中国医药工业杂志 Chinese Journal of Pharmaceuticals 2012, 43 (12) 

# 阿瑞匹坦静脉注射乳剂的研制

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**摘要**：通过单因素试验和正交设计优选了阿瑞匹坦静脉注射乳剂的最佳处方，并考察了形态、粒径、ζ电位和稳定性等基本性质。结果表明，乳化剂的种类和用量及油酸的用量对制品稳定性有较大影响。优化得到的阿瑞匹坦乳剂的处方为：阿瑞匹坦 0.25%、大豆油 15%、蛋黄磷脂 E80 2.5%、油酸 0.125%。所得制品外观呈乳白色，灭菌前、后的平均粒径、PDI、离心稳定常数（$K_e$）、ζ电位、pH 和 1 含量（标示量%）分别为（218.71±1.26）和（220.3±0.53）nm、0.203±0.016 和 0.210±0.009、0.21±0.01 和 0.34±0.01、(−37.8±0.2) 和 (−37.6±0.8) mV、7.21±0.23 和 7.31±0.30、(101.03±0.89) %和 (101.12±1.20) %。

**关键词**：阿瑞匹坦；注射用乳剂；制备；离心稳定常数；粒径；ζ电位
**中图分类号**：R944.1　　**文献标志码**：A　　**文章编号**：1001-8255 (2012) 12-1003-04

# Preparation of Aprepitant Emulsion for Intravenous Injection

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**ABSTRACT**: The formulation of aprepitant emulsion for intravenous injection was optimized by single factor test and orthogonal design. The properties, such as appearance, particle size, ζ potential and stability of the product were investigated. The results showed that the type and amount of emulsifier as well as the amount of oleic acid had the greater effect on stability. The optimal formulation of aprepitant emulsion was as follows: aprepitant was 0.25%, soybean oil was 15%, egg yolk lecithin E80 was 2.5% and oleic acid was 0.125%. The appearance of the product was ivory. The average particle size, PDI, centrifugal stability constant ($K_e$), ζ potential, pH value and drug content of the product before and after sterilization were (218.71±1.26) and (220.3±0.53) nm, 0.203±0.016 and 0.210±0.009, 0.21±0.01 and 0.34±0.01, (−37.8±0.2) and (−37.6±0.8) mV, 7.21±0.23 and 7.31±0.30, (101.03±0.89) % and (101.12±1.20) %, respectively.

**Key Words**: aprepitant; emulsion for injection; preparation; centrifugal stability constant; particle size; ζ potential

阿瑞匹坦（aprepitant，**1**）是美国 FDA 于 2003 年批准上市的第一个神经激肽 -1（NK-1）受体阻滞剂，通过与 NK-1 受体（主要存在于中枢及外周神经系统）结合来阻滞 P 物质的作用。本品可通过血脑屏障，对脑中 NK-1 受体具有选择性和高亲和性，而对 NK-2 和 NK-3 受体亲和性很低。同时本品对其他用于治疗化疗诱发的恶心和呕吐症状的药物靶点（如多巴胺受体、5-$HT_3$ 受体）亲和作用也很低，减少恶心、呕吐的效果优于其他药物。临床研究表明，**1** 可有效预防化疗引起的急性恶心和呕吐，而其突出优点是还可以同时预防化疗中的呕吐和延迟性呕吐[1]。

药用静脉注射乳剂是当今药物释放系统研究中的热点之一[2]。它可增加难溶性药物的溶解度，提高注射剂的载药量；提高化学不稳定药物的稳定性；具有缓释、延效作用；具有明显的靶向性；减轻药物不良反应；且制备工艺较成熟，产品质量稳定，适于工业化生产[3]。

有些呕吐患者口服药物时易产生恶心呕吐，导致吐而不纳，用药无效[4]。且 **1** 口服制剂的生物利用度为 60%～65%[5]，因而考虑将其制成静脉注射剂，以提高疗效。由于 **1** 不溶于水，须制成乳剂

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来保证药物的溶解度。

## 1 仪器与试药

UV-2450 型紫外分光光度计（日本 Shimadzu 公司）；AH100D 型高压均质机（加拿大 Seeker 工业公司）；Zetasizer Nano-ZS90 型激光粒子测定仪（英国 Malvern 公司）；JEM-2010 型透射电镜（日本电子公司）。

**1** 原药（照文献[6]自制，含量 99.4 %，批号 20110812）；注射用大豆油（广州汉方现代中药研究开发有限公司）；泊洛沙姆 188（德国 BASF 公司）；注射用蛋黄磷脂 E80、注射用油酸（德国 Lipoid 公司）；甘油（中国医药集团化学试剂有限公司）。

