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Chloé Pierson

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UNIVERSITE JOSEPH FOURIER

FACULTE DE PHARMACIE DE GRENOBLE

Année : 2009

MOLECULES SENSIBLES A L'OXYGENE : ETUDE ET OPTIMISATION DES PROCEDES D'INERTAGE. APPLICATIONS AUX SERINGUES PRE-REMPLIES

THESE PRESENTEE POUR L'OBTENTION DU TITRE DE DOCTEUR EN PHARMACIE

DIPLOME D'ETAT

par

Chloé PIERSON

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HP2 : Hypoxie Physiopathologie Respiratoire et Cardiovasculaire
IAB : Institut Albert Bonniot
LBFA : Laboratoire de Bioénergétique Fondamentale et Appliquée
LCIB : Laboratoire de Chimie Inorganique et Biologie
LR : Laboratoire des Radio pharmaceutiques
PAST : Professeur Associé à Temps Partiel
PRAG : Professeur Agrégé
UVHCI: Unit of Virus Host Cell Interactions

Mise à jour le 03/09/2009

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UVHCI: Unit of Virus Host Cell Interactions

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« L'esprit critique ne s'use que si l'on ne s'en sert pas »

Richard Monvoisin

A mon père,

qui m'a donné le goût des sciences et la persévérance

A ma mère,

qui a développé mon ouverture culturelle et sociale et qui m'a toujours soutenue dans les moments difficiles

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Table des matières

Remerciements **6**

Table des matières **8**

Liste des figures **11**

Liste des tableaux **13**

Glossary **14**

Introduction (Français) **16**

Développement **18**

Introduction (English) **25**

1. Context and bibliography **27**

1.1 Context **27**

1.2 Bibliography **30**

1.2.1. Formulation and engineering aspects 30

1.2.2. Oxygen measurement 33

1.2.3. Container gas permeation 35

1.2.4. Oxygen and dissolution physics 36

2. Material: oxygen measurement **37**

2.1 Requirements **37**

2.2 Comparison of the 4 devices selected: **40**

2.3 Conclusions **42**

3. Studies at Laboratory scale **43**

Introduction **43**

3.1 Dissolved oxygen equilibrium under various conditions **44**

Introduction and objectives 44

Material and Methods 44

Results and discussion 45
Conclusion 46
3.2 Long-term study of oxygen reuptake in syringes 47
Introduction and objectives 47
Material and Methods 47
Results and discussion 49
Conclusion 52
3.3 Silicone tubing permeation 53
Introduction and objectives 53
Material and Methods 53
Results and discussion 54
Conclusion 55
4. Optimization of the manufacturing process 56
Introduction 56
4.1 Test 1 of inerting optimization on the filling line 59
Introduction and objectives 59
Material and Methods 59
Results and discussion 64
Conclusion 68
4.2 Test 2: real filling conditions and consequences of a long machine stopping 70
Introduction and objectives 70
Material and Methods 70
Results and discussion 73
Conclusion 79
4.3 Test 3: impact of machine rate, nitrogen flow rate at the filling station and of dead volumes 81
Introduction and objectives 81
Material and Methods 81
Results and discussion 85
Conclusion 91

Conclusion 94

Bibliography 98

Appendix 101

- **Appendix 1** 101
- **Appendix 2** 103
- **Appendix 3** 105
- **Appendix 4** 107
- **Appendix 5** 109
- **Appendix 6** 114
- **Appendix 7** 118
- **Appendix 8** 119
- **Appendix 9** 124
- **Appendix 10** 126

Serment des Apothicaires 135

Liste des figures

Figure 1 – BD Hypak® 28
Figure 2 – Syringe diagram 28
Figure 3 – Packaging of SCF syringes 29
Figure 4 – BD Hypak® SFC Nest diagram 29
Figure 5 – Oxydot 38
Figure 6 – OxyNeedle fixed on the Oxysense pen 39
Figure 7 – Comparison of oxygen concentrations in syringes according to the conditions of inerting and corking (n = 12) 45
Figure 8 – Numbering of syringes according to the test conditions 48
Figure 9 – Oxygen concentration evolution in 1.2 mL syringes, function of the stopper nature and the syringe position (n = 3) 49
Figure 10 – Oxygen concentration evolution in 2.2 mL syringes, function of the stopper nature and the syringe position (n = 3) 50
Figure 11 – Average oxygen concentration in 1.2 and 2.2 mL filled syringes at the beginning and the end of the experiment (n = 12). 51
Figure 12 – Filtration transfer system of the filling machine 53
Figure 13 – Facility for the measure of the silicone tubing permeation 54
Figure 14 – Oxygen reuptake in silicone tubing compared with usual oxygen rate in WFI 54
Figure 15 – Oxygen reuptake in silicone tubing the 30 first minutes. 55
Figure 16 – Inerting manufacturing process optimization 56
Figure 17 – Filling and stoppering of syringes with the pilot filling machine 58
Figure 18 – Diagram of the tank 62
Figure 19 – Position of sampled tub for Experiments 1 to 9 63
Figure 20 – Sampled syringes for each sampled tub (Exp 1 to 6) 64
Figure 21 – Pareto Chart of the standardized Effects for oxygen partial pressure in syringes from line 20 65

Figure 22 – Normal Plot of the standardized effects for oxygen partial pressure in syringes from line 20 65
Figure 23 – Main effects of each factor on oxygen partial pressure..... 66
Figure 24 – Graphs for interactions between the factors 67
Figure 25 – Nitrogen purge in the filtration system 72
Figure 26 – Sampled tubs 72
Figure 27 – Sampled syringes for each sampled tub..... 73
Figure 28 – Oxysense dissolved oxygen measure in silicone tubing 74
Figure 29 – Oxygen partial pressure in syringes of line10, function of the tub number 76
Figure 30 – Sampled syringes in Tub 51 78
Figure 31 – Sampled syringes in Tub 52 78
Figure 32 – Oxygen measurement in tubs just after a long stopping machine 78
Figure 33 – Position of sampled tub for Experiments 1 to 6..... 83
Figure 34 – Sampled syringes for each sampled tub (Exp 1 to 6) 83
Figure 35 – Sampled tubs for Experiment n°7..... 84
Figure 36 – Sampled syringes for each sampled tub (Exp 7)..... 84
Figure 37 – Influence of nitrogen flow rate at the filling station on oxygen partial pressure (n=10) 85
Figure 38 – Influence of the machine rate on oxygen partial pressure (n=10)..... 87
Figure 39 – Influence of the machine rate on oxygen partial pressure without the results of Needle 5 (n=8)..... 88
Figure 40 – Oxygen partial pressure in the syringes of line 10, function of the tub number (n=5) 89
Figure 41 – Oxygen partial pressure in the syringes of line 10, function of the tub number, with and without needle 5 (n=5 with A5, n=4 without A5) 91
Figure 42 – Correlation between oxygen partial pressure and oxygen concentration according to the temperature..... 102

Liste des tableaux

Table I – Comparison of oxygen meter devices 40

Table II – Test Matrix 44

Table III – Design of Experiment factors..... 59

Table IV – Experimental matrix with coded values 61

Table V – Experiment matrix with real values..... 63

Table VI – Experimental matrix for the first part of Test 3 83

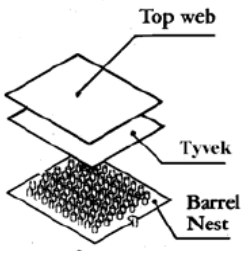
Table VII – Average oxygen concentrations in syringes according to the time after corking 103

Table VIII – Standard deviations on oxygen concentrations in syringes according to the time after corking..... 104

Table IX – Results of Oxygen partial pressure in line 10 syringes every sixth tub .....**Erreur ! Signet non défini.**

Glossary

API	**A**ctive **P**harmaceutical **I**ngredient
BD	**B**ecton **D**ickinson
BDM-PS	BD **M**edical **P**harmaceutical **S**ystems
cGMP	**c**urrent **G**ood **M**anufacturing **P**ractices
CPMP	**C**ommittee for **P**roprietary **M**edicinal **P**roducts
GC	**G**as **C**hromatography
Hypak®	Model of glass syringe developed by BD and manufactured in Pont de Claix site
IPC	**I**n **P**rocess **C**ontrols: controls performed during the production.
MTG	Monothioglycerol
Nest	Component of the packaging. Plastic support for SCF syringes in tubs



PPH	**P**ont de Claix **P**harmaceutical **H**ome center
PRTC	**P**lastic **R**igid **T**ip **C**ap

Rondo Plastic medium in which the syringes can be stored. A rondo can handle 18 syringes positioned horizontally.

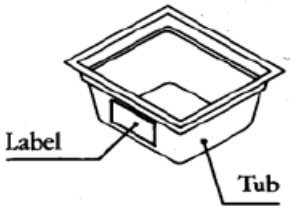
SCF **S**terile **C**lean and Ready to **F**ill

SPU **S**topper **P**lacement **U**nit. Laboratoy apparatus used to cork syringes with stoppers. The stoppering can be performed with air, or with nitrogen.

Stopper Rubber element that can slide in the barrel of the syringe to inject the drug. It has a function of physical barrier (water tightness, sterility).

Also called Plunger Stopper.

Tub Component of the packaging. Plastic container for SCF syringes. The syringes are placed with their nest into the tub. A Tyvek® sheet will seal the tub to sterilize and maintain sterility into the tub.



Tyvek® Brand name given to the film used to cover and seal the tub. The Tyvek® prevents the contamination of sterile syringes by micro-organisms.

VHP **V**apor **H**ydrogen **P**eroxide

WFI **W**ater **F**or **I**njection

Introduction (Français)

Les expériences décrites dans cette thèse sont été réalisées durant une étude de huit mois dans l'entreprise américaine Becton Dickinson (BD) sur le site de Pont de Claix, en France.

BD est une société qui développe, fabrique et commercialise des fournitures et du matériel pour le secteur médical, des instruments de laboratoire, des anticorps, des réactifs et des produits de diagnostic. Le site de Pont de Claix est spécialisé dans le développement et la production de seringues en verre, à destination des industries pharmaceutiques et des professionnels de la santé.

Le marché des seringues pré-remplies est grandissant et constitue une vraie opportunité pour une entreprise spécialisée dans les seringues en verre telle que BD de développer des nouveaux marchés et de signer des nouveaux contrats avec des clients.

La première étape pour formuler une solution injectable est de considérer sa stabilité. L'étude présentée ci-après concerne la sensibilité à l'oxygène : comment contrôler les taux d'oxygène en solution et comment maitriser le procédé de fabrication pour protéger les molécules sensibles à l'oxygène.

Dans un premier temps, le cadre de cette thèse va être présenté avec une brève description de la seringue BD Hypak®, support aux expériences décrites par la suite. Les recherches bibliographiques seront ensuite présentées.

La seconde partie concerne le matériel utilisé et la recherche d'un équipement adéquat pour mesurer les taux d'oxygène dans les seringues.

Nous verrons ensuite les résultats d'études réalisées à petite échelle au sein du laboratoire de formulation. Ces études ont pour but d'étudier les phénomènes physiques

en lien avec l'oxygène ainsi que de préparer des tests à plus grande échelle sur une ligne pilote de remplissage.

La quatrième partie détaille trois essais réalisés sur une ligne pilote de remplissage, dans le but d'en fixer les paramètres intrinsèques d'inertage à l'azote.

Enfin, la conclusion et les perspectives de cette étude seront présentées.

Développement

1. Contexte et bibliographie

Les études présentées dans cette thèse ont été réalisées au sein de la société BD à Pont de Claix, dans le service de Recherche et Développement dans le cadre d'un projet appelé PI. Ces études avaient pour objectif final le remplissage de seringues avec des solutions injectables comprenant des molécules sensibles à l'oxygène. Les seringues servant de support à ces essais sont des seringues en verre de type BD Hypak® de 2,25 ml fabriquées sur le site.

Le chapitre Bibliographie de cette thèse développe quatre points concernant la problématique de la sensibilité à l'oxygène des molécules.

Il est d'une part possible de jouer sur la composition des solutions à proprement parler et sur leur procédé de fabrication pour limiter les dégradations liées à l'oxygène en solution. Les principaux moyens cités par les auteurs sont soit des moyens physiques (bullage à l'aide d'un gaz inerte, augmentation de température de la solution, etc.) soit des moyens chimiques avec l'utilisation d'un agent antioxydant, soit la combinaison des deux.

Le deuxième point de cette étude bibliographique porte sur les moyens de mesurer l'oxygène dans la solution médicamenteuse ou dans l'espace de tête. Les auteurs utilisent différents appareils et techniques de mesure en fonction de leurs besoins et du type d'échantillon analysé (seringue pré-remplie, flacon).

Ensuite, nous verrons que certains auteurs ont détecté et évalué une perméabilité à l'oxygène des contenants tels que seringues pré-remplies et flacons en verre renfermant des solutions inertées.

Enfin, un dernier point de bibliographie explique les lois physiques régissant les phénomènes d'oxygénation et notamment la loi de Henry, qui stipule qu' *« À température constante et à saturation, la quantité de gaz dissous dans un liquide est proportionnelle à la pression partielle qu'exerce ce gaz sur le liquide ».*

2. Matériel : mesure de l'oxygène

La deuxième partie de cette thèse concerne la recherche d'un appareil pour mesurer les concentrations en oxygène dans les seringues, pré-requis à toutes les expériences qui suivront.

L'appareil devait satisfaire aux exigences suivantes :

- être capable de mesurer des concentrations en oxygène dans des seringues BD Hypak® de 2,25 ml ;
- donner des résultats fiables et répétables ;
- être simple d'utilisation et donner une mesure rapide ;
- pouvoir être calibré facilement ;
- réaliser des mesures non destructives ;
- être utilisé à la fois dans un laboratoire de formulation à une petite échelle que dans un laboratoire de contrôle de qualité pour réaliser des contrôles en cours de production et sur le produit fini ;
- être qualifiable et respecter les Bonnes Pratiques de Fabrication américaines (cGMP).

Plusieurs appareils ont été testés, certains étant déjà disponibles au laboratoire et d'autres en location. Ils fonctionnaient selon des principes différents : mesure polarographique, mesure optique basée sur un détecteur de fluorescence sensible à l'oxygène ou encore électrode ampérométrique.

Le choix final s'est orienté vers un appareil de marque Radiometer, mesurant les concentrations en oxygène grâce à une électrode ampérométrique et exprimant les résultats en pression partielle en oxygène. Un appareil ABL5 a été acheté et installé au laboratoire R&D tandis qu'un appareil ABL80 est en cours d'acquisition pour le laboratoire de contrôle de qualité pour réaliser les contrôles en cours de fabrication ou sur les produits finis. Cet appareil, fonctionnant sur le même principe que l'ABL5 sera qualifié et fonctionnera selon les cGMP.

3. Etudes à l'échelle du laboratoire de formulation

Durant cette étude de huit mois, de nombreux essais ont été réalisés au laboratoire de formulation dans plusieurs buts.

D'une part ces tests ont permis de mieux comprendre les phénomènes liés à l'oxygène en solution (l'inertage, qui consiste à remplacer l'oxygène par un gaz neutre tel que l'azote ou l'argon, les phénomènes de réoxygénation consécutifs à un contact avec l'air…).

D'autres part, ils ont permis de préparer des tests réalisés à plus grande échelle sur une ligne pilote de remplissage.

Enfin ces tests ont permis de préparer des études de pré-stabilité et de stabilité pour des molécules sensibles à l'oxygène, afin de vérifier leur stabilité dans les conditions de stockage recommandées par l'ICH (*International Conference of Harmonization*).

Trois expériences sont détaillées dans cette thèse.

La première expérience a permis d'étudier les équilibres entre l'oxygène dissous en solution et l'oxygène gazeux dans l'espace de tête de la seringue, selon différentes conditions d'inertage et de bouchage. Des seringues ont été remplies par une solution isotonique inertée ou non puis bouchées sous azote ou sous air. La concentration de la solution en oxygène a été ensuite régulièrement mesurée dans les seringues pendant 24 heures. L'appareil utilisé pour boucher ces seringues était un *Stopper Placement Unit* (SPU), présent au laboratoire de formulation.

Les résultats ont montré une forte augmentation de la concentration en oxygène dans les solutions inertées et bouchées sous air et réciproquement une forte diminution de la concentration en oxygène dans les solutions non inertées et bouchées sous azote.

D'après ce test, il est important et nécessaire d'attendre un certain temps d'équilibrage entre l'oxygène dissous en solution et l'oxygène de la bulle. Si ce délai n'est pas respecté, la concentration pourrait être mal interprétée.

D‘autre part, nous notons que le volume et la composition du gaz dans l’espace de tête de la seringue est critique et devra être maitrisé.

Enfin, ce test a permis de maîtriser les différents paramètres de réglage de l’équipement de laboratoire (SPU) de manière à contrôler la concentration en oxygène dans les seringues ; il a ainsi été possible de préparer des échantillons ayant différents niveaux d’inertage..

La deuxième expérience a consisté à mesurer les concentrations en oxygène au cours du temps dans des seringues remplies à l’eau inertée. Les facteurs étudiés étaient : la nature du stopper (deux stoppers de composition différentes), la position de la seringue (couchée ou debout) et le volume de remplissage (1,2 et 2,2 ml).

Sur une période de trois mois, une forte reprise en oxygène a été observée au cours du temps, indépendamment de la position de la seringue et de la nature du stopper.

Nous observons par contre une reprise en oxygène plus rapide pour des seringues remplies à 2,2 ml par rapport à celles remplies à 1,2 ml. Ceci n’a pu être expliqué sans expériences complémentaires mais nous pouvons déduire que le volume plus important (2,2 ml) représente un cas critique.

Plusieurs hypothèses sont énoncées pour expliquer cette reprise en oxygène au cours du temps : un passage des molécules d’oxygène entre les lèvres du stopper et la surface intérieure du corps de la seringue ; une perméabilité du caoutchouc composant le stopper ; ou enfin une légère dépression dans l’espace de tête de la seringue (due au système de fermeture des seringues au laboratoire), responsable d’un appel d’oxygène vers l’intérieur. Des tests supplémentaires seront nécessaires pour confirmer ou infirmer ces hypothèses.

La troisième expérience présentée était une mesure de la perméabilité à l’oxygène des tubulures en silicone. De l’eau, préalablement inertée par bullage à l’azote sous poche protectrice, a été introduite dans une section de tuyau en silicone identique à celui utilisé en zone pilote pour transférer et filtrer les solutions médicamenteuses avant le remplissage. Le tuyau, bouché de part et d’autre, a été sorti de la poche et la concentration en oxygène de l’eau a été mesurée grâce à un appareil Oxysense 4000B.

Les résultats de ce test ont montré une augmentation importante et rapide de la concentration en oxygène de l'eau préalablement inertée. Au bout de deux heures, la concentration en oxygène était comparable à celle mesurée dans une solution non inertée (environ 8 ppm).

Cet aspect sera un point critique à prendre en compte lors du remplissage des seringues en zone pilote, et son impact sera étudié dans le paragraphe suivant.

4. Optimisation du procédé de fabrication

Au niveau de la ligne pilote de remplissage des seringues, plusieurs facteurs ont été identifiés comme pouvant avoir un impact pour le remplissage de molécules sensibles à l'oxygène.

Il est d'abord possible de jouer sur l'inertage des solutions par bullage à l'azote au niveau des cuves de formulation et de stockage, au cours des premières étapes du procédé.

Le transfert de la solution vers la remplisseuse peut ensuite se faire sous pression d'azote. Durant ce transfert, la solution est filtrée par un système contenant deux filtres avec de part et d'autre des tubulures en silicone. Comme démontré dans le chapitre précédent, les tubulures en silicone étant perméables à l'oxygène, cette étape est critique et fera l'objet d'une étude plus précise dans ce chapitre.

La solution passe ensuite par une cuve dite « tampon » avant d'atteindre la remplisseuse à proprement parlé qui va remplir puis boucher les seringues grâce à cinq « aiguilles de remplissage » et cinq « bras de bouchage ». A ce niveau du procédé, il est possible d'agir sur le degré d'inertage par injection d'azote lors du remplissage et lors du bouchage. Les paramètres considérés comme pertinents et étudiés par la suite sont : la cadence globale de la machine, le débit d'azote en station de remplissage et le débit d'azote en station de bouchage.

Trois expériences ont été réalisées sur cette ligne pilote de remplissage pour déterminer et fixer les paramètres impactant les taux d'oxygène finaux dans les

seringues. L'impact de la tubulure en silicone en amont du procédé de remplissage a également été étudié au cours de ces expériences.

Plusieurs conclusions peuvent être tirées de ces tests :

Les paramètres intrinsèques de la remplisseuse ont été optimisés : le débit en station de bouchage est le paramètre le plus critique et devra être fixé à son niveau maximum (5,5 l/min.) lors du remplissage de solutions d'actifs sensibles à l'oxygène. Le débit d'azote en station de remplissage est beaucoup moins critique, mais une différence significative a toutefois été montrée entre un niveau minimal et maximal, il faudra donc également le fixer à son niveau maximal (5,5 l/min.). La cadence de la machine n'ayant pas montré d'impact sur les taux d'oxygène dans les seringues, elle sera maintenue à son niveau usuel (50 cycles/min.).

Les mesures des taux d'oxygène dans les seringues, lorsque ces paramètres sont optimisés, sont prometteuses, avec des taux relativement bas.

Toutefois, la remplisseuse s'est arrêtée plusieurs fois au cours des essais pour des raisons inconnues et en indiquant des défauts de gazage. Dans ce cas, les concentrations en oxygène dans les seringues impactées augmentent. L'origine de ces défauts doit être investiguée. Si ces défauts étaient amenés à se reproduire, les seringues impactées devraient être éliminées.

Une étude comparative des taux d'oxygène dans les seringues remplies et bouchées par chacune des cinq aiguilles de remplissage et chacun des bras de bouchage montre une différence significative, avec des taux d'oxygène supérieurs, pour les seringues remplies en station numéro 5 pour deux des trois expériences. Ce défaut doit être investigué et corrigé pour assurer un inertage homogène des seringues.

Une mesure de l'oxygène dans la tubulure silicone du système de filtration a été réalisée au cours d'un remplissage, pendant et après un arrêt prolongé de la machine. Ces mesures confirment la ré-oxygénation importante et rapide de la solution contenue dans la tubulure puisqu'après un arrêt machine de quatre vingt minutes, la solution est

totalement réoxygénée. L'impact sur les concentrations en oxygène dans les seringues est néanmoins limité.

Lors d'une de ces expériences, l'eau inertée servant au remplissage des seringues a été remplacée en cours de manipulation par de l'eau non inertée, afin d'étudier l'impact de ce changement sur les taux d'oxygène dans les seringues. Après le changement de solution, la concentration en oxygène a d'abord augmenté légèrement pour se stabiliser (plateau) à une valeur supérieure à celle obtenue avec l'eau inertée mais néanmoins relativement basse.

De nouveaux essais sont envisagés sur la ligne pilote de remplissage notamment pour étudier la robustesse du procédé et confirmer les capacités de la machine, ainsi que pour réaliser une investigation des défauts rencontrés.

Introduction (English)

The experiments described in this thesis were conducted during an eight months study performed within the US Company Becton Dickinson (BD), at Pont de Claix site, in France.

BD develops, manufactures and sells medical supplies, devices, laboratory instruments, antibodies, reagents and diagnostic products. The site of Pont de Claix is specialized in the development and production of glass syringes, designed for the pharmaceutical industries and for health care professionals.

The market of the pre-filled syringe is growing and for a company specialized in the production of glass syringes such as Becton Dickinson, it represents a real opportunity to develop new markets and sign new contracts with clients.

The first step to formulate injectable solutions is to consider their stability characteristics. The study specifically dealt with sensitivity to oxygen: how to monitor oxygen rates in a solution and how to control the process to protect oxygen-sensitive drugs.

First, the extent of this thesis will be introduced with a brief description of the syringe BD Hypak® which was the support for the experiments. The bibliography will then be exposed.

The second part deals with the material used and the research of suitable equipment to measure oxygen rates in syringes.

In a third part, some of the results from experiments performed on a small scale in the formulation laboratory will be presented. They are aimed at understanding the physical phenomena linked to oxygen and at preparing the tests performed at a higher scale in a pilot manufacturing filling line.

The fourth part details three tests carried out in a pilot production line on a larger scale, in order to fix its intrinsic parameters for inerting.

The conclusions and perspectives of this study will finally be presented.

1. Context and bibliography

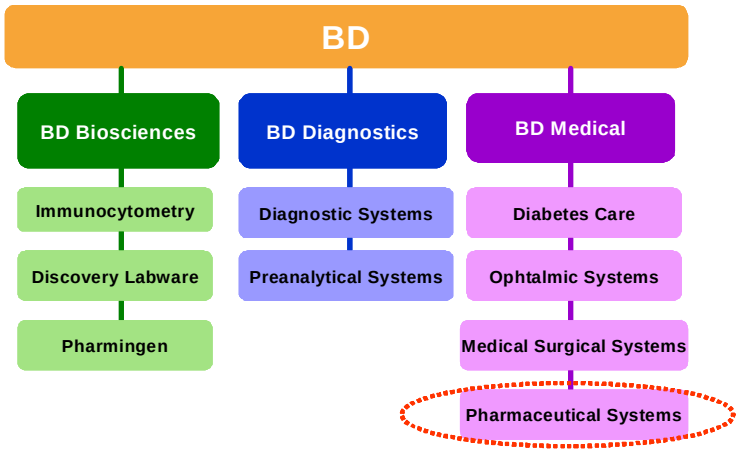
1.1 Context

BD is a medical technology company that serves healthcare institutions, life science researchers, clinical laboratories, industry and the general public.

