A modular management production system based on the ANSI ISA S88.01 standard provides a comparison of Equipment Module and Control Module Options.

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The ANSI ISA S88.01 Standard: A Case Study of its Application in the Pharmaceutical Industry

by M. Mangiarotti and M. Rizzi

Introduction

In the light of an innovative production systems development project, and in line with the guidelines issued by its US parent company, an Italian company has based its new model for a modular management production system on the ANSI ISA S88.01 standard.

The system is intended to guarantee that the control of process-related activities are safely performed and to ensure that a highly flexible management of recipes and equipment is achieved, as well as a substantial improvement in the management of the production units.

S88.01 Standards

In February 1995, following an extended period of preparation, the final version of the ANSI ISA S88.01 standard was published.

The standard is intended to "*define reference models for batch control as used in the process industries and terminology that helps explain the relationship between these models and terms.*" (ANSI/ISA-S88.01, Batch Control Part 1: Models and Terminology, October 23, 1995.)

More specifically, the ANSI ISA S88.01 standard outlines standard models and terminologies aimed at defining the requirements of

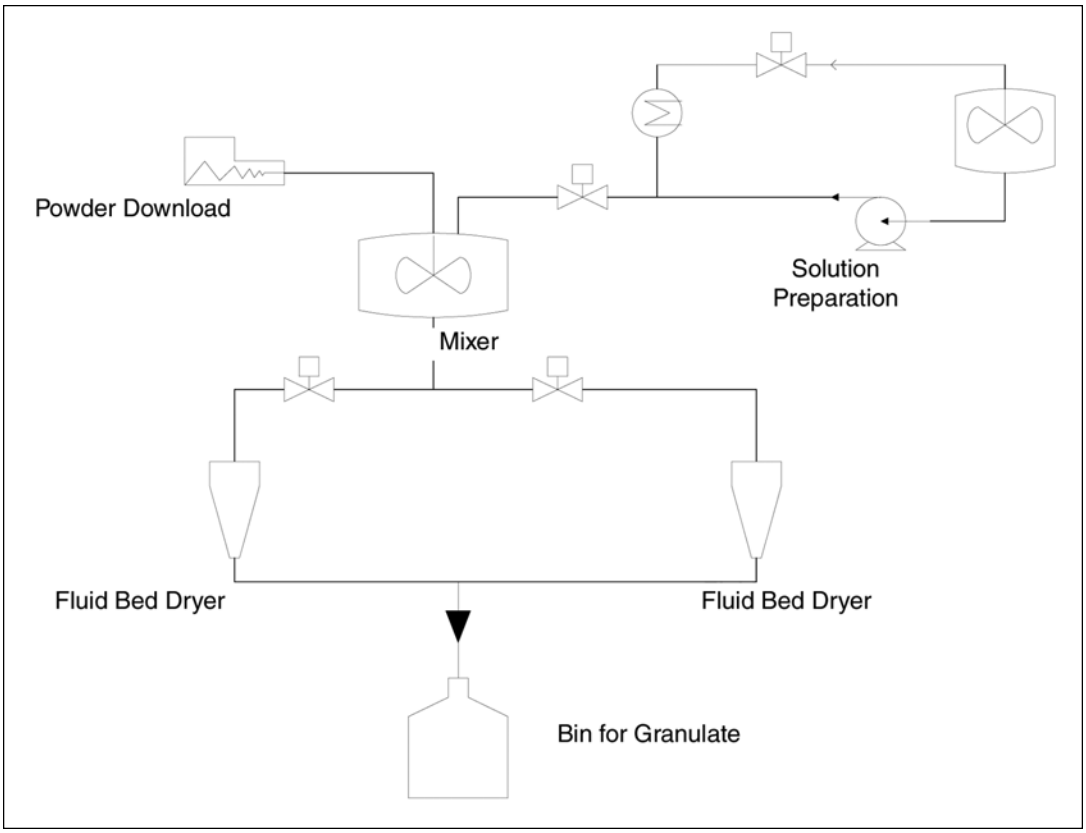

Figure 1. The fluidized-bed granulation process.

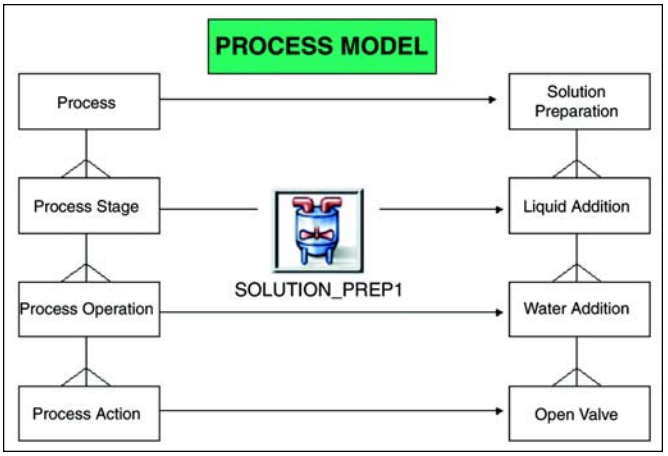

Figure 2. Process model.

batch control for a manufacturing plant. These models and the terminology help emphasize the Good Manufacturing Practices (GMPs) for the implementation and running of a batch-type manufacturing plant.

The standard may be used to improve control of existing batch-type manufacturing plants, and in particular, may be applied regardless of the automation level of the plant.

The ANSI ISA S88.01 standard does not:

- suggest a single way to implement batch control and does not guarantee the outcome of a project
- require a change in the habitual way of developing batch processes
- limit the scope of new developments relating to batch controls

Set Up and Management of Batch Processes

Batch processes manage the production of predetermined quantities of material (batches) using preset quantities of raw materials within established operation cycles, but employing different equipment.

The set up and management of batch processes in a pharmaceutical production facility raises several questions in relation to process automation. A common example is whether to have prescriptions managed by a Programmable Logic Controller (PLC) or a Personal Computer (PC).

Although they are certainly safer than personal computers during the process, PLCs are less flexible, i.e., the higher flexibility linked to recipes within a PLC-based system results in a more complex code and the associated issues that this can bring to the development and testing processes.

Conversely, a PLC/PC interface-based system may yield better results in terms of flexibility and control although two other issues need to be considered:

1. computer reliability and performance, which become critical parts of the process
2. the potential need to implement bespoke batch management software, resulting in an additional workload during development and validation of the system

To provide batch-type manufacturing plants with a practical solution, a few software companies have studied and developed the implementation model outlined by the ANSI ISA S88.01 standard, creating applications that are able to meet the requirements of ANSI ISA S88.01 standard. The opportunity of using such applications in automated pharmaceutical

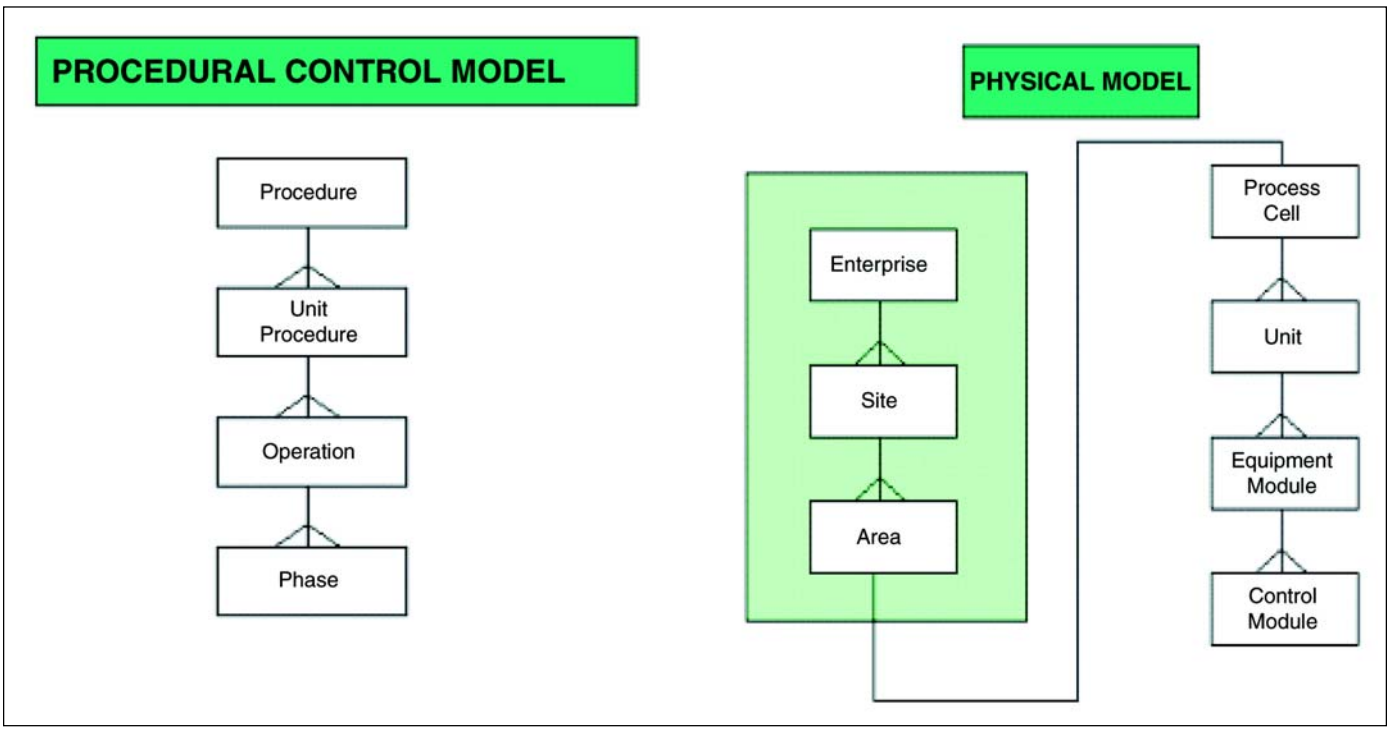

Figure 3. Procedural and physical model.

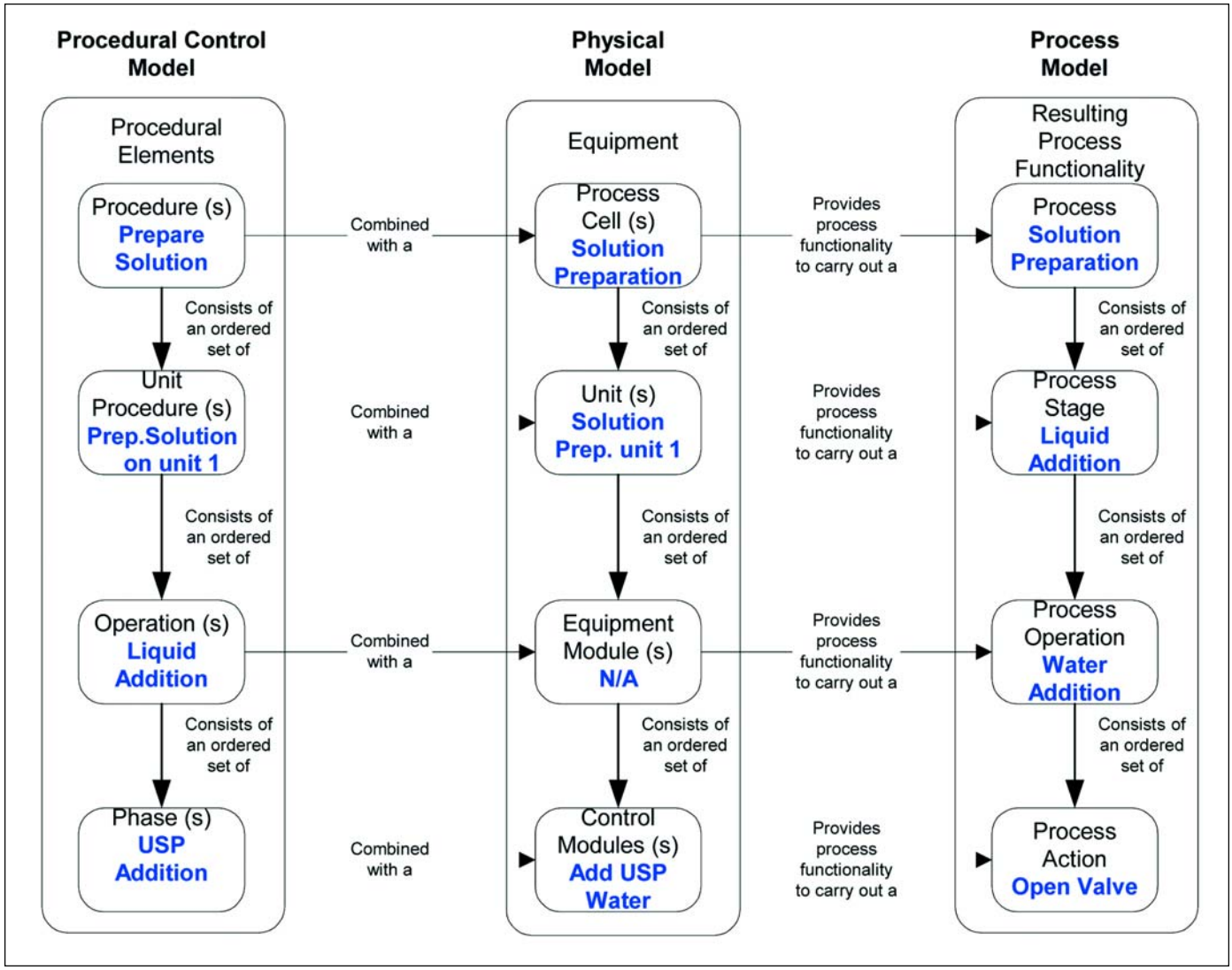

Figure 4. Relation between physical/procedural model and process.

batch-type manufacturing plants is becoming increasingly important.

The structure of a batch-type production plant must be tightly linked to its control system and consists of two main components:

- the physical components of the plant (Physical Model)
- the operating processes (Procedures)

The physical components (Physical Model) are defined as:

- Process Cell - a functionally complete plant area
- Unit - a plant unit with independent functions (e.g., a reactor or a mixer)
- Equipment Module - set of devices with specific functions (e.g., temperature control, pressure control)
- Control Module - device (e.g., a valve, an agitator, or a pump)

Case Study Description

This case study uses as an example a fluidized-bed granulation process - *Figure 1*. Without analyzing the pharmaceutical process in detail, it will be sufficient to say that fluidized-bed granulation is used in the manufacture of tablets, and primarily requires a solution preparation tank, a hopper or a powder loading system, a granulator, one or more desiccators (fluid bed driers), and a system unloading granulate into steel containers.

The solution production tank is fitted with a solution temperature control system, a mixer, and a loading system for liquids. Once they have been added and properly heated by means of a recirculation system, the liquids are poured into the mixer.

Here, the solution is mixed with the powder. After a predetermined time, the resulting granulate is transferred to the desiccators where it is air-dried under controlled dew point conditions.

After drying, the product is placed in containers and conveyed onto the next phase of the process.

Studying and Identifying the Phases of the Process

Studying and identifying the phases of the process is essential for successful plant automation. Both the process engineer and the automation engineer should be involved in this activity (it's a milestone project phase based on S88.01 modeling) and it is considered beneficial for them to collaborate from the start of the project.

The first step involves identifying the individual phases, which compose the process, and analyzing each phase individually.

In the example given, for the solution preparation tank, the process can be subdivided into a number of related actions (The action is the part of the process related to the phase, as shown in Figure 4) (Process Stage):

1. addition of liquids
2. recirculation
3. mixing
4. unloading into the mixer

To prepare a solution, each of the four actions is required. Each of these actions can be further divided into a number of operations and each operation can, itself, comprise a number of actions. Figure 2 shows an example of the liquid (water) addition phase.

Production Prescription Analysis

After studying the process, the production recipes and the equipment interactions are analyzed, taking into account the physical (site/plant/equipment) model and the procedural (process) model, as shown in Figure 3.

This analysis will result in the definition of the most critical part of the whole system: the Area Model or basic system configuration. The Area Model describes the equipment necessary to perform the process by means of recipes.

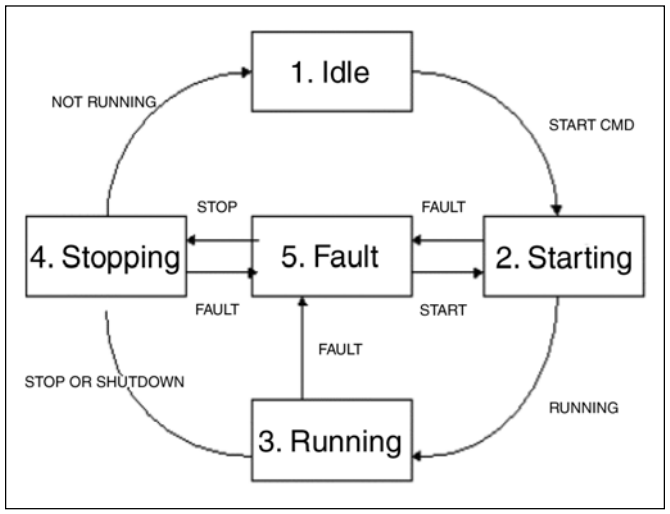

Figure 5. Equipment module.

Within the area model, every process management and implementation phase is identified along with the equipment necessary to perform the process on site.

This defines the next steps, which are:

- to define the process to be carried out (down to the level of defining individual 'actions')
- to define the equipment necessary to execute the process (Area Model)
- to define the "procedures," i.e., the production prescriptions which will be issued by means of the area model

Relation Between Physical/Procedural Model and Process

Figure 4 shows the connections between the various parts which comprise the correct definition of a batch process.

