Chinese Manufacturer’s Perspective on Excipient Monograph Specifications for Polysorbate 80 used for Injection
Design of the polysorbate 80 for injection

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About Well Chemical

01 Specialized manufacturer of pharmaceutical excipients in China.

02 All the excipients of Well Chemical have been approved by CFDA.

03 For polysorbate 80, we have pharma grade and for injection grade.
Introduction of Polysorbate 80

Definition: Polysorbate 80 is a mixture of partial esters of fatty acids, mainly oleic acid, with sorbitol and its anhydrides ethoxylated with approximately 20 moles of ethylene oxide for each mole of sorbitol and sorbitol anhydrides. (USP-39, NF34)

Structure:
Introduction of Polysorbate 80

Actual composition:

polysorbate 80
polyoxyethylene(12)isosorbide monooleate
polyoxyethylene(12)isosorbide dioleate
polyoxyethylene(24)sorbitan monooleate
polyoxyethylene(24)sorbitan dioleate
polyoxyethylene(24)sorbitan trioleate
polyoxyethylene(24)sorbitan tetraoleate
and so on ..................
Introduction of Polysorbate 80

Widely used in many formulations:

- Solubilizer-injection, oral solution, topical lotions
- Emulsifier-ointment, cream, gel
- Dispersing agent-biological product
Polysorbate 80 is added as solubilizer in dozens of injections and the proportion ranges from 0.1% to 50%;

## Typical formulation

<table>
<thead>
<tr>
<th>EPO injection</th>
<th>Vitamin k1 injection</th>
<th>Docetaxel injection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin supplements</td>
<td>Monoclonal antibody</td>
<td>Isosorbide Dinitrate for injection</td>
</tr>
<tr>
<td>Triamcinolone acetonide injection</td>
<td>Amiodarone hydrochloride injection</td>
<td>Traditional Chinese injections</td>
</tr>
</tbody>
</table>
The raw materials of polysorbate 80

- Ethylene Oxide (EO)
- Oleic Acid
- Sorbitol
The composition of polysorbate 80:

- **EO**
- **Complex system**
  - **Sorbitol**
  - **Sorbitan**
  - **Isosorbide**
  - **glycan**
  - **Various isomers**
- **Oleic acid**
  - Stearic acid
  - Linoleic acid
  - Linolenic acid
  - myristic acid
  - Palmitic acid
  - palmitoleic acid
  - Other fatty acids
Two synthetic routes

First esterify, then polymerize

1. Esterify sorbitol with oleic acid to form sorbitan oleate.
2. Polymerize sorbitan oleate with ethylene oxide to form Polysorbate 80.

First polymerize, then esterify

1. Polymerize sorbitol with ethylene oxide to form polymer.
2. Esterify polymer with oleic acid to form Polysorbate 80.
Two synthetic routes

1: First esterify, then polymerize

(1) After dehydrating partly, sorbitol becomes the mixture of sorbitol and sorbitol anhydride, then esterify with oleic acid to produce sorbitan oleate (span80).

(2) Sorbitan oleate polymerize with about 20 mol ethylene oxide and produce Polysorbate 80.

Current problems:

(1) The source and purity of oleic acid have not been controlled. The purity of oleic acid is about 70% generally, the source has still not been determined.

(2) Sorbitan oleate keeps dehydrating incessantly in the stage of polymerization, which results in uncontrollable manufacturing procedure, many by-products and poor inter batch stability.
Two synthetic routes

2: First polymerize, then esterify

Ethylene oxide react prior with sorbitol and sorbitol anhydride and occupy hydroxyl group site, which avoid the uncontrollable incessant dehydration in the stage of polymerization between sorbitol and sorbitol anhydride in the former route.
Dehydration reaction of sorbitol

Two synthetic routes
Current status of Polysorbate 80

We carried out the test using HPLC-ELSD to analysis the internal composition of polysorbate 80 and found products from different manufacturers are various.