BD manufactures and sells a broad range of medical supplies, devices, laboratory equipment and diagnostic products. BD is headquartered in Franklin Lakes in the United States and has offices in nearly 50 countries worldwide. It counts 28,000 employees.

The company is listed on the NYSE (New York Stock Exchange) & Dow Jones Sustainability World Index. BD revenues for Fiscal Year 2008 were of about $7,000,000.

BD is divided into 3 worldwide segments and 9 Units:



Pont de Claix site is the worldwide head office of BD Medical – Pharmaceutical Systems (BDM-PS).

With a 65,000 m² surface and more than 1600 employees, the site offers: General Management, Customer-Facing Team, Research & Development, Marketing, Medical Affairs, Quality, Regulatory Affairs, Technical Support and Industrial Operations.

The study was performed within a project called “PI”. It took place mainly in a small entity of BD Pont de Claix site called PPH for Pont de Claix Pharmaceutical Home center. PPH includes people from Pharmaceutical Systems Laboratories and from the PI project. It contains laboratories (formulation lab, microbiology, chemistry) and a pilot manufacturing line where syringes are filled.

The study of the phenomenon linked to drugs sensitive to oxygen in pre-filled syringes was performed in syringes manufactured in BD Pont de Claix site: **BD Hypak® SCF**. A brief description will allow a better understanding of the tests performed.

Figure 1 – BD Hypak®

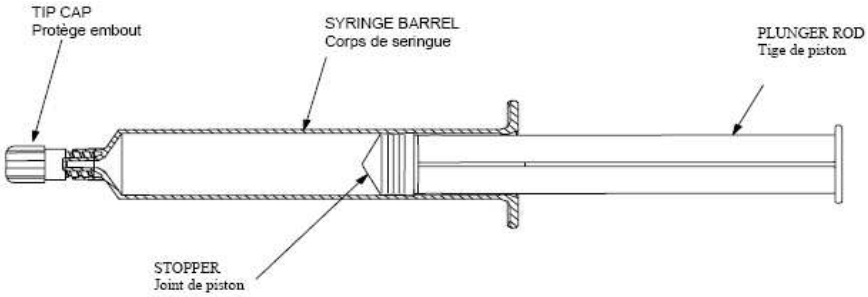


Figure 2 – Syringe diagram

The Hypak® SCF (**S**terile, **C**lean and ready to **F**ill) is composed of a glass barrel in which the injectable drug is contained, a rubber stopper (to maintain the integrity of the drug) and a Tip Cap that hermetically closes the Tip and where a needle or a catheter can be fixed.

All of the tests performed during my training dealt with the BD Hypak® 2.25 mL, filled at 2 usual filling volumes for the PI project: 1.2 and 2.2 mL.

The SCF syringes are packaged in a Nest (polypropylene plate that supports the barrel of syringes) maintained in a tub (polystyrene) covered by 2 sheets of Tyvek® (a strong material that allows the entrance of vapor water but not liquid water). The Tyvek® allows to sterilize the syringes and to maintain sterility. The last layer is the Steri bag that surrounds the tub and is also used to sterilize the syringes.

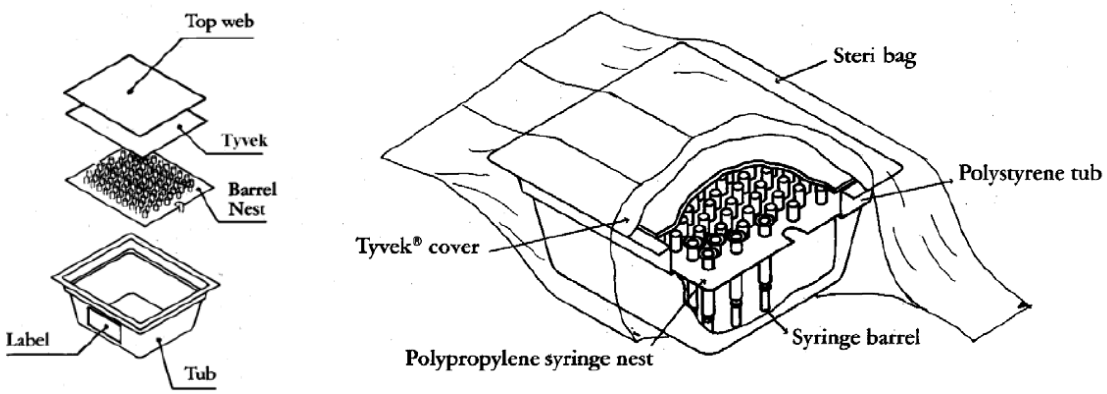


Figure 3 – Packaging of SCF syringes

Each nest can carry 100 syringes, distributed on 20 lines of 5 syringes. The lines are organized as shown on Figure 4 in order to optimize the space in the tub:

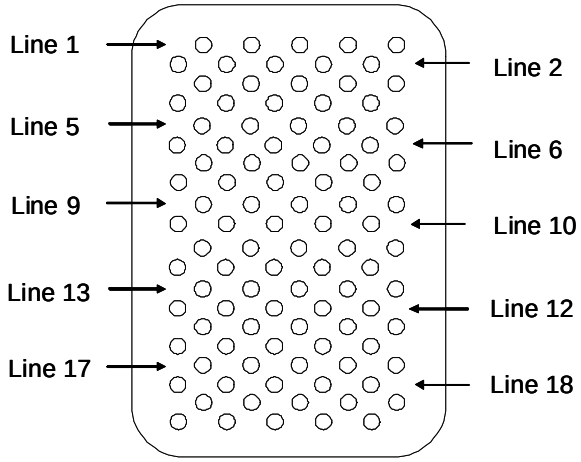


Figure 4 – BD Hypak® SFC Nest diagram

1.2 Bibliography

1.2.1. Formulation and engineering aspects

The stability of oxygen-sensitive drugs depends on various factors.

Different methods described by Dietlin F and Fredj D [2] can be carried out to prevent oxidation phenomena:

- Oxygen elimination by increasing the temperature of the aqueous solution, vacuum or bubbling with an inert gas such as nitrogen;
- Dissolved oxygen neutralization by an antioxidant;
- Combination of the 2 previous methods.

Some authors recommend the use of antioxidant excipients:

Sklar S [5] recommends using Monothioglycerol (MTG) to improve the shelf life of injectable drugs, in the case of phenothiazines, which are molecules particularly sensitive to oxygen. The importance of the nature of the antioxidant is shown: a series of promethazine formulations containing different antioxidants were tested for their oxidation potentials and Sklar S demonstrates that not only the use of antioxidant is important but their nature is primordial as well.

Kasraian K *et al.* [10] go further in their demonstration. They observed that trace impurities from two different antioxidants significantly contributed to color change associated with oxidation in a Danofloxacin injectable solution. The minimization of oxygen levels alone in the solution and headspace was not sufficient to completely eliminate the product instability. The addition of MTG, resulted in a formulation less sensitive to processing variables.

The impact of pH on the performance of MTG was also studied. At pH 7.5, MTG resulted in significant improvement in stability; however, at pH 6.0 it was not effective as an antioxidant.

Thus, they conclude that process modifications alone may not be sufficient to prevent oxidation and chemical approaches, such as pH control, addition of an antioxidant, and control of components should be considered first as means of enhancing the stability of oxygen-sensitive solutions.

Kalifa Madar D and Dietlin F [4] developed dobutamine injectable solution containing ascorbic acid instead of classical sulfites used in the pharmaceutical industry, which present toxicity issues. Their solution provides enough stability to allow heat sterilization and extent to its shelf life.

Other authors prefer to act on process steps to protect oxygen-sensitive drugs:

Indeed, Current CPMP (Committee for Proprietary Medicinal Products) guidelines [18] require that the manufacturing process be optimized prior to inclusion of an antioxidant, because of the potential toxicity of these products.

For Zanon R *et al*. [16], the extent of degradation of their Injectable Lipid Emulsions of Compound A was correlated to oxygen level present in the headspace of vials. Building from this, process improvements and in-process monitoring were implemented, resulting in minimal impact of this degradation on subsequent emulsions. The same way, Brown M and Leeson JL [7] recommend the use of nitrogen (inert gas) to prevent oxidation.

Zhang JA *et al.* [17] studied the stability of famotidine injection. They saw that oxygen content present in the headspace promoted further oxidation and formed sulfoxide impurity and they raised the problem of intensive exposure to light that enhanced oxidative reaction.

Dietlin F and Fredj D [2] observed that oxidation induces a decrease of the API content, particularly when the drug needs a terminal sterilization (autoclave) and even infinitesimal quantities of oxygen (approximately 2ppm) can induce an oxidation at these temperatures. Dinnequin B [3] also observed this increased risk of altering molecules.

For Dietlin F and Fredj D, in the specific example of Paracetamol, the process chosen is a reduction of the residual oxygen (<2ppm) by bubbling with an inert gas and a final vacuum (≤65kPa) to maintain the low oxygen level.

Needham GF *et al.* [12] go further with a description of the manufacturing process and storage optimization to fill vials containing a new parenteral product sensitive to oxygen: a filling machine which included nitrogen pre-purge needles to purge the vials prior to liquid filling was used for this study. The critical variables that affected oxygen headspace during the vial filling process were identified as the presence of a tunnel (newly designed to provide nitrogen blanketing for the vial headspace between filling and stoppering), line speed and nitrogen flow rate.

The level of anti-oxidant in the formulation was finalized once the manufacturing capability was optimized.

Dinnequin B [3] also analyzed the manufacturing features linked to the deoxygenating process. The water is permanently deoxygenized thanks to nitrogen (Dissolved Oxygen rate ≤0.02 ppm), and the temperature of solution and of the water is maintained at 8-10°C to avoid its reoxygenation. A dense inert gas such as Argon is also used for deoxygenating fabrication tanks and blanketing, deoxygenating empty vials and inerting residual volume of full vials. The most critical point is inerting the vial. It can be managed by the speed of the repartition line (oxygen is better removed into the vials when the filling line goes slowly).

Dinnequin B fixed special conditions to be respected:

- The collecting tank must be immediately placed under argon after filtration and maintained under argon pressure for the full duration of filling.
- The line must be cleared carefully at the beginning of the filling.
- Whenever the machine stops, it is necessary to reject the uncorked filled products and the empty products having previously receiving gas.

In the same way, Zanardo V *et al.* [6] describe a manufacturing process to control oxygen concentration in injectable pharmaceutical composition containing a cathecolamine and a peptidic hormone.

Contrary to Dinnequin B, Al-Dandachi Atassi K [1] recommends to perform the complete preparation of the liquid formulations, up to the filling of glass bottles or vials,

at a temperature between 60°C and 105°C and low oxygen concentration conditions, avoiding an initial oxygen removal step at the initiation of the preparation of the liquid formulations.

The tanks are therefore preferably being kept under an inert gas pressure to replace any air by the same inert gas. The air in the filtration unit is replaced by an inert gas.

Advantageously, the aqueous solvent is not cooled prior to admixturing of the active principle, which constitutes not only a gain in the preparation time of the formulation (no need to use heat exchangers to cool the aqueous solvent), but also permits to obtain a suitable low oxygen concentration.

1.2.2. Oxygen measurement

In the literature, various methods and devices are used, depending on the type of sample tested and the step of the process.

In pharmaceutical packages, the determination of headspace oxygen can be done using Gas Chromatography (GC):

Dinnequin B [3] determines residual oxygen concentration by GC, measuring the residual oxygen concentration in the other gases.

One important advantage of gas chromatography compared to other techniques is small sample volume requirements [13]. But the main difficulty in this technique lies in removing, transferring and injecting a sample without contamination from oxygen present in atmospheric air. This is why Xu H *et al.* [15] developed a µGC method, which requires extremely small sample volumes, sampling advantages and shorter times for analysis. They determined headspace oxygen and moisture in packaging of solid drugs (glass vials or blisters containing tablets and capsules). It has not be tested on liquid formulation but the µGC can not handle liquid samples. This should probably make the technique unsuitable for our use.

Wu L *et al.* [14] provide a convenient GC method for measuring the levels of headspace oxygen in vials of pharmaceutical formulations. The GC, coupled with a mass spectrometry is simple, rapid and convenient and requires very small injection volume

(10µL). It can be used to determine oxygen contents in the headspace of injectable formulation containers.

In their experiments, Zanon R *et al.* [16] determined oxygen level in the headspace of individual emulsion vials using a Mocon Pac Check headspace analyzer [22]. The measure is made though a zirconium sensor: due to oxygen vacancies in the ceramic lattice structure of this material, oxygen ions are able to move in the solid material at a high temperature. This property enables the measurement of oxygen in a gas of unknown composition. Other oxygen analyzers use the same sensor (Illinois instruments [20] for example) or a similar electrochemical cell (Systech Instruments [24]).

These kinds of devices that measure oxygen concentration in gas need a minimum of 1 mL headspace (they are usually used in food-processing industries). The average headspace volume in syringes filled on the BD pilot filling line (0.3 to 0.5 mL) makes them unsuitable.

Lighthouse Instruments [21] and Wilco [26] propose at-line and in-line analyzers and are based on a laser absorption technology. Monitoring headspace oxygen concentration in sealed parenteral containers gives rapid analysis of 100% of the product. These devices could be integrated in the pilot production line but are not adapted for laboratory scale tests.

Zanardo V *et al.* [6] measured the dissolved oxygen concentration with a machine type ABL-5, Radiometer brand. This device was tested during the study and its principle and characteristics will be detailed in paragraph 2.1 of chapter 2.

To evaluate oxygen ingress into parenteral container closure systems during storage, Fukuyama et al. [9] used a Fibox 3-Trace oxygen meter (PreSens Precision Sensing GmbH [23]), whose principle is based on the effect of dynamic luminescence quenching by molecular oxygen. The system consisting of an optic fiber probe and a trace oxygen sensor spot enables oxygen measurement in a non-destructive way. The Oxysense

4000B used for a lot of experiments decribed below is based on the same principle. It will be detailed in paragraph 2.1 chapter 2.

In the pilot filling line isolator, a cleaning cycle consists in introducing Vaporized Hydrogen Peroxide (VHP). This quantity of high oxidative product must be carefully controlled for oxygen-sensitive drugs. Corveleyn S *et al*. [8] have developed a Near-infrared (NIR) spectroscopy method using a gas cell with optic fibers to monitor VHP sterilization cycles. This oxidating risk is therefore limited in the case of small headspace volumes in prefilled-syringes.

1.2.3. Container gas permeation

Fukuyama T *et al.* [9] evaluated oxygen ingress into parenteral container closure systems (glass vials sealed with rubber stoppers) during storage, particularly important for aqueous parenteral formulations which are subject to oxidative degradation. Their work demonstrated the ingress of trace oxygen, greatly influenced by the types of stoppers and their processing conditions.

The effect of oxygen permeation in glass prefilled syringe through rubber stoppers on the stability of an epinephrine solution was studied by Li M *et al*. [11]. The stability appeared to be better in syringes filled higher or with less oxygen in headspace. Inclusion of syringes into N_2-flushed aluminum foil pouches afforded better stability whereas use of a less oxygen-permeable tip cap did not significantly improve stability. They concluded that the permeation of oxygen through rubber stopper in the prefilled syringes appeared to cause the degradation in epinephrine solution with low fill volume.

1.2.4. Oxygen and dissolution physics

The physical law that describes the solubility of a gas in a liquid as a function of temperature is Henry's law [25]:

At a constant temperature, the amount of a given gas dissolved in a given type and volume of liquid is directly proportional to the partial pressure of that gas in equilibrium with that liquid.

It can be put in mathematical terms: $p = K_H c$ where *p* is the partial pressure of the solute in the gas above the solution, *c* is the concentration of the solute and K_H is Henry's law constant, which depends on the solute, the solvent and the temperature.

For oxygen dissolved in water at 298 K, K_H=769.2 L·atm/mol

Henry's law constant depends on the temperature through the following equation: $K_H(T) = K_H(T_0) \cdot e^{-c\left(\frac{1}{T} - \frac{1}{T_0}\right)}$ where *T* is the sample temperature and T_0 refers to the standard temperature (298 K), C (O_2) = 1700 K.

The air naturally contains about 21% of oxygen.

If a gas (in our case oxygen) is dissolved in a solution (it is the case for injectable drugs), the contact between drug molecules and dissolved oxygen is increased. This can lead to chemical reactions leading to unwanted degradation products that can be allergenic or toxic, a loss of activity of the active ingredient because of the decrease of its title in an uncontrollable manner, or the formation of colored compounds, making the aqueous solution unsuitable for therapeutic applications.

2. Material: oxygen measurement

2.1 Requirements

One of the objectives of the study was to look for a device that would best fit our needs:

A List of the different devices for measuring oxygen rates in liquid and gas phases that exist on the market was established, describing their working methods and their characteristics.

The advantages and drawbacks were studied, for a R&D use and later for quality control use (IPC). Some devices were concretely tested and a selection was operated among this panel.

A new apparatus was acquired and installed for the formulation laboratory.

As presented in the previous chapter, a lot of devices are available on the market to measure oxygen concentration.

The device had to satisfy the following requirements:

- Be able to measure oxygen concentrations in liquid and/or in gas and more precisely in the Hypak® 2.25 mL syringes that would be studied.
- Give reliable, accurate and repeatable data.
- Be easy to use and give a rapid measure.
- Get calibrated easily.
- Make non destructive measurements.
- Be used on a laboratory scale as well as during manufacturing for In Process Controls (IPC) and final product control.
- Be qualified as cGMP equipment.

Among the devices quoted below, some of them were immediately rejected.

Four devices were tested in the formulation laboratory. The detail of how they work and their main characteristics is provided hereunder. Representative pictures are presented in Table I p. 40.

- **Oxygen meter Mettler-Toledo Seven Go Pro with InLab® 605 polarographic oxygen sensor:**

  This device was already used in the laboratory to measure oxygen concentrations in bulk solutions. It is designed for the simultaneous measurement of dissolved oxygen and temperature in aqueous samples (plunging probe). The connectors provide waterproof performance. The InLab®605 DO sensor incorporates a technology in polarographic dissolved oxygen determination based on Clark principle.

- **Oxysense® 4000B:**

  This device was also already present in the laboratory at the beginning of the study.

  The OxySense® 4000B Oxygen Analyzer is a portable, fully integrated instrument that enables the non-destructive measurement of oxygen in transparent sealed packages or bottles.

  The system utilizes a non-invasive optical measurement methodology with an Oxydot, (a separate fluorescent oxygen-sensitive sensor) that must be manually glued inside a transparent container to measure oxygen concentration inside this container.

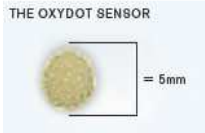


Figure 5 – Oxydot

The system is based on the effect of oxygen on fluorescence lifetime of an optically excited Ruthenium complex immobilized in a highly stable polymer. An optical fiber probe is used to emit and receive light. The partial pressure of oxygen is derived from the fluorescence lifetime of the Ruthenium complex.

Figure 6 – OxyNeedle fixed on the Oxysense pen

The Oxyneedle is an accessory that can be fixed to the tip of the probe. It consists in a penetration needle containing a sensor (composed of the same material as the Oxydot) that fits directly onto the optical fiber probe assembly. When the Oxydot cannot be glued inside the system to test, the Oxyneedle can be inserted in the sample.

- **ABL5 Radiometer:**

It is a blood gas analyzer that measures pH, pCO_2 and pO_2 starting from a minimum of 85 µL of liquid sample. The sample is sucked through a small pipe. In the case of filled syringes, the measure is destructive and the stopper must be manually removed before performing the measure.

Oxygen partial pressure (pO_2) is measured by an amperometric electrode in a thermostated chamber. The results are given in pressure unit: mmHg or kPa.

This device was used by Zanardo *et al*.[6] in their patent and we were able to test it for three months (lease agreement).

- **ABL80 FLEX Radiometer:**

It works on the same principle that the ABL5 but is equipped with a customizable user interface using Windows XP operating system. The electrode chambers are located on a removable cassette that allows a specific number of measures (50 to 300) and has to be changed monthly. The solution pack containing the calibrating solutions has to be changed monthly too.

I was able to test this device for one month (lease agreement).

2.2 Comparison of the 4 devices selected:

Table I – Comparison of oxygen meter devices

Device	Oxygen meter probe	Oxysense (Oxydot and Oxyneedles)	ABL5	ABL80
Picture				
Measure-ment/Unit	Bulk solution % or ppm	Gas and solution % or ppm	Solution mmHg or kPa	Solution mmHg or kPa
Advanta-ges	° Easy to use ° Mobile ° Good ergonomics ° Easy and rapid 1-point calibration or 2-points calibration ° Cheap device	° Results in ppm unit ° Good reliability of results ° Fast response time ° Doesn't consume oxygen ° Can perform automatic measures and record the results on excel format sheet ° OxyDot: long shelf life of syringes with the Oxydot sensor → long-term studies possible ° Oxyneedles: can be used when Oxydot gluing is not possible, taking of the stopper	° Easy to use ° Good reproducibility ° Minimum maintenance ° Automatic calibration ° Cheap device and consumables	° Intuitive touch screen ° Automatic copy of all data ° USB Connection to collect data ° Solution can be sampled directly from the tip of the syringe, taking off the PRTC (without removing the stopper) ° Easy identification of sample and operator (possible access by password)

Device	Oxygen meter probe	Oxysense (Oxydot and Oxyneedles)	ABL5	ABL80
Drawbacks	° Only on bulk solution (large probe) ° Low precision	° Not accurate for high oxygen levels ° OxyDot: Must be formerly glued Gluing tricky and not possible in all equipments (i.e. IPC not possible) ° Oxyneedles: Long response time, very bad reliability (at prototype stage when tested)	° Blood gas analyzer (not specific to our use) ° Results in mmHg unit (partial pressure) ° Destructive: long-term studies impossible ° Stopper must be removed manually before measure ° Few possibilities of identifying the sample (only figures) and for ticket printing	° Consumables expensive and with short shelf life ° Results in mmHg unit (partial pressure)
Price (HT)	Device: 872€	Device : 7,700 € Application kit: 480 € Oxydots x 100: 578 € OxyNeedles x 5: 1,110 €	Device: 9604 €	Device: 20,000 € Consumables: 530 €/month
Possible use	Formulation tank only	Oxydot: lab studies only	Lab studies	In Process Control, cGMP use

Note: Oxygen concentration (in ppm) can be approximately converted into an oxygen partial pressure (mmHg) with Henry's law. For details, see Appendix 1.

2.3 Conclusions

Oxygen **meter probe Mettler-Toledo Seven Go Pro** will still be used for the determination of oxygen rates in bulk solution (during formulation) in lab-scale. We can notice that this type of oxygen meter is permanently present in the formulation tank of the pilot production line (50 liters tank) and allows to check oxygen rate during formulation (feedback to the Programmable Logic Controller).

The **Oxysense® 4000B** will still be used, with the Oxydot sensors for studies at laboratory scale. The promising Oxyneedles turned out to be unreliable with non repeatable results between each needle (prototype accessory that need supplier improvement). This accessory will not be used anymore.

The ABL5 **Radiometer** was tested for 3 months and purchased on August 2009. It will be used only for R&D tests and therefore will not be specifically qualified. The problematics of the mmHg unit can be treated in 2 ways: either we use as a reference an oxygen concentration with a partial pressure unit and then the referential for product specifications is in mmHg; or the results in mmHg can be translated in concentration unit (ppm) with Henry's law.

The **ABL80 FLEX Radiometer** was tested during one month and represents the best choice. Its major drawback being the very expensive consumables, this device cannot be used daily for R&D tests. We plan to purchase this device later to equip the manufacturing area and qualify it for cGMP use (quality control on final product and IPC).

3. Studies at Laboratory scale

Introduction

During this eight months study, numerous tests were performed in the formulation laboratory.

These tests had three main purposes:

- To better understand the physical phenomena linked to oxygen;
- To prepare the tests performed at a bigger scale in the pilot manufacturing filling line;
- To prepare the pre-stability and stability tests that will be launched for oxygen-sensitive drugs to control their stability in accelerated conditions of temperature and humidity. These conditions are described in guidelines from the International Conference of Harmonization (ICH).

Some of these tests are detailed below:

3.1 Dissolved oxygen equilibrium under various conditions

Introduction and objectives

Before starting the tests at a bigger scale (pilot manufacturing line), it was important to predict the impact of the operating conditions on oxygen concentration in syringes at the equilibrium. The conditions tested are the composition of the gas headspace and the way the solution is inerted. They are important to define a worst case volume for pilot experiments.

This test was also conducted to know the capacity of our laboratory equipment for corking syringes: Stopper Placement Unit (SPU).

Material and Methods

The solution to test was isotonic saline solution (NaCl 0.9%), in order to get closer to the pharmaceutical isotonic solutions. The syringes studied were BD Hypak® SCF 2.25 mL syringes described in chapter 1.1. The filling volume was 2.2 mL and the headspace volume was about 0.25 mL.

Oxygen measurement was performed with the Oxysense device (see chapter 2), and the Oxydots were first glued on the inner surface of the syringes.

