# A batch procedure for preparation of factor Ⅸ complex concentrates

YU Rong[1]*, LI Xiao-hong[1], QI Hui[1], WU Yang-bin[2], ZENG Rong[1] (1. *West China School of Pharmacy, Sichuan University, Chengdu, 610041, China*; 2. *Ningbo Asia-Pacific Biotechnology Ltd, Ningbo, 315803, China*)

**ABSTRACT: OBJECTIVE** To produce factor Ⅸ(FⅨ) complex concentrates with high purity and low potential thrombogenicity. **METHOD** Fresh-frozen plasma was adsorbed successively by domestic DEAE-Sepharose Fast Flow gel and $Ca_3(PO_4)_2$, and then solvent-detergent (S/D) treatment was used. **RESULTS** The activity recovery rates of the two adsorption processes were (89.94±1.31)%($n$=7) and (82.27±2.18)%($n$=6). The specific activity of the product is (16.28±2.80) IU/mg. The content of the FⅨ was (126.82±11.60) IU/200PE. **CONCLUSION** This method is highly practicable, and it can be used in producing high-quality Factor Ⅸ complex concentrates in low cost.

**KEY WORDS:** Factor Ⅸ complex; concentrates; DEAE-Sepharose Fast Flow; $Ca_3(PO_4)_2$; adsorption

# 批式吸附法制备人凝血因子Ⅸ复合物

余蓉[1]*,李晓红[1],祁辉[1],邬杨斌[2],曾蓉[1](1.四川大学华西药学院,四川 成都 610041;2.宁波亚太生物有限公司,浙江 宁波 315803)

摘要:目的 制备纯度较高,潜在血栓性小的凝血因子Ⅸ(FⅨ)复合物。方法 以人新鲜冰冻血浆为原料,采用SD病毒灭活工艺,经国产DEAE-Sepharose Fast Flow胶批式吸附和$Ca_3(PO_4)_2$吸附制备凝血因子Ⅸ复合物。结果 两步的活性收率分别为(89.94±1.31)%($n$=7),(82.27±2.18)%($n$=6),制品的FⅨ:C比活为(16.28±2.80) IU/mg。FⅨ的量为(126.82±11.60) IU/瓶(200PE)。结论 本工艺实用性较高,可用于制备低成本,高质量的凝血因子Ⅸ复合物。

关键词:人凝血因子Ⅸ复合物;国产DEAE-Sepharose Fast Flow胶;$Ca_3(PO_4)_2$;吸附

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Factor Ⅸ complex concentrates (Prothrombin Complex Concentrates, PCC) has always been used for a replacement therapy in hemophilia B patients. This application was reported in the United States Pharmacopeia, British Pharmacopeia, Europe Pharmacopeia, and Chinese Rules of Biological Products (2000 edition). The main problems, including the virus infection, the potential thrombosis and high cost prevent its clinical use. Therefore, we have established a method, i.e. that two steps of batch adsorption to produce Factor Ⅸ complex concentrates. As a result, the cost is much lower than that of other existing techniques in China. The final recovery of the total potency is over 70%, the quality of the product is greatly improved, and the potential of leading to thrombosis is much lower.

## 1 Materials and methods

### 1.1 Materials

Fresh-frozen human plasma, offered by Xindu Plasma Station, stored at -30℃; domestic DEAE-Sepharose Fast Flow, supplied by Hangzhou Zhengguang colophony LTD; normative plasma, complex concentrates substrate plasma, Factor Ⅸ-deficient plasma, obtained from Chinese Academy of Medical Sciences, Institute of Blood Transfusion; low molecular weight protein, obtained from Shanghai Dongfeng Biotechnology LTD; Comassie Brilliant Blue G-250, Fluka reagent.

### 1.2 Preparations

Preparation Ⅰ, Traditional domestic Factor Ⅸ complex concentrates, prepared by the bettered gel adsorption[1].

Preparation Ⅱ, Domestic Factor Ⅸ complex concentrates, prepared by the domestic DEAE-Sepharose Fast Flow chromatography and $Ca_3(PO_4)_2$ adsorption[2].

