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API Chemical Synthesis Trends in Reactor Heat Transfer Design

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Introduction

Heat transfer is a fundamental operation in chemical synthesis. This article explores basic and advanced concepts, presents some calculations, and compares different methods for controlling heat transfer systems.

Functional requirements are discussed for these specific heat transfer requirements:

- heating and cooling the reactor
- condensing vapors in the overhead system
- providing heating and cooling for various reasons such as cooling vacuum pumps, controlling temperature in overheads piping, and servicing mixers and pumps

Various fluids (and non-fluids) are used to provide heating or cooling. These include steam, water, water/glycol mixtures, synthetic fluids, liquid nitrogen, and electrical heating tape. The alternatives are compared with guidance given for selecting among them.

Most facilities have heat transfer systems that service multiple reactors. There are several ways to configure and control single-fluid systems. They are compared.

Functional Requirements

Each part of a reactor system must include the definition of its heat transfer functional requirements. What are the required end-point temperatures (maximum and minimum)? How fast should the temperature change? Is there a specific heating or cooling duty needed? Are exothermic or endothermic reaction conditions expected? Can material solidify in the reactor overhead system? What are the environmental emission limits? Consider these and other questions.

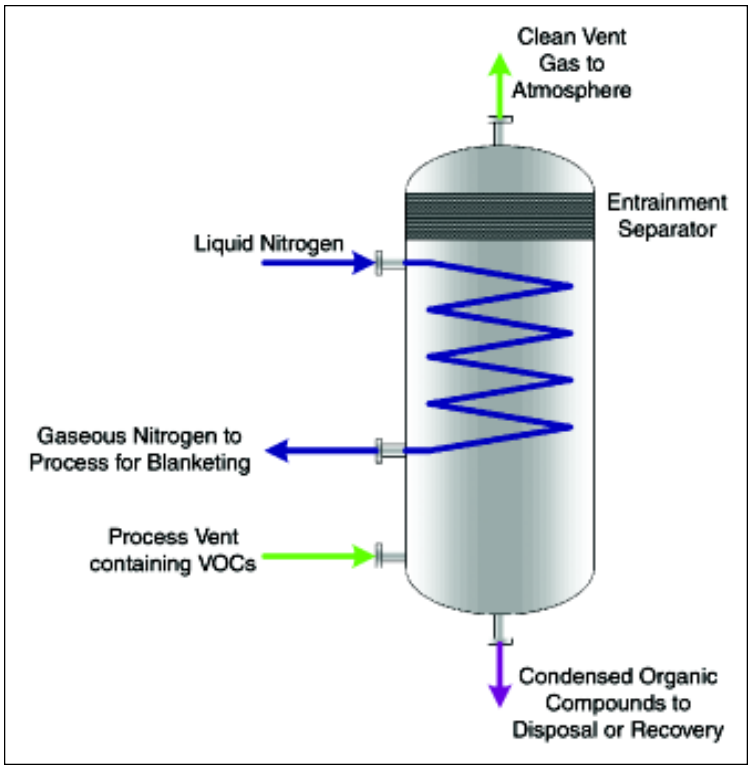

Figure 1. Simplified diagram of a VOC condenser. The liquid nitrogen used to condense VOCs is vaporized in the unit, and can be used to blanket the reactors.

Reactor Functions

Reactors need heating and cooling for several reasons. Reactions may proceed best at specific temperatures, taking advantage of the relationship between kinetics and temperature. Temperature is often used to control solubility, heating to dissolve solid ingredients then cooling to crystallize product in the solution. Exothermic or endothermic reactions require cooling or heat-

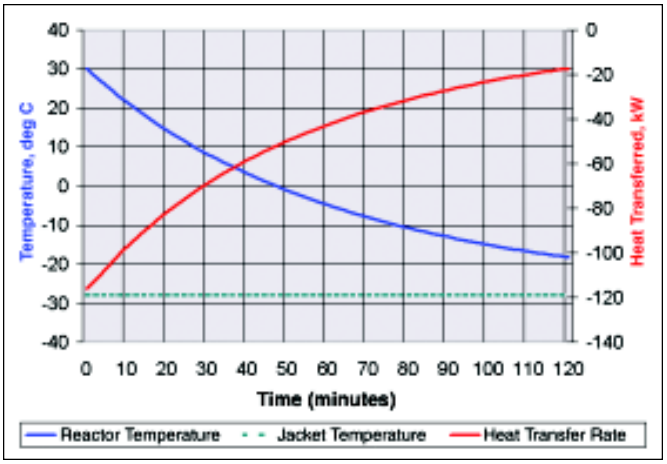

Figure 2. Temperature and Rate of Heat Transfer in 2000 liter glass-lined reactor with Dowtherm J at -28°C.

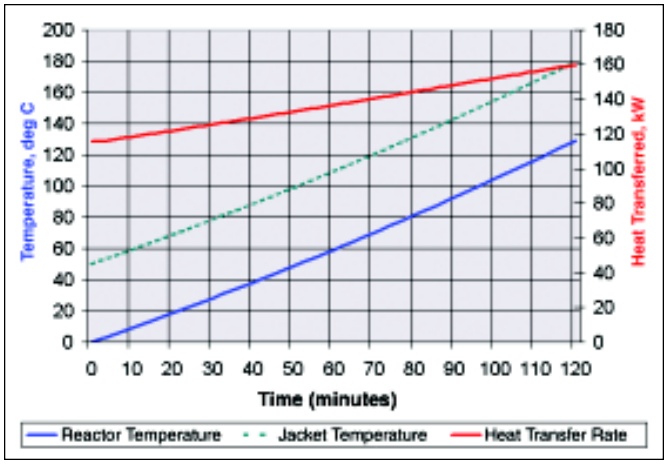

Figure 3. Temperature and Rate of Heat Transfer in 4000-liter glass-lined reactor with Syltherm XLT ramped 50°C hotter than reactor contents.

ing to control isothermal conditions. Boiling for distillation or solvent removal demands a heat source. Smaller cooling needs might be identified for removing heat of mixing or work energy (e.g., pumping).

Temperature ramping is often required. It is very important to know how fast temperature changes should occur, and whether the rate of change must be precisely controlled. For example, if a desired reaction occurs at a certain high temperature, but a competing side reaction dominates at lower temperatures, there would be a desire to raise the reactor temperature "as quickly as possible." Using kinetic data for the two reactions at the two temperatures, the relationship between ramp rate and reaction results could be quantified. This would be very useful when analyzing heat transfer factors such as jacket area, internal coil, the temperature of the heat transfer fluid, agitation, etc.

Reactor Temperature Limits

The traditional temperature limits of a glass-lined carbon steel reactor are -30°C (-20°F) to 230°C (450°F). The reasons for this range are rooted in the physical capabilities of reactor systems. Scientists would prefer larger envelopes and often express frustration when confronted with these temperature limitations. Cryogenic temperatures as low as -100°C (-150°F) are encountered in most API facilities.[1,2] However, the typical plant has only a few reactors equipped to achieve those low temperatures.

The lower end of the range is dictated by two factors: 1) the availability of low temperature cooling sources and 2) the behavior of ordinary carbon steel in response to temperature.

Traditionally, glycol/water mixtures cooled by mechanical chillers provided cooling. Ethylene glycol is out of favor due to its harmful effect on the environment; propylene glycol is usually preferred. Concentrations of 60 to 90 volume % glycol in water have a useful lower temperature range of -30°C (-20°F). Allowing for a temperature gradient between the jacket and the reactants, this results in a working temperature in the reactor of about -25°C (-13°F).

Some formulated heat transfer fluids use propylene or ethylene glycol as their primary ingredient, mixed with stabilizers and corrosion inhibitors. The additives are designed to maximize the service life of the fluid and equipment. In addition, the technical support provided by fluid manufacturers justifies the selection of a proprietary product versus generic glycol.

Mechanical chillers lose efficiency at low temperatures. Cascade units are available to operate as low as -70°C (-94°F), but a more common limit is around -30°C (-20°F). Lower temperatures are most often obtained using cryogenic gases such as nitrogen or carbon dioxide.

Modern heat transfer fluids can be used at much lower temperatures. For example, silicone polymer fluids such as polydimethyl siloxane are recommended for use from -100°C (-150°F) to 260°C (500°F). Factors to consider when choosing fluids and designing systems are given later in this article.

Carbon steel is generally avoided for service temperatures below -45°C (-50°F) because its body-centered crystalline structure becomes brittle.[3] Since standard glass-lined carbon steel reactors utilize this material, the temperature cannot be safely reduced. When lower temperatures are needed, high-nickel steel can be specified, such as ASTM A645 5% Ni alloy. However, it is more common to use a 300 series stainless steel in low temperature service.

The American Society of Mechanical Engineers' Boiler and Pressure Vessel Code, Section VIII Unfired Pressure Vessels may be used as a specific guide to the selection of materials to be used in low temperature service. Some metals suitable for cryogenic temperatures are stainless steel (300 series and other austenitic series), copper, brass, bronze, Monel® (nickel-copper alloy), and aluminum. Non-metal materials that perform satisfactorily in low temperature service are Dacron®, Teflon®, Kel-F® (PCTFE), asbestos impregnated with Teflon®, Mylar®, and Nylon. Once the materials are selected, the method of joining them must receive careful consideration to ensure that the desired performance is preserved by using the proper soldering, brazing, or welding techniques and materials.

New reactors, using the available low temperature heat transfer fluids, can achieve near-cryogenic temperatures if suitable materials are chosen. These include A203 Grade D

carbon steel, austenitic stainless steel, Hastelloy, and other high-nickel alloys.

In the early 1980s, Teflon®-lined stainless steel reactors were specified for service at -75°C (-100°F). The lining consisted of multi-layer chemically bonded PFA resin.

Upper temperature limits also are dictated by materials of construction and performance of heat transfer fluids. Glass linings, elastomers, and Teflon® each contribute to a typical maximum of about 260°C (500°F).

Duty Calculations

Detailed calculation procedures are given later in this article. Be aware that heat transfer is dynamic; it changes significantly over the course of a batch. This is primarily due to two factors: 1) the temperature difference between jacket fluid and reactants changes as the reactor heats or cools which reduces the driving force behind heat transfer and 2) physical properties that affect the efficiency of heat transfer (especially viscosity) change with temperature. Therefore, it is prudent to determine maximum, minimum, and average duty for each reactor system. If you have a central heat transfer system serving multiple reactors, it is important to analyze the production schedule in conjunction with heat transfer. This diversity determines the sizing of the central system for heat duty, volume of heat transfer fluid, and flowrate.

Determination of instantaneous heat flux at the reactor is dependant on the assumed temperature difference between the jacket and reactor. Glass-lined reactors are limited to about 100°C (180°F) temperature difference due to the limited ability of the lining to withstand thermal stress. Consult the equipment manufacturer for limitations specific to your equipment.

Volatile Organic Compounds (VOC) Emissions Control

It is common practice to install two overhead condensers. The first one is used for refluxing solvent to the reactor or to capture it in a receiver for reuse. The second is for controlling emissions of volatile organic compounds (VOCs).

The effectiveness of a VOC condenser is determined by three factors: 1) the vapor pressure of the VOCs, 2) the quantity of VOCs relative to non-condensable gas (e.g., nitrogen), and 3) the temperature that the vapor stream is reduced to. It is easy to predict the performance of a VOC condenser if the amount of non-condensable gas is known.[4] Very cold temperatures (< -40°C) may be necessary to achieve the desired reduction.

VOC condensers for emission control can be installed locally, dedicated to a single reactor, or centrally. Local condensers are usually shell-and-tube type, but "cold fingers" are also used. A cold finger is designed to permit extensive icing within the condenser. After the batch is complete, the unit is de-iced by circulating warm heat transfer fluid through it to melt the ice for collection.

Central VOC condensers are specialized units that use liquid nitrogen to reduce the temperature of combined vent streams to about -75°C (-100°F). The liquid nitrogen is vaporized in the tubes of the condenser (while condensing VOCs from the process vent stream) then used in the process for blanketing reactors and tanks. Ideally, the nitrogen usage for cooling balances that consumed in the process. To the extent this ideal is reached, the VOC condenser operating costs are virtually nil[5] - *Figure 1.*

Heat Transfer Fluids (HTF)

Whether designing a system to utilize an existing heat transfer fluid, or having the luxury of selecting the fluid, be aware of fluid properties that affect the design of your equipment. Important properties include:

- recommended temperature range
- viscosity at operating temperatures and ambient temperature
- vapor pressure at highest foreseeable operating temperature
- flash and fire points
- chemical composition

HTF manufacturers publish recommended operating temperature ranges for their fluids. Excursions beyond the published limits are permissible although excessively high temperature will accelerate oxidation and discoloration in most fluids. However, the allowable film temperature is higher than the recommended maximum bulk temperature. This is important because a fired heater will induce high film temperatures in the fluid.

Pumpability usually determines the low end of the recommended temperature range. Other properties also are considered, such as freezing point and viscosity. Viscosity has a significant effect on the heat transfer coefficient, and should be carefully considered throughout the desired temperature range. Viscosity and surface tension also are measures of how easily a fluid will leak past gaskets and seals.

The importance of vapor pressure might be overlooked because heat transfer calculations don't consider this property. However, vapor pressure at the upper temperature limit (use the highest film temperature in the heater) can exceed standard pressure ratings for piping and equipment. For example, at 280°C (536°F), the vapor pressure of a popular alkylated aromatic HTF is approximately 6 bar (90 psig). Assuming a safety factor of 1 bar (15 psi) to prevent boiling, and pressure drop through the distribution piping and equipment of 2 bar (30 psi), it would be necessary to have a pressure at the discharge of the circulating pump of at least 9 bar (135 psig) in order to prevent the fluid from vaporizing.

Designing a System

Here is a procedure for designing a heat transfer system. Follow these steps to achieve an optimal design.

1. ***Define the User Requirements Specifications (URS).*** The URS describes what the system is supposed to do, defining the functions to be carried out, the data on which the

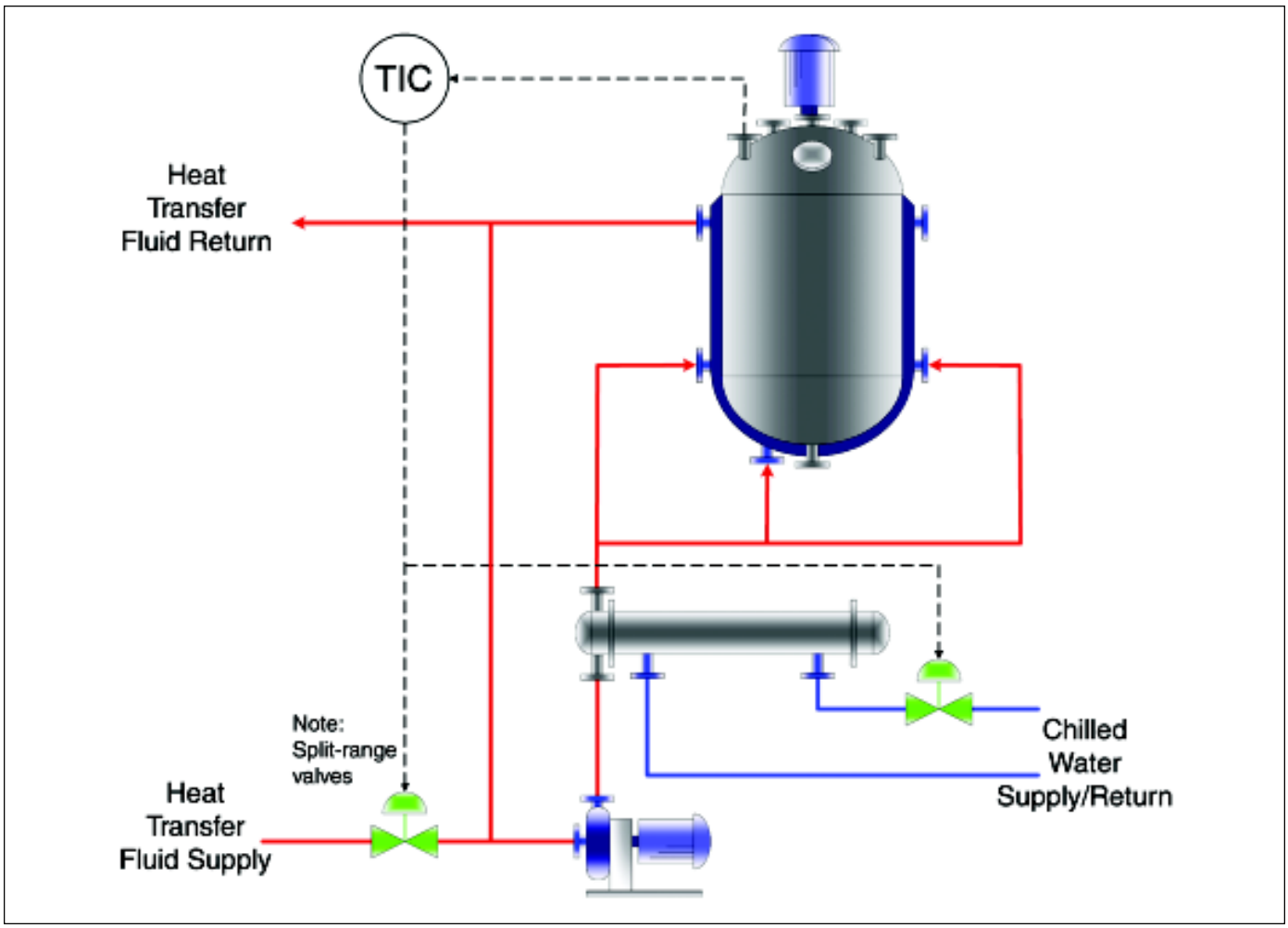

Figure 4. Hot central HTF system is cooled locally at the reactor.

system will operate, and the operating environment. It also defines non-functional requirements, constraints, and deliverables. With an emphasis on required functionality, rather than the method of implementing the functions, the URS is the basis for determining if the completed system meets the user's needs.[6]

2. *Begin developing schematic drawings.* A common approach is to start with block diagrams that show the various heating and cooling loads such as reactors and condensers. As details are determined, process flow diagrams with heat and material balances are created. The ultimate schematic drawing is the Process and Instrumentation Diagram (P&ID) that illustrates every piece of process equipment, pipelines, valves, instruments, and control logic.

3. *Determine maximum theoretical heat transfer loads.* Each load is listed (e.g., Reactor 1, Reactor 2, Condenser 1). The heating and cooling duties for each are defined and quantified. There are usually several different conditions to consider; each is listed along with the basis including frequency and duration. Calculations are done to compute the instantaneous loads.

4. *Account for diversity.* Timelines for each major unit (i.e., reactor) are prepared to illustrate the change in heat transfer load as reactions progress. See examples later in this article. Then, an analysis is performed to determine both the average and peak heating and cooling requirements, considering the individual major units independently and together. For instance, if four reactors are included in the project, the diversity analysis would determine whether peak heating is required simultaneously for all four or only one or two of them.

5. *Select the heat transfer fluid and distribution scheme.* Heat transfer fluid(s) are selected using criteria listed in Figure 4, and block diagrams are used to map out a distribution scheme. Some guidance is given later on selecting between central or local heating and cooling. The distribution scheme analysis should consider the size of the HTF storage tank (if any), used for a "flywheel" effect, and the flowrates for circulating pumps. If the reactors are not yet specified or purchased, the design of reactor jackets are reviewed to optimize heat transfer area and pressure drop and ensure that heat transfer "zones" are incorporated where necessary.

6. *Establish the control philosophy.* Create Piping and Instrumentation Diagrams (P&IDs) and at the same time

begin development of Functional Requirements Specifications (FRS). The FRS defines the system explicitly, including sensors, controllers, interlocks, alarms, and user interface. Performance requirements are specified in quantitative and unambiguous terms. Any user-configurable functions are described. The completed FRS is used as the basis for writing controls software; tests developed for commissioning or validating the system (OQ) will reference the specifics listed in the FRS.[1]

7. *Take care of details.* Steps 1 through 6 provide a solid foundation for building the system. Careful attention to details as the design, fabrication, and installation proceed will ensure that the requirements and expectations are fully realized.

System Calculations

Important calculations include the heat flux from reactor jacket, time for heating or cooling a reactor, duty for the overhead condenser, and overall system capacity considering diversity and heat losses.

Heat Flux

Heating or cooling from a reactor jacket or internal coil is described by the equation:

$$Q = U\ A\ \Delta T_{LM}$$

Q is the heat flux in watts (Btu/hr)
U is the overall heat transfer coefficient, watts/(m^2 °C) [Btu/(hr ft^2 °F)]
A is the effective heat transfer area, m^2 (ft^2)
ΔT_{LM} is the log-mean temperature difference between jacket and reactor, °C (°F)

Determination of U is somewhat inexact. It depends on many factors, including the geometry of the reactor, physical properties of the heat transfer fluid and the reactor fluid, flowrate through the jacket or coil, and agitation in the reactor.

Several correlations exist for calculating the film coefficients that are combined into U, the overall heat transfer coefficient. The correlations are specific to different jacket types. Some have been published[8-10]; others are proprietary. Low cost software packages are available that do the detailed calculations. Be aware that the correlations are only approximate; actual results deviate up to 30% from the calculations.

"Typical" heat transfer coefficients are published in many handbooks and texts. For instance, Perry's gives an overall U of 140 to 370 watts/(m^2°C) [25-65 Btu/(hr ft^2 °F)] for a glass-lined carbon steel vessel with heat transfer oil on the jacket and organics in the vessel.[11] Generalized tables of this type should be used with great caution because the actual coefficients are dependant on a large number of factors that the tables cannot account for. For example, 50% propylene glycol can be used down to about -20°C (4°F), but the viscosity at that temperature is over 50 cP reducing the overall heat transfer coefficient to below 50 watts/(m^2°C) [9 Btu/(hr ft^2 °F)].[12]

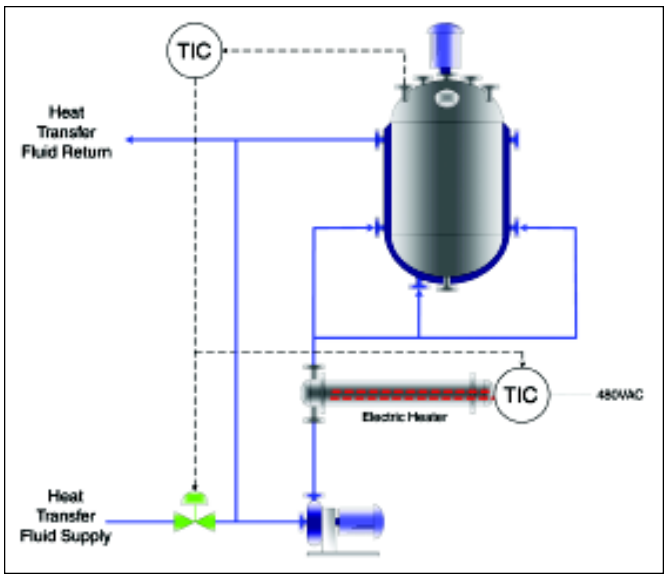

Figure 5. Cold central HTF heated locally using electric immersion heater.

Another important consideration is the amount of turbulence in the jacket and in the reactor. The flowrate of fluid through the jacket is usually designed to ensure turbulence. A pump is often used to circulate fluid through the jacket.