The solution was inerted (nitrogen bubbling under an atmosbag pocket) or not inerted. The stoppering with the SPU was conducted under a double nitrogen cycle or under air.

The operating conditions are summed up in Table II below. For each condition, 12 syringes were filled and corked.

Table II – Test Matrix

	Solution inerted with N_2	Stoppering with SPU
Test 1	No	**Under N_2**
Test 2	**Yes**	Under air
Test 3	**Yes**	**Under N_2**

The measures of oxygen concentrations in the syringes were performed during 24 hours. The T0 point corresponds to the measure just before the SPU corking.

Results and discussion

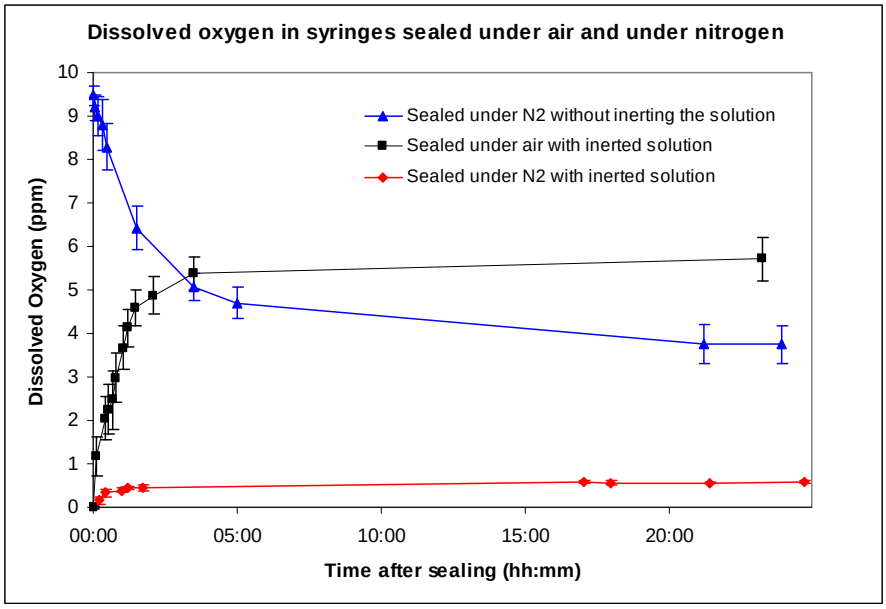


Figure 7 – Comparison of oxygen concentrations in syringes according to the conditions of inerting and corking (n = 12)

This test shows an important oxygen reuptake in solution for syringes filled with inerted solution and sealed under air (from about 0 to 5.7 ppm) and an important oxygen decrease for syringes not inerted and sealed under nitrogen (from 9.5 to 3.5 ppm).

On the one hand, it means that even if the solution is not very well inerted, oxygen level in the syringe can be quite low if the corking is done with nitrogen. And on the other hand, if the solution is well inerted, the final oxygen concentration in syringes can be high if the corking is not optimized with nitrogen.

This test shows that three to five hours are necessary to reach equilibrium in oxygen concentration in the syringes. Another experiment not presented in this manuscript showed that this time can be reduced to about twenty minutes using a vortex agitator.

Conclusion

This test highlights several points.

At first the necessity of waiting for the equilibrium between oxygen dissolved in the solution and the gaseous oxygen in the headspace.

Then we notice that the headspace volume and its gas composition are critical to achieve low oxygen level. This is a very important parameter that will impact the filling process at a higher scale. From this test, the 1.2 mL filled syringes seems to be the worst case because as the headspace is the same in the 1.2 and 2.2 mL syringes, the composition of the gas will have a higher impact.

Finally we have an idea of the capacity of our laboratory equipment to maintain a low oxygen concentration in syringes or to impact this concentration, according to the parameter fixed.

3.2 Long-term study of oxygen reuptake in syringes

Introduction and objectives

As we saw previously in the bibliography, an important method to protect oxygen-sensitive drugs from oxidation in filled syringes is to remove oxygen from the solution during formulation and to conduct the process under an inert gas protection (nitrogen most of the time). Previous experiments performed showed that an equilibrium time of about 24 hours was necessary to equilibrate oxygen present in the gaseous headspace of the syringe and oxygen dissolved in the solution. After 24 hours, oxygen concentration seemed stable but some other tests showed an increase after a few days.

The objective of this test was a long-term study of oxygen reuptake in syringes filled with inerted water and corked with the SPU, beyond these 24 hours of equilibrium time. This study was also aimed at determining the influence on this oxygen reuptake of the stopper nature, the filling volume and the position of the syringes during the test.

Material and Methods

The syringes studied were BD Hypak SCF 2.25 mL syringes described in chapter 1.1. The solution used for the test was water for injection (WFI). Two volumes of WFI were compared in this test: 1.2 mL and 2.2 mL. These volumes are usually used to fill syringes within PI project.

Two different types of stopper were tested: stopper A and stopper B. The difference between these stoppers is the composition of the rubber.

Oxygen concentration in the syringes was measured by oxygen meter Oxysense (concentration in part per million).

Work instructions

The Oxydots were first glued on the inner surface of 24 syringes. The spots were glued at a same distance from the bottom of the syringes to obtain comparative results

for all the syringes filled with 1.2 mL and the same for the syringes filled with 2.2 mL of water.

After purging an Atmosbag pocket (plastic pocket that maintain an inert atmosphere) with nitrogen, oxygen was removed from the WFI by nitrogen bubbling.

The syringes were then filled with 1.2 mL or 2.2 mL of deoxygenated water under the pocket and corked with a SPU with stoppers A and B according to Figure 8 below. The stoppering was performed under nitrogen atmosphere.

Oxygen concentration was measured in all the syringes with the Oxysense. To see the evolution of this oxygen concentration according to the storage condition, half of the syringes were placed horizontally in a rondo, whereas half were left vertically in a nest (see Figure 8).

Note: The stopper was in contact with the solution only in syringes kept in a rondo.

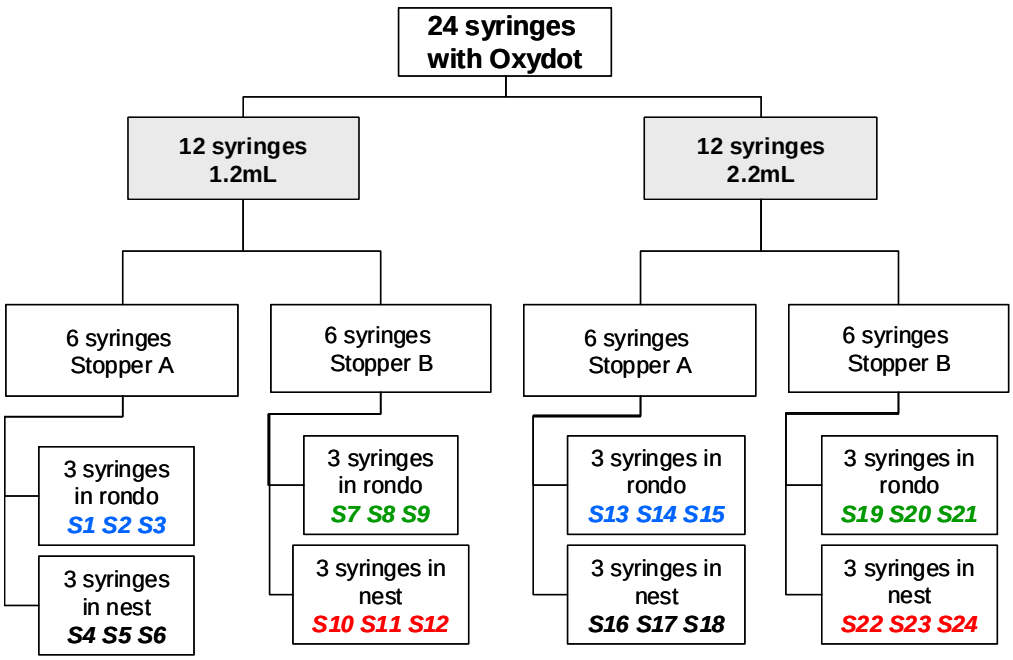


Figure 8 – Numbering of syringes according to the test conditions

All of the syringes were stored at room temperature (between 22 to 24°C).

Measurements were performed on the 24 syringes with the Oxysense every 2 or 3 days during 2 months. A last measurement was made 3.5 months (103 days) after stoppering.

The data registered into the Oxysense was collected to determine oxygen reuptake function of the stopper and the position of the syringe during the test (rondo or nest)

Results and discussion

A table of gross results registered in the Oxysense and a table that sums up the standard deviation for each condition are available in Appendix 2.

Results for 1.2 mL syringes:

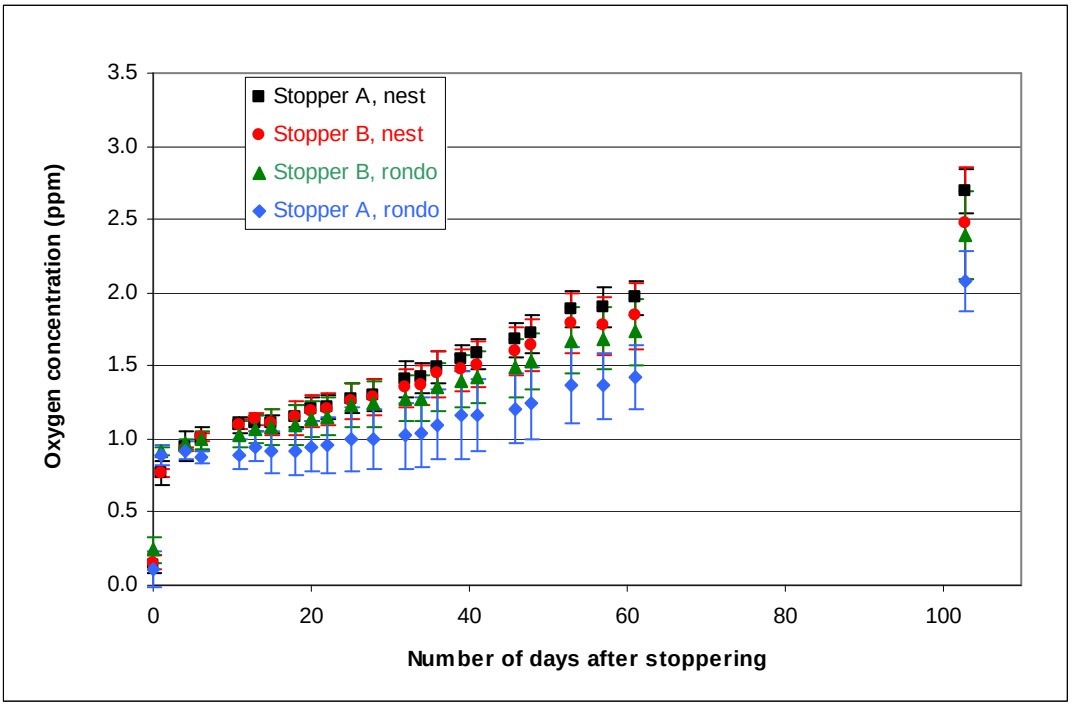


Figure 9 – Oxygen concentration evolution in 1.2 mL syringes, function of the stopper nature and the syringe position (n = 3)

The results show a sharp increase of oxygen concentration from T0 to T1. This increase was already seen in lab-scale tests and corresponds to the equilibrium time between oxygen in the gas headspace and oxygen dissolved in the solution.

After the first days, oxygen concentration continues to increase slowly but steadily and the Y-bar errors increase at the same time. This means that the difference between each syringe for a same condition is quite important.

To compare the results obtained, an ANOVA two-factor test was performed (see Appendix 3 p105)

This test states that, for the 1.2 mL filled syringes, there is no significant difference between stoppers A and B (*p-value*=0.75>0.05) and no significant difference between the syringes conserved in rondo and in nest (*p-value*=0.06>0.05).

Results for 2.2 mL:

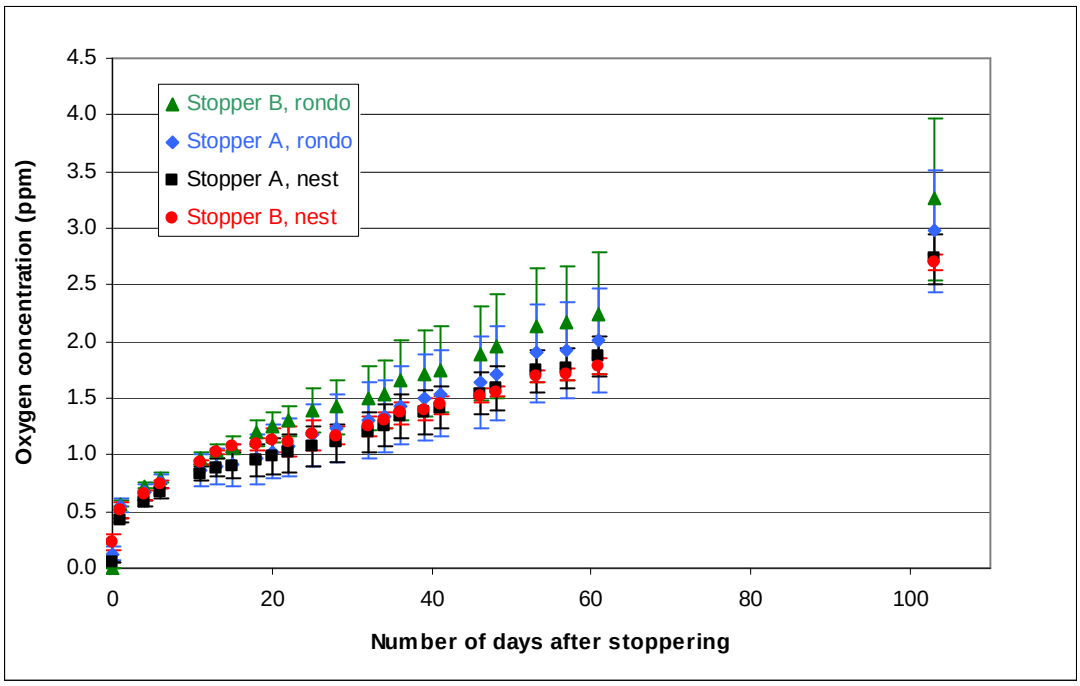


Figure 10 – Oxygen concentration evolution in 2.2 mL syringes, function of the stopper nature and the syringe position (n = 3)

This graph is very similar to the previous one: a sharp increase of oxygen concentration from T0 to T1 corresponding to the equilibrium time between oxygen in the gas headspace and oxygen dissolved in the solution and a regular increase of oxygen concentration and Y-bar errors in a long-term.

However, the results show responses that differ from the 1.2 mL syringes. Here, the syringes with the highest oxygen increase are the ones conserved in rondos.

To compare the results obtained, an ANOVA two-factor test was performed (see Appendix 4 p107)

This test states that, for the 2.2 mL filled syringes, there is no significant difference between stoppers A and B (*p-value*=0.65>0.05) and no significant difference between the syringes conserved in rondo and in nest (*p-value*=0.17>0.05).

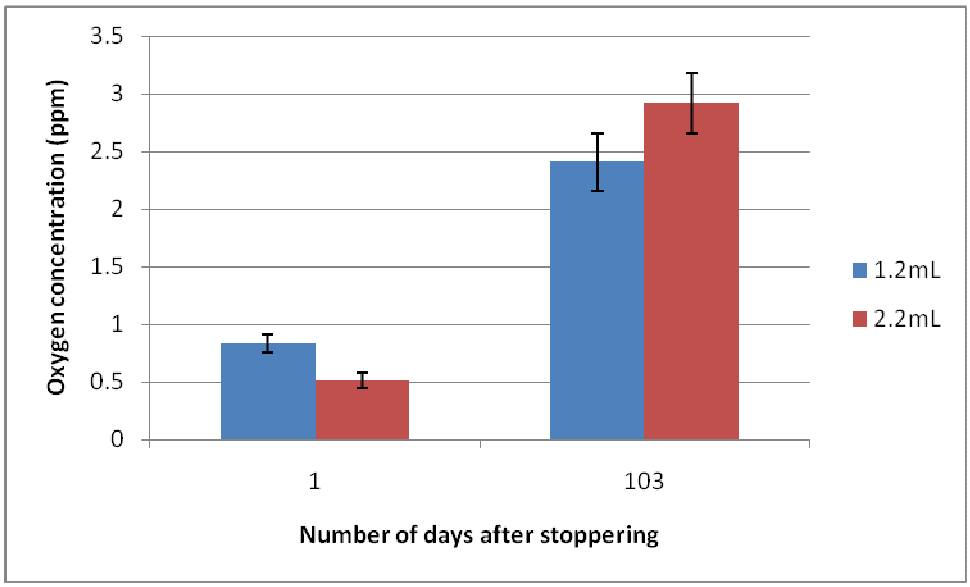


Figure 11 – Average oxygen concentration in 1.2 and 2.2 mL filled syringes at the beginning and the end of the experiment (n = 12).

After one day, the difference of oxygen content between the 1.2 and 2.2 mL filled syringes is explained by the importance of the headspace volume compared to the solution volume.

We can notice that the slope of oxygen increase is sharper for 2.2 mL filled syringes (concentration lower than 1.2 mL syringes at the beginning of the experiment and higher at the end). This means that for oxygen reuptake, the worst case is 2.2 mL. The reason of this remains unclear. The number of syringes in each group (n=3) was probably too small in order to provide significant differences, given that a lot of variability occurred during measurements.

Conclusion

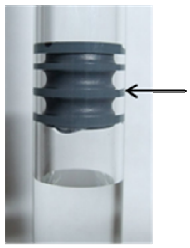
The phenomenon of oxygen reuptake observed in a long-term study is very important for the development of oxygen-sensitive drugs. This increase goes beyond the classical stabilization time during which the equilibrium between the dissolved oxygen and the gaseous oxygen is reached.

This study demonstrates that oxygen concentration increases sharply in syringes, regardless of the stopper (A or B) or the position of the syringe during the test (rondo or nest).

The origin of this increase is not determined for sure. Some hypotheses are proposed.

This increase could be linked to a leak of gas. Glass is known to be impervious to gases so researches should turn to the stopper or the tip cap. The permeability of silicone has been demonstrated in a previous test.

Actually, the stopper and the inner surface of the syringe barrel are covered with silicone. We can suppose that the leakage comes from that part of the device. This should be confirmed or invalidated by other tests.



Another hypothesis incriminates the vacuum in the headspace of the syringe. Indeed, to cork the syringes with the stoppers, the SPU creates a vacuum. The importance of the vacuum determines the volume of headspace in syringes. The depression makes the stopper lower but when the friction force gets equilibrate with the depression, the stopper stops and a slight depression remains in the headspace. If the stopper has a slight permeability to gas (it remains to be demonstrated), this residual depression could attract gas and increase oxygen level.

The last hypothesis is the presence of oxygen in the stopper before stoppering. Oxygen can then diffuse into the syringe and increases oxygen content in solution. This could explain the increasing oxygen concentration at the beginning of the experiment but does not explain the increase in a long-term.

The 2.2 mL syringes saw their oxygen level increase more than the 1.2 mL syringes. This volume seems therefore more critical than the 1.2 mL volume.

3.3 Silicone tubing permeation

Introduction and objectives

During the process of filling in the pilot manufacturing area, an important step for the product is the transfer from the storage tank to the filling machine, through an intermediate tank called buffer tank. For more details on the process, see chapter 4.

This transfer is performed with a specific filtration system composed of silicone tubing and 2 sterilizing filters type Millipore Opticap, as shown on Figure 12 below:

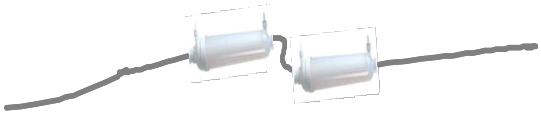


Figure 12 – Filtration transfer system of the filling machine

In the rest of the process, the solution is conducted in the filling machine through metal pipes, impervious to oxygen transfers. Thus, this silicone tubing represents a critical part of the process and I performed this test to evaluate its permeability to oxygen.

Material and Methods

The material to test is a silicone tubing Silicone Pharma 50 with an internal diameter of ½ inch (1.27 cm) and an external diameter of ¾ inch (1.90 cm). An Oxydot was glued in the inner surface of the tubing and a rapid test was performed to verify the capacity of the Oxysense to capture light through the slightly opaque tubing.

The solution tested was water for injection (WFI).

Work instructions

- Oxygen was removed from the water by bubbling with nitrogen inert gas under an Atmosbag pocket in an oxygen free environment.
- The silicone tubing: Silicone Pharma 50 was filled with this deoxygenated water.
- The pipe was then taken off from Atmosbag.

- The dissolved oxygen was at first measured every 30 minutes during 24 hours (automatic capture) with the Oxysense device.

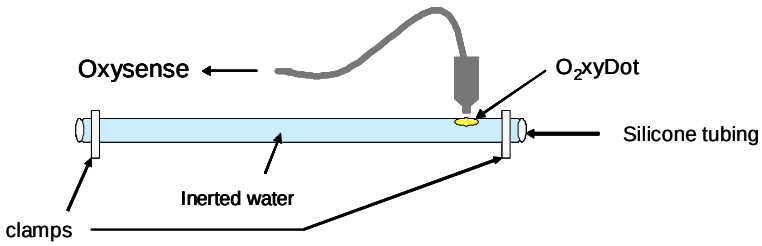


Figure 13 – Facility for the measure of the silicone tubing permeation

Results and discussion

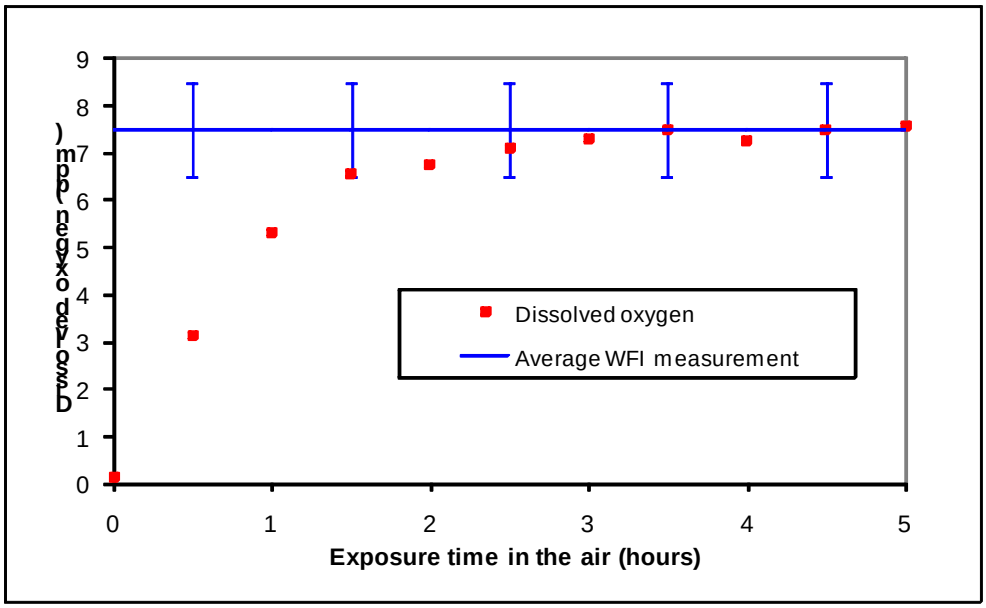


Figure 14 – Oxygen reuptake in silicone tubing compared with usual oxygen rate in WFI

Note: the average WFI measurement gives information on the concentration of dissolved oxygen in water. The Y-error bar is important because it varies a lot depending on the measuring conditions (water at rest or agitated for example). In addition, the accuracy of the Oxysense measure is relatively bad for high oxygen levels.

The aim of this first test was to observe an increase on 24 hours, but oxygen reuptake is very fast and high. Indeed, in about 2 hours, oxygen concentration in the tubing reaches the level of non inerted water. With one measure every 30 minutes, we

were not able to see what really happens in the first minutes. The same test was then repeated again, measuring oxygen concentration every minute during 30 minutes, in order to observe the phenomenon in the first instants:

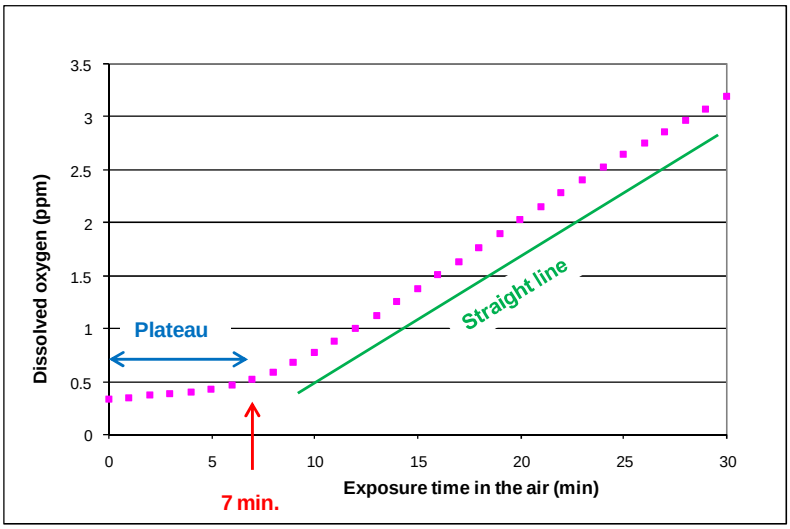


Figure 15 – Oxygen reuptake in silicone tubing the 30 first minutes.