This analysis of the identified process phases provides the complete definition of the physical model of the process and its associated phases.

Addition of Liquids

The liquid addition phase [PROCESS] (three types of liquids) requires a physical system.

The physical system is made up of three valves (one per type of liquid) and, e.g., three dosing pumps.

- This physical system (composed of the three valves and three pumps) is considered a **single phase** [PHYSICAL MODEL]

The recirculation phase [PROCESS] requires one pump, several valves, and one heat exchanger.

- The system (composed of one pump, several valves, and one heat exchanger) is considered a **single phase** [PHYSICAL MODEL].

The mixing phase [PROCESS] requires a mixer.

- The mixer is considered a **single phase** [PHYSICAL MODEL].

The mixer unloading phase [PROCESS] is made up of an unloading valve and a Mixer loading valve.

- The unloading valve and mixer loading valve are considered a **single phase** [PHYSICAL MODEL].

The next step in the procedure is to translate the Physical Model into an appropriate PLC-based automation system.

There are two types of approaches, based on the same theory:

1. the "Equipment Module" approach

2. the "Control Module" approach

Equipment Module Approach

The "Equipment Module" approach requires the creation of a coded structure within the PLC that enables the operation of the relevant part of the physical model. In the example given, the three loading valves and the three dosing pumps are the components of a single "Equipment Module" within the PLC, i.e., a single coding structure that has parameters and operating controls which are summarized in the schematic diagram shown in Figure 5.

The equipment module starts from an idle state, and if every condition enabling it to start has been implemented, the module is brought into a running state after receiving a start command. The module will stop following if it receives an alarm signal or a stop command.

Control Module Approach

Using the example given, at the point that the water addition phase must start: the control module shown in Figure 6 will behave as follows:

If the starting conditions are met (module in idle state, i.e. valves in place and pump at rest) the start command (given by a supervisor or by the prescription management system, depending on whether the system is in semi-automatic or automatic mode) will bring the module into a starting state.

In this state, the water dosing valves receive the positioning command (by managing a parameter which could result from the prescription) and the pump receives the running command. If the feedback signals from the field are correct, i.e. if the valves are properly positioned and the pump is running, the module will go into running mode. Upon reception of a stop command, the module stops the pump and closes the valves.

If no fault arises, the module will go into an idle state again, ready to restart. An emergency condition will put the module into a fault mode. In order to set the module running again, either the restart control needs to be initiated (e.g., if the fault has been identified and is easily removed), or the stop control operated (e.g., if the fault requires maintenance).

The "Control Module" approach is based on a different structure, i.e., on the operation of single devices or physical components.

Individual pumps and valves are operated in a standard manner, as shown in Figure 6.

The two Manual/Automatic command signals originating from the supervising system are filtered by a code structure which is able to control the safety interlocks/alarms, as well as the devices to be operated first (e.g., a device driver).

The manual command is given by the Supervisory Control and Data Acquisition (SCADA) system (e.g., for the forcing of single devices) whereas the automatic command is generated by the batch software which operates the various devices in accordance with the prescription steps. A fault in the batch system will cause the devices associated with the faulty step to stop immediately.

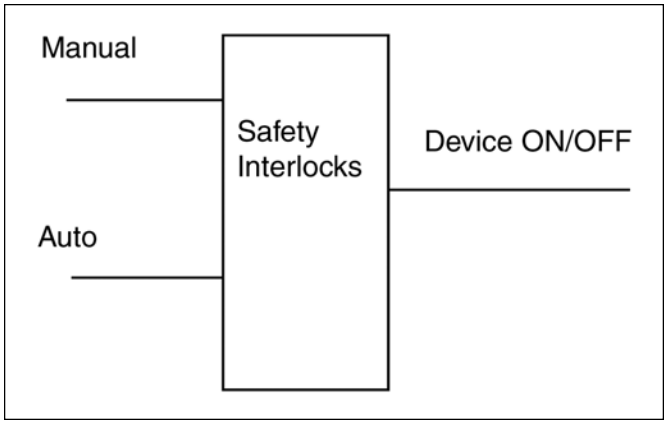

Figure 6. Control module.

Defining Phase Modules

Both the *equipment module* and the *control module* structure need that part of the PLC code to interface with the batch system. The structure is called a *phase module*.

The main components of the phase module are the Commands, Parameters, and Reports. The phase module sends Commands and Parameters to the *equipment module.* The *equipment module* sends the Reports to the phase module.

In the example given, the liquid addition *equipment module* receives the following parameters from the phase module:

- type of liquid (water)
- quantity to be added
- start/stop commands

The *equipment module* returns the following report:

- added quantity (back to the phase module)

The report will then be used by the batch report structures in conjunction with the report from the equipment module to generate the batch records for the manufacturing process.

Analysis of the discussion to this point indicates that the *equipment module* parameters are nothing more than the recipe parameters of the pharmaceutical process.

Conclusion

Comparison of the Equipment Module and the Control Module Options

The *control module* structure guarantees considerably simpler PLC software which on the one hand reduces its capability, but on the other provides a greater storage capacity. The greatest disadvantage is the dependence on the batch program when executing the recipes. There is no alternative. Any faults in the recipe management program will result in an inevitable production standstill which can only be reduced by means of robust back-up structures, e.g., single devices could be manually operated (forced) by means of the SCADA system. However, this would only enable relatively simple processes to be recovered.

On the other hand, although the *equipment module* structure provides a complex PLC code structure, it ensures that

process recipes can be manually completed with subsequent steps even if the batch system crashes. In such case, the operator will use each single equipment module following the master formula to achieve the final product. This type of structure is recommended for highly critical processes such as pharmaceutical manufacturing processes.

In order for the batch control system and its validation to be successful, it is worth stressing that it is essential that the various players involved in the project need to collaborate during every phase, that the process traceability is achieved by means of a common systematic approach, and that an appropriate batch system development process is used in order to avoid having to repeatedly develop a new methodology. Since pharmaceutical manufacturers no longer operate in local markets, the recommendations of the various boards in charge of guaranteeing observance of those regulations cannot be neglected.

Reference

1. ANSI/ISA - S88.01 - 1995.

About the Authors

Maurizio Mangiarotti is Manufacturing Automation Manager for the Pavia Engineering Department of Merck Sharp & Dohme Italy. With an educational background in Technical Studies, he received a Degree in electronic engineering from the Università di Pavia with a thesis on the GSM data transmissions. He worked as automation project engineer in various application fields such as mechanics, food, petrochemical; and in the pharmaceutical industry working with engineering companies, production companies, plant maintenance, and validation services including every aspect of automation engineering. He has shared his experience by publishing articles and presenting papers at international conferences. He is an active member of the Associazione Italiana per l'Automazione (ANIPLA).

Merck Sharp & Dohme Italy S.p.A., Automation Department, Pavia, V. Emilia 21, 27100 Italy, tel: +39 0382 433371, e-mail: maurizio_mangiarotti@merck.com.

Marco Rizzi joined Rockwell Automation Italy five 5 years ago as application manager and then assumed the marketing responsibility for the Process Business. Currently, he is Application and Marketing Manger, Process Control Italy. He has 15 years of experience in the process control industry with positions such as system engineer, project leader, and project manager. He has worked in countries such as the Peoples Republic of China and Saudi Arabia. He has been involved in the MS&D Pavia project, spoken at international conferences, and published articles in technical Italian magazines.

Rockwell Automation Italy, Viale De Gasperi, 126-20026 Mazzo Di Rho, Italy.

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Manufacturing and Quality Partnership in Support of Product Development

by Carmen M. Wagner and Frank S. Kohn

Introduction

Vaccine companies are entrusted with the responsibility to develop vaccines that are safe, efficacious, meet regulatory compliance, and are not too costly. This is a tall order, considering that companies must risk up to 15 years, and as much as $800 million, knowing these investments of time and dollars may never pay off.[1] In fact, many of the products now under development will never actually reach the market. Consequently, meeting these time and investment requirements, while remaining profitable, requires creative and "out of the box" thinking.

The above business demands are just a part of the story. In order to be successful, vaccine companies also must invest heavily in regulatory compliance. They must apply the appropriate level of current Good Manufacturing Practices (cGMPs) to the development of new vaccines, and must ensure the right level of quality oversight and the application of appropriate manufacturing principles.[2] As stated in the FDA guidance document,[3] "when drug development reaches the stage where the drug products are produced for clinical trials in hu-

Figure 1. Key factors for successful manufacturing and QA partnership.

mans or animals, then compliance with cGMPs is required. For example, the drug product must be produced in a qualified facility, using laboratory and other equipment that has been qualified, and processes must be validated." According to FDA expectations, the quality and manufacturing functions must not only ensure the availability of sufficient and adequate product, but also must assure their compliance with the appropriate regulatory requirements.

Business, technological, and regulatory demands continue to pressure companies to search for more efficient and cost-effective ways to develop and market new products. In our experience, one of the answers lies in the organization of effective teamwork and in the constructive relationship between the manufacturing and quality portions of the organization. All teams, including the manufacturing/quality team must work synergistically to shorten timelines and contain cost. We believe it is never too early to start involving the quality and manufacturing specialists in the product development process. Successful companies should start thinking of these two functions as soon as a candidate is considered to be a potential commercial product. The role of the quality function should increase in step-wise fashion, along with the increase in level of cGMPs. Quality oversight should be in full mode by Phase III, when full cGMPs should apply.

Given the potential for the Manufacturing and Quality functions to significantly impact the outcome of the development process, we believe that a partnership between quality and manufacturing can increase the potential for companies to achieve their goal of getting to market quickly, while marketing vaccines that meet pre-determined regulatory requirements and quality standards, and are not too costly.

This article focuses on one aspect of product development teamwork, namely the partnership between quality and manufacturing. It includes a discussion of key business, technical, and regulatory challenges that may impact product development and describes the attributes of the effective quality and manufacturing partners. It ends with a presentation of the Total Product Quality concept, and a discussion of how Quality and Manufacturing must work together to achieve product quality and support product development.

The Vaccine Business - Key Challenges

For those who are unfamiliar with the vaccine industry, it is worth mentioning that being first to market is often key to success and profitability. To remain competitive, it is often critical to pay strict attention to activities that can shorten the time it takes to achieve product commercialization. Shortening the time to market may be difficult since this is usually tied to a company's willingness and ability to dedicate the proper resources and budget to a given project.

As discussed below, the key challenges can be classified into technical, regulatory, and business. These challenges must be addressed keeping in mind the overall project goals and timelines. This may seem simple, but in large companies, with many departments and with the same people involved in several projects, it is easy for project team members to be confronted with conflicting assignments, project target completion dates and goals that are not clearly defined.

The information below outlines the issues classified as technical, regulatory, and business; and illustrates how quality and manufacturing can work together to maximize the chances for business success.

1. Technical

The manufacturing areas in a vaccine plant often resemble a laboratory, including specialized equipment such as the ones used in fermentation, purification and/or conjugation of vaccines. This kind of operation generally requires:

- high level of technical expertise
- use of automated and customized equipment
- costly and complex processes
- long lead times for manufacturing

These technical requirements tend to add to the overall operational cost, and impose long lead times that greatly impact materials management, production planning, and product distribution. Effective communication between Quality and Manufacturing can help overcome some of the technical issues by ensuring that quality systems take into consideration the appropriate manufacturing requirements.

Our experience: In order to deal with some of the existing conflicts between manufacturing and quality, and to address manufacturing's technical needs, we instituted weekly meetings between the two groups. These meetings lasted from 30-60 minutes (sometimes less) and focused on technical and compliance issues that were often resolved before they became a problem. The meetings helped the Quality Department design and update systems, taking into consideration the technical needs of the manufacturing process, but with attention to regulatory requirements. The interaction helped develop better understanding of each group's function and helped build mutual respect between the quality and manufacturing staff. Quality was able to prevent certain concerns from escalating into full compliance problems.

2. Regulatory Compliance

An overriding imperative for a company in the vaccine industry is to stay ahead of the competition, while dealing with technological changes in a highly regulated environment. The nature of these complex biological processes, and the resources needed to comply with increasing regulatory demands are numerous and costly.

The Quality/Manufacturing partnership can help achieve compliance with regulatory expectations by developing a joint strategy to ensure the following:

- that the Quality oversight is part of every step of product manufacturing

- that cGMP increases in a step-wise manner, as recommended by FDA

- that product manufacturing complies with the appropriate level of cGMP at different stages of development
- that full cGMP compliance is achieved by Phase III Clinical Trials.
- that in the case of global commercialization, the requirements imposed by the Center for Biologics Evaluation and Research (CBER) in the US, and by several Boards of Health worldwide, are understood and applied
- validation of critical processes is completed as early as possible, no later than Phase III (examples: sterilization, lyophilization, and media fills)
- cGMP activities are segregated from research and from development
- change control is in place for cGMP manufacturing of clinical supplies and consistency runs
- preliminary validation data for cleaning manufacturing equipment is available as early as possible

Our experience: Quality encouraged Manufacturing to propose solutions to problems associated with manufacturing control. These solutions were then discussed with Quality to ensure that they complied with regulatory requirements. Manufacturing's active participation in the troubleshooting process lead to a feeling of ownership, made them feel part of the solution, and encouraged greater compliance with the implemented solution.

3. Business

The development of new vaccines is often lengthy and costly. A company can lose as much as $1 million a day for every day that market entry is delayed. Besides aggressive timelines and pressures to be first-to-market, additional business issues include:

- capital intensive facilities, calling for specialized equipment and work area design
- pricing strategy is difficult
- complex and varied customer requirements
- integrating quality requirements in the business plan

Our experience: Manufacturing and Quality agreed on an approach for self-audit in the manufacturing department. This did not replace the Quality audits, but encouraged the manufacturing department to discover their own deficiencies, and propose their own solutions to correct them. The deficiencies and proposed solutions were then discussed with Quality and joint solutions were frequently agreed upon. However, it is important to note that Quality did reserve the right to disagree and impose alternative solutions, when appropriate.

Technical, business, and regulatory considerations must be integrated into the overall development plan to prevent delays in timelines and prevent profit loss. Moreover, as a practical consideration, when the common conflicts between manufacturing and quality occur, if dealt with constructively and quickly, these potential roadblocks can be turned into successes.

As mentioned previously, addressing business, technological, and regulatory demands requires more effective partnership between quality and manufacturing. These two functions need to develop a joint strategy and work to prevent problems, develop proactive solutions, and practice cost avoidance. The remainder of this article will focus on two Models that illustrate the effective quality/manufacturing partnership attributes.

Quality as an Effective Partner

The scope and list of tasks associated with product management and control can seem overwhelming, but a well designed, cross-functionally derived quality program, based on proactive quality thinking, can help break the tasks into manageable pieces. It also can help promote strong partnerships with Manufacturing.

Quality principles integrated into routine product manufacturing can be great problem prevention and cost avoidance tools. However, for this to happen, quality needs to be integrated into routine manufacturing planning. This integration should start with clinical manufacturing and should be focused on product and package development, from clinical evaluation through scale-up, to full-scale manufacturing start-up and commercialization/distribution. Thus, quality must be an integral part of the development and the commercial supply chain.

Achieving total product quality requires consideration of customer requirements and several other critical factors as illustrated in the proposed TPQ model - *Figure 2*. The ultimate goal in process development and routine manufacturing is to build efficiency and cost effectiveness, while maintaining product quality.

To emphasize the point, bringing quality and manufacturing into the development process as early as possible, helps ensure that the processes are carried out in a controlled and documented way, thus facilitating technology transfer and expediting launching of new products. This in turn helps establish effective quality systems and practices, applicable to routine product manufacturing and quality control during the commercial phase.

Manufacturing as an Effective Partner

The primary mission of the Manufacturing Department is to produce high quality products, help control cost, and meet production schedules. Quality's responsibility is to develop quality systems, test, audit, release or reject products, and assure that released lots meet all quality attributes and regulatory requirements. The Quality Department cannot build quality into the process or product, but it can help set the policies, quality culture, and practices that encourage the

manufacturing staff to meet acceptable quality standards. In the ideal situation, product quality must start within the manufacturing function in partnership with the Quality Department. Manufacturing management can ensure a successful partnership with Quality by helping foster an environment of cGMP compliance as part of the department's daily routine.

Our experience has demonstrated that the factors identified in Figure 1 are essential for a successful partnership. Without clear partnership goals, communication, strong leadership, clearly defined and implemented policies and procedures, staff training, of cross-functional team membership, and Standard Operating Procedures (SOPs), there can be no successful partnership between Manufacturing and Quality.

Figure 1 identifies the necessary factors to ensure a successful Manufacturing – Quality partnership.

Manufacturing, Quality, and the Total Product Quality (TPQ) Concept

Figure 2 shows the different components of the Total Product Quality (TPQ) concept. Both Quality and Manufacturing must integrate the TPQ concept into their routine practices in order to ensure the effective application of cGMPs to product development. The first and central component of the model identifies the factors that influence the overall product quality. These factors or disciplines should be initiated in Phase I with focus on safety related aspects of the cGMPs. As the product moves through the development stages, the emphasis on cGMPs also should increase. Documentation Management and Control is a key discipline since documentation is critical even in the early stages of product development.

The second component illustrates the timeline from discovery to commercialization. It also depicts the GMP continuum and the Technology Transfer (TT) steps during the product development life cycle. Tracking and trending, the third component is used to indicate that all the factors shown should be monitored and their performance documented in order to measure the effectiveness of the concept, and its application to the development process. The more automated the tracking and trending process, the easier it is to gather and access the information.