HPLC-ELSD

Result of four manufacturers
Safty comparison of polysorbate 80 from different sources

The degree of allergic reactions

Test animal: guinea pig

<table>
<thead>
<tr>
<th>scale</th>
<th>phenomenon</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No obvious reaction</td>
</tr>
<tr>
<td>1</td>
<td>catching nose slightly, trembling or pilo-erection</td>
</tr>
<tr>
<td>2</td>
<td>cough, nose-catching frequently, trembling or pilo-erection, ear vasodilation, etc.</td>
</tr>
<tr>
<td>3</td>
<td>high-frequency or continuous cough, complicated by difficulty in breathing or spasms, convulsions</td>
</tr>
<tr>
<td>4</td>
<td>spasm, tic, gatism, shock and death</td>
</tr>
</tbody>
</table>
The differences in compositions and safety of Polysorbate 80 from different sources induce the disparities in the application.

According to the reference, Docetaxel injection’s abnormal phenomenon was induced by the quality of polysorbate 80, whose fatty acid composition and solidifying point are the crucial standards.
The concept of our design:

- The intrinsic composition of polysorbate 80 is complex. It consists of many substances of the same character.

- We can not separate them by purification reaction after synthetic process.

- We start from the preprocessing of raw materials and try our best to reduce the content of impurities.

- It is our aim that to acquire higher stability and safety.
Features of manufacture procedure of polysorbate 80 for injection

(1) Purification of the raw materials

Purify the Oleic acid and make the content of Oleic acid not less than 98%;
The Oleic acid must be plant source;
Purify the Sorbitol & Sorbitan and fix their reaction proportion;

(2) Process route (First polymerize, then esterify)

Ethylene oxide react prior with sorbitol and sorbitol anhydride and occupy hydroxyl group site, which avoid the uncontrollable incessant dehydration in the stage of polymerization between sorbitol and sorbitol anhydride in the former manufacturing procedure.

(3) Production control

High efficient esterification technology and narrow molecular distribution technology of ethoxylation.
## Comparison of specifications of polysorbate 80

<table>
<thead>
<tr>
<th>Test item</th>
<th>USP 39, NF 34</th>
<th>JP 16</th>
<th>EP 8.0</th>
<th>Well spec &amp; CHP 2015 polysorbate 80 for injection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peroxide value</td>
<td>max 10</td>
<td>max 10</td>
<td>max 10</td>
<td>max 2</td>
</tr>
<tr>
<td>UV absorption</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>≤1.0 (225nm) ≤0.1 (267nm)</td>
</tr>
<tr>
<td>Viscosity</td>
<td>300 - 500 cSt</td>
<td>345 - 445 mm²/s</td>
<td>about 400 mPa·s</td>
<td>350 - 450 mm²/s</td>
</tr>
<tr>
<td>Water content</td>
<td>≤3.0%</td>
<td>≤3.0%</td>
<td>≤3.0%</td>
<td>≤0.5%</td>
</tr>
<tr>
<td>Acid value</td>
<td>≤2.0%</td>
<td>≤2.0%</td>
<td>≤2.0%</td>
<td>≤1.0%</td>
</tr>
<tr>
<td>Colour</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>lighter than yellow #2</td>
</tr>
<tr>
<td>Ethylene glycol</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>max 100ppm</td>
</tr>
<tr>
<td>Diethylene glycol</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>max 100ppm</td>
</tr>
<tr>
<td>Triethylene glycol</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>max 100ppm</td>
</tr>
<tr>
<td>Fatty Acid Composition</td>
<td>oleic acid≥58%</td>
<td>oleic acid≥58%</td>
<td>oleic acid≥58%</td>
<td>oleic acid≥98% other acid ≤0.5%</td>
</tr>
<tr>
<td>Bacterial endotoxin</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>≤12EU/g</td>
</tr>
<tr>
<td>Sterile</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>common test method in CHP 2015</td>
</tr>
</tbody>
</table>
Purpose on setting up or modifying these tests

**Peroxide value**: no more than 2

The peroxide value has a negative correlation with the stability of the formulations. High peroxide value will lead to the decrease of stability and the raise of related substances during the storage, especially some protein-drugs. It should be lowered as much as possible.

