出國報告（出國類別：參訪）

參訪法國 SEPPIC 佐劑上海青浦工廠

服務機關：行政院農業委員會家畜衛生試驗所
姓名職稱：施雨華助理研究員、謝政橘助理研究員
派赴國家/地區：中國
出國期間：**108** 年 **10** 月 **16** 日**-108** 年 **10** 月 **19** 日
報告日期：**108** 年 **12** 月 **27** 日

摘要

為推動我國動物用疫苗產業的發展，家畜衛生試驗所自 109 年起至 112 年建置動物用疫苗先導工廠，以符合國際 PIC/S GMP 標準，提供國內高品質的動物疫苗，並扶植國內動物用疫苗產業，提升國際競爭力，擴展新興市場。而在動物用疫苗研發中，佐劑於疫苗扮演重要的角色，主要功能為協助誘發、延長或增強對目標抗原產生特異性免疫反應。法國 SEPPIC 公司係屬於全球著名之佐劑供應商，於中國上海市設有佐劑生產工廠。此次特別聯繫安排參訪法國 SEPPIC（上海）特殊化學品公司，藉以深入瞭解疫苗佐劑的生產流程及管理、品管實驗室檢測項目及乳化過程的擴展：從實驗室到工廠等級等，了解佐劑與動物疫苗的應用，促進動物疫苗研發的動能，並有助於規劃畜衛所 PIC/S GMP 動物用疫苗廠之建置。此外，藉由參訪交流，緊密扣合彼此關係與帶動未來合作的契機，以落實將來研發成果的商品化及外銷國際的目標。

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壹、參訪目的

為推動我國動物用疫苗產業的發展，家畜衛生試驗所（畜衛所）自 109 年起至 112 年建置動物用疫苗先導工廠，以符合國際 PIC/S GMP 標準，提供國內高品質的動物疫苗，並扶植國內動物用疫苗產業，提升國際競爭力，擴展新興市場。而在動物用疫苗研發中，佐劑於疫苗扮演重要的角色，主要功能為協助誘發、延長或增強對目標抗原產生特異性免疫反應。SEPPIC 公司是法國最大的佐劑研發製造公司，生產製造人類與動物疫苗佐劑，在中國上海成立亞洲的製造研究工廠，主要生產動物用疫苗佐劑。此次參訪上海青浦的疫苗佐劑基地，參觀佐劑製造商的製程及管理，以及了解佐劑與動物疫苗的應用，促進疫苗研發的動能。

貳、行程紀要

本次赴中國參訪期間自 108 年 10 月 16 日至 19 日，參訪行程詳列如下行程表（表一）。

天數	日期	星期	行程內容
1	10/16	三	抵達中國上海虹橋國際機場
2	10/17	四	參訪法國 SEPPIC（上海）特殊化學品公司（青浦工廠：上海市青浦工業園區勝利路 1098 弄 59 號）
3	10/18	五	參訪法國 SEPPIC（上海）特殊化學品公司（市區辦公室:上海市南京西路 819 號中創大廈 1508 室）
4	10/19	六	返抵國門

參、法國 SEPPIC（上海）特殊化學品公司介紹

SEPPIC 是一家知名的跨國公司，總部設於法國巴黎，主要生產特殊化學品，廣泛地用於化妝品和醫藥行業（圖 1）。法國 SEPPIC 公司的研發，提供超過 70 年的創新專業佐劑和活性成分化妝品，SEPPIC 公司致力於開發，生產和銷售類別的

賦形劑，表面活性劑及活性成分，用於製藥、食品、化妝品和其他特殊化學工業生產中。

圖 1：SEPPIC 於全球的服務分布。（資料來源：法國 SEPPIC（上海）特殊化學品公司）

SEPPIC 公司自 1996 年在上海成立亞洲首個辦事機構-SEPPIC（上海）特殊化學品有限公司，是亞太地區總部，包含所有的業務部門，是亞洲首個工廠基地。自 2007 年開始運營，位於上海青浦工業園區內，擁有 2,300 平方公尺的廠房和實驗室。SEPPIC 公司主要生產動物用疫苗佐劑，獲得 ISO 9001/2008 和工業管理系統等認證，另於 2016 年獲得中國佐劑 GMP 認證。

目前臺灣、中國和香港的業務由 SEPPIC（上海）銷售團隊負責，亞太區業務則由設立在印度（孟買）、日本（東京）和新加坡的公司分別負責。

圖 2：SEPPIC 於亞洲的服務分布。（資料來源：法國 SEPPIC（上海）特殊化學品公司）

自 1974 年以來，SEPPIC 公司整合專業技術與服務，開發出 MONTANIDE™ 疫苗佐劑。這些佐劑用於改善疫苗的安全性、功效性和穩定性。這些即用型佐劑目前廣泛應用於禽類、反芻類動物、豬和魚類疫苗之中，SEPPIC 公司致力於不斷創新，對抗各種疾病，以創建更加健康的未來生活。

此次參訪上海青浦的疫苗佐劑基地，主要在於瞭解佐劑製程及管理，以及了解佐劑與動物疫苗的應用，促進後續動物用疫苗的研發動能。

肆、疫苗佐劑的生產流程參訪

10 月 17 日參訪的第一個行程是至法國 SEPPIC（上海）特殊化學品公司青浦工廠（上海市青浦工業園區勝利路 1098 弄 59 號）（圖 3），主要目的在於參觀佐劑製程管理及品管實驗室品管檢測樣品操作。

圖 3：於法國 SEPPIC（上海）特殊化學品公司青浦工廠門口合影。

此次參訪，另有日本、韓國及馬來西亞等國派員。至廠區辦公室後，先由 SEPPIC 公司亞太區商務經理 Sébastien DEVILLE 先生說明 SEPPIC 公司的簡介及目前該公司所生產的動物用疫苗使用的佐劑品項。續由 SEPPIC 公司中國區銷售和市場經理孔美萍女士說明實驗室內疫苗佐劑的乳化調配方式，另說明疫苗佐劑乳化後的品管檢測方式。

參觀工廠前，工廠管理經理說明需穿著實驗衣，配戴護目鏡與安全帽，並穿戴參觀用工作鞋，且須聽從現場人員，遵照參訪動線行走，避免意外發生，後續由廠區人員帶領參觀廠房。而佐劑的主要製程，是由礦物油及表面活性劑依一定的比例配製而成，礦物油和表面活性劑等原料均來自於法國，至青浦工廠後進行混和、過濾和包裝，然後出售（圖 4）。

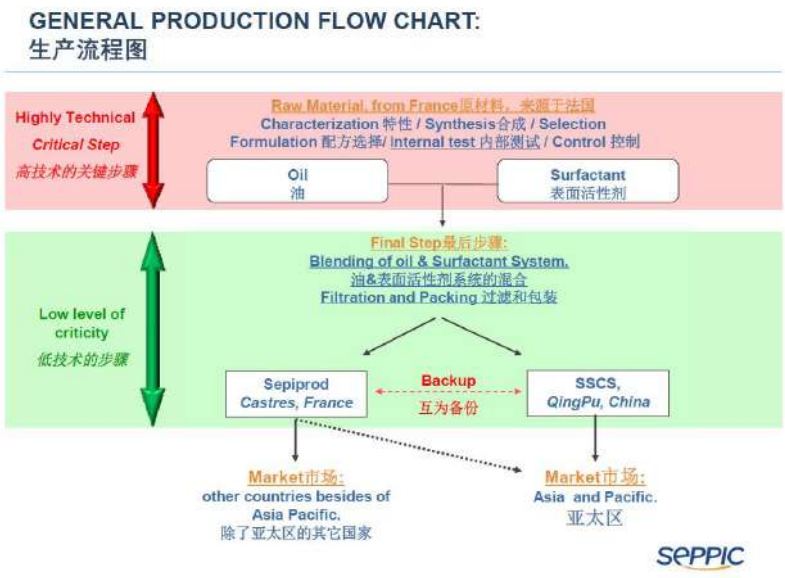


圖 4：佐劑生產流程。（資料來源：法國 SEPPIC（上海）特殊化學品公司）

參觀廠房的第一站，見到液態氮儲存桶，用於充填於佐劑內，避免佐劑的氧化酸敗（圖 5）。

圖 5：液態氮儲存桶，用於充填於佐劑內，避免氧化。

第二站至礦物油儲存區域，內有 3 組礦物油儲存槽（SS 316L, 50 m^3），礦物

油卸入儲存槽前會先經過初濾步驟（SS, 180μm），儲存槽內充填氮氣保存，另有電腦系統進行監控管理（圖 6-8）。

圖 6：礦物油儲存區域。

圖 7：儲存區內有 3 組礦物油儲存槽（SS 316L, 50 m^3）。（資料來源：法國 SEPPIC（上海）特殊化學品公司）

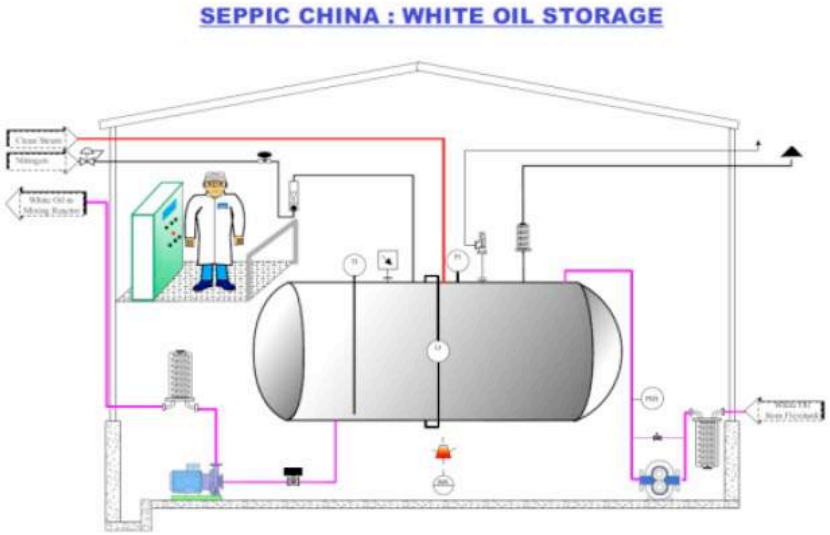


圖 8：使用電腦系統進行監控管理。（資料來源：法國 SEPPIC（上海）特殊化學品公司）

礦物油（原料）存放於儲存槽，將進行一系列的品管檢測，經由品管實驗室檢測合格後，給予合格證，准予放行使用（圖 9）。

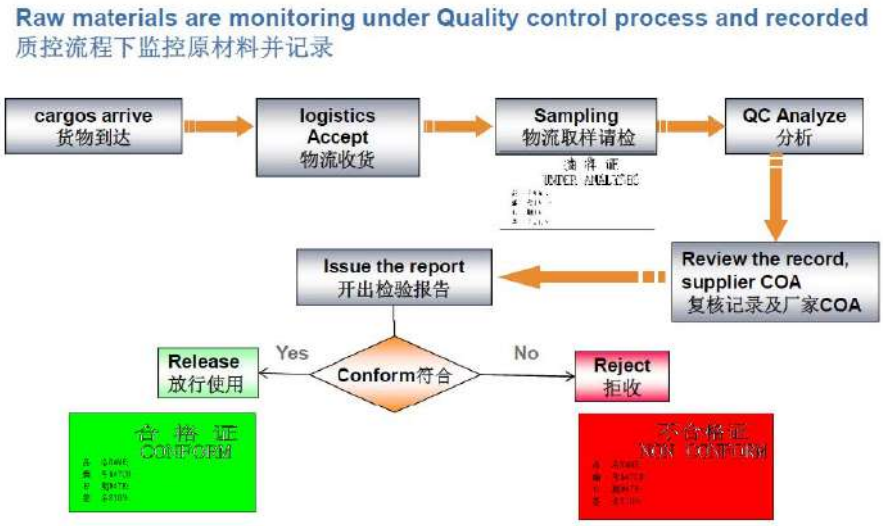


圖 9：品質管控流程下監控原物料並紀錄。（資料來源：法國 SEPPIC（上海）特殊化學品公司）

最後參觀佐劑產製的廠房（圖 10-11）。另動物用疫苗佐劑的生產流程，首先領取原料，因廠區已皆設置不鏽鋼管傳輸，由不鏽鋼管傳輸投入原料（人員禁止接觸），在高純氮氣保護下混合，進行製程的品管監控。接著利用 3 μm PP 濾芯過濾，再進行 0.2 μm PTFE 濾芯過濾，於 100 級潔淨區內灌裝。最後包裝並置於待