We can observe here a short plateau of about 7 minutes where oxygen permeation rate almost doesn't increase, followed by a sharp and linear increase.

Conclusion

This study demonstrates the high oxygen permeability of silicone tubing. It raises questions for the manufacturing process, because if the solution stays a certain time in this tubing in contact with the air, the solution will rapidly reuptake oxygen and it will be crucial to determine whether or not it impacts the final oxygen rate in syringes. This will be the subject of chapter 4.

4. Optimization of the manufacturing process

Introduction

The production of syringes filled with a solution comprising an oxygen-sensitive drug raises some process issues.

The manufacturing process in PPH is summarized on Figure 16 below. The important points for inerting issues are highlighted.

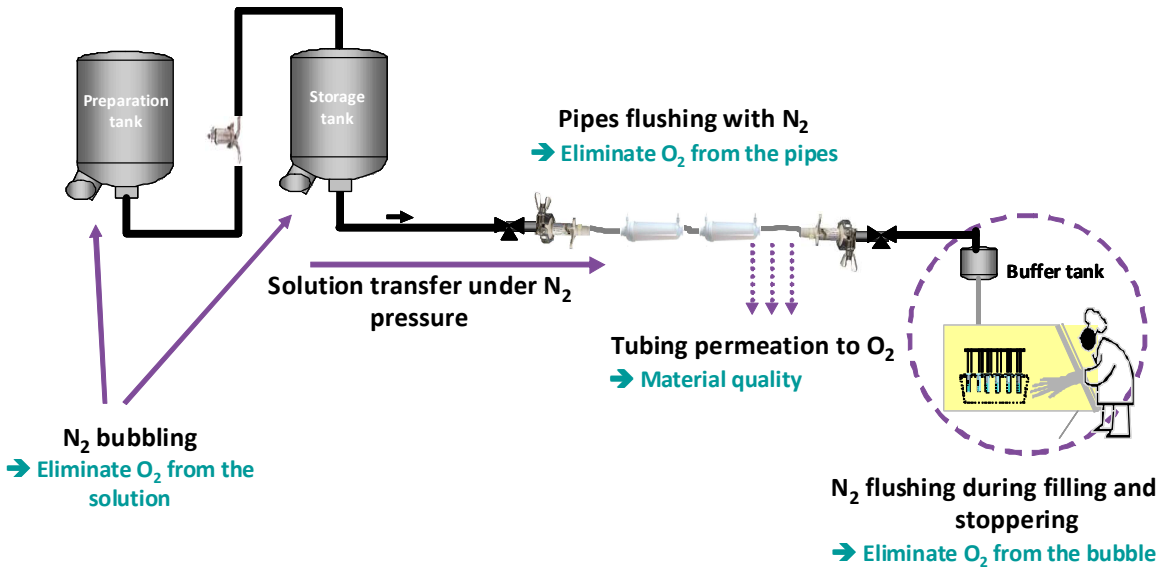


Figure 16 – Inerting manufacturing process optimization

- The first and second steps of the process consist in the formulation in a preparation tank and the storage of the prepared solution in a storage tank. These tanks are connected by metal pipes that are not submitted to oxygen reuptake. A diving wand enables to bubble nitrogen into the solution to eliminate the dissolved oxygen into the two tanks. An oxygen meter working on the same principle as the Mettler-Toledo Seven Go Pro (see chapter 2.2 above) is inserted in the tank. It is connected to the diving wand. When oxygen concentration into the tank increases up to a specific level (determined by the user), the wand automatically bubbles into the tank to reach a lower oxygen

concentration. This part of the process can be easily controlled and won't be the subject of the following tests.

- The transfer of solution through the metal pipes and through the filtration system can be done under nitrogen pressure.

- The following point is the one discussed on paragraph 3.3 and concerns the silicone tubing. The silicone material is well known for its high permeation to oxygen. During the process, the solution can stay some time in this tubing and be re-oxygenated. This tubing can possibly be replaced by less permeable materials (for example thermoplastic elastomers).

- Then the solution reaches a buffer tank before being distributed, via silicon tubing, into the syringes through filling needles. The detail of this part of the process is important to understand the tests performed:

  - The buffer tank has two sensors, corresponding to a lower and an upper limit, that determine the amount of solution contained in the tank. During the filling of the syringes, the volume of solution in the buffer tank decreases and when this volume reaches the lower level, the buffer tank sends an automatic feedback to open the on-off valve that controls the entrance of solution from the storage tank and fills the buffer tank up to the upper limit. Then the on-off valve closes.

  - The filling of the syringes consists in two mains stages: the filling and the stoppering or corking. The machine counts 5 filling needles and 5 stoppering "arms". As we saw in the introduction (chapter 1.1), a tub of 100 syringes is decomposed in 20 lines of 5 syringes, there is an interval of 1 line between the filling and the stoppering station, that means that while the syringes from line 3 are filled, the syringes from line 1 are corked. This is explained on Figure 17.

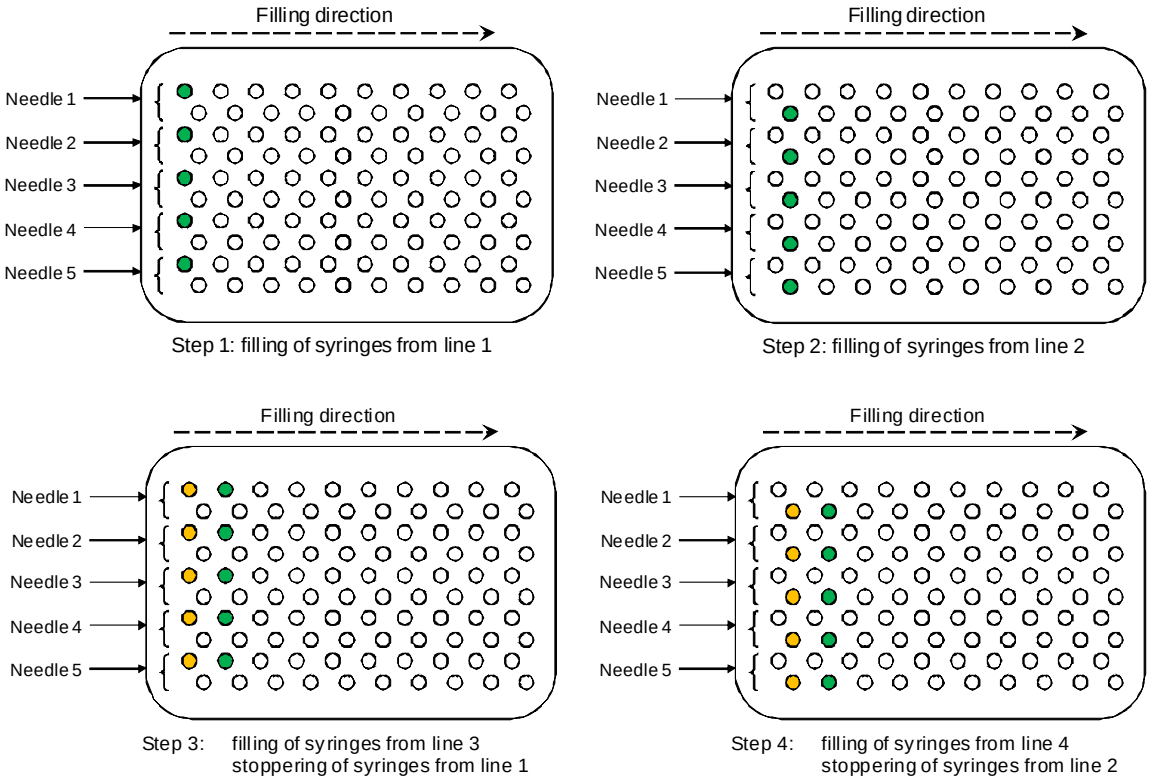


Figure 17 – Filling and stoppering of syringes with the pilot filling machine

At this stage, the filling machine parameters can impact oxygen levels thanks to the possibilities of flushing nitrogen through the filling needles during filling and at the stoppering station during the corking. These nitrogen flow rates have to be optimized; this will be one of the aims of the following tests.

Three tests were carried out on the pilot filling line. The aim of these tests was to determine intrinsic parameters of the filling machine itself. The tests presented below were not carried out with the preparation and storage tanks but with a laboratory 20 liters tank, directly connected to the machine.

4.1 Test 1 of inerting optimization on the filling line

Introduction and objectives

The aim of this test was to optimize the process to reach the best inerting of the syringes during filling and stoppering to obtain pre-filled syringes containing the minimum oxygen concentration.

This test was also conducted to identify potential inerting differences into a tub at each filling station (needles 1 to 5) or between the beginning and the end of the tub.

Material and Methods

The solution used to fill the syringes was WFI.

7,200 syringes BD Hypak® 2.25 mL (72 tubs of 100 syringes) were filled during this test, and they were corked with 7,200 stoppers.

The machine (Groninger brand) can fill and cork 100 syringes each minute. Among the parameters that can be modified, the relevant factors influencing the inerting were determined by brainstorming. As there were many of them and considering the complex manipulations, the study was conducted with the Design of Experiment (DOE) methodology.

Table III – Design of Experiment factors

Factors		Level -	Level +
A	Nitrogen flow rate at filling station (L/min)	2.5	5.5
B	Nitrogen flow rate at stoppering station (L/min)	2.5	5.5
C	Global machine rate (cycles/min)	45	55

The global machine rate determines the speed of the filling line. The usual rate fixed for this machine is 50 cycles per minute.

These parameters were also admitted as critical in the bibliography. For example for Needham GF *et al.* [12], the critical variables that affected oxygen headspace during the vial filling process were identified as the presence of a tunnel (newly designed to provide

nitrogen blanketing for the vial headspace between filling and stoppering), line speed, and nitrogen flow rate. In our case, the blanketing between filling and stoppering was not possible and not considered as critical thanks to the very short time between the two operations.

The 3 factors studied will vary during the test and other important parameters have to be fixed:

- The filling volume must be fixed at 1.2 or 2.2 mL (usual volumes for the project). The previous tests conducted in the formulation laboratory (see chapter 3 Paragraph 3.1) led us to consider the 1.2 mL as a worst case. It will be the filling volume in this test.
- To connect the laboratory tank to the filling machine, we had to use silicone tubing. The impact of the silicone tubing permeation will be studied in the next test. The length will be therefore minimized to 60 cm (minimum length) to restrict its effect. It is a tubing Silicone Pharma 50 with an internal diameter of ½ inch (1.27 cm) and an external diameter of ¾ inch (1.90 cm), similar to the one in the filtration system.

Two devices were used to measure oxygen concentration during this test: oxygen meter Mettler Seven Go Pro, to control oxygen level in the tank; and the ABL5 to measure the final oxygen partial pressure in syringes.

Choice of the experimental model:

The model used was a linear model with all interactions (Full factorial experiment at 2 levels: 2^3), a model traditionally used when searching for optimum conditions out of the experimental domain. The nine experiments were performed in the same test.

Table IV below indicates the experiments conducted with the coded value of each factor.

Table IV – Experimental matrix with coded values

Experiment Number	**Factor A** Nitrogen flow rate at filling station (L/min)	**Factor B** Nitrogen flow rate at stoppering station (L/min)	**Factor C** Machine rate (cycles/min)
1	-1	-1	+1
2	+1	-1	+1
3	-1	+1	+1
4	+1	+1	+1
5	**0**	**0**	**0**
6	-1	-1	-1
7	+1	-1	-1
8	-1	+1	-1
9	+1	+1	-1

Note 1:

Experiment 5 corresponds to the conditions in the center of the experimental domain. It will be important to check the linearity of the model.

Note 2:

The experiments were performed in a partially randomized order. The machine rate was changed only once to better separate the importance of the different effects.

Work instructions

- 18 liters of WFI were collected into the tank and oxygen was removed by bubbling during 20 minutes with nitrogen from BD utilities (Oxygen control with oxygen meter Mettler Seven Go Pro).

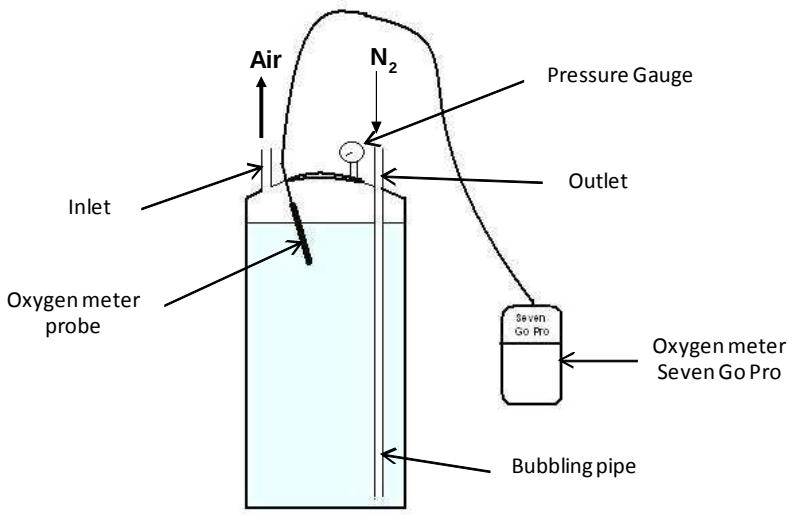


Figure 18 – Diagram of the tank

- A 0.6 bar nitrogen pressure was then applied inside the tank to transfer it (and all the material required) in the pilot manufacturing area.

- The filling machine was purged with nitrogen from BD utilities from the buffer tank to the filling needles. The silicone tubing was also purged and the tank was connected to the filling machine.

- The filling program was started with the 1st set of experiment parameters:
  - Syringes filled with 1.2 mL of water
  - Machine rate: 55 cycles/min
  - Nitrogen flow rate at filling station: 2.5 L/min
  - Nitrogen flow rate at stoppering station: 2.5 L/min.

- The nine experiments were then performed according to the order defined by the design of experiment below, filling eight tubs per experiment and sampling the required syringes.

Table V – Experiment matrix with real values

Exp Number	**Factor A** Nitrogen flow rate at filling station (L/min)	**Factor B** Nitrogen flow rate at stoppering station (L/min)	**Factor C** Machine rate (cycles/min)
1	2.5	2.5	55
2	5.5	2.5	55
3	2.5	5.5	55
4	5.5	5.5	55
5	**4.0**	**4.0**	**50**
6	2.5	2.5	45
7	5.5	2.5	45
8	2.5	5.5	45
9	5.5	5.5	45

- The analysis of oxygen partial pressure was done in the syringes with the ABL5 at least 24hours after the filling to have stable measures.

- Among each series of 8 tubs filled, 30 syringes were sampled, taken from lines 1, 10 and 20 of the seventh and eighth tubs filled, as described below.

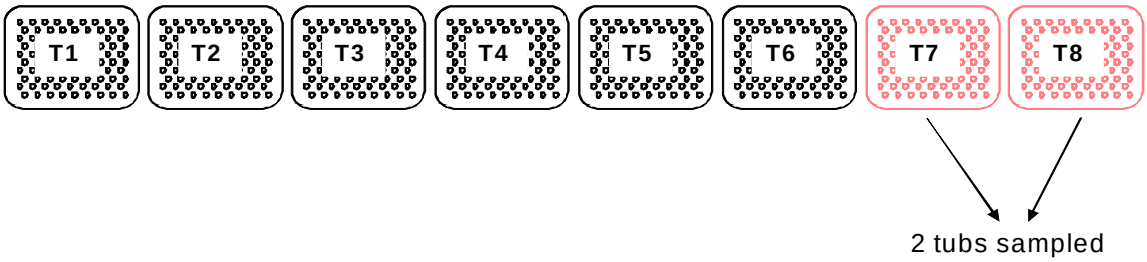


Figure 19 – Position of sampled tub for Experiments 1 to 9

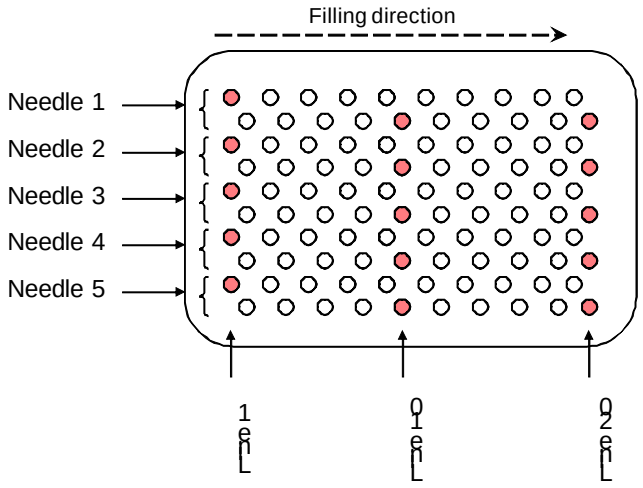


Figure 20 – Sampled syringes for each sampled tub (Exp 1 to 6)

- For experiment 5 (center of the experimental domain) the measures were doubled (sampling of syringes from line 19)

Results and discussion

Measurements were performed with the ABL5, 24 to 48 hours after corking. Fifteen syringes were analyzed for each sampled tub, line by line: every syringe from line 1 first, then every syringe from line 10 and finally every syringe from line 20. The results are given in Appendix 5.

An increase of oxygen partial pressure between each line measured was rapidly observed, meaning that oxygen content increased during the time required to perform the measures.

Note: the test “Long-term study of oxygen reuptake in syringes” described in chapter 3.2 had not been performed yet and the increase was perceived, at that time, as an important equilibrium time between oxygen dissolved in the water and the gaseous oxygen from the headspace.

The following analysis was therefore done only on the syringes from line 20 (oxygen partial pressure better stabilized).

Statistical analysis with Minitab® Software

The impact of the 3 factors and their interactions are shown on the Pareto chart and the Effects Plot below.

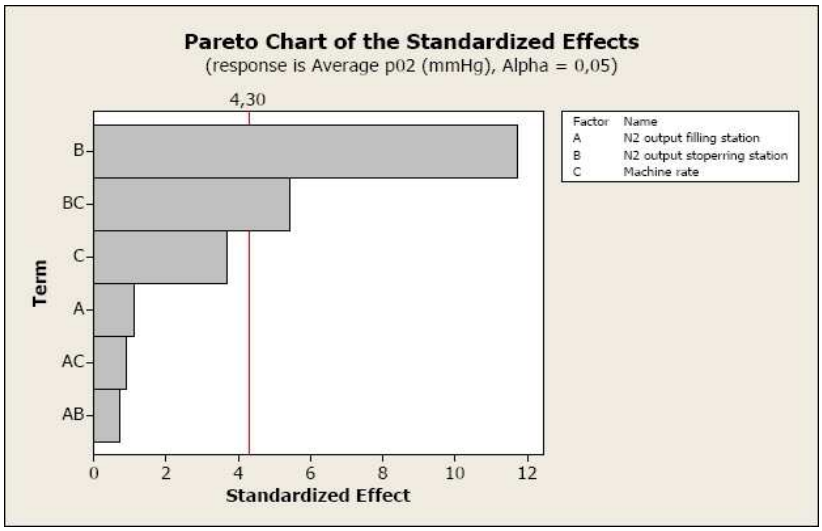


Figure 21 – Pareto Chart of the standardized Effects for oxygen partial pressure in syringes from line 20

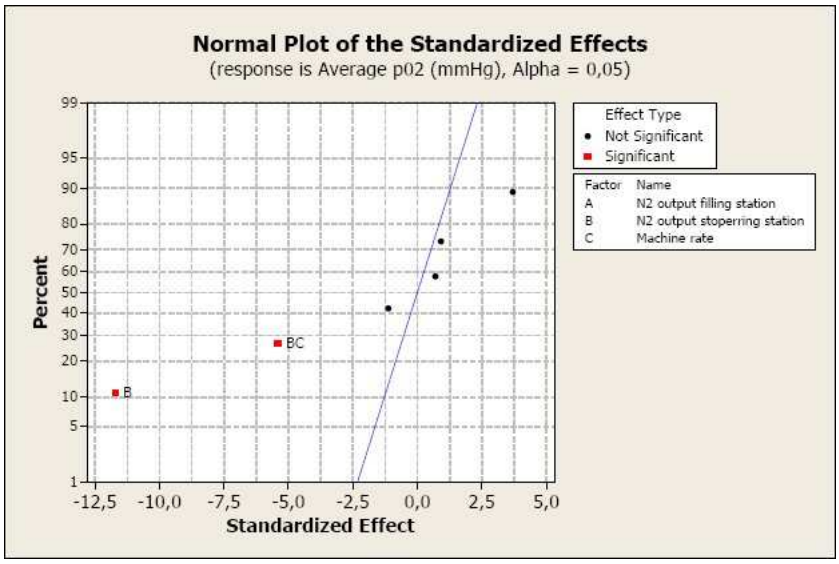


Figure 22 – Normal Plot of the standardized effects for oxygen partial pressure in syringes from line 20

The significant factors (with a 0.05 alpha level) are factor B (nitrogen flow rate at the stoppering station) and the interaction between factor B and C (nitrogen flow rate at the stoppering station and machine rate)

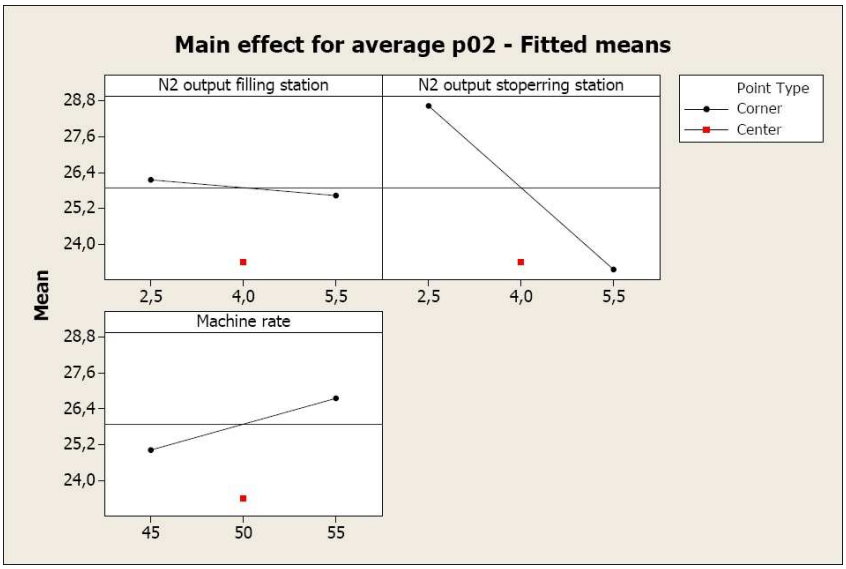


Figure 23 – Main effects of each factor on oxygen partial pressure

This graph shows the global effect of each factor on the average oxygen partial pressure measured in the syringes. These graphs will be interpreted in the following subsections

Factor A: Nitrogen Flow rate at filling station

In this study, the effect of factor A appeared to be not significant.

Nevertheless, we can see a tendency to reduce oxygen level when nitrogen flow rate at the filling station is increased.

According to these results the relevant issue is whether this factor has to be increased at its higher level or if it can be let at its minimum level (cost saving).

Another test was performed to optimize the value of this factor. (See Test 3, p81)

Factor B: Nitrogen Flow rate at stoperring station

Factor B is the one with the effect on average oxygen partial pressure.

The optimum of the response (lowest oxygen partial pressure) is out of the experimental domain. To achieve this lowest level, factor B must be at its maximum level: 5.5 L/min.

Factor C: Machine rate

The impact of factor C couldn't be determined because its effect on the response was not high enough. Other tests are needed to optimize the value of this factor.

Interactions between factors

Only one interaction is significant: the interaction between the Nitrogen flow rate at the stoperring station and the machine rate (Figure 24).

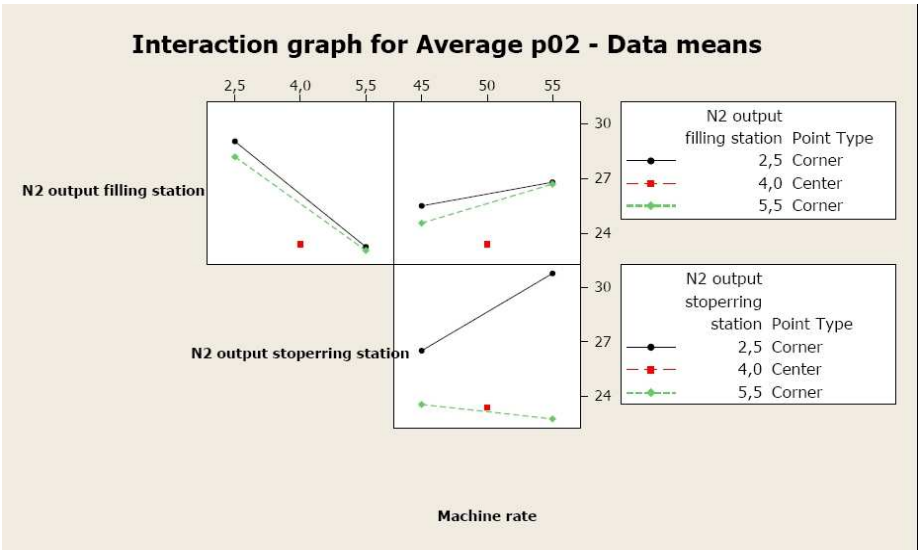


Figure 24 – Graphs for interactions between the factors

We can see on the lower graph above that when nitrogen flow rate at the stoppering station is low (2.5 L/min.), the machine rate has a big incidence on oxygen level: the machine rate must be lowered to decrease oxygen level. On the contrary, when the nitrogen flow rate at the stoppering station is high (5.5 L/min.), the machine rate has a very small incidence and should be increased to obtain a lower oxygen rate in the syringes.