The Manufacturing and Quality Departments must consider the following essential factors to achieve TPQ:

Customer Requirements **-** before a product can be developed, it is important to have an understanding of what the market needs and wants. What kind of product, for what

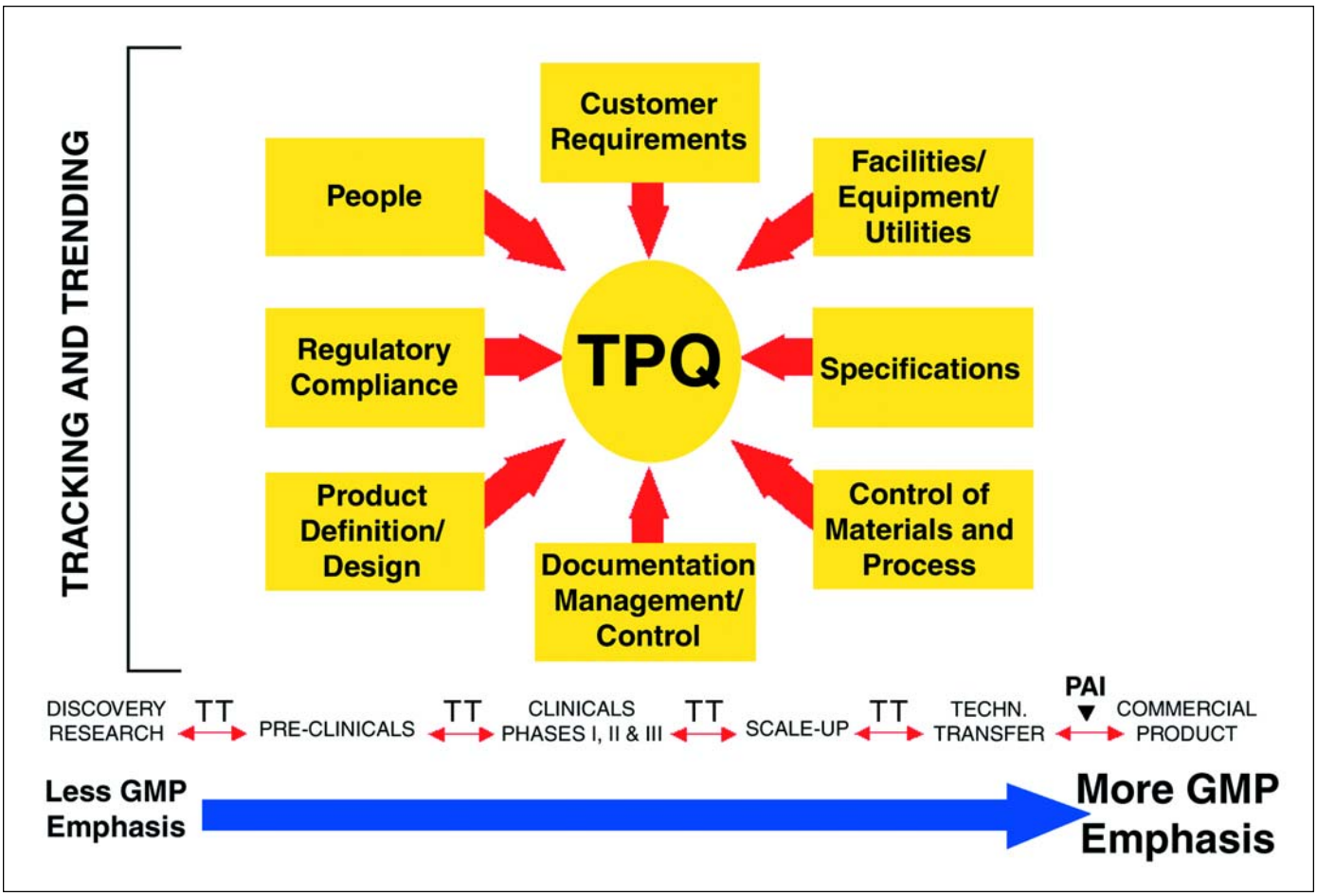

Figure 2. This figure shows the different components of the Total Product Quality (TPQ) concept. These components must work in synergy, and must be embraced by both quality and manufacturing so that they can contribute to the goal of shortening product commercialization timeline, and to control cost.

purpose, in what kind of container presentation, and by when. This is a major challenge since market input is not always clear, and market forecasts are seldom accurate. When your lead manufacturing time is as long as 9 to 12 months, it is critical to be able to predict how much product you can make by when and what technical and business challenges you will have to deal with in the future. Quality and manufacturing must work together to identify the needs of the process and fit regulatory compliance requirements into this process.

People **-** people are the key to success in any organization. The success of this model is dependent on full participation of all employees, including senior management. The establishment of a "win-win" relationship and effective teamwork is critical to control quality input and ensure continuous evaluation and improvement in the manufacturing process. Proper team structure, leadership, and communication strategy should be in place to support team members.

There is a need to clearly define roles, responsibilities, and accountabilities. It should be clear to all concerned that the final decision relative to product quality acceptance lies with the senior management in the Quality Group. It should be evident that the manufacturing department must produce products that are of quality, properly validated, designed to meet the regulatory expectations, and properly documented for effective and efficient product distribution. The product must consistently demonstrate quality performance throughout its shelf life. Finally, the Production Department should understand their role in product development and technology transfer, pulling the process into manufacturing and checking at every step to ensure that all information they need is available, including the rationale for critical decision points in the manufacturing process.

Product Definition/Design **-** quality attributes must be part of the product design from its inception, but it is also important to take into consideration the Manufacturing Department's needs. Product attributes should be evaluated and confirmed during pre-clinical and clinical evaluations. Finally, during manufacturing of consistency runs, these attributes should be controlled through the use of quality systems to monitor and measure performance throughout the product supply chain.

Facilities, Equipment, and Utilities **-** the infrastructure must be designed, calibrated, validated, used, and maintained according to a continuum of quality principles and regulatory compliance expectations. Control of equipment and facility is the first line of defense against problems and cost increases during development. The Quality and Manufacturing Departments should be involved in every step, from purchasing through calibration, to validation, and should have approval signature in critical decision points to ensure that the final facility and equipment will meet all requirements.

Specifications **-** specifications must be designed based on scientific rationale. Quality and Manufacturing should ensure that documented rationale is available for raw materials, intermediates, packaging components, labeling, and the final vial. The acceptance program for raw materials receipt, testing, and release should be defined in writing, and vendors of critical materials and components should be properly qualified. The program should be designed jointly between Manufacturing and Quality, together with R&D and the Materials Management Department.

Control of Material and Processes **-** all raw materials, components, product intermediates, and final packaging should be properly controlled through the establishment of an effective lot numbering system that will enable lot identification and traceability. This system should be designed by both Manufacturing and Quality and should help establish the historical documentation for the entire production, testing, and release of the final product. Bar coding should be seriously considered.

In addition to lot identification and control, it is necessary to ensure that the process is capable of consistent, reproducible, and reliable performance. Process manufacturing data should help minimize reliance on product testing and help address questions that may arise during routine manufacturing. A joint effort between Quality and Manufacturing can help ensure the development of a program that works to troubleshoot problems during routine production.

Auditing/Monitoring **-** auditing and monitoring are important factors in the assurance of quality. More specifically, manufacturing's involvement in auditing and documentation review should be considered. A written procedure should be available to describe the auditing function. At a minimum, the quality department should audit and monitor the following:

- control of sterility assurance
- process, equipment, utilities, and cleaning of manufacturing equipment
- final product release criteria
- container vial integrity studies
- control monitoring program - the plan and implementation
- development of documentation, including adequate batch records and associated release documents.
- establishment of change control procedures
- establishment of investigation documentation
- preparation for licensing inspection
- documented training

Again, working with manufacturing to practice self-auditing and commitment tracking of findings is an effective way to help them accept responsibility for the first line of defense against quality problems and product waste.

Last, it is necessary to comment on documentation. All the factors discussed require documentation. One must remember the rule of thumb *"if it isn't documented, it isn't done."* The

partnership team must identify all documents needed for filings, technology transfer and ultimately, to support the Pre-Approval Inspection. The Quality Department must ensure that all documents are available, are approved, and secured/controlled according to company and regulatory agencies' requirements. However, the Manufacturing Department, working with the development scientist, also must take responsibility for ensuring the accuracy, security, and ready access to such documentation**.**

The following are examples of documentation/reports to be considered:

- raw materials specification
- in-process specifications
- SOPs
- development batch records
- manufacturing batch records
- raw data to support clinical batches
- deviation reports
- investigation reports
- stability protocols
- validation reports
- stability data report
- assay method development and transfer reports, and associated training documents
- manufacturing summary reports for submission
- development history reports
- final product specifications (rationale)
- testing monographs
- training records

In summary, the proposed illustration (Figure 2) identify the factors and associated activities that teams require for bringing quality and manufacturing together into a winning partnership. The effective quality partner should be prepared to understand the constraints of the manufacturing process, but also should be prepared to provide the proper guidance and systems to facilitate the integration of the quality process into product manufacturing. On the other hand, the manufacturing partner should accept responsibility for ensuring that manufacturing resources also are focused on quality in addition to maintaining production schedule and achieving cost control.

Our experience clearly showed us that effective communication between Quality and Manufacturing, together with our management leadership, appropriate staff training, strong emphasis on documentation, clear goals, policies and procedures, could indeed help prevent problems and expedite the development process.

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About the Authors

Carmen M. Wagner, PhD, is President of Strategic Compliance International, Inc., in Cary, NC. She provides specialized services in the area of regulatory compliance, quality assurance, regulatory affairs, training, and technology transfer support for the biotechnology and vaccine industries. Dr. Wagner has held a variety of quality related positions in the health industry and has worked in the pharmaceutical industry for more than 20 years. Her work experience includes tenures at E.I. DuPont, Johnson & Johnson, American Cyanamid, and Wyeth. More recently, she was vice president, Quality and Technical Services at MERIX Bioscience, Durham, NC, and vice president, Regulatory Compliance and Technology Transfer at Serentec Inc., Raleigh, NC. While at Wyeth, Dr. Wagner worked closely with Dr. Kohn, director of manufacturing, to develop a quality philosophy and quality systems that worked well and lead to the successful launching of new vaccine products. Dr. Wagner is an active member of PDA, ISPE, and ISCT.

Strategic Compliance International, Inc., 2500 Regency Pkwy., Cary, NC 27511.

Frank S. Kohn, PhD, is currently President of FSK Associates, a consultancy for the biotechnology and vaccine industries. Prior to starting FSK Associates, Dr. Kohn held technical and managerial positions at Wyeth, Schering Plough Corp., Armour Pharmaceutical, and Sanofi Inc. with experience in R&D, Manufacturing, Validation, and Quality. He has more than 30 years of experience with pharmaceutical and biological products. Dr. Kohn holds graduate degrees in environmental microbiology and operations management. He is certified as a Specialist Microbiologist (SM) by the American Academy of Microbiology. He is a frequent conference speaker and has given more than 150 technical seminars and conferences. He also has published in the area of technology transfer, cleaning validation, and microbiology in the US and Europe. He is a member of the ISPE Publications/Internet Committee, current Chair for the Vaccine Interest Group of PDA, and a member of the Technical Council of PDA.

FSK Associates, Inc., 1899 N. Twin Lakes Rd., Manson, IA 50563.

This article discusses items that need to be considered when using the overencapsulation process as a blinding option from component selection and comparator purchase to the availability of trained personnel.

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Blinding Clinical Supplies Utilizing Overencapsulation

by Robert G. Myers and Colleen M. Cratty

During the preparation of clinical studies, the method for visually blinding the dosage form is a decision that needs to be made early on in the process. While there are many blinding options available today, i.e., deprinting, mill and fill, manufacture of generic drug, and overencapsulation to name a few, the overencapsulation method still seems to be the most popular method. Perhaps overencapsulation is not the least complex, but it is the most commonly chosen option for blinding clinical supplies today.

The following paragraphs detail some of the items that need to be taken into consideration when utilizing the overencapsulation process as a blinding option. There are many items that need to be addressed to ensure that the operation runs smoothly, from component selection and comparator purchase to the availability of trained personnel.

Overencapsulation is basically hiding another dosage form, tablet, or capsule inside a capsule shell. It is important to select the appropriate components that will be needed to support the overencapsulation of the tablet or capsule unit. Once the unit has been identified, the first thing to determine is what size capsule shell will need to be utilized to properly blind each unit. Although it is not completely necessary, it is recommended that the unit that is being encapsulated does not protrude above the body of the capsule shell when inserted. If the unit does not "sit" properly inside the body shell, and backfilling is required, it may become necessary to backfill the capsule in a manner that will produce a considerable amount of backfill as waste.

There are various size capsule shells available for blinding and perhaps the most popular is the DB CAPS™ capsule shell.[1] This style shell is typically shorter and larger in diameter than the standard capsule shell sizes. An additional feature of this style is the double layer of shell that is created upon closing the capsule. This is due to the walls of each piece of the shell being almost identical in length. There is little area for the patient to grab a hold of at either end of the capsule, making it very difficult for the patient to pull the capsule apart. These two features not only make the capsule more user friendly from a manufacturing stand point, but assist in keeping the drug blinded at the patient level.

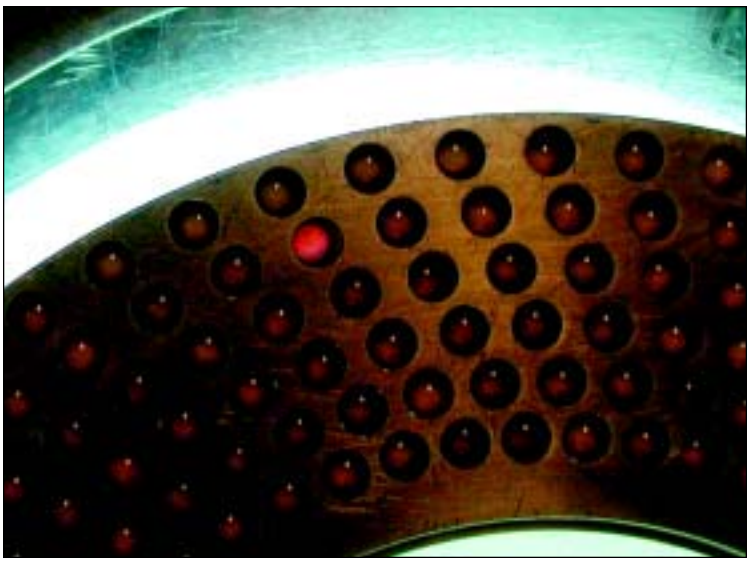

Figure 1. Light from beneath the capsule ring illuminates the shell that does not contain the unit to be encapsulated. Here, the unit being encapsulated is another capsule.

While the DB CAPS™ style shell is the most frequently used, it is not unusual to see the standard size capsule shells used as well. Size 0 and size 00 capsules are most commonly used for overencapsulation in this case. In some instances, the larger size 000, and smaller size 1, 2, and 3

capsules also are chosen for blinding. Generally, a study will involve the breaking of tablets to fit them into the smaller capsule sizes. When tablets are broken, it is critical to ensure that all of the tablet fragments are collected and accurately placed into each associated capsule shell. If all of the fragments are not collected, the final dose of the blinded tablet could be altered. Other matters to consider when choosing a capsule size is your study population. Is the study geared toward children or the geriatric population both of whom may have difficulty swallowing larger size capsule shells?

Besides the appropriate capsule size, capsule color is an extremely important detail that requires a lot of insight. It is critical to choose a color that will completely hide your enclosed unit. One that does not show any shadowing or air pockets due to the backfill encompassing the unit, or allow for any printing or coloring of the encapsulated tablet or capsule to be seen, is the color that should be utilized. These are generally opaque capsules in nature and are usually not the same color or shade of the unit being blinded, but rather slightly darker or more opaque in color.

Not only do we need to ensure that the capsule color will effectively blind the enclosed unit, but also that the color dyes and pigments used in the color formulation are accepted wherever the study is being conducted. Many countries have restrictions on particular colors. This needs to be researched prior to selecting a color. There are several colors that are accepted worldwide and capsule vendors should be able to provide any information with these selections. Capsule vendors are capable of producing capsule colors in any imaginable shade.

Once the color and size of the capsule shell have been selected, one must be aware of the lead times involved with ordering capsule shells. It is possible to have lead times of two to three months depending on size and color requirements. Vendors normally stock a few colors in quantities of a few million; however, it would be impossible for them to stock every color and size combination conceivable. This is usually the main obstacle, which can delay the start of most studies. Upon receiving the capsule shells, storage becomes an important issue as well. Be sure that the capsules are stored under the manufacturer's recommended conditions. Generally, if capsules are stored for more than two years, it is not a bad practice to replace them with a fresh supply, especially if one cannot store them at the recommended temperature and humidity conditions. Extended periods of storage can create brittle and distorted capsules. This creates its own difficulties once encapsulation begins.

Now that capsule details have been determined, the selection of backfill material becomes the next critical step in the overencapsulation process. Backfilling the capsules is required to eliminate the rattle of the unit inside the capsule shell so that the patient is not able to determine the presence of another dose inside the capsule. If the rattle is not eliminated, the patient can possibly break the blind. In rare cases, backfill may not be used and both the placebo and the active doses contain overencapsulated tablets for similar rattle between the doses.