Another trick is to divide the jacket into parallel zones. Each type of jacket (annular, dimple, and half-pipe) can be constructed in independent stacked sections. Half-pipe coils also can be constructed in multiple interleaved spirals with each of the zones covering the entire reactor sidewall. Zoning is done for one of these reasons:

- Different fluids can be used in different zones, preventing cross contamination. Steam is segregated from chilled water. Or, heat transfer fluid is kept away from liquid nitrogen.

- Pressure drop is managed. High jacket flow may result in excessive pressure drop if the flow path is long. Instead of pumping 100 gpm through a single half-pipe coil, by dividing the loop into two zones and putting 50 gpm through each of two parallel circuits the pressure drop is cut by about 85%. Or, 100 gpm could be pumped through the two parallel loops (total flow rate of 200 gpm), cutting pressure drop in half since each of the loops is only half as long as the single loop alternative.

- Temperature change in the fluid is reduced. With sufficient time, the temperature of the jacket and reactor contents equilibrate. It is very easy to approach this equilibrium in jacketed vessels; dividing the flow to multiple zones decreases the effect.

- Heat transfer can be localized to a specific portion of the reactor. This is useful when working with reduced volumes of reactant. It is also helpful if the goal is to maintain the reactor contents at a temperature just below boiling.

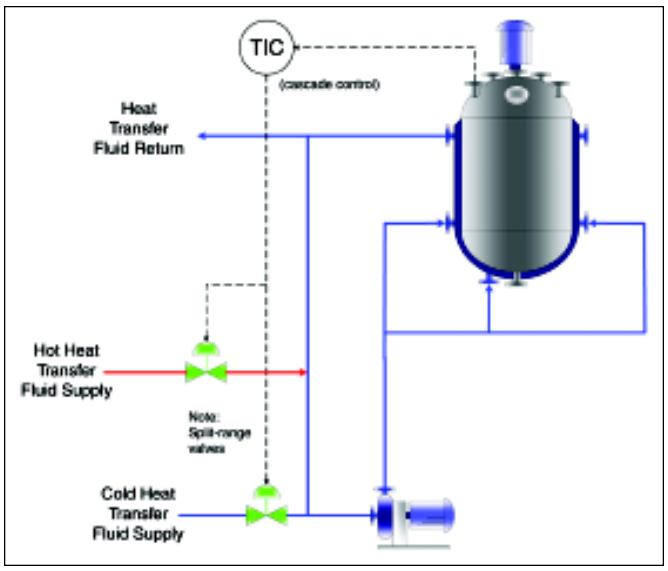

Figure 6. Centralized hot and cold HTF. See text for explanation of the HTF return path.

Time to Heat or Cool

Since U changes as the jacket and reactor temperatures change, the best way to estimate the time for heating or cooling is to recalculate U and Q at increments over the time period. Figure 2 shows a cooling curve for a reactor with jacket fluid at constant temperature. The rapid decrease in heat transfer is due primarily to the decreasing temperature difference between jacket and reactor.

In Figure 3, a heating curve is shown. In this case, the jacket temperature is constantly adjusted to maintain a 30°C difference with the reactor. The increase of the red heat transfer curve as the blue reactor temperature curve increases represents improvement in the overall heat transfer coefficient with temperature since the other parts of the equation remain approximately constant.

Overhead Condenser Sizing

The overhead condenser is sized to handle all vapor evaporated in the reactor. The condenser duty, in terms of heat exchange (watts or Btu/hr), is about equal to the heat input during the vaporization process (i.e., boiling). Therefore, estimate the duty requirements by calculating reactor boil-up under various conditions, including alternative solvents in the reactor (such as water, methanol, and acetone) and differing pressures (which will affect the boiling temperature).

Condenser sizing accounts for the temperature driving force (vaporization/condensation temperature less the heat transfer fluid temperature) as well as the presence of non-condensibles such as nitrogen that is used to blanket the head space of the reactor. The detailed calculations are well beyond the scope of this article; heat exchanger designers utilize one of several commercially available computer programs to size and configure condensers.

System Capacity Considering Diversity and Heat Losses

The total heating and cooling duties are determined on both peak and average bases. Individual loads are calculated as described above, but assembling them into a whole requires more than simply summing the parts.

The diversity analysis is done using a spreadsheet or graphical format. With a spreadsheet, you can indicate the approximate percentage of time that each load is on line. Then multiply the loads by their corresponding percentages and sum. A better method is to create a stacked graph of load versus time. For example, if the plant has three reactors that behave like Figure 2, simply offsetting the initiation of cooling by 30 minutes (Reactor 1 starts at, say, 9:00; Reactor 2 at 9:30; and Reactor 3 at 10:00) results in about 200,000 watts of peak cooling for the three reactors, compared with 300,000 watts if all three were to start cooling simultaneously.

When computing system capacities, consider miscellaneous heating and cooling duties and losses. Heating energy may be consumed by endothermic reactions, jacketed piping, thermal inertia (heating the metal in the reactor in addition to the process fluids), etc. Miscellaneous cooling duties include heat of mixing and pumping, secondary condensers, vacuum pumps, mechanical seals, etc. Energy losses occur through insulation, by radiation from hot surfaces, and other inefficiencies.

Heat transfer is driven by the temperature difference between HTF and process fluid. The assumptions made when computing individual loads should be reviewed when determining the overall system capacity. It is important that the design is consistent, yet flexible to accommodate changing conditions. Assumptions can be somewhat arbitrary; conduct a "what if...?" exercise to see the effect of a different set of assumptions.

Temperature Control

This section discusses alternative concepts for centralized and localized heat transfer systems. Central systems can be comprised of one, two, or three different temperature loops (hot, cold, ambient) with local heaters or coolers if necessary. We give advantages and disadvantages for different permutations.

At the localized level, there are various ways to transfer heat to/from reactors and overhead condensers. Energy efficiency, safety, and effectiveness are impacted. Our guidance will help you assess the choices.

We have chosen a typical jacketed glass-lined reactor for illustration purposes. The reactor has a full annular conventional jacket with agitating nozzles to improve heat transfer performance. Each nozzle requires about 120 liter/minute flow (32 gpm) for a pressure drop of 100 kPa (15 psi). A typical 4,000 liter reactor (nominally 1,000 gal) has three nozzles so the total flow through the jacket is about 360 liter/minute (100 gpm).

The way to control heat transfer for this reactor is to adjust the temperature of the jacket fluid. This is preferable to adjusting the flowrate because it results in more uniform temperature transfer throughout the jacket and can be finely controlled at the setpoint temperature.

Single Temperature Central HTF System

A central heat transfer system that circulates a single temperature fluid, either hot or cold, is very commonly encountered. There is often a buffer tank incorporated in the central circulating system that provides a "fly wheel" effect for smoothing transient loads. Cooling (or heating if the central system is cold) is provided at the reactor using a secondary fluid. The secondary fluid is not mixed with the central HTF (compare with the next scenario). Thus, for cooling it can be chilled water, tower water, another heat transfer fluid, liquid nitrogen, or a self-contained refrigeration machine - *Figure 4*. The advantage of doing this is that the indirect cooling (or heating) permits the use of different fluids for heating and cooling. However, there is a penalty in that the achievable temperature in the HTF circulation loop cannot reach the temperature of the chilled water due to the necessity of having a temperature driving force across the heat exchanger.

Local heating is economically achieved with an electrical immersion heater (up to about 600 kW power input). Steam also is used. The advantage of electric heat is its simplicity and reliability. If steam is used for local heating, then provision must be made to either drain the heat exchanger shell or bypass it during periods of cooling to prevent freeze-up in the exchanger.

Dual Temperature Central HTF System

A dual-temperature central system, using the same fluid, is advantageous for larger facilities because the heating and chilling equipment can be centralized in a mechanical room. Heating is typically done with a gas or oil fired heater, capable of reaching temperatures above 350°C (650°F). Special environmental permits may be required due to the potential for emitting nitrogen oxides. Central chilling is usually accomplished with mechanical refrigeration machines, either air or water cooled. Central chilled HTF systems usually operate at about -30°C (-22°F) - *Figure 6*.

For lower temperatures, a secondary heat exchanger can be installed at the reactor with liquid nitrogen being the cold source.

Note: Figures 6 and 7 show a generalized "Heat Transfer Fluid Return." In practice, there are three ways to control the return to ensure that the supply loops retain their volume. The simplest way is to return all fluid, hot or cold, to a buffer tank from which the supply loops draw. The second method is to have two or three buffer tanks (corresponding to the number of central loops), and return all fluid to a tank selected on the basis of return fluid temperature. The fluid supplies are equalized by interconnecting the buffer tanks. The third method is to provide modulating control valves that correspond to the modulating supply valves.

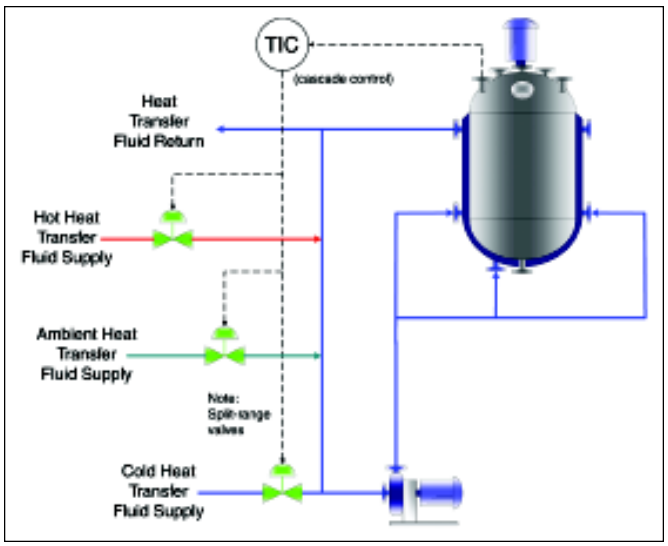

Figure 7. A 3-temperature central HTF system can be energy efficient.

Three Temperature Central HTF System

Some facilities use central HTF systems operating at three temperatures: hot, cold, and ambient. The hot and cold loops are the same as described above. The ambient loop is tempered in a closed cooling tower, interchanging its heat with the atmosphere without exposing the HTF to the environment. This is comparatively inexpensive and can result in significant energy savings.

There are several ways to pipe such a system. We like the method depicted in Figure 7 with modulating control valves on each of the three central supplies. The three valves in Figure 7 are controlled by split ranging the output of the temperature controller in three segments:

- An output in the range of 0 to 33% modulates the supply valve for the hot HTF from full open to full closed, and holds the return valve open. Above 33% controller output, both valves in the hot line are closed.

- At the other end of the scale, an output from 66 to 100% modulates the supply valve in the cold loop from closed to full open, and holds the return valve open. Below 66% both valves in the cold loop are closed.

- The valves in the ambient line operate when the output is between 33% and 66%, but the action depends on whether this range was entered from the hot or the cold side. In either case, the valves will be closed until the controller output enters the 33% to 66% range. If the range is entered from below, increasing the output from 33% to 66% causes the control valve to stroke proportionally open; as the output increases above 66% the valve closes. Conversely, if the range is entered from above, decreasing the output from 66% to 33% causes the valve to open. As the output falls below 33%, the valve is closed. In both cases the return valve is held open throughout the range 33% to 66%, and is closed otherwise.

- In steady state, when the reactor calls for no heat transfer, the HTF will circulate through the pumped sub-loop at the reactor batch temperature. The temperature controller output will settle at either 33% or 66% and all valves will be closed.

The advantages of this arrangement are:

- simple to design, install, and control
- maintains hydraulic balance among the three systems
- energy efficient

Local Heat Transfer Alternatives

All of the examples described above assume that a conventional reactor jacket is used, fed with a single fluid HTF. Conventional jackets also are used with steam/chilled water systems; most designs of this type include provision for removing residual liquid from the jacket prior to introducing steam so that glycol, heat transfer oil, and water treatment chemicals are not returned to the steam boiler.

Half-pipe coil jackets are often preferred for alloy reactors. Half-pipe jackets can easily be fabricated into independent loops, either stacked or interleaved, as discussed earlier. Heat transfer is more reliably predicted, and due to the smaller cross section of the half-pipe compared to an annular jacket, velocity (and heat transfer coefficient) are higher for a given flow rate.

An interleaved coil jacket allows for different heat transfer fluids without cross contamination. One of the best applications for this is to put liquid nitrogen into a dedicated coil for cryogenic reaction temperatures. This is being done at an API pilot plant in Pennsylvania (confidential source).

When additional heat transfer is required, beyond the capability of a reactor jacket, there are a few ways to provide it. Internal coils are often specified for this purpose. The drawback is that the coils present a significant cleaning problem in the reactor. Similarly, the baffles can be constructed with heat transfer capability. A method for achieving cryogenic temperatures is to directly inject liquid nitrogen into the reactor. This is very efficient from a heat transfer viewpoint, but can create undesirable temperature gradients within the reactor.

Another method is to pump the reactants through an external heat exchanger. Ed Steve provided a detailed discussion with calculation procedures in a recent article.[13] His approach is to use the external exchanger in conjunction with the vessel jacket to provide much greater heat transfer than is possible with the jacket alone, while avoiding the expense of adding internal coils to the reactor.

Final Words

Every API chemical synthesis relies on heat transfer systems. The systems are costly to design, install, and operate. It is well worth the effort to fully understand the capabilities and limitations of a proposed system before rushing to adopt it. This article provides insight into the methods used to build that understanding.

Optimization requires more than a detailed analysis of the heat transfer system. In a previous article, the advances in reactor technology that are aimed at improving both heat and mass transfer were described.[14] When the benefits of technologies such as spinning disc reactors or process intensification are realized, the size of associated heat transfer systems will be greatly reduced.

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A historical account of Process Analytical Technology and its adoption in pharmaceutical processing.

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An Introduction to Process Analytical Technology

by Nissan Cohen

Process Analytical Technology (PAT) is the usage of sensory data in any process. PAT was developed for telemetry. Telemetry, by definition, is the transmission of sensory data via wire, cable, telephone, radio, or wireless technology. Sensor telemetry has been implemented and employed extensively by NASA for more than 45 years. Remote sensing telemetry in NASA entails everything from the complete operational status of a spacecraft to an individual astronaut's heartbeat. PAT is the usage of on-line, in-line, on-stream, in-stream, real time, or near real time sensors for monitoring continuous and episodic operations.

The pharmaceutical industry has relied on laboratory testing methods in pharmaceutical manufacturing for the past 100 years. A "Laboratory Centric" view of pharmaceutical manufacturing became the norm during the last century. All manufacturing processes, product, and quality assurance issues were resolved, approved, or rejected via laboratory testing. The lag time for a laboratory's issuance of test data, approvals, and product releases was hindered by the testing bottleneck in the laboratory. The lab tests and preparations were arduous and time-consuming, with the need for repetitive testing and sampling. PAT removes laboratory testing from the manufacturing process.

The Spring 2003 issue of *Pharmaceutical Manufacturing* magazine mentions the FDA's interest in PAT. Dr. Ajaz S. Hussain, Deputy Director of the Office of Pharmaceutical Science within CDER, presented his views of PAT during the 17th International Forum on Process Analytical Chemistry (IFPAC/2003). Dr. Hussein offered a regulator's perspective of PAT. Emphasis was placed on the following comments: "Quality cannot be tested in pharmaceutical products. Rather, it results from a quality-oriented process design... without specific on-stream analytical instruments, final product quality is neither predictable nor controllable."[1]

Dr. Carmel Egan was a speaker at the same conference as Dr. Hussain. Dr. Egan commented

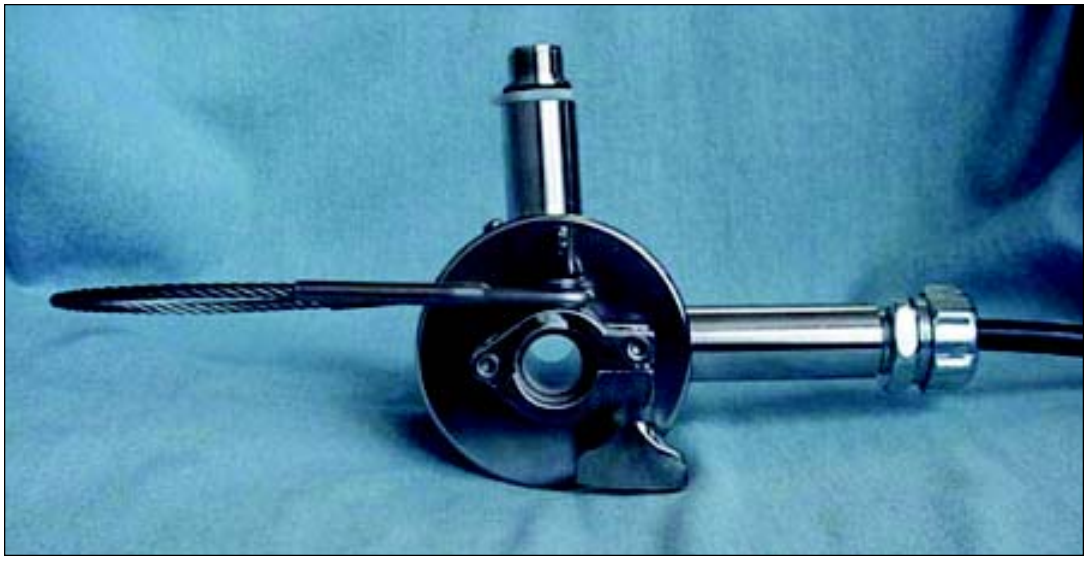

Figure 1. Stainless version of a sanitary flow through electrodeless conductivity sensor for either USP Purified or WFI water.

"...the PAT sensor will function in various ways to maintain the quality of the process. The process will never get out of control or meet a specification limit as threshold alarms will trip long before the process approaches a specification alarm."

on how "PAT can improve and possibly revolutionize drug development and manufacturing," and she noted, conversely, the laboratory testing bias for quality control by elucidating the following often-heard comments:

"On-stream measurement is not as good as in the laboratory; on-stream measurements cannot be validated; on-stream analyzer reliability is inadequate; a small skill-base exists for this highly specialized technology."

The statements above about "on-stream measurement" are inaccurate. PAT sensors are verifiable, accurate, and can be validated with repeatable results with less drift and less frequent calibrations than most laboratory instrumentation.

Historical Development and Introduction of PAT

The advent of sensors with telemetric capabilities harbored the beginning of PAT. Thermometers are not PAT devices. Thermometers are indicators of temperature, but without telemetry. The thermometer reading cannot be transmitted. Temperature sensors are indicators of temperature with telemetry. An analog signal is transmitted from the temperature sensor to a controller, indicator, or display. The development of microprocessor-based Programmable Logic Controllers (PLCs) in the 1970s bridged the final gap for the maximum deployment of PAT. Before the advent of PLCs, sensory data was displayed with limited action. Limited control schemes were based on simple logic using relays or delimiter schemes. Only when a threshold value was exceeded was the logic employed. PLCs instigated control schemes using feedback loops to monitor or initiate actions based on an analog value or digital status. As real time conditions changed, controllers could react to the changing conditions, in real time to prevent excursions in values, tolerances, or limits.

Figure 2. Calibration is inline, non-invasive, accurate, with no stoppage of water flow. Calibration unit is mated to the conductivity sensor as shown above.

Distributed Control (DCS) and Supervisory Control and Data Acquisition (SCADA) systems became the harbingers of integrated sensory data and control systems used in large process industries such as petrochemical, pulp and paper, and nuclear power plants. During the early 1980s, a new type of device was introduced called Process Analytical Instrumentation (PAI). These PAIs were on-line microprocessor-based instruments measuring single and multiple parameters with serial communications ports to export the multi-stream data. PAIs noted as on-line Total Organic Carbon (TOC) analyzers, conductivity/resistivity sensors, and particle counters with ultra-sensitive detection limits became instrumentation standards in the semiconductor industry 10-15 years before introduction and acceptance in the pharmaceutical industry. All continuous 24/7 utilities and processes in a semiconductor production facility use PAT and PAI technology. No laboratory tests are administered.

In 1997, the US Pharmacopeia published in USP 23 Addendum 5, the first PATs for on-line or laboratory usage.[2] USP <643> and USP <645> monographs were specifically written for the measurement of TOC and conductivity in USP Purified Water (PW) and Water for Injection (WFI). This was the first time the same test method was authorized for both on-line and laboratory applications.

Typically, PAT sensors offer immediate data and information on a given process. Parameters can be monitored at all times with information on excursions exceeding thresholds. These thresholds and alerts need not be specification alarms only. Thresholds can be intermittent limits within a Statistical Process Control procedure. Trending towards a specification limit can be mitigated by placing intermittent control alarm limits below the specification thresholds. In this manner, the PAT sensor will function in various ways to maintain the quality of the process. The process will never get out of control or meet a specification limit as threshold alarms will trip long before the process approaches a specification alarm.

The conductivity sensor displayed below is installed in a USP Purified Water system. The sensor continually measures conductivity and has been calibrated. The conductivity

sensor measures the conductivity of the water and compares its readings to the threshold values for trending analysis. As long as the measurement of the conductivity remains below a customer-ordained threshold value, the process continues unabated. If a threshold alarm should occur the water system can continue to provide USP Purified Water to the process, as the threshold value is a control value only, not a specification value. Only if the water system exceeds the specification value is there jeopardy in the supply of USP Purified Water.

Increases in conductivity can manifest in many different ways. The causes of increased conductivity can be attributable to Ion Exchange depletion, changes in the pretreatment steps, different water sources, Reverse Osmosis (R/O) membrane issues, etc. The conductivity sensor as a PAT will indicate the water quality at all times. Judicial usage of PAT can be used in feedback loops to a PLC to initiate other actions. If the Ion Exchange is depleting over time, change out and regeneration procedures can be initiated, manually or automatically, using the conductivity sensor and threshold values to engage the PLC for regeneration.

Figure 1 depicts a stainless steel version of a sanitary flow through electrodeless conductivity sensor for either USP Purified or WFI water. This sensor is available in various bore sizes from ½ - 4 inch. The device can be calibrated in-line, without removal, and without stoppage of the water flow. Figure 2 shows the in-line calibration technique for the conductivity sensor in Figure 1. This PAT device not only sends data to another device but prevents downtime associated with line stoppage for calibration. Additionally, no recertification is needed, the calibration procedure is non-invasive, is not affected by flow rates, and has the least possible affect on process runtime.

Pharmaceutical Manufacturing Today

Today, pharmaceutical manufacturing employs PAT sensors, PLCs, feedback loops, controllers, displays, and man-machine interfaces (MMI) with sensitive calibrated sensors in continuous and batch processes with 24/7 uninterruptible operations. In addition, Web-based devices allow the usage of an internal network to transmit the data to reception devices in the facility, to a remote central location, or to any location worldwide. PAT has improved product quality, throughput, and the product uniformity. These highly controlled and real-time monitored processes produce excellent and repeatable products. The scrap and product destruction due to laboratory testing is virtually non-existent. Previously, laboratory tests could fail a production lot for any reason.

In the near term, laboratory testing will remain a fixture in the pharmaceutical industry. However, the amount of tests and the types of tests are diminishing as more sophisticated PAT and PAI devices are deployed.

Future PAT Applications

Many of the exclusive laboratory tests performed routinely today for pharmaceutical manufacturing will be eliminated in the future. Bacterial Endotoxin Testing (BET), microbial growth and presence testing, and ion chromatography will be converted to on-line or at-line testing methods with no human intervention. Labor-intensive laboratory preparation and testing will be outmoded as the automation sequencing is refined and standardized.