驗區儲存，由品管進行檢測決定是否放行，若符合標準，再至外倉庫（圖 12）。

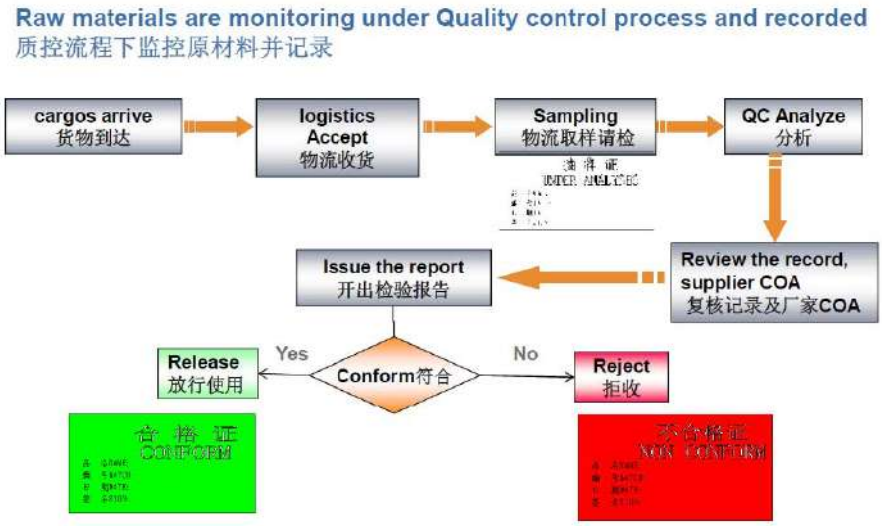


圖 10：廠房外觀。（資料來源：法國 SEPPIC（上海）特殊化學品公司）

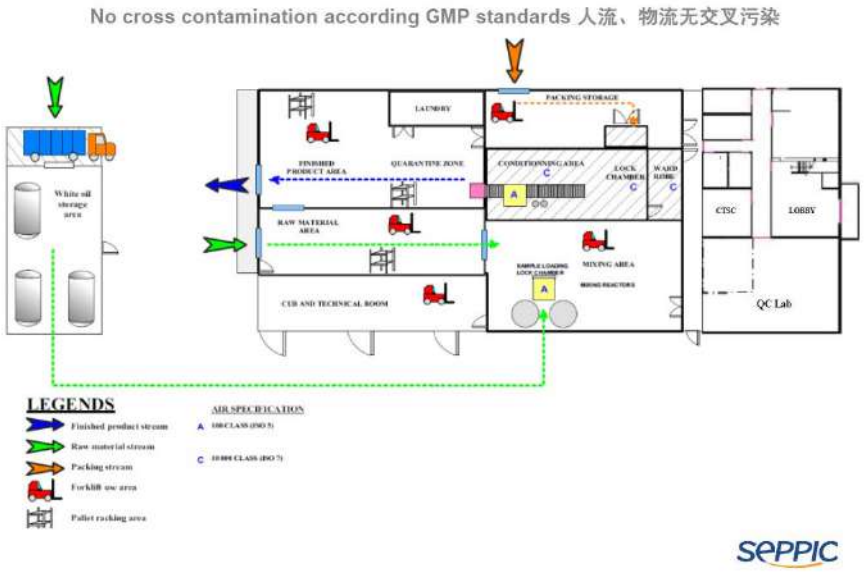


圖 11：廠房內平面圖。（資料來源：法國 SEPPIC（上海）特殊化學品公司）

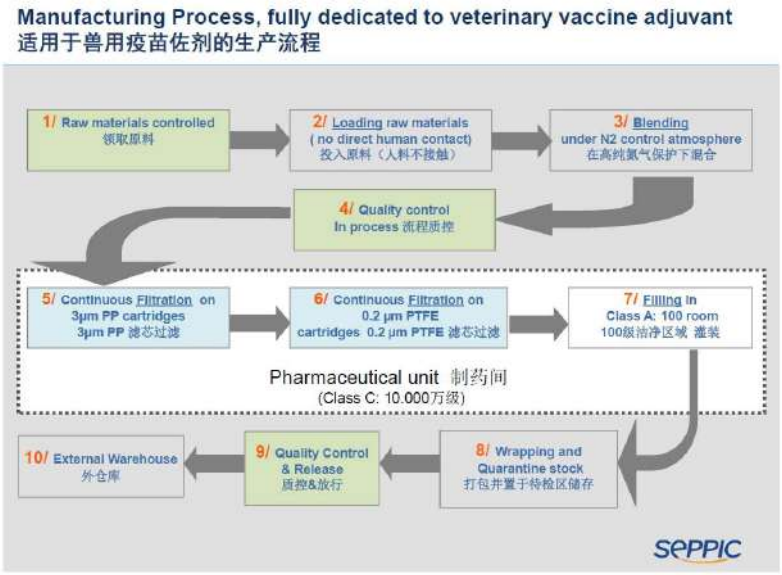


圖 12：動物用疫苗的生產流程。（資料來源：法國 SEPPIC（上海）特殊化學品公司）

進入廠區內，首先見到表面活性劑（原料）及水（原料）的儲存區域，配合不同的佐劑型號，加入不同的表面活性劑及水的比列混合而成。再進入下一個區域就是佐劑混合區域，將原料經由不鏽鋼管道輸入至混合反應器內，低速攪拌，並將氮氣以 0.1 μm 過濾加入保存（圖 13-14）。

圖 13：配製佐劑的混合反應器。（資料來源：法國 SEPPIC（上海）特殊化學品公司）

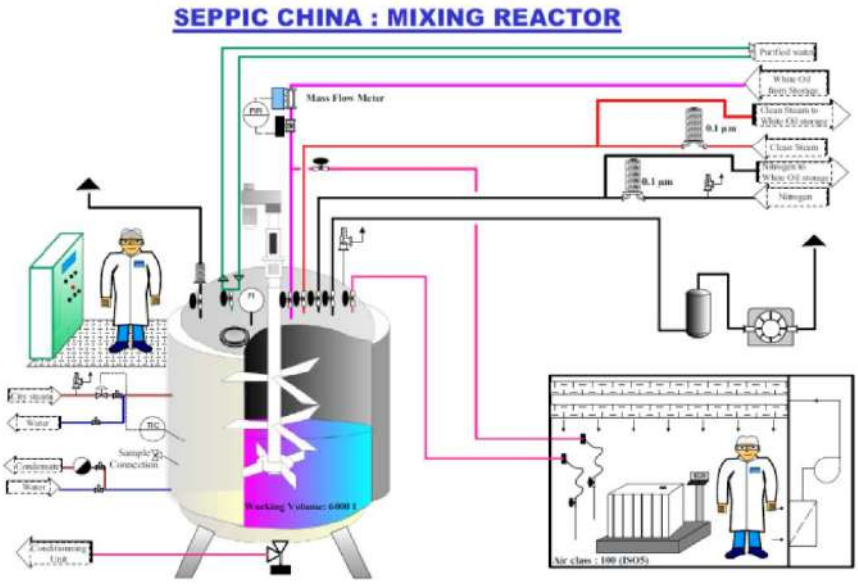


圖 14：佐劑配製混合室。（資料來源：法國 SEPPIC（上海）特殊化學品公司）

接著於 100 級潔淨區內進行灌裝，並於封桶前加入 0.1 µm 過濾的氮氣保護佐劑：並於每日檢測濾芯，以確保過濾的安全性（圖 15）。

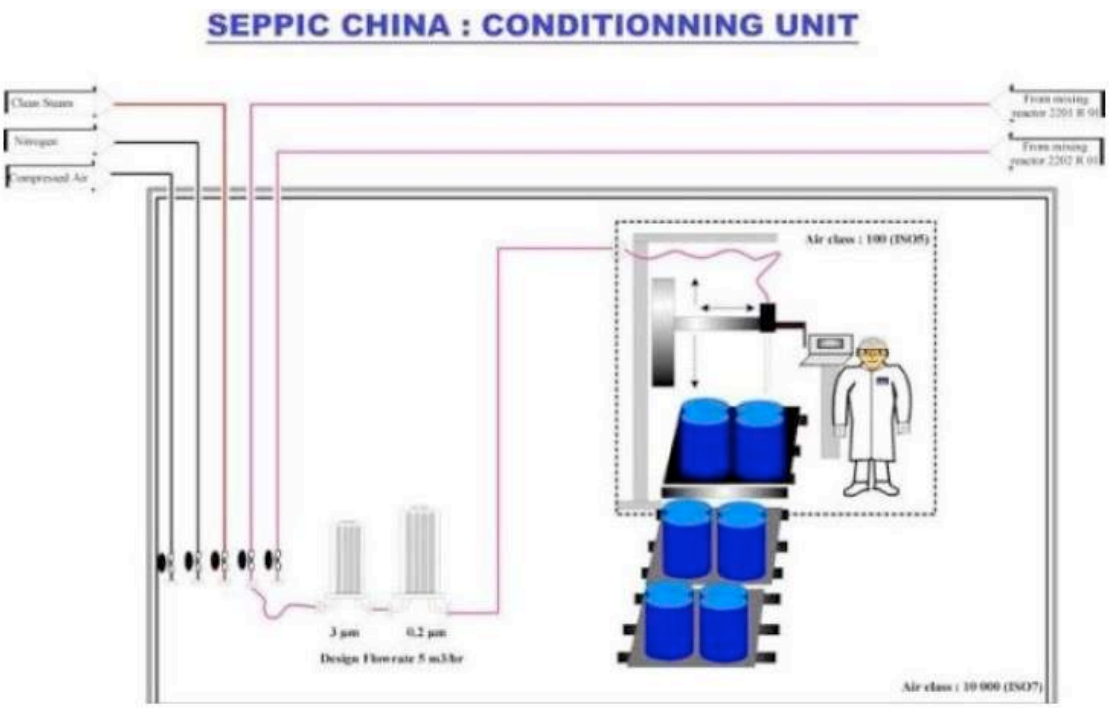


圖 15：過濾和灌裝區域。（資料來源：法國 SEPPIC（上海）特殊化學品公司）

分裝結束後，會暫存於待驗區，等待品管檢測（圖 16），決定是否放行。

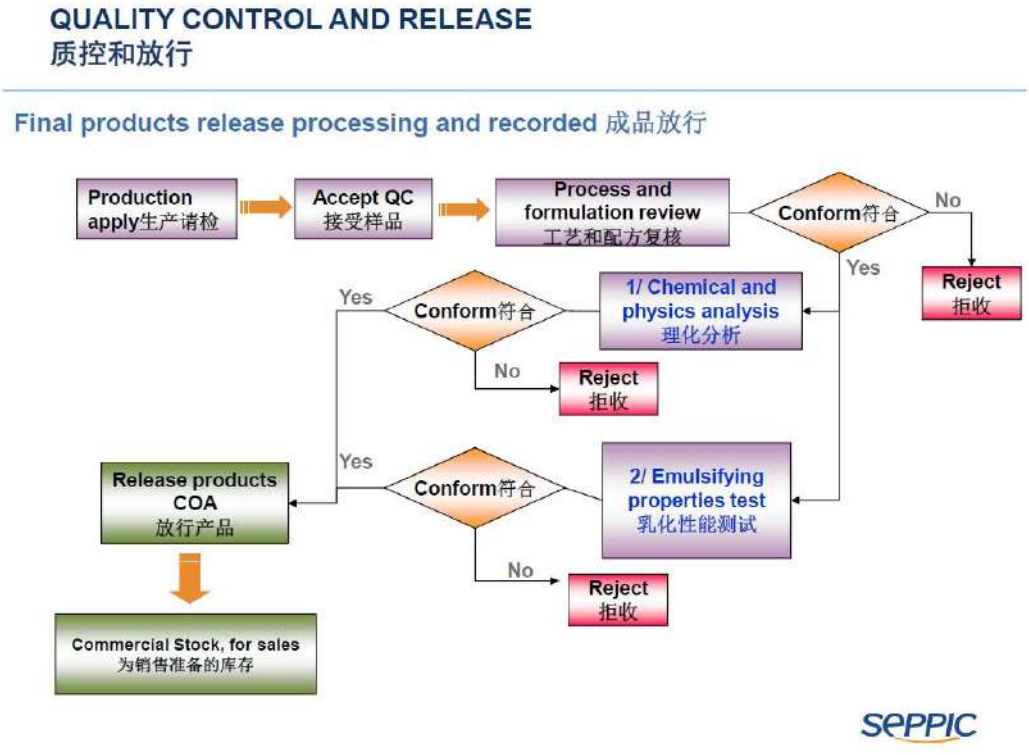


圖 16：品管檢測流程。（資料來源：法國 SEPPIC（上海）特殊化學品公司）

品管放行後，會由待驗區域移至成品區域（圖 17-18）；雖然最終產品不屬於溫度敏感產品，但整個廠房仍須配有通風和空調系統：另可聘請合格的除蟲滅鼠公司來負責控制蟲鼠等。

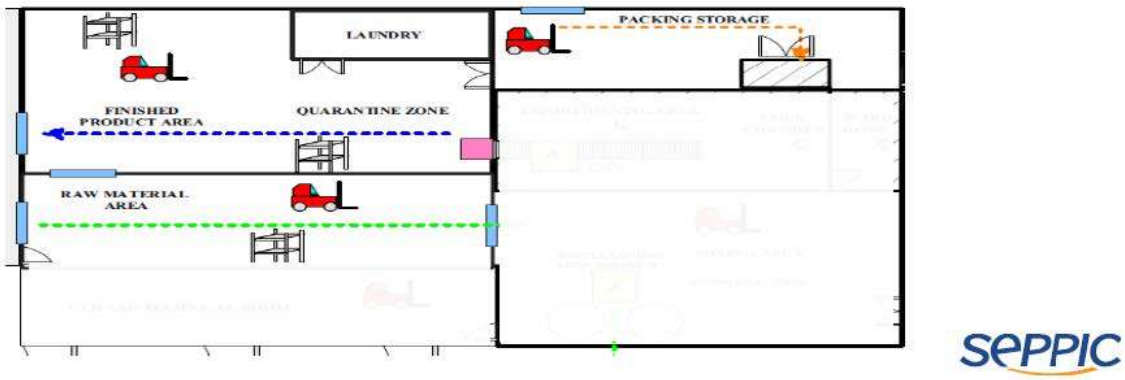


圖 17：待驗區及成品之倉儲分布圖。（資料來源：法國 SEPPIC（上海）特殊化學品公司）

圖 18：成品儲存倉庫。（資料來源：法國 SEPPIC（上海）特殊化學品公司）

伍、疫苗佐劑品管實驗室參訪

疫苗佐劑品管實驗室參訪部分，由 SEPPIC 公司中國區銷售和市場經理孔美萍女士帶領參訪。主要了解佐劑乳化流程、檢測方法及使用相關儀器設備。實驗室工作區域

可分為準備與乳化，有各類均質機依佐劑類型選擇合適之均質機進行乳化作用。技術人員示範油質佐劑乳化過程，完成乳化步驟後可利用跌落測試（Drop test）、微觀方面（Microscopics aspect）、粒度分析（Granulometry）等，檢測佐劑的性質及乳化程度（表 2、圖 19-25）。

表 2：一般佐劑品管檢測常用的方法。

	Property	Test Method
1	乳化類型 Emulsion type	跌落測試 Drop test
2	粒徑 Particle size	微觀方面 Microscopics aspect 粒度分析 Granulometry
3	流變學 Rheology	黏度 Vixcosity 可注射性 Syringeability
4	穩定性 Stability	離心試驗 Centrifugation test 即時穩定性研究 Real time stability study

技術人員說明乳化條件會因製備體積而有不同，且不同抗原種類之乳化條件，亦需再進行測試與調整。有關乳化條件部分，SEPPIC 公司可提供完成之乳化資訊供客戶參考；另有關佐劑的選擇方面，可因抗原之種類不同，SEPPIC 公司會給予適當的佐劑建議。