## 2 方法与结果

### 2.1 乳剂的制备

取适量 **1**、蛋黄磷脂和油酸加至大豆油中，加适量乙醇至全溶，70 ℃水浴减压蒸除乙醇，得澄清油相。搅拌下（17 000 r/min）将预热至 70 ℃的水相（注射用水；若处方中含泊洛沙姆和甘油，则将其先溶于注射用水中）缓慢加至油相（8.5 ∶ 1.5）中，得初乳，采用高压均质机在一定压力下均质数次后，充氮灌封于 2 ml 安瓿中，121 ℃灭菌 15 min，即得。

### 2.2 乳剂的质量评价指标

形态：通过透射电镜观察乳液的乳滴大小及分布情况。

粒径和 ζ 电位：通过激光粒子测定仪（工作温度 25 ℃，以水为溶剂）测定乳滴的平均粒径和 ζ 电位，从而评价乳剂粒子分布状况和乳滴的表面电荷。

稳定性：参考文献[7]采用离心法评价制品稳定性。取乳剂适量置离心管中，离心（1 000×*g*）10 min，取底部液体 0.1 ml，用水稀释至 10 ml，以水为空白，在 600 nm 处测定吸光度 *D*。取未离心乳剂 0.1 ml，同法稀释后测定吸光度 $D_0$，按下式计算离心稳定常数（$K_e$）。$K_e$ 越小则乳剂越稳定。

$$K_e=[(D_0-D)/D]\times100\%$$

另取适量乳剂置离心管中，离心（1 800×*g*）1 h，取底部溶液用水稀释一定倍数镜检，查看有无结晶，考察乳剂是否存在破乳现象。

### 2.3 乳剂的处方优化

#### 2.3.1 正交设计

经预试验及单因素考察，初步确定乳剂的处方为：**1** 0.25%，大豆油 15%，蛋黄磷脂（*A*）2%～4%，油酸（*B*）0 ～ 0.2%，泊洛沙姆（*C*）0 ～ 0.5%，甘油（*D*）0 ～ 3%。采用正交设计继续考察因素 *A*、*B*、*C*、*D* 的影响，各因素水平见表 1。照“**2.1**”项下方法制备 **1** 乳剂，考察各组 $K_e$ 的大小（试验结果存编辑部）。

**表 1 因素水平表**

| 水平 | 因素 | | | |
|---|---|---|---|---|
| | *A*/% | *B*/% | *C*/% | *D*/% |
| 1 | 2 | 0 | 0 | 0 |
| 2 | 2.5 | 0.125 | 0.2 | 1.5 |
| 3 | 3 | 0.15 | 0.3 | 2.5 |
| 4 | 4 | 0.2 | 0.5 | 3 |

直观分析和极差分析表明，最佳处方组合为 $A_2B_2C_1D_1$，即 10 ml 制剂中含蛋黄磷脂 0.25 g，油酸 12.5 mg，泊洛沙姆和甘油无需加入。该处方制得的乳剂外观呈乳白色，振摇后乳液迅速从瓶壁滑落，不黏壁。

#### 2.3.2 单因素试验

乳化温度：将按上述优化处方配制的油水两相分别预热至 50、60、70 和 80 ℃，再保温混合得初乳，冷却后立即取初乳 0.1 ml，用水稀释至 10 ml，在 600 nm 处测得 $D_0$，室温静置 24 h 后，取下层液 0.1 ml，同法测得 *D*，计算 $K_e$。结果表明，50、60、70 和 80 ℃下制得乳剂的 $K_e$ 值分别为 22.54±0.86、17.74±0.40、13.66±0.25、13.63±0.31（$n$=3）。可见随着温度的升高，初乳稳定性提高，当温度达到 70 ℃以上，初乳稳定性变化不明显，且温度太高可能会造成蛋黄磷脂和油相氧化加快，所以选择 70 ℃作为乳化温度。

乳化时间：将按优化处方配制的油水两相于 70 ℃混合，分别搅拌 1、3、6、10、15 min 制备初乳。所得初乳室温静置 12 h，结果乳化 1 min 的初乳分层最不明显，稳定时间最长。但乳化时间不影响终乳的稳定性和粒径大小（见表 2）。所以选择乳化时间为 1 min。



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表 2 乳化时间对终乳粒径及 $K_e$ 的影响 ($\bar{x}\pm s$, $n$=3)

| 参数 | 乳化时间/min | | | | |
|---|---|---|---|---|---|
| | 1 | 3 | 6 | 10 | 15 |
| 粒径/nm | 218.63±0.31 | 218.70±0.20 | 218.57±0.15 | 219.03±0.35 | 219.50±0.20 |
| $K_e$ | 0.33±0.01 | 0.35±0.01 | 0.33±0.01 | 0.33±0.01 | 0.35±0.01 |