Nitrogen flow rate at the stoppering station will be fixed at its higher level (5.5 L/min). Thus, the machine rate will have a very small incidence on the results and may therefore be fixed at its usual value, that is to say 50 cycles/min.

Study of variability of the results between the needles

An ANOVA two-factor test was first performed to compare the variability between the experiments and a probable significant difference on oxygen concentration in the syringes according to the filling needle (See Appendix 6 below). The p-values for the factors and their interaction show that all of them are very significant (5.91×10^{-32}). This makes the interpretation of the results difficult.

The results of Experiments 1 and 2 show a great variance, and the results of these experiments should probably skew the test.

For this reason, a new ANOVA two factors test was performed without considering Experiments 1 and 2. See Appendix 6 p116.

In this test, the p-values for the interactions and for the needles are not significant (respectively 0.96 and 0.46) which means that there is no significant difference in oxygen partial pressure depending on the 5 needles.

A confirmation of the significance of the differences between the different experiments was also obtained.

Conclusion

The first test conducted in the pilot manufacturing line brought to light the following points:

- Importance of the nitrogen flow rate at the stoppering station. This is the main factor, and it has to be maintained at its highest level (5.5 L/min) in order to achieve the lowest oxygen partial pressure in the filled syringes.
- However, the design of experiment considered the nitrogen flow rate at the filling station and the machine rate as non significant. This will be studied with another test on the filling line (See section 4.3)
- Without considering experiments 1 and 2, the results of oxygen partial pressures obtained in the syringes filled by the 5 needles didn't show significant differences.
- Experiments 1 and 2 showed a high variability with significant differences between the needles. We can suppose that during these experiments, the filling machine was not working in its optimized conditions and needs a certain time to get stabilized.

This point is very important because it indicates that stabilized and repeatable oxygen rates are not reached before the filling of 16 tubs. This point should be confirmed or invalidated on next tests.

- Oxygen partial pressures achieved on this test (22 to 23 mmHg) are very promising; especially for the experiments in which the nitrogen flow rate at the stoppering station is 5.5 L/min.

This means that the process is efficient and it has now to be tested with a real transfer system. This is the purpose of the next test on the filling line (See Test 2 p70 below).

4.2 Test 2: real filling conditions and consequences of a long machine stopping

Introduction and objectives

The aim of this second test was to study oxygen partial pressure rates in syringes in real manufacturing conditions, and especially to analyze the impact of the silicone tubing. In this test, the laboratory tank was still used, but contrary to Test 1 it was connected to the filling machine through a filtration system similar to the one used during filling campaigns.

This test will also show the impact of an extended stopping of the machine on oxygen level in syringes.

Material and Methods

The solution used to fill the syringes was WFI.

10,000 syringes BD Hypak® 2.25 mL (100 tubs of 100 syringes) were filled during this test, and they were corked with 10,000 stoppers.

Some of the relevant factors influencing the inerting were determined by the first test conducted on the filling line. In this study, to get as close as possible to the reality, parameters were fixed in the domains mentioned below:

- Filling volume: 1.2 mL, for the same reason as previously
- Nitrogen flow rate at the filling station: 5.5 L/min. The results of the previous test showed little significance of this parameter, but it was decided to maintain it at its higher level.
- Nitrogen Flow rate at the stoppering station: 5.5 L/min (Factor optimized in the previous test).
- Machine global rate: 50 cycles/min. The effect of this factor on oxygen rate in syringes was not clearly identified by the results of the previous test. In this test, the rate was maintained at 50 cycles/min which is the usual rate during manufacturing campaigns.

- Transfer tubing: Tubing Silicone Pharma 50, 30.5 cm long x 2 (tubing on both sides of the filter) + 2 filters Millipore Opticap 4'' 0.22 µm
  This filtration system is similar to the one used on the filling line.

Three devices were used to measure oxygen concentration during this test:

- Oxygen meter Mettler Seven Go Pro, to control oxygen level in the tank;
- Oxysense 4000B, to check the evolution of oxygen contained in the silicone tubing of the filtration system;
- ABL5 to measure the final oxygen partial pressure in syringes.

Work instructions

The first steps of this test are exactly the same as for the previous test:

- 18 liters of WFI were collected in to the tank and oxygen was removed by bubbling for 20 minutes with nitrogen from BD utilities (Oxygen control with oxygen meter Mettler Seven Go Pro).

- A 0.6 bar nitrogen pressure was then applied inside the tank to transfer it (and all the material required) into the pilot manufacturing area.

- In this test, oxygen concentration was measured during the filling into the silicone tubing thanks to the Oxysense 4000B. The Oxydot was formerly glued inside of the silicone tubing after the second filter (see Figure 25 below).

- Prior to starting, the filling machine was purged with nitrogen from BD utilities from the buffer tank to the filling needles.

- The filtration system was also purged, opening the air vent of the second filter, according to Figure 25.

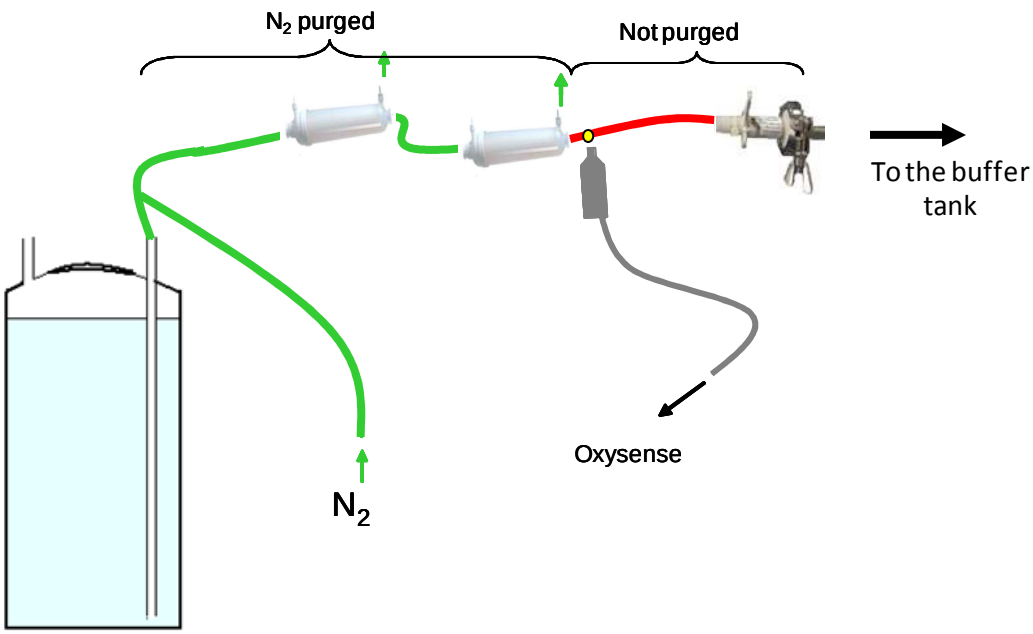


Figure 25 – Nitrogen purge in the filtration system

- The Oxysense measurements were launched with an automatic program, measuring oxygen concentration every minute.

- The filling program was started with the following fixed parameters:
  - Syringes filled with 1.2 mL of water
  - Machine rate: 50 cycles/min.
  - Nitrogen flow rate at filling station: 5.5 L/min
  - Nitrogen flow rate at stoppering station: 5.5 L/min

- 50 tubs were filled and 5 syringes every second tub were sampled as described in Figure 26 and Figure 27 below (syringes from line 10, filled by the 5 filling needles)

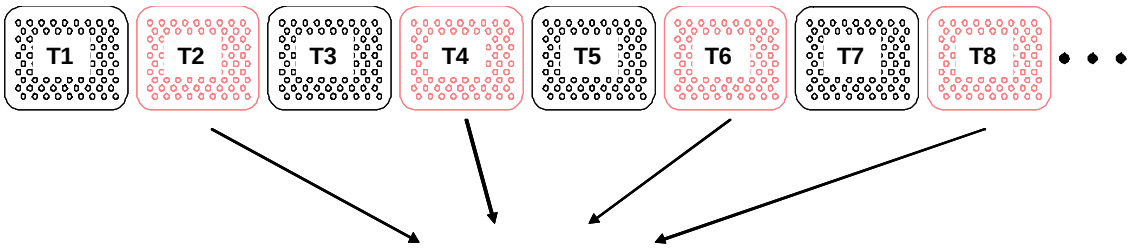


Figure 26 – Sampled tubs

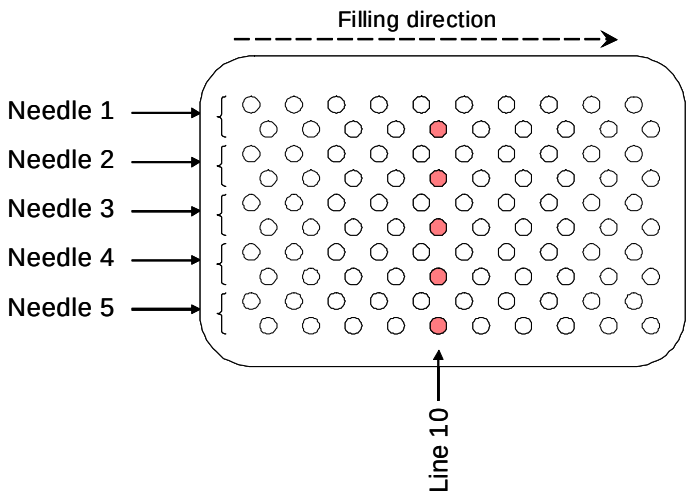


Figure 27 – Sampled syringes for each sampled tub

- After 50 tubs filled, the machine was stopped during one hour and 23 minutes (1h23) and restarted, the continued the filling and sampling of 50 other tubs, in the same way as previously.

- The analysis of oxygen partial pressure was done in the syringes with the ABL5, at least 24 hours after filling to have stable measures, on the 5 syringes sampled of one every sixth tub (2, 8, 14…). The other syringes were kept for additional measures.

Results and discussion

Different types of results are treated:

- Oxygen measurement in the silicone tubing using Oxysense during the test. The results are given in O_2 concentration unit (ppm).
- Oxygen partial pressure in the syringes after the test, measured with ABL5. The results are given in O_2 partial pressure unit (mmHg).

Measurement of Oxygen concentration in the silicone tubing after the filtration system:

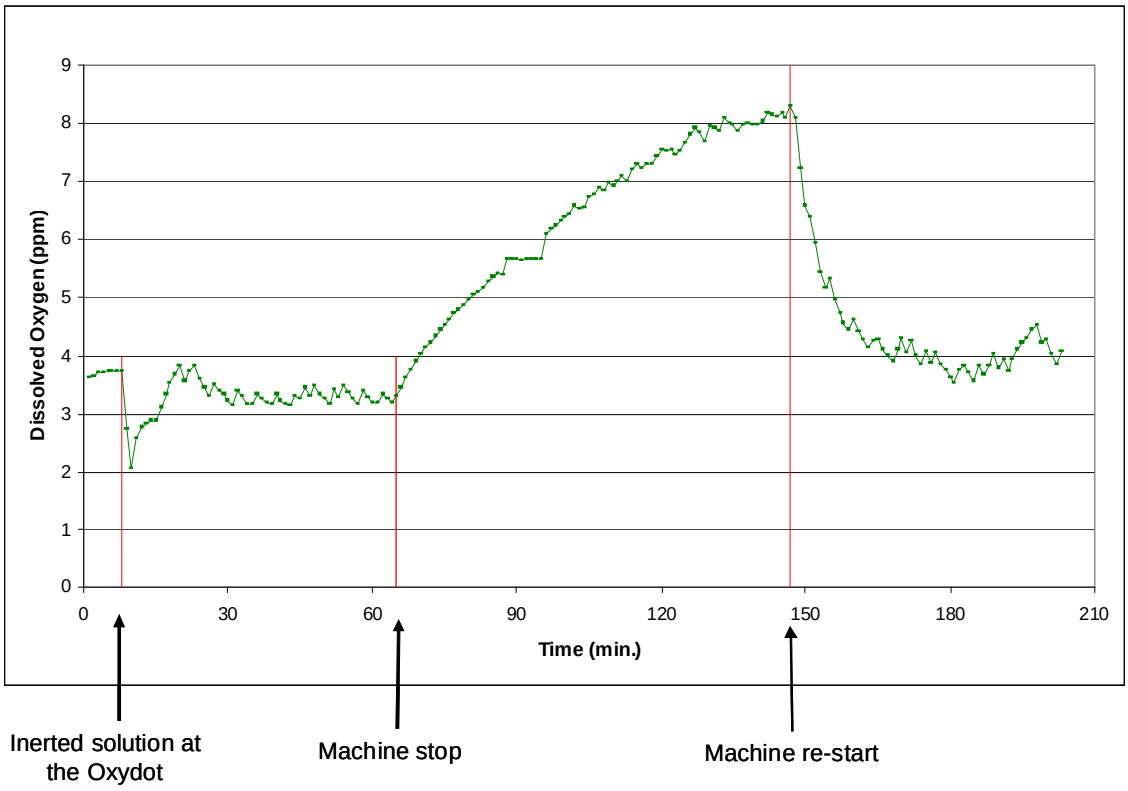


Figure 28 – Oxysense dissolved oxygen measure in silicone tubing

When the Oxysense started the automatic recording, there was no water in the tubing yet. At t + 8 min, the buffer tank made a solution request and the inerted water reached the Oxydot. Oxygen concentration decreased rapidly (inerted water).

During the following minutes, there was no buffer tank request because a manual operation was necessary to connect the filling needle in front of the filling station. This explains the increase in oxygen concentration.

The filling started at t + 20 min and the regular requests of the buffer tank is seen on the saw-toothed part of the graph, until the machine stopped at t + 64 min. Indeed, oxygen level increased on the silicone tubing (air permeation) during stagnancy and decreased at each request (when inerted water got in contact with the Oxydot).

We can observe a rapid and sharp increase f oxygen concentration while the machine was stopped from about 3 ppm to about 8 ppm. Once more, this demonstrates the high air permeation of silicone material.

Before the machine re-started (t + 147 min), oxygen concentration reached 8 ppm, which corresponds approximately to the value of non inerted water.

When the machine re-started, the oxygen concentration fell and the saw-tooth phenomenon could be observed again.

The curve is however less stable than before the machine stopped and never reached the same low level. This can be explained by numerous unintended stops (mainly because of gas failures) and a manual filling at the end.

Measurement of Oxygen concentration in the syringes

Analysis all along the filling:

Oxygen partial pressure was first measured in the 5 syringes sampled of every 6th tub (tubs 2, 8, 14…).

The results are represented on **Erreur ! Source du renvoi introuvable.** of Appendix 7 p118 and on Figure 29 below.

During the filling of tub 92, the machine gave an alert message of gas failure for lines 1 to 11. Line 10 was therefore impacted and this explains the very high oxygen level and average deviation.

Oxygen values of this tub are not taken into account for the total average calculation (average oxygen concentration in all the syringes analyzed during this test).

From **Erreur ! Source du renvoi introuvable.** of Appendix 7 we can assume that the filling needle n°5 gives higher oxygen partial pressure than the other needles and this can be demonstrated by a statistical test.

An ANOVA Single-factor test was performed to study the possible difference between the filling needles. The p-value obtained (8.41×10^{-6}) indicates that there is a significant difference in oxygen level between the needles (for details, see Appendix 8, p119).

A Scheffé test was performed to make individual comparisons.

Needle n°5 gives significantly different results compared to needles 1, 2 and 3 but not significant compared to needle 4.

Factors compared	A1-A2	A1-A3	A1-A4	A1-A5	A2-A3	A2-A4	A2-A5	A3-A4	A3-A5	A4-A5
F_{12}	0.53	0.59	1.65	7.32	0.07	0.62	4.99	0.28	3.88	2.09
Significance	NO	NO	NO	YES	NO	NO	YES	NO	YES	NO

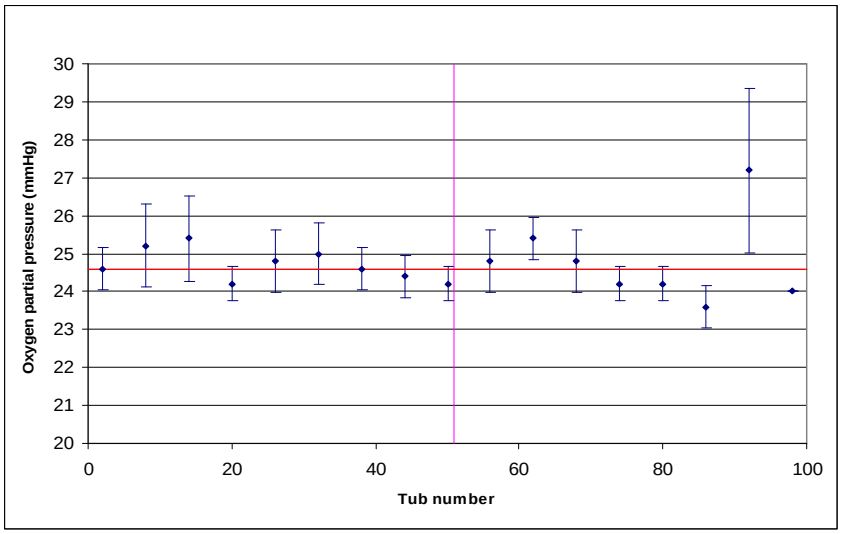


Figure 29 – Oxygen partial pressure in syringes of line10, function of the tub number

This graph shows the evolution of oxygen partial pressure in the tubs with usual filling conditions.

The red line represents the total average (25 mmHg) and does not include the results of Tub 92.

The pink line represents the stop of the machine (after the 50th tub).

The case of Tubs 51 and 52 (just after the stop) will be studied later in this report.

Interpretation:

The machine stop does not seem to affect the global oxygen partial pressure in the following tubs: the average *before* the machine stop (tubs 1 to 50) is 25 mmHg and the average *after* the machine stop (tubs 56 to 98, excepted tub 92) is 24 mmHg.

Except for Tub 92, oxygen partial pressure measured never exceeds 27 mmHg. These results are promising, and the behavior after the machine stop is a sign of a robust process.

However, we can note a low variability for a quite large batch (about 10,000 syringes) with a global RSD of 3%.

By a statistical analysis (ANOVA single factor test) we can confirm that there is no significant difference between the measures in tubs 2 to 50 (p-value = 0.17). For details see Appendix 8 p121.

However some statistical significant differences are found in the second part of the test (from tubs 56 to 98, except tub 92): p-value=8.91×10^{-4}. See Appendix 8 p122.

The only statistical difference is between the lowest and the highest oxygen value average that is to say between tubs 62 and 86.

If we compare the average values obtained for each tub before and after the machine stop, there is no significant difference (p-value=0.29). See Appendix 8 p123.

Analysis of Tubs 51 and 52 after the machine stop:

Just after the machine stop, 2 tubs (51 and 52) were sampled to study their oxygen partial pressure with ABL5.

These measures were done for needle 3 all along tub 51 and until the middle of tub 52 as shown on Figure 30 and Figure 31 below:

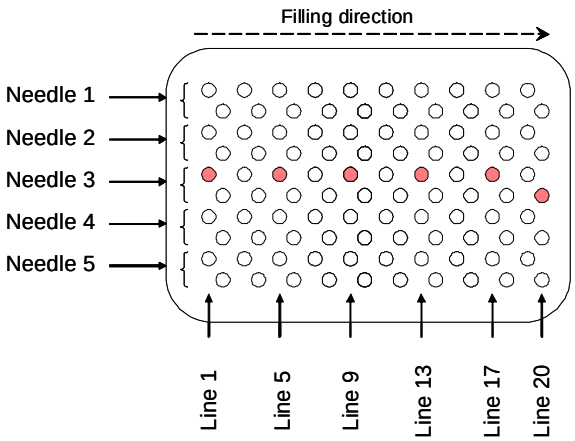


Figure 30 – Sampled syringes in Tub 51

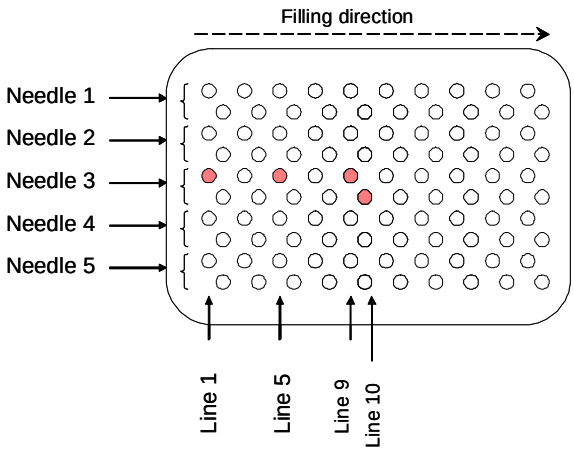


Figure 31 – Sampled syringes in Tub 52

Figure 32 below shows the results obtained:

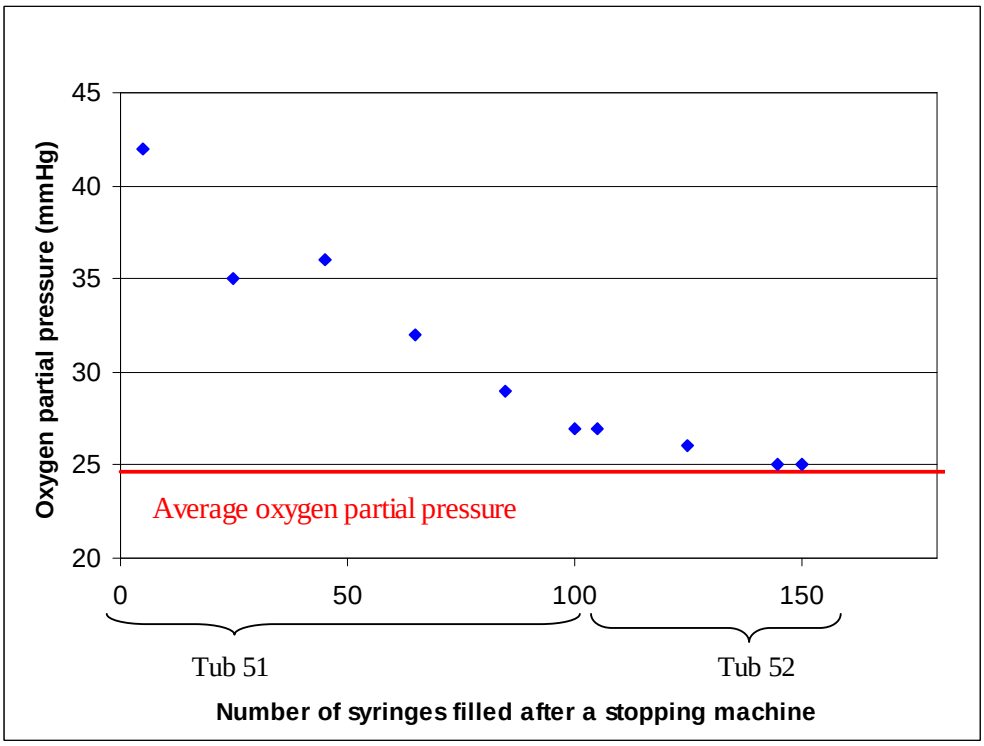


Figure 32 – Oxygen measurement in tubs just after a long stopping machine

Oxygen partial pressure decreases until it reaches the average measure in the middle of the second tub after a machine stop.

The increase of oxygen in the tubs following the machine stop is explained by the presence of silicone tubing just upstream the filling needles. This volume can be calculated:

Tubing between the collector and the pump:

62 cm in length for 0.4 cm in diameter x 5 pumps = 38.96 cm^3

Tubing between the pump and the filling needles:

100 in of length for 0.4 cm in diameter x 5 pumps = 62.83 cm^3

The total silicone tubing represents 102 cm^3, that is to say. 85 syringes filled with 1.2 mL. This is in accordance with the results of Figure 32.

These results show once more the importance of oxygen permeation through silicone tubing. In case of long stop of the machine, the first two tubs should be removed (for 2.2 mL filled syringes, only one tub should be removed).

Conclusion

This second test performed in the filling line led us to the following conclusions:

- The measures on the silicone tubing confirm the high oxygen uptake observed in a previous test (See chapter 3.3). In about 80 minutes, the solution contained in the tubing is totally re-oxygenated.
- Despite this important oxygen uptake, the average oxygen partial pressure in syringes stays constant after the machine stop, until the 48th tub after stopping.

The average oxygen partial pressure measured during the whole test (25 mmHg) is superior to the one measured in Test 1 of inerting optimization on the filling line which was about 23 mmHg in similar conditions. This is probably due to the transfer system composed of the 2 filters and the fact that the system couldn't be purged totally. But it is also important to notice that the measures with the ABL5 were performed 4 days after the filling in this test versus 2 days in the previous test. The fact that oxygen concentration increases day after day was shown in paragraph 3.2.