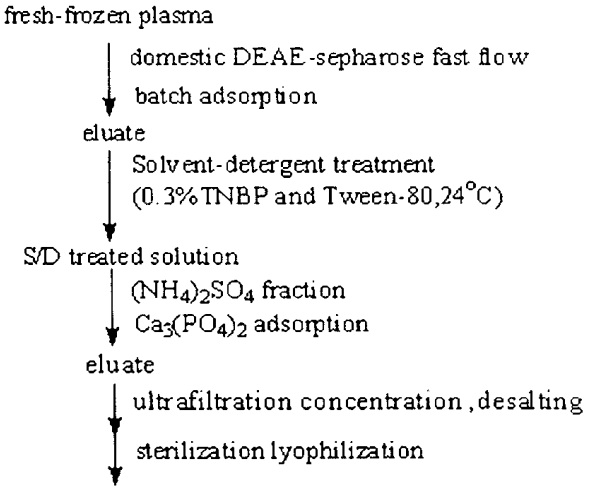
### 1.3 Methods

**1.3.1** Preparation technology: Fresh-frozen plasma was adsorbed by domestic DEAE-Sepharose Fast Flow and $Ca_3(PO_4)_2$ successively, and then solvent-detergent (S/D) was used. The scheme is described as follows:

**1.3.2** Factor Ⅸ complex concentrates: Total potency of factor Ⅱ, Ⅶ, Ⅸ, and Ⅹ was determined by the one-stage clotting

* corresponding author

assay[3].




**1.3.3** Factor Ⅸ was assayed as described in the direction for use of the kit.

**1.3.4** Total protein was determined using the method of Li Juan *et al*[4].

## 2 Results

### 2.1 Preparation of factor Ⅸ complex concentrates

**2.1.1** DEAE-Sepharose Fast Flow adsorption: Many factors, including the ratio of resin volume to plasma volume, time, pH value, and ionic strength that affect adsorption, washing, and elution were optimized. Optimum technology was repeated, with the recovery of the total potency of factors Ⅱ, Ⅶ, Ⅸ, and Ⅹ of (89.94±1.31)%($n$=7). The results are shown in Table 1.

**2.1.2** $Ca_3(PO_4)_2$ adsorption: All of the elements, including time, pH of the buffer solutions, ionic strength, and temperature of $Ca_3(PO_4)_2$ adsorption are optimized, and then the best technique was achieved. The recovery of the potency of factor Ⅸ complex concentrates was (82.27±2.18)%($n$=6).

### 2.2 Purity of the Product

**Tab 1** Results of factor IX complex concentraterecovery of potency with optimum technology.

表 1 最佳工艺条件的收率

| Test number | Recovery of potency (%) | |
|---|---|---|
| | Adsorption of DEAE-Sepharose Fast Flow | adsorption of $Ca_3(PO_4)_2$ |
| 1 | 92.08 | 80.14 |
| 2 | 90.46 | 79.60 |
| 3 | 90.21 | 82.31 |
| 4 | 88.35 | 82.49 |
| 5 | 87.89 | 82.70 |
| 6 | 90.56 | 86.35 |
| 7 | 90.00 | |
| $\bar{x}\pm s$ | 89.94±1.31($n$=7) | 82.27±2.18($n$=6) |

**2.2.1** The total specific potency of factors Ⅱ, Ⅶ, Ⅸ, and Ⅹ (PE/mg), factor Ⅸ content and specific activity (IU/mg) are listed in Table 2. The total specific potency of the product was (25.70±3.78) PE/mg, much better than traditional domestic factor Ⅸ complex concentrates. It means that the purity of the production is greatly improved. The content of factor Ⅸ was (126.82±11.60) IU/200PE, and the specific avtivity of FⅨ:C was (16.28±2.80) IU/mg, which was also higher than that in preparation Ⅱ.

**Tab 2** The content and specific activity of factor Ⅸ in preparation

表 2 制品中因子Ⅸ的含量和比活

| Test number | Purity (PE/mg) | FⅨ:C (IU/mg) | FⅨ content (IU/200PE) |
|---|---|---|---|
| 1 | 29.95 | 16.52 | 110.30 |
| 2 | 25.02 | 15.87 | 126.86 |
| 3 | 18.98 | 12.67 | 133.50 |
| 4 | 23.79 | 13.40 | 112.67 |
| 5 | 26.53 | 18.25 | 140.00 |
| 6 | 29.91 | 20.94 | 137.58 |
| $\bar{x}\pm s$($n$=6) | 25.70±3.78 | 16.28±2.80 | 126.82±11.60 |

**2.2.2** SDS-PAGE According to the chromatogram we could find the purity of the factor Ⅸ complex concentrates produced in the new way was much higher than that in the traditional production. Compared with preparaton Ⅱ that was produced by chromatography technology, the purity was at the same level, and distribution of molecular weight was centralized at 50,000 ~ 70,000, but obviously different. The product was less various than preparation Ⅱ.