水相加入速度：将按优化处方配制的水相分别缓慢或一次性加至油相中，照“2.1”项下方法制备初乳，乳化时间 1 min。所得初乳静置 12 h，观察初乳分层情况，再制备终乳。目测结果表明，一次性加入法制得的初乳分层情况比缓慢加入法所得初乳明显，但不影响终乳的稳定性和粒径大小，一次性加入法和缓慢加入法所得的终乳粒径为 (219.07±0.76) 和 (219.40±0.50) nm，$K_e$ 为 0.21±0.01 和 0.21±0.01 ($n$=3)。所以选择水相缓慢加入油相，边加边搅拌、振摇。

均质次数和均质压力：按上述优选的处方和工艺制备初乳，在均质压力分别为 300、400、500、600 和 700 bar 条件下进行均质，所得制品的粒径见表 3。

表 3 均质压力和次数对终乳平均粒径和多分散系数 (PDI) 的影响[1] ($\bar{x}\pm s$, $n$=3)

| 均质压力/bar | 均质次数 | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| 700 | 292.40±0.90 | 239.07±0.59 | 231.07±0.59 | 218.13±0.96 | 228.67±0.68 | | |
| | (0.278±0.001) | (0.219±0.002) | (0.185±0.003) | (0.207±0.003) | (0.150±0.005) | | |
| 600 | 296.20±2.61 | 252.30±2.27 | 234.90±0.20 | 222.13±1.57 | 210.80±3.15 | 213.50±2.38 | |
| | (0.282±0.005) | (0.180±0.004) | (0.202±0.005) | (0.195±0.009) | (0.157±0.012) | (0.169±0.005) | |
| 500 | 298.60±2.17 | 253.87±0.06 | 233.63±0.38 | 236.57±0.85 | 216.80±0.85 | 229.03±0.90 | 218.63±0.67 |
| | (0.358±0.005) | (0.210±0.004) | (0.226±0.004) | (0.181±0.003) | (0.209±0.005) | (0.091±0.008) | (0.217±0.003) |
| 400 | 276.50±1.29 | 241.71±0.85 | 227.15±3.53 | 232.40±1.23 | 218.71±1.26 | 223.97±1.95 | |
| | (0.226±0.001) | (0.163±0.005) | (0.203±0.008) | (0.097±0.012) | (0.203±0.016) | (0.146±0.010) | |

注：[1] 括号内外分别为 PDI 和平均粒径 /nm

结果表明，300 bar 均质 5 次及以上时，制品粒径都只能达到 249.4 nm，说明均质压力不够。综合平均粒径和 PDI 结果，最终选择 400 bar 均质 5 次，可连续均质。

### 2.4 1 静脉注射乳剂的优化处方和工艺

通过正交设计与单因素考察，确定的 1 静脉注射乳剂的最优处方与工艺如下：称取 1 原药 (0.25%)、蛋黄磷脂 (2.5%)、油酸 (0.125%)，加适量乙醇溶解，再加大豆油 (15%)，在 70 ℃水浴下减压蒸除乙醇，得澄清油相。以 1.5 ∶ 8.5 的比例缓慢加入预热至 70 ℃的注射用水，边加边搅拌，17 000 r/min 搅拌 1 min 得初乳，采用高压均质机于 400 bar 连续均质 5 次，充氮灌封于 2 ml 安瓿中，121 ℃高压蒸汽灭菌 15 min 即得。所得制品灭菌前、后的平均粒径、PDI、$K_e$、ζ 电位、pH 和 1 含量 (标示量%) 分别为 (218.71±1.26) 和 (220.3±0.53) nm、0.203±0.016 和 0.210±0.009、0.21±0.01 和 0.34±0.01、(−37.8±0.2) 和 (−37.6±0.8) mV、7.21±0.23 和 7.31±0.30，(101.03±0.89)% 和 (101.12±1.20)% ($n$=3)。外观和粒度分布见图 1 和 2。

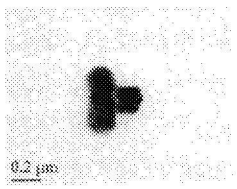


图 1 1 乳剂灭菌后的透射电镜照片 (×250 000)

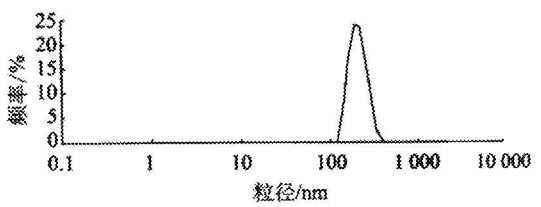


图 2 1 乳剂灭菌后的粒度分布图



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**2.5** 稳定性考察

考察了照“**2.4**”项下条件制得的乳剂在灭菌后及灭菌后贮存 3 个月后的稳定性。结果表明，制品外观无显著改变，仍呈乳白色，未出现分层和破乳的情况。质量评价结果见表 4。

