Solubilization Effects of Tinidazole by β-Cyclodextrin

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Abstract Tinidazole was included in β-cyclodextrin (β-CD) using the spray-drying and heating methods. The larger number of molecules of tinidazole included in β-CD was obtained from the initial molecular ratio 1:3 with both the two methods. More evidence of a complex formation between tinidazole and β-CD was obtained from the analysis of scanning electron microscope, X-Ray diffraction and the solubility study. The calculated apparent stability constant was 0.1925 $(mol/L)^{-1}$ at 25℃ and 0.1089 $(mol/L)^{-1}$ at 37℃. The dissolution rate of tinidazole was enhanced. After 5 minutes in 500 ml of distilled water at 37℃, it was more than 90% for the inclusion products but reached only 80% for the pure product after 60 minutes. The complexation increased the tinidazole solubility, decreased its crystallinity and improved its wettability in water.

Key words tinidazole; β-Cyclodextrin; solubility; stability constant

Complexation of the drug with cyclodextrins (CDs) may increase its water solubility and consequently improve the bioavailability. A great number of cyclodextrin (CD) derivatives have been synthesized from natural CDs[1], but most of them have no use in the pharmaceuticals or food because of their toxicity and/or their cost. β-cyclodextrin (β-CD) is the most commonly used CD. It is the less soluble, less expensive and commercially available from a number of sources[2]. Many researches reported the possibility of obtaining inclusion complex with β-CD. In this work, the possibility of including tinidazole in β-CD molecule was investigated.

Tinidazole is a derivative of 5-nitroimidazole, which has strong antimicrobial actions. It is used efficiently in the treatment of various infections such as trichomonas vaginalis[3] mainly by oral administration or venous injection, which often lead to the gastro-intestinal or systemic side actions. About 180 million of women worldwide are infected with trichomonas vaginalis every year[4].

Tinidazole is slightly soluble in water, darkens on exposure to light and has an unpleasant bitter taste. If tinidazole can be included into the CD cavity, some of these drawbacks may be altered. This alteration may lead to suitable formulations such as vaginal topical gel in which tinidazole may dissolve better and faster, and has a better efficiency in the therapy of trichomonas vaginalis. Furthermore, the stability to light can be expected. Not much literature and work on tinidazole in this area have been reported.

The spray-drying and the heating processes were used to obtain the complexes of tinidazole and β-CD.

The inclusion formation and its physical characteristics were evaluated by using scanning electron microscope, X-ray diffraction and solubility analysis. The dissolution behavior of the solid complexes in water was also determined.

1 MATERIALS AND METHODS

1.1 *Materials*

° Tinidazole with a purity of 99.5% for venous injection used was purchased from Beijing Third Pharmaceutical Factory (China) and used without any preliminary treatment.

° β-CD was purchased from Tianjin Huabei (Special Region) Chemical Reagent Development Centre (China).

° The solvents employed were of analytical

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grade.

1.2 *Methods*

1.2.1 *Tinidazole assay* From the scanning ultraviolet spectroscopy analysis (Perkin Elmer UV/MS spectrophotometer Lambda 2), the maximun absorption of tinidazole in distilled water was at wavelength of 317 nm, while β-CD had no UV absorption in this wavelength. The tinidazole assay was carried out by ultraviolet absorption analysis [5] using a UV 755B UV-VIS spectrophotometer (China). The Standard calibration curve equation obtained from the dilution of tinidazole in the concentration range of 6μg/ml to 22μg/ml was $Y = 0.0359X + 0.0058$, $r = 0.9999$ ($n = 5$).

1.2.2 *The determination of tinidazole phase solubility* The phase solubility determination of tinidazole in β-CD solution was carried out according to the method of Higuchi and Connors[6]. Excess amounts of tinidazole were added to aqueous solutions containing different concentrations of β-CD and stirred in water bath at 25±0.5℃ and 37±0.5℃ for 5 days. After equilibrium, an aliquot was filtered with a 0.45μm millipore filter. Then, the filtered solutions were analyzed spectrophotometrically to define the solubility characteristics of tinidazole.

