 1% tryptone, 0.5% yeast extract powder and

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1% NaCl. For the preparation of experiment, it was sterilized in high pressure steam for 20 min at 121°C.

The *Amoxicillin Sodium* and *Cefuroxime Sodium* sterile powder for injection were kindly donated by the Hunan Institute of Drug Detection, P.R. China.

*Methods*

A twin-type, isothermal microcalorimeter TAM Air (Thermometric AB, Sweden), was used to measure the power–time curves of the metabolism of *E. coli* DH5α. The microcalorimeter was periodically calibrated using an electrical substitution method and the experiments were performed isothermally at 37°C. The power–time signals were recorded in situ by a computer. The full details about the performance and construction of the instrument have been described by Wadsö [8].

The metabolic power–time curves of *E. coli* DH5α were determined using the ampoule method in this study. After the 20 mL reaction ampoules was cleaned and sterilized, 10 mL LB culture medium was put into the ampoules containing antibiotics of different concentrations, and 200 μL *E. coli* DH5α suspension (optical density is about 0.6 at λ=600 nm) were inoculated into each ampoules. Then the ampoules were sealed and put into the microcalorimeter. The metabolic power–time curves of *E. coli* DH5α were determined. The temperature of the calorimeter was controlled at 37°C. The power–time signals were recorded at intervals of 1 min.

## Results and discussion

*Thermokinetics*

The power–time curves of *E. coli* DH5α cell show that the log phase of growth obeys the exponential equation [22]:

$$n_t=n_0\exp(kt) \quad (1)$$

where $t$ is the incubation time, $n_t$ is the cell number at time $t$, $n_0$ is the initial cell number and $k$ is the constant of cell growth rate. If the power output of each cell is denoted as $P_w$, then:

$$n_tP_w=n_0P_w\exp(kt) \quad (2)$$

where $P_0$ and $P_t$ are defined as the power output at time 0 and $t$, respectively. Then Eq. (2) can be rewritten as:

$$P_t=P_0\exp(kt) \quad (3)$$

or

$$\ln P_t=\ln P_0+kt \quad (4)$$

Using the Eq. (4), the rate constant of cell growth $k$ was calculated by analyzing the experimental data of $P_t$ and $t$ obtained from the bacterial growth curves, and its values are shown in Tables 1 and 2.

The antibiotic inhibitory ratio ($I$) is defined as:

$$I=[(k_0-k_c)/k_0]\cdot 100\% \quad (5)$$

where $k_0$ is the rate constant of the control, $k_c$ is the rate constant of *E. coli* DH5α growth inhibited by the antibiotic with a concentration of $C$. When the inhibitory ratio ($I$) is 50%, the corresponding half-inhibitory concentration of the antibiotics is represented as $IC_{50}$. It can be regarded as the inhibiting concentration causing a 50% decrease of the *E. coli* DH5α growth rate constant. The analysis results are also listed in Tables 1 and 2.

*The effect of the Amoxicillin Sodium on E. coli DH5α growth*

The thermokinetic parameters of *E. coli* DH5α growth at various concentrations of *Amoxicillin Sodium* are listed in Table 1. And parts of the power–time curves are exhibited in Fig. 1a.

The values of $k$ in Table 1 illustrate that the inhibition of *Amoxicillin Sodium* on *E. coli* DH5α was clearly dose-dependent. The rate constants decreased gradually with increasing concentration of *Amoxicillin Sodium*. Figure 2 shows the relationship between the $C$ and $k$ which can be expressed as the following equation by using least square method:

$$k=(1.457\text{E-}4)C^2-(7.977\text{E-}4)C+0.001840 \quad R^2=0.99385$$

**Table 1** Parameters of *E. coli* DH5α growth at different concentrations of *Amoxicillin Sodium*

| $C$/μg mL$^{-1}$ | $k$/10$^{-3}$ min$^{-1}$ | $R$ | $P_m$/μW | $t_m$/min | $I$/% | $IC_{50}$/μg mL$^{-1}$ |
|---|---|---|---|---|---|---|
| 0.0 | 1.85 | 0.9997 | 542.7 | 2271 | 0 | |
| 0.4 | 1.56 | 0.9996 | 500.5 | 2391 | 15.7 | |
| 0.5 | 1.44 | 0.9993 | 494.2 | 2420 | 22.2 | |
| 1.0 | 1.19 | 0.9988 | 383.7 | 2445 | 35.7 | 1.6 |
| 1.5 | 0.95 | 0.9997 | 356.0 | 2504 | 48.8 | |
| 2.0 | 0.88 | 0.9990 | 398.1 | 2662 | 52.4 | |
| 2.5 | 0.73 | 0.9979 | 236 | 3650 | 60.4 | |
| 3.1 | 0 | – | – | – | 100 | |

$k$ – growth rate constants, $R$ – correlated coefficients for $k$, $P_m$ – maximum power output, $t_m$ – the time of $P_m$, $I$ – inhibitory ratio

**Table 2** Parameters of *E. coli* DH5α growth at different concentrations of *Cefuroxime Sodium*

| $C$/μg mL$^{-1}$ | $k$/10$^{-3}$ min$^{-1}$ | $R$ | $P_m$/μW | $t_m$/min | $I$/% | IC$_{50}$/μg mL$^{-1}$ |
|---|---|---|---|---|---|---|
| 0.0 | 1.85 | 0.9997 | 542.7 | 2271 | 0 | |
| 0.1 | 0.87 | 0.9995 | 474.6 | 2371 | 52.9 | |
| 0.5 | 1.54 | 0.9996 | 540.2 | 2350 | 16.8 | |
| 1.0 | 2.18 | 0.9995 | 552.9 | 2363 | –17.8 | |
| 1.5 | 1.25 | 0.9996 | 534.7 | 2388 | 32.4 | 2.0 |
| 2.0 | 0.91 | 0.9997 | 461.4 | 2620 | 50.9 | |
| 2.5 | 0.73 | 0.9996 | 396.7 | 3662 | 60.4 | |
| 3.0 | 1.63 | 0.9982 | 540.8 | 3996 | 11.9 | |
| 3.5 | 1.75 | 0.9997 | 690.7 | 4009 | 5.4 | |
| 4.1 | 0 | – | – | – | 100 | |

When the concentration of *Amoxicillin Sodium* reached 3.1 μg mL$^{-1}$, the growth of *E. coli* DH5α was completely inhibited during the measurement time, and the inhibitory ratio ($I$) is 100%.

By analyzing the Table 1 and Fig. 3, one can see that the maximum power output $P_m$ of growth phase decreased with the increasing of the *Amoxicillin Sodium*'s concentration. The relationship between $P_m$ and $C$ is nearly linear which is as follows:

$$P_m = -106.6C + 536.7 \quad R = -0.92297$$

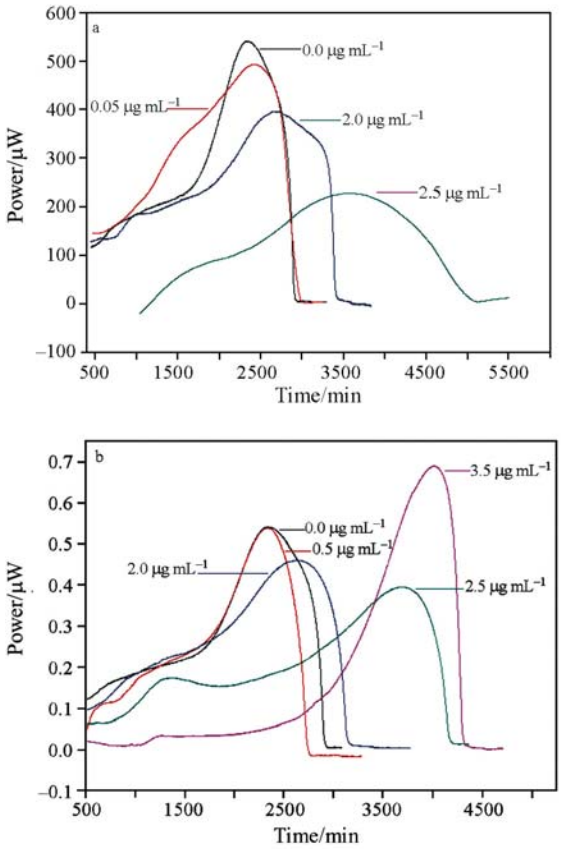


**Fig. 1** Power–time curves of *E. coli* DH5α growth at various concentrations of a – *Amoxicillin Sodium* and b – *Cefuroxime Sodium*

Usually, the time ($t_m$) corresponding to maximal power output $P_m$ for growth phase is delayed with the increasing of concentration of *Amoxicillin Sodium*. In the concentration range of 0.4–2.0 μg mL$^{-1}$ of the *Amoxicillin Sodium*, the increase of $t_m$ is very gently. When the concentration reaches 2.5 μg mL$^{-1}$, the $t_m$ increases drastically as shown in Fig. 4. It demonstrates that the inhibitory activity of *Amoxicillin So-*