**表 4 优化处方质量评价测定结果及稳定性考察结果** ($\bar{x}\pm s$, $n$=3)

| 条件 | 粒径/nm | PDI | $K_e$ | ζ电位/mV | pH | 含量(标示量%) |
|---|---|---|---|---|---|---|
| 灭菌后 | 220.3±0.53 | 0.210±0.009 | 0.343±0.012 | −37.6±0.8 | 7.31±0.30 | 101.12±1.20 |
| 室温贮存3个月 | 219.1±0.95 | 0.205±0.008 | 0.333±0.015 | −37.8±0.3 | 7.08±0.23 | 100.99±0.78 |
| 4～8 ℃冷藏3个月 | 219.2±0.889 | 0.199±0.010 | 0.347±0.025 | −37.9±0.9 | 6.83±0.43 | 100.14±0.04 |

## 3 讨论

**3.1** 磷脂种类的选择

曾考察了用不同磷脂（大豆磷脂、蛋黄磷脂 E80 和蛋黄磷脂 E PC S）配制的初乳静置 4 d 后的稳定性。结果表明，用大豆磷脂和蛋黄磷脂 E PC S 配制的初乳均有分层，前者制得的乳剂在高温灭菌时颜色变黄；而蛋黄磷脂 E80 所制得的初乳无分层现象，且进行高温灭菌时颜色不发生变化。同时蛋黄磷脂 E PC S 所制得的乳剂 ζ 电位约为 −29 mV，不利于乳剂的稳定。所以选用蛋黄磷脂 E80。

有报道比较了含量为 98%、80%和 30%的卵磷脂产品的乳化效果和综合质量指标，结果纯度较高或较低的卵磷脂都不利于脂肪乳的形成和稳定性，只有含量为 80%的卵磷脂制成的脂肪乳最稳定，这种卵磷脂含少量酸性脂质，可增加乳滴的表面电荷，使乳剂具有较好的稳定性；而进一步纯化后的磷脂产品中磷脂酰胆碱或磷脂酰乙醇胺的含量可达 95%～100%，这些磷脂中几乎没有酸性脂质，乳化后乳粒的表面电荷减少，稳定性下降，因此并不是理想的乳化剂[8]。因此，本试验中选用含量为 80%的卵磷脂 E80。

磷脂一般作为乳化剂，但在本处方中还起到助溶作用。**1** 溶于乙醇、不溶于水，配制油相时如仅将处方量的 **1** 和油相混合后加乙醇溶解，再减压抽干乙醇，结果得不到澄清油相，须加入磷脂才能得到澄清油相，可见蛋黄磷脂起到助溶效果。

**3.2** 油酸的使用

由于蛋黄磷脂中含有的溶血磷脂较少，对某些药物的乳化效果不好，油酸可作为助乳化剂。油酸的加入能增加乳剂膜的刚性，同时也能增加乳剂的表面电荷，使其分散性增强，减小聚集能力，更易得到粒径较小的乳剂，增强了乳剂的稳定性。油酸的用量一般为磷脂用量的 2.5%～5%，本处方筛选得到的最优油酸用量是磷脂用量的 5%。

**3.3** 本试验中的一些注意点

以离心法判定乳剂的稳定性时，由于取样点对于结果的判定有较大影响，所以每次取样部位为离心管最底部，平行操作，这样可避免由于取样点不同而对结果产生的影响。

由于 **1** 不溶于水，所以一旦乳剂发生破乳，药物就会从油相漏至水相中，从而产生结晶。所以可用有无结晶来判断是否破乳。

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APREPREF_0000207

# Preparation of Aprepitant Emulsion for Intravenous Injection

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Aprepitant (**APT**) is the first neurokinin-1 (NK-1) receptor antagonist approved by the United States Food and Drug Administration (FDA) in 2003 to be brought to the market, and it blocks the effects of substance P by binding with neurokinin-1 (NK-1) receptors (primarily in existence in the central nervous system and its peripheries). Aprepitant (APT) is capable of crossing the blood-brain barrier and occupying neurokinin-1 (NK-1) receptors in the brain, and it possesses binding selectivity and high affinity with NK-1 receptors, but its affinities with neurokinin-2 (NK-2) and neurokinin-3 (NK-3) receptors are very low. In addition, the affinity of aprepitant (APT) to other drug targets used for treating chemotherapy-induced nausea, vomiting and other symptoms (e.g. dopamine receptors, 5-hydroxytryptamine (5-$HT_3$) receptors) is also very low, and the antiemetic and anti-nausea effects of APT are better than those of other drugs. Clinical studies have indicated that APT is capable of effectively preventing acute chemotherapy-induced nausea and vomiting (CINV), and an outstanding advantage of APT is its ability to prevent vomiting during chemotherapy and delayed chemotherapy-induced nausea and vomiting (CINV)[1].