圖 19：跌落測試 Drop test。

圖 20：微觀方面 Microscopics aspect。

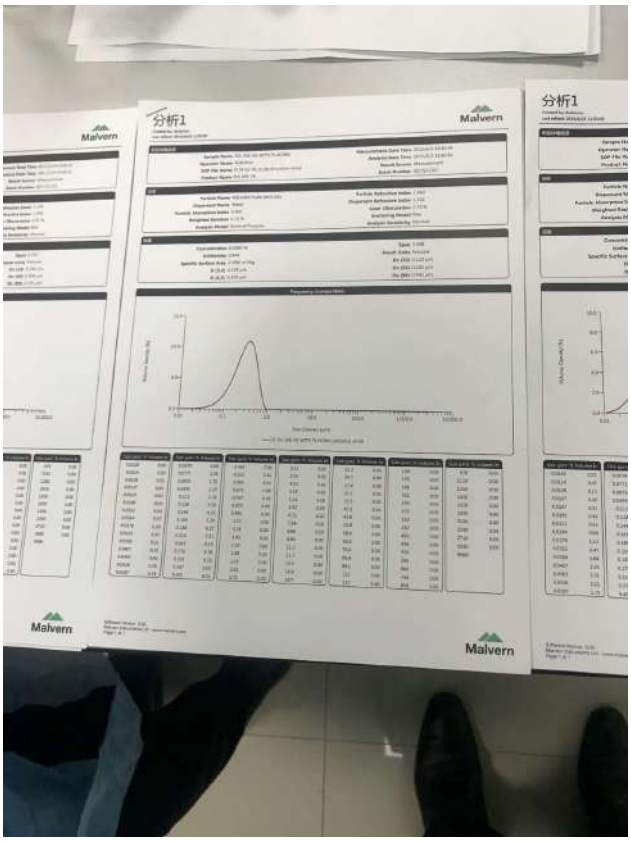


圖 21：粒度分析 Granulometry。

圖 22：黏度分析 Vixcosity。

圖 23：可注射性分析 Syringeability。

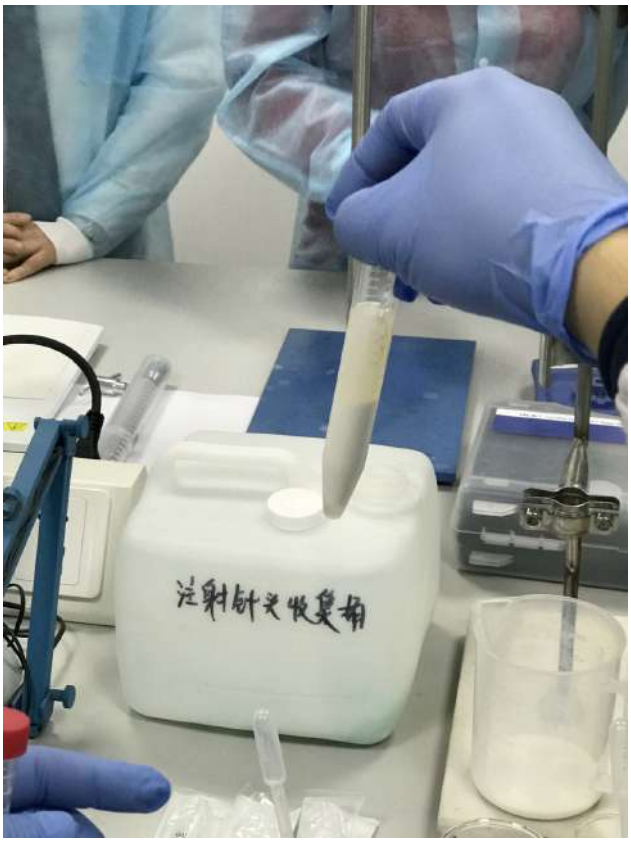


圖 24:離心試驗 Centrifugation test。

圖 25：與孔美萍經理討論佐劑的應用策略。

陸、佐劑乳化過程的擴展：從實驗室到工廠等級介紹

10 月 18 日參訪法國 SEPPIC（上海）特殊化學品公司市區辦公室（上海市南京西路 819 號中創大廈 1508 室）（圖 26），參觀 SEPPIC 公司內部及營運模式，並由孔美萍經理講解佐劑乳化過程的擴展；從實驗室到工廠等級（圖 27）。舉例以油包水（W/O）說明乳化過程，於預乳化（Pre-emulsion），將水加入油質佐劑中，以低速攪拌，形成較大的水滴；另於乳化（Emulsification）時，利用高速而在攪拌，水滴會變小，乳化會變得穩定。而在過程擴展中，關鍵的參數通常包括具有高的剪切能（high shear energy）、儀器具有均質的功能、控制乳化的溫度、使用預乳化（Pre-emulsion）的步驟等等皆須注意。而以油包水（W/O）佐劑乳化放大過程中可以分為幾個等級，如表 3。

表 3：油包水（W/O）佐劑乳化放大過程分級。

	Grade	Scal
1	200 g	Lab scale
2	10 Kg	Small pilot
3	70 Kg	Pilot scale
4	1,000 L	Industrial scale example

SEPPIC 公司依不同規模大小，提供相關的儀器機型、設定條件及實驗數據供參，如附錄參。

圖 26：於法國 SEPPIC（上海）特殊化學品公司市區辦公室門口合影。

圖 27：由孔美萍經理講解乳化過程的擴展。

柒、參訪心得及建議

一、 疫苗之效力除與抗原有關外，佐劑亦扮演重要的角色。良好的佐劑可以減少抗原的使用，亦可以延長疫苗的保護效果。疫苗研發過程中，佐劑的添加應選擇高安全性，在低濃度下就可協助抗原誘發免疫反應、方便使用、易於儲藏及價格便宜。目前國內動物用疫苗製造藥廠所使用的佐劑來源主要有國內生產之鋁膠佐劑及油質佐劑；購置國外的水質、油質及凝膠等佐劑。參訪之 SEPPIC 公司是全球知名的佐劑供應商，所生產之佐劑產品也已廣泛的應用到動物疫苗產品中。此次的參訪，瞭解佐劑的製造流程及品管檢測，雙方也彼此交流意見，可作為未來疫苗研發時佐劑選用的參考依據。

二、 目前大多的佐劑只能應用於不活化的抗原上，近年 SEPPIC 公司已研發出可運用於活毒疫苗的 IMS 佐劑，將來對於畜衛所活毒疫苗研發，可增加其免疫效力，具有加乘效果。

三、 畜衛所將自 109 年至 112 年建置動物用疫苗先導工廠，將符合 PIC/S GMP 的規範，使產程優化及提高生產品質，帶動國內動物疫苗產業的發展。而當

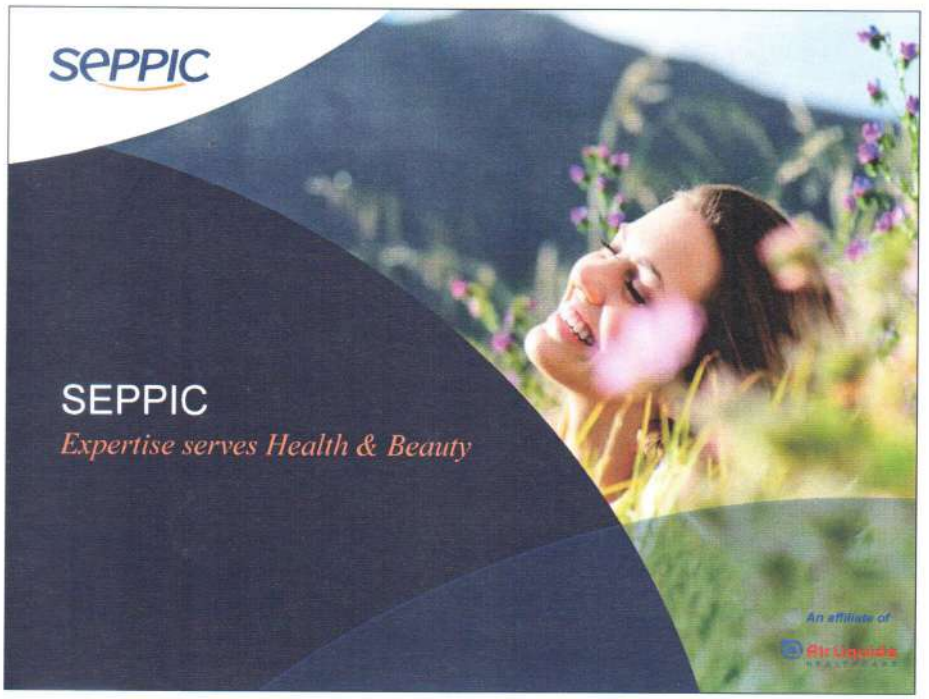
疫苗產量增加，品質提高時，佐劑乳化技術放大建立，有助於規劃將來 PIC/S GMP 動物用疫苗廠的建置。

四、 本次的參訪有助於佐劑品管檢驗技術之建立，包含檢測儀器的建置、檢測項目的確認、實驗參數的建立等，可供未來畜衛所佐劑疫苗的品管檢測。

五、 藉由本次參訪交流，可與各國相關人才相互交流（圖 28），並與 SEPPIC 公司緊密扣合彼此關係與帶動未來合作的契機，以落實將來研發成果的商品化及外銷國際的目標。

圖 28：各國參訪人員合照。

捌、附錄



Within Air Liquide Healthcare

Industrial Merchant | Large Industries | Air Liquide HEALTHCARE | Electronics | Other activities

Specialty Ingredients	Hygiene	Home Healthcare	Hospital Care
SEPPIC	schülke	VitalAire	AIR LIQUIDE Healthcare

Air Liquide Healthcare's 2017 revenues

€3.4 billion

50% in home healthcare

30% in medical gases, equipment and services

20% in hygiene and **specialty ingredients**

Within Air Liquide, world leader in gases, technologies and services

SEPPIC

Designer of specialty ingredients

❝ Seppic is a designer of specialty ingredients for health and beauty care.

As a subsidiary of Air Liquide Healthcare, it contributes to protecting vulnerable lives through prevention and well-being. ❞

SEPPIC in ASIA

Tokyo

Qing Pu

Veterinary vaccine adjuvants
疫苗佐剂
Asia Pac. Management & Marketing
Production, QC/QA, Supply Chain, HR

Established in 2008. 2008年成立

Singapore

Shanghai

Representative Office
Sales & Marketing, Finance & HR

Established in 1996 1996年成立

- 50 employees
- 4 locations, 1 production unit
- 16 countries
- 1,800 Customers

SEPPIC

MANUFACTURING UNITS:

Worldwide, 2 plants are qualified to manufacture
Montanide adjuvants (veterinary market)

- MONTANIDE adjuvants, a unique quality, worldwide
- Best customer service:
  - Stock and flexibility
  - Faster delivery, reactivity
- Reliability of supply: mutual backup

Headquarter
Production sites
AVI Customer Technical Service Centers

SSCS QINGPU OPERATIONS & SERVICES

CTSC
Customer Technical Service Center

PRODUCTION UNIT
VETERINARY VACCINE ADJUVANT
-
MONTANIDE™ ISA

TRADING
Montanide™ adjuvant from France (Castres)

Animal Health

FROM CHINA, FLEXIBILITY AND QUICKER DELIVERY
从中国发货，灵活、迅速

QP PRODUCTION 青浦工厂生产

Qualification by Castres's Expert for:
Castres专家认证产品：

- MONTANIDE™ ISA 206 VG
- MONTANIDE™ ISA 201 VG
- MONTANIDE™ ISA 50 V2
- MONTANIDE™ ISA 61 VG
- MONTANIDE™ ISA 70 VG
- MONTANIDE™ ISA 71 VG
- MONTANIDE™ ISA 71 R VG

Importation and storage of Montanide produced only in Castres (FRANCE):
进口存储Castres产品：

- MONTANIDE™ ISA 11 R VG
- MONTANIDE™ ISA 15 A VG
- MONTANIDE™ ISA 28 VG
- MONTANIDE™ ISA 760 VG
- MONTANIDE™ GEL 01
- *others, depending on market request*
  市场需要的其他产品

TRADING 贸易

Deliveries to Asian countries
满足亚洲所有国家的物流送达.