The new sciences of micromachining, nanobots, and engineering at the atomic level will leapfrog the in situ process environment and redefine Process Analytical Technology. In the future, microdesigned machines, a few micrometers or less in size, will be able to fabricate, synthesize, and produce genetic components in microproduction facilities enabling vast quantities of pharmaceuticals to be produced in 24/7/365 non-stop manufacturing environments.

Summary

Process Analytical Technology is a vast pool of sensors and technology allowing immediate feedback and control of any given process. PAT sensors range from single channel output examples of temperature, humidity, and flow sensors up to and including sophisticated Process Analytical Instruments offering many channels of information through a single communications port. PAT sensors can be validated, calibrated, and employed in the pharmaceutical manufacturing environment.

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About the Author

Nissan Cohen has over 29 years of experience in Instrumentation and Monitoring in Semiconductor Manufacturing, Pharmaceutical Process and Production, Ultrapure Water, Industrial Water, Chemical Systems, and Fossil and Nuclear Power Plants. Cohen has written over 25 technical and peer reviewed articles for various publications including *Pharmaceutical Engineering, Pharmaceutical Technology, Ultrapure Water, Semiconductor International, Microcontamination, A2C2,* and *The Journal of the Institute for Environmental Sciences.* A recognized worldwide expert in TOC and water systems, Cohen is a member of the International Society for Pharmaceutical Engineering (ISPE), the Institute for Environmental Sciences (IES), an editor for the *Journal of the Institute for Environmental Sciences,* and Chairman of the Web Site Subcommittee of ISPE. Cohen received a BS from the University of Wisconsin and a MS from Hebrew University, Israel. He can be reached at tel: 1-303/926-1866 or email: coguy@eudoramail.com.

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With the multitude of challenges facing the Food and Drug Industry, compliance to 21 CFR, Part 11 need not be a difficult one, particularly with the advancement of the Smart Chart Recorder.

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Next Generation Chart Recorders: Part 11 in a Box

by Matt Bothe

Among the multitude of systems serving the food and drug industry comes a practical solution for those acknowledging and seeking the benefits of electronic recording while maintaining the required level of FDA compliance to 21 CFR, Part 11 regulation: the Smart Chart Recorder. However, before we detail the reasons why chart recorders have evolved into practical approaches to 21 CFR, Part 11 (or simply Part 11), an introduction to the "rule" is provided followed by a cross-reference between the code components and attributes of the chart recorder. Additional detail is provided differentiating the more prominent aspects of conventional data historians from the chart recorder alternative with regard to achieving and maintaining Part 11 compliance, while applying methods with which the end user feels comfortable.

Introduction

The Code of Federal Regulations 21, Part 11, has been in effect as an enforceable component of validation compliance in the Food, Drug, and Medical Devices industry since August of 1997. Its primary focus covers the handling of records in electronic form (Erecs) and the authentication of such information through electronically produced or reproduced signatures, user codes, and biometrics (Esigs). As with all FDA-mandated regulations, the fundamental purpose of Part 11 is to protect the health and well being of the public from the consequences of fraudulent or accidental manipulations of electronic data. Unlike data that has been erased and rewritten on paper copies, manipulations of data in electronic form are considered far more difficult to track. Part 11 establishes the criteria whereby electronic records and signatures will be considered to be AS TRUSTWORTHY AS, AS RELIABLE AS, AND EQUIVALENT TO paper records and handwritten signatures. The functional purpose of the regulation is to allow for the use of new computer-based technologies. Pharmaceutical companies strive to remain in competitive positions, reduce costs of operations, and quicken product release time. For companies to take advantage of the latest electronic recording, compliance to current regulations must be met. Selection and designing the most cost effective reliable recording solution that creates electronic records containing reportable data makes good business sense.

There are many reasons why Pharmaceutical companies should opt for Erecs (in lieu of paper records) seven of which are listed below:

1. Data Processing and Storage

Data collected electronically has a far greater potential to be easily processed and managed than their paper counterparts. Large amounts of electronic data can be stored, sorted, grouped, categorized, and maintained with relative ease using a variety of software applications readily available. The storage of historical data alone can result in significant savings in real estate, hence adding valuable space for lab or production equipment.

2. Statistical Computations

Data can be compared to each other and other parameters (such as time) in an attempt to establish relationships and patterns for the purposes of quality control and process improvements - impractical feats for the paper alternatives.

3. Inferential Determination

Applying the principles of relationships (from item number 2) and balances (i.e. material and energy), savings in field device purchases and maintenance can be realized from calculations using related variables. Two common applica-

tions that make extensive use of Inferential Determination involve analyzers and flow reconciliation. Monetary savings are typically derived through cost avoidance and added reliability.

4. Improved Control and Optimization (Academic Utilization of Electronic Process Data)

With electronic data, practical models can be identified that closely define the current behaviors of particular processes. From these models, non-intrusive tests can be run to determine optimum operating conditions as well as highly effective control methodologies (particularly those derived from dynamic modeling).

5. Interfaceability

Data can be much more easily shared in electronic form than paper records, not only among the users of a particular control system, but also interdepartmentally throughout the entire organization. The Internet has added another, highly flexible layer of communications thereby enabling Business-to-Business and similarly structured transactions.

6. Retrievability

Applicable to most any database environment, and large repositories of process data, the presentation of information in readable and useable forms is paramount. With a relatively small amount of effort, enormous databases with a plethora of inter-related tables and fields can be quickly searched and easily presented through the use of such high level languages as SQL (Structured Query Language) or VB (Visual Basic).

7. Electronic Submissions

Considering the concepts of Interfaceability and Retrievability, electronic submissions to regulatory agencies are greatly simplified. Of course this added simplicity breeds increased vulnerability to data integrity and consequently a greater concern for data security. The increased concern for data security is precisely the focus of Part 11.

The desires of many companies that currently use or wish to apply automated systems for control and data processing include the ability to take full advantage of the benefits listed above - some of which are not even feasible without computer-based systems and networks. Although past interpretations of Part 11 pressured many in the industry to over-engineer their compliance strategy, the risk-based approach to the regulation should not be perceived as a deterrent from automation, and certainly not be an excuse not to automate. Even without Part 11 in the mix of validation requirements, a company can still take full advantage of items 1 through 6 listed above as long as their procedures detail the use of such electronic data stored long term (greater than 24 hours).

Two factors that profoundly affect Part 11 compliance are: 1) does the data collected directly or indirectly impact product quality and consistency, and 2) will the data collected be used on a batch or production record to be submitted to a regulatory body such as the FDA? According to the currently accepted version of the FDA "Final Guidance on Part 11 Scope and Applicability", the implementation of Part 11 by those subject to its influence is expected to take a more risk-based approach to compliance. In other words, the producers of human-consumable products, recognized as being closest to and most familiar with their respective methods of production, should be allowed to decide the most suitable approach to Part 11 compliance, as well as decide the representative set of measurements that provides the required proof of product quality and consistency. Note that not all data collected on a historian need be reported to a regulating body. Since the quantity of data subject to Part 11 compliance is nearly always a relatively small subset of the total tag list of archived information, it is up to the End User to determine what information is required – as long as the information used (for Part 11 compliance) can be proven as accurate and reliable. With this in mind, does it really make sense to validate a data historian to the Part 11 regulation if the vast majority of data collected need not be compliant? For a control system with 100 points, for example, and 18 of which possess FDA-significance, it would be far easier to protect the integrity of only 18 points as opposed to the entire gamut of 100. The chart recorder - an isolated, independent component of overall data recording - can be easily configured to process and retain FDA-sensitive data in a compact, flexible, and secure environment, with Part 11 compliance about as "off-the-shelf" as one can come. If the recorder is securely interfaced via Ethernet to a Process Control System or Local Area Network, the data can still be made available for other uses, if such is desired, without violating compliance.

Aside from the rogue administrator, one of the primary concerns of Part 11 compliance involves data retention, keeping in mind that there are numerous options as long as meaning and content of archived information are preserved. Over a period of 30 years or more, the obsolescence of applications can be a greater concern. In other words, if vital batch records are stored on removable media applying a particular data format (say v3), how will the data be retrieved (in readable and useable form) 20 years from now using an application compatible with a later data format (say v7A)? Although a chart recorder storing data on removable media is faced with similar challenges, the evolution of chart recorders can be such that the preservation of such formats is maintained. Therefore, even if the application is no longer available, applications can continue to be developed around an earlier format thereby maintaining compatibility from one generation to the next (i.e. backwards compatibility). Perhaps much of this perceived rigidity of the chart recorder could be attributed to its specific, non-generic nature.

Implementation

The new generation of chart recorders have the capacity to not only be configured to collect a considerable amount of data (i.e. up to 80 parameters plus considerable text for long-term storage), but also be interfaced to a control system network

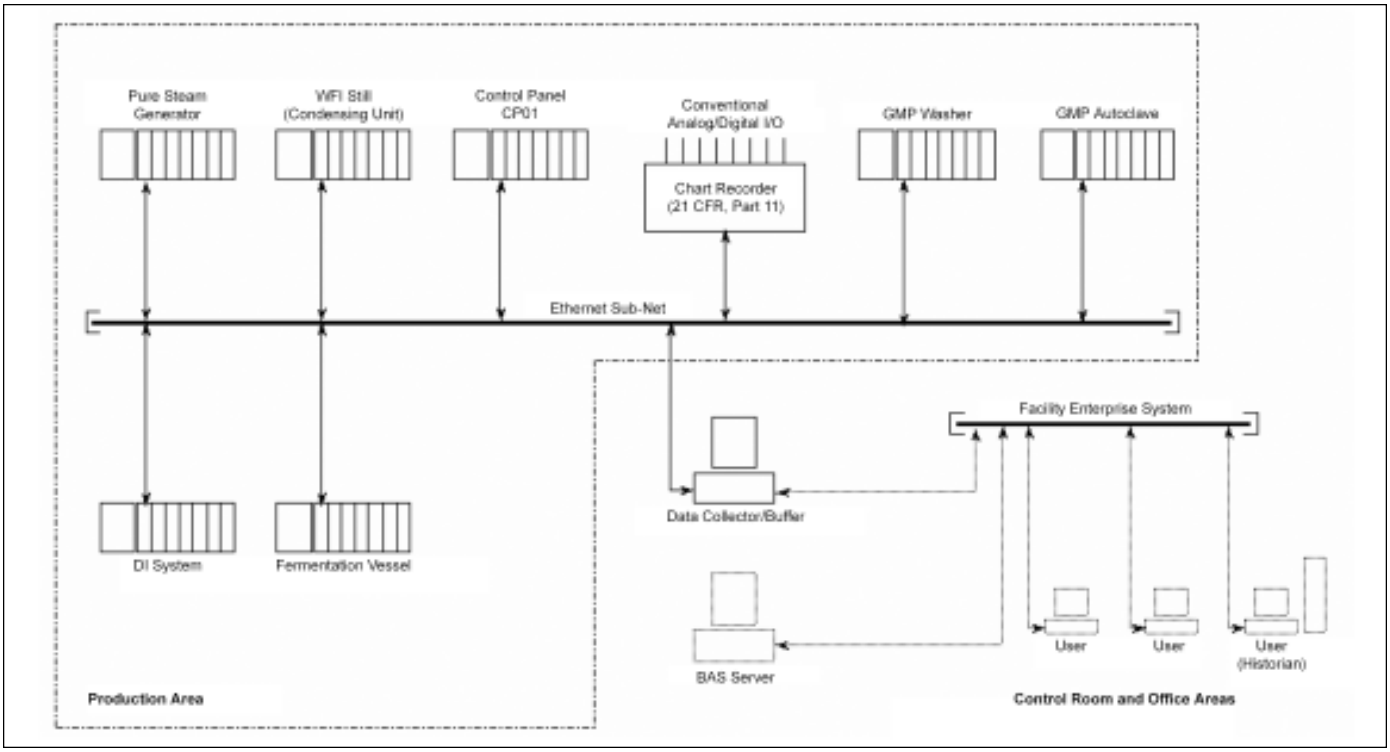

Figure 1. Typical Networking Architecture for Process Monitoring.

with regard to data availability and sharing. The illustration in Figure 1 depicts a smart chart recorder tied to a distributed monitoring system network.

For the network depicted in Figure 1, the Part 11-compliant digital chart recorder collects data either directly from the field devices via conventional I/O, along the Ethernet highway from the PLC contained in control panel CP01 as well as those from the packaged units (if configured to do so), or both. Through secure links, limited by (read only) data fetches from the chart recorder, the PLCs need not be Part 11-compliant (since no long term dynamic data is stored), but must be validated as viable and reliable sources of process data. Developmental and maintenance procedures are applied to cover the changes made to PLC control code and other system loadables to satisfy the validation compliance expectations per 21 CFR, Parts 210, 211, and other associated regulations. These efforts are very similar to the validation expectations of related field devices (i.e. intelligent transmitters).

Chart Recorder Part 11 Compliance

In an effort to justify Part 11 applicability, many claimants to compliance prepare White Papers cross-referencing various features and functions to interpretations of the code - placing much emphasis on access security and activity logs via audit trails. Compliance references to application obsolescence, rogue administrator, and open system topologies rarely exist, or are often not supported. Table A provides a brief cross-reference to each of the more significant Part 11 compliance issues. Among those compliance issues most often avoided by alternative systems may be easily addressed through procedures. Chart recorder data storage media and manual input devices have the capacity to be physically locked in place, accessible only with an appropriate key. Additionally, chart recorders may be totally isolated components of a process control network, not imbedded in mainstream operations. To compliment the Part 11 compliance issues addressed in Table A, other advantages attributed to the use of chart recorders as Part 11 compliant data repositories include the following:

1. Ethernet interface availability for high-speed integration into new or existing network systems and the internet, also configured with web-based data transmit/receive formats, protocols, and methods

2. Multi-functional displays configured for data specifically identified for Part 11 compliance

3. Easy differentiation between data vital for product quality and consistency, and information with purely academic and business significance. It is important to note that the chart recorder is not intended to replace the more conventional data historian. The chart recorder applications emphasized in this paper address specifically the information required for validation compliance as defined by the End User and deemed important through End User/FDA corroboration.

4. Addresses obsolescence of databases by preserving the on-disk data format in an environment not directly influenced by PC-based industry practices.

5. Addresses the Rogue Administrator issues by providing both physical and software access restrictions in an envi-

ronment that maintains accessibility by operations and quality personnel.

6. Although not considered an open system, the chart recorder maintains its highly configurable status to the point flexibility is not ultimately compromised.

7. By adding one or more Part 11 compliant chart recorders to a Process Control Network along with a conventional Data Historian, developing procedures to regulate the use of historian data as presentable to the FDA is easily managed (i.e. "Any and all data derived from the subject historian is not Part 11 compliant and can therefore not be used as a validated data source for batch or production records to be submitted to the FDA as reflections of process performance and quality").

Of course, linking a Part 11 compliant chart recorder to a Process Control Network (Ethernet or otherwise) is only an option. Many of the newer chart recorders can be configured to serve as totally stand-alone repositories of process data sets (selected by the manufacturer of human-consumable products as evidence of quality and consistency), complete with a multitude of display capabilities and reporting functions. As the chart recorder evolves, much, if not all, event trapping and interpretations of events through custom text messages and prompts can be easily imbedded into process data and trigger reports generated during batch execution cycles. Batch numbering schemes, already provided with configurable features to auto-increment, may be tightly associated with and carried throughout real-time batch operations. These identifiers may in turn be used to securely reference batch information for reporting purposes as well as within records to be submitted for FDA review.

Assessment of Alternatives

With conventional historians, the complexities and challenges are numerous, and are often shrouded in a veil of confusion and uncertainty. This should be no surprise considering the more noticeable attributes of current server technologies…namely:

1. Security Management systems that can never "really be truly" validated (i.e. validation is often implied through wide-spread use of the SMS tightly inter-twined within a proprietary operating system with enormous utilization).

Reg Item	Requirement	Chart Recorder	Remarks
11.10(a)	System Validation	Included	Data integrity checks for invalid data identification
11.10(b)	Data retrieval and presentation	Included	Via screen configuration of trends and event logs
11.10(c)	Retrievable throughout retention period	Included	Capabilities include data transfers from/to removable disks for future retrieval/presentation
11.10(d)	Access Control	Included	Via Username/Password prompting
11.10(e)	Audit Trails retention and retrieval	Included	Audit trail to record any manipulation of data (both measured and configuration changes) and events formalized for periodic retrieval
11.10(f)	Enforcement of pre-defined execution of sequential steps	Not applicable	Although possible, no control is managed directly by a recorder. However, sequence of events data collected from a discrete control (or logic) device such as a PLC can be analyzed through recorder applications if configured to do so.
11.10(g)	Authority checks for accesses	Included	Usernames and passwords assigned for multiple users and associated functions/rights.
11.10(h)	Validation of Data Sources	Included	Via data input ranges and reconciliation techniques or comparisons
11.10(i)	User training and experiences	Procedure-based	User profiles to assess access levels
11.10(j)	Deterrence to record falsification	Procedure-based and Physical lockout capabilities	Physical access controls associated with recorder compliance provide much of this deterrence.
11.10(k)	Document Controls	Procedure-based and configuration of documentation tools	Measures to limit presentation and printout of recorder data may be configured. Revision controls via audit trail.
11.30	Data encrypted and signatures used	Included	Signatures required and retained throughout configuration and data retrieval sessions. Data files are in a binary format conducive to tamper-resistance.
11.50	Required signature data	Included	User profiles developed and accessible by document utility and audit trail
11.70	Signatures linked to Erec	Included	Refer to item 11.50
11.100	Signature uniqueness/controls	Included	Via duplicate signature identification and rejection
11.200	Signature collaboration	Included (procedure-based protocols required for format)	Password aging and number of access attempts-to-lockout configurable
11.300	Lost device and altered data identification	Included	Via procedures (lost devices), access security, and audit trails

Table A. Chart Recorder Cross-References to Part 11 Regulatory Compliance.

2. The database structures and tables most often used to manage data are highly dynamic resulting in a high probability of obsolescence issues arising over the course of the retention period. Note that some retention periods can span a time period in excess of 30 years.

3. Since many of the security measures applied are similar to those among facility enterprise network systems (vis-à-vis LAN), the rogue administrator concern should not be ignored.

4. Considering item 3, the conventional data server may have a close association with Information Technology (IT), and therefore must abide by their rules. These rules are often established under a set of priorities not necessarily consistent with those most closely associated with the validation effort.

5. Among the more prevalent and difficult complexities linked to conventional data servers include the challenges associated with managing data, as well as differentiating archived data that provides evidence of product quality and consistency from all the rest (a situation that becomes more exacerbated as data densities and diversity increase). If such a historian exists, pressures from Process and Maintenance Engineers, among others, also may desire data for academic or business purposes accessible from a single source. Multiple historians with differentiable uses may be difficult to justify, and validation of both (in principle) may be both formidable and costly.

6. For any existing or proposed open automation systems with regard to Part 11, suppliers and contributors impacting the development of a related compliance strategy (as a corroborative effort with the end user) for a data repository, and associated components thereof, should be familiar with the expectations of the regulation, as well as have verifiable experiences in such related areas. The rules outlined and referenced in 21 CFR, Part 11, section 11.10(i) apply.

7. Conventional servers are generally networked via standard media and protocols (i.e. Ethernet) as the most practical means available to collect data. These linking characteristics place the server in a position of vulnerability to unauthorized accesses and subsequent manipulation of records (accidental or otherwise). Chart recorders, on the other hand, possess onboard converters designed to accept a variety of discrete and analog signals less likely to be adversely affected by remote accesses.

In addition to the seven items listed above, chart recorders may be dedicated to specific purposes (i.e. repository for Part 11 data as one) thereby facilitating the maintenance of the device and protecting the integrity of its data - resulting in significantly lower implementation, validation, and maintenance costs. The conventional historian, due to its generic nature and diversity of oftentimes unrelated functions, may be more difficult to explicitly dedicate to Part 11 compliant data and justify the cost to develop and maintain the environments in which the data reside.

Conclusion

There are many interpretations of the Part 11 code, and at least an equal number of methods by which Part 11 can be applied. This diversity should not be met with resistance (particularly to automation), but to an understanding that the code was written to not provide the "HOW to secure and authenticate data", but rather the "WHAT is required to be compliant." This inherently places the responsibility of code interpretation, implementation, validation, and maintenance into the hands of the End User. Since the End User may not be familiar with all the technologies available to execute such a compliance effort, it is usually advisable for the End User to consult with technology providers who not only understand the Code's intent, but also are familiar with the popular hardware platforms and software applications available to assure End User compliance to Part 11.

Since tangible benefits are generally difficult to quantify, cost carries a considerable amount of weight while deciding on a Part 11 compliance strategy, and whether to even automate. The application of chart recorders considers both complexities of implementation (including validation) and the maintenance of user-selected, product-critical data stored over the entire retention period (following implementation) as the basis for assessing cost vs. benefit relationships. Since the chart recorder, as an independent repository for Part 11 data, addresses only data with Part 11 significance, and with all the features and functions of a Part 11-compliant data server, they (chart recorders) can easily be viewed as a practical, cost-effective approach to a potentially over-engineered and costly alternative. The inherent simplicity and manageability of the chart recorder only increases its cost advantages.

Another benefit includes the concept that if one chart recorder is successfully validated (per Part 11 et. al.), and satisfies the requirements of the End User (per User Requirements Specifications, or URS), the validation efforts of subsequent chart recorders are reduced to only validating the data inputted to and read from the devices - greatly simplifying the Part 11 validation efforts of larger, more complex systems. The required additional validation effort may be confined to verifying the integrity of the input data as well as the sources from which the data is derived (as confirmed during IQ). The only exception may include any changes to the way the data is pre-processed prior to being logged – in which case a simple "code review" may be in order.

Remember, it is important to note that the chart recorder is not intended to replace the conventional server; it is merely a highly practical and cost-effective solution toward Part 11 compliance for entities of any size working on or maintaining a position to take full advantage of the benefits of automation. The current risk-based approach to Part 11 compliance, dealing only with product data that influence product quality

and consistency, only further enhances the use of the chart recorder as a viable repository of Part 11 compliant data.

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About the Author

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This article discusses the effects that stainless steel bottomed trays have on the heat transfer process during primary drying.

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The Influence of Bottomed Trays on Sublimation Rates

by Edward H. Trappler, Wendy B. Sunderland, and Timothy F. Gentilcore

Introduction

Lyophilization is a method of preservation in which a solution is first frozen with subsequent removal of the solvent, generally water, initially by sublimation (primary drying), followed by desorption (secondary drying) to a moisture content that no longer supports biological activity or chemical reactions.[1] In the pharmaceutical industry, an important objective of batch operations is to achieve reproducibility in processing and assure product uniformity. Specific to lyophilization, the objective of this low temperature vacuum drying is to effectively remove sufficient solvent to promote adequate stability, while retaining the original attributes and activity of the drug. Efficiency is also desirable for processing in the shortest amount of time to minimize the costs of freeze-drying. The greatest success is achieved when all three of these objectives are accomplished.