1.2.3 *Preparation of physical mixture* The physical mixture was prepared by a simple blending of tinidazole and β-CD at 1∶1, 1∶2, and 1∶3 molar ratios at room temperature. These mixtures were compared with the corresponding solid complex powders.

1.2.4 *Preparation of inclusion complex*

1.2.4.1 *Spray-drying method* Tinidazole was dissolved in acetone. One, double or three times molar quantities of β-CD were dissolved at 70℃ in the lowest volume of water necessary to obtain a solution then cooled to 40℃. The acetone solution of tinidazole was added to the solution of β-CD and maintained under stirring for 30 minutes at 40℃. The proportion of acetone to water in the solution was 1∶9. The solutions were spray-dried using a Buchi 190 Mini Spray Dryer (Switzerland) under the following conditions: feed rate 10 ml/min, inlet temperature 95℃, outlet temperature 65℃, pressure 5 bar. The collected powders were dried, then washed with acetone and dried again at 50℃ overnight, stored in a desiccator for 3 days and then analyzed.

1.2.4.2 *Heating method* The physical mixture of tinidazole and β-CD in series proportions was put in a glass respectively, which was heated at a fixed temperature of 145℃ in the oven for about 6 hours. At interval times the glass was shaken to mix the powder and was then replaced into the oven. The content of the vessel was washed with acetone to remove the excess of tinidazole and dried at 50℃ overnight, then analyzed.

1.2.5 *Evidence of inclusion formation in solid phase*

1.2.5.1 *Scanning electron microscope (SEM)* The experiment was carried out using an ISI-SX-40 scanning electron microscope (Japan). The microscopic aspects of the product obtained through the spray-drying and heating methods were compared with those of tinidazole and β-CD individually viewed under the SEM.

1.2.5.2 *X-ray diffraction* Powder X-ray diffractograms were recorded with a Rigaku D/Max-RC diffractometer (Japan) using Ni-filterd Cu Kα radiation detector for the tinidazole, β-CD, physical mixture and the tinidazole inclusion respectively.

1.2.6 *Dissolution study*

Dissolution study was performed using an Intelligent Dissolution Tester ZRS-4 apparatus (Tianjin University Radio Factory, China), paddle method, and 500 ml of distilled water maintained at 37℃ with a stirring speed of 50 r/min. An amount of tinidazole alone, an amount of physical mixture 1∶3 and an amount of complexes 1∶3, each powder containing 350 mg of tinidazole, were dispersed on the surface of the medium.

After 5, 10, 20, 30, 40, 50, 60 minutes 2 ml of samples was passed through a 0.45μm millipore membrane filter and assayed spectrophotometrically. The initial water volume of the vessel was maintained by adding 2 ml of distilled water after each sampling. The results were the

mean of 5 determinations.

2 RESULTS AND DISCUSSIONS

2.1 *Phase solubility diagrams*

Higuchi and Connors[6] divided solubility diagrams into two major types: type A and type B phase solubility diagrams. In type A phase diagram the complex formed is soluble and does not form a precipitate regardless of the amount of ligand added. In type B, the inclusion formed has a definite and limited solubility.

Type A can be further subdivided into A_L, A_P and A_N types. There are also various possibilities in type B: B_s and B_t. Figure 1 shows the phase solubility diagrams of the tinidazole-β-CD system. The solubility of tinidazole increased slightly with the increasing of β-CD concentration and reached a plateau region, which appeared at higher concentration of β-CD.