GENERAL PRODUCTION FLOW CHART:
生产流程图

Highly Technical
Critical Step
高技术的关键步骤

Raw Material, from France原材料，来源于法国
Characterization 特性 / Synthesis合成 / Selection
Formulation 配方选择/ Internal test 内部测试 / Control 控制

Oil 油 — Surfactant 表面活性剂

Final Step最后步骤:
Blending of oil & Surfactant System,
油&表面活性剂系统的混合
Filtration and Packing 过滤和包装

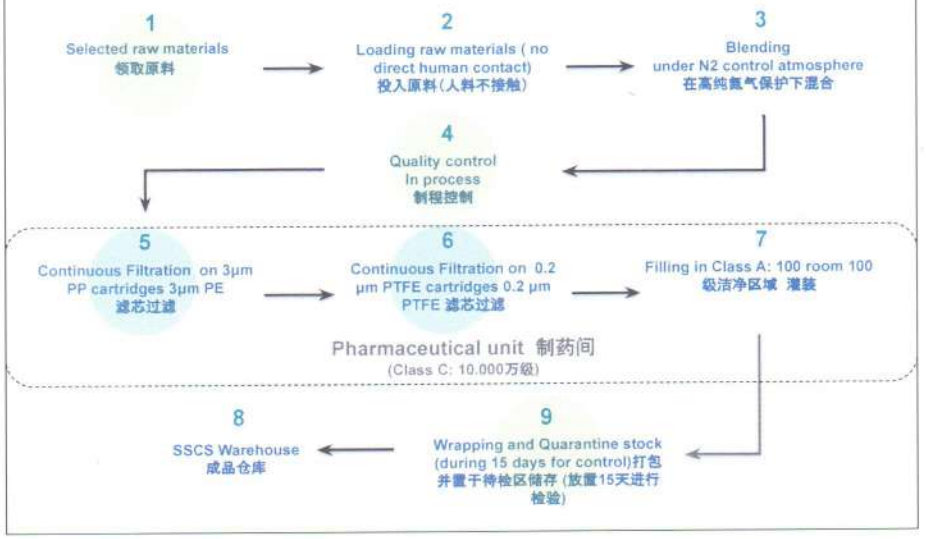
Low level of criticity
低技术的步骤

Sepiprod *Castres, France* — Same quality 互为备份 — SSCS, *QingPu, China*

Market市场:
other countries besides of Asia Pacific.
除了亚太区的其它国家

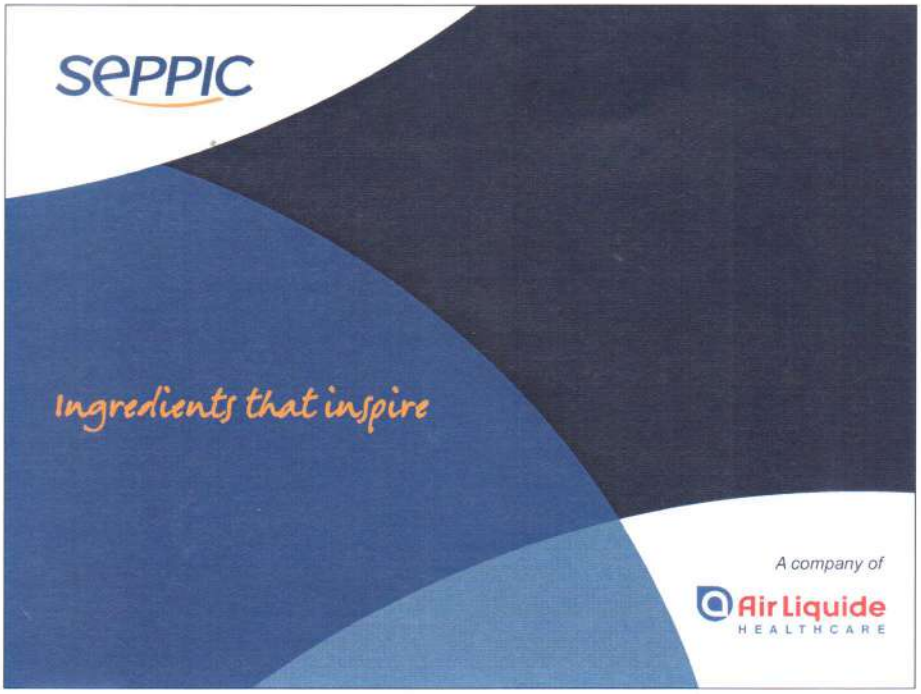
Market市场:
Asia and Pacific.
亚太区

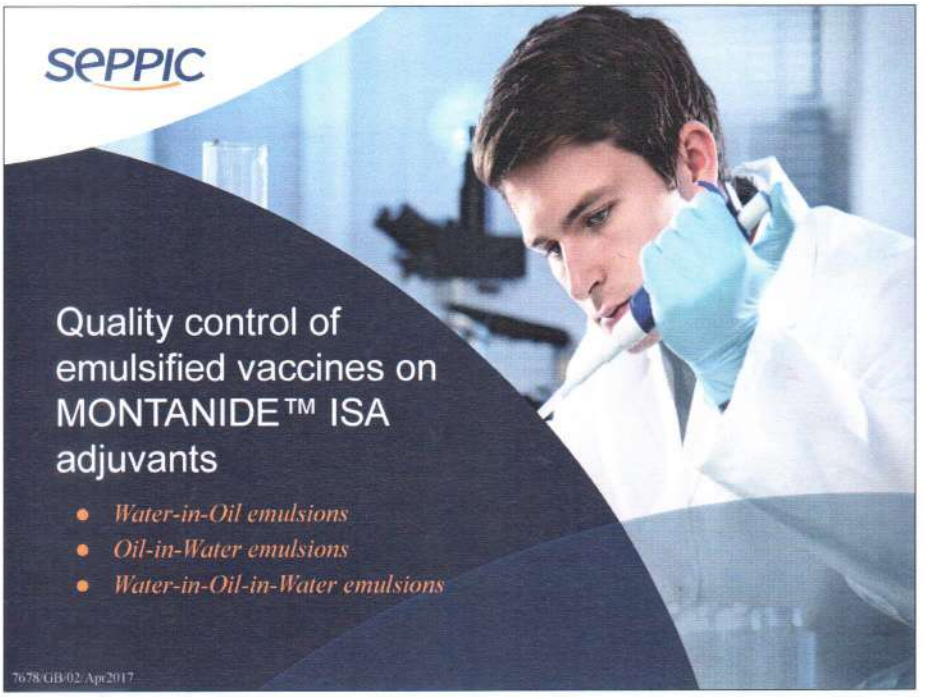
Manufacturing Process, fully dedicated to veterinary vaccine adjuvant
适用于兽用疫苗佐剂的生产流程



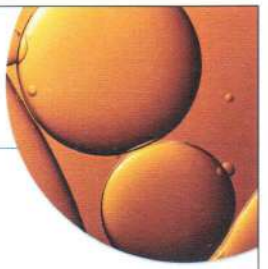
QingPu QHSE standards

CTSC: Excellence of assistance to our customers (AVI & COS)
客户技术服务中心:为客户提供帮助(AVI & COS)


SEPPIC
Ingredients that inspire
A company of
Air Liquide
HEALTHCARE



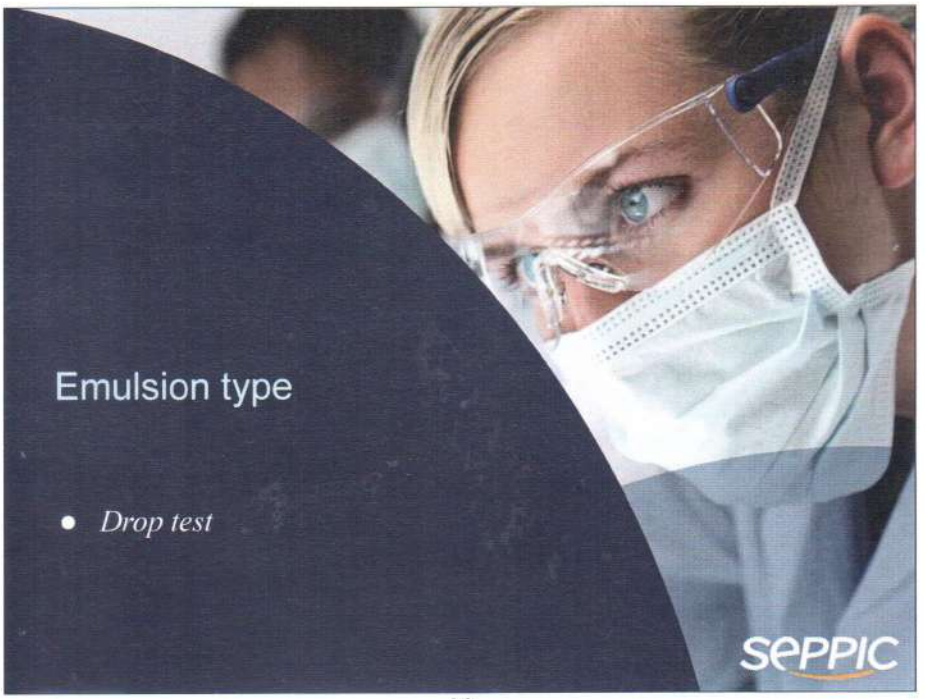
Control of vaccines



1. Emulsion type
   - **Drop test**
2. Particle size
   - **Microscopic aspect**
   - **Granulometry**
3. Rheology
   - **Viscosity**
   - **Syringeability**
4. Stability
   - **Centrifugation test**
   - **Real time stability study**

Emulsions need some time to mature so characterisation tests are always performed the day after manufacture.

ingredients that inspire SEPPIC



1. Emulsion type: *Drop test*

In order to check the type of emulsion:
- pour carefully 1-2 droplets in a beaker of water
- give a gentle stir

W/O emulsion ➢ The drop stays at the surface

O/W emulsion ➢ The drop diffuses into water

W/O/W emulsion ➢ One part stays at the surface
➢ One part diffuses into water

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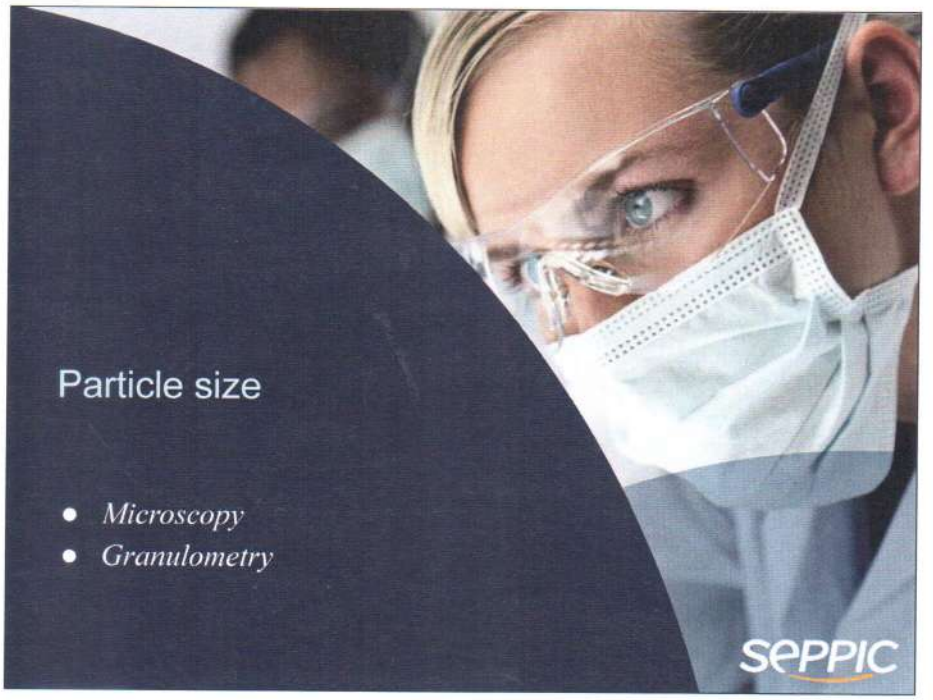
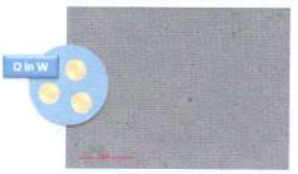


2. Particle size: *microscopic aspect (1)*

- Example of ideal W/O emulsion with optimised formulation and good process
- Homogeneous and thin emulsion,
- Particle size around 1 µm
- Slow release of antigen
- Expectation of good vaccine stability

- Example of W/O emulsion with non optimised formulation and/ or wrong process
- Heterogeneous emulsion, with some large droplets
- Risk of immediate release of antigen
- Expectation of poor vaccine stability



- Example of good W/O/W or O/W emulsion
- Homogeneous and thin emulsion,
- Particle size < 1 µm
- Expectation of good vaccine stability

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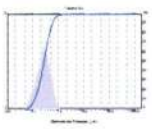
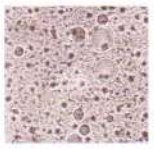
2. Particle size: *microscopic aspect (2)*



- Example of good W/O/W emulsion

- Homogeneous and thin emulsion, particle size below 1 µm
- Expectation of good vaccine stability



TEST SCALE RECORDED AND SCANNED WITH THE SAME RATIO THE SPACE BETWEEN EACH BAR IS 10µm

- Example of poor W/O/W emulsions

- Heterogeneous emulsion, with some large droplets
- Expectation of poor vaccine stability

2. Particle size: *granulometry*

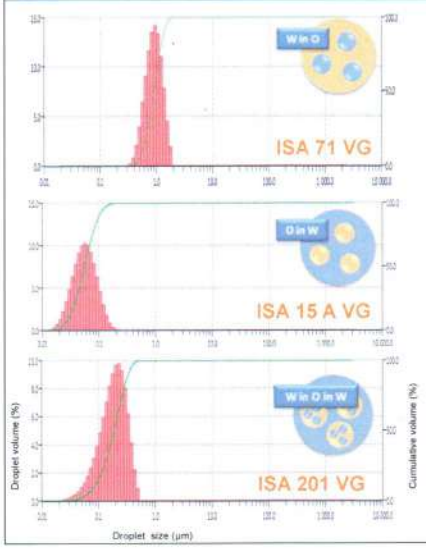
- Malvern MasterSizer 3000

- Measure of particle size distribution of the emulsion by laser diffraction
- Particle sizing from 0.01 µm to 3500 µm

- Particle sizing results are highly dependent on:

- Equipment, method, sampling, diluent...

2. Particle size: *granulometry*



Emulsion particle size distribution

- One population of droplets
- 2 values of note:

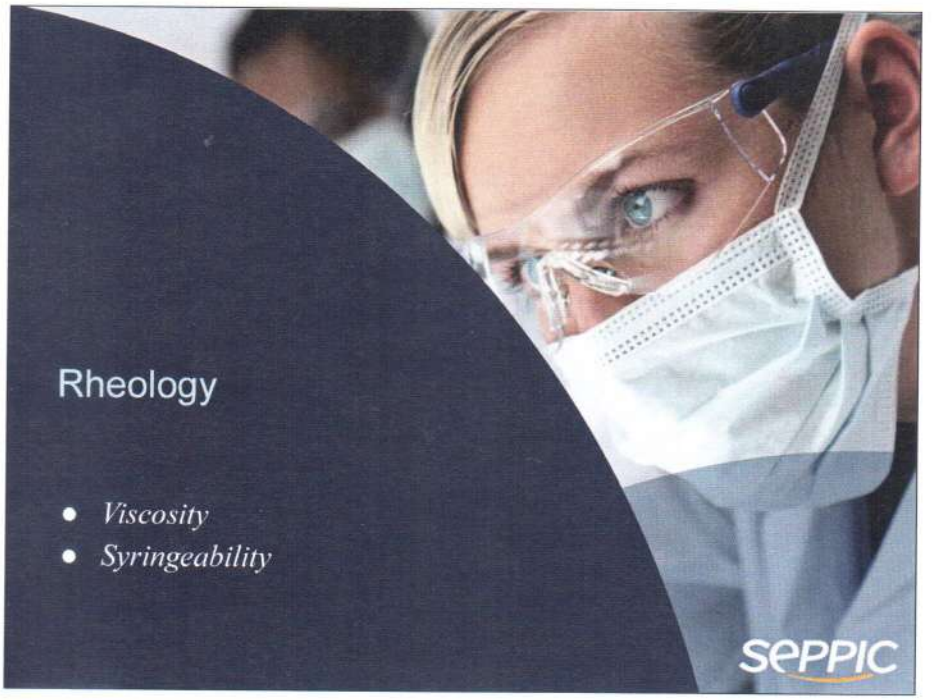
➢ D(v; 0.5): Volume median diameter

50% of the volume of the antigenic phase is in droplets larger than this value and the other half is smaller.