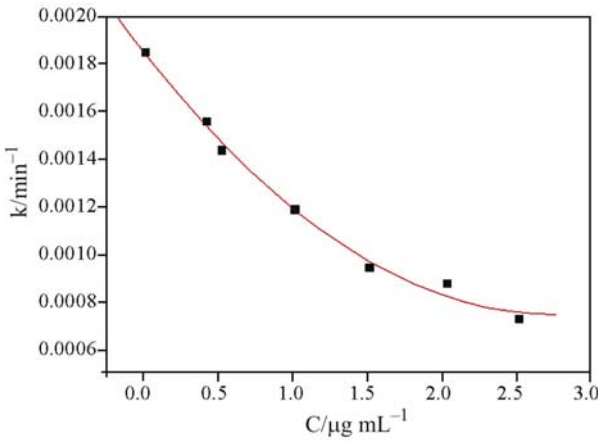


**Fig. 2** Relationship between $k$ and $C$ for *Amoxicillin Sodium*

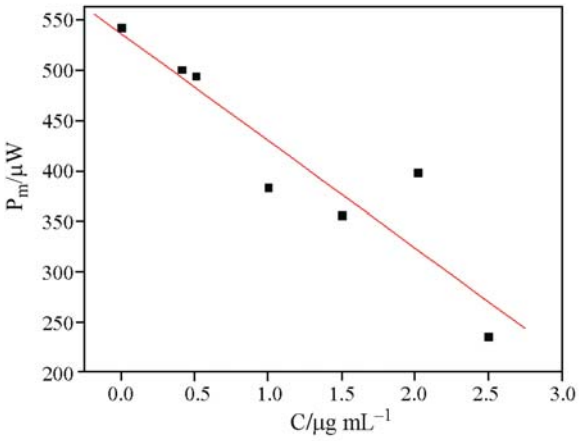


**Fig. 3** Relationship between $P_m$ and $C$ for *Amoxicillin Sodium*

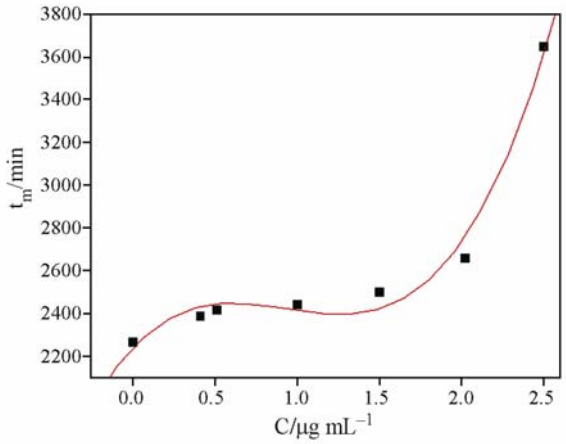


**Fig. 4** Relationship between $t_m$ and $C$ for *Amoxicillin Sodium*

*dium vs. E. coli* DH5α enhances with the increasing of the concentration of *Amoxicillin Sodium*, especially when the concentration is above 2.5 μg mL$^{-1}$. The relationship between $t_m$ and $C$ is as follows:

$$t_m=357.8C^3-1003C^2+821.9C+2242 \quad R^2=0.98432$$

*The effect of the Cefuroxime Sodium on E. coli DH5α growth*

The power–time curves of *E. coli* DH5α growth under the action of *Cefuroxime Sodium* at different concentrations are displayed in Fig. 1b. The power–time curves show that the shapes of the metabolic power–time curves changes slightly when the *Cefuroxime Sodium* is at low concentration. But when high concentration of *Cefuroxime Sodium* was added, the shapes changed markedly, and there are two peaks on the metabolic power–time curves when its concentration is above 2.5 μg mL$^{-1}$.

From data in Table 2 and Fig. 5, one can see that the metabolism of *E. coli* DH5α showed an interesting behavior for *Cefuroxime Sodium*. At concentrations of *Cefuroxime Sodium* below 1.0 μg mL$^{-1}$, the *E. coli* DH5α growth was inhibited. But when the concentration of *Cefuroxime Sodium* reached 1.0 μg mL$^{-1}$, the growth of *E. coli* DH5α was facilitated, the rate constant $k$ became a high value than that of control, and the inhibitory ratio was –17.8%. With the concentration increasing further, the rate constant $k$ became smaller originally and then increased again, but it still showed inhibition. The phenomenon may attribute to this reason: at the beginning of the experiment, the growth of the *E. coli* DH5α was depressed by *Cefuroxime Sodium*, then it may adjust itself and adopt the environment with the lapse of time. It is proved that the *Cefuroxime Sodium* has inhibitory effect but not bactericidal effect on the *E. coli* DH5α under this concentration. When 4.1 μg mL$^{-1}$ of *Cefuroxime Sodium*

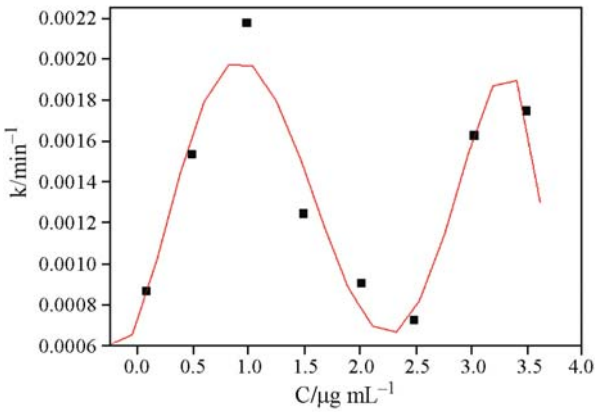


**Fig. 5** Plot of $k$ *vs.* $C$ for *Cefuroxime Sodium*

concentration is reached, the growth of *E. coli* DH5α was completely inhibited during the measurement time. The $k$–C equations can be described as below:

$$k=(-2.287\text{E-}4)C^5+0.00182C^4-0.00447C^3+$$
$$+0.00284C^2+0.00133C+(6.931\text{E-}4)$$
$$R^2=0.93547\ (0.1\text{–}3.5\ \mu g\ mL^{-1})$$