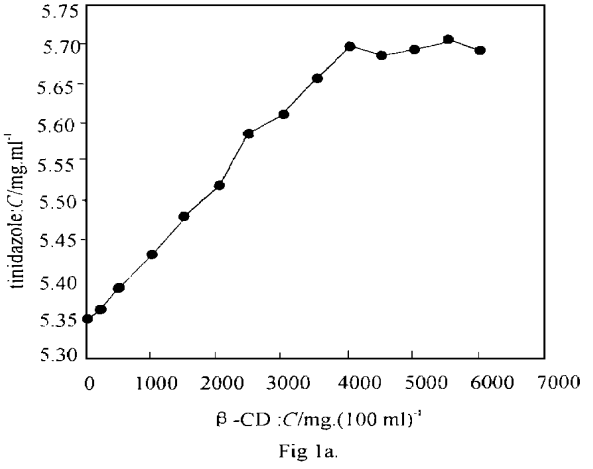

Fig 1a.

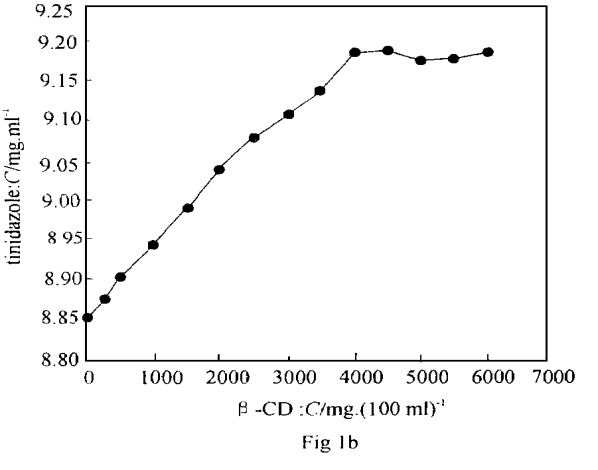

Fig 1b

Fig 1. Phase solubility diagrams of tinidazole-β-CD in distilled water: Fig 1a at 25℃ and Fig 1b at 37℃

The appearance of the solubility curve can be classified as A_N type solubility diagram. The phase diagram suggested that the molar ratio of complex in the solid state, theoretically, can not be completely explained in terms of a stoichiometric relationship[7]. The spray dried and heated powders, after being washed with acetone and dried, were quantitatively analyzed by ultraviolet absorption. It was found that about 0.85 mole of tinidazole from the initial molecular ratio 1∶3 (tinidazole-β-CD) was included using the heating process and about 0.92 mole from the initial molecular ratio 1∶3 (tinidazole-β-CD) was included using the spray-dried method. Experimentally the larger number of molecules of tinidazole included in β-CD was obtained from the initial molecular ratio 1∶3 with both spray-drying method and heating method. The heating method proved to be the most advantageous as an easy and simple operating process and the stability of tinidazole at higher conditions of heating, will be reported in another paper, was not affected.

2.2 *Crystalline properties of tinidazole inclusion in solid phase*

Previous studies reported that molecular encapsulation with β-CD may occur both in solution and in solid state[8~10]. In solution there is equilibrium between complexed and non-complexed guest molecules. In the solid state, guest molecules can be enclosed within the cavity or may be aggregated to the outside of the CD molecule. More evidence of complex formation was obtained by scanning electron microscope, X-ray diffraction and from solubility study.

The analysis, using the scanning electron microscope, revealed that the crystallization of tinidazole appeared in relatively large polyhedral crystal (Fig 2a). β-CD appeared also in large polyhedral crystal (Fig 2b). The products obtained after spray-drying or heating methods appeared substantially different from tinidazole and β-CD (Fig 2c and Fig 2d).