Nevertheless, the average oxygen partial pressure is still relatively low and the process seems to be robust as no increase is observed after a long stop. This value of partial pressure has to be tested on syringes with an oxygen-sensitive drug to confirm its long term stability.

Contrary to the previous test oxygen concentration seems to stabilize very quickly, without a big variance between the filling needles, even in the 2nd tub.

- This test also shows oxygen uptake in silicone tubing just before the filling needle that impacted the 140 first syringes filled after the machine stop. We can confirm that it is imperative to eliminate at least the first tub after a long machine stop and the second tub should also be eliminated in the case of 1.2 mL filled syringes.

Nevertheless, oxygen partial pressure obtained in the first syringes from the tub 51 doesn't exceed 45 mmHg, which is relatively low considering that the water in the silicone tubing just before the filling needle was not inerted anymore.

- The next test of inerting on the filling line will consist in the study of the time required to impact oxygen level in syringes when the solution changes from inerted to non inerted water. The production parameters will be maintained like in this test (real conditions).

4.3 Test 3: impact of machine rate, nitrogen flow rate at the filling station and of dead volumes

Introduction and objectives

This test is divided in two distinct parts that were performed on the bounce:

- The first part of this test is aimed at concluding on the importance of two parameters tested during the first test that were not determined for sure: the importance of the nitrogen flow rate at the filling station and the machine rate.
- The second part of this test is meant to determine the time necessary to impact oxygen level in the syringes filled with a non inerted solution after filling with an inerted solution. In other words: what is the impact of the dead volumes of the filling machine and of the dilution of non inerted water in the buffer tank?

Material and Methods

Like previously, the solution used to fill the syringes was WFI.

9,400 syringes BD Hypak® 2.25 mL (94 tubs of 100 syringes) were filled during this test, and they were corked with the same number of stoppers.

The aim of this test was to get close to the reality but also to test the parameters studied on the 1st test in a broader range to fix the conditions that should be applied in a production campaign. The parameters are:

- Filling volume: 1.2 mL, for the same reason as previously
- Nitrogen flow rate at the filling station: 2 to 5.5 L/min.
- Nitrogen Flow rate at stoppering station: 5.5 L/min. Factor optimized in the first test.
- Machine global rate: 35 to 55 cycles/min. The effect of this factor on oxygen rate in syringes could not be clearly identified and will be tested once more in this test.
- Transfer tubing: Tubing Silicone Pharma 50, 30.5 cm long x 2 (tubing on both sides of the filter) + 2 filters Millipore Opticap 4’’ 0.22 µm

  This filtration system is similar to the one that will be used on the filling line.

Two devices were used to measure oxygen concentration during this test:

- Oxygen meter Mettler Seven Go Pro, to control oxygen level in the tank;
- ABL5 to measure the final oxygen partial pressure in syringes.

Work instructions

The first steps of this test are similar to the previous tests:

- 10 liters of WFI were collected into the tank and oxygen was removed by bubbling for 15 minutes with nitrogen from BD utilities (Oxygen control with oxygen meter Mettler Seven Go Pro).

- A 0.6 bar nitrogen pressure was then applied inside the tank to transfer it (and all the material required) into the pilot manufacturing area.

- The tank was connected to the filling machine through the filtration system. The filling machine was purged with nitrogen from BD utilities from the buffer tank to the filling needles. The filtration system was also purged, opening the air vent of the second filter

  .

- The filling program was started with the fixed following parameters:
  - Syringes filled with 1.2 mL of water
  - Machine rate: 50 cycles/min.
  - Nitrogen flow rate at filling station: 5.5 L/min
  - Nitrogen flow rate at stoppering station: 5.5 L/min.

- Six experiments were performed. The parameters were changed meanwhile according to the order defined by the experimental table below. Four tubs were filled per experiment.

Table VI – Experimental matrix for the first part of Test 3

Exp. Number	Nitrogen flow rate at stoppering station (L/min)	Nitrogen flow rate at filling station (L/min)	Machine rate (cycles/min)
1	**5.5**	**5.5**	**50**
2	**5.5**	**1.5**	**50**
3	**5.5**	**3.0**	**50**
4	**5.5**	**3.0**	**55**
5	**5.5**	**3.0**	**45**
6	**5.5**	**3.0**	**35**

- Among these four tubs, ten syringes were sampled, taken from tline 10 of the third and fourth tubs filled, as described below.

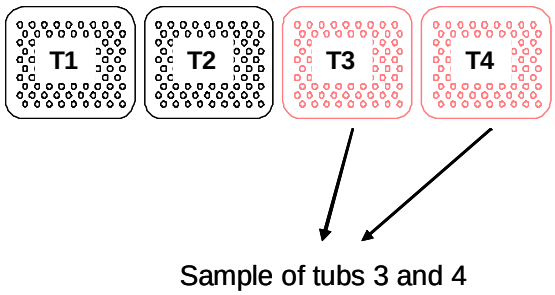


Figure 33 – Position of sampled tub for Experiments 1 to 6

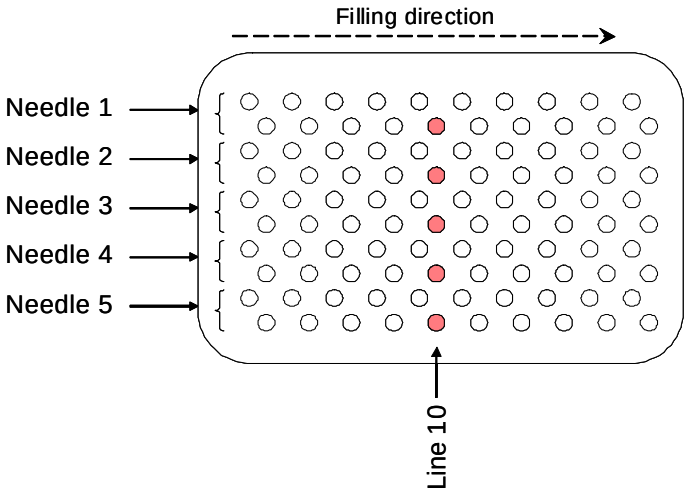


Figure 34 – Sampled syringes for each sampled tub (Exp 1 to 6)

- After the 6 experiments, the machine was stopped and a plastic drum containing non inerted water was connected.

- The machine was restarted, and went on with the filling. Seventy other tubs were filled. In this second part, called **Experiment 7**, the fixed parameters are:
  - Syringes filled with 1.2 mL of water
  - Machine rate: 50 cycles/min.
  - Nitrogen flow rate at filling station: 5.5 L/min
  - Nitrogen flow rate at stoppering station: 5.5 L/min.

- 5 syringes every second tub were sampled as described in Figure 35 and Figure 36 (syringes from line 10, filled by the 5 filling needles)

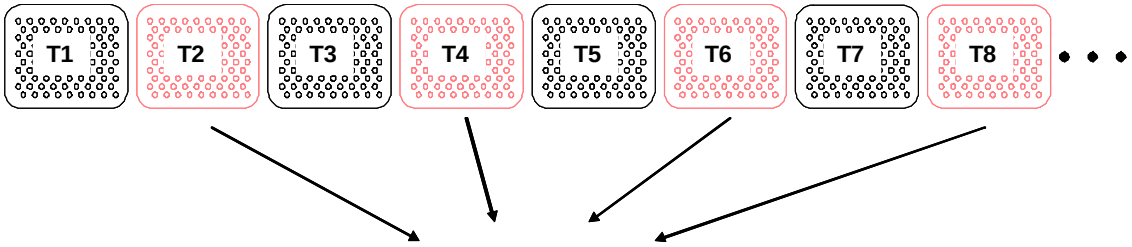


Figure 35 – Sampled tubs for Experiment n°7

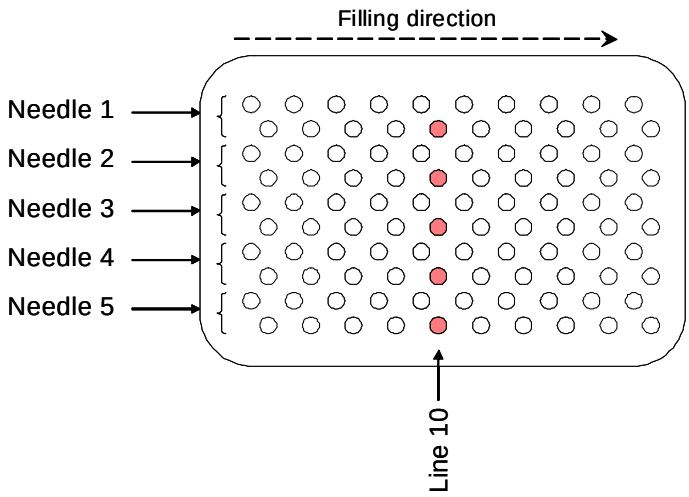


Figure 36 – Sampled syringes for each sampled tub (Exp 7)

- The analysis of oxygen partial pressure was done in the syringes with the ABL5, at least 24 hours after the filling to have stable measures

- Experiments 1 to 6: measurement in the 10 sampled syringes

- Experiment 7: measurements in 5 syringes sampled of one every sixth tub (tubs 2, 8, 14…). Additional measures were performed in the other sampled syringes.

Results and discussion

a. Measurement of Oxygen rate in the syringes for experiments 1 to 6:

The measures were performed with the ABL5, 7 days after the filling and can be consulted in Appendix 9 p 124.

The influence of nitrogen flow rate at the filling station (Experiments 1 to 3) and the influence of the machine rate (Experiments 4 to 6) are shown on Figure 37 and Figure 38 below.

Influence of nitrogen flow rate at the filling station:

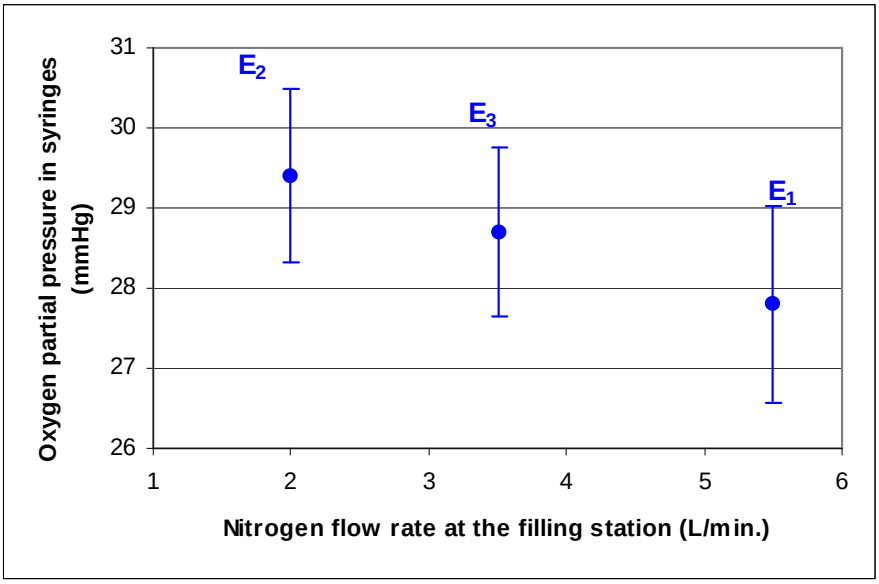


Figure 37 – Influence of nitrogen flow rate at the filling station on oxygen partial pressure (n=10)

In this graph we can observe that the average oxygen partial pressure decreases when nitrogen flow rate at the filling station increases.

To see if this effect is significant or not, an ANOVA Two-Factor test with replication was made. Thus the effect of the needles and of the experiments can be analyzed at the same time.

The 2 replications corresponds to tubs 7 and 8. The ANOVA analysis is detailed in Appendix 10 p126.

The p-values obtained indicate that there is a significant difference in oxygen level depending on the needles (1.51×10^{-2}) and also on the experiments (6.60×10^{-3}).

To make individual comparisons between experiments, a Scheffé test was performed:

Experiments	**E1-E2**	E1-E3	E2-E3
F_{12}	7.11	2.25	1.36
Significance	**YES**	**NO**	**NO**

There is only a significant difference between experiments 1 and 2, which corresponds to the extremes of the experimental domain (2 and 5.5 L/min.)

For the needles, a Scheffé test was also performed to make individual comparisons:

Needles	A1-A2	A1-A3	A1-A4	**A1-A5**	A2-A3	A2-A4	**A2-A5**	A3-A4	**A3-A5**	**A4-A5**
F_{12}	0.00	0.74	0.05	3.75	0.74	0.05	3.75	0.42	7.82	4.63
Significance	**NO**	**NO**	**NO**	**YES**	**NO**	**NO**	**YES**	**NO**	**YES**	**YES**

Needle 5 gives significantly different results compared to all the other needles.

Influence of the machine rate:

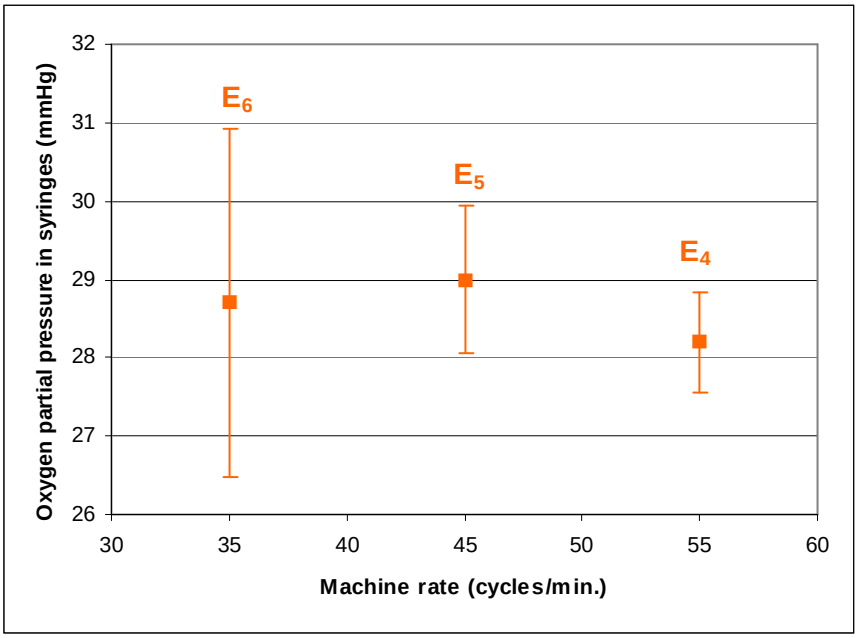


Figure 38 – Influence of the machine rate on oxygen partial pressure (n=10)

We can't observe a particular correlation between the machine rate and the average oxygen partial pressure in the syringes by looking at this graph.

However, the error bars are very high and decrease with the machine rate which represents big differences between the needle measures.

To see if the effect of the machine rate is significant or not, an ANOVA Two-Factor test with replication was made. Thus the effect of the needles and of the experiments can be analyzed at the same time.

The two replications correspond to tubs 7 and 8.

The ANOVA analysis is detailed in the Appendix 10 p128.

The p-value obtained for the interaction A x E (needle x experiment) is lower than 0.05 (4.79×10^{-2}) which means that an interaction between the effect of the needle and the rate makes the test difficult to interpret. The very low p-value for the needles (6.27×10^{-4}) and the big error bars still mean that there are differences between oxygen levels according to the filling needle.

Considering the previous results we can assume that the filling needle n°5 gives higher results than the others and we can make a second ANOVA analysis, without considering the results of this needle.

The test is detailed on Appendix 10 p130.

The p-values obtained indicate that there is now no significant difference in oxygen level depending on the needles (0.31) but there is a significant difference for the experiments (2.36×10^{-2}). No interaction is seen here.

To make individual comparisons between experiments, a Scheffé test was performed:

	E4-E5	E4-E6	E5-E6
F_{12}	2.70	0.30	4.80
Significance	**NO**	**NO**	**YES**

Only one difference is noticed, between Experiment 4 and 5 that is to say between 35 and 45 cycles/min machine rates.

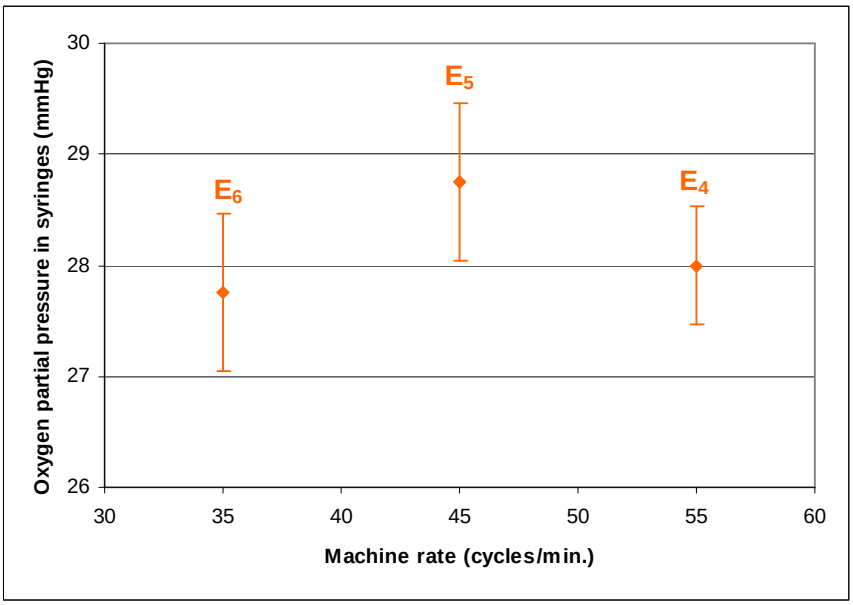


Figure 39 – Influence of the machine rate on oxygen partial pressure without the results of Needle 5 (n=8)

Note: The graph above shows that without the result of needle 5 the profile of oxygen partial pressure function of the machine rate is very different. from the one of Figure 38.

Oxygen partial pressure is significantly higher for a machine rate of 45 cycles/min compared to 35, but there is no significant difference between 45 and 55 cycles/min.

b. Measurement of Oxygen rate in the syringes for Experiment 7:

The measures were performed with the ABL5, 8 days after the filling.

The results are represented in the Appendix 9 p125.

Figure 40 below represents oxygen measurements in the syringes from lines 10, filled by the 5 filling needles.

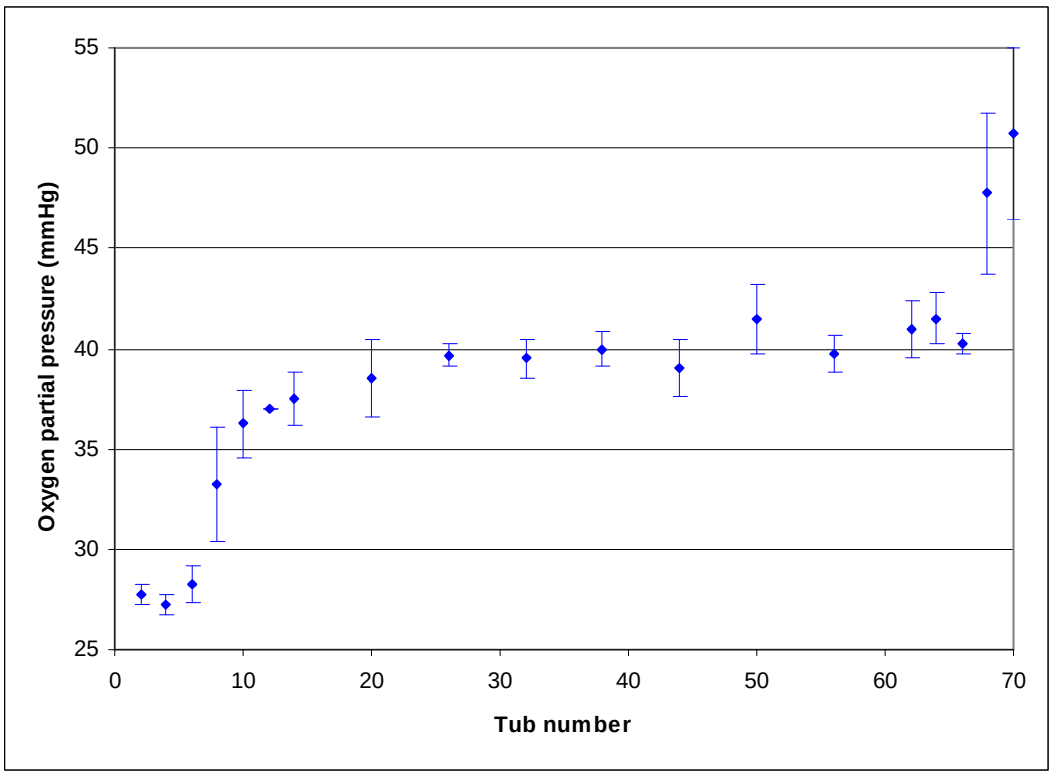


Figure 40 – Oxygen partial pressure in the syringes of line 10, function of the tub number (n=5)

The first tubs filled after changing the solution (from inerted to non inerted water) contains the same oxygen partial pressure than the tubs filled with inerted water with the

same parameters (Experiment E1): average of 28 mmHg. This corresponds to dead volume in the filling machine.

Then oxygen increases until it reaches a plateau at about 40 mmHg, from tub 26 to 62.

From tub 66 on, the oxygen level increases rapidly. This phenomenon is not explained.

Considering that the dead volume of the filling machine, including the buffer tank and the tubing corresponds to 4.7 L, 3,916 syringes at 1.2 mL can be filled with this dead volume, i.e. about 40 tubs. We should therefore see an increase of oxygen level from the 40th tub.

An ANOVA two-factor test is performed to simultaneously compare the results given by the 5 needles and if there is a significant difference between the results from tubs 26 to 62 (plateau).

The analysis is detailed in Appendix 10 p132.

This test demonstrates significant differences for the needles (needle 5 gives different results compared to all the other needles) and for the tubs of the plateau.

Considering these significant differences, the same analysis was performed without considering needle 5.

The results are detailed in Appendix 10 p134.

This test concludes that there is here no significant difference in oxygen partial pressure in the syringes according to the tubs from the tubs 26 to 62.

We can also notice that there is now no significant difference between the filling needles when needle 5 is not considered.

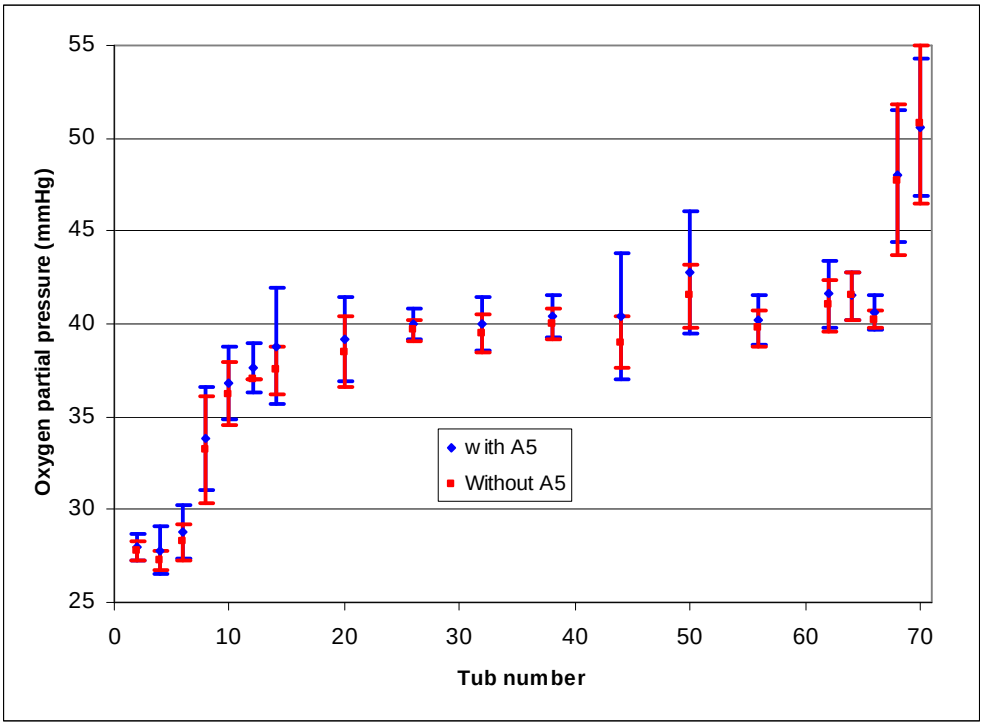


Figure 41 – Oxygen partial pressure in the syringes of line 10, function of the tub number, with and without needle 5 (n=5 with A5, n=4 without A5)

Figure 41 represents the results of the test without considering the filling needle n°5. We can confirm that the average oxygen level is lower and the error-bar smaller when it is not considered.

Conclusion

The main elements that we can retain from this test are summed up below:

- The first part of this test confirms the small incidence of the nitrogen flow rate at the stoppering station. However, it concludes that there is a significant difference between 2.5 and 5.5 L/min, and the maximum (5.5 L/min) should be recommended to achieve the lowest oxygen level in syringes.

  The study of the machine rate confirms that its effect is not really relevant, and that it can be maintained at the usual production rate: 50 cycles/min.

- All along the test, the filling needle number 5 gave results (oxygen partial pressure) significantly higher compared to the other needles. It will be necessary to investigate on this phenomenon that was not observed in Test 1 on inerting optimization on the filling line and to correct it.