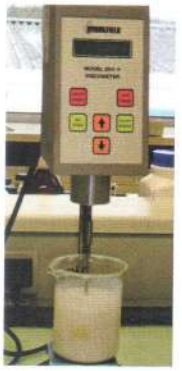
➢ D(v; 0.9)

90% of the volume of the aqueous phase is in droplets smaller than this value.

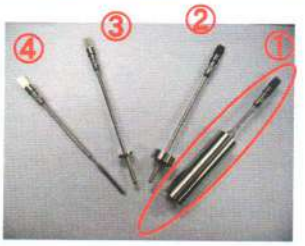
MONTANIDE adjuvant	Emulsion type	Adjuvant content in emulsion	D(v;0.5) (µm)	D(v;0.9) (µm)
ISA 71 VG	W/O	70% wt	0.8	1.3
ISA 15 A VG	O/W	15% wt	0.06	0.12
ISA 201 VG	W/O/W	50% wt	0.15	0.45



3. Rheology: *viscosity*



Measure of a fluid resistance to a flow



Viscosity measure highly dependent on method
- Brookfield viscometer DVI+
- LV spindle *(1, 2, 3, 4: model depending upon viscosity range)*
- 60 rpm

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3. Rheology: *syringeability*

- Test designed by SEPPIC to give an idea of the dynamic viscosity through a needle 21 G
- Materials:
  - 10 mL syringe *(piston without rubber)*
  - Needle: 21G / 25 mm
  - Calibrated weight : 3.306 kg
- Method:
  Time necessary to release 10 mL of emulsion from a 10 mL syringe with 21G needle when a specific weight is applied on the piston

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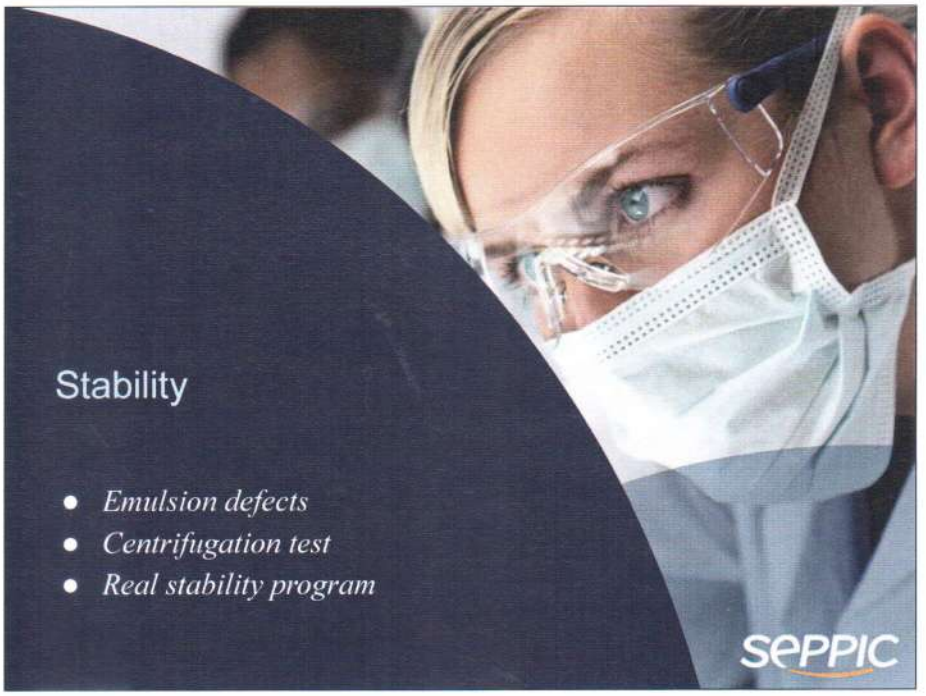
3. Rheology: *viscosity and syringeability*

MONTANIDE adjuvant	Emulsion type	Adjuvant content in emulsion	Brookfield viscosity (mPa.s)	Brookfield spindle/ speed	Syringeability Approximate value (s)
ISA 70 VG, ISA 71 VG		70% wt	30-45	LV spindle 2 60 rpm	25 s
ISA 61 VG		60% wt	35-50		25 s
ISA 50V2		50% vol.	220-340		50-55 s
ISA 15 A VG		15% wt	2-6	LV spindle 1 60 rpm	< 6 s
ISA 25 VG		25% wt	2-6		< 6 s
ISA 201 VG		50% wt	~ 30	LV spindle 2 60 rpm	7 s
ISA 206 VG		50% wt	~ 30		7 s

SEPPIC



4. Stability

- **Stable** emulsion → **No** defect
  → **Non critical** defect

Non critical *defect* :
- when the properties of the emulsion are conserved
- hand reversible defect

Critical *defect* :
- severe coalescence happens with permanent modification of droplet size
- breakage => impact on vaccine properties or process reproducibility?

- Stability: combination of ***intensity*** of defect and ***time of apparition***
- Emulsion stability assessed by
  - Resistance to ***centrifugation***
  - ***Real time stability*** program

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4. Stability: *resistance to centrifugation*

Vaccine stability → Physical stability *(gravity)*

Vaccine stability → Chemical/biological stability *(antigen/surfactant interactions)*

- **Centrifugation**: simulation of gravity accelerated stability
  - Qualitative assessment of process efficacy at time 0
  - Not a quantitative prediction of shelf life.

- **Protocol**
  - 10 mL samples
  - speed: **2570 G** (3700 rpm)
  - 30 min at 20°C
  - record the defects

- **Centrifugal force**

$$G = 1.119 \times 10^{-5} \times R \times N^2$$

R: centrifuge radius (cm)
N: rotational speed (rpm)

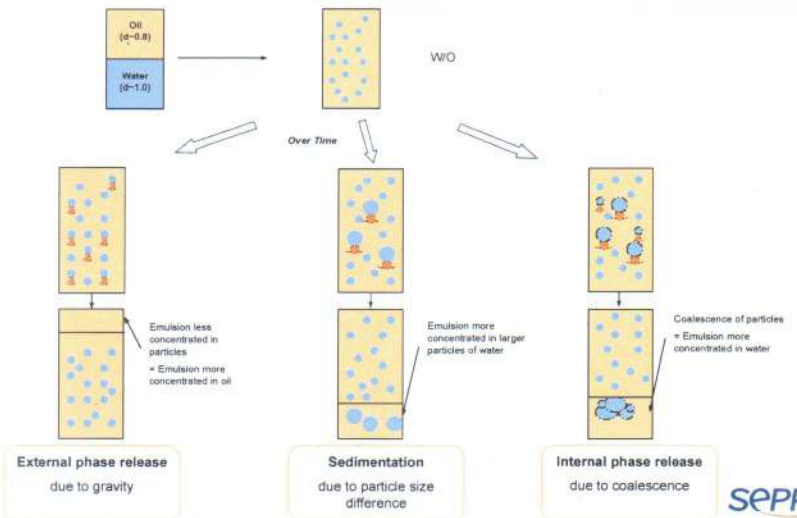
4. Stability: *real time stability program*

- Emulsion appearance checked at time 0
- Evolution of emulsion aspect under stressed conditions:
  - Samples stored in glass bottles at 4/ 20/ 37°C
  - Aspect checked over time and defects recorded
  - *WOW emulsions made in direct process ⇨ 37°C stability not performed, as it is not correlated to emulsion long term stability*

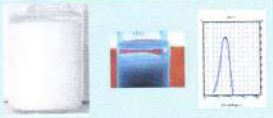
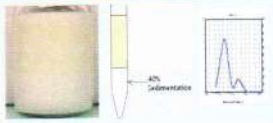
Purpose	Temperature storage	15 days	1 month	3 months	6 months	1 year	As per needs
Process efficiency assessment	4°C	✓	✓				
	20°C	✓	✓				
	37°C (not WOW)	✓	✓				
Shelf life definition	4°C	✓	✓	✓	✓	✓	...
	20°C	✓	✓	✓	✓	✓	...
	37°C (not WOW)	✓	✓				

➢ Vaccine shelf life definition, routine control, process optimisation

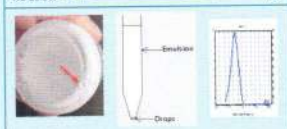
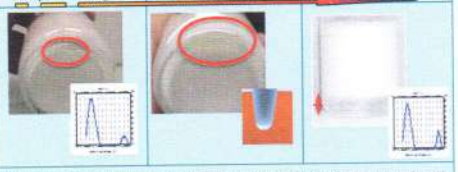
4. Stability: *possible defects of W/O emulsions*



4. Stability: *defects of W/O emulsions (1)*

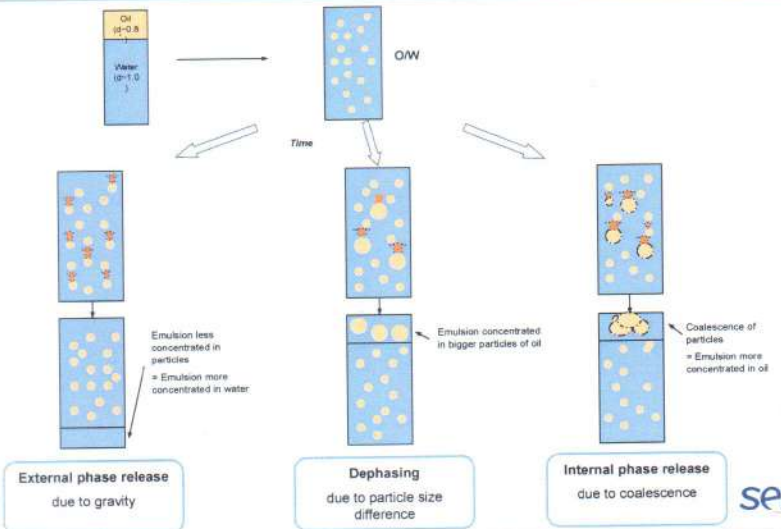
Defect name	Non critical defects	Critical defects
	0% → 30%	30% → 100%
External phase release **Oil layer**	 **Non critical defect** - easily reversed by hand shaking- - back to initial homogeneous concentration of antigen droplets after shaking	 - Large oil layer: still no difference in vaccine behaviour as hand shaking will provide homogenous vaccine again. - **Critically aesthetic defect** : process could need improvement.
Two layers of emulsion **Sedimentation**	 **Non critical defect** - easily reversed by hand shaking- - back to initial homogeneous concentration of antigen droplets after shaking	- Colour contrast might increase over time - Upper layer could potentially evolve towards oil layer over time. - Strong sedimentation might be improved by process optimisation.

4. Stability: *defects of W/O emulsions (2)*

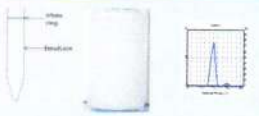
Defect name	Non critical defects	Critical defects
	No defect → Drop	Very small breakage / Small breakage (<1%) / Breakage (>1%)
Internal phase release **Water layer / coalescence**	 - Sometimes difficult to see - if the drop does not evolve in larger breakage over time, it can be tolerated	 - Release of aqueous phase is **critical**, as it needs high shear energy to get re-homogenised: hand shaking is insufficient. - Needs process improvement

- Importance of the defect depends on the time of apparition:
  - Presence of a drop after one year storage at 20°C is not a problem
  - Presence of a drop after one month storage at 4°C is a warning, as this can remain the same for one year or get worse over time

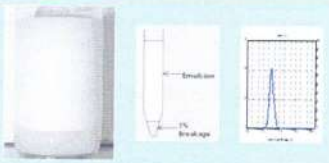
4. Stability: *possible defects of O/W emulsions*



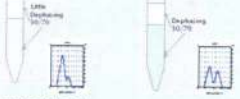
4. Stability: *defects of O/W emulsions (1)*

Defect name	Non critical defects	Critical defects
Internal phase release **Oil layer**	0% — 5-10%  **Non critical defect** - easily reversed by hand shaking- - back to initial homogeneous concentration of antigen droplets after shaking	>5-10% - Tolerated up to 5-10%, depending on storage temperature and time of apparition
Two layers of emulsion **Creaming**	0% — 20%  **Non critical defect** - easily reversed by hand shaking- - back to initial homogeneous concentration of antigen droplets after shaking	>20%  - Large creaming: still no difference in vaccine behaviour as hand shaking will provide homogenous vaccine again. - **Critically aesthetic defect** if important defect.

4. Stability: *defects of O/W emulsions (2)*

Defect name	Critical defect
External phase release **Water layer**	 - Looks like **breakage** (critical defect of W/O emulsion) - Presented as critical and not acceptable for O/W emulsions - Not frequent with our O/W emulsions

4. Stability: *defects of W/O/W emulsions (1)*

Defect name .	Non critical defects	Critical defects
Internal phase release **Oil layer**	0% — 5%  **Non critical defect** - easily reversed by hand shaking- - back to initial homogeneous concentration of antigen droplets after shaking	5-10% — 100%  - Presence of oil layer can be tolerated up to 5-10%, depending on storage temperature and time of apparition - Needs to be improved.
Two layers of emulsion **Creaming**	 **Non critical defect** - easily reversed by hand shaking- - back to initial homogeneous concentration of antigen droplets after shaking	 - Still not critical defect as reversible by hand shaking, but cannot be accepted if very heterogeneous aspect.