All the test result were provided by National institutes food and drug control center of China.
High peroxide value will lead to the increase of impurity I in nimodipine injection.
Purpose on setting up or modifying these tests

Composition of fatty acid: oleic acid ≥98%, other individual fatty acid ≤0.5%

Ensure product reproducibility and decrease other fatty acid and unknown impurities to the minimum.

Strict restriction on the fatty acid of high freezing point (stearic acid and palmitic acid), which is not suitable for injection.

The composition of fatty acid has influences on the other attributions: colour, viscosity, freezing test, peroxide value and solubility.
Purpose on setting up or modifying these tests

The higher the purity of oleic acid is, the less the unknown impurities exist.

content of oleic acid 72.98%
unknown impurities 13.51%

content of oleic acid 62.50%
unknown impurities 18.81%

content of oleic acid 98.88%
unknown impurities 0.09%
Purpose on setting up or modifying these tests

**Absorbance:** $\leq 1.0$ (225nm) $\leq 0.1$ (267nm)

To control the content of the “C=C” and “C=O” in peroxy radical, organic peroxide acid and other fatty acid.
Purpose on setting up or modifying these tests

**Colour**: lighter than yellow #2 (common test method in CHP 2015)

Polyosorbate 80 is colourless come from raw materials with high purity.

Conjugated double bond and unsaturated carbonyl-compound are impurities, which come out in the extremely high temperature and will make product darker.

The product will become darker when there are too much glycan, for glycan will be carbonized in the esterification.
# Purpose on setting up or modifying these tests

<table>
<thead>
<tr>
<th>Test Category</th>
<th>Parameter</th>
<th>Limit Value</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid value</td>
<td>≤1.0%</td>
<td></td>
<td>Improve the stability and application performance</td>
</tr>
<tr>
<td>Water content</td>
<td>≤0.5%</td>
<td></td>
<td>Ensure the safety</td>
</tr>
<tr>
<td>Freezing test</td>
<td>Improve the stability and application performance</td>
<td>Ethylene glycol ≤100 ppm</td>
<td>Ensure the safety</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Diglycol ≤100 ppm</td>
<td>Ensure the safety</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Triethylene glycol ≤100 ppm</td>
<td>Ensure the safety</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ethylene oxide ≤1 ppm</td>
<td>Ensure the safety</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dioxane ≤5 ppm</td>
<td>Ensure the safety</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bacterial endotoxin &lt;12EU/g</td>
<td>Ensure the safety</td>
</tr>
<tr>
<td>Sterility</td>
<td></td>
<td></td>
<td>Ensure the safety</td>
</tr>
</tbody>
</table>
Features of polysorbate 80 for injection

- Extremely low peroxide value
- Ultra-high purity (>98%)
- Improve the stability of drug formulation
- Hypoallergenic
- Excellent producibility between batches
1. Docetaxel injection

According to stability test, Polysorbate 80 may increase the impurity D in accelerated testing, nevertheless, Polysorbate 80 for injection could reduce the impurity D apparently.
The influence on the preparation by different source of polysorbate 80

2. Vitamin k1 injection

The comparison of volatile constituents in Vitamin k1 injections made of different Polysorbate 80s shows that the polysorbate 80 for injection decrease volatile constituents remarkably.
Conclusion and Suggestion

01 Injections are very important to the treatment of the patients.

02 The criteria compiling of the pharmacopoeia should pay more attention for the essential attributes of the product as much as possible.

03 The specific items of injections should be added on the basis of the general pharmacopoeia as criteria of excipients of injections.
THANK YOU!