Pharmaceutical emulsions for intravenous injection are one of the hot spots among the studies on drug delivery systems (DDS)[2]. Pharmaceutical emulsions are advantageous in that they: increase the solubility of insoluble drugs and increase the drug-loading efficiency of injections; improve the stability of chemically unstable drugs; have the functions of sustained release and prolonged release; have obvious targetability; alleviate adverse drug reactions; and the preparation processes thereof are technically mature, product quality thereof is stable, and they are therefore suitable for industrial production[3].

For some patients with vomiting, taking oral medication is prone to causing nausea and vomiting and this results in non-absorption and ineffective drug administration[4]. Given that the bioavailability of oral aprepitant (APT) is 60~65%[5], it is contemplated that APT be prepared in the dosage form of intravenous injection so as to improve the therapeutic effects thereof. As aprepitant (APT) is not water-soluble, it must be prepared in the form of emulsion to ensure drug solubility.

## 1. Instruments and Reagents

UV-2450 Ultraviolet Spectrophotometer (Shimadzu Corporation, Japan); AH100D High-pressure Homogenizer (Seeker Industry Limited, Canada); Zetasizer Nano-ZS90 Laser Particle Size Analyzer (Malvern Panalytical Ltd., United Kingdom);

JEM-2010 Electron Microscope (JOEL Ltd., Janpan).

Aprepitant (**APT**) starting material (self-prepared according to literature reference[6], content 99.4%, Batch No. 20110812); soybean oil for injection (Guangzhou Hanfang Modern Chinese Medicine Research and Development Co., Ltd.); poloxamer 188 (BASF SE, Germany); egg yolk phospholipid for injection E80, oleic acid for injection (Lipoid GmbH, Germany); glycerol (Sinopharm Chemical Reagent Co., Ltd.).

## 2. Method and Results

### 2.1 Preparation of Emulsion

Suitable amounts of aprepitant (APT), egg yolk phospholipid and oleic acid were taken and added into soybean oil, an adequate amount of ethanol was added until the mixture was fully dissolved, the fluid was put in a water bath at a temperature of 70°C and underwent decompression to remove ethanol by evaporation to obtain a substance in the clarified oil phase. While stirring was being performed (17,000 RPM), the aqueous phase preheated to 70°C (water for injection; if the recipe contained poloxamer and glycerol, the substance would first be dissolved in water for injection) was slowly added into the oil phase (8.5: 1.5) to obtain a primary emulsion, homogenization was performed multiple times with the high-pressure homogenizer at a specific pressure, nitrogen-filled packaging was performed to pack into 2ml glass ampoules, sterilization was performed at 121°C for 15 minutes to obtain the finished product.

### 2.2 Quality Evaluation Indicators for Emulsion

Appearance: Sizes and distribution of emulsion droplets were observed using the electron microscope.

Particle size and Zeta (ζ) potential: The average particle size and Zeta (ζ) potential of the emulsion droplets were determined using the laser particle size analyzer (working temperature 25°C, water used as solvent), thereby evaluating the particle size distribution of emulsion and the electrical charge of emulsion droplets.

Stability: The use of centrifugation for the evaluation of product stability is mentioned in literature reference[7]. An adequate amount of emulsion was taken and placed in a centrifugal tube, and was subjected to centrifugation (at 1,000xg) for 10 minutes, 0.1ml of bottom liquid was taken and diluted with water to 10ml, and with water used as a blank the absorbance at 600nm, $D$ was measured. 0.1ml of emulsion having not been centrifuged was taken and diluted using the same method, and the absorbance $D_0$ was then measured, the centrifugal stability constant ($K_e$) was computed using the following formula. The smaller the value of $K_e$, the more stable the emulsion.

$K_e = [(D_0 - D) / D] \times 100\%$

An adequate amount of emulsion was taken and put into a centrifugal tube, and was subjected to centrifugation (at 1,800xg) for 1 hour, bottom liquid was taken and diluted with water to a certain multiple and underwent microscopic examination to check for crystallization and investigate any de-emulsification.