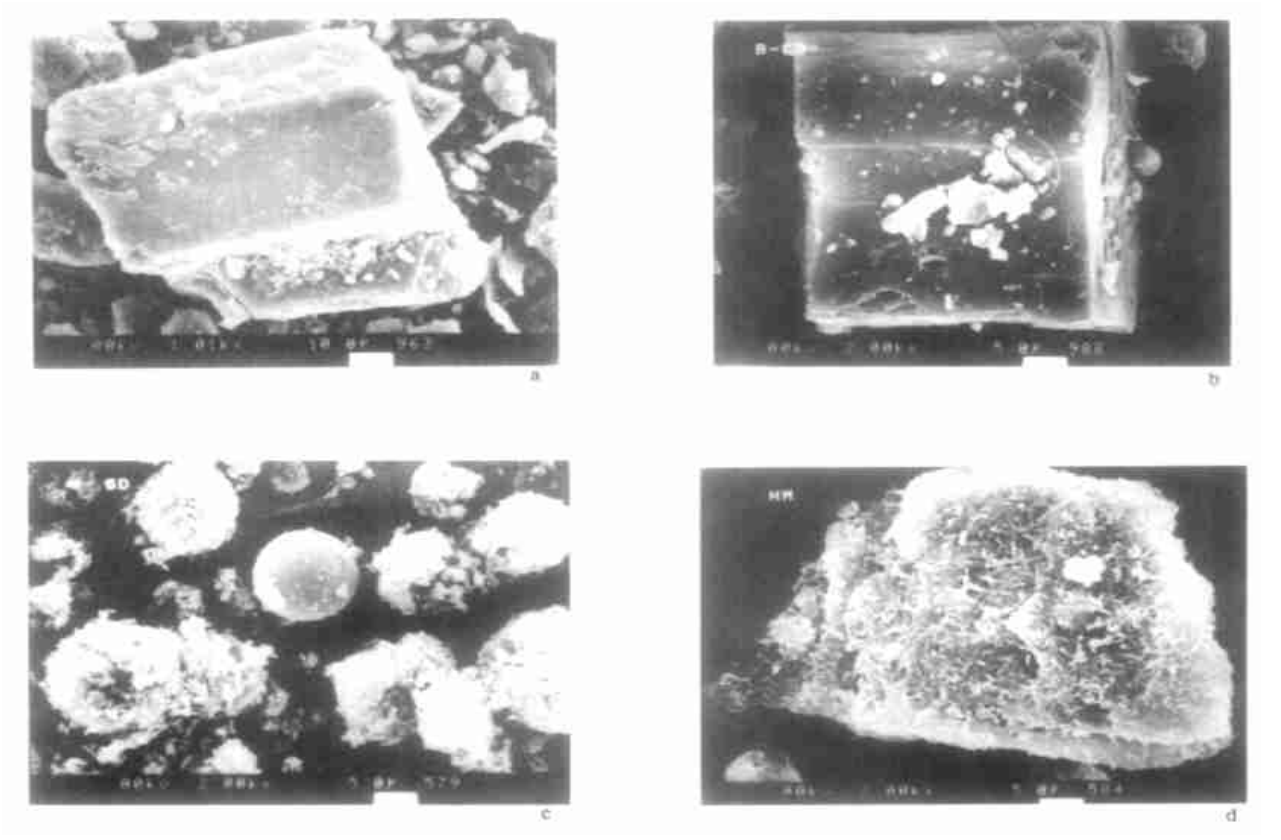

Fig 2. Scanning electron microscope photographs
a. tinidazole, b. β-CD, c. complex tinidazole-β-CD 1∶3 (Spray-drying method), d. complex tinidazole-β-CD 1∶3 (Heating method).

The X-ray diffraction analysis of powder samples is shown in Fig 3. The diffractogram of the physical mixture was characterized by the superposition of individual diffractograms of tinidazole and β-CD with the peaks having lower intensity. The diffractograms of the solid complex were different from those of the physical mixture and the two components, confirmed an interaction between tinidazole and β-CD. The diffractograms revealed less crystallinity in the complex system as evidenced by fewer and broader peaks of lower intensities. This indicated that inclusion complexes are markedly less crystallinity than the physical mixture or the pure components. Powder obtained from the spray-drying method showed more fewer and broader peaks of lower intensities indicating less crystallinity than the powder obtained from the heating method.