The initial drying step in the lyophilization process is sublimation, with converting ice from the solid state to the vapor state, without passing through the liquid phase; this step is commonly known as primary drying. In order for this event to readily occur, the pressure surrounding the product is reduced to well below that of the vapor pressure of the ice in the product. The addition of heat is then required in order to supply the ice with sufficient energy for sublimation to continue at an appreciable rate.

Heat to promote continued sublimation is provided to the product by warming the shelves with circulating heat transfer fluid. The heat is then transferred via conduction from the shelf to the bottom of the product container, or when using a bottomed tray, from the shelf to the tray bottom, through the tray and from the tray to the container. Heat energy passes through the container and frozen product, ultimately to the sublimation front. Figure 1 depicts the heat transfer from the shelf to the product for both bottomed and bottomless trays. Heat is consumed as the ice is converted to water vapor at the ice-vapor interface forming the sublimation front. As the quantity of heat supplied to the sublimation front dictates the quantity of ice that will sublime, effectiveness in heat transfer from that supplied by the circulating heat transfer fluid to the sublimation front becomes a rate limiting factor.

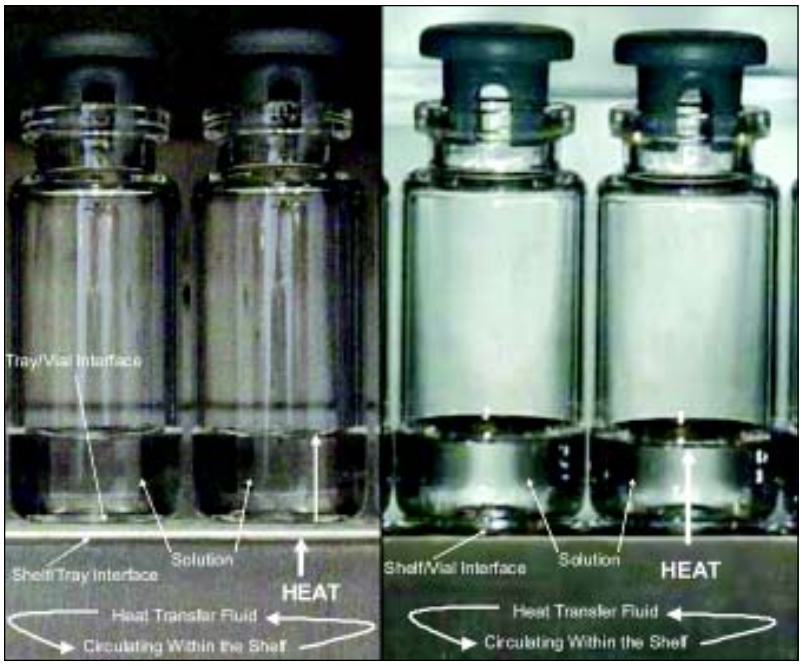

Figure 1. Heat transfer from the shelf to the product using a bottomed tray (left) and a bottomless tray (right).

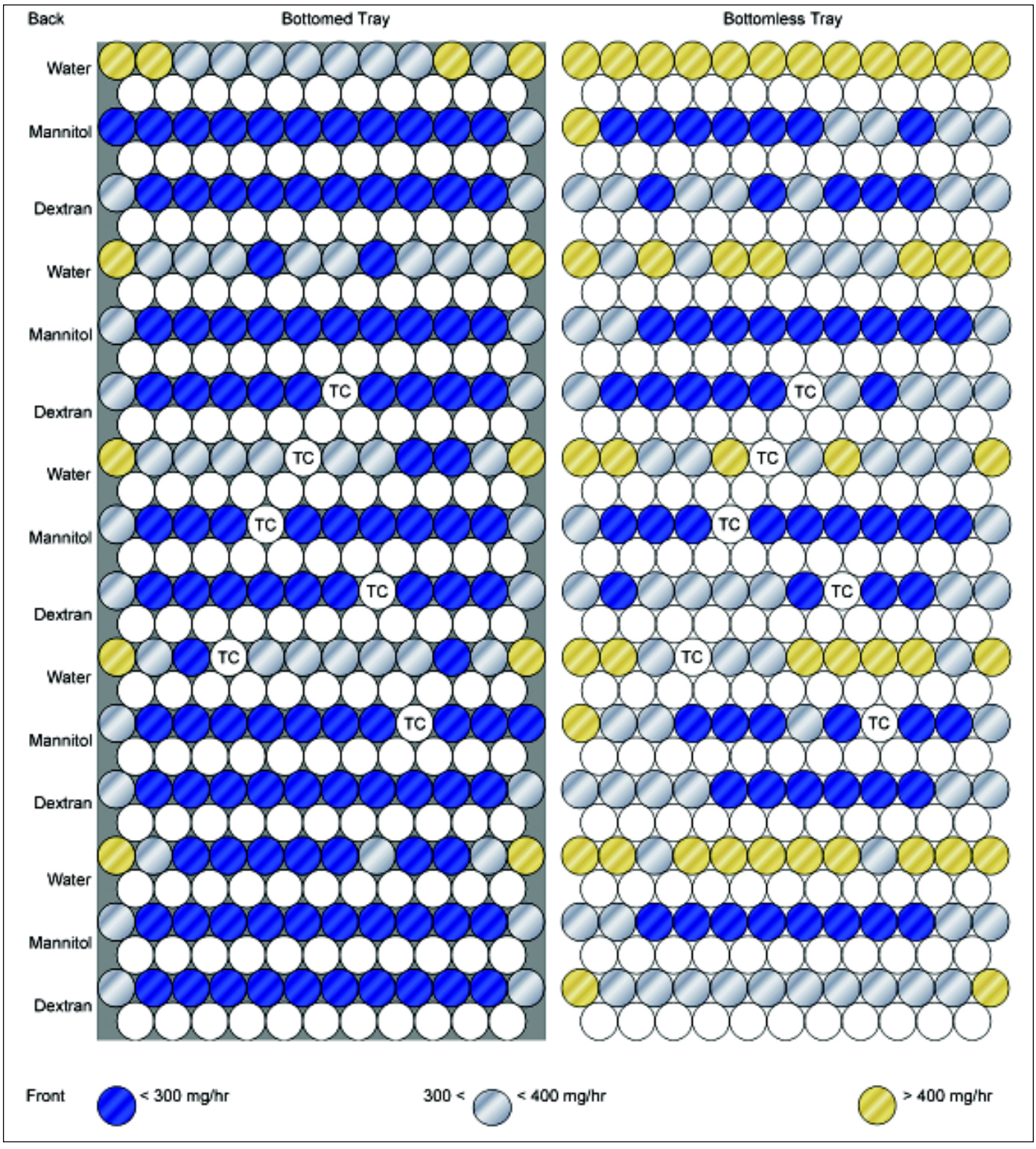

Figure 2. Sublimation rates of individual vials during two separate studies.

In the string of avenues for heat transfer, presence of a tray adds supplementary thermal resistance to the heat transfer process and in turn hinders the supply of heat to the containers. In addition to influencing overall heat transfer by their presence, tray bottoms become warped with repeated use over time and consequently result in very diverse drying rates for product containers within a tray, with corresponding variation in product temperature profiles. For improved heat transfer and vial-to-vial uniformity, trays have been constructed where the bottoms forming the trays can be removed after the vials have been placed onto the dryer shelf, leaving only a ring to contain the vials. It is hypothesized that the removal of this thermal resistance will result in greater heat transfer from the shelf to the vial, leading to an in-

creased sublimation rate along with improved uniformity during primary drying.

Heat transfer from the heat source to the sublimation interface has been purported to be the rate-limiting step in sublimation.[2] The rate of sublimation would therefore increase by the transfer of additional heat energy with decreased resistances in overall thermal conductivity. The purpose of this investigation was to determine if product containers placed directly on the shelf with the use of a bottomless tray would result in more efficient heat transfer and improved uniformity as compared to processing with containers left on a stainless steel bottomed tray. Effects of the differences in overall thermal conductivity as a result of the type of tray used were quantified by comparing the sublimation rates of the two studies.

Materials and Methods

Three preparations were frozen and primary drying conducted for three hours using what may be considered moderate processing conditions to show variation in sublimation rates using either a bottomed or bottomless tray. The preparations included Purified Water, USP as a control and model preparations representing amorphous and crystalline solutes: 5% w/v Dextran 3,000 and 5% w/v Mannitol, USP. Purified water was used as prepared. The Dextran solution was pre-filtered through a 0.45µm syringe filter, and then filtered through a 0.2µm sterilization filter. The Mannitol solution was filtered directly through a 0.2µm sterilization filter.

The three liquids were each filled into sixty 10cc Type 1 glass tubing vials to a target fill volume of 3mL. The vials were then partially stoppered with grey butyl 20mm stoppers. Each filled vial and stopper was weighed individually on an analytical balance to the nearest tenth of a milligram prior to being loaded into the lyophilizer. Thermocouples were precisely placed at the bottom center of six specified vials and randomly positioned throughout the tray in order to monitor product temperatures during processing. Identical placement of thermocouples and location of the monitored vials in the freeze dryer occurred for both studies. The vials with thermocouples were positioned toward the middle of the tray in order to negate any edge effects that would influence the product temperature had the vials been placed along the outer boundaries of the lyophilizer. The three formulations were positioned on the shelf in individual rows, which alternated with rows of open vials containing purified water - *Figure 2*. The open vials were present simply as fillers and were not used for analytical purposes. The first study included vials placed into a dryer with a single 12" x 24" shelf and six thermocouples for monitoring product temperatures. The bottom of the 0.06" thick 304 stainless steel tray, shown in Figure 3, remained on the shelf under the vials for the initial study. The second study utilized a bottomless stainless steel tray where the tray bottom was removed prior to processing, leaving the vials in direct contact with the shelf and the ring surrounding the vials. The samples were processed using the same lyophilizer as in the first study.

Figure 3. A lyophilization tray with an inner ring; the bottom can be removed while the ring remains in place during the lyophilization process.

The shelf was pre-chilled to 5°C before the vials were loaded. The samples were allowed to equilibrate for one hour after loading. The shelf was then chilled at an average controlled rate of 0.1°C min^{-1} to a final temperature of -45°C and controlled at the target set point for six hours. After the completion of freezing, the chamber was evacuated to 200 µHg (26.66 Pa) and the shelf warmed to 0°C at an average controlled rate of 0.5 °C min^{-1}. The vials were then held under these conditions for three hours while sublimation progressed. After the allotted time, the chamber pressure was raised to one atmosphere using filtered nitrogen; the vials were stoppered, warmed to 20°C, and then removed from the chamber. The vials were once again individually weighed and their differences in weight calculated. The difference of the final weight to the initial weight was then divided by the three-hour drying time producing an average sublimation rate of ice from each formulation.

Results

Shelf temperature and chamber pressure were controlled within a nominal range to ensure comparable methods. Shelf temperature was controlled by a controller within a range of 3°C of the set temperature. Another controller maintained the chamber pressure at 200 ±7 µHg (26.66 ±0.93 Pa). The two controlled variables of shelf temperature and chamber pressure dictated the resultant dependent variables of product temperature and sublimation rate throughout the cycle.

Sublimation rates were calculated and compared to assess differences between the two tray types. The average sublimation rates of fifty-eight of the sixty vials of each solution, not including vials with thermocouples used for monitoring product temperature, were included in the comparison. It should be noted that the average sublimation rate of the formulations containing only purified water are slightly greater not only due to the lack of mass transfer inhibition, but also because the row of vials positioned in the rear of the dryer were prone to higher sublimation rates due to the edge effect. Table A contains the average sublimation rates for the three solutions in mg/vial/hr along with their corresponding stan-

	Dextran	Mannitol	Water
Bottomed	244.9 ±36.85	243.0 ±34.98	337.5 ±62.28
Bottomless	320.2 ±35.70	297.6 ±40.21	432.3 ±69.12

Table A. Sublimation rates (mg/vial/hr) of Dextran, Mannitol, and Water after three hours of primary drying, with respective deviations.

dard deviations. The table displays the increase in sublimation rates in all three formulations when the tray bottom was removed as a heat transfer barrier. The average rates of sublimation for Dextran 3,000, Mannitol, and Water respectively were 30.7%, 22.5%, and 28.1% greater with the bottomless tray than with the bottomed tray. A statistical difference between sublimation rates of vials on a bottomed tray versus a bottomless tray was confirmed by a Student's t-test with p-values for the three solutions of less than 0.001. There was also direct comparison of the same vials in the same location in the tray during each run in order to differentiate uniformity and sublimation rates as a function of location on the shelf and within the chamber. Figure 2 shows the comparative sublimation rates of the individual vials.

By monitoring product temperatures throughout the cycle, several different factors contributing to the overall sublimation of ice were observed. The data in Figures 4 and 5 shows the product temperature profiles during primary drying for the three models processed using bottomed and bottomless trays, respectively. There is more separation between the average product temperatures of the product models in the bottomless tray than that for the bottomed tray. In addition, key product averages for each solution are presented in Tables B and C.

Discussion

Noting there was a statistical difference between the sublimation rates of vials on the bottomed stainless steel tray and vials setting directly on the shelf, the comparative heat transfer and rate limiting factors for sublimation were evaluated. The independent variables of shelf temperature, chamber pressure, and time were identical in both studies. The resistance to mass transfer from the area above the dried layer to the condensing surface would also be the same. It was thus evident that there was no direct impact from these variables on the differences in sublimation rates. Composition of the filled solution and the resulting resistance to mass transfer through the dried layer above the ice-vapor interface as sublimation progressed would be expected to impart differences in measured sublimation rates. The effect of coupled rate limiting factors of heat and mass transfer could then be evaluated by differences in both product temperature and sublimation rates for the three model formulations studied.

Heat transfer, one of the two rate limiting factors during sublimation, was first analyzed. Relative differences in the sublimation rates for the three systems studied could be directly correlated to the quantity of heat transferred and therefore the relative increase in efficiency of heat transfer when the vials were in direct contact with the shelf by utilizing the bottomless tray. Pikal has shown that the drying time when the vials rest on a tray is slower by a factor of two than that of a study where the vials were directly on the shelf.[3] The explanation of his findings is that the tray bottom adds additional thermal resistance to heat transfer leading to a decrease in heat transfer and consequently lower sublimation rates. The required heat energy for ice to sublime travels from the shelves to the ice/vapor interface of the frozen product. Barriers interfering with the conduction of heat add resistance to heat transfer, causing the product to receive a reduced amount of energy to result in a slower sublimation rate during primary drying. Impedances to heat transfer include the vapor between the vial bottom and the shelf, the glass vial, and the frozen matrix between the vial and the sublimation front.[4] Adding more thermal resistance with the use of a bottomed tray would introduce additional barriers for heat transfer to the product including the metal of the tray and any additional gaps between the tray and shelf and between the tray and vial - *Figure 1*. The effect this has on heat flux warrants analysis as a single variable.

The equation describing the amount of heat energy transferred through a material can be quantified as heat flux using Equation 1, representing heat flux under steady state conditions within a constant heat flow environment:

$$Q = \frac{kA(\Delta T)}{L} \qquad \text{(Eq1)}$$

where k represents the thermal conductivity of the material, A is the cross-sectional area of the material perpendicular to the flow of heat energy, ΔT is the difference between the temperature at the heat source and that measured at the opposite end, and L is the distance through which the heat travels from the heat source.

A qualifier in the application of Equation 1 is steady state conditions where there is no change in the temperature of the

	Water	Mannitol	Dextran
Pre-nucleation cooling rate (°C min^{-1})	0.10	0.10	0.10
Shelf temperature at target set point	-31.9°C	-29.8°C	-30.1°C
End of 3 hours of sublimation	-32.2°C	-28.1°C	-28.3°C

Table B. Pre-nucleation cooling rate, product temperature at shelf temperature set point, and product temperature at the end of three hours of primary drying with bottomed trays.

	Water	Mannitol	Dextran
Pre-nucleation cooling rate (°C min^{-1})	0.13	0.12	0.12
Shelf temperature at target set point	-30.9°C	-26.5°C	-28.9°C
End of 3 hours of sublimation	-31.4°C	-25.1°C	-26.4°C

Table C. Pre-nucleation cooling rate, product temperature at shelf temperature set point, and product temperature at the end of three hours of primary drying with bottomless trays.

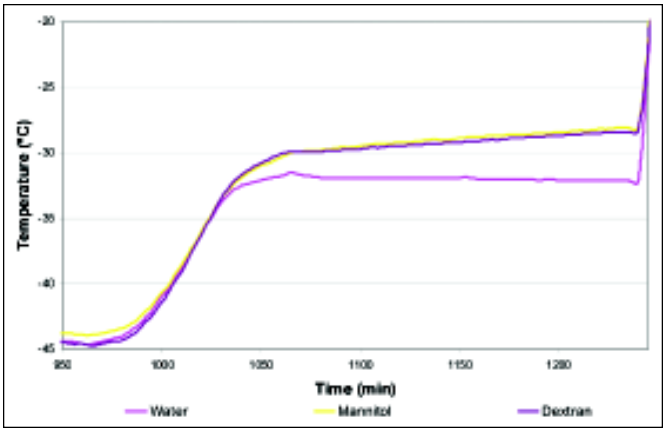

Figure 4. Average temperature at the bottom center of model preparations during primary drying utilizing a bottomed tray.

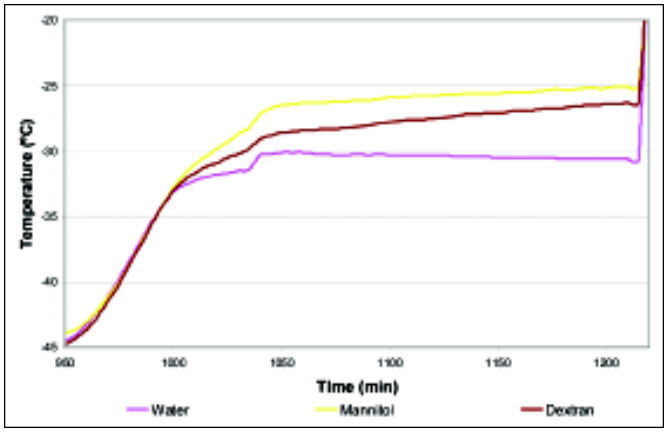

Figure 5. Average temperature at the bottom center of model preparations during primary drying utilizing a bottomless tray.

heat source and mass of the material. With the realization that there are several factors to be considered in the proper application of the equation, accommodation for such differences needs to be taken into account in calculating heat flux during sublimating in primary drying. In the application of the equation, the thermal conductivity k becomes cumulative for the different materials through which the heat travels. As outlined previously, the thermal conductivity of the tray, glass vial, ice within the vial, along with points of contact between the materials, and gas/vapor composition for any gaps that may exist between the shelf, tray and glass vial is treated as a cumulative value for the thermal conductivity constant k. In addition, L varies as a constantly changing distance for the ice layer during the sublimation process. Most prominent is the change in mass due to the sublimation of ice and the associated heat of sublimation as the ice is converted to water vapor.

The equation necessary to calculate the heat flux during dynamic conditions needs to accommodate the factors that vary during sublimation of ice. In determining the heat flow from the shelf to the sublimation interface the following equation applies:

$$Q = \frac{k_c A(\Delta T)}{\Delta L} + \frac{dm}{dt}\ \Delta Hs \qquad \text{(Eq2)}$$

where k_c represents the cumulative thermal conductivity constants of the materials in the path of heat flow, A is the cross-sectional area of the vial, ΔT is the difference between the shelf temperature and the product temperature, ΔL is the distance through the ice to the sublimation front, $^{dm}/_{dt}$ is the change in mass of the quantity of ice in the vial due to sublimation with respect to time, and ΔHs is the heat of sublimation. ΔL is continuously changing as sublimation progresses and the thickness of the remaining frozen layer decreases with time. ΔT also will be hanging as sublimation proceeds when heat not consumed by sublimation causes an increase in product temperature. Equation 1 provides the instantaneous heat flux at constant temperature with no change in mass instead of heat flux over some interval throughout primary drying during the lyophilization cycle. Equation 2 yields the average heat flux per unit time when including the change in mass over a specific interval. Calculating the heat flux that occurred for each experiment and comparing the quantity of heat transferred using Equation 2, reveals the presence of the metal tray causing a decrease in the quantity of heat that was provided to the product. The removal of the tray results in a greater cumulative thermal conductivity due to elimination in the additional material of the stainless steel tray and the gap that exists between the tray and the lyophilizer shelf.

Mass transfer, another rate limiting factor in primary drying, was also evaluated. The condenser averaged -43°C during both studies, and the chamber pressure was maintained within a reasonable variation of ±7 µHg (0.93 Pa), therefore there was no notable difference in mass transfer from the chamber to the condenser. As shown by Pikal, the expected major factor on mass transfer of water vapor for the crystalline and amorphous preparations studied would be effect of resistance to vapor transport through the dried product layer above the sublimation front.[4] Differences would therefore be expected between the absence of any dried layer for the Purified Water samples when comparing results for the amorphous and crystalline models. Identical processing conditions for these two studies would provide comparative results in assessing the impact of the differences in heat transfer and relative influence on mass transfer. Product temperature data presented in Figures 4 and 5 and Tables B and C, were analyzed to distinguish how the tray bottom, or lack thereof, affected the temperature of the products throughout the run. Figures 4 and 5 revealed that the product temperatures of the Mannitol and Dextran solutions during the study using the bottomless tray were higher than those when using the bottomed tray. In addition, there is a difference in the temperatures associated with the different solutes studied. Combined with the increase in sublimation rates and greater quantity of heat removed by the sublimation of a quantity of ice, associated with $^{dm}/_{dt}\ \Delta Hs$, the differences reflect the increase in heat flux when the vials are in direct contact with the shelf. Conversely, under the influences of similar processing con-

"Concerns often arise about changing the style of the tray due to compatibility with current equipment, validation, and cost issues. Only minor changes in equipment and handling procedures would need to be implemented assuming the same sized tray is utilized."

ditions during primary drying, a lower product temperature reflects a decrease in heat flux when the cumulative resistance increases due to the presence of the tray bottom and the limited intimate thermal contact between the tray and the shelf. This consideration assumes that the contact between the vial and the tray would be the same as that of the vial and the shelf.

Mass transfer resistance increases as the thickness of the dried product layer above the frozen product increases with the progression of the sublimation front through the contents of the vial. In addition, relative mass transfer resistance differs with the nature of the solute. Pikal categorized such differences into various types of behavior in studies comparing crystalline and amorphous model compositions.[3] With a bottomed tray, both Mannitol, a relatively low molecular weight crystalline form, and Dextran, a high molecular weight amorphous solute, exhibit similar product temperatures and average sublimation rates. This suggests that the overall rate limiting step is heat transfer and the effect of mass transfer diminishes; different preparations expected to impart different resistances to mass transfer through the dried layer behave the same when the rate of vapor evolution is reduced because of decreased heat flux. When heat transfer is improved, greater quantities of water vapor are evolved however the mass flow through the dried layer cannot increase at a proportional rate to the increased heat flux.

Water vapor transport is also influenced by the molar concentration and nature of the solute. This is well demonstrated by similar temperature profiles and sublimation rates for both Mannitol and Dextran preparations on a bottomless tray. Because the solutions were prepared as weight per volume, the lower molecular weight solute Mannitol actually has a greater molar concentration and therefore molecular density as compared to the Dextran solution. The impedance to sublimation leading to higher product temperatures is illustrated by the temperature profile for Mannitol presented in Figure 5. Consequently, Mannitol exhibited the slowest sublimation rate of the three models when on a bottomless tray - *Table A*. Improved heat transfer, as discussed previously, still allows sublimation rates to increase for all presentations on a bottomless tray as compared to a bottomed tray. Therefore, it is concluded that more efficient heat transfer causes mass transfer to become a more prominent rate limiting factor to sublimation.