4. Stability: *defects of W/O/W emulsions (2)*

Defect name	Non critical defects	Critical defects
External phase release **Water layer**	No defect → Drop  	Breakage (>1%) 
	- Sometimes difficult to see - if the drop does not evolve in larger breakage over time, it can be tolerated	- Looks like severe breakage for a W/O emulsion - For W/O/W emulsions, defect often linked with non respect of 31°C temperature during emulsification process

- Importance of the defect depends on the time of apparition:
  - Presence of a drop after one year storage at 20°C is not a problem
  - Presence of a drop after one month storage at 4°C is a warning, as this can remain the same for one year or get worse over time

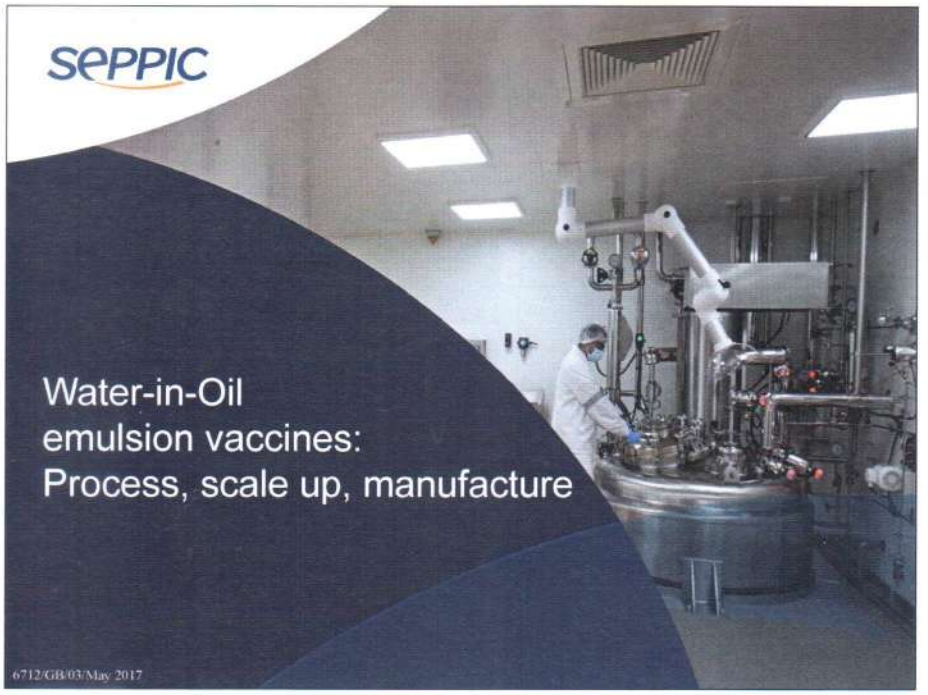
4. Stability: *influencing factors*

The stability of an emulsion can be influenced by:

- Oil and surfactant
  - Each oil can be defined with a required HLB value
  - Surfactant must be adapted to the oil and to the type of emulsion
  - Concentration and HLB of the surfactant can influence stability with a defined oil phase

MONTANIDE™ ISA adjuvant, ready to use

- Oil/water ratio
  - Each adjuvant is optimised for one oil to water ratio (or range of ratio for some flexible adjuvants)
  - With more dispersed phase (antigen), emulsion viscosity will increase, but stability will be modified
- Antigenic phase
  - Can have surfactant properties which can modify the HLB value
  - Other components (LPS, PEG) can also affect stability
- Manufacturing process
  - Duration, mixing speed, homogeniser type have to be optimised
- Temperature storage of the vaccine
  - Store and transport refrigerated (2-8°C)

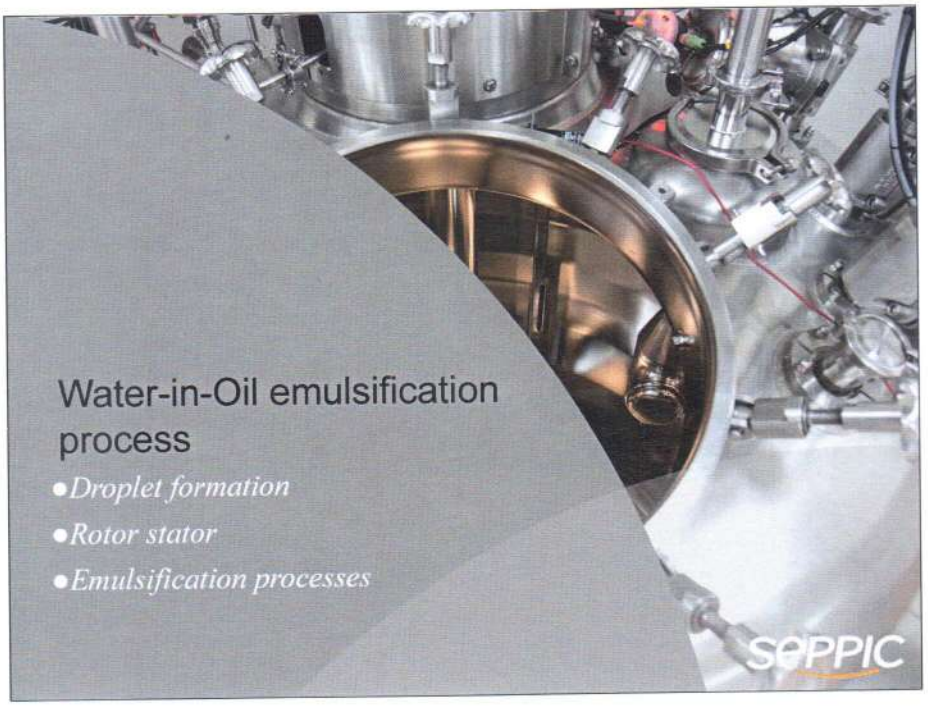


Vaccine adjuvant and emulsions

Our adjuvant development aims to obtain:

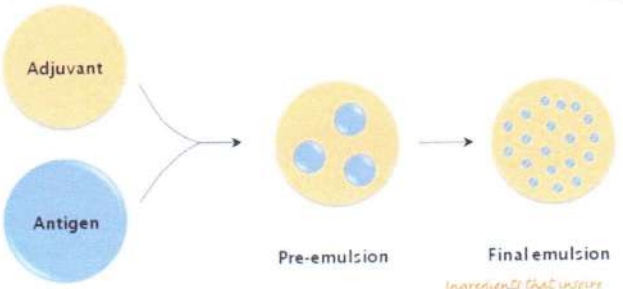
- a **product**
  - injectable, homogeneous and sterile,
  - efficient and safe
  - robust and stable, able to smoothen antigen variability
- a **process**
  - robust and reproducible at large scale
  - optimised for equipment
  - compatible with manufacturing needs

1. **Water-in-oil emulsification process**
2. **Process scale up**
3. **Water-in-oil processes at different scales**



W/O emulsification

- **Pre-emulsion**
  - Addition of water in oil
  - Low shear agitation
  - Formation of large droplets
- **Emulsification**
  - High shear energy
  - Droplet size reduction
  - Emulsion stabilisation



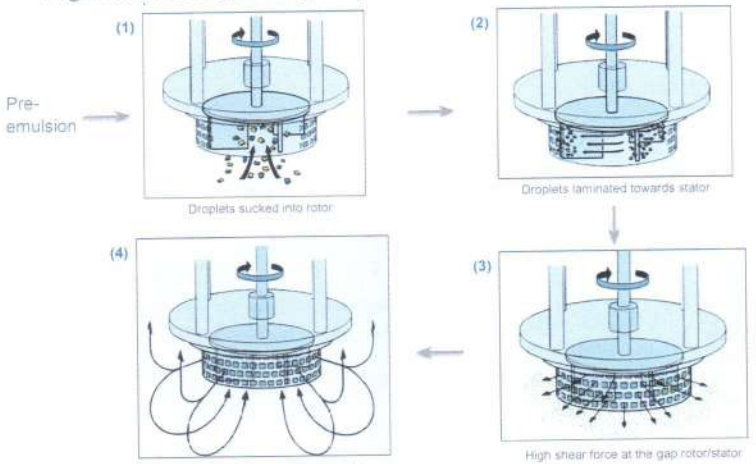
5

SEPPIC ingredients that inspire

Rotor stator mixer principle

High shear energy brought by rotor stator mixer:

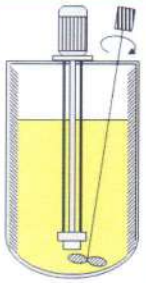
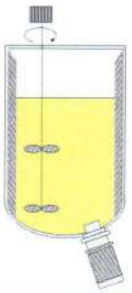
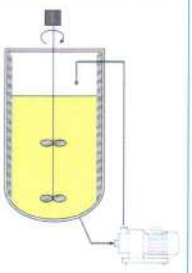
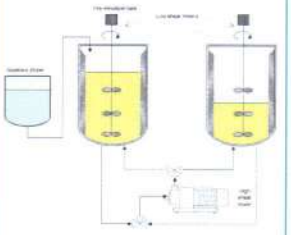
Pictures courtesy of Silverson Ltd



6

SEPPIC

Subtypes of rotor stator processes

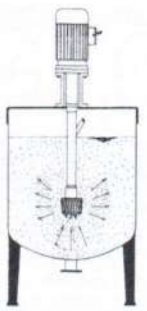
Batch process		Inline process	
Overhead / Immersion	Bottom driven	Recycling	Transfer
			

Ingredients that inspire SEPPIC

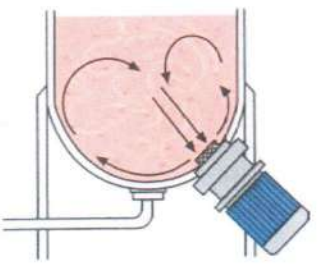
Batch processes

- Easy to use
- Reproducible
- Expensive at large scale (high power consumption)

Overhead mixer



Bottom driven mixer

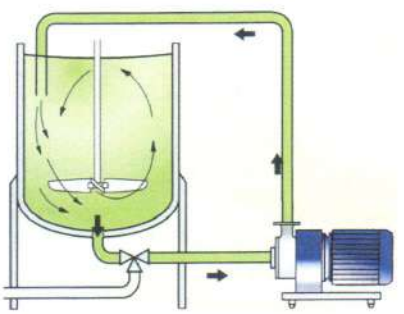


Picture courtesy of Silverson Ltd

Ingredients that inspire SEPPIC

Inline processes: Recycling

- Efficient process if good recycling
- Agitation critical to emulsion homogeneity
- More complex to clean/sterilise than batch process
- Longer process than batch process
- Statistical recirculation times for each droplet

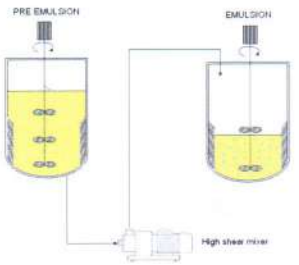


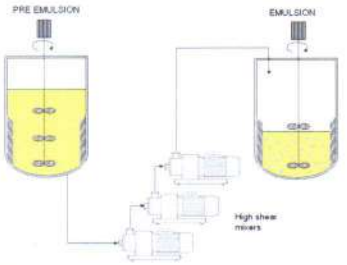
Picture courtesy of Silverson Ltd

Ingredients that inspire SEPPIC

Inline processes: Transfer

- Process needs two manufacturing tanks
- Full control of process
- More complex to clean/sterilise
- Longer process than batch process
- Several passes are required





- Or less passes with serial mixers

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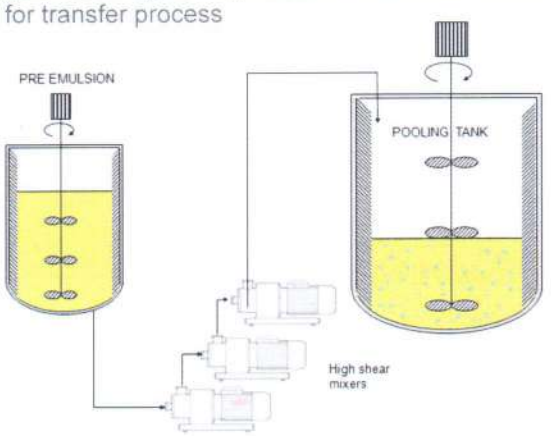
Ingredients that inspire SEPPIC

Pooling

- Final batch size increased by combining several sub-lots
- Can be applied to all types of processes mentioned before
- Example for transfer process



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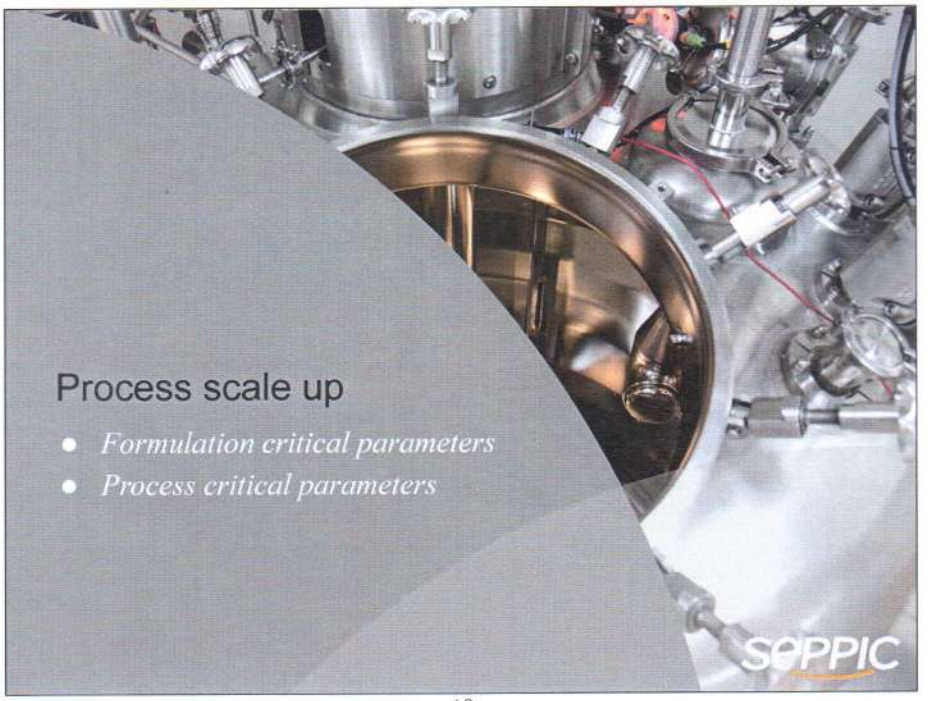
SEPPIC



Process scale up

- *Formulation critical parameters*
- *Process critical parameters*

SEPPIC

Process scale up

- Insure vaccine production from lab to industrial scale
  - Transfer from R&D to manufacturing
  - Optimisation to answer sales increase or cost optimisation
- Increase chances of success
  - Accommodating existing tools or finding the right balance between equipment investment and process efficiency
  - Minimising risks (feasibility, maintenance, forecasts)
  - Optimising working conditions: safe process, compatible with industrial needs (duration, consistency, records)
- Scale up: guaranty for long life of vaccine production process

Ingredients that inspire SEPPIC

System critical parameters

- Scale up = reproduce similar quality and properties at different scales
- Need to characterise the **system {equipment + product}**

➢ What are the critical parameters?