When selecting a backfill material, it is best, but not required, to choose an excipient that is present in the dosage form of which you are blinding. This information can usually be found on the package insert as well as in the Physicians' Desk Reference.[2] Dissolution profiles and stability work should be conducted to verify that the material selected does not interfere with or create any bioavailability issues in the overencapsulated dosage form. The most commonly used excipients for backfilling are Microcrystalline Cellulose and Lactose Monohydrate. These materials are used both independently of one another as well as combined in a blend. In some cases, research has shown that the combination of the two may improve the dissolution results.[3] Depending on the grade of the material chosen, a lubricant, usually, Magnesium Stearate, present usually less than 0.5%, is added as part of the backfill formulation. Not all grades of these two materials require such lubrication and the choice of adding the Magnesium Stearate is usually based on its presence in the formulation of the unit being encapsulated. Lead times are usually not an issue with regards to backfill when compared to those that may be encountered with ordering capsule shells.

When running a trial and overencapsulating a commercial product, there are additional things to consider besides what capsule size the unit will fit in or what backfill formulation should be utilized. Perhaps the most important thing you need to keep in mind is who is going to order the comparator. It is important to protect the confidentiality of the company doing the study, and therefore consideration needs to be taken because suppliers may become aware of what compounds the company has involved in such trials. When the company conducting the trial orders the comparator, this can create the potential for various outside parties to know which compounds are being considered. If a second party orders the comparator, the potential for the manufacturer of the compound to know that a study is being conducted against their compound, as well as confidentiality issues can practically be eliminated.

In conjunction with ordering supplies, be sure that the proper size change parts to run the capsule size on the equipment are on hand. Change part availability could become an issue. Typically, there are long lead times and the parts can become rather costly. It is advantageous to have an adequate supply of parts on hand for the machinery. If you are in the middle of a run and the equipment fails, the down time that can be saved by having items on hand is immeasurable. In this article, a semi-automated capsule filling machine is utilized for the encapsulation process. Additional equipment such as a loading ring and a light table will enhance the process making it easy for operators to determine and eliminate defects in the drug as well as the capsule shells. These items are discussed in more details within the actual process below.

Personnel also can be an issue during the overencapsulation process. One must ensure that there are an adequate number of trained operators available to support the project(s). The overencapsulation process can be quite complex when per-

formed properly, and requires a dedicated team of trained personnel that are familiar with the common issues that occur during the process, from equipment problems to recognizing issues created with the materials being used.

From this point forward, we will assume that the equipment, room, and personnel are adequately prepared for manufacturing, the components for the project are released accordingly, and all production records have been written and approved. With everything in place, we can start to remove the drug from its commercial package. As the drug is removed, it is important to perform a 100% gross inspection on the drug that is being overencapsulated. Although these products are commercially manufactured, packaged, and released, there are occasions where you may need to reject entire lots of product due to anomalies found in the product. Examples of these anomalies include everything from broken tablets, crushed capsules, and broken or missing induction seals, to foreign materials compressed directly into tablets. Once these units are eliminated from the commercial lot, the overencapsulation process is ready to begin.

When overencapsulating any drug product, the use of a tablet or capsule loading ring will enhance the efficiency of the process and assist in ensuring that only one unit is placed into each capsule shell at a time. There are a number of different style loading systems available. However, the design of the loading ring must be carefully chosen. The loading ring is utilized by flooding the ring with product and then manually working one unit into each cavity of the loading ring. This ring is then placed on top of the lower portion of the capsule ring that contains the bodies of the capsule shells. The drug product is then released into the bodies. If additional units are required in each capsule shell, the process is then repeated as required.

There are several things to consider when selecting the proper loading ring:

1. The ring should not be made of aluminum. Aluminum has the potential to leave black markings on tablets when traveling across the surface of the ring.
2. The ring should be designed so that each cavity of the ring is size specific to the shape of the tablet or capsule. Each cavity also should accommodate only one unit at a time.
3. If the loading ring utilizes offset holes to load the units into the capsule shells, be cautious when working with caplet or oval shaped tablets. The ends of the units can get stuck in the cavities and when the spring mechanism is triggered to align the holes, the ends of the tablets can be broken and/or chipped. It is extremely difficult to tell if the entire single unit went into the same capsule shell. You may not even know that the tablet has been damaged.
4. Outsourcing of loading rings can sometimes take several weeks, leading to delays in starting the project.

Prior to placing the loading ring onto the capsule bodies, utilizing a light table underneath the bodies can have many benefits. The first advantage is that the light will draw immediate attention to any cavity that is missing a capsule shell. Second, if the capsule bodies contain any defects such as pinholes due to a thin gelatin area, usually found on the capsule ends, the light magnifies these holes and the capsules can be removed prior to filling. This capsule defect is extremely difficult to detect otherwise. If this defect is not detected prior to filling, it could result in capsules leaking powder out of the ends of the capsule shell. If this defect is present, it is usually not noticed until the product is packaged and/or distributed, long after the capsules are filled and closed. Third, once the capsules are filled with the drug product, the light will illuminate any empty capsules without the product – *Figure 1*. Even though the loading ring will release a unit into each shell, human error can still result in an empty capsule. It is highly recommended to perform an additional 200% visual, documented inspection with the final check being completed by the operator responsible for backfilling the capsules, totaling a 300% inspection.

With the units loaded into the capsule shell bodies, the capsule ring is then transferred to the filling machine. Upon completion of filling the first set of capsules, several capsules should be checked prior to formally closing the capsules. This is to determine if any "rattle" or movement can be felt or heard from the encapsulated drug inside the capsule shell. To do this, remove several capsules by hand, closing them as they are removed from the ring. If there is noticeable movement, there are several routes to take to "lock" the drug in the capsule shell. However, be aware that depending on the backfill and the shape of the unit being overencapsulated, there is a possibility that the movement will not be completely eliminated. If this point is reached, a decision needs to be made on how to proceed. Two options for increasing the amount of backfill present in the capsule are as follows:

1. The ring of capsules may be tapped to settle the backfill around the drug and a second filling can be done to add more backfill to the capsule.
2. The auger on the capsule filling equipment can be changed to assist in forcing additional backfill into the capsule shell, as well as altering the fill settings on the equipment.

Once the capsules are filled, it is typically necessary to polish the capsules to remove any residual backfill material from the outside of the capsule. Having an empty capsule eliminator attached to the discharge area on the polisher will eliminate any possibility of an empty shell making its way into the finished product container. An empty capsule can occur if a capsule is crushed during closing or if a capsule does not close properly and opens during the polishing process. This is possible due to the turbulence created inside the polisher.

In low humidity conditions, the residual backfill may become difficult to remove due to static. If this situation arises, determine the relative humidity in the room, and if possible, raise the humidity to at least 25%, or move the operation into a room at a higher relative humidity without jeopardizing operating conditions.

At this point, the overencapsulation process is basically complete. A final weight check and inspection to assure

proper closure and no visual defects are the closing segments of production. During the entire overencapsulation operation, there are many quality checks at integral parts of the process:

- gross inspection of the drug to be encapsulated
- inspection of empty capsules for pinholes,
- three documented, visual checks for the presence of the proper number of units in each capsule body
- a formal check for drug movement inside the capsule at the initiation of powder filling to determine the final filling requirements.

A final confirmation in the process is weighing a minimum of 10 capsules from every ring to ensure that they are within the desired range and that the operators are in control of the filling operation. Samples may be pulled from each ring to create a mini-batch of the entire process. Retains and release samples are then pulled from this composite.

The finished product upon final inspection should be placed into a properly lined, tared, and labeled container. Labels should be present on the inside as well as the outside of the containers for identification purposes. Pre-numbered tamper seals should be placed on the containers and the number recorded within the batch documentation. The manufacturing operation is now complete and the material can be released for further processing.

Overencapsulation involves many individual operations that can create a variety of complex situations. Additional complexities arise when tablets need to be broken to fit into capsule shells, or half of a tablet needs to be placed into a capsule shell. Even different doses may be combined into the same capsule to meet specified dose requirements. Analytical support becomes extremely important when creating a new dose or altering the original form.

With the potential of using any of these different scenarios, overencapsulation remains the most sought after method of blinding drugs for clinical trials. The key to success during each of these operations is to remember that each segment of the process is extremely important. Each requires proper planning and careful execution. From capsule color and size selection to having a well-trained team dedicated to the manufacturing process, taking the time to make sure each segment is managed properly will result in minimal problems related to the encapsulation portion of the study, as well as curtail problems that could occur once the patient receives the supplies.

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About the Authors

Robert G. Myers is currently the Manager of Clinical Manufacturing for Fisher Clinical Services Inc. He has 25 years of experience in the areas of manufacturing of solid dosage forms, clinical manufacturing, sterile operations, formulation, and development.

Colleen M. Cratty is currently a Manager in Client Services for Fisher Clinical Services Inc. Her experience is in the area of project management and manufacturing of clinical supplies for solid dosage forms. Cratty holds a BS in chemical engineering from The Pennsylvania State University.

Fisher Clinical Services Inc., 7554 Schantz Rd., Allentown, PA 18106.

Enhanced Design Review/ Design Qualification is an investment that pays dividends not only to enhance regulatory compliance, but also to improve project delivery and streamline qualification efforts.

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Enhanced Design Review/Design Qualification

by Robert E. Chew, PE

European Regulatory Agencies, and now the US FDA, have an expectation for design qualification for certain types of pharmaceutical and biotech projects. Design qualification is more than a standard owner engineering approval, and it is more than evaluating the design against a generic checklist of common GMP/GEP attributes and practices. Design qualification is an examination of the design against stated requirements. Design qualification can help focus IQ/OQ/PQ efforts on those aspects of the design (fabrication, installation, operational, performance) that could impact process performance and product quality. Enhanced design review/design qualification should be conducted in a manner that helps focus and streamline the overall project delivery process. Efforts expended on enhanced design review/DQ early in the project will pay dividends during the final stages of the project.

Background

The ISPE Commissioning and Qualification (C&Q) Baseline® Guide espouses the concept of an *Enhanced Design Review*, which is defined as "a documented review of the design, at an appropriate stage in a project, for conformance to operational and regulatory expectations."[1] The Guide further explains the concept as:

- structured review of the design of facilities, utilities, and equipment
- a smart way to prepare for IQ and OQ activities
- a method of examining the design for impact of the system, system complexity, and degree of novelty or familiarity
- a review to verify that design links construction to the User Requirement Specification
- a review to verify that the owner gets what he asked for

Annex 15 of the EC Working Party on Control of Medicines and Inspections, *Qualification and Validation*, states under Design Qualification:[2]

> "The first element of the validation of new facilities, systems or equipment could be design qualification (DQ)."[3]
>
> "The compliance of the design with GMP should be demonstrated and documented."

The ICH Q7A Good Manufacturing Practice Guidance for Active Pharmaceutical Ingredients was adopted last year by CBER and CDER.[4] This document states under *Qualification* §12.3:

> "Design Qualification (DQ): documented verification that the proposed design of the facilities, equipment, or systems is suitable for the intended purpose."

The FDA has indicated that it will have an expectation for design qualification to be performed on API (and bulk biotech) facilities.

Finally, IEEE has standards on software quality assurance which include the concept of a *Functional Audit*. A Functional Audit is defined as verification that all requirements have been implemented in the design.

Importance of Requirements vs Design

Ultimately, the facility, equipment, and systems must meet the cGMP *requirements*. The

selected design is merely a particular method of achieving or implementing those requirements. And, there may be many ways to meet a requirement. Hence, whether or not the design is met is secondary; if cGMP *requirements* are met, then the facility, equipment, and systems are in fact *qualified*, whether or not they met each and every design attribute contained in drawings and specifications.

In most cases, there are aspects of the design which are critical to meeting a particular requirement. For example, in order to achieve cleanroom conditions, HEPA filter efficiency and integrity are important. In order to achieve low microbial levels in water systems, a combination of design features is necessary. Although it is only necessary to satisfy requirements, it is prudent to develop a solid design which provides a high degree of assurance those requirements will ultimately be met.

Of course, to emphasize the importance of requirements means that rigorous and thorough requirements definition must occur, these requirements must be documented and supported by development data, and they must be maintained under strict QA change control throughout the project.

What is Enhanced Design Review/ Design Qualification?

The ISPE Commissioning and Qualification Baseline® Guide, ICH Q7A, and EC Annex 15 all explain Enhanced Design Review (EDR) or Design Qualification (DQ) in terms of reviewing the design for conformance to operational and regulatory expectations, GMPs, and suitability for the intended purpose, as well as the User Requirements and Functional Design as outlined in the Guide. The ISPE Commissioning and Qualification Baseline® Guide contains additional guidance and suggestions on how to implement and integrate an EDR within the overall design development and review process.

Specific regulatory language, which should be used to define and structure the EDR/ DQ process, includes:

- …*first element* of validation…(Annex 15); …*proposed* design…(Q7A): this indicates that EDR/DQ is performed before the design is purchased or implemented in the field.

- …*documented* verification…(Q7A); … and *documented* (Annex 15): this indicates EDR/DQ is a documented activity.

- …*verification*… (Q7A); …*demonstrated*…(Annex 15): this implies an additional level of documentation detail over and above a simple sign-off that the design has been reviewed and approved for implementation. It implies review by persons who can evaluate the design from a technical point of view as adequately addressing each requirement.

- …*compliance of the design with GMP*…(Annex 15); …*design is suitable for the intended purpose*...(Q7A): this indicates that in order for EDR/DQ to proceed, the GMP requirements and intended purpose, e.g., user requirements, must have been previously defined.

Enhanced Design Review/ Design Qualification Approach

As indicated above, the EDR/DQ cannot be only an engineering judgment-based (hip-shot) review of a design with some form of QA participation and formal sign-off. It should be centered about a more structured analysis based on a foundation of well-defined requirements with corresponding documentation. In addition, the EDR/ DQ process can include assessment of component impact on product quality (as described in the ISPE C&Q Guide).[5] Outputs of this process can form the basis of IQ/OQ protocols, inspections, and testing, as well as identification of critical instruments, ranges, accuracies, etc. against Acceptance Criteria established in Functional Design.

Well-defined requirements are the foundation of any basis of design. However, pure requirements differ from the traditional Basis of Design (BOD) packages issued by many engineering firms. Differences include:

- User, process, safety, environmental, quality, and regulatory requirements should be established long before commencing the design, whereas much of the engineering BOD is the Preliminary Design or a skeleton thereof.

- The engineering BOD typically consists of text paragraphs, preliminary drawings, equipment lists, etc. Well-defined requirements consist of a set of statements that could form the basis of inspection and test acceptance criteria.

- It should be straightforward to establish a process to manage changes to individual requirements, whereas managing changes to a BOD package may be more difficult or complex.

A suggested Best Practice is to define a comprehensive set of requirements, first at the user level, and later at the more detailed functional requirements level. Requirements should encompass process, quality, maintainability, capacity, general facility, operability, safety, environmental, and regulatory topics. Requirements should be short, distinct statements with measurable goals that can later be verified through a commissioning or qualification program. Each requirement should be uniquely identified to facilitate change management.

Requirements must be comprehensive. Each and every requirement relating to product safety, identity, strength, purity, and quality must be identified. Hence, QA must have a significant role in reviewing and approving the final set of requirements, and must be an approver of changes to any requirement that can affect the above product or process attributes, e.g., GMPs.

Given a comprehensive set of requirements that has been approved by QA and is under project change management, the EDR/DQ process then can be reduced to two key objectives:

1. Documented verification that the overall design appears to address, by some means, each and every requirement affecting the product and performance of the manufacturing process. Or, in the case of unknown product or multi-product manufacturing facility, the required equipment/system performance capabilities.

2. Identification (and documentation) of the critical individual physical components, attributes, and operational features that directly support meeting each requirement.

Figure 1 illustrates how the EDR/DQ and component impact assessment process can work within the context of the "V-model" which describes the relationship between requirements, design, and IQ/OQ/PQ.

Objective #1 satisfies the requirements of Annex 15 and Q7A. A suggested method for accomplishing Objective #1 is to present the requirements in the form of a spreadsheet or database, and document the components of systems and their particular drawing(s) and specification(s) section(s) that appear to meet the given requirement. This annotated spreadsheet can then be attached to a generic DQ protocol, either on a system by system basis or for the project as a whole, and formally approved by engineering, user, QA, and other affected groups.

Objective #2 may be used to streamline qualification efforts and the design change control process. According to the C&Q Guide, IQ, OQ, and PQ efforts are focused on "those attributes…that can affect product quality." Objective #2 seeks to identify the attributes (critical parameters and direct impact components.) that can affect product quality (e.g., which support meeting a GMP quality requirement); hence those and ONLY those attributes need be included in any IQ, OQ, or PQ protocol, as appropriate. Other aspects of the design, which may, in the past, have been included in IQ/OQ/PQ protocols (and may have resulted in inconsequential deviations to be laboriously processed), can be excluded from those protocols and instead incorporated into non-GMP commissioning efforts under control of Good Engineering Practice (such items might include electrical checks, lubrication and alignment, continuity checks, utility supply checks, make/model/serial number data, nameplate data, purchase orders, IOM manuals, etc.).

There are several suggested methods by which Objective #2 may be accomplished, while at the same time meeting the C&Q Guide expectations for component level impact assessment and definition of critical instruments:

- Using the same mechanism as described for meeting Objective #1 (annotated spreadsheet), identify the critical attributes, components, operational features, etc., that appear to impact or support meeting each GMP requirement, i.e., a list of critical components in direct impact systems.

- Using drawings, instrument lists, equipment lists, and specifications, annotate the product or process requirement (by number(s) that the particular component supports or impacts).

The DQ protocol also should include a list of the drawings, specifications, etc., that were reviewed as part of the design review/qualification process. The completed protocol should then be approved by QA. QA should participate in the design qualification process to ensure that the design meets quality-related requirements including critical parameters, sanitary construction, cleanability, environmental controls, etc.