Uniformity of the batch was also evaluated to determine if the bottomed tray caused a larger variation in sublimation rates and product temperatures. Warping of bottomed trays can occur with repeated use, and if there is variation in flatness of the tray then there can also be variation in cooling and warming rates within individual vials at different locations on the tray. Some vials may be resting in concave regions of the tray where there would be direct shelf contact, whereas others may be resting on convex regions of the tray that are not in intimate contact with the shelf. If warping increases the gap between the shelf and the tray, thermal resistance is increased and heat transfer will be reduced. There is no consistency in tray warping, with the extent of warping varying from tray to tray, and from region to region, differing in corners and in the middle of the tray. An additional aspect of the differences in heat transfer is in the cooling of the samples during freezing. In tables B and C, the average pre-nucleation cooling ramps are summarized. The samples on the bottomless tray appear to cool more quickly indicated by the relative differences in cooling rates. This difference would be expected to increase with greater cooling rates. The relative differences measured are most likely attributed to the slower cooling ramp of 0.5°C min^{-1}.

Conclusion

The results of these studies confirm that the stainless steel bottoms of freeze-drying trays add thermal resistance to the heat transfer process during lyophilization. This extra thermal resistance in turn hinders the amount of energy that is relayed from the shelf to the sublimation front, decreasing the sublimation rate. With the removal of the bottom of the tray, heat transfer efficiency increases dramatically, causing mass transfer resistance to play a more prominent role during primary drying. The removal of bottomed trays will allow for an increased rate of sublimation and therefore shorter processing times.

Concerns often arise about changing the style of the tray due to compatibility with current equipment, validation, and cost issues. Only minor changes in equipment and handling procedures would need to be implemented assuming the same sized tray is utilized. This is due to the fact that a bottomless tray is simply a bottomed tray with an inner ring - *Figure 3*. The bottom of the tray remains under the vials during filling, loading, and unloading, but is removed prior to beginning the lyophilization cycle. Freezing and drying rates may change significantly when a bottomed tray is removed and heat transfer efficiency is improved. Modifications in the cycle parameters, such as shelf temperature or chamber pressure, may be required to accommodate these improved efficiencies. It is for these reasons that studies should be completed to ensure the finished product is comparable.

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This article describes a holistic approach to Process Analytical Technology (PAT).

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Use PAT to Gain a Fundamental Understanding of Your Process and then Move Forward to Knowledge Development

by Jean-Marie Geoffroy, PhD

The freedom afforded to manufacturers by the FDA is driving innovation of new technologies in order to obtain a more fundamental understanding of pharmaceutical products and processing. Appropriate implementation of PAT will require a transition from summary batch information to stratified batch sampling and testing. The roles of data acquisition at all levels throughout the enterprise are described, from R&D and post-commercial product development data to commercial lot manufacturing information (real-time and batch history). Examples demonstrate the advantages of electronic systems for obtaining product knowledge and rapid troubleshooting exercises. Finally, the role of the ideal pharmaceutical scientist is shifting to include not only pharmaceutical sciences and chemical engineering, but also applied (i.e. SPC) statistics, instrumentation engineering, and data-

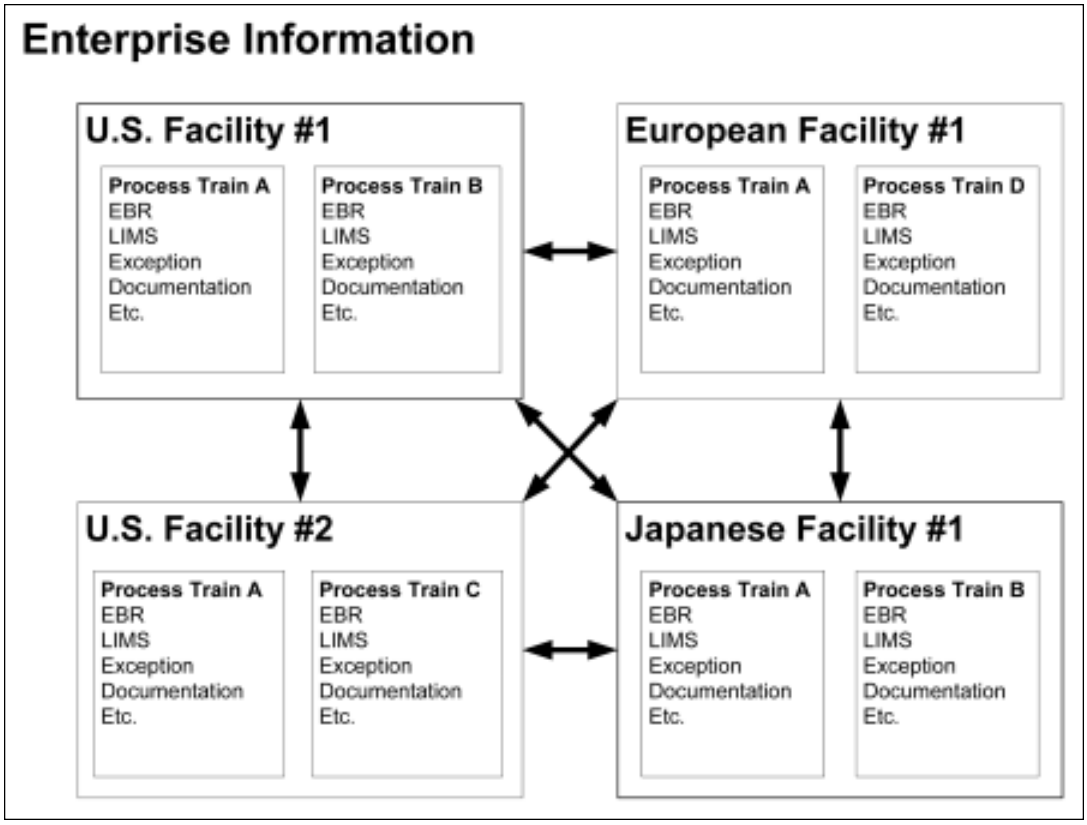

Figure 1. Real-time coating conditions for a non-functional coating aqueous process.

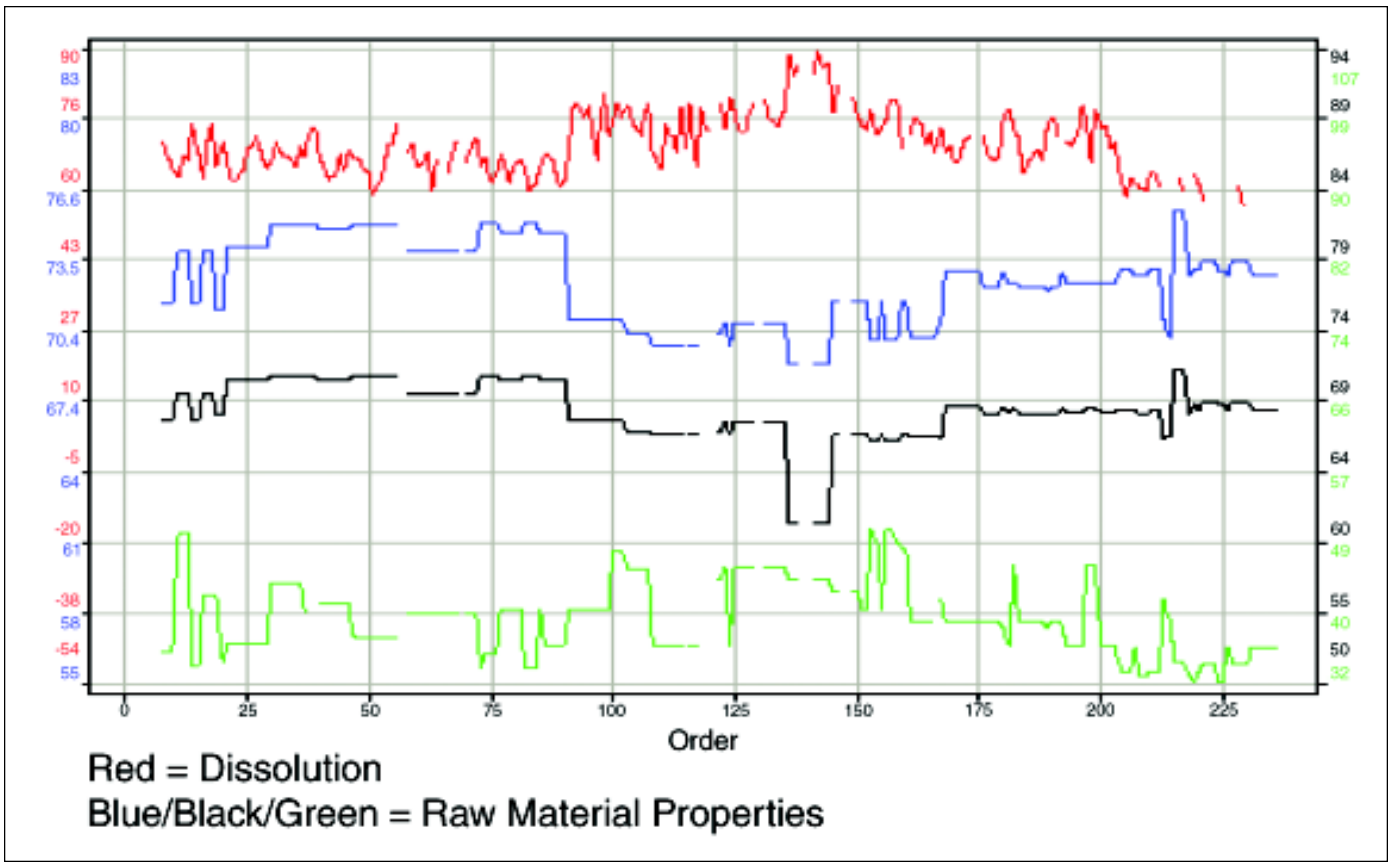

Figure 2. Real-time coating conditions for a non-functional coating aqueous process.

base design, management and querying. As a result, the pharmaceutical engineer will have to become even more aware of the cross-functional aspects of pharmaceutical data sampling, testing and analysis. PAT is a positive and necessary trend for the 21st century.

Systems & Operations Understanding	**Goals:** Operational and Quality Systems Analysis **Activities:** Product to Product Analysis of Operations, Consistency of Unit Operations, Plant Efficiency; Impact of Quality Systems on Operations		
	Product Understanding	**Goal:** Product Specific Operations and Quality **Activity:** Data Analysis of All Unit Operations for a Product: Real-Time and Aggregate (Batch) Product Analysis; Product Quality Review; Continuous Review of Product Design Specifications; Refinement of Mechanistic Understanding of Formulation & Process	
		Unit Operations	**Goal:** Process Understanding & Optimization by Each Individual Unit Operation of a Product **Activity:** Real-Time Data Analysis of Each Individual Unit Operation (Process Modeling, Control & Capability); Continuous Real-Time Quality Assurance

Table A. New tasks for pharmaceutical manufacture as a result of PAT-like initiatives.

Introduction

Process Analytical Technology (PAT) consists of:

> *"Systems for continuous analysis and control of manufacturing processes based on real-time measurements, or rapid measurements during processing, of quality and performance attributes of raw and in-process materials and processes to assure end product quality at the completion of the process."*[1]

The message being sent by the FDA to the pharmaceutical industry is quite clear. If an organization can demonstrate a thorough understanding of its products and processes, and if this organization can show that they are responsible with GMPs and compliance, the FDA will reward these companies by giving them more flexibility and leeway in managing their facilities and products. FDA initiatives such as Risk-Based Management, Comparability Protocols, Quality by Design, and Process Analytical Technologies are all indicators of this premise.[2]

The FDA is advocating Risk-Based Management for Risk-Assessment, Risk Management and Pharmacovigilance[3], and is now further developing the concept to properly balance the FDA's resources towards higher risk firms and products. Risk-Based Management is and will continue to be utilized to manage the manufacture of pharmaceuticals.

Though the technologies utilized in Process Analytical Technology are not necessarily new, many additional technologies are being developed for implementation within the

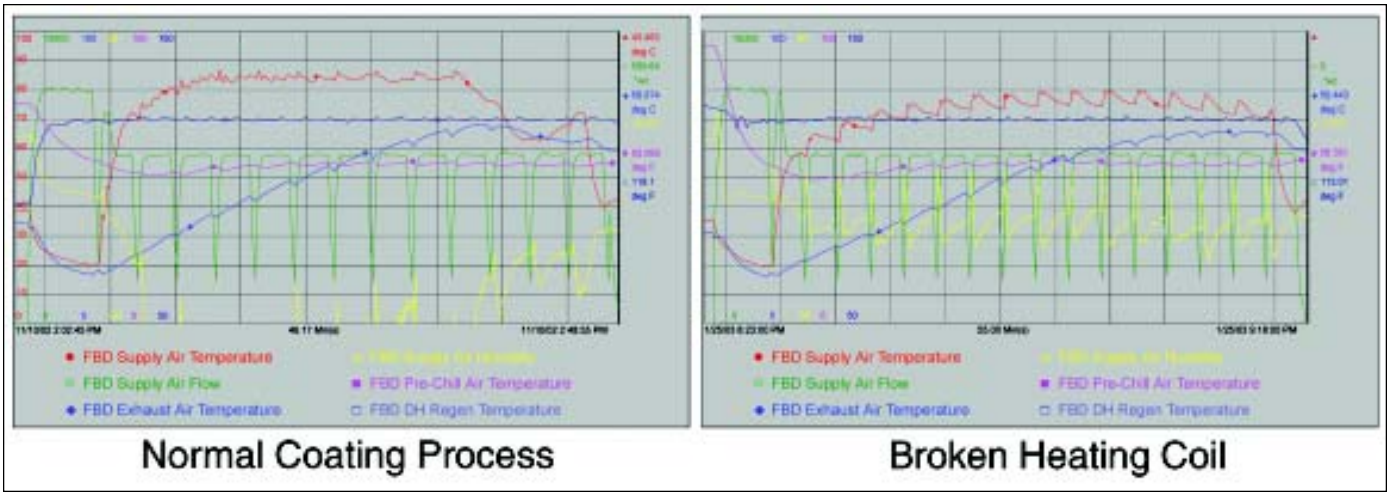

Figure 3. Real-time fluid-bed drying conditions for a granulation demonstrating the impact of a broken heating cell.

pharmaceutical industry. Typical examples include Near Infrared Spectroscopy, Laser Induced Fluorescence, Thermal Effusivity, Laser Induced Breakdown Spectroscopy, Raman Spectroscopy and many more. Petrochemical/chemical, electronics, aviation, aeronautics and other industries have utilized PAT technologies for some time either for cost reduction, product improvement, or risk reduction. In the case of these industries, deployment was required simply to compete within an adequate level of risk, cost and quality. With longer development times, increased competition from generic manufacturers, and enhanced compliance initiatives driven by both regulatory bodies and pharma itself, profit margins are decreasing while additional resources are required to deal with compliance and quality issues simply to maintain a product on the market. Pharma in general is beginning to feel significant pressure to reduce costs to maintain historical levels of profitability by using more advanced analytical methods (not necessarily technologies) to improve product quality.

The time to capitalize on PAT is now. FDA clearly is attempting to promote risk-based, science, while keeping the consumer and industry in mind. The FDA has created a PAT subcommittee from industry, academia and government; a Steering Committee of senior FDA managers to oversee the PAT initiative; a PAT reviewing team charged with overseeing issues, technologies and PAT reviews/inspections; and a PAT Research Team which conducts research to provide information for policy development.[2]

Furthermore, the FDA has issued a draft guidance on PAT.[4] This guidance provides an initial foundation for the advancement of PAT. In this guidance, the FDA discusses PAT tools, Process Understanding, Risk-Based Management, Integrated Systems Thinking, and Real-Time Product Release.

Enterprise-Wide Deployment of PAT

There are many sources of product information that can be leveraged in developing a complete and thorough understanding of a product and product line. The knowledge gained by development batch analysis, historical commercial data analysis and real-time data analysis can be utilized to determine process performance. Minimally, all three of these components should be employed to gain a fundamental understanding of product processing performance. Developing techniques to deal with the large amount of data will be critical to surviving information overload.

However, most discussions to date relate only to performance of a specific product. One should also consider deploying an enterprise-wide approach to PAT - *Figure 1 and Table A*. Consider, for example, a high-volume product manufactured at more than one facility. A direct comparison of multiple manufacturing trains within and between facilities becomes possible. The performance of raw materials can also be assessed across multiple product lines in which they are used and additional understanding obtained relative to their performance for the company. Similar assessments could be made of manufacturing equipment across multiple product lines.

R&D and Post-Commercialization Development Data

R&D and post-commercialization batch development data are a critical source of useful information for obtaining product and process understanding. Formulation and process variables that are highly likely to have an impact on product performance are within the scope of research and development, sometimes at several manufacturing scales. However subtle manufacturing variables which cannot be studied at smaller scale or which require a significant amount of manufacturing experience cannot be evaluated during product development. Therefore, the utility of R&D data is limited because of its small size and limited scope. Because of product and process "creep" which is a result of normal process variation, process optimization, drift in raw materials' quality over a number of years, minor changes in equipment, etc., the relevance of the development dossier also decreases. Therefore, careful retention of commercial manufacturing data becomes increasingly important.

Post-commercialization development data are also limited in that they cannot represent process shifts over extended periods of time. They can, however, focus on more

Granularity	Lot Example	Specific Example
Low Granularity	Multi-Product Information	Generalization(s) on LOD
	Product Historical Information	Average LOD Across All Lots
	Lot Specific Information	Average Lot Granulation LOD
High Granularity	Unit Operation Specific Information	Granulation LOD

Figure 4. Level of granularity for in-process LOD information.

subtle process variables that were not known or discovered during R&D development. Often the goal at this stage is to remedy a problem situation (e.g. coating failures, negative trends in granulation, etc.) that can lead to either more detailed manufacturing instructions or to tightened raw material or product specifications.

Commercial Data History

The technical transfer of a product from R&D to commercial operations does not have the benefit of frequent and repeated batch manufacture. In addition, the amount of data actually collected is often small and typically does not include real-time data that is available in the operation's organization of some pharmaceutical companies. As a result, the current concept of validation as a three-lot exercise is slowly shifting to a more comprehensive approach that also includes extensive or continuous post-validation monitoring.

The advantages of an Electronic Batch Record management tool are obvious. Such EBR systems provide for automation of batch calculations, data storage and retrieval, and decreased review, among many other features. However, one large advantage with EBRs is the fact that such systems typically capture much more detailed data (operator information, signature dates and times, etc.) that is collected automatically. When an organization is interested in evaluating the exact time or exact order of operations (to within seconds), it is significantly more difficult if not impossible to recreate events if the data are not accurately detailed. Such features allow for more detailed analyses of process performance such as cycle times that provide a more fundamental understanding of the business itself. They also allow the pharmaceutical process engineer to quickly summarize data by using database (SQL) queries and reports on these systems without the time intensive, manual data re-entry.

Imagine a dissolution investigation for one product. The time to manually re-enter data from a paper batch record would take two pharmaceutical process engineers approximately 2 months to collect and verify process information and test results from approximately 150 lots of recently manufactured product (approximately 1 year of information) before analysis could take place. By designing query tools to retrieve data from the LIMS, Electronic Batch Record (EBR) systems, data could now be quickly retrieved, typically within a few minutes. In addition, the effect of process variables on re-

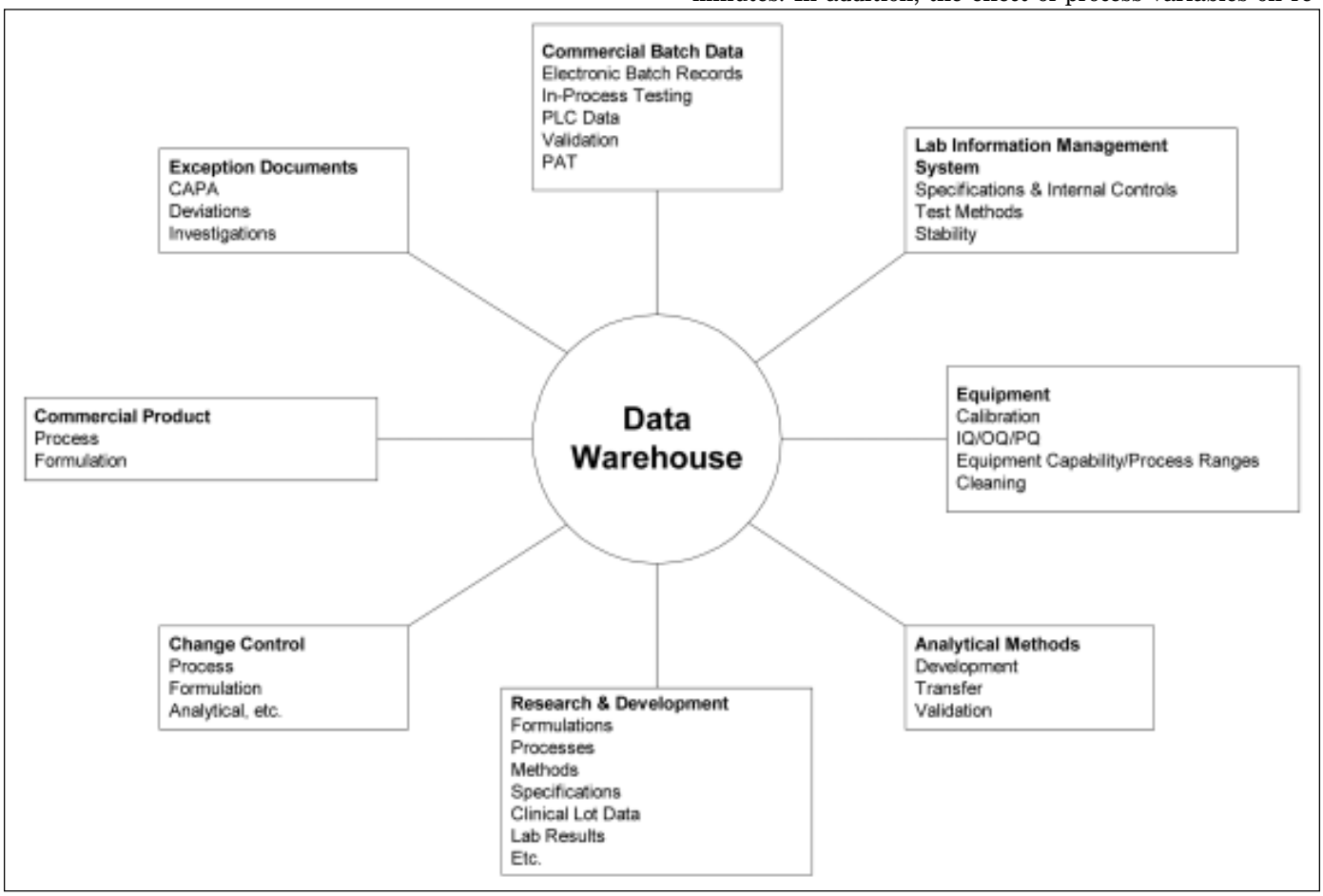

Figure 5. Potential data sources for a PAT Data Warehouse.