From Figs 5 and 6, one can see that the tendency of change of $P_m$, with the increasing of *Cefuroxime Sodium* concentration, is similar to that of $k$. During 0.1–1.0 μg mL$^{-1}$ for *Cefuroxime Sodium*, the $P_m$ increased with the increasing of $C$, but when the concentration was in the range of 1.5–2.5 μg mL$^{-1}$, the $P_m$ decreased with the increasing of $C$, when the concentration increasing further, the $P_m$ increased drastically. The relationship between $P_m$ and $C$ was established as follows:

$$P_m=1.373C^4+52.43C^3-278.2C^2+345.7C+438.0$$
$$R^2=0.93536$$

The relationship between the $t_m$ and $C$ is shown in Fig. 7. It is seen that the $t_m$ increased slowly in the range of 0.0–1.5 μg mL$^{-1}$. When the concentration of *Cefuroxime Sodium* is beyond 1.5 μg mL$^{-1}$, the $t_m$ was sharply increased.

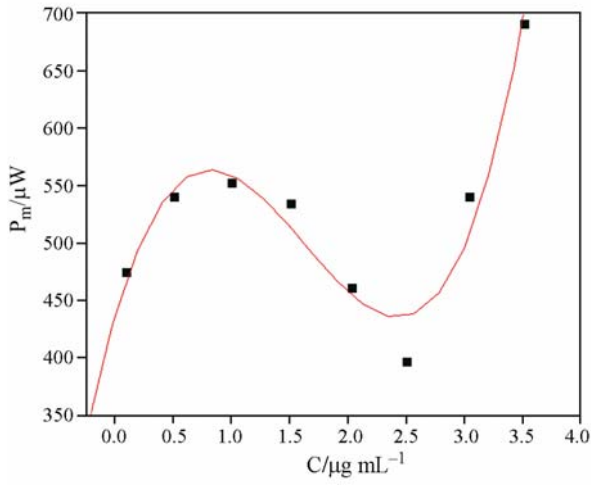


**Fig. 6** Plot of $P_m$ *vs.* $C$ for *Cefuroxime Sodium*

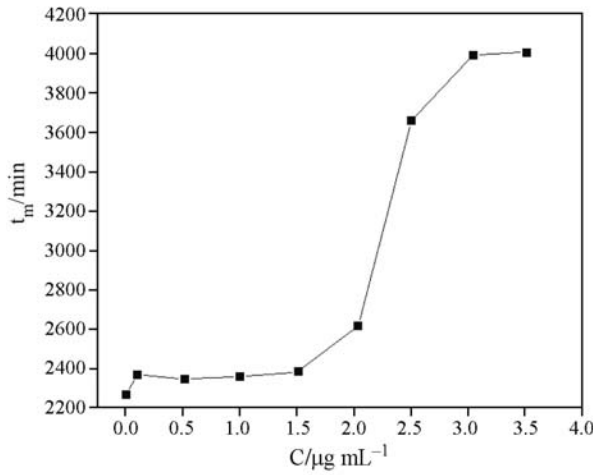


**Fig. 7** Plot of $t_m$ *vs. C* for *Cefuroxime Sodium*

## Conclusions

This study demonstrated the use of microcalorimetric method online tests of bioactivity screening. The effects of *Amoxicillin Sodium* and *Cefuroxime Sodium* on the growth of *E. coli* DH5α have been interpreted in metabolic power–time curves, and they both have inhibition on the growth of *E. coli* DH5α. When the concentration of the *Amoxicillin Sodium* and *Cefuroxime Sodium* reached 3.1 and 4.1 μg mL$^{-1}$, respectively, the growth of *E. coli* DH5α could be completely inhibited during the detection time. Moreover, comparing the $IC_{50}$ of *Amoxicillin Sodium* and *Cefuroxime Sodium* (1.6 μg mL$^{-1}$ for *Amoxicillin Sodium*, 2.0 μg mL$^{-1}$ for *Cefuroxime Sodium*), one can draw the conclusion that the *E. coli* DH5α is more sensitive to *Amoxicillin Sodium*.

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