2.3 *Apparent stability constant*

A CD inclusion complex is always in equilibrium with its free components in the solution. The equilibrium state of an 1∶1 (host∶guest) system is given by the following scheme:

$$\mathrm{Drug}_{free} + \mathrm{CD}_{free} \overset{K_c}{\rightleftharpoons} \mathrm{Drug}\cdot\mathrm{CD}_{complex}$$

The general definition of the complex stability constant is given by the equation:

$$K_c = \frac{[\mathrm{Drug}\cdot\mathrm{CD}]_{complex}}{[\mathrm{Drug}]_{free}\,[\mathrm{CD}]_{free}}$$

Higuchi et al have proposed the following equation to estimate the apparent stability constant K_c

$$K_c = \frac{(S_t - S_o)}{S_o\{[CD]_t - (S_t - S_o)\}}$$

Considering the phase-solubility curve, the initial linear portion was fitted to a straight line. The intercept gave the aqueous solubility S_o and the slope gave $S_t - S_o$. S_t is the total solution molar concentration of substrate and $[CD]_t$ is the total molar concentration of ligand. Assuming that a 1∶1 complex was formed at the initial stage of solubility diagram, the apparent stability K_c can be obtained by the equation:

$$K_c = \frac{\text{slope}}{\text{intercept}\,(1 - \text{slope})}$$

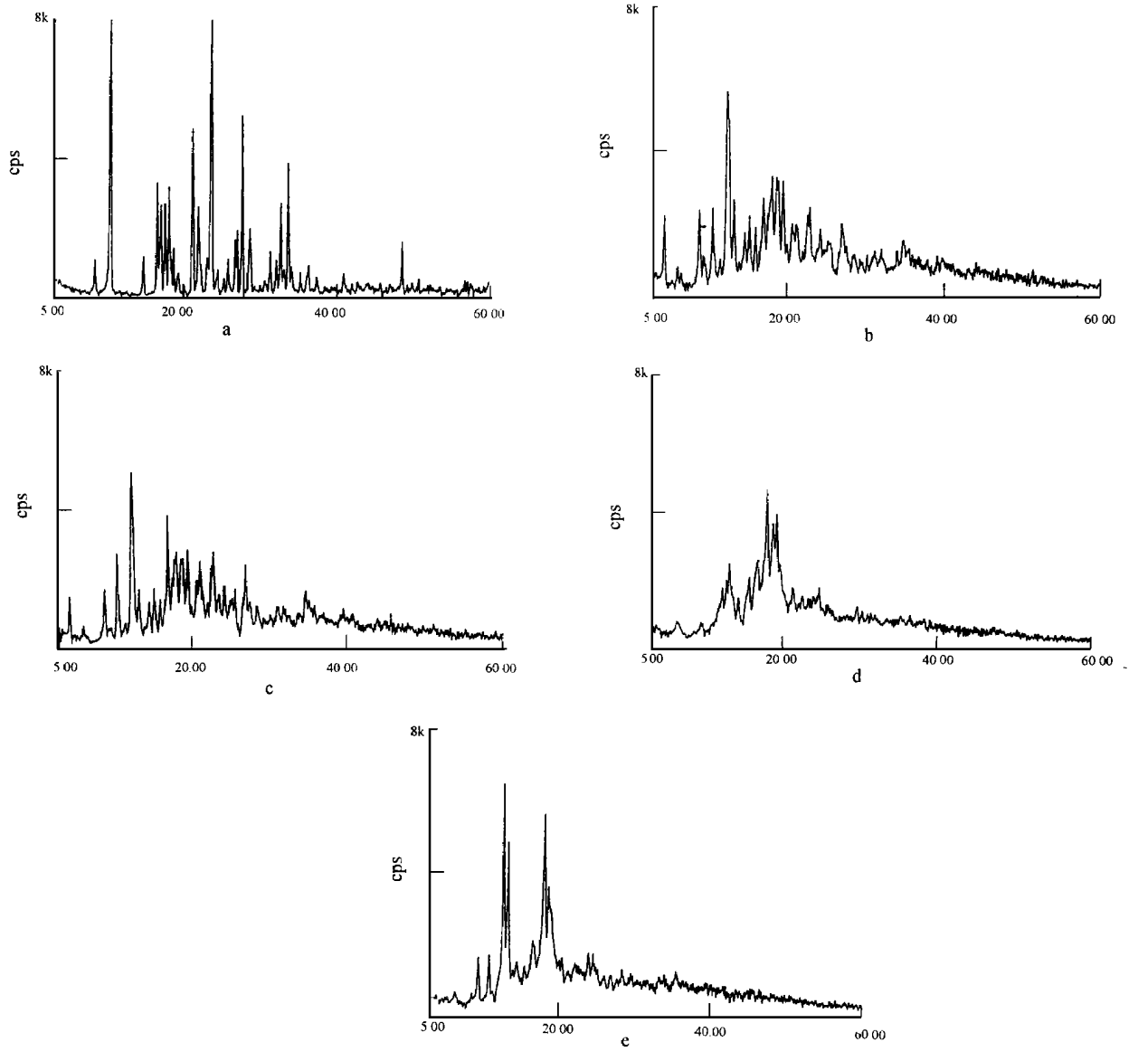

Fig 3. Powder X-ray diffraction patterns

a. tinidazole, b. β-CD, c. physical mixture tinidazole-β-CD 1∶3, d. complex tinidazole-β-CD 1∶3 (Spray-drying method), e. complex tinidazole-β-CD 1∶3 (Heating method).

The apparent stability constant K_c estimated from the concentration solubility data of β-CD versus the concentration of tinidazole (phase solubility diagram) was 0.1925 $(mol/L)^{-1}$ at 25℃ and 0.1089 $(mol/L)^{-1}$ at 37℃. The value of K_c for tinidazole with β-CD at 25℃ was greater than that at 37℃. The apparent stability constant K_c related with the inclusion