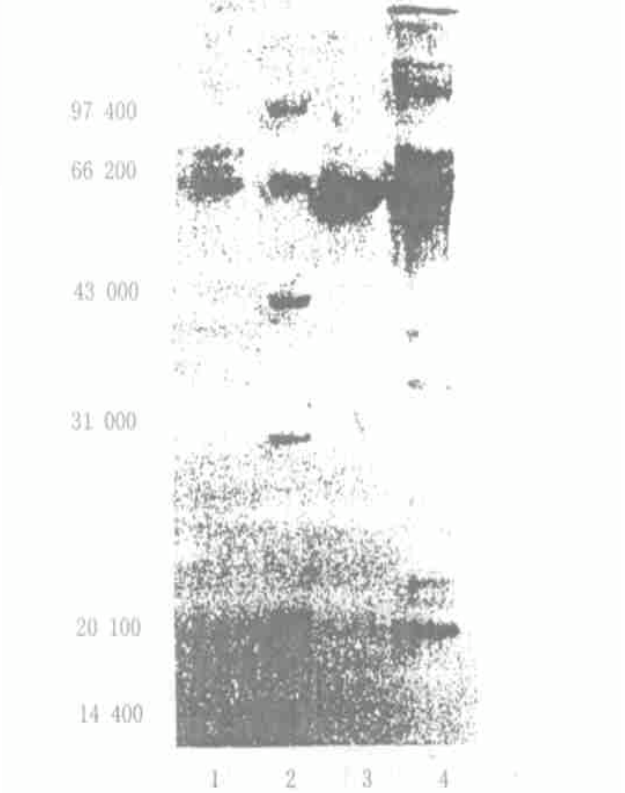



**Fig 1** SDS-PAGE chromatograms (1) newly prepared pro-

duet; (2) low molecular weight standard; (3) preparation Ⅱ; (4) preparation Ⅰ.

图 1 SDS-PAGE 图谱(1)新产品;(2)低分子量;(3)制品Ⅱ;(4)制品Ⅰ

## 3 Discussion

The final recovery rate is above 70 % by this batch adsorption technique. It is much higher than that in the early report of the adsorption of chromatography method[5], and is also much higher than that in any other report inland. The batch adsorption technique has reduced the requirements of the equipment, and the requisite time and solutions. It is beneficial to cut down the cost, shorten the cycle, improve the efficiency.

Because of the low content of FⅨ in the traditional productions, hemophilia B patients must be injected in a high dose. So it will lead to relative excessive content of FⅡ and FⅩ, and occurrence of various antibodies, while the danger of forming thrombus is greater[6]. Compared with the traditional methods, the purity of factor Ⅸ complex concentrates made in our way is higher. And compared with the chromatography methods, the total specific potency of the production is slightly enhanced, but FⅨ: C is enhanced by 100 %. That means that when the purity of the product was slightly enhanced, the scale of the effective ingredient (factor Ⅱ、Ⅶ、Ⅸ、and Ⅹ) is much improved. This is a benefit to lower potential thrombosis.

Although there is no quality standard of FⅨ content of factor Ⅸ complex concentrates in Chinese Rules of Biological Products (2000 edition), it is described in Europe Pharmacopeia. Recently, the research by Shen Qi[7] showed that the factor Ⅸ complex concentrates were about 50 % of labeled total complex concentrate potency. The factor in our preparation was 63 %, which is better than the average level in China.

At present, hemophilia B patients are advised to be treated with high purity factor Ⅸ complex concentrates abroad. However, high purity factor Ⅸ complex concentrates are often made by affinity chromatography, and the cost is very high. Our study indicates that the FⅨ content of our product is higher. With further improvement and optimization of the technology, it may be used to produce high purity factor Ⅸ complex concentrates.

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