- Another time in this test, we noticed some alert messages of gas failure at the stoppering station from the filling machine. These alerts are once again correlated with an increase in oxygen level and an important variability between the filling needles. This time the phenomenon is restricted to the concerned lines.

- The second part of the test (Experiment 7) showed an increase in oxygen partial pressure in syringes followed by a plateau at about 40 mmHg and a sharp increase starting from tub 66. Two hypotheses can be considered to explain this plateau.

1st hypothesis:

The plateau value (40 mmHg) is relatively low. If the water was really not inerted anymore, it would mean that the capacity of the machine to decrease oxygen level is very high and almost only due to the nitrogen flow rate at the stoppering station (see Test 1 on inerting optimization on the filling line, paragraph 4.1). In this case, the increase from tub 66 is not explained and should be investigated (reduction of nitrogen supply for example).

This hypothesis is in agreement with the results of oxygen partial pressure obtained in the syringes from the first lines of tub 51 filled during Test 2 on inerting optimization on the filling line (paragraph 4.2). In these syringes the filling water was considered as totally re-oxygenated (oxygen uptake in silicone tubing during more than one hour) and oxygen measured was less than 45 mmHg.

In this case, the volume and the composition of the gas headspace in the syringes are critical to achieve a low oxygen level. The volume of the headspace will have to be strictly controlled to prevent the degradations of oxygen-sensitive drugs.

Consequently, we can say that the 1.2mL filling volume is not necessarily the worst case as was formerly believed. New tests should be performed with a 2.2 mL filling volume.

2nd hypothesis:

Another hypothesis states that there is an important dilution of a small amount of non inerted water on a quite important quantity of inerted water. Indeed, this test permitted to calculate the volume of solution requested by the buffer tank: 290mL; and the dead volume of the buffer tank was previously determined at about 4,266mL. The 7% contribution of inerted water in the buffer tank might not be significant. Furthermore, the buffer tank is permanently under nitrogen pressure. These two factors should enable oxygen level to stay quite low during a certain time. This would explain the important increase in oxygen level only at the end of Experiment 7.

This should be confirmed or invalidated by a later test at the filling machine: filling of non inerted water directly at the beginning of the test with the highest nitrogen flow rates for stoppering and filling stations (5.5L/min) to obtain comparable results.

Conclusion

This thesis presents the results of experiments carried out during an eight-month study in Becton Dickinson Company, in Pont de Claix. The goals of the study were to prepare and anticipate on the development of injectable solutions with oxygen-sensitive drugs in prefilled-syringes.

One of the goals was an assessment of the different devices existing on the market to measure oxygen concentrations. The equipment is to be used in the laboratory, where formulation tests and stability studies are conducted. These devices were also assessed to equip a pilot manufacturing line that fills syringes to realize in process controls.

The oxygenating and inerting phenomena were then studied at laboratory-scale and at higher scale on a pilot filling line. The critical parameters to be set up in order to fill and cork syringes under nitrogen have been determined and optimized. Among these parameters are the importance of the volume and the composition of the gas headspace and the nitrogen flow rate at the stoppering station of the filling machine. This flow rate will indeed condition the nitrogen composition of the headspace gas and so oxygen concentration in syringes. Another important parameter is the high gas permeability of the silicone tubing that impacts the filling process. The consequences should be considered on the final product, eliminating the syringes impacted by a large oxygen reuptake.

In addition, oxygen reuptake with time was underlined in glass prefilled syringes inerted beforehand. The reasons for this reuptake are under investigation. Its impact on solutions of oxygen-sensitive drugs will also have to be assessed by new tests. Several factors such as rubber porosity from both sides of the syringes and a potential permeable junction between the stopper and the barrel of the syringe are incriminated.

Stability studies are in progress for oxygen-sensitive drugs to evaluate the stability with time with accelerated conditions, with or without antioxidant and at different inerting levels.

New tests are planned on the pilot filling line to study the robustness of the process and to confirm the capacity of the machine. These tests will particularly consist in filling syringes and varying the filling volume and the gaseous headspace volume. Some syringes will also be filled with non inerted water and inerting parameters should be at their higher level to determine the capacity of the machine. Finally, the anomalies observed in the process should be investigated, notably one of the filling needles that leads to higher oxygen concentrations in syringes compared to the other filling needles.

THESE SOUTENUE PAR : Chloé PIERSON

TITRE : Molécules sensibles à l'oxygène : étude et optimisation des procédés d'inertage. Applications aux seringues pré-remplies

CONCLUSION

Cette thèse présente les résultats d'expériences réalisées dans le cadre d'une étude de huit mois au sein de la société Becton Dickinson, à Pont de Claix. Les objectifs de l'étude étaient de préparer et d'anticiper le développement de solutions injectables de principes actifs sensibles à l'oxygène en seringues pré-remplies.

Un des objectifs était d'évaluer les différents appareils de mesure de concentration en oxygène existant sur le marché. Il fallait trouver un appareil pour équiper le laboratoire où les essais de formulation ainsi que les études de stabilités de molécules sensibles à l'oxygène sont réalisés. Ces appareils ont également été évalués pour équiper une ligne pilote de remplissage de seringues dans le but de réaliser des tests en cours de fabrication.

Les phénomènes d'oxygénation et d'inertage ont ensuite été étudiés à l'échelle du laboratoire ainsi qu'à une plus grande échelle sur une ligne pilote de remplissage. Les paramètres critiques pour remplir et boucher des seringues sous azote ont été déterminés et optimisés. Il s'agit d'une part du volume de l'espace de tête des seringues pré-remplies et du débit d'azote en station de bouchonnage au niveau de la remplisseuse. Ce débit va en effet conditionner la composition en azote du gaz présent dans cet espace de tête et donc la concentration en oxygène dans les seringues. D'autre part, la perméabilité aux gaz des tubulures en silicone a montré son importance et impacte le procédé de remplissage et il faudra prendre en compte ses conséquences sur le produit fini en éliminant les seringues impactées par une forte reprise en oxygène.

Par ailleurs, une reprise en oxygène au cours du temps a été mise en évidence dans des seringues pré-remplies en verre, préalablement inertées. Les causes de cette reprise sont en cours d'évaluation. Il faudra évaluer également son impact sur des solutions d'actifs sensibles à l'oxygène.

Des études de stabilité sont en cours sur des molécules sensibles à l'oxygène pour évaluer cette stabilité au cours du temps et en conditions accélérées, avec et sans antioxydant et à différents degrés d'inertage.

D'autres études seront à prévoir pour déterminer la cause de la reprise en oxygène observée au long cours dans les seringues. Plusieurs facteurs comme la porosité du caoutchouc d'un côté ou de l'autre de la seringue ainsi que la jonction entre le stopper et le corps de la seringue potentiellement perméable aux gaz sont incriminés.

De nouveaux essais sont envisagés sur la ligne pilote de remplissage notamment pour étudier la robustesse du procédé et confirmer les capacités de la machine. Ces tests correspondront particulièrement à remplir des seringues en modifiant les volumes de remplissage et les volumes gazeux d'espace de tête ainsi qu'à remplir des seringues à l'eau non inertée avec les paramètres d'inertage à leurs plus hauts niveaux. Il faudra par ailleurs investiguer sur des anomalies observées au niveau du procédé, notamment une des aiguilles de remplissage qui induit des concentrations en oxygène dans les seringues supérieures aux autres aiguilles.

VU ET PERMIS D'IMPRIMER

Grenoble, le 17/11/09

LE DOYEN

UNIVERSITE JOSEPH FOURIER U.F.R. DE PHARMACIE GRENOBLE 1

Professeur Renée GRILLOT

LE PRESIDENT DE LA THESE

Professeur Aziz BAKRI

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Appendix

Appendix 1

To convert an oxygen concentration (ppm) to an oxygen partial pressure (mmHg) (or the contrary), it is necessary to use Henry's law: $p = K_H c$

Henry's constant KH that depends on the temperature can be calculated by the following equation:

$$K_H(T) = K_H(T_0) \cdot e^{-C\left(\frac{1}{T} - \frac{1}{T_0}\right)}$$ ➔ Unit: L·atm/mol

with $K_H(T_0, O_2) = 769.23$L·atm/mol and C(O2)=1700 K

1 atm=760 mmHg

$$K_H(T) = K_H(T_0) \cdot e^{-C\left(\frac{1}{T} - \frac{1}{T_0}\right)} \cdot 760$$ ➔ Unit: L·mmHg/mol

Transformation of oxygen partial pressure **(mmHg)** to mass concentration **(ppm)**:
O2 concentration (ppm) = pO2 (mmHg) x O2 molar mass (g/mol) / KH /weight of 1L of water (g) x 10^6 for ppm

➔ $$[O_2] = \frac{pO_2 \cdot 32}{K_H \cdot 1000} \cdot 10^6$$ or $$pO_2 = \frac{[O_2] \cdot K_H \cdot 1000}{32 \cdot 10^6}$$

The graph below represents the correlation between oxygen concentration (ppm unit) and partial pressure, at 25°C and 37°C (the ABL5 measures oxygen partial pressure at 37°C).

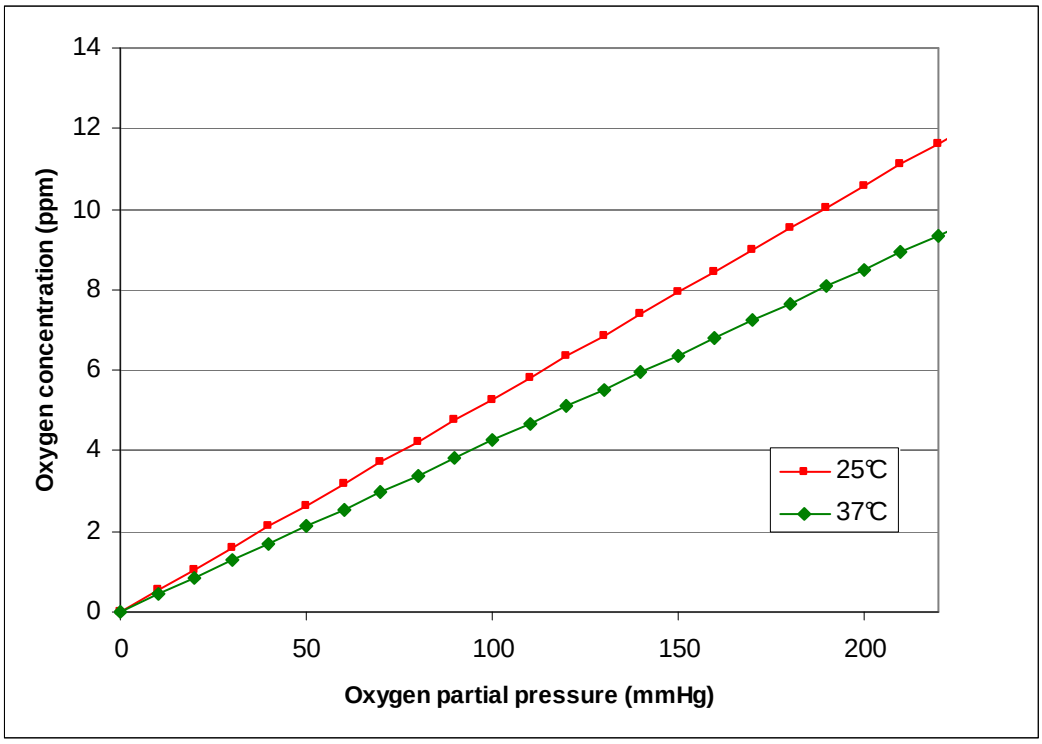


Figure 42 – Correlation between oxygen partial pressure and oxygen concentration according to the temperature

Appendix 2

Gross results: measurements performed with the Oxysense. The results are given in concentration unit: part per billion.

Table VII – Average oxygen concentrations in syringes according to the time after corking

	Syringe number (1.2 mL syringes)				Syringe number (2.2 mL syringes)				
Stopper/ Position	**A Rondo**	**A Nest**	**B Rondo**	**B Nest**	**A Rondo**	**A Nest**	**B Rondo**	**B Nest**	
Time (days)	**1, 2, 3**	**4, 5, 6**	**7, 8, 9**	**10, 11, 12**	**13, 14, 15**	**16, 17, 18**	**19, 20, 21**	**22, 23, 24**	
0	109	140	242	155	131	49	NA	234	Averages (ppb)
1	885	765	914	767	554	425	573	511	
4	923	946	966	934	671	579	732	648	
6	877	996	992	1011	762	665	799	744	
11	894	1092	1032	1100	869	832	966	934	
13	949	1105	1071	1134	901	891	1033	1021	
15	912	1104	1077	1113	913	906	1084	1074	
18	912	1154	1094	1142	962	947	1191	1095	
20	949	1205	1135	1193	1026	994	1245	1127	
22	960	1216	1154	1206	1070	1018	1299	1115	
25	998	1276	1231	1257	1180	1076	1389	1178	
28	1003	1303	1238	1288	1235	1105	1425	1172	
32	1021	1408	1277	1349	1303	1200	1497	1252	
34	1045	1419	1278	1370	1342	1259	1540	1307	
36	1095	1488	1351	1443	1437	1343	1657	1369	
39	1162	1550	1395	1471	1509	1377	1717	1396	
41	1162	1585	1425	1510	1544	1419	1749	1439	
46	1208	1675	1486	1605	1643	1544	1897	1525	
48	1241	1716	1528	1639	1720	1590	1957	1557	
53	1364	1884	1672	1791	1901	1740	2142	1695	
55	1343	1855	1646	1758	1891	1746	2125	1684	
57	1363	1903	1685	1775	1923	1767	2164	1709	
103	2075	2688	2394	2475	2978	2733	3260	2704	

Note: for syringes 17, 18, 19, 20 and 21, the 1st measurement failed and there is no result for the T0 point. For syringes 16, 17 and 18, the "average" value marked represents the oxygen concentration in syringe 16 only.

Table VIII – Standard deviations on oxygen concentrations in syringes according to the time after corking

	Syringe number (1.2 mL syringes)				Syringe number (2.2 mL syringes)				
Stopper/ Position	**A Rondo**	**A Nest**	**B Rondo**	**B Nest**	**A Rondo**	**A Nest**	**B Rondo**	**B Nest**	
Time (days)	**1, 2, 3**	**4, 5, 6**	**7, 8, 9**	**10, 11, 12**	**13, 14, 15**	**16, 17, 18**	**19, 20, 21**	**22, 23, 24**	
0	122	60	88	44	62	NA	NA	71	SD (ppb)
1	69	82	26	31	60	16	28	67	
4	61	101	34	15	64	28	31	43	
6	37	84	56	25	59	40	39	35	
11	108	56	84	29	141	61	54	20	
13	108	54	97	38	159	83	58	24	
15	147	58	127	93	187	116	75	26	
18	155	75	132	118	223	139	117	55	
20	176	79	120	108	237	156	134	105	
22	195	85	132	112	252	168	138	136	
25	214	101	157	121	275	173	198	131	
28	207	108	156	122	294	165	237	73	
32	226	129	157	129	330	172	278	81	
34	238	101	159	139	319	191	300	72	
36	239	110	161	154	347	199	349	91	
39	296	89	178	148	373	190	375	96	
41	252	102	177	158	383	190	378	76	
46	241	114	196	164	401	187	418	63	
48	246	125	194	177	410	200	462	48	
53	260	123	223	199	434	188	504	51	
55	236	139	207	182	446	172	510	49	
57	221	138	209	197	421	177	504	50	
103	207	152	298	387	536	219	714	70	

Appendix 3

Long-term study of oxygen reuptake in syringes. Anova two-factor with replication for 1.2 mL filled syringes 103 days after stoppering.

Oxygen concentration in syringes (ppb):

	Stopper A	Stopper B
Rondo	2243	2064
Rondo	1844	2476
Rondo	2139	2643
Nest	2526	2083
Nest	2827	2858
Nest	2710	2482

SUMMARY	**Stopper A**	**Stopper B**	**Total**
Rondo			
Count	3	3	6
Sum	6226	7183	13409
Average	2075	2394	2235
Variance	42657	88808	83115
Nest			
Count	3	3	6
Sum	8063	7424	15487
Average	2688	2475	2581
Variance	22989	150020	82845
Total			
Count	6	6	
Sum	14290	14607	
Average	2382	2434	
Variance	138760	97458	

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	359681	1	359681	4.73	**0.06**	5.32
Columns	8390	1	8390	0.11	**0.75**	5.32
Interaction	212460	1	212460	2.79	**0.13**	5.32
Within	608948	8	76119			
Total	1189479	11				

Conclusion: For 1.2 mL filled syringes, there is no significant difference between stoppers A and B (*p-value*=0.75>0.05) and no significant difference between the syringes conserved in rondo and in nest (*p-value*=0.06>0.05)

Appendix 4

Long-term study of oxygen reuptake in syringes. Anova two-factor with replication for 2.2 mL filled syringes 103 days after stoppering.

Oxygen concentration in syringes (ppb):

	Stopper A	Stopper B
Rondo	3523	2480
Rondo	2958	3881
Rondo	2452	3419
Nest	2984	2639
Nest	2631	2693
Nest	2584	2778

SUMMARY	Stopper A	Stopper B	Total
Rondo			
Count	3	3	6
Sum	8933	9780	18713
Average	2978	3260	3119
Variance	287164	509422	342576
Nest			
Count	3	3	6
Sum	8199	8111	16310
Average	2733	2704	2718
Variance	47898	4893	21378
Total			
Count	6	6	
Sum	17132	17891	
Average	2855	2982	
Variance	151969	298659	

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	481377	1	481377	2.27	**0.17**	5.32
Columns	48004	1	48004	0.23	**0.65**	5.32
Interaction	73011	1	73011	0.34	**0.57**	5.32
Within	1698755	8	212344			
Total	2301148	11				

Conclusion: For 2.2 mL filled syringes, there is no significant difference between stoppers A and B (*p-value*=0.65>0.05) and no significant difference between the syringes kept in rondo and in nest (*p-value*=0.17>0.05).

Appendix 5

Test 1 on inerting optimization on the filling line: oxygen partial pressure in syringes for E1 to E9 (values in mmHg)

Condition (Experiment number) : E1							
Tub n°7							
Filling needle / Line	1	2	3	4	5	Average	SD
1	27	34	30	30	30	30	2
10	32	31	32	28	25	30	3
20	29	34	30	33	27	31	3
				Tub average		30	3
Tub n°8							
Filling needle / Line	1	2	3	4	5	Average	SD
1	32		34	30	28	31	3
10	31	34	31	30	26	30	3
20	31	33	32	30	28	31	2
				Tub average		31	2
General average		**30**					
General SD		**2**					

Condition (Experiment number) : E2							
Tub n°7							
Filling needle / Line	1	2	3	4	5	Average	SD
1	32	35	30	24	30	30	4
10	32	35	32	25	26	30	4
20	31	36	33	24	28	30	5
				Tub average		30	4
Tub n°8							
Filling needle / Line	1	2	3	4	5	Average	SD
1	32	35	30	24	32	31	4
10	33	34	33	23	27	30	5
20	32	35	34	25	30	31	4
				Tub average		31	4
General average		**30**					
General SD		**4**					

Condition (Experiment number) : E3							
Tub n°7							
Filling needle / Line	1	2	3	4	5	Average	SD
1	23	23	24	25	23	24	1
10	23	24	23	22	23	23	1
20	24	23	23	22	23	23	1
				Tub average		23	1
Tub n°8							
Filling needle / Line	1	2	3	4	5	Average	SD
1	22	24	22	22	24	23	1
10	23	23	23	22	23	23	0
20	22	24	23	22	23	23	1
				Tub average		23	1
General average		**23**					
General SD		**1**					

Condition (Experiment number) : E4							
Tub n°7							
Filling needle / Line	1	2	3	4	5	Average	SD
1	22	22	22	22	22	22	0
10	22	22	21	23	23	22	1
20	24	22	23	22	23	23	1
				Tub average		22	1
Tub n°8							
Filling needle / Line	1	2	3	4	5	Average	SD
1	22	22	23	22	22	22	0
10	22	22	22	22	22	22	0
20	23	23	22	22	22	22	1
				Tub average		22	0
General average		**22**					
General SD		**1**					

Condition (Experiment number) : E5							
Tub n°7							
Filling needle / Line	1	2	3	4	5	Average	SD
1	22	22	23	23	23	23	1
10	22	22	22	22	23	22	0
19	25	24	25	24	23	24	1
20	22	24	23	24	23	23	1
				Tub average		23	1
Tub n°8							
Filling needle / Line	1	2	3	4	5	Average	SD
1	23	23	25	24	22	23	1
10	24	23	23	24	23	23	1
19	24	23	23	23	24	23	1
20	22	23	22	23	24	23	1
				Tub average		23	1
General average		**23**					
General SD		**1**					

Condition (Experiment number) : E6							
Tub n°7							
Filling needle / Line	1	2	3	4	5	Average	SD
1	26	25	25	26	26	26	1
10	26	25	26	25	26	26	1
20	28	27	28	29	28	28	1
				Tub average		26	1
Tub n°8							
Filling needle / Line	1	2	3	4	5	Average	SD
1	26	26	24	24	24	25	1
10	27	26	26	25	26	26	1
20	26	27	27	27	27	27	0
				Tub average		26	1
General average		**26**					
General SD		**1**					

Condition (Experiment number) : E7							
Tub n°7							
Filling needle / Line	1	2	3	4	5	Average	SD
1	26	26	25	25	26	26	1
10	26	25	27	25	27	26	1
20	25	25	26	26	26	26	1
				Tub average		26	1
Tub n°8							
Filling needle / Line	1	2	3	4	5	Average	SD
1	24	24	23	24	25	24	1
10	26	26	24	25	25	25	1
20	25	26	26	26	25	26	1
				Tub average		25	1
General average		**25**					
General SD		**1**					

Condition (Experiment number) : E8							
Tub n°7							
Filling needle / Line	1	2	3	4	5	Average	SD
1	22	21	22	24	23	22	1
10	23	22	23	23	24	23	1
20	23	24	23	24	23	23	1
				Tub average		23	1
Tub n°8							
Filling needle / Line	1	2	3	4	5	Average	StDev
1	21	21	22	22	23	22	1
10	23	23	24	23	23	23	0
20	24	24	24	23	24	24	0
				Tub average		23	1
General average		**23**					
General SD		**1**					

Condition (Experiment number) : E9							
Tub n°7							
Filling needle / Line	1	2	3	4	5	Average	SD
1	21	22	23	22	22	22	1
10	23	23	22	23	24	23	1
20	24	24	23	23	24	24	1
				Tub average		23	1
Tub n°8							
Filling needle / Line	1	2	3	4	5	Average	SD
1	22	21	22	21	23	22	1
10	22	23	22	22	23	22	1
20	24	23	23	23	24	23	1
				Tub average		23	1
General average		**23**					
General SD		**1**					

Appendix 6

Test 1 on inerting optimization on the filling line: Anova analyses.