phenomena interaction between the hydrophobic cavity of CD and the hydrophobic part of the drug. It is well recognized that the drug-CD interactions are not limited to the inclusion phenomena, but also included intermolecular forces such as Van der Walls, hydrogen bonding, electrostatic forces[11,12], which are involved in the complex stability.

2.4 *Dissolution rate*

A compound in the crystalline state dissolves more slowly than that in the amorphous state. The process to prepare inclusion complexes leads to a decrease in crystallinity or even to amorphous powders. This explained the increase of water solubility of tinidazole, as shown in Fig 4.

After 5 minutes the dissolution rate of tinidazole in 500 ml of distilled water at 37℃ was 44%

for the pure product, increased to 53% for the physical mixture, and reached 94% and 95% for the heated and spray dried inclusion complexes. The dissolution rate of pure drug reached 80% after 60 min. The wetting of the solid phase by a solvent is the first step of any dissolution process. The fast dispersion of the inclusion in dissolution media revealed that β-CD complexation of tinidazole improved the wettability in water considerably and the simple addition of β-CD enhanced its wettabilty.

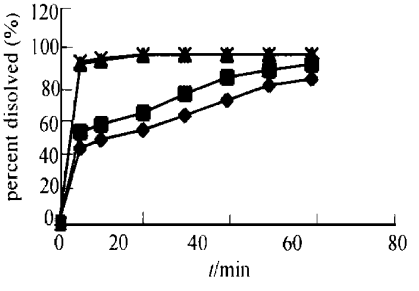

Fig 4. Dissolution profiles

—◆— tinidazole, —■— physical mixture, —×— complex tinidazole-β-CD 1∶3(Spray-drying method) and —▲— complex tinidazole-β-CD 1∶3 (Heating method)

3 Conclusion

The feasibility of preparing an inclusion complex of tinidazole in β-CD occured using spray-drying and heating methods. This complexation increased the tinidazole solubility, decreased its crystallinity and improved its wettabilty in water. These alterations may lead to suitable formulations for potential drugs.