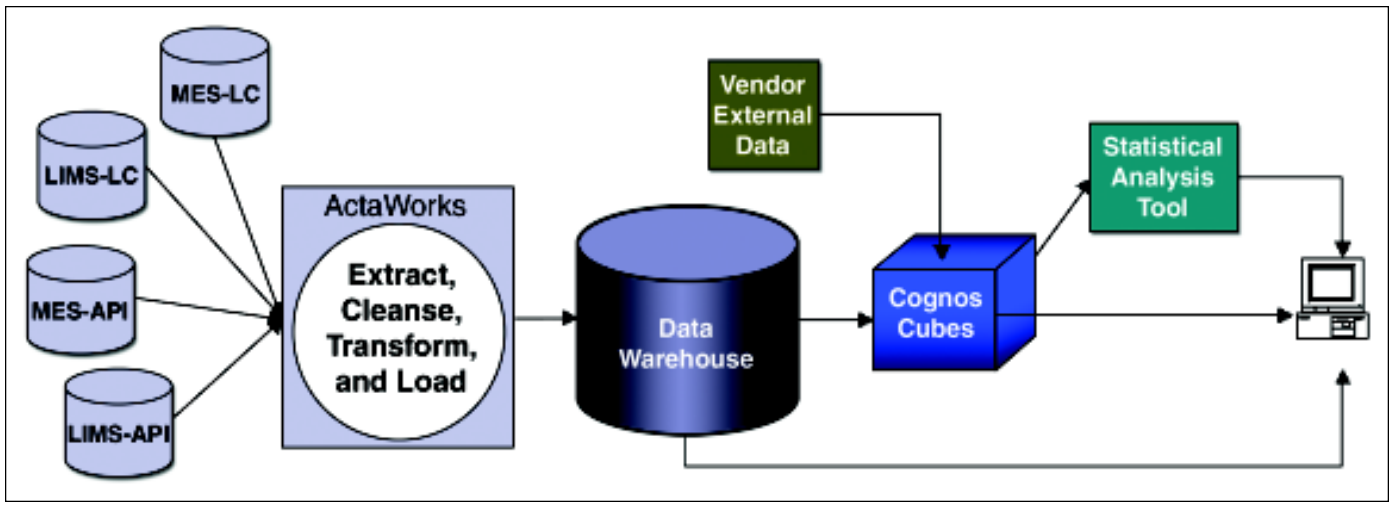

Figure 6. Data architecture for a potential manufacturing PAT Data Warehouse.
*Data Cleaning refers to standardization of data in order to be able to perform data analyses.

sponse or test data can be accurately and quickly made. For a large investigation, it is not unusual to investigate 80 or more process variables in search of the root cause(s). Quickly obtaining, formatting, and visualizing the data to separate the useful many parameters from the vital few parameters is critical to timely resolution of the issue(s).

Tracking raw material data against drug product performance can be extremely insightful for process understanding. Figure 2 is an example of product dissolution (red) for a sustained releasing dosage form and indicates that dissolution trends with three different physicochemical properties of a raw material. Monitoring process performance against raw material properties could be easily performed to ensure product acceptability. Furthermore, predictive models could be developed in order to improve the performance of the drug product by minimizing variability in the dissolution rate. This would be accomplished by ensuring that the modified release excipient is tightly controlled.

Ideally, the EBR dataset should be stored in a database that also contains other information such as in-process and lot release data, exception documentation, and real-time data (e.g. data stored in a data historian). Integration of these data stores facilitates the process of developing queries and search tools, and allows analysis of process and test data without having to manually enter data. Re-entry or manipulation of data also increases the probability that an error will occur in the manipulation of the data.

Application of Data Historians in Process Monitoring and Understanding

Modern pharmaceutical manufacture utilizes fully integrated PLC and APC systems which monitor and control processes. These units are key to maintaining operational effectiveness and control; however, they are not designed for data collection and trending on a large scale (i.e. multiple lots for months/years of data).

A data historian is an analytical tool that simply collects PLC or similar data into a central repository where it can be reviewed real time or at a later date. The frequency of data collection is determined by the specific application.

Deployment of a data historian can strengthen an organization's ability to track, trend and diagnose pharmaceutical manufacturing performance. Figure 3 is an example of the output that can be gained with a data historian. In this example, granulation-drying conditions are recorded for each drying run. The effect of a broken heating coil can be seen in the second example. Similar examples could be shown for granulation operations, facilities utilities (e.g. compressed air pressure, nitrogen flow rates, etc.), milling conditions, compressing conditions, and coating conditions. Application of analytical tools to analyze real-time data can be further extended to improve process understanding and control.

Transitioning From Highly Granular Data To Product Knowledge and Understanding

The granularity of data is defined as the level of detail that it contains. This is best demonstrated by example. Tablets tested for dissolution are typically randomly selected from a composite lot sample. Thus, dissolution data provide an estimate of the lot average and lot variation for dissolution. However, tablet hardness data which are generated at frequent intervals throughout the manufacture of a lot provide not only an estimate of the lot average and variation for hardness, but also information about average hardness and variation at a particular point in time during manufacture. Therefore, tablet hardness data contain more detailed information about the process; tablet hardness has a higher level of granularity than dissolution data. See Figure 4 for an example of granularity for Loss On Drying data.

PAT will result in an increase in the granularity of data available for analysis, providing a more fundamental understanding of process and product performance. A single NIR test will provide several hundred data points that must be converted to useful information. If a complete NIR scan of every tablet in a 1 million-tablet lot were to be performed to "validate every tablet," several hundred million data points could become available for analysis just for one lot.

Data Storage and Querying

PAT will drive the collection and storage of significant amounts of data. Proper maintenance of each dataset in a usable and retrievable format will be required to enhance organizational effectiveness. There are numerous data sources (see Figure 5) which a pharmaceutical company should take into consideration, including commercial batch manufacturing information (EBR/MES), lab data (LIMS), equipment, exception documentation, change control, R&D data, analytical methods, etc. An enterprise-wide data warehouse would also include inventory, financial, marketing, sales, and other data stores.

Figure 6 shows an example of a small data warehouse consisting of batch release data from a Laboratory Information Management System (LIMS) and data from three different Manufacturing Execution Systems (MES) which are separated geographically. Each of these databases is uploaded into one larger data warehouse. The data warehouse is refreshed on a nightly basis to ensure timeliness of the information. From this data warehouse, reports are generated and frequently automatically updated to reflect the new information entered into the data warehouse. Using specialized software, subsets of the data warehouse (cubes) are retrieved and refreshed nightly and can either be analyzed with the analytical tools or can they can be downloaded to a separate application which may fulfill a more specific need. Finally, external data or data not collected as part of the warehouse can be uploaded to the data cubes so that additional parameters can be tracked and trended against data contained within the warehouse. In addition, the pharmaceutical engineer can also upload the data manually.

Skill Sets of the Ideal Pharmaceutical Scientist

Today's pharmaceutical support or technical services scientist must be trained in many areas in order to fully leverage and integrate development, product and process information. The breadth of knowledge required for an ideal candidate spans from the application of pharmacokinetics and pharmacology, applied chemistry utilized in formulation development, PAT methods development, chemical engineering principles utilized in unit operations development and execution, electronics and instrumentation, design and structure of databases, database querying, and a strong background in applied statistics.

Many compounds are now being developed using "exotic" technologies that include modified release dosage forms, aerosols, liposomes, etc. Understanding the pharmacological and pharmacokinetic impact of process changes on the characteristics and performance of the final dosage form is crucial to process development and refinement as well as biological implications.

Formulation development and optimization requires an understanding of the interplay between physical and chemical properties of drug actives and excipients. Detecting an excipient which is misbehaving in a commercial product requires that the process engineer know how to identify and prioritize likely raw material characteristics which may explain the change in quality or process performance of the in-process or final drug product.

The pharmaceutical scientist responsible for methods development will face new challenges as PAT evolves. New innovations and new applications of existing technologies for pharmaceutical manufacture are already in full swing. Since the FDA's pronouncement of PAT as a concept, instrument vendors and several companies have begun to explore and innovate with new technologies. As a result, new challenges in methods development and deployment are surfacing. The recent FDA draft guidance[5] on Process Analytical Technology goes a long way to help dispel some of the fears and misconceptions on the deployment of PAT, clearing the way for manufacturers to experiment actively and without the threat of enforceable action. The qualification of unconventional PAT instruments (e.g. NIR) and validation of their methods will require experienced analysts to create and utilize new and potentially highly technical methods (e.g. chemometrics).

The impact of modifying process parameters for each unit operation is important for development and optimization of a pharmaceutical manufacturing process. Mill speeds and screen sizes, granulation processing variables such as liquid addition rate, impeller and chopper speeds; coating variables such as solution flow rate, atomization pressure, nozzle configuration, etc. are a very small selection of potential process parameters which must be understood.

Instruments to be placed near or on-line will require the skill-sets of an instrumentation or electrical engineer. Setup of the equipment and subsequent networking and data collection will be key skills that these engineers can bring to the organization.

Finally, a pharmaceutical scientist that has a fundamental understanding of the supportive database structure, and has knowledge relative to retrieving the data in an efficient, usable and timely manner, will provide his/her organization with valuable skills, and opportunity for organizational learning. Those versed in datamining will also be able to provide an organization with knowledge that would otherwise not be self-evident. Datamining is an exercise where the pharmaceutical scientist does not know up front what knowledge he is seeking; however, the methodology allows one to find relationships in the data that are not self-evident.

Conclusion

Process Analytical Technology is the logical extension of the current state of pharmaceutical manufacture. It represents a transition from summary batch data to a more holistic approach to product manufacture and analysis. It also represents technological advances from which an organization can capitalize to improve quality, decrease costs and potentially gain a competitive advantage. As a result, the pharmaceutical engineer will have to become even more aware of the cross-functional aspects of pharmaceutical data sampling, testing and analysis. PAT is a positive

and necessary trend for the 21st century.

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As the heart of the bioreactor system, agitators must be designed differently at production scale than pilot scale.

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Advances in Bioreactor Agitation

by Mark Brothers, Tony Kocienski, Bill Smith, and Brad Yundt

Introduction

For such a critical part of scaling up a cell culture system, bid specifications don't pay enough attention to the bioreactor agitator. Typically, the specs furnish little more than the impeller type, mounting orientation, whether the vessel is baffled, materials of construction and surface finish. Although vessel baffles and agitator mounting orientation are truly a matter of customer preference—impeller type affects process scale-up. It may not be possible to obtain the desired performance in a production bioreactor while using the same impeller type successfully used at pilot scale. Furthermore, process parameters needed for scaling up are almost always missing.

Virtually all bid specifications are silent on design features of the shaft seal. This is unfortunate, especially given the wide variation in price and advances in ease of maintenance, cleanability and sterile operation between seal models.

Process Requirements

The agitator has two jobs in a cell culture system. First, it must produce enough shear to obtain the desired oxygen and carbon dioxide mass transfer. Second, it must keep the vessel well-blended to minimize variations in temperature, pH and additive concentration. The first requirement may be characterized by the gas mass transfer effectiveness (k_La) and the second by the blend time. At the same time, the peak shear rate must be kept low enough to avoid cell damage or stress—contradictory to the first two requirements. Ideally, all three parameters would be specified by the end-user.

The oxygen mass transfer coefficient or effectiveness k_La is defined by:

$$OTR = k_{\mathrm{L}}a\ \frac{(C^* - C)_{IN} - (C^* - C)_{OUT}}{1\mathrm{n}\left|\frac{(C^* - C)_{IN}}{(C^* - C)_{OUT}}\right|}$$

where OTR is the oxygen transfer rate (mmol/L-hr), C and C^* are the local and saturation concentrations of oxygen in the culture medium. Bigger is better for k_La, because reducing the fraction of oxygen in the sparge gas or flow of sparge gas can lower the operating cost and reduce cell damage in certain cell lines.

The k_La may be measured experimentally using either sodium sulfite addition or nitrogen stripping to reach 0% dissolved oxygen (DO) concentration. The k_La is proportional to the rate of rise in DO concentration as air sparging continues. The sodium sulfite method is more practical for shop-testing large vessels because it doesn't release large quantities of nitrogen into the test room and because it reaches baseline values quickly.[1] This method does obtain 30~50% higher $k_{\mathrm{L}}a$ values than the nitrogen stripping method (performed in deionized water) because of the higher ionic strength—but various agitator types and configurations may still be compared. It is worth noting that typical culture media have even higher ionic strength, so k_La values measured by either method will usually be conservative.

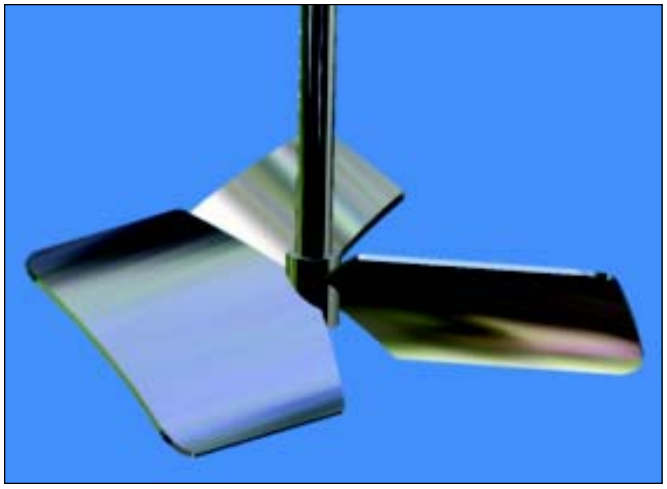

Figure 1. High solidity impeller.

	Pumping #	Power #	Mixing Rate Constants		Shear Coefficient (in^{-1}; m^{-1})	
	N_Q	N_P	a	b		
Marine propeller, 1.0 pitch ratio	0.44	0.32			0.42	16.5
Rushton turbine, 6 blades	0.80	5.75	1.06	2.17	4.8	189
High solidity, 3 blades	0.68	0.65				
High efficiency,3 blades	0.56	0.30	0.272	1.67		
4 blades pitched	0.79	1.27	0.641	2.19	0.8	31.4

Table A. Impeller performance parameters vary with impeller type.[3,4] These coefficients apply for cell culture and fermentor systems with low viscosity and standard vessel geometry.

For geometrically similar vessels and agitators, the k_La depends on the superficial gas velocity F rising through the bioreactor, power per unit volume P/V for the agitator, size of the bubbles and ionic strength. Flooding occurs when the agitator is turning too slowly for the gas rate; k_La won't increase further if gas load exceeds this flooding point. The superficial gas velocity F is calculated as though the vessel were empty (i.e. sparge volumetric flow rate/vessel cross-sectional area). Most published correlations of k_La follow the form (where A, α and β are experimentally determined constants):

$$k_La = A\left(\frac{P}{V}\right)^{\alpha} F^{\beta}$$

One very interesting thing about the k_La equation is the fact that all of the variation with impeller type, tip speed and diameter appears in the term for power per unit volume. Except for the two extremes (flooded impeller or zero gas flow), one may change impeller types as equipment is scaled up, yet still be confident of obtaining the required k_La. If the proposed production bioreactors are geometrically similar to existing pilot scale equipment, similar k_La performance may be expected at the same superficial gas velocity and power per unit volume.

Note that the appearance of the free surface in geometrically similar sparged vessels will change with diameter. If the gas flow/working volume ratio or VVM is held constant, then superficial gas velocity will increase linearly with vessel diameter. Production bioreactors therefore have a more violent looking surface than small seed bioreactors.

The shear produced by an agitator impeller may be measured by Doppler velocimetry using a laser beam to scan the instantaneous velocity at points throughout the vessel.[6] Leading agitator vendors have characterized the performance of their impellers using this technique. Peak shear rates occur at the tip of the impeller, and vary linearly with tip speed for radial impellers and linearly with rpm for high efficiency and high solidity impellers.[7] Coefficients are shown in Table A. Shear rates at the baffles are at least an order of magnitude lower.

Peak shear = kND *radial impellers*
Peak shear = kN *high efficiency, high solidity impellers*

Because the peak shear coefficients have been characterized, it is possible to measure the maximum shear from the agitator that a cell line can tolerate. To do this at pilot scale, slowly increase the agitator rpm while measuring lactate dehydrogenase (LDH) and cell viability. The breakpoints in the plot of LDH and cell viability vs. rpm indicate the peak shear tolerated by the cell line. This value may then be provided to the bioreactor vendor as a scale-up limit.

Mixing time may be defined as the time required to achieve a specified degree of uniformity in batch concentration, temperature, pH or conductivity. The uniformity of mixing is defined as:

$$U = 1 - \frac{[\Delta C(t)]_{max}}{[\Delta C(0^+)]_{max}}$$

Where $[\Delta C]_{max}$ is the maximum deviation from average concentration, either at time t or an instant after the beginning of the test. Typically, uniformity increases exponentially up to the average concentration:

$$U(t) = 1 - e^{-k_m t}$$

where k_m is the mixing rate constant. Assuming 95% uniformity, this latter equation may be solved for mixing time as:

$$t_u = \frac{-1n(1-U)}{k_m} - \frac{2.996}{k_m}$$

For standard baffled tanks with Reynolds number over 10,000 (typical of cell culture), the mixing rate constant is a function only of geometry and rpm:

$$k_m = aN\left[\frac{D}{T}\right]^b\left[\frac{T}{Z}\right]^{0.5}$$

where a and b are constants that vary with impeller design, D is impeller diameter, T is vessel diameter and Z is ungassed liquid depth.[2] When scaling up geometrically similar vessels, D/T and T/Z are constants, so mixing time varies as a/N.

Another way of looking at mixing time is to calculate the ratio of vessel working volume to agitator pumping rate. This approach is useful because the impeller pumping number N_Q is more readily available than the mixing rate constants a and b. Pumping rate is given by:

$$Q = N_Q N D^3$$

Once again, mixing time varies inversely with rpm (as $1/N_QN$).

Figure 2. Flat blade pitched impeller.

Figure 4. Rushton impeller (also called 6 blade disk).

To complete the picture, agitator power in turbulent flow conditions is given as:

$P = N_P SpGrN^3D^5$

Scale-Up

Armed with the parameters, one may now study the scale-up of a Rushton and marine impeller system which had satisfactory performance in a 2500 L vessel operating at 150 ft/min (0.76 m/sec) tip speed - *Figures 1-5*. Rather than suffering performance degradation after scaling up the Rushton to 15,000 L scale, one should consider using either a high solidity or high efficiency axial turbine instead.

Owing to concern over shear damage, it made sense to keep peak shear rate at or below the values found in the existing vessel. With a Rushton turbine, that meant keeping the tip speed at 150 ft/min, with slightly improved k_La as the vessel scale up, but worsened mixing time as undesirable consequences. Had the existing vessel used a high efficiency or high solidity impeller, then scaled up vessels using the same impeller would always have a lower peak shear rate than the reference vessel (because the rpm is lower).

If tip speed ND is held constant, then power per unit volume varies as D^2/T^3. In geometrically similar vessels, the net result is power per unit volume decreasing inversely as vessel diameter T. If sparging in the large vessel occurs at the same VVM as in the smaller one, then the superficial gas velocity F increases proportional to tank diameter T. After taking into account the exponents in the k_La equation, the k_La increases as $T^{0.33}$, thereby offering the possibility of reducing oxygen concentration in the sparge gas.

Vessel turnover time (working volume/pumping rate) will vary in geometrically similar vessels designed for constant shear. Pumping rate increases as D^2, but vessel volume goes as T^3. Since impeller diameter D and vessel diameter T have a fixed ratio, the turnover time increases proportional to vessel diameter. Blending time for a Rushton impeller designed for constant shear varies as $Z^{0.5}T^{1.67}/D^{1.17}$. If the vessels are geometrically similar, then impeller diameter D and liquid depth Z are proportional to tank diameter T, so blending time increases proportional to vessel diameter.

The variation of k_La and blending time with working volume are shown in Figures 6 and 7. As mentioned above, k_La improves with increasing scale, but blend time gets worse. At the same peak shear rate, axial flow impellers (marine, high solidity, high efficiency or 4 blade pitched) offer both higher k_La and shorter blend times than the Rushton/

Figure 3. High efficiency impeller.

Figure 5. Marine impeller.

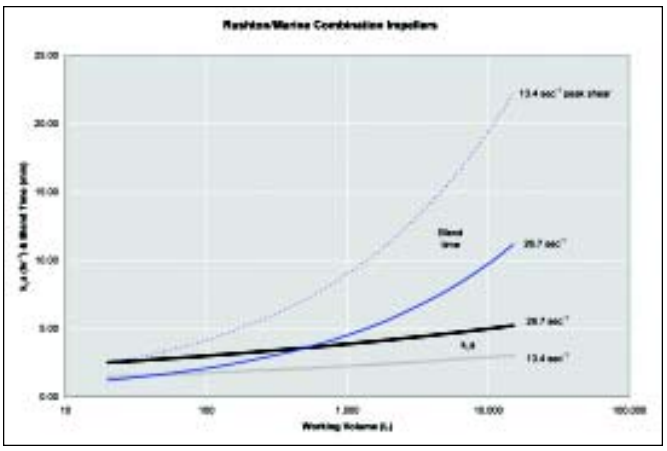

Figure 6. k_La and blend time variation with bioreactor working volume for Rushton/marine impellers.

marine combination.

To obtain similar blending time in the production scale bioreactor, one must either increase agitator rpm or change the impeller. Rather than conduct process development experiments to see if the cell strain could tolerate higher shear (possibly with the aid of polyol surfactants), one may study the use of high solidity and high efficiency impellers. These impellers convert a bigger fraction of their power input into fluid pumping rather than shear, and so can operate at much higher tip speeds. Table B shows the results of such a study (all agitators used two impellers).

After considering the k_La requirements of high density mammalian cell cultures together with managing the shear level of such a culture, it is clear that the Rushton/marine combination cannot perform as well in large scale production bioreactors as in the smaller reference system.

As a result, it became necessary to consider dual axial flow impellers instead of the Rushton/marine combination that worked well at smaller scale. Though any of the axial flow impellers offer viable performance, a high solidity impeller has good performance at a low tip speed. Peak shear was half that of the Rushton, k_La 130% higher, and blend time over seven times faster.

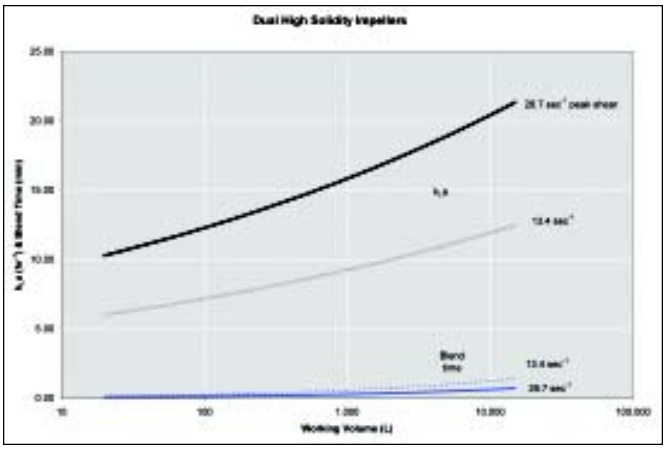

Figure 7. k_La and blend time variation with bioreactor working volume for high solidity impellers operated at similar shear rates. Note superior performance compared to Rushton/marine impellers.