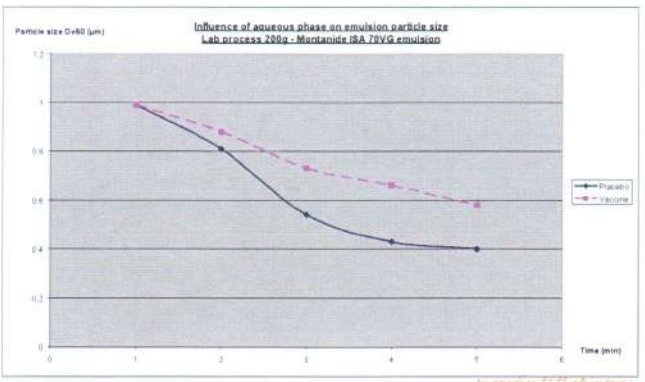
Formulation critical parameters
- Ratio oil / water,
- Aqueous phase composition *(concentration, surfactant compatibility…)*

Process critical parameters
- Exposure to high shear energy *(mixer, rotor speed, position, process duration),*
- Equipment homogenisation capability *(tank geometry, agitators, deflectors),*
- Emulsion temperature,
- Pre-emulsion step

Ingredients that inspire SEPPIC

Formulation critical parameters
Aqueous phase composition (1)

- Influence of aqueous phase (antigen, placebo…) on vaccine:
  - **Physical properties (droplet size…)**
  - **Chemical properties (stability…)**



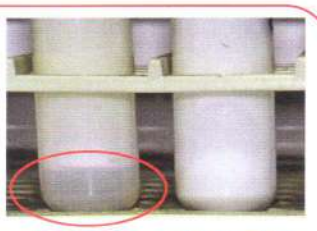
Ingredients that inspire SEPPIC

Formulation critical parameters
Aqueous phase composition (2)

- Interaction antigen/adjuvant can change the stability

W/O emulsion

Aqueous phase: model destabilising antigenic medium



Stability result after 6 months at 20°C

ISA 71 VG **Broken**

ISA 71 *R* VG Stable

- Vaccine: synergy {antigen/adjuvant}

Compatibility/ stability is to be assessed at lab scale along with efficacy and safety.

ingredients that inspire SEPPIC

Process critical parameters

- Exposure to **High Shear (HS) energy**: rotor-stator
  - HS mixer: geometry, tip speed (radius x rotation speed)
  - Mixing duration
- Equipment **homogenisation** capability
  - Tank geometry (dimensions, baffles...)
  - Agitation : speed, type/ number/ size of low shear agitators
  - Speed of aqueous phase addition
- Emulsion **temperature**
- Use of a **pre-emulsion** step

➢ Scale factor
  - Maintain homothety: keep geometric ratios constant as much as possible between the scales
  - Need to adapt to logistic constraints with available equipments at lab scale and workshop

➢ Validate the process with **industrial trials** and real time stability.

➢ **Changes** in phase ratio, batch size, antigenic phase (multivalent, concentration) are process deviations and require **additional validation work**.

ingredients that inspire SEPPIC

Process critical parameters
Tip speed

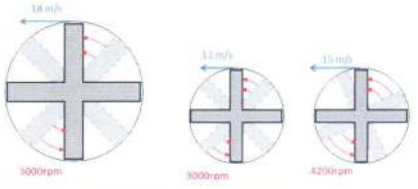
A W/O emulsion is stabilised through droplet size reduction by high shear energy, which depends on rotor speed.

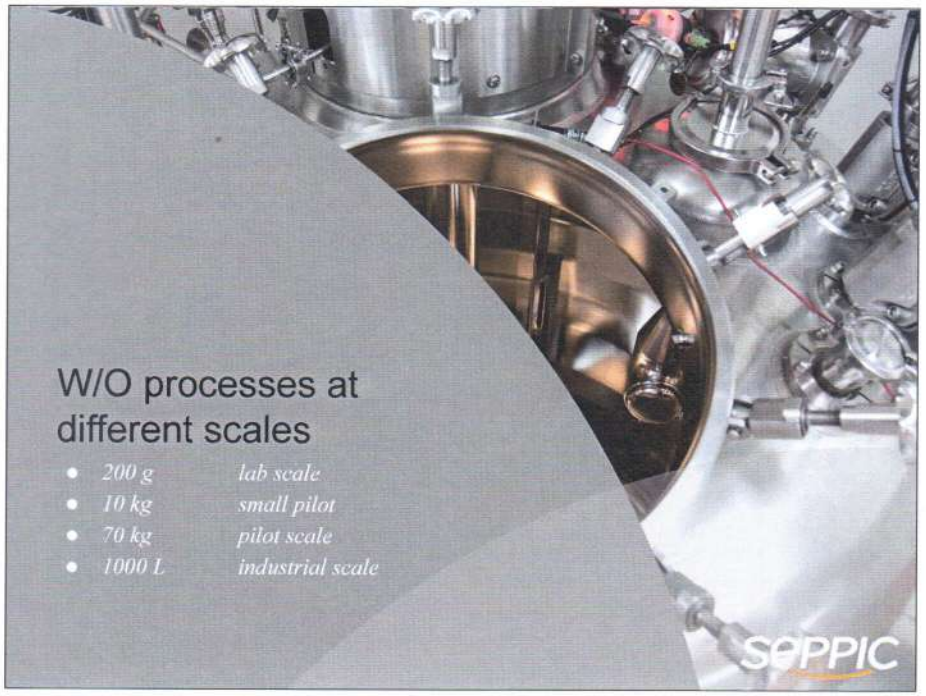
For process comparison, key parameter is **tip speed**, not rpm speed.

At same rpm speed, tip speed (m/s) can be very different:
Target **≥15 m/s** at large scale

High shear mixer	Rotor diameter	Rotor angular speed	Rotor tip speed
Silverson 450	114 mm	3000 rpm	18 m/s
Silverson 275	70 mm	3000 rpm	11 m/s
		4200 rpm	15 m/s
IKA DR 2000/4	55 mm	7950 rpm	23 m/s
Fluko FDC1/180	180 mm	2000 rpm	18 m/s
		2800 rpm	26 m/s
Fluko FDC1/100	100 mm	3500 rpm	18 m/s

→ Acceptable but defects appear faster





W/O emulsion scale up

- **200 g Lab scale**

200 g SEPPIC lab scale (Silverson/ IKA/ Fluko)

- 10 kg Small pilot
- 70 kg Pilot scale
- 1000 L Industrial scale example

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ingredients that inspire SEPPIC

200 g W/O lab process

- **Silverson**

- Silverson L4 or L5
- Standard emulsion grid
- 250 mL low form beaker

Process
- Addition of aqueous phase in adjuvant at 1000 rpm
- High shear step: 3 min at 4000 rpm (7m/s)

- **IKA Ultra Turrax**

- IKA Ultra Turrax T25
- S25-18G working head
- 400 mL tall form beaker

- Process
- Addition of aqueous phase in adjuvant at 7000 rpm
- High shear step: 3 min at 15600 rpm (10m/s) (level 4)

- **Fluko**

- Fluko FA25
- 20G working head
- 400 mL tall form beaker

- Process
- Addition of aqueous phase in adjuvant at 7000 rpm
- High shear step: 3 min at 14000 rpm (9m/s) (level B)

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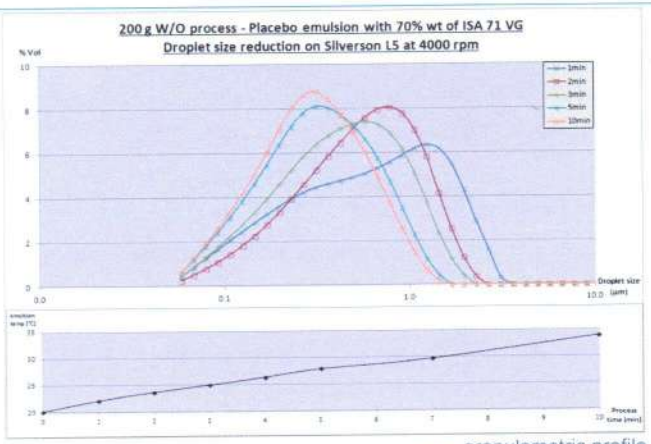
W/O process scale up
200 g scale – lab Silverson



Process optimisation = finding best compromise → granulometric profile, emulsion temperature, emulsion stability

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W/O emulsion scale up

- 200 g Lab scale
- **10 kg Small pilot**

Batch, Transfer Silverson L5

- 70 kg Pilot scale
- 1000 L Industrial scale example

ingredients that inspire SEPPIC

10 kg W/O process
Batch / transfer

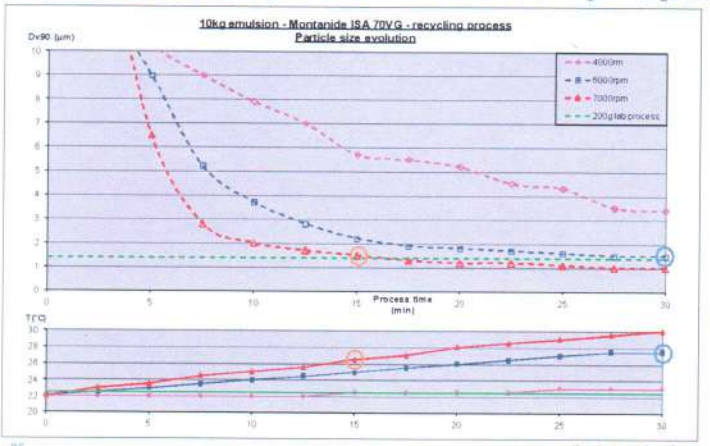
- **10 kg batch process**
  - Silverson L4 or L5
  - **Standard** emulsion grid (**EMSC**)
  - MONTANIDE™ ISA 70 VG
- Process
  - 10 min premix
  - **10 min** at **7000 rpm** (11m/s)

- **10 kg transfer process**
  - Silverson L4 or L5
  - **Fine** emulsion grid (**FEMSC**)
  - MONTANIDE™ ISA 70 VG
- Process
  - 10 min premix
  - **10 passes** at **6000 rpm** (10m/s)

ingredients that inspire SEPPIC

W/O process scale up
10 kg batch process

Higher mixing speed and time: particle size ↘ but temperature ↗
→ OK at small scale / short processes but need T control for larger / longer batches



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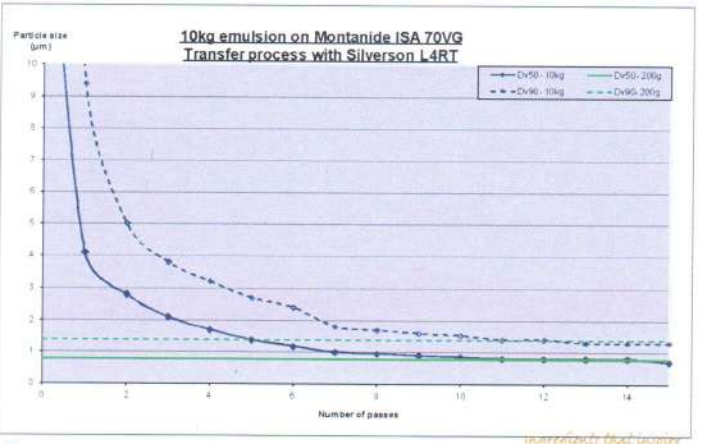
W/O process scale up
10 kg transfer process

Particle size decreases with number of passes
10 kg emulsion similar to lab emulsion after 10 passes



ingredients that inspire SEPPIC

W/O emulsion scale up

- 200 g Lab scale
- 10 kg Small pilot
- **70 kg Pilot scale**

Silverson 275

- 1000 L Industrial scale example

ingredients that inspire SEPPIC

70 kg W/O process
Recycling

- **Equipment**
  - Silverson 275 UHS
  - Fine emulsion grid (FEMSC)
  - Frequency modulator (ProxiDrive IP66)
  - Double jacketed vessel with low shear agitation
- **Process**

1/ pre-emulsion step
- Aqueous phase addition: 8 L/min
- 15 min paddle agitation (100 rpm)

2/ HS emulsification step
- High shear recirculation : 50 min at 4200 rpm (70 Hz, 15 m/s)
- Vessel under low shear agitation
- Maintain emulsion temperature < 25°C

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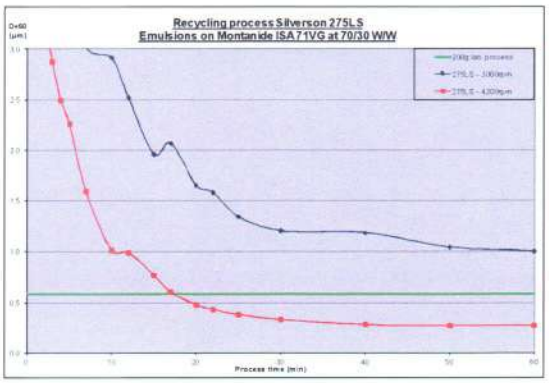
ingredients that inspire SEPPIC

W/O process scale up
70 kg recycling process

70 kg process optimisation with Silverson 275 LS



At larger scale, it is possible to achieve a better emulsion than the lab process, with smaller particle size and better stability.

Process	Stability 1 month			Stability 12 months	
	4°C	20°C	37°C	4°C	20°C
Lab process	10% sedim.	5% OL 15% sedim.	20% OL 30% sedim.	10% sedim.	25% sedim.
60 min 3000 rpm 275LS	10% sedim.	10% OL 10% sedim.	20% OL 30% sedim.	10% sedim.	25% sedim.
17 min 4200 rpm 275LS	10% sedim.	5% OL 10% sedim.	15% OL 25% sedim.	5% sedim.	25% sedim.
40 min 4200 rpm 275LS	No defect	2% OL	15% OL 15% sedim.	2% OL	25% sedim.
60 min 4200 rpm 275LS	No defect	2% OL	10% OL	2% OL	25% sedim.