2.3 Optimization of Emulsion Recipe

2.3.1 Orthogonal Design

Through pre-experimental and single factor analysis, it was primarily determined that the emulsion recipe was: 0.25% of APT, 15% of soybean oil, 2~4% of egg yolk phospholipid (*A*), 0~0.2% of oleic acid (*B*), 0~0.5% of poloxamer (*C*) and 0~3% of glycerol (*D*). Orthogonal design method was used to continue investigating the impacts of the factors *A*, *B*, *C* and *D*, and the factor levels were as shown in Table 1 below. The method mentioned in Section 2.1 was employed to prepare aprepitant (APT) emulsion, and the magnitudes of $K_e$ in various groups were examined (experimental results kept by the Editorial Department).

Table 1 - Factor Levels

| Level | *A* (%) | *B* (%) | *C* (%) | *D* (%) |
|---|---|---|---|---|
| 1 | 2 | 0 | 0 | 0 |
| 2 | 2.5 | 0.125 | 0.2 | 1.5 |
| 3 | 3 | 0.15 | 0.3 | 2.5 |
| 4 | 4 | 0.2 | 0.5 | 3 |

Intuitive analysis and range analysis indicated that the optimal recipe composition should be $A_2B_2C_1D_1$, i.e. 10ml of preparation containing 0.25g of egg yolk phospholipid (*A*) and 12.5mg of oleic acid (*B*) without any poloxamer (*C*) or glycerol (*D*). The emulsion prepared following that recipe had a milky white appearance, and the emulsion rapidly slid off the wall of the bottle and did not stick to the wall.

2.3.2 Single Factor Experiment

Emulsification temperature: the oil phase and aqueous phase prepared according to the abovementioned optimized recipe were heated to 50°C, 60°C, 70°C and 80°C respectively, and were then thermally insulated and mixed to obtain a primary emulsion; immediately after cooling, 0.1ml of primary emulsion was diluted with water to 10ml, and the absorbance at 600nm, $D_0$ was measured; after being placed at ambient temperature for 24 hours, 0.1ml of subnatant was taken and the

centrifugal stability constant ($K_e$) was computed using the same method. The results indicated that the $K_e$ values of the emulsions prepared at 50°C, 60°C, 70°C and 80°C were 22.54±0.86, 17.74±0.40, 13.66±0.25 and 13.63±0.31 (n=3), respectively. It could be seen that the stability of the primary emulsion increased along with temperature rise and when the temperature was above 70°C the change in the stability of the primary emulsion was inconspicuous, and that excessively high temperature might result in accelerated oxidation of egg yolk phospholipid and the oil phase, therefore 70°C was selected as the emulsification temperature.

Emulsification time: the oil phase and aqueous phase prepared according to the optimized recipe were mixed at a temperature of 70°C, and stirring was performed for 1min, 3min, 6min, 10min and 15min respectively to prepare primary emulsions. The primary emulsions obtained were placed at ambient temperature for 12 hours, eventually the primary emulsion having undergone 1min of emulsification had the least visible stratification but the longest time of stability. However, the emulsification time did not affect the stability and particle size of the eventual emulsion (see Table 2). Therefore 1 minute was selected as emulsification time.

Table 2 - Impact of Emulsification Time on the Particle Size and $K_e$ of the Eventual Emulsion ($\bar{x}$ ±s, n=3)

| Parameter | Emulsification Time *(minutes)* | | | | |
|---|---|---|---|---|---|
| | 1 | 3 | 6 | 10 | 15 |
| Particle Size *(nm)* | 218.63±0.31 | 218.70±0.20 | 218.57±0.15 | 219.03±0.35 | 219.50±0.20 |
| $K_e$ | 0.33±0.01 | 0.35±0.01 | 0.33±0.01 | 0.33±0.01 | 0.35±0.01 |

Rate of addition of aqueous phase: the aqueous phase prepared according to the optimized recipe was added to the oil phase slowly or on a one-off basis, primary emulsion was prepared using the method mentioned in Section 2.1 and the emulsification time was 1 minute. The primary emulsion obtained was left alone for 12 hours, stratification of the primary emulsion was observed, and eventual emulsion was then prepared. Visual observation results indicated that stratification of the primary emulsion prepared using the one-off addition method was more conspicuous than that of the primary emulsion obtained by the slow addition method, but that did not affect the stability and particle size of the eventual emulsions, and the particle sizes of the eventual emulsions obtained using the one-off addition method and the slow addition method were 219.07±0.76nm and 219.40±0.50nm respectively, and the $K_e$ values thereof were 0.21±0.01 and 0.21±0.01 (n=3) respectively. Therefore, a selection was made whereby the aqueous phase was slowly added to the oil phase, during which stirring and shaking were performed.

Number of homogenization cycles and homogenization pressure: primary emulsions were prepared according to the abovementioned optimized recipe and techniques, homogenization was performed under homogenization pressure conditions of 300 bar, 400 bar, 500 bar, 600 bar and 700 bar respectively, and the particle sizes of the products obtained were as shown in Table 3.