The DQ process including pre-requisite requirements definition, as integrated with the overall project, might proceed as follows:

1. Users define requirements, typically as part of the conceptual design phase.

2. Requirements baseline (rev 0) are approved and placed under project change control, approximately the same point as conceptual design is complete. QA is key approver of requirements.

3. Requirements are added, modified, or deleted as the project proceeds. QA approves any changes to GMP requirements.

4. As major equipment is ordered, the bid packages are reviewed to confirm the appropriate user requirements have been included. This is the first step in EDR/DQ, and should be documented. QA involvement is not required, but may be advisable at this point.

5. Functional requirements definition proceeds in parallel with preliminary design.

6. Detail designs are received from equipment vendors and engineering design firms. Formal DQ is performed on the detail design to confirm the designs appear to meet all requirements, and to identify critical components. Requirements that cannot be met by the design should be evaluated, and acceptable solutions (which may mean deleting or changing a requirement) reviewed and approved by the group which defined the original requirement. Such decisions are documented in the DQ report and reflected in updates to the user requirements. QA is involved at this stage and approves the completed DQ report, along with the system owner/user. Ultimate responsibility for ensuring the design incorporates all requirements, and for identifying critical components, rests with the manufacturing firm as represented through QA and owner/user.

7. Software detailed design is reviewed against the functional requirements in a similar manner.

8. The detail design is placed under project change management.

9. Qualification confirms that the critical components of the design meet specification, and that the user and functional requirements have been achieved in the delivered system. Given a thorough DQ with component impact assessment, generation of IQ/OQ protocols is much easier, faster, less expensive, and less controversial as to what should be included.

Controlling Changes to a "QA-Qualified/ Approved" Design

Now that QA has participated in the EDR/DQ process, and has presumably approved the design as a result, how should we control changes to the design? QA, via EDR/DQ, has confirmed that the design appears to meet all requirements and cGMPs. How do we assure that the final design implementation also will accomplish this? One school of thought is that since QA has approved it, then any changes thereafter, e.g., to any drawing, specification, or vendor design submittal also must be approved by QA. This also assumes that there are no errors in the design and that everything will go together without field changes. This is unrealistic. Implementation of such a process is probably cumbersome and unnecessary.

The ISPE Water and Steam Systems Baseline® Guide, which has been favorably reviewed by the FDA, offers alternatives and options regarding the design of high purity water systems. In other words, there is no single design solution; many may be acceptable. The Agency's comments to the Water and Steam Systems Guide stated that the degree to which GEPs are included in any design is a risk assessment on the part of the manufacturer;[6] QA should be involved in that risk assessment as part of EDR/DQ. In reviewing the ISPE C&Q Guide, the Agency stated that the engineering change management system shall allow for QA review and approval of changes that: (i) change the User Requirements Specification; (ii) fundamentally change the operational concept; (iii) alter a system's potential impact on product quality. Hence, QA should be involved in approving all changes to requirements, and approving a selected subset of all design changes.

Second, QA is involved in the following two key project processes:

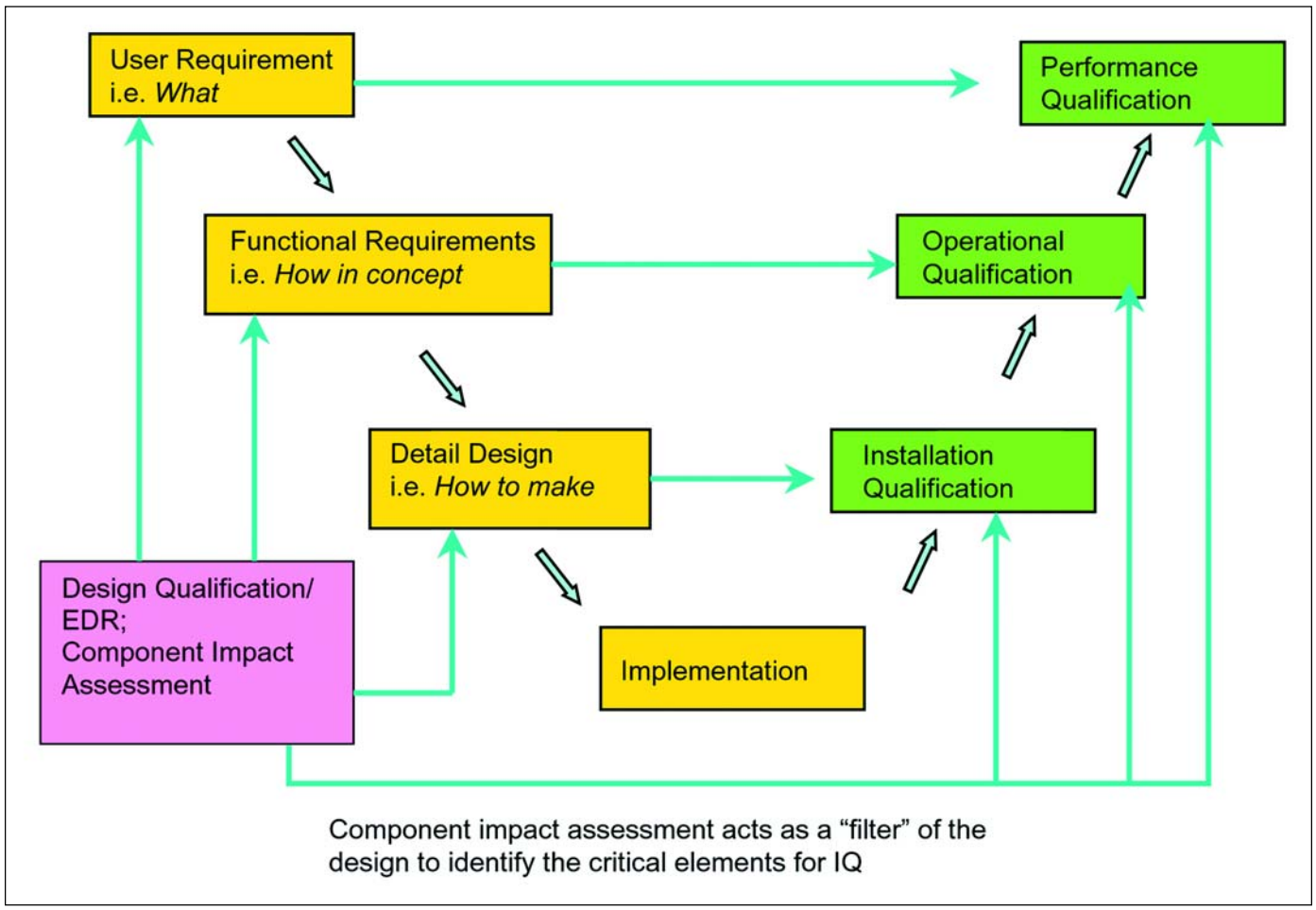

Figure 1. Design qualification confirms that all requirements and general cGMPs have been included in the design.

1. definition, approval, and modifications to the comprehensive set of requirements, especially those tagged as "GMP" requirements, critical parameters, and the like (changes to non-GMP items are controlled by good engineering practices, e.g., project change management)

2. approval of qualification and process validation protocols which should verify that each and every GMP requirement has been met by the installed facility

The exact mechanism by which a given requirement is met is secondary, provided the requirement is ultimately satisfied. The mechanism (the "how") is the design. Qualification protocols will confirm that all requirements and critical design features have been met.

Therefore, it is not necessary for QA to approve every design change. Instead, the onus should be placed upon design engineers, given a completed and approved EDR/DQ that identifies critical aspects of the design, to recognize when a proposed design change might affect the ability to meet a requirement. When this is the case, the designers should request QA involvement in approving that particular change (thus meeting FDA expectations as stated in their review of the C&Q guide). If the design group fails in this regard, the QA approved qualification protocols should cause inspections and/or testing to identify any non-conformance to requirements or critical design features when those protocols are executed.

Suggestion: If the requirements are maintained in a searchable database, and if the EDR/DQ process is conducted in accordance with the best practices described above, then the database could reference those drawings/specification sections which meet each requirement. If a design engineer wants to change a drawing or specification section, he or she could search the database for any listing of that particular drawing or specification and would then be made aware of the potential for the change to impact the ability to meet the particular requirement. Identification of design changes that could have GMP impact then becomes easy with electronic aids.

A more comprehensive (and yet simple) solution would be to include QA on the distribution of every change. Since the project is still under construction and no product is being manufactured, the change management process could allow for QA notification in all cases, but not require QA pre-approval of changes; QA can be afforded the opportunity to question any change of their choice and make objections as they see fit without implementing a pre-approval requirement during the design and construction phases of projects.

Finally, the IQ/OQ/PQ process will examine the installed, operational version of the ultimate design including changes; this will demonstrate acceptable conformance to requirements and will force evaluation of any deviations of critical components from specifications. This provides sufficient controls and quality assurance of the delivered system prior to product validation.

Timing of EDR/DQ

The ISPE C&Q Guide places EDR/DQ after detailed design and prior to implementation in the field. However, there may be reasons to conduct a preliminary EDR/DQ as part of reviews of the Basis of Design and Preliminary Design. The preliminary EDR/DQ could be restricted to Objective #1, verification that every requirement has been factored into the design at that stage of the design development. This prevents incomplete or inaccurate design packages from being issued to groups engaged in detailed design work. EDR/DQ should be structured around individual systems, major equipment, support utility systems, HVAC, facility architectural, etc. And, the EDR/DQ may need to be revisited when significant field changes are to be implemented. Hence, EDR/DQ is an ongoing process that ends when the design is implemented in the field. Once implemented, the remaining "Q" processes (IQ, OQ, PQ) take over to verify that the final design, as implemented, meets requirements.

Case Study

A recent project to expand the fermentation capacity of an established process used the concepts of thorough user requirements definition followed by formal design qualification. User requirements were derived from sources including:

- voided production tickets from the existing process
- existing fermentation requirements for the DCS
- process flow diagrams for the existing system
- P&IDs from the existing system
- process qualification report for the existing system
- control charts
- periodic product quality evaluation that was done in 2000
- key process, product, and user requirements (interviews)
- regulatory requirements (OSHA, FDA, NDA, Regulatory commitment documents)
- [company] corporate standards
- industry requirements (NIST, NFPA, ISO)

Interactive meetings were held where requirements were displayed and edited to incorporate comments. The final set of requirements were captured in a database (spreadsheet) and made available to the project team on a shared file server with read-only access.

Lessons learned: (1) There were no drawbacks to having well-defined user requirements. They formed the basis for the entire project, through design, construction, and qualification. (2) There is a fine line between too general and too specific. They must be specific enough to serve as a basis for inspections and testing, but not too specific such that they are dictating design. For example, the user requirement should not specify the material of construction as 316SS, but rather that "materials of construction should be non-corrosive, non-absorptive, non-additive…" (3) Because of the tight link between user requirements and design qualification, more training of participants on the DQ process prior to defining user requirements would have been helpful.

Design qualification was an organized approach at evaluating the design. It was a systematic way to make sure the user requirements were being met before the materials were actually ordered. DQ caught several design errors and prompted discussions that the technical operations group and the engineering group might not have had for months down the road. DQ was conducted by completing worksheets and archiving in a design notebook that will serve as a reference of design decisions and design evaluation, as well as a record that the design appeared to meet all requirements.

Summary and Conclusion

Enhanced design review/design qualification is both an emerging regulatory requirement and a tool for project success. If structured and executed properly, it can help assure the design is robust and complete, giving the project team and QA reviewers a high degree of confidence that every requirement has been factored into the design. EDR/DQ can identify and document the critical, direct impact components, and operational features. If these items are clearly identified with a sound basis, then IQ, OQ, and PQ protocols can be streamlined and focused, yielding a more effective and efficient qualification process. Finally, EDR/DQ cannot be performed without first formally defining requirements. Meeting requirements is the ultimate objective of the project; verifying requirements have been met is the role of qualification.

References

1. ISPE, Commissioning and Qualification Baseline® Guide, Volume 5, pgs 75-81.

2. European Commission, Working Party on Control of Medicines and Inspections, "Revised Draft Qualification and Validation," pg. 5.

3. Note that in this context, validation is used as a broad term encompassing IQ, OQ, PQ, and process validation. Qualification, as defined in the ISPE C&Q Guide, includes IQ, OQ, and PQ and is focused on facilities, equipment, and systems. And, by extension, DQ also would be included as a fourth element of qualification. Process validation is not part of qualification.

4. Guidance for Industry, "Q7A Good Manufacturing Practice Guide for Active Pharmaceutical Ingredients," US FDA, August 2001, pg. 38.

5. ISPE Commissioning and Qualification Baseline® Guide, pgs 30-32.

6. ISPE Conference, Principles and Applications of Water and Steam Systems Baseline® Guide.

About the Author

Robert E. Chew, PE is Vice President of Commissioning Agents, Inc. He has 12 years of experience in the pharmaceutical industry in areas of compliance, commissioning, and validation. He is a former Navy submarine officer and is a registered professional engineer. He has a degree in chemical engineering from Case Western Reserve University. He can be contacted at: robert.chew@CAgents.com, or phone: 1-317/ 710-1530.

Commissioning Agents, Inc., 1515 Girls School Rd., Indianapolis, IN 46214.

The characterization of soybean powder and the adsorption profile of insulin on the surface of the powder are presented in this article.

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Human Insulin Interaction with Soybean Powder

by Antoine Al-Achi, Thomas J. Clark, III, Robert Greenwood, and Shylock Sipho Mafu

The objective of this study was to elucidate the mode of interaction between human insulin and soybean powder. Soybean has been shown to contain substances that have anti-trypsin and anti-chymotrypsin activities, which may potentially protect insulin from degradation. Soybean powder had an average size particle of d_{vs} = 45.5 mm (an average diameter for a sphere having the same volume and surface area as the particles) and d_{vn} = 31.6 µm (an average diameter for a sphere having the same volume and number of particles per unit weight as that of the tested powder). The powder (mean ± s.d., n = 6) had a bulk volume of 44.5 ± 0.55 ml and a bulk density of 0.625 ± 0.0085 g/ml. The angle of repose of the powder was 38.33 ± 1.106° and its Carr index was 26.72 ± 1.06%. The findings from this study showed that the mode of interaction between human insulin and soybean powder is of an adsorption type. Langmuir and Freundlich isotherms, two commonly used models for adsorption, showed that human insulin was able to adsorb on the surface of the particles, interacting weakly with its adsorption sites. Thus, soybean particles can act as a physical carrier for insulin, yet insulin is easily released from the carrier due to its weak bonding with adsorption sites.

Introduction

Human insulin is a hormonal drug that is a product of biotechnology. It contains 51 amino acids with a molecular weight of 5,808 daltons. Endogenous insulin is secreted by β cells of the pancreas which is then delivered to the circulation via the portal vein. The entire human pancreas contains at any given time about 8 mg

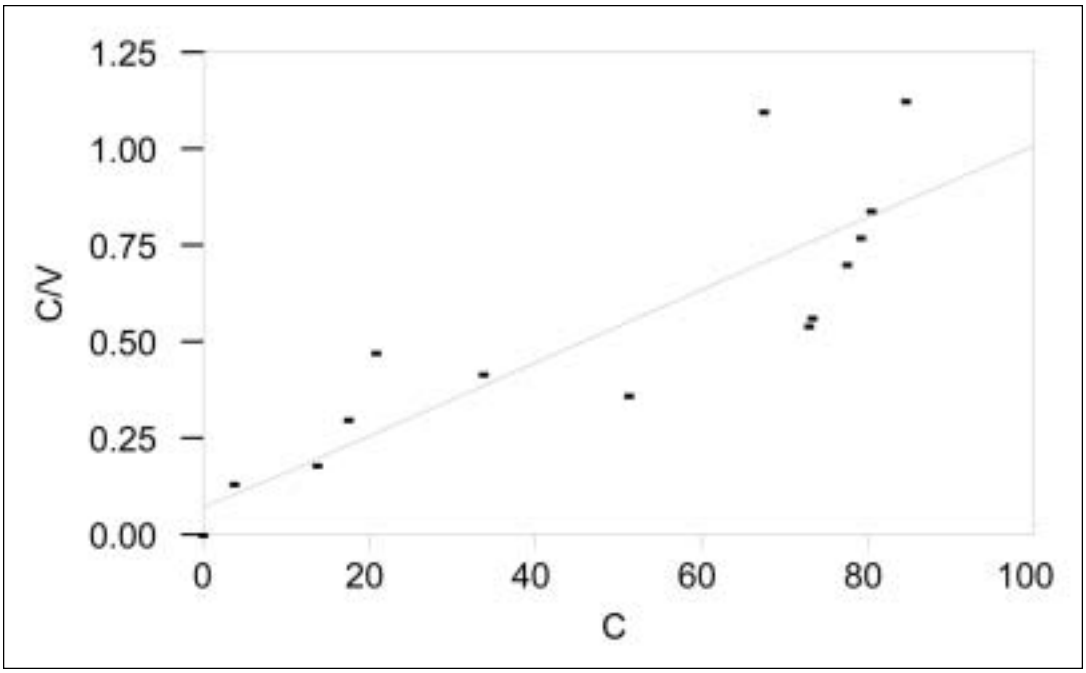

Figure 1. Langmuir isotherm: C/V = 0.0704 + 0.0094 C (r = 0.87, $p < 0.0001$). V is the amount of insulin (U) adsorbed per 1 g of soybean powder at any given equilibrium insulin concentration C.