OL oil layer
Sedim sedimentation

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ingredients that inspire SEPPIC

W/O emulsion scale up

- 200 g Lab scale
- 10 kg Small pilot
- 70 kg Pilot scale
- **1000 L Industrial scale example**

Recycling Silverson 450
Transfer Fluko FDC3-100
Top mixer Dedong - Beide

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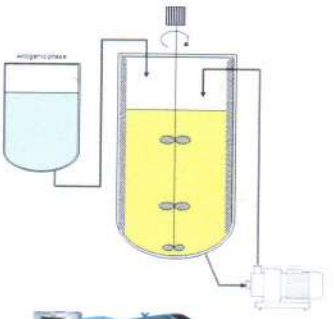
ingredients that inspire SEPPIC

1000 L W/O process
Recycling

- **Equipment**
  - Silverson 450 UHS
  - Standard emulsion grid (EMSC)
  - Double jacketed vessel with low shear agitation



- **Process**

1/ pre-emulsion step
- Aqueous phase addition : 15L/min
- 45 min agitation (400 rpm)

2/ HS emulsification step
- High shear recirculation : 45 min at 3000 rpm (18 m/s)
- Vessel under low shear agitation
- Maintain emulsion temperature < 25°C

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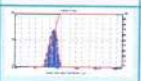
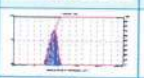
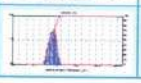
ingredients that inspire SEPPIC

W/O process scale up
1000 L recycling process

Process optimisation on 1000 L vaccine with 70% weight content of MONTANIDE™ **ISA 71 VG** with a **Silverson 450 UHS**

Process	Centri test 60 min	Granulometry			Stability 1 month			Stability 12 months	
		Dv50 (µm)	Dv90 (µm)	Profile	4°C	20°C	37°C	4°C	20°C
30 min	2% OL 5% sedim.	0.8	1.2		5% sedim.	5% OL 5% sedim.	10% OL 5% sedim.	10% OL 5% sedim.	10% OL 5% sedim.
45 min	2% OL 5% sedim.	0.8	1.3		No defect	2% OL 5% sedim.	5% OL	5% OL 5% sedim.	10% OL 5% sedim.
60 min	2% OL 5% sedim.	0.8	1.4		No defect	2% OL	5% OL	5% OL 2% sedim.	5% OL 5% sedim.

OL oil layer
Sedim. sedimentation

A longer process is improving stability, even if there is only a small difference of particle size.

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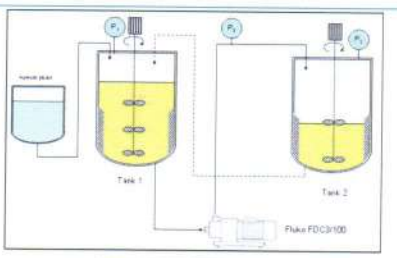
ingredients that inspire SEPPIC

1000 L W/O process
Transfer

- **Equipment**
  - Fluko FDC3/100 (3 double rotor stator)
  - Double jacketed vessel with low shear agitation



- **Process**

1/ pre-emulsion step
- Aqueous phase addition : 15L/min
- 45 min agitation (100 rpm)

2/ HS emulsification step
- High shear transfer: 2 passes at 3550 rpm (18 m/s)
- Vessel under low shear agitation
- P1 = 0.8 bar; P2 = 0.2 bar
- ~60 min per pass for 50/50 w/w emulsion
- Maintain emulsion temperature < 25°C

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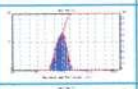
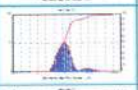
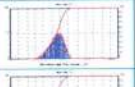
W/O process scale up
1000 L transfer process

Process optimisation on 1000 L vaccine with 50% volume content of MONTANIDE™ **ISA 50 V2** with a **Fluko FDC3/100**

Process	Centri test 60 min	Granulometry			Stability 1 month			Stability 12 months	
		Dv50 (µm)	Dv90 (µm)	Profile	4°C	20°C	37°C	4°C	20°C
Placebo lab control	5% OL	0.9	1.5		5% OL	10% OL	5% OL	20% OL	20% OL 10% creaming
Pass 1	5% OL	0.8	1.2		5% OL	10% OL	5% OL	25% OL	10% OL 10% creaming
Pass 2	2% OL	0.8	1.3		5% OL	5% OL	5% OL	20% OL	10% OL 10% creaming
Pass 3	2% OL	0.8	1.4		5% OL	5% OL	2% OL	15% OL	10% OL 10% creaming

OL oil layer

A longer process can help reducing the oil layer.

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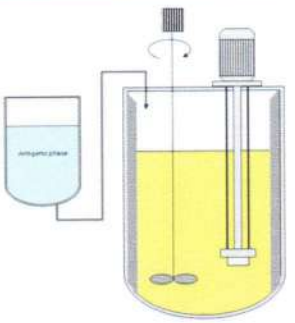
1000 L W/O process
Batch - top mixer

- **Equipment**
  - **Top mounted high shear mixer** (Dedong or Beide)
  - Double jacketed vessel with low shear agitation



- **Process**

1/ pre-emulsion step
  - Aqueous phase addition : 15-20 L/min
  - 20 min under agitation (impeller prefered; if not available, reduced high shear speed to allow good mixing)

2/ HS emulsification step
  - High shear speed : **60 min** at **2800 rpm**
  - Vessel under low shear agitation if possible
  - Maintain emulsion temperature < 25°C

Batch size	300 L	1000L
Rotor diameter	20 cm	30 cm
Max speed	2800 rpm	1500 rpm or 3000 rpm (model dependant)

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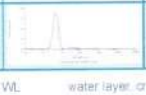
W/O process scale up
1000 L top mixer process

Process optimisation on 300 L and 1000 L vaccine with 70% weight content of MONTANIDE™ **ISA 71 VG** with a **Dedong** top high shear mixer

Process		Centri test 60 min	Granulometry			Stability 1 month			Stability 6 months	
			Dv50 (µm)	Dv90 (µm)	Profile	4°C	20°C	37°C	4°C	20°C
300 L	20 min 2800 rpm	2% OL	0.5	0.8		No defect	2% OL 5% sed.	5% OL	No defect	2% OL 5% sed.
1000 L	40 min 1500 rpm	2% OL	0.7	1.3		10% sed	10% sed	5% OL **2% WL**	10% sed	2% OL 5% sed.
1000 L	60 min 2800 rpm	2% OL	0.5	0.8		No defect	2% OL	5% OL	No defect	2% OL

OL oil layer / Sedim Sedimentation / WL water layer, critical at 4°C or early at 20°C

- **Top mixer** process: not always successful, but good stability possible.
- Large scale rotor speed: **2800-3000 rpm** prefered, 1500 rpm acceptable but not optimal (larger droplet size can cause reversible apparition of sedimentation).
- **Independant low shear agitation prefered** (optimal premix step, vaccine homogeneity)

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Process optimisation

- **High shear mixing time is dependant on:**
  - High shear mixer
  - Type of process
  - Vessel
  - Batch size
  - Antigen

- **Process optimisation**

Take emulsion samples at different time points and perform QC tests in order to select the most appropriate conditions.

- SEPPIC is happy to provide help and support to help with the process optimisation.

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W/O processes through the scales

Process examples, more available on request to fit specific configurations

Batch size	Process	High shear mixer	Rotor speed	Process duration
200 g	Batch	Silverson L5	4000 rpm *7 m/s*	3 min
200 g	Batch	IKA T25 *S25N-18G*	15600 rpm *10 m/s*	3 min
200 g	Batch	Fluko FA25	14000 rpm *9 m/s*	3 min
10 kg	Batch	Silverson L5	7000 rpm *11 m/s*	10 min
10 kg	Transfer	Silverson L5	6000 rpm *10 m/s*	10 passes
70 kg	Recycling	Silverson 275 UHS	4200 rpm *15 m/s*	50 min
1000 L	Recycling	Silverson 450 UHS	3000 rpm *18 m/s*	45 min
1000 L	Transfer	Fluko FDC3/100	3550 rpm *18 m/s*	3 passes

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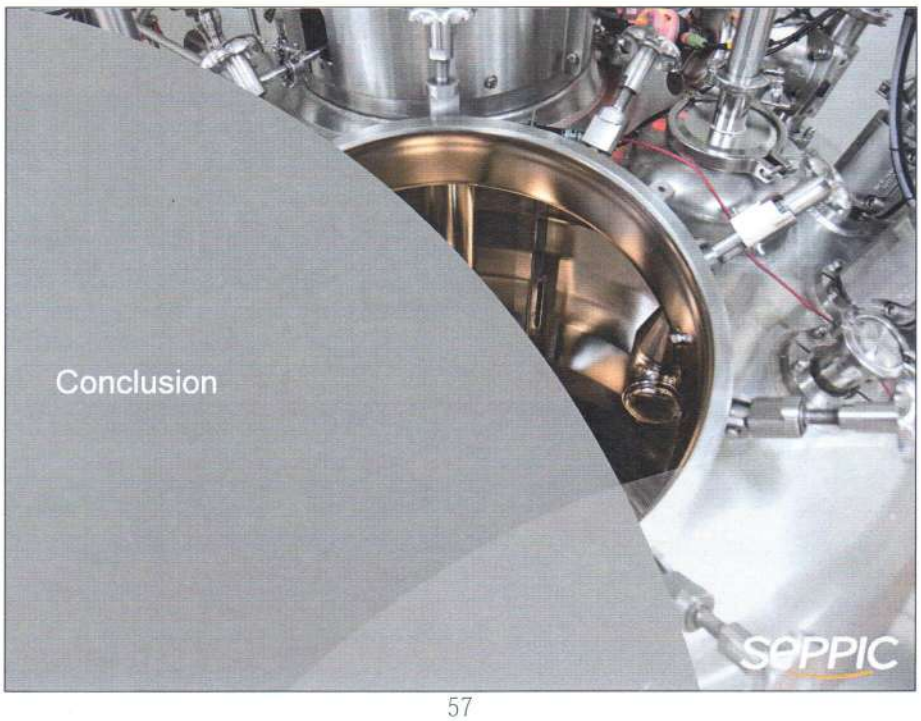
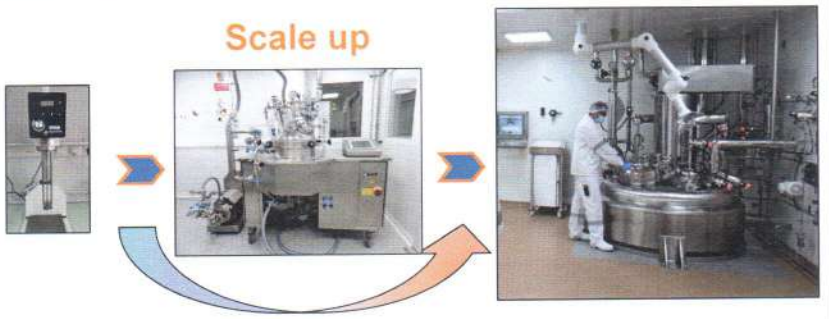


Conclusions (1)



- ➢ Formulation
- ➢ Process
- ➢ Control

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Conclusions (2)

- W/O emulsion
  - High shear energy
- **Before scale up**, we need to:
  - Understand well the formulation behavior (robustness, limits, critical parameters)
  - Set up QC tests to evaluate vaccine quality
- **Industrial scale up** is a **compromise** between:
  - Critical process parameters
  - Existing / new equipments (cost)

➢ to obtain a **sustainable process** able to insure **efficiency** and **consistency**

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Conclusions (3)

- QC controls
  - Assessment of process efficacy during scale up
  - Routine tests during daily manufactures
- **Future modifications** might be needed in the system: phases ratio, batch size, antigenic phase (multivalent, concentration):

➢ Change in the system previously optimised: need for **additional validation work**

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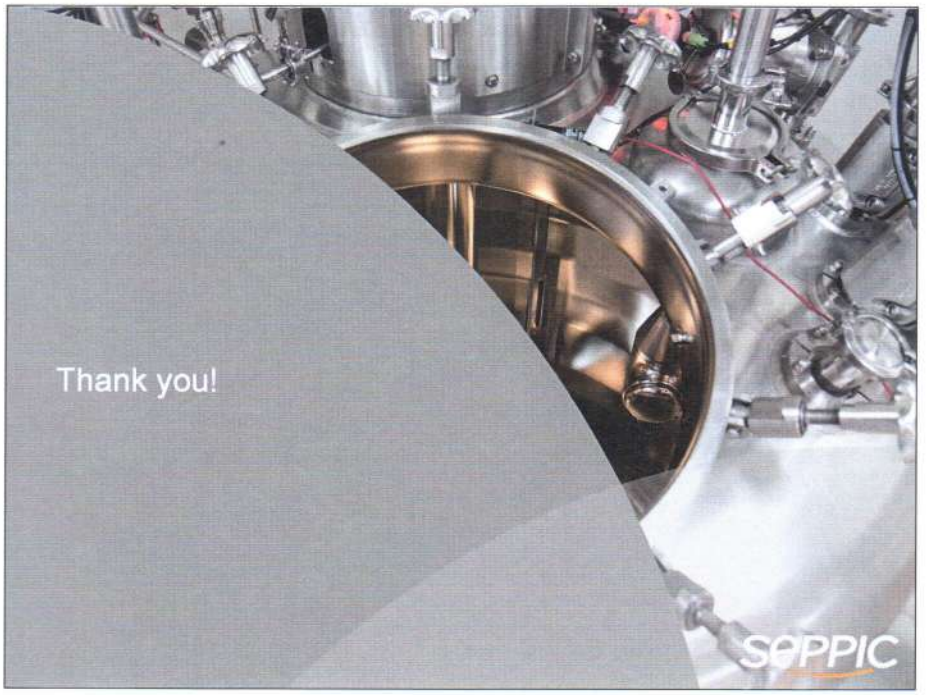
Thank you!
SAPPIC