It is worth noting that marine impellers from many manufacturers are made from castings rather than fabrications. As such, the impeller surface will almost always have some porosity. Even if not apparent after mechanical polishing, electropolishing will frequently expose the pits. These pits render the marine impeller unacceptable for most cell culture applications. For this reason, agitator manufacturers may suggest a high solidity impeller (which is a formed and welded assembly) when specifications call for a marine impeller.

Agitators for a seed train can be designed as scaled down versions of the one for the largest bioreactor. The same impellers and geometry ratios apply, though the smallest seed train vessels might use a single impeller. Agitator rpm would then be varied to obtain the desired k_La. It is worth noting that the required k_La is frequently lower in the seed train than in the production bioreactor because of lower cell density. The seed train vessels also may be operated economically at higher VVM sparge flow, thus letting the superficial gas velocity F partially substitute for agitator power to volume ratio P/V.

Optimizing Agitator Geometry

Agitation orientation is generally specified by the customer. On small vessels, such as used in pilot scale or seed train bioreactors, top-mounted agitators are very common. They are easier to seal, but do require longer shafts and larger diameter to control runout and vibration. If the vessel does not have baffles, then the agitator must be mounted either on an angle or offset from the vessel centerline. Baffled vessels with center top-mounted agitators are more common.

Bottom-mounted agitators are more prevalent in large production bioreactors. They need much shorter shafts—perhaps as much as 3 meters shorter on a production scale bioreactor. The shorter and smaller diameter shaft saves money and is easier to handle during servicing.

Bottom-mounted agitators also require much less headroom to remove the impeller. Rather than remove the shaft assembly through a ceiling hatch (and thereby expose the bioreactor suite to outside air), most users build their bioreactor suite with enough ceiling height (or height in a ceiling bump-out) to keep all the maintenance activity within the classified room area. There are considerable construction cost savings with the lower ceilings that still provide enough headroom to service a bottom-drive agitator. There also are significant savings in mechanical systems because a smaller volume room needs less HEPA filtered air to meet air change requirements.

Maintenance on bottom-mounted agitators in production bioreactors is simplified because it is easy to support the shaft while removing the mechanical seal and because vessel entry is not required. With a top-mounted agitator, you must support the shaft with slings around the impeller to remove the mechanical seal. This process often requires vessel entry and the shaft assembly may be unstable if supported below its center of gravity.

Bigger impeller diameters (for the same k_La) result in

	Tip speed (ft/sec, m/sec)	K_La (hr^{-1})	Peak shear (sec^{-1})	Time for 3 vessel turnovers (min)
Rushton/marine 2500 L	150 0.77	6.6	46.1	3.12
Rushton/marine 15,000 L	150 0.77	8.2	46.1	5.93
Marine 1.0 pitch ratio	771 3.92	19.2	20.6	1.00
4 Blade pitched turbine	482 2.45	19.0	24.5	0.89
High solidity	602 3.06	19.0	23.0	0.83
High efficiency	784 3.98	19.1	19.9	0.78

Table B. Comparison of key process parameters for different impellers. Note the decided inferiority of the Ruston/marine combination.

shorter blend time and lower peak shear. Once the vessel diameter and working volume have been set, blend time varies as D^{-b}. The outer 20% of the vessel ID is occupied by baffles (typically T/12 in width, T/36~T/72 off the wall), thus setting an upper physical limit for D. Against these process benefits must be weighed the higher cost, increased vibration and larger runout and more robust design requirements associated with larger diameter impellers and greater fluid forces. After considering all these factors, impeller diameter in cell culture systems is usually set at half the vessel ID.

Multiple impellers are required when the liquid level/vessel ID ratio Z/T exceeds 1~1.1. They are optional at lower liquid levels. At the typical cell culture Z/T of 1.0, it is common for small seed train agitators to use a single impeller, with the production unit agitators having two impellers.

Minimum vessel level significantly affects agitator design and performance. To avoid drawing gas into the impeller, it is undesirable to operate the agitator when ungassed liquid level is close to the impeller (less than a coverage to impeller diameter ratio of 0.5). One should not compromise performance by specifying a low minimum operating level if there is no intention of operating that way. Normally, the minimum operating level is about 80% of maximum. Rebatching (partial harvest of batch) and fed batch operating strategies require lower operating levels.

Typical off-bottom clearance for the lower impeller is ½ ~1 D; lower than that, an increasing proportion of the input power translates into energy dissipation (shear) as the impeller discharge flow decreases with little change in power input. There is also typically ¾~1 D spacing to the next impeller. If the impeller diameter is ½ the vessel ID, the upper impeller horizontal centerline will be somewhere between 5/8 and 1 times the vessel ID. This is equivalent to 37 ~ 74% of working volume. To permit rebatching in the future (with operation at 50% of normal working volume), impellers could be positioned at the low points of their normal range.

Testing

Scale-down tests can be run to confirm scalability. Such tests are frequently run by agitator vendors, with initial screening runs in small transparent vessels using a variety of impeller designs and locations. Once the geometry has been optimized, a confirmation test may be run in a large metal test tank.

Blend time in a 70 L transparent test tank may be determined by observing the color change of a phenolphthalein indicator after acid or base addition to the liquid free surface. The last portion of the vessel to reach uniformity is about 2 inches (5 cm) under the free surface about halfway between the centerline and baffles. In a 15,000 L metal test tank, blend time is determined by noting the time required to reaching 95% uniformity using multiple probes for in-situ conductivity measurements. Blend times on the 15,000 L vessel generally matched those on the 70 L vessel when using the same impeller and rpm.

The k_La was measured at the 15,000 L scale using the sodium sulfite addition method.[5] With a perforated sparger, the three constants A, a, and b were found by least squares. This empirical equation was then used in the calculations for Table B.

Mechanical Seals

Mechanical seals are relatively easy to implement on top drive agitators. Bottom drive agitators offer more of a challenge to comply with biotech industry needs.

The oldest and most common seal design for bottom drive agitators places an elastomeric bellows and spring in the culture medium. These seals were originally developed as a

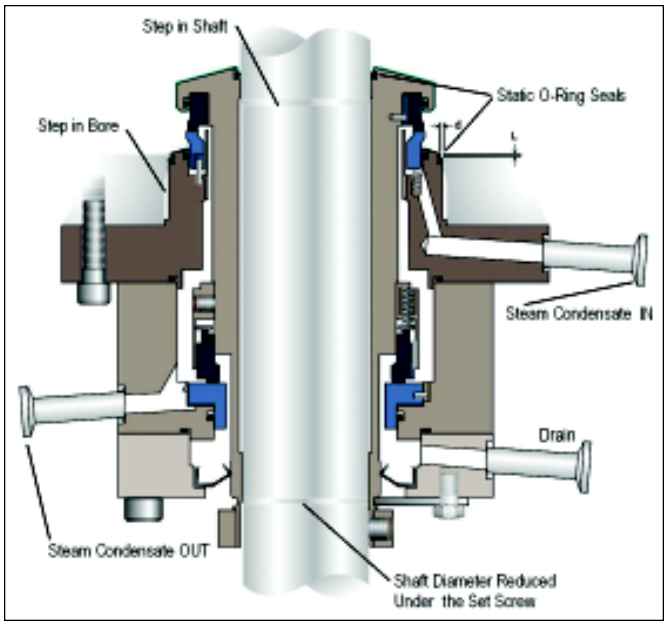

Figure 8. Cross-section of mechanical seal designed for bottom entry cell culture agitation. Note the "clean" appearance of penetrations into the vessel and typical L/d relationship.

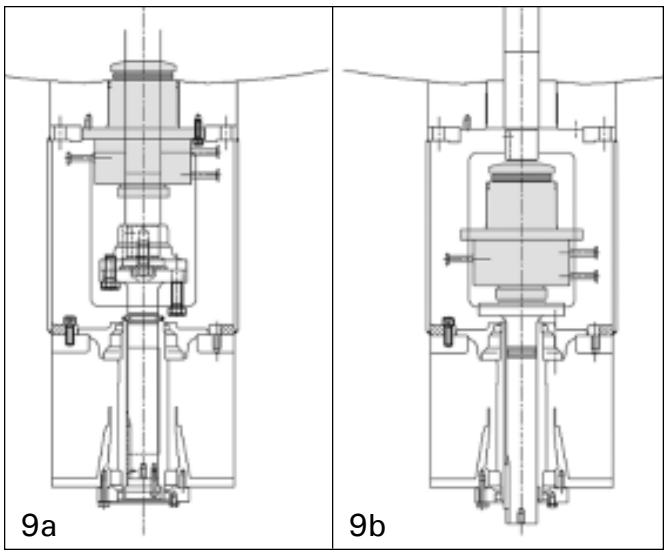

Figure 9a and b. Cross-section of Pedestal between gearbox and vessel. Seal cartridge in Figure 9a shown in operating position; in Figure 9b seal cartridge has been dropped onto coupling half for removal for servicing.

low-cost end-face seal for general industrial use. They are not marketed by their manufacturer to the biopharm industry, but various equipment manufacturers have adapted them somewhat successfully in numerous cell culture applications. The bellows and spring, however, can trap soil if not cleaned very carefully.

Because these older design seals don't use a cartridge design, installation is somewhat tricky and there is always the possibility of nicking the elastomers or cracking the seal faces during installation or removal of the mechanical seal components. The inboard seal components (spring, elastomer bellows, retainer and seal rotor) must be installed loose onto the agitator shaft, which is then dropped through the outboard seal housing. The agitator shaft must be located precisely because its axial position "sets" the correct spring pressure on the inboard seal faces. An internal shaft coupling can simplify the assembly procedure, but adds yet another potential contamination area that may be difficult to clean.

Recognizing the unique concerns of the biotech industry, a special double mechanical seal has been developed for bottom mount agitators. This seal is lubricated with condensed clean steam. As shown in Figure 8, this seal uses a cartridge-canister design that can be pre-assembled and statically tested before installation. Only the housing, seal sleeve, O-rings and inboard seal faces contact the culture medium. None of the springs are in contact with the culture medium. Static O-ring seals are located between the equipment shaft and seal sleeve, and between the seal housing and vessel flange (or adapter) as shown. These O-ring seals are designed with 2:1 L/d ratio (or less) for more effective CIP. Increased bores below the O-rings in the equipment flange and reduced shaft diameter at the set screw (and below) simplify installation and reduce the possibility that O-rings might get nicked during disassembly/reassembly procedures. A drain port removes any seal fluid that leaks through the outboard seal faces.

A pedestal between the gearbox and vessel bottom head simplifies maintenance on large agitators by leaving the motor and gearbox in place when the seal is serviced. An external coupling inside the pedestal joins the gearbox stubshaft and impeller/shaft assembly. This pedestal is shown in Figure 9a with seal in operating position.

To prepare the seal for removal, the seal setting plates are reinstalled, setscrews at the shaft loosened, and external coupling unbolted. The agitator shaft is then lifted 4 inches (10 cm) and blocked in place so the upper coupling half of the external shaft coupling can be removed. The seal housing may then be unbolted from the vessel pad flange and lowered down the agitator shaft - *Figure 9b*. When the seal housing reaches the lower coupling half (on the gearbox stubshaft), lift the shaft enough to clear the seal housing. The seal cartridge may now be removed through the pedestal access

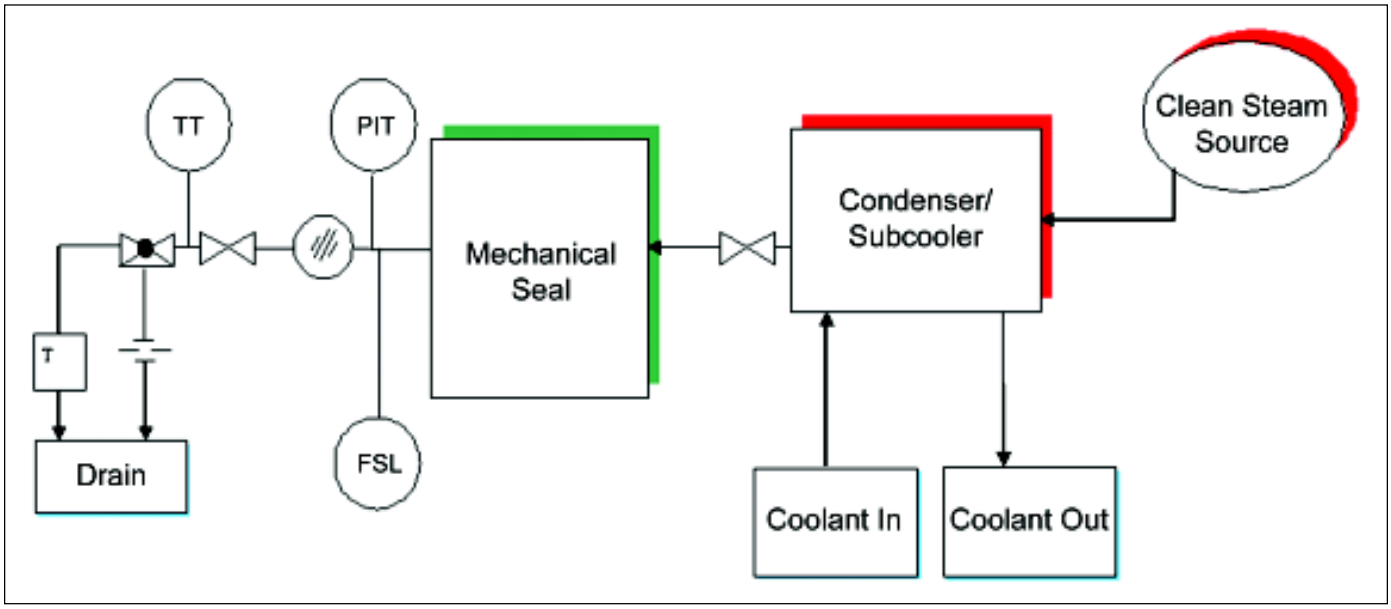

Figure 10. Typical schematic for agitator seal using condensed clean steam as a barrier fluid.

"Scale-down tests can be run to confirm scalability. Such tests are frequently run by agitator vendors, with initial screening runs in small transparent vessels using a variety of impeller designs and locations."

window and taken to the maintenance shop for inspection and service.

Seal face material selection depends on drive size, normal operating conditions, CIP/SIP temperatures, shaft speeds, seal fluid pressure and auxiliary support system used. Because cell culture agitators run at relatively low speeds, frictional heating at the seal faces is not as much of an issue as in bacterial or yeast fermentors. Some users therefore prefer silicon carbide vs. silicon carbide for the inboard seal faces to eliminate the possibility of carbon wear particles entering the culture medium. If this approach is followed, however, seal fluid flow and temperature must be sufficient to lubricate the seal faces during elevated temperature CIP. Use of dissimilar materials (alpha sintered silicon carbide vs. reaction bonded silicon carbide) will help in this regard.

The outboard seal faces, which do not contact culture medium, are recommended to be carbon vs. silicon carbide. This combination provides the most reliable performance under both normal and adverse lubrication conditions.

The static seals between the shaft and seal sleeve and between the cartridge housing and equipment flange can be provided either with double O-rings or single. Some users favor the single O-ring design so any failure will be detected by integrity testing before the batch begins. Others favor the double O-ring design for the shaft static seal because culture medium leaking past the O-rings will be trapped between the shaft and seal cartridge housing. This type of failure is hard to detect during the vessel integrity test, leaked culture medium can't be sterilized during SIP (it's not exposed to steam), and may make seal cartridge removal difficult if hardened deposits form on the shaft.

It may be desirable to have USP Class VI elastomers, despite their higher cost. This certification is available for peroxide-cured EPDM and perfluoroelastomers, both of which have broad chemical resistance, high maximum temperature rating and good suitability for steam sterilization. Silicone O-rings are not recommended because their surface gets hard and cracked after repeated SIP cycles. If USP Class VI certification is not required, FDA-compliant (food grade) peroxide-cured EPDM elastomers are widely available.

Metal parts are typically made from 316L stainless steel. Metal components contacting culture medium are electropolished. Those components contacting only seal fluid may be passivated and/or electropolished if desired.

The greatest amount of cooling to the mechanical seal is required during elevated temperature CIP. Even at very low rpm, the seal faces must receive enough lubrication to prevent surface spalling. For this reason, the auxiliary support system must be sized for this condition. Typical flows are 6.5~13 gal/hr (25~50 L/hr) at 115°F (46°C) or less.

Barrier fluid pressure is the subject of much debate. The seal can be designed with the barrier fluid pressure consistently either higher or lower than hydrostatic pressure of the culture medium at the bottom of the vessel. Seal internals will vary slightly, so the user preference must be known when the seal is being designed.

The authors recommend a dual pressurized sealing system, in which the seal fluid pressure always exceeds vessel hydrostatic pressure by at least 21 *kPa* (3 psi). Higher pressure differential is better—170 *kPa* (25 psig). Even at these elevated pressure differentials, leakage rates into the culture medium are insignificant. The main advantage of the dual pressurized design is the longer and more predictable life due to lower wear rates (only clean fluid between the seal faces).

With the dual pressurized seal, integrity testing before each batch will identify even the smallest leak. Before each batch, close valves upstream and downstream of the seal to trap a known volume of condensed clean steam. While the agitator is running, monitor the seal pressure decay; the rate of barrier fluid pressure decline is proportional to the leakage rate. Halt the test after the pressure decays 5 psi (35 *kPa*) or 30 minutes, whichever comes first. The rate of decline should be compared to the baseline value measured when the seal was first installed.

Some users may prefer to run the seal unpressurized, i.e., seal pressure is always less than vessel hydrostatic pressure. This ensures that any leakage through the seal faces will not result in batch contamination. Furthermore, if a carbon face is used on the inboard seal, any wear particles will be pushed into the seal chamber. Seal life with this approach is both shortened and made unpredictable because clean water is no longer used as the seal face lubricant. In addition, integrity testing is much less sensitive, because leaks are detected only when enough culture medium passes through the seal to change seal fluid color or increase its conductivity.

A typical schematic for seal fluid piping is shown in Figure 10. Clean steam is condensed and subcooled in a double pipe heat exchanger. Coming out of the seal housing, seal fluid flows through a sight flow indicator and low flow switch to either a steam trap or restriction orifice. During normal operation, this block valve is closed and the restriction orifice controls seal flow. Seal fluid backs up into the condenser until the remaining surface area condenses enough clean steam to match the flow through the orifice. The flooded portion of the condenser produces enough subcooling to cool the seal faces and avoid thermal damage to the cells.

Agitator Fabrication

The surface finish for the agitator should match that specified for the vessel. Typically, this would be 20 microinch (0.5 micron) Ra with electropolish. The required quality of finish for cell culture often exceeds the requirements for bacterial fermentation or those common in the food process industry.

The usual industry practice of applying the finish after completing impeller and shaft assembly does not produce consistently satisfactory results. Corner areas cannot be polished orthogonally and it is difficult to obtain good electrode geometry for electropolishing. Even though the finish met Ra requirements, the end result was an agitator finish visibly inferior to the vessel finish.

The far better procedure is to apply the mechanical polish to blades, hubs and shaft before welding. In so doing, you can use successive grits at right angles to the previous polish, without concern for interference by other parts of the assembly. The components should then be electropolished and protected by paper during forming and assembly. After the rotating assembly is complete, the welds are ground and then spot electropolished.

Agitator specialist firms routinely use CFD (computational fluid dynamics) to study agitator performance in the customer's vessel. The CFD analysis has been validated by laser Doppler velocimetry data collected in test vessels, and so represents a good predictor of actual agitator performance. The CFD study greatly reduces the need to adjust impeller centerlines. If CFD studies will be performed, it is recommended that the impellers be welded to the shaft, thus eliminating two hard-to-clean O-ring seals at the hub of each impeller.

Conclusions

As cell culture systems get larger, the key process parameters (k_La, blend time and peak shear rate) do not remain constant. The equations offered, supported by test data and CFD analysis, can guide scale-up and optimization. Large cell culture systems may require a different impeller from existing pilot scale equipment, with high efficiency and high solidity axial flow impellers being recommended.

Production-scale systems frequently use bottom-drive agitators to reduce ceiling height in the bioreactor suite. Double mechanical seals lubricated by pressurized condensed clean steam are needed for bottom-drive agitators. The cartridge seal improves on past practice through fewer parts contacting the culture medium, design features that meet ASME BPE requirements and ability to be removed for service without entering the vessel or removing the gearbox.

An agitator can be built with visual appearance matching the vessel provided the shaft assembly is welded from prepolished components.

Symbols Used in Equations

A	Constant in k_La equation
a	Constant in equation for mixing rate constant
b	Exponent in equation for mixing rate constant
C	Concentration of solute (e.g. dissolved gas)
C^*	Saturation concentration of solute
D	Impeller diameter
d	Width of a crevice (e.g. the gap leading to an O-ring)
F	Superficial gas velocity
k	Shear coefficient
k_La	Gas mass transfer effectiveness
k_m	Mixing rate constant
L	Depth of a crevice (e.g. distance between vessel surface and O-ring)
N	Agitator rotational speed
NQ	Impeller pumping number
NP	Impeller power number
OTR	Oxygen transfer rate
P	Agitator power
Q	Agitator (volumetric) pumping rate
$SpGr$	Specific gravity of culture medium
T	Vessel ID
t_u	Time to reach a specified degree of mixing uniformity
U	Mixing uniformity
Z	Ungassed liquid depth (measured from inside of bottom head)

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This article discusses the fundamentals of ultrasound contrast agents including new innovations like targeted imaging.

This article represented the Delaware Valley Chapter as a finalist in the International Student Poster Competition/ Graduate Division held at the 2002 ISPE Annual Meeting in Orlando.

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Surface Modification of Polymeric Contrast Agents for Cancer Targeting

by Justin D. Lathia, Dalia El-Sherif, Nikhil O. Dhoot, and Margaret A. Wheatley

Background

Ultrasound Imaging

Ultrasound is a popular medical imaging technique that has gained increased usage over the past 10 years. Some well-known uses of ultrasound include the in utero imaging of a fetus and the imaging of the heart to evaluate heart function. However, the applications and potential of ultrasound imaging extend well beyond pre-natal and cardiovascular imaging, including major human health issues such as tumor detection.[1]

Ultrasound is a non-invasive medical imaging technique that relies on high-frequency sound waves to produce an image. Since most tissues are heterogeneous in structure, a sound wave sent via a transducer acting as both a transmitter and a receiver will be scattered or reflected.[2] It is this scattered energy, or backscatter, that returns to the transducer and from which an image can be produced. Ultrasound was first used for medical imaging of soft tissues in the 1970s when the technology was capable of capturing and displaying backscatter.[2] The first images that were captured were static, but as the technology improved, real time images were possible, as seen today in the imaging of a moving fetus in the uterus.

Ultrasound energy can produce many types of images depending on the mode by which the sound wave is sent and received. The first images that were produced used line of sight displays, such as in radar and sonar, and are referred to as A-mode. A-mode images are displayed as the amplitudes of the signals received and could initially be seen only on oscilloscopes. A-mode images recorded side by side to show motion are referred to as M-mode, which were produced on special thermal paper. The sweeping of the transducer, either electronically or mechanically, over an area to produce a two-dimensional image is referred to as B-mode imaging.[2] B-mode images are different than A-mode images because they display images that vary in brightness instead of amplitude, in relation of the strength of the signal received. B-mode images could be seen on television monitors as a result of the build up of multiple scans of static images. Using more sophisticated equipment and image analysis techniques, it is now possible to see all three imaging modes on television monitors.