Powder	d_{vs} (µm)[a]	d_{vn} (µm)[b]
Soybean	45.5 ± 12.8[c]	31.6 ± 5.9
Avicel	25.3 ± 8.4	14.1 ± 7.6
Calcium Carbonate	18.9 ± 4.0	16.0 ± 2.8
Lactose	29.6 ± 5.2	19.7 ± 2.9

[a] Volume-surface mean.
[b] Volume-number mean.
[c] Mean ± S.D. of 6 observations.

Table A. Average particle size of soybean powder as compared to that of Avicel, calcium carbonate, and lactose.

Powder	Bulk Volume (ml)	Bulk Density (g/ml)
Soybean	44.5 ± 0.55[a]	0.629 ± 0.0085
Avicel	47.8 ± 0.75	0.416 ± 0.0064
Calcium Carbonate	42.7 ± 1.03	0.375 ± 0.0108
Lactose	45.0 ± 0.89	0.620 ± 0.0130

[a] Mean ± S.D. of 6 observations.

Table B. Bulk volume and bulk density for soybean powder compared to those of Avicel, calcium carbonate, and lactose.

of insulin (about 200 biological units). Human insulin, being a peptide, cannot be given orally due to its degradation by proteolytic enzymes such as pepsin, trypsin, and chymotrypsin. Its main mode of administration has been the parenteral route.

Soybean (*Glycine max*), also commonly known as soy or soya bean, is a leguminous plant that is important in Asian culture. Soybean seeds are almost spherical in shape and yellow. Some varieties of the seeds are found to be brown or green in color. Both the seeds and the plant itself are referred to by the same name, soybean.

There are two main components of the soybean seed: protein and oil. The protein accounts for about 40% while the oil is about 20%. Soybean contains various kinds of proteins that were shown to inhibit trypsin and chymotrypsin proteolytic activities. The whole soybean contains between 16.7 to 27.2 mg of trypsin inhibitors per gram.[2] The major inhibitors are Bowman-Birk (up to 4.9 mg/g, molecular weight 7848 daltons) and Kunitz (1.1 - 19 mg/g, molecular weight 20083 daltons).[2,3] Other major constituents in soybean are phytic acid (1.0 - 2.3 g/100 g dry matter), saponin (0.09 - 0.53 g/100 g dry matter), and isoflavone (1200 - 4200 µg/g).[2] Lyophilized soybean powder obtained from Sigma (St. Louis, MO) contains approximately 80% protein. One milligram of this powder inhibits 3-5 mg of trypsin and 2-5 mg of chymotrypsin. Crude soybean soluble powder (1 mg) inhibits about 1 mg of trypsin.

The main objective of this project focuses on elucidating the mode of interaction between human insulin and soybean powder. This study is an important step in examining a method to potentially protect insulin from degradation if given orally.

Materials and Methods

Materials

Soybean (*Glycine max*) was purchased from a local store in North Carolina. Human insulin (Humulin R, 100 U/ml, Lilly) was obtained from NC Mutual, North Carolina. Avicel was obtained from FMC Corporation (Newark, DE). All other chemicals were from Sigma, St. Louis, MO, except for acetonitrile which was from Fisher Scientific (Pittsburgh, PA). All reagents were of analytical grade, except those used in the HPLC assay were HPLC grade.

Methods

1. Preparation of soybean powder: a total of 2 kg of soybean were ground in a coffee grinder (Mr. Coffee, Sunbean Products, Hatiesburg, MS) in 57 ± 3.59 g weights for 50 seconds to yield a fine "expresso" powder. The resulting powders after each grinding procedure were combined and blended together using a V-blender set at 25 r.p.m. for 20 minutes. The resulting soybean powder mixture was stored at 4°C until further experimentation.

2. Characterization of soybean powder: the soybean powder was subjected to various tests in order to establish its characteristics.[4] Also, as reference points, Avicel, calcium carbonate, and lactose were subjected to the same tests as those applied to soybean powder.

2A. Average particle diameter: 10 mg of powder were dispersed in 10 ml of light mineral oil. One drop of the resulting dispersion was then examined under light microscopy using a reticle (10 µm/division) at 10x lens. Six different samples from the powder were examined; the horizontal width of 100 particles from each sample was recorded, and the volume-surface diameter (d_{vs}) and the volume-number diameter (d_{vn}) were estimated:

$$d_{vs} = \Sigma\, d_i\, n_i^3 / \Sigma\, d_i\, n^2 \quad (1)$$

$$d_{vn} = [\Sigma\, d_i\, n_i^3 / \Sigma\, n_i]^{1/3} \quad (2)$$

where d_i is the midpoint of the interval i and n_i is the number of particles within the interval i. Since the particles within the powder vary in shape, size, and form, methods have developed to relate the average diameter of particles in a powder to that of a sphere. These equivalent diameters describe diameters for a sphere having the same volume and surface as that for the particles (d_{vs}) or the same volume and number of particles per unit weight as that of the tested powder particles (d_{vn}).

2B. Bulk volume and bulk density determination: soybean powder was introduced inside a 100-ml graduated cylinder to the 50 ml mark. The cylinder was then dropped three times on a bench-top from a distance of about 1 inch height at 2-second intervals. The tapped final volume of the powder was tapped bulk volume (V_b) in milliliters.

Powder	tan θ[a]	θ (°)
Soybean	0.79 ± 0.03[b]	38.33 ± 1.106
Avicel	0.68 ± 0.03	34.45 ± 0.948
Calcium Carbonate	1.17 ± 0.06	49.35 ± 1.525
Lactose	1.02 ± 0.03	45.63 ± 0.918

[a]The Coefficient of Friction.
[b]Mean ± S.D. of 6 observations.

Table C. Angle of repose (θ) of soybean powder compared to that of Avicel, calcium carbonate, and lactose.

Powder	Carr Index (%)
Soybean	26.72 ± 1.06[a]
Avicel	16.00 ± 0.63
Calcium Carbonate	32.83 ± 3.82
Lactose	25.80 ± 2.23

[a]Mean ± S.D. of 6 observations.

Table D. The Carr index of soybean powder compared to Avicel, calcium carbonate, and lactose.

The tapped bulk density (g/ml) of the powder was calculated from:

$$\text{Tapped } \rho_b = \text{Weight (g)}/V_b \quad (3)$$

The bulkiness (ml g^{-1}) of the powder was estimated by taking the inverse of the tapped ρ_b.

2C. Angle of repose: glass, stemmed funnel was placed into a clamp of a ring-stand, 5.7-cm off the bench-top. A paper was centered and placed underneath the funnel's stem. Soybean powder was poured through the funnel, making a mound until it touched the funnel's stem. A circle around the mound was then drawn, and its diameter was measured using a ruler. The angle of repose (θ) was estimated from

$$\tan \theta = 2h/D \quad (4)$$

where tan θ is the coefficient of friction, h is the height of the mound, and D is the diameter of the mound.

2D. Carr index: soybean powder was introduced into a 100-ml graduated cylinder to the 100 ml mark. The cylinder was then dropped 75 times on a bench-top from a distance of about 1 inch at 2-second intervals. The tapped volume was recorded in milliliters. Carr index was calculated from:[5]

$$\text{Carr Index} = [(\text{Initial volume} - \text{Final volume})/\text{Initial volume}] \times 100 \quad (5)$$

3. HPLC assay for human insulin: human insulin concentration was determined using a HPLC assay with the

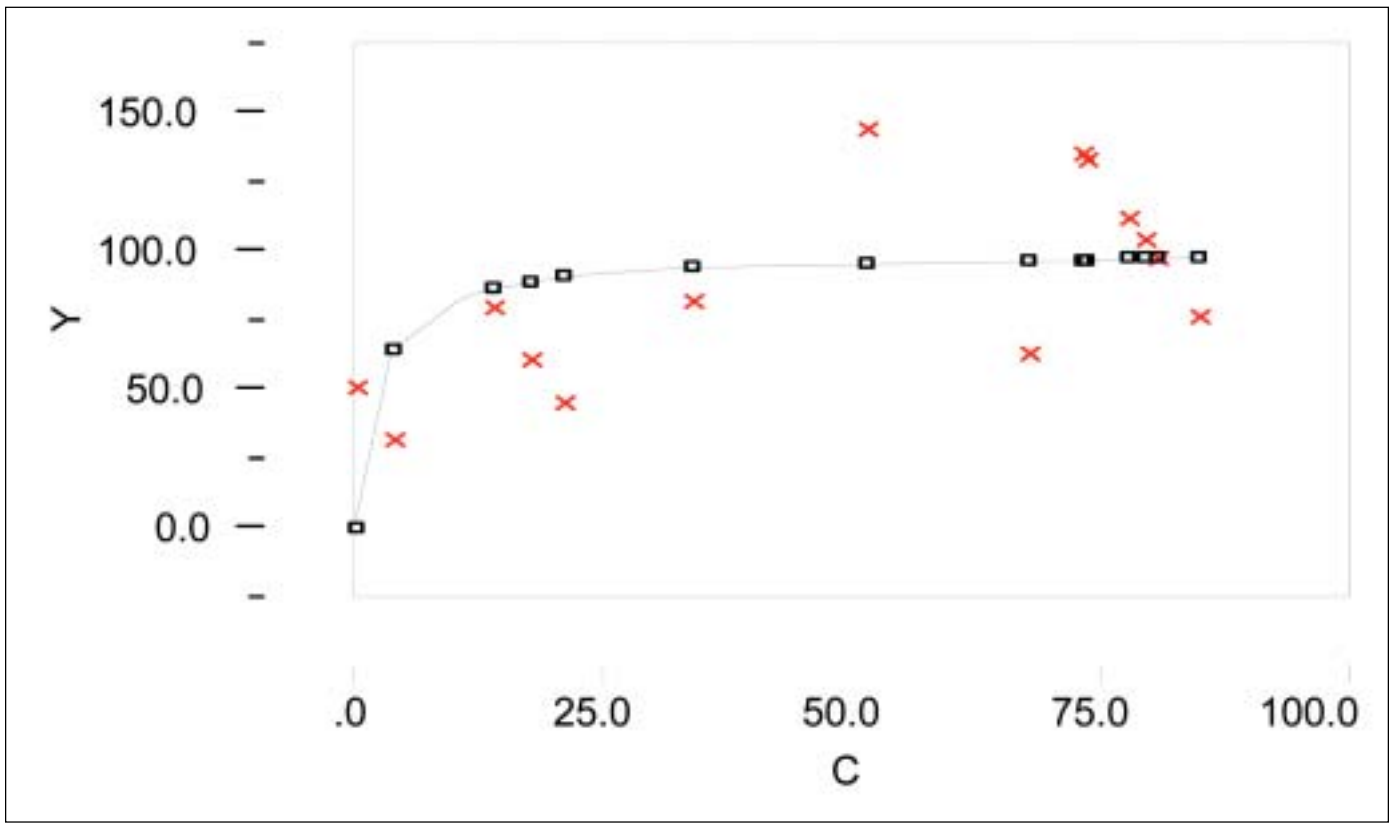

Figure 2. Langmuir isotherm: x experimental data and ςfitted curve: Y = θ = (b C)/(1 + b C). Where θ is the fraction of the surface covered, b is fraction of the adsorption rate constant to desorption rate constant, and C is equilibrium concentration.

following specifications: Protein C4 column; ConstaMetric 4100 solvent delivery system; Waters Lambda Max Model 481 LC Spectrophotometer; Waters 712 WISP; Waters 740 Data Module; flow rate: 1 ml/min; wavelength: 215 nm; injection volumes: 5-20 µl; and a mobile phase of acetonitrile, water, trifluroacetic acid, and hexanesulfonic acid-sodium salt (30:70:0.1:0.1). The absorbance of human insulin was linear over a range of 0.5-50 U/ml (r = 0.99).

4. Incubation of human insulin with soybean powder: 1 ml of human insulin solution (100 U/ml) was mixed with varying amounts 5, 10, 50, 100, and 200 mg of soybean powder dispersed in 1 ml of water. The mixture was then incubated for 1 hour at 37°C. Following the incubation period, the mixture was centrifuged, and the amount of insulin remaining in the supernatant was determined by HPLC.

   In a second set of experiments, varying concentrations (10, 30, 50, 80 U/ml) of insulin were used with a fixed amount of soybean powder (200 mg). 1 ml of insulin solution was mixed with a dispersion of 200 mg soybean powder in 1 ml of water. The mixture was then incubated for 1 hour at 37°C. The dispersion was then centrifuged and the concentration of insulin in the supernatant was determined by HPLC.

Results and Discussion

The average particle size, d_{vs}, is the average diameter corresponding to a sphere with the same volume and surface area of those of soybean particles. Similarly, the average d_{vn} corresponds to a sphere's diameter with the same volume and number of particles as those of soybean particles.[4] Table A shows the average particle size of soybean powder as compared to those of Avicel, calcium carbonate, and lactose. Soybean particles are significantly larger (d_{vs} = 45.5 mm and d_{vn} = 31.6 µm) than any of those other powders investigated in this study. In theory, given everything else is the same, the larger the particles, the better is the flowability of a powder. The bulk volume of soybean powder was similar in magnitude to that of the other powders - *Table B*. However, its bulk density (ρ_b = 0.63 g/ml) was similar to that of lactose, and significantly higher than that of Avicel or calcium carbonate - *Table B*. The coefficient of friction (tan θ) of soybean powder was closer in magnitude to Avicel than the other two powders - *Table C*. This is significant, since Avicel is known to be a good flowing powder. The Carr index (CI) (Table D), also known as the compressibility index, is a measure of the powder's ability to compress into a compact mass under pressure. It is also an indication of the powder's flowability; the smaller the CI the better the flowability. Soybean powder's CI value is similar to that of lactose; its value is better than that of calcium carbonate, but worse than that of Avicel. Powder flowability is an important parameter in tableting and capsuling dosage forms, where the powder's ability to flow from one compartment of a tablet press, e.g., the hopper, to another is extremely important during the tableting process.

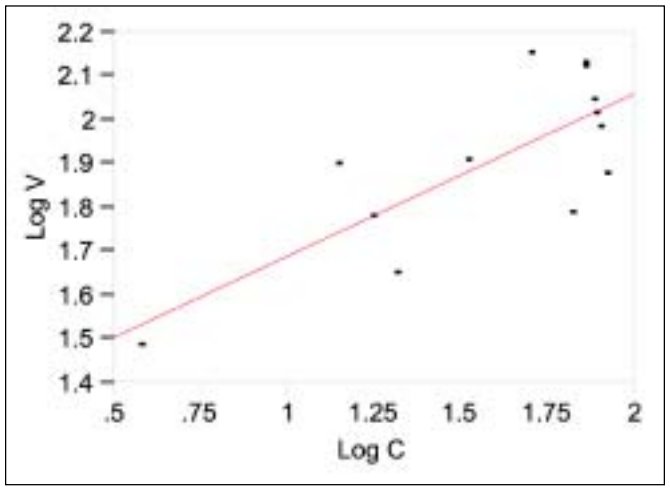

Figure 3. Freundlich isotherm model: log V = 1.32 + 0.37 log C (r = 0.75, *p* = 0.002). V is the amount of insulin (U) adsorbed per 1 g of soybeans powder at any given equilibrium insulin concentration C.

When human insulin was incubated with soybean powder, some of the insulin was found to be associated with powder. To examine the type of association between insulin and soybean, two models were selected. Freundlich isotherm model was originally derived for describing the adsorption of gas molecules on the surface of solid. Similarly, Langmuir isotherm was used to describe the adsorption of the gas molecules forming a monolayer on the surface. These two models are traditionally used in pharmaceutical applications to describe the adsorption of drug molecules on the surface of solid materials. Parameters obtained from these two models can explain in part the mode of interaction between insulin with its binding sites on soybean. The data was fitted to a Langmuir isotherm (Figures 1 and 2):

$$C/V = 1/(b\ V_m) + (1/V_m)\ C \qquad (6)$$

where C is the equilibrium concentration of insulin, V is the amount of insulin (U) adsorbed per 1 g of soybean powder at any given C, b is the ratio of the adsorption rate constant to the desorption rate constant, and V_m is the maximum amount of insulin adsorbed per 1 g of powder. Based on this model, the b value for human insulin adsorption on soybean powder's surface was 0.13, indicating that the rate of desorption is faster than that of adsorption, which implies that the strength of interaction of insulin with the adsorption sites on the surface of the particles is relatively weak. The amount adsorbed reaches a maximum value V_m of 106.0 U/g.

The data was also fitted to a Freundlich isotherm model (Figure 3):

$$\log V = \log k + (1/n) \log C \qquad (7)$$

where k is the amount of insulin (U) adsorbed per 1 g of soybean powder at an equilibrium insulin concentration equal to unity (1 U/ml), and n is a constant. The value of the slope (1/n) reflects the strength of the interaction between

insulin molecules and the adsorption sites on the surface of soybean particles, and V and C are as defined above. The higher the value of the slope, the stronger is the interaction between insulin and the adsorption sites. This model showed that the value of k was 20.9 U/g and n = 2.7. This also leads to the conclusion that the strength of the interaction between insulin molecules and adsorption sites on the surface was relatively weak (implies a negligible affinity less steric interactions). This weak interaction is favorable since soybean powder acts as a carrier for insulin and is able to adsorb the hormone, but yet capable of releasing it when needed. Further studies are in progress to examine the interaction between the soybean-insulin complex and the various digestive enzymes in vitro.

Conclusion

This study demonstrated that human insulin adsorbs on the surface of soybean powder particles. This adsorption is a physical one since it was reversible. The strength of the interaction between insulin and the adsorption sites was weak. This may allow soybean powder to act as a carrier system for insulin easily capable of releasing the hormone.