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β-环糊精对替硝唑的溶解度的影响

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摘 要 用喷干法和加热法可制备得到替硝唑的β-环糊精包合物。当初始分子比例为 1∶3时,采用这两种方法可使更多量的替硝唑分子包合到β-环糊精中。通过扫描电子显微镜观察,X光衍射,溶解性等研究,都证明了有包合物形成。经计算,包合物稳定常数 25℃时为 0.1925 $(mol/L)^{-1}$,37℃时为 0.1089 $(mol/L)^{-1}$。包合物的形成提高了替硝唑的溶出度。包合物在 37℃ 500 ml的蒸馏水中 5 min即可溶出 90%以上,而药物本身 60 min溶出只能达到 80%。药物溶出度的增加是由于包合物增大了药物的溶解性,降低了药物的结晶性和提高了它的可湿性。

关键词 替硝唑;β-环糊精;溶解性;稳定常数

本校 1999年 9~12月国际学术交流信息

9月 10日 中药学院博士研究生张庆文赴香港科技大学进行为期六个月的合作研究。

9月 11日 荷兰籍华人张明强博士应我校邀请来校作有关组织化学等方面的系列学术讲座。

10月 10日 中药学院周荣汉教授、宗大颀副研究员应邀赴香港科技大学参加"植物化学分类及中药资源学术研讨会"。

10月 12日 根据我校和日本近畿大学药学部校院交流协议,中药学院王峥涛教授应邀赴近畿大学进行学术访问并作学术报告。

10月 14日 美国卫生实验员 Limit先生借来宁访问之机顺访我校,与我校药理教研室有关教师进行了会谈。

10月 18日 根据我校和日本岐阜药科大学及长崎大学的校际交流协议,李丰文副校长、药学院朱庆振副院长以及张奕华教授赴上述两校进行为期一周的学术访问。

10月 22日 应新西兰 Otago大学药学院的邀请,药学院药剂教研室孙国庆副教授赴该院进行为期五个月的合作研究。

10月 25日 由我校和南京药学会共同举办的"99南京-名古屋药学学术研讨会"在南京古南都饭店举行,会议邀请了日本近畿大学、长崎大学、名城大学、千叶大学、岐阜药科大学等校的来宾50余人参加该会,并在会上作了学术报告。

10月 26日 根据我校和日本长崎大学的校际交流协议,日本长崎大学药学部田中隆助教授以及山本浩文助教授应邀来校访问。

10月 26日 根据我校和日本岐阜药科大学的校际协议,日本岐阜药科大学永井博式教授、永濑元光教授以及足立哲夫助教授应邀来校访问。

10月 28日 应越南河内药科大学的邀请,赵鸣副书记、中药学院封昌邓书记及余国奠教授赴越南进行为期五天的学术访问。

11月 15日 巴西米那斯州科技代表团 D. M. S. andrade先生等一行四人来校访问,与药学院有关教师进行了会谈。

11月 16日 应我校邀请日本长崎大学名誉教授古川淳夫妇来校进行短期学术访问。

11月 18日 由我校主办的"'99中国科技期刊进入国际权威检索系统国际学术研讨会"在南京国际会议中心举行,共有 8个国家的 22位国外代表和 3位驻华商社的代表出席了会议。

11月 25日 药学院平其能院长应邀赴新加坡国立大学进行为期三天的学术访问。

11月 29日 药学院平其能院长应邀赴香港科技大学进行为期四天的学术访问。

12月 8日 本校制药有限公司王强总经理随南京市科委组织的"企业培育及运行机制赴德培训团"赴德国进行为期 21天的培训考察。

(高冬梅)