Table 3 - Impacts of Homogenization Pressure and Number of Homogenization Cycles on Average Particle Size and Polydispersity Index (PDI) of Eventual Emulsion ($\bar{x}$ ±s, n=3)

| Homogenization Pressure *(bar)* | Number of Homogenization Cycles | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| 700 | 292.40±0.90<br>(0.278±0.001) | 239.07±0.59<br>(0.219±0.002) | 231.07±0.59<br>(0.185±003) | 218.13±0.96<br>(0.207±0.003) | 228.67±0.68<br>(0.150±0.005) | | |
| 600 | 296.20±2.61<br>(0.282±0.005) | 252.30±2.27<br>(0.180±0.004) | 234.90±0.20<br>(0.202±0.005) | 222.13±1.57<br>(0.195±0.009) | 210.80±3.15<br>(0.157±0.012) | 213.50±2.38<br>(0.169±0.005) | |
| 500 | 298.60±2.17<br>(0.358±0.005) | 253.87±0.06<br>(0.210±0.004) | 233.63±0.38<br>(0.226±0.004) | 236.57±0.85<br>(0.181±0.003) | 216.80±0.85<br>(0.209±0.005) | 229.03±0.90<br>(0.091 ±0.008) | 218.63±0.67<br>(0.217±0.003) |
| 400 | 276.50±1.29<br>(0.226±0.001) | 241.71 ±0.85<br>(0.163±0.005) | 227.15±3.53<br>(0.203±0.008) | 232.40±1.23<br>(0.097±0.012) | 218.71±1.26<br>(0.203±0.016) | 223.97±1.95<br>(0.146±0.010) | |

Note: (1) The figures in brackets and outside brackets are Polydispersity Indices (PDI) and average particle sizes (nm) , respectively.

The results revealed that when homogenization was performed five (5) times and above at 300 bar, the product particle size could only reach 249.4nm, which indicated that the homogenization pressure was inadequate. Considering the average particle size and PDI results, it was determined that 400 bar and five (5) times be selected as the homogenization pressure and number of homogenization cycles respectively, and continuous homogenization was possible.

2.4 Optimized Recipe and Processes of Aprepitant (APT) Emulsion for Intravenous Injection

The optimal recipe and processes of APT emulsion for intravenous injection determined by means of orthogonal design and single factor experiment were as follows: APT starting material (0.25%), egg yolk phospholipid (2.5%) and oleic acid (0.125%) were weighed and added with an adequate amount of ethanol to have the mixture dissolved, soybean oil (15%) was then added, the fluid was put in a water bath at a temperature of 70°C and underwent decompression to remove ethanol by evaporation to obtain a substance in the clarified oil phase. Water for injection preheated to 70°C was slowly added at a ratio of 1.5: 8.5, while stirring was performed (17,000 RPM) for 1 minute to obtain a primary emulsion, homogenization was performed five (5) times with the high-pressure homogenizer at a pressure of 400 bar, nitrogen-filled packaging was performed to pack into 2ml glass ampoules, sterilization was performed at 121°C for 15 minutes to obtain the finished product. The

average particle sizes; polydispersity indices (PDIs); $K_e$ values; Zeta (ζ) potentials; and APT contents (indicated in %) of the obtained product before and after sterilization were 218.71±1.26nm and 220.3±0.53nm; 0.203±0.016 and 0.210±0.009; 0.21±0.01 and 0.34±0.01; -37.8±0.2mV and -37.6±0.8mV; 7.21±0.23 and 7.31±0.30; and 101.03±0.89% and 101.12±1.20% (n=3), respectively. The appearance and particle distribution were as shown in Figure 1 and Figure 2, resepctively.

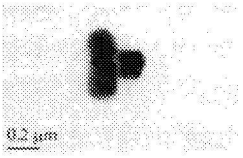

Figure 1 - Electron Microscopic Picture of Aprepitant (APT) Emulsion after Sterilization (x 250,000)

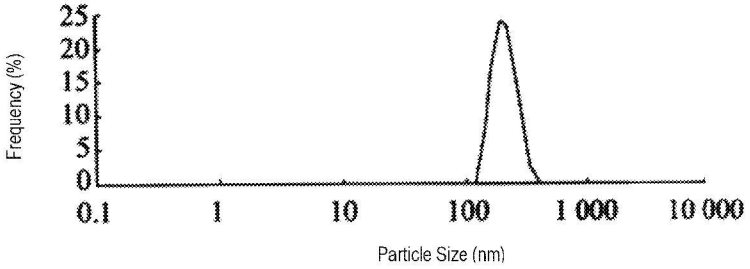


Figure 2 - Particle Distribution of Aprepitant (APT) Emulsion after Steriliztion

2.5 Stability Analysis

The stability of the emulsion prepared under the conditions specified under Section 2.4 after sterilization and after 3 months of storage after sterilization was analyzed. The results indicated that the product appearance had no significant change and was still milky white, and no stratification or de-emulsification was detected. The quality evaluation results were as shown in Figure 4.