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About the Authors

Antoine Al-Achi is an Associate Professor of Pharmaceutics at Campbell University School of Pharmacy. He received his PhD form Northeastern University in Boston, Massachusetts. His area of research interest is in the drug delivery systems for peptides and proteins. Before joining Campbell, Al-Achi had his postdoctoral training at Dana Farber Cancer Institute in Boston, MA. He is a member of Sigma Xi, Controlled Release Society, and the American Society of Clinical Pathologists.

Thomas J. Clark III received BS in pharmaceutical sciences from Campbell University School of Pharmacy. He works at Eckerd as a pharmacy intern and head technician.

Robert B. Greenwood is an Associate Professor of Biopharmaceutics/Pharmacokinetics at Campbell University School of Pharmacy. He received his BS in pharmacy and PhD from University of North Carolina. Dr. Greenwood has broad research interest in biopharmaceutics, pharmacokinetics, drug and additive stability, and dosage formulation and design. He is a member of AACP, NCAP, and APhA.

Shylock Sipho Mafu received his MS in industrial pharmacy from Campbell University School of Pharmacy. He is a member of ISPE and Student National Pharmaceutical Association (SNPhA).

Campbell University School of Pharmacy, PO Box 1090, Buies Creek, NC 27506.

A model was developed to describe the thermal response of large plastic bioreactors in incubators with variable air speed, and then used to provide a general guide for incubator design.

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Incubator Design for Optimal Heat Transfer and Temperature Control in Plastic Bioreactors

by William Adams, Colette Ranucci, Sara Diffenbach, Kim Dezura, Charles Goochee, Abraham Shamir, and Scott Reynolds

Culture of mammalian cells for the manufacture of vaccines for human use has traditionally been performed in small plastic bioreactors such as T-flasks and roller bottles. Generally, the reactors are manipulated at ambient temperature for process operations such as cell plant, refeed, or infection. Subsequently, the reactors are transferred into incubators which warm and then maintain the cells at the optimal temperature for cell growth or viral propagation (e.g., ~37°C for human cell lines). The incubators are generally designed to control temperature using forced air circulation, and may range in size from a small tabletop unit to full walk-in rooms capable of storing thousands of reactors. To achieve process consistency and control, the time required for warming to optimal temperature must be satisfactory relative to the time scale of biologically significant events in the reactor, such as cell settling and attachment, initiation of growth, or viral transmission.

In recent years, the design of bioreactors has advanced in two ways. Firstly, to provide greater cell culture surface area within a given manufacturing footprint, and secondly to reduce the number of container openings and thereby provide increased sterility assurance. In so doing, however, it is apparent that the heat transfer necessary to provide the desired warming rates has become a bigger challenge. Table A shows the impact of design on the ratio of heat transfer surface area to cell culture area for several commercially available bioreactors, including the 40-tray Nunc Cell Factories (NCFs) reactors studied in this work.

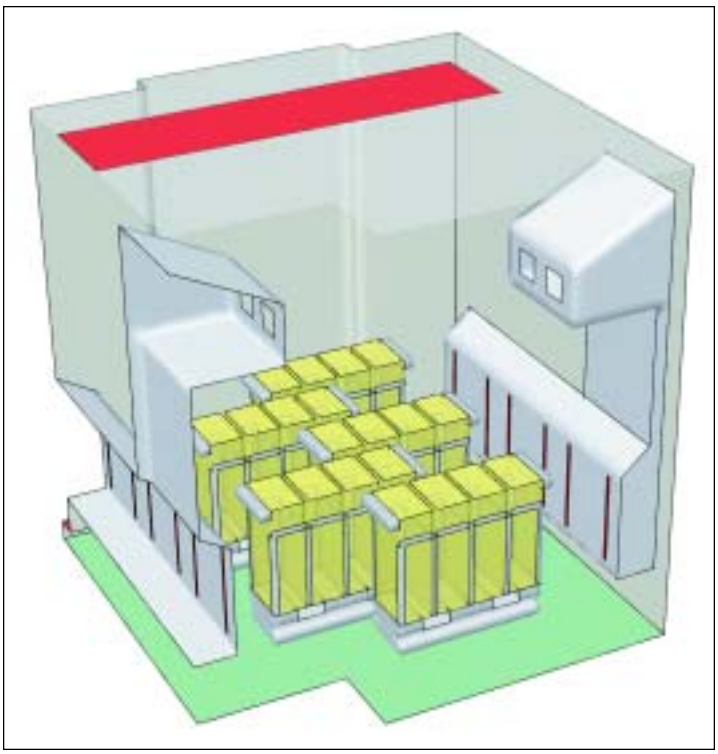

Figure 1. Isometric view of incubator layout and typical load pattern.

These design properties suggest that heat transfer into the 40-tray units will be ~10 times slower than into a T-flask if all other factors are equivalent.

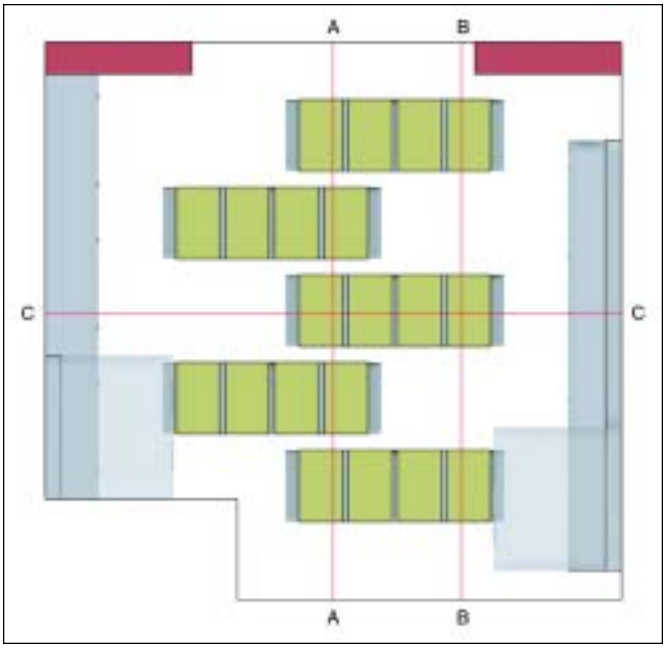

Figure 2. NCF load pattern and numbering key plan.

While the impact of warming rates has been shown to be important in some applications of cell and virus cultivation, heat transfer and temperature control of the largest reactor listed in Table A has not been well understood. Standard incubator designs generally promote heat transfer by recirculating air controlled at a specific temperature set point. Air speed and direction have essentially been driven by cGMP design factors rather than rigorous thermal performance analysis. To provide a class 10,000 environment, for example, walk-in incubators would generally be designed to provide HEPA-filtered airflow from the ceiling into the room with low wall returns to the HVAC system, typically at a minimum of 35 air changes per hour. Achieving a consistent warming time among bioreactors having very different surface area to volume ratios; however, will require different specific incubator airflow conditions.

The focus of this work was to analyze the transient thermal characteristics of 40-tray NCFs to determine the dependence of warming rates on airflow conditions in incubators. The results of this study were then generalized to facilitate incubator design and operation to enable optimal bioreactor performance and process robustness. More specifically, this article describes an experimental approach to assess the thermal response of 40-tray NCFs in walk-in incubators along with the development and use of Computational Fluid Dynamics (CFD) to model the thermal behavior, considering both steady state (fixed temperature) and transient (warming) modes. The utility of the model was to:

1. calculate air velocity and direction around the NCFs as a function of incubator design features

2. provide the lumped transient thermal model parameters for the NCFs that produce the same predicted transient response as measured data

Bioreactor Type	Cell Growth Area cm^2	Heat Transfer Area cm^2	Ratio
T-flask	175	546	3.1
Roller Bottle	850	1,050	1.2
NCF (2-tray)	1,264	1,741	1.4
NCF (40-tray)	25,280	8,718	0.36

Table A. Relative heat transfer considerations.

3. define the impact of air flow conditions on NCF warming rates in a general way to facilitate future incubator design optimization

Experimental and Computational Methods

Temperature Mapping in NCFs

The 40-tray polystyrene NCFs were positioned in sets of four on the stainless steel carts used for automated cell culture manipulations. Small holes were drilled in the sidewalls of selected trays to allow for the insertion of thermocouples. The tubing assemblies for vent and plant operations were placed into the applicable ports of each NCF, and the NCFs were filled with 0.33 mL/cm^2 of Water-For-Injection (WFI) (equivalent to 210 ml per tray). Thermocouples were inserted midway into the selected trays, manipulated until the end of each thermocouple was submerged in water, and subsequently taped in place to prevent inadvertent displacement during the mapping study. The temperature of up to 20 thermocouples available for use per mapping was monitored and recorded using standard data logging software. The thermocouples were calibrated pre- and post-use to ensure accurate data acquisition. The NCF carts were maintained at room temperature in an attempt to provide a uniform temperature for all trays. To start the experimental mappings, the carts containing the NCFs were wheeled into the 37°C incubator, and temperature data was then collected at five-minute intervals from each of the thermocouples. Temperature mapping studies were typically continued until all trays reached within 0.5°C of the 37°C incubator control set point. The data was transferred to spreadsheets for numerical and graphical analysis.

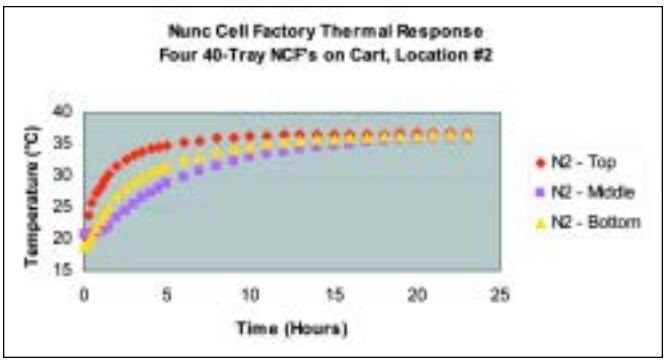

Figure 3. NCF thermal response at low air speed. Average local air speed ~28 FPM. Side wall air circulation units providing 0 FPM at supply slot inlets (off).

NCF Number and Layer	Time to Within 1°C (hours)
Cart1-N2-Top	7.1
Cart1-N2-Middle	19.0
Cart1-N2-Bottom	14.0

Table B. Thermal response time for trays at low air speed.

NCF Number and Layer	Time to Within 1°C (hours)
Cart5-N4-Top	5.0
Cart5-N4-Middle	9.0
Cart5-N4-Bottom	6.5

Table C. Thermal response time for trays at higher air speed.

Incubator Design Features

Two different-sized walk-in incubators were evaluated in the study. The first incubator was 9 ft wide by 9 ft long with overhead HEPA-filtered air supplied at ~1240 CFM (cubic feet per minute) at 37°C. Typically, five NCF carts, each holding four 40-tray NCFs, were placed in the incubators. Two low wall air returns were located along one wall. The second incubator measured 20.6 ft long by 9.8 ft wide with overhead HEPA-filtered air supplied at ~2,400 CFM and 37°C, and with low wall returns positioned at each corner of the room (four total). The bulk average air velocity in the middle of the incubators resulting from these supply and return arrangements was about 15 feet per minute (FPM). Both incubators had a stainless steel interior finish with well insulated wall panels. To provide for a wide range of air velocities in the vicinity of the NCFs, additional wall-mounted air recirculation units were installed. The fans in these units drew air in at an elevation of ~7 ft and drove it through a bank of 2 ft by 2 ft HEPA filters positioned at the level of the NCFs (approximately 1.1 ft to 3.1 ft above the floor). The flow from the filters was forced through slots about 0.08 ft wide by 1.8 ft tall (positioned about 1.2 ft apart), through which the velocity was increased. The output of the fans was designed to provide an air speed of up to ~1,000 ft per minute (FPM) at the slot outlet. By adjusting the output of the fan-powered recirculation units, the air speed in the vicinity of the NCFs could be significantly varied. Volumetric air flow rates were measured using standard portable flow hoods, and local air velocities were measured using a hand-held hot wire anemometer.

Computational Fluid Dynamics Modeling

The two incubator designs were modeled using Computational Fluid Dynamics (CFD). Large-scale three-dimensional CFD models were created from the physical domains described in the previous section. These models were built using commercially available general purpose CFD software. Parameters used for the model included the following: an unstructured solver, k-ε RNG turbulence capabilities, and a domain descretized with tetrahedral cells throughout. Approximately 620,000 computational cells (small incubator) and 910,000 cells (large incubator) were employed to solve for the three velocity directions, two turbulence terms, one pressure term, and one energy term.

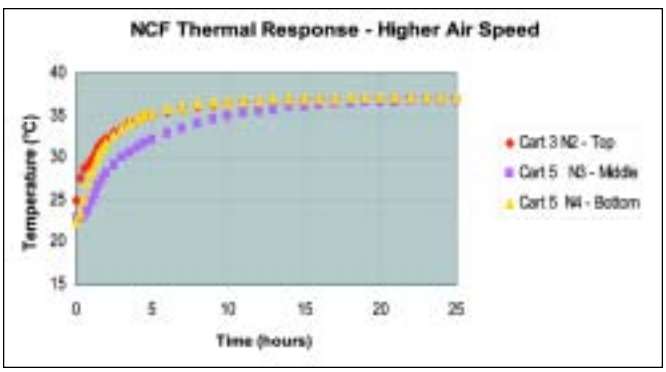

Figure 4. NCF thermal response at higher air speed. Average local air speed ~ 40 FPM. Side wall air circulation units providing ~ 550 FPM at supply slot inlets.

Experimental Results

More than 30 distinct temperature mappings of NCF warming were performed covering various NCF locations, airflow speeds, and directional patterns. An isometric view of the small incubator with five NCF carts is shown in Figure 1. A typical experimental load pattern along with the numbering scheme for carts and specific NCFs on each cart is indicated in Figure 2. The bulk average thermal performance of the 40-tray NCFs on the carts in low air flow conditions is shown in Figure 3 which shows the temperature versus time profiles for the top, middle, and bottom trays (numbers 1, 20, and 40 respectively). The data was taken from NCF #2 among the four on cart #1 (identifying nomenclature Cart1-N2). All trays exhibit the expected first order dependence on the difference between the tray and incubator temperature. The response is notably asymmetric; however, with the top tray warming the fastest, the bottom tray notably slower, and the middle tray warming the slowest following an initial lag phase. This pattern in the relative thermal response for the different trays was observed in all studies although the specific response times and magnitude of disparity between trays was influenced by airflow and heat transfer conditions.

To summarize the performance in a way most meaningful to the cell and virus cultivation, we evaluated the time to reach a temperature within 1°C of the incubator control point. Referring to the data in Figure 3, the performance is summarized in Table B.

For this experiment, the incubator temperature was 36.7°C, and the times listed above therefore represent the time to achieve 35.7°C. Within a group of experimentally mapped NCFs, the response time of comparable trays could vary by a few hours. The standard deviation (σ) in time required to reach within 1°C of the control temperature ranged from ~three hours for top trays to ~six hours for the slowest responding middle trays.

Representative performance within the incubator at higher air speed conditions is shown in Figure 4 which plots the temperature versus time profiles for the top, middle, and bottom trays from Cart 5-N4 in the load pattern. In this case, the sidewall air circulation units were providing ~550 FPM at

"Therefore, CFD was deemed as the more accurate and convenient means to solve the transient response problem as long as suitable values for R and C could be estimated."

the supply slot outlets. Although the NCF trays warm more rapidly in the higher air flow conditions, the asymmetric trend in response between trays remains prevalent. The response times can be summarized in Table C.

The faster response and reduced absolute deviation between layers would be expected to provide improved consistency and control of the cell culture.

The asymmetric thermal response between top and bottom layers in the 40 tray NCFs is in contrast to performance in the smaller two tray and 10 tray NCFs. In the 10-tray bioreactor, for example, both top and bottom trays lead the response of the middle tray by an equivalent margin. Apparently the cart used to support the 40 tray NCFs provides a significant additional heat capacity and thermal resistance. Interestingly, there was not any significant difference between the NCFs at different positions on the carts. That is the performance was essentially the same between the outside and inside positions (i.e. positions #1 through #4 are all similar). This was determined through analysis of variance applied to all the data from studies covering all positions in the load patterns. Warming rates were not significantly affected by cart location within the incubators studied, most likely due to the similar proximity of carts to airflow sources.