- **Anova two-factor with replication (Needles A1-A5, Experiments E1-E9)**

SUMMARY	A1	A2	A3	A4	A5	Total
E1						
Count	6	6	6	6	6	30
Sum	182	196	189	181	164	912
Average	30.3	32.7	31.5	30.2	27.3	30.4
Variance	3.9	3.1	2.3	2.6	3.1	5.8
E2						
Count	6	6	6	6	6	30
Sum	192	210	192	145	173	912
Average	32.0	35.0	32.0	24.2	28.8	30.4
Variance	0.4	0.4	2.8	0.6	5.0	15.6
E3						
Count	6	6	6	6	6	30
Sum	137	141	138	135	139	690
Average	22.8	23.5	23.0	22.5	23.2	23.0
Variance	0.6	0.3	0.4	1.5	0.2	0.6
E4						
Count	6	6	6	6	6	30
Sum	135	133	133	133	134	668
Average	22.5	22.2	22.2	22.2	22.3	22.3
Variance	0.7	0.2	0.6	0.2	0.3	0.3
E5						
Count	6	6	6	6	6	30
Sum	135	137	138	140	138	688
Average	22.5	22.8	23.0	23.3	23.0	22.9
Variance	0.7	0.6	1.2	0.7	0.4	0.7
E6						
Count	6	6	6	6	6	30
Sum	159	156	156	156	157	784
Average	26.5	26.0	26.0	26.0	26.2	26.1
Variance	0.7	0.8	2.0	3.2	1.8	1.5

E7						
Count	6	6	6	6	6	30
Sum	152	152	151	151	154	760
Average	25.3	25.3	25.2	25.2	25.7	25.3
Variance	0.7	0.7	2.2	0.6	0.7	0.9

E8						
Count	6	6	6	6	6	30
Sum	136	135	138	139	140	688
Average	22.7	22.5	23.0	23.2	23.3	22.9
Variance	1.1	1.9	0.8	0.6	0.3	0.9

E9						
Count	6	6	6	6	6	30
Sum	136	136	135	134	140	681
Average	22.7	22.7	22.5	22.3	23.3	22.7
Variance	1.5	1.1	0.3	0.7	0.7	0.8

Total					
Count	54	54	54	54	54
Sum	1364	1396	1370	1314	1339
Average	25.3	25.9	25.4	24.3	24.8
Variance	13.1	21.2	14.5	6.9	5.9

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*	
Sample	2546.5	8	318.3	263.80	**8.9E-110**	1.98	→ Exp (E1-E9)
Columns	72.4	4	18.1	15.01	**6.9E-11**	2.41	→ Needles (A1-A5)
Interaction	442.5	32	13.8	11.46	**5.91E-32**	1.50	→ A x E
Within	271.5	225	1.2				
Total	3333.0	269					

- **Anova two-factor with replication (Needles A1-A5, Experiments E3-E9)**

SUMMARY	A1	A2	A3	A4	A5	Total
E3						
Count	6	6	6	6	6	30
Sum	137	141	138	135	139	690
Average	22.8	23.5	23.0	22.5	23.2	23.0
Variance	0.6	0.3	0.4	1.5	0.2	0.6
E4						
Count	6	6	6	6	6	30
Sum	135	133	133	133	134	668
Average	22.5	22.2	22.2	22.2	22.3	22.3
Variance	0.7	0.2	0.6	0.2	0.3	0.3
E5						
Count	6	6	6	6	6	30
Sum	135	137	138	140	138	688
Average	22.5	22.8	23.0	23.3	23.0	22.9
Variance	0.7	0.6	1.2	0.7	0.4	0.7
E6						
Count	6	6	6	6	6	30
Sum	159	156	156	156	157	784
Average	26.5	26.0	26.0	26.0	26.2	26.1
Variance	0.7	0.8	2.0	3.2	1.8	1.5
E7						
Count	6	6	6	6	6	30
Sum	152	152	151	151	154	760
Average	25.3	25.3	25.2	25.2	25.7	25.3
Variance	0.7	0.7	2.2	0.6	0.7	0.9
E8						
Count	6	6	6	6	6	30
Sum	136	135	138	139	140	688
Average	22.7	22.5	23.0	23.2	23.3	22.9
Variance	1.1	1.9	0.8	0.6	0.3	0.9
E9						
Count	6	6	6	6	6	30
Sum	136	136	135	134	140	681
Average	22.7	22.7	22.5	22.3	23.3	22.7
Variance	1.5	1.1	0.3	0.7	0.7	0.8

Total					
Count	42	42	42	42	42
Sum	990	990	989	988	1002
Average	23.6	23.6	23.5	23.5	23.9
Variance	3.1	2.6	2.7	2.8	2.4

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit	
Sample	397.7	6	66.3	76.57	**2.39E-46**	2.15	→ Exp. (E3-E9)
Columns	3.2	4	0.8	0.91	**0.46**	2.42	→ Needles (A1-A5)
Interaction	11.4	24	0.5	0.55	**0.96**	1.58	→ A x E
Within	151.5	175	0.9				
Total	563.8	209					

Appendix 7

Test 2 on inerting optimization on the filling line: results in syringes from lines 2 to 98, Needles A1 to A5

Table IX – Results of Oxygen partial pressure in line 10 syringes every sixth tub

Oxygen partial pressure in line 10 syringes								
Filling Needle / **Tub Number**	**1**	**2**	**3**	**4**	**5**	Average (mmHg)	SD (mmHg)	RSD (%)
2	24	25	24	25	25	25	1	2
8	25	24	25	25	27	25	1	4
14	24	25	25	26	27	25	1	4
20	24	24	25	24	24	24	0	2
26	24	24	25	25	26	25	1	3
32	NA	25	24	25	26	25	1	3
38	24	24	25	25	25	25	1	2
44	24	24	24	25	25	24	1	2
50	24	24	24	24	25	24	0	2
56	24	25	24	25	26	25	1	3
62	25	25	26	25	26	25	1	2
68	24	25	24	25	26	25	1	3
74	24	24	24	24	25	24	0	2
80	24	24	24	24	25	24	0	2
86	23	23	24	24	24	24	1	2
92	28	29	26	24	29	27	2	8
98	24	24	24	24	24	24	0	0

Average total (without T92) (mmHg)	**25**
SD (mmHg)	**1**
RSD (%)	**3**

Note: **Tub 92:** the machine gave an alert message of gas failure for lines 1 to 11. Line 10 was therefore impacted and this explains the very high oxygen level and average deviation. The report of gas failure won't be detailed on that report.
Oxygen values of this tub are not taken into account for the Total average calculation.

The orange color represents the maximum oxygen partial pressure measured for each analyzed tub depending on the filling needle.

Appendix 8

Test 2 on inerting optimization on the filling line: Anova analyses

- **Anova single-factor (Needles A1-A5, Tubs 2-98)**

Tub Number	A1	A2	A3	A4	A5
2	24	25	24	25	25
8	25	24	25	25	27
14	24	25	25	26	27
20	24	24	25	24	24
26	24	24	25	25	26
32	25	25	24	25	26
38	24	24	25	25	25
44	24	24	24	25	25
50	24	24	24	24	25
56	24	25	24	25	26
62	25	25	26	25	26
68	24	25	24	25	26
74	24	24	24	24	25
80	24	24	24	24	25
86	23	23	24	24	24
98	24	24	24	24	24

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
A1	15	361	24.1	0.2
A2	16	389	24.3	0.4
A3	16	391	24.4	0.4
A4	16	395	24.7	0.4
A5	16	406	25.4	0.9

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	15.7	4	3.9	8.68	**8.41E-06**	2.50
Within Groups	33.5	74	0.5			
Total	49.2	78				

Scheffé Test:

F (Scheffé) are calculated for individual comparisons

2 groups are significantly different if Fmeasured>Fcrit

$$F_{12} = \frac{(\overline{X}_1 - \overline{X}_2)^2}{MS_W\left(\frac{1}{n_1} + \frac{1}{n_2}\right)(K-1)}$$

Critical F	**2.50**

	A1-A2	A1-A3	A1-A4	A1-A5	A2-A3	A2-A4	A2-A5	A3-A4	A3-A5	A4-A5
F_{12}	0.53	0.59	1.65	7.32	0.07	0.62	4.99	0.28	3.88	2.09
Significance	**NO**	**NO**	**NO**	**YES**	**NO**	**NO**	**YES**	**NO**	**YES**	**NO**

- **Anova single factor with replication (Tubs T2-T50)**

	T2	T8	T14	T20	T26	T32	T38	T44	T50
A1	24	25	24	24	24	N/A	24	24	24
A2	25	24	25	24	24	25	24	24	24
A3	24	25	25	25	25	24	25	24	24
A4	25	25	26	24	25	25	25	25	24
A5	25	27	27	24	26	26	25	25	25

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
T2	5	123	24.6	0.3
T8	5	126	25.2	1.2
T14	5	127	25.4	1.3
T20	5	121	24.2	0.2
T26	5	124	24.8	0.7
T32	4	100	25.0	0.7
T38	5	123	24.6	0.3
T44	5	122	24.4	0.3
T50	5	121	24.2	0.2

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	7.2	8	0.9	1.57	**0.17**	2.22
Within Groups	20.0	35	0.6			
Total	27.2	43				

- **Anova single factor with replication (Tubs T56-T98)**

	T56	T62	T68	T74	T80	T86	T98
A1	24	25	24	24	24	23	24
A2	25	25	25	24	24	23	24
A3	24	26	24	24	24	24	24
A4	25	25	25	24	24	24	24
A5	26	26	26	25	25	24	24

Anova: SingTe Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
T56	5	124	24.8	0.7
T62	5	127	25.4	0.3
T68	5	124	24.8	0.7
T74	5	121	24.2	0.2
T80	5	121	24.2	0.2
T86	5	118	23.6	0.3
T98	5	120	24.0	0.0

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	11.0	6	1.8	5.33	**8.91E-04**	2.45
Within Groups	9.6	28	0.3			
Total	20.6	34				

<u>Test de Scheffé :</u>

F (Scheffé) are calculated for individual comparisons

$$F_{12} = \frac{(\overline{X}_1 - \overline{X}_2)^2}{MS_W\left(\frac{1}{n_1} + \frac{1}{n_2}\right)(K-1)}$$

Critical F	**2.45**

	T62-T86	T62-T98	T56-T86
F_{12}	3.94	2.38	1.75
Significance	**YES**	**NO**	**NO**

- **Anova single factor with replication for averages (Tubs 2 to 50 and 56 to 98)**

Averages for tubs 2 to 50	Averages for tubs 56 to 98
24.6	24.8
25.2	25.4
25.4	24.8
24.2	24.2
24.8	24.2
25.0	23.6
24.6	24.0
24.4	
24.2	

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Averages for tubs 2 to 50	9	222.4	24.7	0.2
Averages for tubs 56 to 98	7	171.0	24.4	0.4

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.3	1	0.31	1.20	**0.29**	4.60
Within Groups	3.6	14	0.26			
Total	4.0	15				

Appendix 9

Test 3 on inerting optimization on the filling line:

- **Results of oxygen partial pressure measured in the syringes from experiments 1 to 6 (in mmHg):**

				Exp Number	E1	E2	E3	E4	E5	E6
Experimental conditions				N_2 output at filling station (L/min.)	**5.5**	**2**	**3.5**	3.5	3.5	3.5
				Machine rate (cycles/min.)	50	50	50	**55**	**45**	**35**
					Oxygen partial pressure (mmHg)					
Tub	T3	Filling needle	A1		28	28	29	27	28	28
			A2		26	29	29	28	29	29
			A3		26	28	29	28	28	28
			A4		28	29	28	28	29	28
			A5		30	31	31	29	31	31
	T4		A1		28	29	29	28	29	28
			A2		28	31	28	28	29	27
			A3		28	29	27	28	28	27
			A4		27	30	28	29	30	27
			A5		29	30	29	29	29	34
Average (mmHg)					**28**	**29**	**29**	**28**	**29**	**29**
SD (mmHg)					1	1	1	1	1	2

The results correspond to measurements performed in syringes from line 10 of tubs 3 and 4 by the 5 filling needles.

- **Results of oxygen partial pressure measured in the syringes from experiments 7 (in mmHg):**

Filling needle / Tub number	1	2	3	4	5	Average	SD
2	28	28	27	28	29	**28**	1
4	27	27	27	28	30	**28**	1
6	27	28	29	29	31	**29**	1
8	29	34	35	35	36	**34**	3
10	34	36	38	37	39	**37**	2
12	37	37	37	37	40	**38**	1
14	38	36	37	39	44	**39**	3
20	36	38	40	40	42	**39**	2
26	40	39	40	NA	41	**40**	1
32	40	40	38	40	42	**40**	1
38	39	41	40	40	42	**40**	1
44	38	38	41	39	46	**40**	3
50	41	44	40	41	48	**43**	3
56	39	39	40	41	42	**40**	1
62	39	41	42	42	44	**42**	2
64	42	40	41	43		**42**	1
66	41	40	40	40	42	**41**	1
68	42	48	50	51	49	**48**	4
70	45	50	54	54	50	**51**	4

The results correspond to measurements performed in syringes from line 10 of tubs 2 to 70 by the 5 filling needles.

Appendix 10

Test 3 on inerting optimization on the filling line.

- **Anova two-factor with replication (Experiments E1-E3, Needles A1-A5)**

Results in mmHg, ordered by experiments and needles (Tubs 3 and 4):

	E1	E2	E3
A1	28	28	29
A1	28	29	29
A2	26	29	29
A2	28	31	28
A3	26	28	29
A3	28	29	27
A4	28	29	28
A4	27	30	28
A5	30	31	31
A5	29	30	29

SUMMARY	**E1**	**E2**	**E3**	**Total**
A1				
Count	2	2	2	6
Sum	56	57	58	171
Average	28.0	28.5	29.0	28.5
Variance	0.0	0.5	0.0	0.3
A2				
Count	2	2	2	6
Sum	54	60	57	171
Average	27.0	30.0	28.5	28.5
Variance	2.0	2.0	0.5	2.7
A3				
Count	2	2	2	6
Sum	54	57	56	167
Average	27.0	28.5	28.0	27.8
Variance	2.0	0.5	2.0	1.4
A4				
Count	2	2	2	6
Sum	55	59	56	170
Average	27.5	29.5	28.0	28.3
Variance	0.5	0.5	0.0	1.1
A5				

Count	2	2	2	6
Sum	59	61	60	180
Average	29.5	30.5	30.0	30.0
Variance	0.5	0.5	2.0	0.8

Total			
Count	10	10	10
Sum	278	294	287
Average	27.8	29.4	28.7
Variance	1.5	1.2	1.1

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*	
Sample	15.8	4	3.95	4.39	**1.51E-02**	3.06	→ Needles (A1-A5)
Columns	12.9	2	6.43	7.15	**6.60E-03**	3.68	→ Exp (E1-E3)
Interaction	4.8	8	0.60	0.67	**0.71**	2.64	→ A x E
Within	13.5	15	0.90				
Total	47.0	29					

Scheffé Test:

F (Scheffé) are calculated for individual comparisons

$$F_{12} = \frac{(\bar{X}_1 - \bar{X}_2)^2}{MS_W\left(\frac{1}{n_1} + \frac{1}{n_2}\right)(K-1)}$$

Critical F	**3.68**

Experiments	**E1-E2**	E1-E3	E2-E3
F_{12}	7.11	2.25	1.36
Significance	**YES**	**NO**	**NO**

Needles	A1-A2	A1-A3	A1-A4	**A1-A5**	A2-A3	A2-A4	**A2-A5**	A3-A4	**A3-A5**	**A4-A5**
F_{12}	0.00	0.74	0.05	3.75	0.74	0.05	3.75	0.42	7.82	4.63
Significance	**NO**	**NO**	**NO**	**YES**	**NO**	**NO**	**YES**	**NO**	**YES**	**YES**

- **Anova two-factor with replication (Experiments E4-E6, Needles A1-A5)**

Results in mmHg ordered by experiments and needles (Tubs 3 and 4):

	E4	E5	E6
A1	27	28	28
A1	28	29	28
A2	28	29	29
A2	28	29	27
A3	28	28	28
A3	28	28	27
A4	28	29	28
A4	29	30	27
A5	29	31	31
A5	29	29	34

SUMMARY	**E4**	**E5**	**E6**	**Total**
A1				
Count	2	2	2	6
Sum	55	57	56	168
Average	27.5	28.5	28.0	28.0
Variance	0.5	0.5	0.0	0.4
A2				
Count	2	2	2	6
Sum	56	58	56	170
Average	28.0	29.0	28.0	28.3
Variance	0.0	0.0	2.0	0.7
A3				
Count	2	2	2	6
Sum	56	56	55	167
Average	28.0	28.0	27.5	27.8
Variance	0.0	0.0	0.5	0.17
A4				
Count	2	2	2	6
Sum	57	59	55	171
Average	28.5	29.5	27.5	28.5
Variance	0.5	0.5	0.5	1.1

A5				
Count	2	2	2	6
Sum	58	60	65	183
Average	29.0	30.0	32.5	30.5
Variance	0.0	2.0	4.5	3.9

Total			
Count	10	10	10
Sum	282	290	287
Average	28.2	29.0	28.7
Variance	0.4	0.9	4.9

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*	
Sample	27.8	4	6.95	9.07	**6.27E-04**	3.06	→ Needles (A1-A5)
Columns	3.3	2	1.63	2.13	**0.15**	3.68	→ Experiments (E1-E3)
Interaction	16.4	8	2.05	2.67	**4.79E-02**	2.64	→ A x E
Within	11.5	15	0.77				
Total	59.0	29					

- **Anova two-factor with replication (Experiments E4-E6, Needles A1-A4)**

Results in mmHg ordered by experiments and needles (Tubs 3 and 4):

	E4	E5	E6
A1	27	28	28
A1	28	29	28
A2	28	29	29
A2	28	29	27
A3	28	28	28
A3	28	28	27
A4	28	29	28
A4	29	30	27

SUMMARY	**E4**	**E5**	**E6**	**Total**
A1				
Count	2	2	2	6
Sum	55	57	56	168
Average	27.5	28.5	28.0	28.0
Variance	0.5	0.5	0.0	0.4
A2				
Count	2	2	2	6
Sum	56	58	56	170
Average	28.0	29.0	28.0	28.3
Variance	0.0	0.0	2.0	0.7
A3				
Count	2	2	2	6
Sum	56	56	55	167
Average	28.0	28.0	27.5	27.8
Variance	0.0	0.0	0.5	0.2
A4				
Count	2	2	2	6
Sum	57	59	55	171
Average	28.5	29.5	27.5	28.5
Variance	0.5	0.5	0.5	1.1

	Total		
Count	8	8	8
Sum	224	230	222
Average	28.0	28.8	27.8
Variance	0.3	0.5	0.5

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*	
Sample	1.7	3	0.56	1.33	**0.31**	3.49	→ Needles (A1-A4)
Columns	4.3	2	2.17	5.20	**2.36E-02**	3.89	→ Experiments (E1-E3)
Interaction	2.3	6	0.39	0.93	**0.51**	3.00	→ A x E
Within	5.0	12	0.42				
Total	13.3	23					

Scheffé Essais

Critical F	**3.89**

$$F_{12} = \frac{(\bar{X}_1 - \bar{X}_2)^2}{MS_W\left(\frac{1}{n_1} + \frac{1}{n_2}\right)(K-1)}$$

	E4-E5	E4-E6	E5-E6
F_{12}	2.70	0.30	4.80
Significance	**NO**	**NO**	**YES**

- **Anova two-factor without replication: Experiment 7, Tubs T26-T62, Needles A1-A5**

This statistical analysis is aimed at seeing if there is a significant difference between oxygen partial pressure measured on the plateau of the graph (tubs 26 to 62) and in the same time if there is a difference between the 5 needles.
We can demonstrate the 2 hypothesis by an ANOVA Two-Factor test, without replication:

Results in mmHg ordered by tubs and needles:

	A1	**A2**	**A3**	**A4**	**A5**
T26	40	39	40	40	41
T32	40	40	38	40	42
T38	39	41	40	40	42
T44	38	38	41	39	46
T50	41	44	40	41	48
T56	39	39	40	41	42
T62	39	41	42	42	44

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
T26	5	200	40.0	0.5
T32	5	200	40.0	2.0
T38	5	202	40.4	1.3
T44	5	202	40.4	11.3
T50	5	214	42.8	10.7
T56	5	201	40.2	1.7
T62	5	208	41.6	3.3
A1	7	276	39.4	1.0
A2	7	282	40.3	3.9
A3	7	281	40.1	1.5
A4	7	283	40.4	1.0
A5	7	305	43.6	6.6

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*	
Rows	33.0	6	5.50	2.61	**4.29E-02**	2.51	→ Tubs (T26-T62)
Columns	72.7	4	18.19	8.65	**1.80E-04**	2.78	→ Needles (A1-A5)
Error	50.5	24	2.10				
Total	156.2	34					

The p-values obtained indicate that there is a significant difference for oxygen level depending on the needles (1.80×10^{-4}) and also the tubs (4.29×10^{-2})

For the needles, a Scheffé test is performed to make individual comparisons.

$$F_{12} = \frac{(\overline{X}_1 - \overline{X}_2)^2}{MS_W\left(\frac{1}{n_1} + \frac{1}{n_2}\right)(K-1)}$$

Scheffé Test:

F (Scheffé) are calculated for individual comparisons

Critical F	**2.51**

	A1-A2	A1-A3	A1-A4	A1-A5	A2-A3	A2-A4	A2-A5	A3-A4	A3-A5	A4-A5
F_{12}	0.31	0.42	0.83	14.29	0.02	0.02	8.99	0.07	9.78	8.22
Significance	**NO**	**NO**	**NO**	**YES**	**NO**	**NO**	**YES**	**NO**	**YES**	**YES**

We can observe that needle 5 gives significantly different results compared to all the other needles.

- **Anova two-factor without replication: Experiment 7 Tubs T26-T62, Needles A1-A4**

This ANOVA-2 factors test is performed to eliminate the influence of needle 5 in the comparison between oxygen partial pressures during the plateau phase (Tubs 26 to 62) Results in mmHg ordered by tubs and needles:

	A1	**A2**	**A3**	**A4**
T26	40	39	40	40
T32	40	40	38	40
T38	39	41	40	40
T44	38	38	41	39
T50	41	44	40	41
T56	39	39	40	41
T62	39	41	42	42

Anova: Two-Factor Without Replication without A5

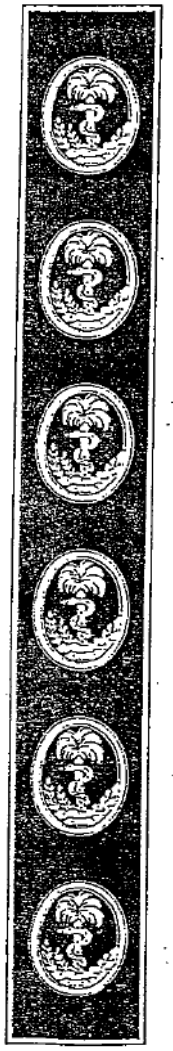
SUMMARY	*Count*	*Sum*	*Average*	*Variance*
T26	4	159	39.8	0.3
T32	4	158	39.5	1.0
T38	4	160	40.0	0.7
T44	4	156	39.0	2.0
T50	4	166	41.5	3.0
T56	4	159	39.8	0.9
T62	4	164	41.0	2.0
A1	7	276	39.4	1.0
A2	7	282	40.3	3.9
A3	7	281	40.1	1.5
A4	7	283	40.4	1.0

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*	
Rows	18.4	6	3.06	2.17	**0.09**	2.66	→ Tubs (T26-T62)
Columns	4.1	3	1.38	0.98	**0.42**	3.16	→ Needles (A1-A4)
Error	25.4	18	1.41				
Total	47.9	27					

This time, there is neither significant difference between the needles nor between the tubs (p-values>0.05).

Serment des Apothicaires



Je jure, en présence des maîtres de la faculté, des conseillers de l'ordre des pharmaciens et de mes condisciples :

D'honorer ceux qui m'ont instruit dans les préceptes de mon art et de leur témoigner ma reconnaissance en restant fidèle à leur enseignement.

D'exercer, dans l'intérêt de la santé publique, ma profession avec conscience et de respecter non seulement la législation en vigueur, mais aussi les règles de l'honneur, de la probité et du désintéressement.

De ne jamais oublier ma responsabilité et mes devoirs envers le malade et sa dignité humaine ; en aucun cas je ne consentirai à utiliser mes connaissances et mon état pour corrompre les moeurs et favoriser des actes criminels.

Que les hommes m'accordent leur estime si je suis fidèle à mes promesses. Que je sois couvert d'opprobre et méprisé de mes confrères si j'y manque.

TITRE

MOLECULES SENSIBLES A L'OXYGENE : ETUDE ET OPTIMISATION DES PROCEDES D'INERTAGE. APPLICATIONS AUX SERINGUES PRE-REMPLIES.

RESUME

Le présent travail porte sur la formulation et le procédé de production de solutions de molécules sensibles à l'oxygène en seringues pré-remplies. Cette problématique est ici principalement axée sur les moyens de contrôle des taux d'oxygène en solution et les paramètres du procédé de fabrication pour protéger ces molécules de l'oxydation.

La première partie présente une étude des appareils servant à mesurer l'oxygène en solution ou dans l'espace de tête des seringues. Un choix final a conduit à l'achat de deux appareils de marque Radiometer, usuellement utilisés pour les analyses de gaz du sang et mesurant des pressions partielles en oxygène dans les solutions.

Dans la seconde partie, des études menées dans un laboratoire de formulation mettent en évidence les points suivants : la composition en gaz et le volume de l'espace de tête sont critiques et ont un fort impact sur la concentration finale en oxygène dissous dans la solution ; des solutions inertées se ré-oxygènent de façon importante au cours du temps dans des seringues en verre bouchées et enfin les tubulures en silicone présentent une perméabilité importante à l'oxygène.

La troisième partie décrit les moyens d'optimiser l'inertage sur une ligne pilote de remplissage de seringues. Les paramètres importants sont les débits d'azote en station de remplissage et de bouchage, qui doivent être maximisés. Un certain nombre de défauts intrinsèques à la machine devront être réglés afin de s'affranchir d'éventuelles augmentations de la concentration en oxygène dans les solutions.

MOTS CLEFS

Oxygène, Inertage, Seringues pré-remplies, Formulation, Procédé, Remplissage, Solutions injectables.

TITRE

OXYGEN-SENSITIVE DRUGS: STUDY AND OPTIMIZATION OF INERTING PROCESSES. APPLICATION TO PRE-FILLED SYRINGES.

ABSTRACT

The present work is about formulation and manufacturing process of oxygen-sensitive drug solutions in pre-filled syringes. This issue mainly deals with the means to evaluate oxygen contents in solution and the process parameters to protect oxygen-sensitive drugs from oxidation.

The first part presents the study of devices to measure the oxygen concentration in solution or in the headspace of the syringes. A final choice led to purchase two devices, Radiometer brand, ordinarily used for blood gas analyses and that measure oxygen partial pressure in solutions.

In the second part, some studies conducted in a formulation laboratory underline the following points: the gas composition and the volume of the headspace are critical and highly impact the final dissolved oxygen concentration; inerted solutions get re-oxygenated in an important way during the time in corked glass syringes, and finally silicone tubing are permeable to oxygen in an important and fast way.

The third part describes the way to optimize inerting on a pilot filling line. The important parameters are nitrogen flow rates at the filling and stoppering stations that have to be maximized. Some defects intrinsic to the filling machine would have to be fixed in order to avoid possible increase of the oxygen concentration in solution.

KEY WORDS

Oxygen, Inerting, Pre-filled syringes, Formulation, Process, Filling, Injectable solutions.