Table 4 - Optimized Recipe Quality Evaluation Results and Stability Analysis Results ($\bar{x}$ ±s, n=3)

| Condition | Particle Size *(nm)* | PDI | $K_e$ | Zeta (ζ) Potential *(mV)* | pH | Content *(%)* |
|---|---|---|---|---|---|---|
| After sterilization | 220.3±0.53 | 0.210±0.009 | 0.343±0.012 | -37.6±0.8 | 7.31±0.30 | 101.12±1.20 |
| Stored at ambient temperature for 3 months | 219.1±0.95 | 0.205±0.008 | 0.333±0.015 | 37.8±0.3 | 7.08±0.23 | 100.99±0.78 |
| Refrigerated at 4~8°C for 3 months | 219.2±0.889 | 0.199±0.010 | 0.347±0.025 | -37.9±0.9 | 6.83±0.43 | 100.14±0.04 |

## 3. Discussions

3.1 Selection of Type of Phospholipid

Stability of primary emulsions prepared using different phospholipids (soybean phospholipid, egg yolk phospholipid E80

and egg yolk phosphatidylcholines (EPCs)) and after 4 days of being left alone was analyzed. The results indicated that the primary emulsions prepared using soybean phospholipid and egg yolk phosphatidylcholines (EPCs) were stratified, and the emulsion made from the former turned yellow when being subjected to thermal sterilization; whereas the primary emulsion made from egg yolk phospholipid E80 was not stratified and its color remained unchanged when being subjected to thermal sterilization. In addition, the Zeta (ζ) potentials of the emulsions made from egg yolk phosphatidylcholines (EPCs) were about -29mV and not conducive to the stability of the emulsions.

There have been reports of comparison of emulsification effects and comprehensive quality indicators of 98%, 80% and 30% phosphatidylcholine products, of which the results indicated that phosphatidylcholines of relatively high or relatively low purity are not conducive to the formation and stability of lipid emulsions and that lipid emulsions made from 80% phosphatidylcholine are the most stable; this phosphatidylcholine contains small amounts of acidic lipids and is capable of increasing the surface charges of the emulsion droplets and causing the emulsion to have better stability; the phosphatidylcholine (PPC) and phosphatidylethanolamine (PE) contents in further purified phospholipid products could be as high as 95%~100%, these phospholipids almost contain no acidic lipid, and after emulsification the surface charges of the emulsion particles and the stability decrease, hence they are not ideal emulsifying agents[8]. Therefore, 80% egg yolk phospholipid E80 was chosen for the experiment.

Phospholipids are often used as emulsifying agents but they play the role of solubilization in this recipe. Aprepitant (APT) is soluble in ethanol but not in water, and if during oil phase formulation only the prescribed amount of APT is mixed with the oil phase before the addition of ethanol for dissolution, after which decompression is performed to remove ethanol, the clarified oil phase will not be obtained and it is necessary to add phospholipid to be able to obtain the clarified oil phase, it can therefore be seen that egg yolk phospholipid has the effect of solubilization.

3.2 Use of Oleic Acid

As the lysophosphatide content in egg yolk phospholipids is relatively low and the emulsification effects on certain drugs are not good, oleic acid can serve as a co-emulsifier. The addition of oleic acid has the advantages of increasing the rigidity of emulsion membrane, increasing the surface charges of the emulsion, causing its dispersivity to be increased and reducing agglomeration, making it easier to obtain emulsion with smaller particle size, and increasing emulsion stability. The amount of oleic acid used is usually 2.5%~5% of that of phospholipid, and the optimal amount of oleic acid to be used is determined by screening of the recipe to be 5% of that of phospholipid.

3.3 Some Points for Attention in the Experiment

When centrifugation was employed to determine emulsion stability, given that sample points would have great impact on the results, each time the sampling location was the extreme bottom of the centrifugal tube and concurrent operation was performed so as to prevent differences in sample points from impacting the results.

As aprepitant (APT) is not water-soluble, once de-emulsification of the emulsion occurs, the drug would leak from the oil phase into the aqueous phase and cause crystallization. Therefore, the presence or absence of crystals can be used to determine whether de-emulsification takes place.

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## Certification

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Date: December 14, 2020

Donald W. Hanley, CEO