Modeling and Discussion of Results

The CFD models were first used to calculate air velocities

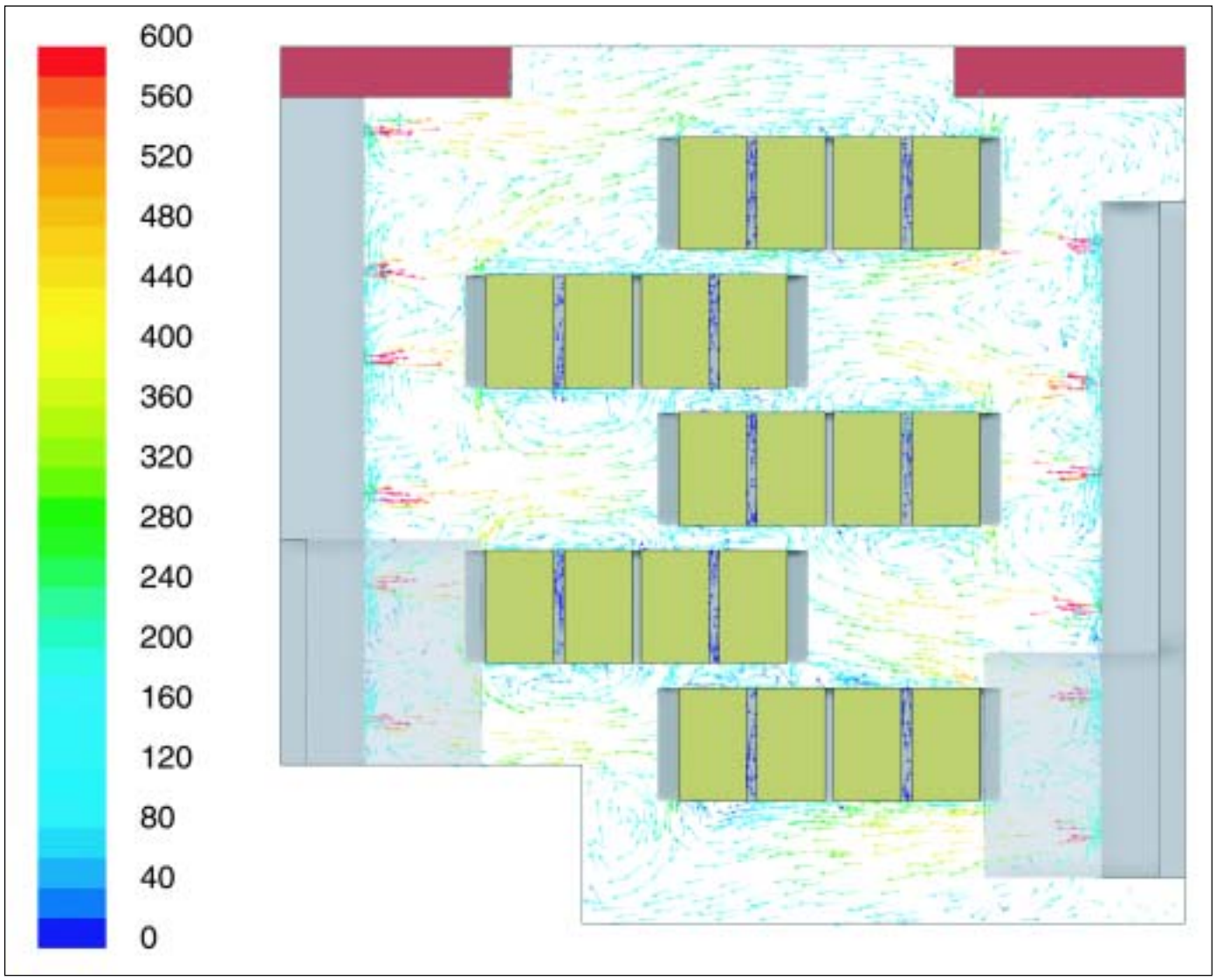

Figure 5. Plan view of velocity vectors in FPM - small incubator at 2.25 ft above floor.

throughout the incubators as a function of air speed settings for the wall-mounted recirculation units. Figure 5 shows a plan view of the predicted velocities at a distance of 2.25 feet above the floor. Upon entry into the room from the sidewall slots, the velocity vectors show a decline from the initial ~1,000 FPM with distance into the room and from impact with the cart obstructions. The areas in between NCFs on any given cart are slightly less than an inch apart and therefore retain relatively low air speeds for the orientation shown. Figure 6 shows a side-view of the same case, highlighting the high local velocities around the NCFs, the relatively lower velocities elsewhere, and the overall air circulation patterns throughout the room.

It was generally desired to isolate a closed-form function relating temperature rise time to various known physical parameters and boundary conditions. By identifying this function, a separate CFD simulation would not be required each time an incubator was designed. From standard transient heat transfer relationships, it was felt that the following equation could be used as a first order approximation:

Air Velocity Avg per slot (FPM)	Bottom trays (hrs)	Middle trays (hrs)	Top trays (hrs)
0	15.5	16.5	9.5
500	6.5	8.5	6.3
1000	4.8	6.8	4.8

Table D. Average time to within 1°C of incubator temperature.

$$T_{nunc} = (T_i - T_\infty)\, e^{-t/RC} + T_\infty \quad (1)$$

where:

T_{nunc} = Transient Nunc Cell Factory temperature

T_i = Initial Nunc temperature (nominally 18°C)

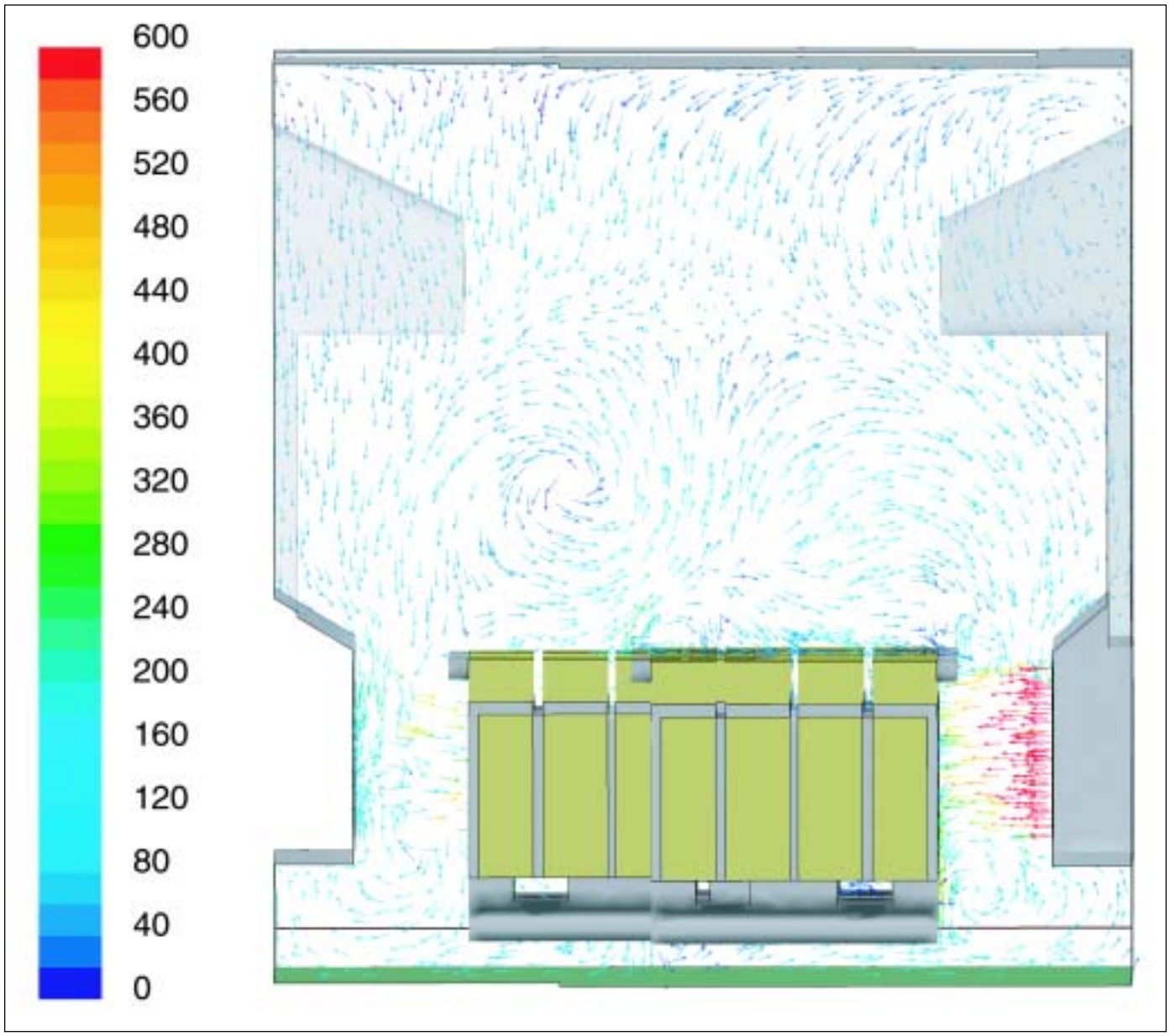

Figure 6. Side view of velocity vectors in FPM - small incubator.

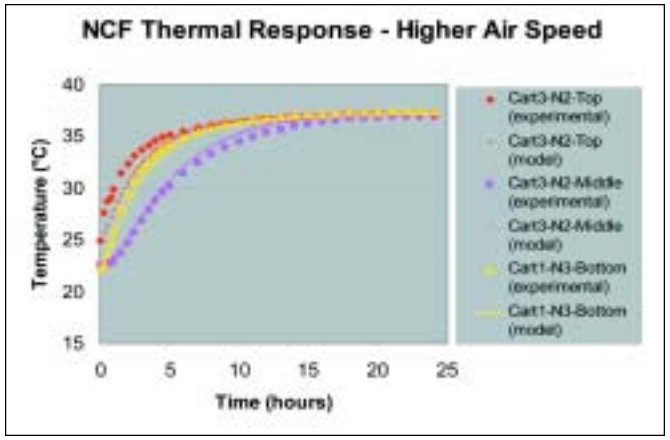

Figure 7. Comparison of model-predicted temperature rise to experimental measurements of a similar incubator layout at high airflow conditions (wall units at ~550 FPM).

T_∞ = Incubator bulk air temperature (37°C)
t = Time (seconds)
R = Thermal Resistance (1/hA)
C = Thermal Capacitance ($\rho C_p V$)
C_p = Specific Heat
ρ = Density
V = volume
h = Heat transfer coefficient
A = Surface area

Initially, it was believed that the parameters of equivalent thermal resistance and thermal capacitance could be directly determined using calculated values for surface areas, volumes, densities, conductivities, specific heats, and heat transfer coefficients for the carts and bioreactors. However, many attempts at solving the function revealed that the relationship was more complex with a strong dependence on other factors such as local airflow velocities, cart orientation, etc. Therefore, CFD was deemed as the more accurate and convenient means to solve the transient response problem as long as suitable values for R and C could be estimated. Along these lines, several transient CFD models were run using closed-form thermal property calculations (to calculate values for R and C), but all such simulations failed to adequately describe the measured transient response of the Nuncs.

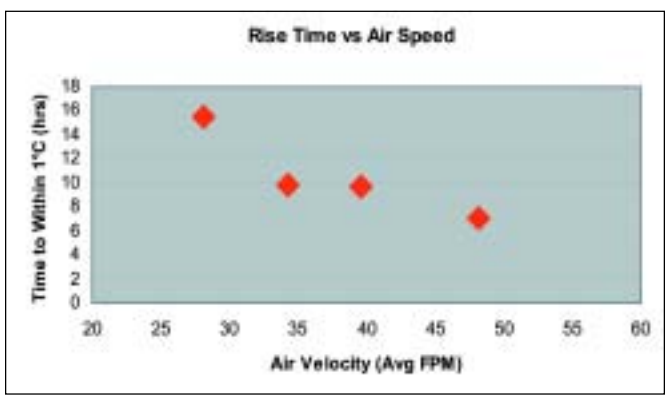

Figure 8. Correlation between NCF warming time and local average air velocity.

After the above technique failed to produce the desired results, an attempt to iteratively "back-calculate" the R and C parameters was made as follows. From the steady state CFD simulation, the heat transfer coefficients were calculated as were the bulk air temperature and the initial NCF layer temperatures. A two-variable least square fit for R and C was made using a power function developed from the experimental data acquired for various air flow rates. This equation used the known variables of temperature and time to provide the best fit values for R and C. These values were then used in a CFD transient model to predict the comprehensive temperature response behavior of the Nuncs. This procedure was further repeated until the calculated thermal resistance and capacitance provided a good emulation of the experimental data for the low air velocity case. Model predictions for the time to reach within 1°C of the incubator control point agreed with experimental data to within two hours for all such cases.

The model was then challenged to predict warming rates under distinctly different air flow conditions. Such an assessment is exhibited in Figure 7 which shows both experimental and predicted thermal response curves for the case in which air flow from the side wall units provides ~550 FPM at the HEPA filter outlet slots. There are three sets of data shown for this faster warming case, corresponding to three representative tray mapping locations among the overall load pattern of five carts containing 20 NCFs. In Figure 7, the dashed lines show the model-predicted temperatures relative to the actual experimental values. Agreement is good in general, the predicted times to reach within 1°C are accurate to within two hours.

The model was used similarly to predict warming rates at three different air flow conditions. The thermal response of the NCFs as a function of the nominal side wall air velocity can be summarized in Table D.

The times listed are average response times for all sample points on the indicated tray location (the model allowed for a total of 11 such points). Table D shows that air speed has less impact on the top trays which have relatively high exposed surface area. The bottom or middle trays have smaller exposed surface areas which leads to a stronger dependence on changes to the heat transfer coefficient.

Incubator Design Guide

An important potential use of this heat transfer analysis and NCF thermal response modeling is to facilitate incubator design. Ideally, incubators could be designed for reliable, predictable performance without the need for developing and running a detailed computer model of thermal response for NCFs (or other bioreactors) in each specific incubator geometry and load pattern. Along those lines, we sought to generalize the model results to enable prediction of NCF warming rates as a function of local average air speed.

More specifically, we developed a correlation for the NCF warming time as a function of the average air velocity which can be experimentally measured and is a physically intuitive parameter. Average air velocities were calculated by the CFD simulation program for the different side-wall velocity cases. The average was calculated by sampling 120 point locations

around the NCF carts. These points were regularly spaced, 2 inches away from the carts, covering the top, middle, and bottom zones of each NCF, along both the long and short sides of the cart. The resulting data for all tray locations was then plotted in Figure 8 as time to achieve 36°C (average time for all trays) versus the average local air speed in FPM. The plot clearly shows a steep decline in warming time between low air speed of ~28 FPM and the more moderate speed of ~48 FPM with a slower decline expected beyond that. The step like appearance in the middle of the plot is believed to be driven by a transition from the laminar to the turbulent flow regime. Figure 8 suggests that reasonably rapid thermal response as well as effective temperature consistency and control will be provided from an incubator environment designed to provide average local air speeds of at least 35 FPM. Note that much higher local point velocities may be needed to achieve an average surrounding velocity of 35 FPM because other areas may be relatively stagnant.

Higher air speeds generally dictate larger air handlers and proportionally greater surface area of HEPA filters since the absolute air speed through the filters should be less than ~100 FPM. As a result, higher incubator air flows will always be more costly to install and operate. The most economical approach to achieve higher local air velocity is to position the dominant room air flow drivers, such as the main supply inlets and/or returns, as close to the bioreactors as is practical. Higher local velocities may require the use of ductwork to provide post-filtration air flow convergence to achieve the desired minimum air speed at important locations in the load pattern. Reasonably rapid and consistent bioreactor warming rates can be achieved through such incubator design approaches.

Conclusion

Incubator air speed was observed to have a significant impact on the warming rate of large plastic bioreactors commonly used for cell and virus cultivation. The relationship between thermal response and air velocity was well correlated through the use of a model utilizing computational flow dynamics. Application of the model indicated that average local velocities in excess of 35 feet per minute were required to achieve timely and consistent response.

Credits

1. 40-tray NCF bioreactors were Nunc Cell Factories procured from Nalge Nunc, Intl.

2. The CFD simulation software used was Fluent®, from Fluent, Inc.

About the Authors

William Adams received his PhD in chemical engineering from Cornell University with a minor in computer science. He joined Merck in 1990, and has progressed through positions in Technical Service, Operations Management, and is currently Associate Director in Vaccine Process Engineering. His current principal focus is on process equipment and facility design for live virus vaccines.

Merck & Co., Inc., WP60T-2, PO Box 4, Sumneytown Pike, West Point, PA 19486.

Colette Ranucci joined the Bioprocess Research and Development department within Merck Research Laboratories in 1992, shortly after receiving BS/BA degrees in chemical engineering and German. After working in the area of vaccine process development for several years, she returned to Rutgers University to complete the PhD program in chemical engineering. Since rejoining Merck in 2000, her primary responsibilities have included process development, scale-up, and process validation related activities. She is an active participant of the BIOT division of ACS, serving as the membership chair.

Merck & Co., Inc., WP17-201, PO Box 4, Sumneytown Pike, West Point, PA 19486.

Sara Diffenbach received a BS in chemical engineering from the Pennsylvania State University in 2000 and joined Merck & Co., Inc., as a staff engineer in the Viral Vaccine Technology and Engineering group, focusing on technology transfer and facility start-up. She recently joined Pfizer, Inc., as a Production Leader in the manufacturing of personal care products.

Pfizer, Inc., 400 W. Lincoln Ave., Lititz, PA 17543.

Kim Dezura is a Senior Process Engineer at Merck.

Merck & Co., Inc., WP62-7, PO Box 4, Sumneytown Pike, West Point, PA 19486.

Charles Goochee is a Director of Bioprocess R&D at Merck.

Merck & Co., WP17-201, PO Box 4, Sumneytown Pike, West Point, PA 19486.

Abraham Shamir joined Merck in 1989 and has held positions of increasing responsibility in technical operations and process engineering. After receiving a BS in biochemistry and a PhD in chemical engineering from Columbia University, he joined Schering-Plough where he progressed to the position of manager for large-scale recombinant protein purification. He went on to join BioTechnology General as director of protein purification - Process Development and Manufacturing.

Merck & Co., Inc., WP60T-2, PO Box 4, Sumneytown Pike, West Point, PA 19486.

Scott Reynolds is a Senior Engineer at Computer Aided Engineering Solutions.

Computer Aided Engineering Solutions, Division of Bearsch Compeau Knudson Architects and Engineers, PC, 41 Chenango St., Binghampton, NY 13901.