The first uses of ultrasound imaging were to study motion, such as in the heart. A-mode and M-mode imaging techniques were used to study many changes in the heart that correlated to clinical conditions. For example, motion of heart valves, thickening of heart chamber walls, and heart motion in relation to pressure. Many of these analyses are still used today for cardiac monitoring. B-mode imaging is two-dimensional and was first used in the imaging of soft tissue and obstetrics.[2] More recent applications of ultrasound imaging include three-dimensional imaging and Doppler imaging, used for flow analysis. With modern ultrasound, blood flow can be imaged to determine abnormalities in blood vessels and cancers can be detected through the vascular changes caused by tumor development. Research is being done on drugs that can be delivered locally using ultrasound energy as a release mechanism.[3]

Ultrasound Contrast Agents

Ultrasound relies on an interface between two different types of tissues to produce an image. At the interface of the two different tissues, there is a difference in properties, which is referred to as an impedance mismatch. Impedance is related to the product of density and the speed of sound through a material. Thus, skin and bone have different impedances and an image of bone can easily be produced. In a

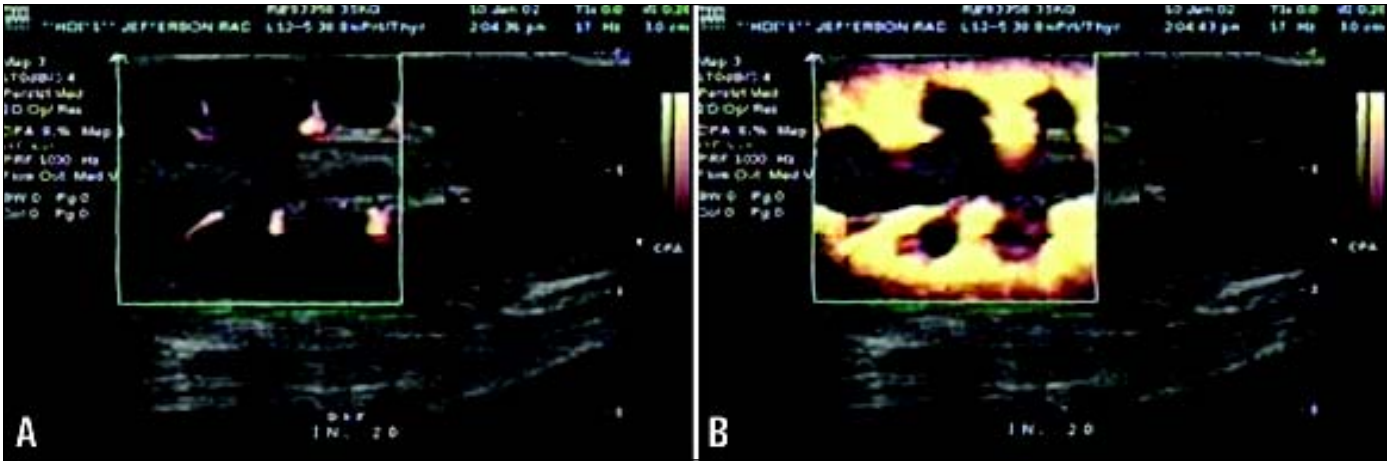

Figure 1. PLGA (50:50) contrast agent injected into a rabbit kidney and imaged in Power Doppler mode (Courtesy of Thomas Jefferson University, Department of Radiology). The Doppler image is inside the box and shows parenchymal enhancement. A) pre-injection, B) Post injection (0.15ml/kg (2.42 x 10^{-6} ft^3)).

situation where the tissue is homogenous and there are no differences in impedances, such as the imaging of breast tumors, an ultrasound contrast agent is needed. A contrast agent provides a difference in impedance and makes imaging which was impossible now possible. Currently, contrast agents are used in two-dimensional imaging and color Doppler imaging, but they are under investigation of use in three-dimensional imaging as well as drug delivery.[4]

Most contrast agents utilize the impedance mismatch between gas and the suspending fluid, usually blood, to cause contrast that can be imaged. In most cases, the contrast agents are spherical, thin shelled, microbubbles. There are five types of systems which were noted to make good contrast agents: free gas bubbles, encapsulated gas bubbles, colloidal suspensions, emulsions, and aqueous solutions.[4] Because contrast agents are injected into the body, there are several requirements which they must satisfy. The contrast agent should be biocompatible, not cause any hemodynamic effects, nor alter blood flow. The contrast agent should be smaller than a red blood cell so it will not block any smaller capillaries and should have similar a physiological transit time and velocity profile to that of a red blood cell. Additionally, the contrast agent should perfuse into the target tissue, stay in the circulation long enough to be imaged, and remain stable long enough for recirculation.[4-5] The backscatter produced by the contrast agent is dependent on the physical characteristics of the microbubble. The reflectivity produced is proportional to the concentration of the microbubbles present at a given site and to the fourth power of microbubble diameter.[6] The most important characteristic of the contrast agent is the resonating ability of the bubble, which can produce up to three orders of magnitude more enhancement. Fortunately, the resonance frequencies of microbubbles of 1 - 7 microns in diameter are in the range of frequency for medical imaging, 2 - 15 MHz.[6]

The first contrast agent was discovered by accident by Gramiac[7] and Joiner in the later 1960s. During an M-mode echocardiogram, Joiner noticed a transient ultrasound signal, which was later discovered to be a result of small bubbles that formed at the tip of the catheter.[6] After this discovery, many hand agitated agents emerged, but their performance was plagued by transient effects and their results were not consistent. It was found that mixing a patient's blood with saline and injecting it back into the body by two syringes through a three-way tap produced bubbles that were stable.[6] It was discovered that sonication of blood produced stable microbubbles of a controllable size. It was later found that the blood protein, albumin, was responsible for the stability. The first contrast agent, Albunex™, was made from sonicated albumin. After Albunex™ emerged onto the market, many other contrast agents based on different principles followed. Levovist™ and Echovist™are made from galactose powder. By adding water, small air microbubbles form on the surface irregularities of the galactose crystals and when injected into the blood stream, the galactose crystals dissolve releasing the air microbubbles.[6] Sonovue™ and Definity™are biological membranes, phospholipid, filled with air.[6] Sonovist™ is made from a biodegradable synthetic capsule filled with sulphur hexafluoride.[6] The newest FDA approved contrast agent is Definity™. It is used for echocardiographic examinations and consists of a perfluoropropane gas encapsulated within a lipid shell.[8]

Polymeric Contrast Agents

As research has advanced in the fields of biomedical engineering and materials science, polymers have emerged as a key biomaterial. Much of the processing technology for polymers is already available though similar manufacturing methods in plastic processing, so production is easily possible. The structure of polymers also is controllable and polymers can be made with a variety of different mechanical properties. Most importantly, many polymers are inert and biocompatible, thus they do not pose a large risk when implanted into the body. For these reasons, polymers have become a useful implant material with applications ranging from joint replacements to drug delivery devices.

With the emergence of polymers as a premier biomaterial, ultrasound contrast agents made from polymers also have

been explored. The novel polymeric contrast agent developed in our laboratory is based on an emulsion and freeze-drying technique principle and uses poly (lactic - co - glycolic) acid (PLGA). PLGA has been FDA approved for use in sutures and drug delivery devices. PLGA is also biodegradable and biocompatible. It degrades into lactic and glycolic acid by non-enzymatic hydrolysis of the ester backbone. Both lactic and glycolic acid are able to be processed by the body.[9] Our PLGA contrast agent is formed by a patented[10] double emulsion technique in which camphor and ammonium carbonate are encapsulated and then later sublimed, creating an echogenic microsphere.[11] The PLGA contrast agent developed by our group has shown good enhancement of the blood flow in a rabbit model - *Figure 1*.

Angiogenesis

One emerging application is the use of ultrasound to detect, diagnose, and monitor angiogenesis. Angiogenesis is the formation of blood vessels from existing vessels and is an early sign in many types of developing tumors. Angiogenesis is also involved in wound repair and inflammation.[12] In the first step in angiogenesis, the parental vessels vasodilate and the basement membrane of the vessels begin to degrade via proteolytic enzymes. Endothelial cells begin to migrate into the extracellular space and proliferate to form a leading edge. The endothelial cells form tubules with lumen and begin to synthesize basement membrane. Finally, the tubules anastamose and recruit smooth muscle cells and pericytes to complete the vessel structure. This process can also be seen in the second half of embryonic vascular development where the immature vessels are stabilized through growth factors such as Vascular Endothelial Growth Factor (VEGF) as well as angiopoietins.

In the 1960s, Dr. Judah Folkman saw the correlation between this process and the development of vasculature during the early stages of cancer. Tumors implanted into isolated organs showed limited growth while tumors implanted into mice grew rapidly and eventually killed the host.[13] These results sparked the idea that for a solid tumor to develop, growing vasculature is necessary. The angiogenesis in tumors is similar to the angiogenesis that occurs in developing vessels, however tumor angiogenesis is not as tightly regulated and is more chaotic.[14] While it is possible to detect a tumor using various techniques, confidently determining if the tumor is malignant is more difficult. In this area, ultrasound can be used in a non-invasive way to assess the state of the tumor. By using the Doppler technique, it is possible to measure blood flow and correlate the results back to the functionality of the developing vascularity.[1] Since Dr. Folkman's observation, the understanding of angiogenesis in relation to cancer has been heavily studied though various techniques such as Doppler imaging, and inhibition of angiogenesis is being researched as an alternative therapy to chemotherapy.

Angiogenesis Targeting

The start of angiogenesis occurs when a signaling molecule, in this case a angiogenic growth factor, binds to the cell via a cell surface receptor and initiates a sequence, which results in new blood vessel formation. Integrins, a type of cell surface receptor, directly associate with the receptors that bind these growth factors and have the capacity to affect the outcome of the cell's behavior.[15] The endothelial cells that line the blood vessels also express receptors for angiogenic growth factors. As a tumor grows and develops it requires vasculature and it has been shown that chemotherapeutic agents bound to the growth factor receptors resulted in localized cytotoxic effects.[16] This observation suggests that if these receptors can be targeted, angiogenesis may be affected and thus, the growth of the developing tumor can be slowed down or even stopped.

Through much experimental evidence, the link between angiogenic growth factors and angiogenesis has been determined to be modulated through the cell surface integrins, $\alpha_v\beta_3$ and $\alpha_v\beta_5$.[17,18] Using an accepted angiogenesis assay, the chick chorioallantoic membrane (CAM) which sprouts vessel during it development and can be used to either induce or inhibit angiogenesis, it was determined that molecules blocking $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrin receptors (anti- $\alpha_v\beta_3$ and anti- $\alpha_v\beta_5$) can slow down the progression of angiogenesis.[17] Since these findings, efforts have been made to find antagonists for these receptors or use the receptors to target angiogenesis in developing tumors.

Cell surface integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$, among many others, have been found to bind to a generic peptide sequence arginine-glycine-aspartic acid (RGD), which is found in the extracellular matrix protein (ECM) fibronectin.[19] The RGD sequence is a versatile peptide sequence and has been used as both a coating to encourage cells to bind to materials as well as a coating to bind smaller, free floating particles to target cells. In a mouse model, an RGD peptide sequence specific to α_v integrins was shown to bind specifically to the blood vessels in human tumors and less in the surrounding normal vasculature.[20] As seen in the literature, RGD containing peptide sequences specific to $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins have been used to target angiogenesis with good success.

Ultrasound Targeting

With recent advances in cell and molecular biology as well as ultrasound imaging, research is being done to identify changes that occur in pathologic condition using targeted imaging. Such imaging can be used to detect a change in tumor vasculature and then correlated back to the malignancy of the tumor. This type of imaging requires that ultrasound contrast agents be modified with molecules, such as antibodies or peptides, which target the pathology and then are viewed using ultrasound imaging. In recent studies, such targeted contrast agents have been shown to be successful at targeting larger pathologies such as clots. Using a monoclonal antibody that targets thrombi formation, a targeted perfluorocarbon emulsion was made to detect thrombi in the vasculature.[21] The detection of thrombus formation could be indicative of pathologic conditions such as coronary artery disease and peripheral vascular disease. In the early stages,

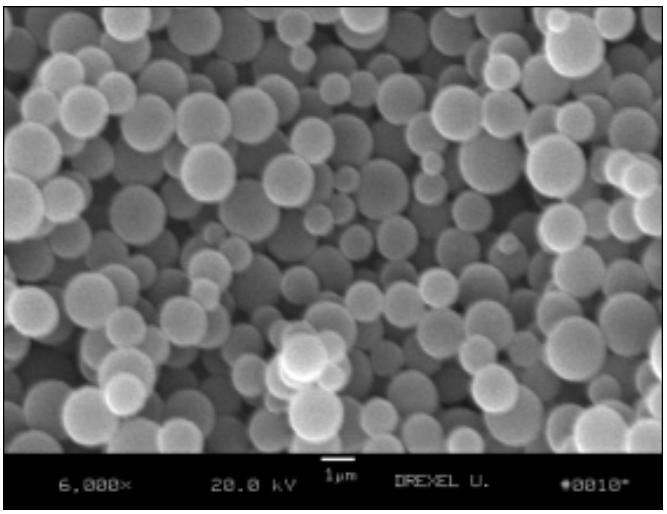

Figure 2. PLGA (50/50) microcapsules imaged with a Scanning Electron Microscope (Amray model 1830D), size bar indicates 1μm (3.94 x 10^{-5} in).

atherosclerosis is marked by an increase in endothelial cell expression of leukocyte adhesion molecule (LAM). Based on this principle, monoclonal antibodies were coupled to lipid-based perfluorocarbon microbubbles and successfully shown to target overexpression of LAM in vitro.[22] These studies show the possibility of coupling a marker and a contrast agent to create a targeted contrast agent, which can ultimately be used in the early detection, diagnosis, and treatment of a variety of pathologies.

In this study, our objective was to create a targeted contrast agent by the surface modification of a polymeric contrast agent with the RGD peptide. The eventual project goal is two fold. First, to create a drug-loaded targeted contrast agent that binds to the vasculature of a developing tumor, which can be imaged to evaluate the vascularity of the tumor. Second, once the drug loaded contrast agent is bound to the tumor vasculature, ultrasonic energy is used to release the drug to the local area. Our strategy is aimed towards the

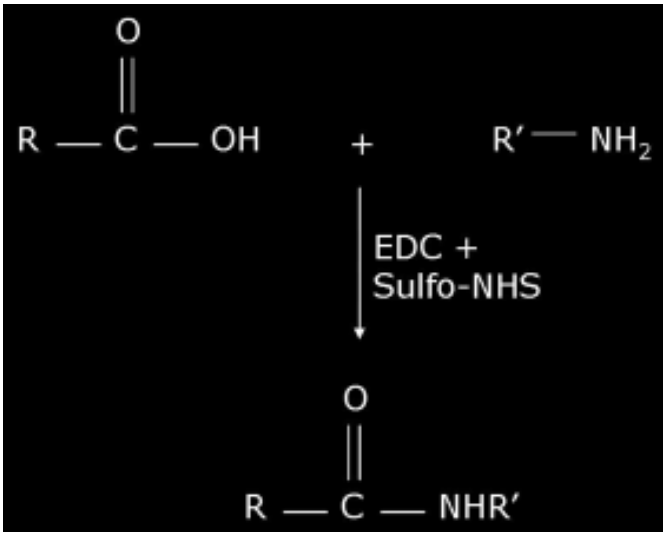

Figure 3. Carbodiimide conjugation of the carboxylic acid group (R-COOH) group of the microspheres to the amine terminal group of the peptide (R′-NH_2).

imaging of tissues that are naturally homogenous and difficult to image, such as the breast. If a contrast agent can be developed that binds to the developing tumor vasculature, it can be visualized and then a drug can be delivered to the local area retarding tumor vasculature growth.

Materials and Methods

Materials

Poly (D,L-lactide-co-glycolic acid 50:50, PLGA) (Medisorb 5050 DL 3A, lot 1010-412) was purchased from Alkermes. Poly (vinyl alcohol) (PVA), 88% mole hydrolyzed, with a M_w of 25,000 was from Polysciences, Inc. (1R)-(+)-camphor, GRGDS (Gly-Arg-Gly-Asp-Ser) peptide complex, EDC (1-Ethyl-3,-3-Dimethylamino-Propyl) carbodiimide, sulfo-NHS (N-hydroxysulfosuccinimide), antibiotics (penicillin and streptomycin), and L-glutamine were from Sigma. Dulbecco's modified eagle medium (DMEM), Hank's balanced salt solution, and fetal bovine serum (FBS) were purchased from Fisher. Ammonium carbonate was purchased from J.T.Baker. All other chemicals were reagent grade from Fisher.

Preparation of Microcapsules

Microcapsules were prepared by a patented double emulsion (W/O)/W solvent evaporation process using camphor as a removable core.[11] Camphor (0.05 g (0.00011 lbs)) and PLGA (0.5 g (0.0011 lbs)) were dissolved in 10 ml (3.53 x 10^{-4} ft^3) of methylene chloride. To generate the first (W/O) emulsion, 1.0 ml of 4% ammonium carbonate solution (w/v) was added to the polymer solution and probe sonicated at 115 watts for 30 seconds. The (W/O) emulsion was then poured into a 5% PVA solution and homogenized for 5 minutes at 9,500 rmp. The double emulsion (W/O)/W was then poured into a 2% isopropanol solution and stirred for 1 hour. The capsules were collected by centrifugation, washed three times with hexane, then once with deionized water. The microcapsules were then frozen at -80°C (-112°F) and lyophilized, using a Virtis Benchtop freeze dryer, to remove the camphor and ammonium carbonate core. After freeze drying, the microcapsules appear spherical in shape and have an average size of 1.2µm (4.72 x 10^{-5} in) - *Figure 2*.

Peptide Conjugation

The platform of our targeted contrast agent is the PLGA, which has a carboxylic acid group that can be used for conjugation. Using carbodiimide chemistry, the carboxylic acid group on the PLGA can be reacted with an amine group, for example an end group on a peptide, and an amide linkage can be formed.[23] This type of reaction can be facilitated using a water-soluble carbodiimide reagent such as ethyl (dimethlyaminopropyl) carbodiimide (EDC) and a catalyst, N-hydroxysuccinimide (sulfo-NHS). The peptide conjugation reaction in this study utilizes carboxylic acid carbodiimide chemistry with EDC and sulfo-NHS as the catalysts. The carboxylic acid group (R-COOH) group of the microspheres is conjugated to the amine terminal group of the peptide (R'-NH_2) - *Figure 3*.

The dried microcapsules (100mg (2.2 x 10^{-4} lbs)) were

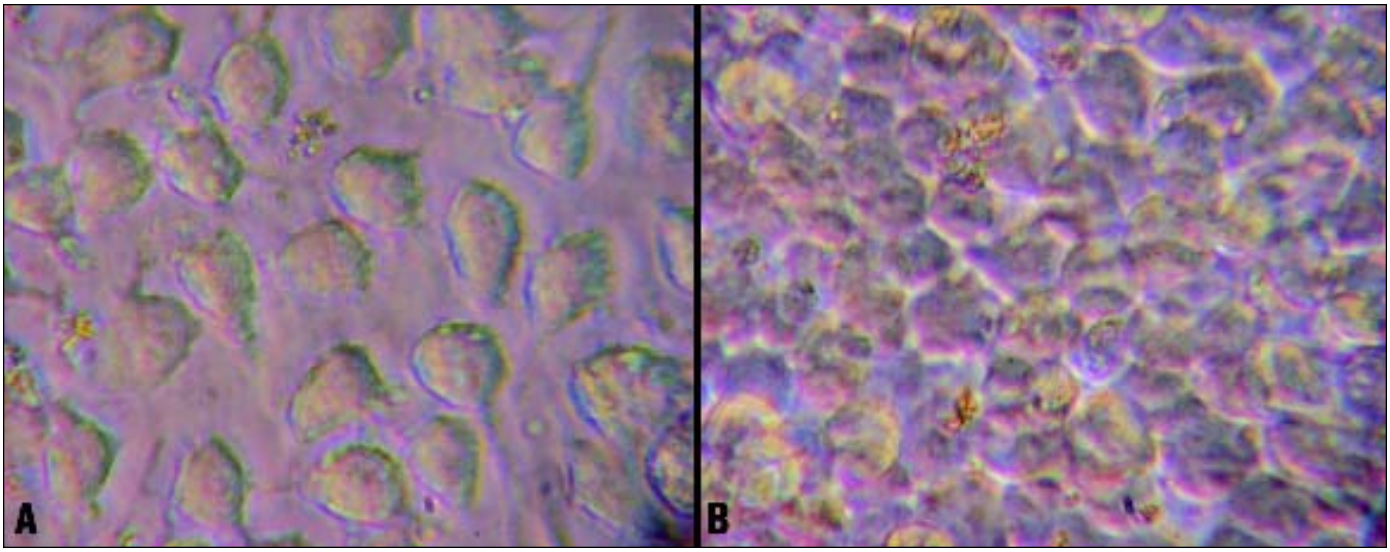

Figure 4. NB2a cells at 0 hours after incubation with PLGA microcapsules, A) unmodified, B) modified. Size bar represents 50μm (0.002 in).

combined with 5 mg (1.1×10^{-4} lbs) EDC (1:1 molar ratio of COOH groups in the microcapsules to EDC), 1.4 mg (3.08×10^{-6}) of sulfo-NHS (1:2 molar ratio to EDC), in 10 ml (3.53×10^{-4} ft^3) of buffer (0.1M MES, 0.3M NaCl, pH 6.5) and stirred for 15 minutes. RGD peptide (150µg (3.3×10^{-7} lbs), in a 1:10 molar ratio of COOH groups in the microcapsules to peptide) was then added and stirred for 3 hours.[24] The microcapsules were then washed with deionized water, frozen, and lyophilized.

Cell Culture

The NB2a mouse neuroblastoma cells were cultured using growth medium containing 90% DMEM, 10% FBS, 2.5ml (8.83×10^{-5} ft^3) of antibiotics (1X) and 2mM L-glutamine. The medium was changed and the cells were split every three days. The experiment was performed on the cells at passage 10. The NB2a cell line was chosen because the cell line was being used in our lab for another project and we were very familiar with the cell culture techniques. Furthermore, since the attachment of the RGD peptide was being examined, the NB2a cell line was acceptable because the RGD peptide sequences bind a variety of receptors, some of which are on the NB2a cell line.

Static Attachment & Microscope Imaging

Cells, ~95,000, were plated in each well of a 12-well cell culture plate with growth medium, total volume 3ml (1.06×10^{-4} ft^3). After three days, the cells became confluent. The cells were then washed with 3ml (1.06×10^{-4} ft^3) of Hank's balanced salt solution and replaced with a 3ml (1.06×10^{-4} ft^3) modified medium containing microcapsules, either PLGA with or without RGD peptide, suspended in the growth medium at a concentration of 0.5 mg/ml (0.031 lbs/ft^3). The cells were then incubated for 0, 1, 6, and 24 hours. After each specified time point, the medium was removed and the cells were washed again with 3ml (1.06×10^{-4} ft^3) of Hank's balanced salt solution. The cells were then viewed under a Wesco Verta 7000 series microscope. Digital pictures were taken using an

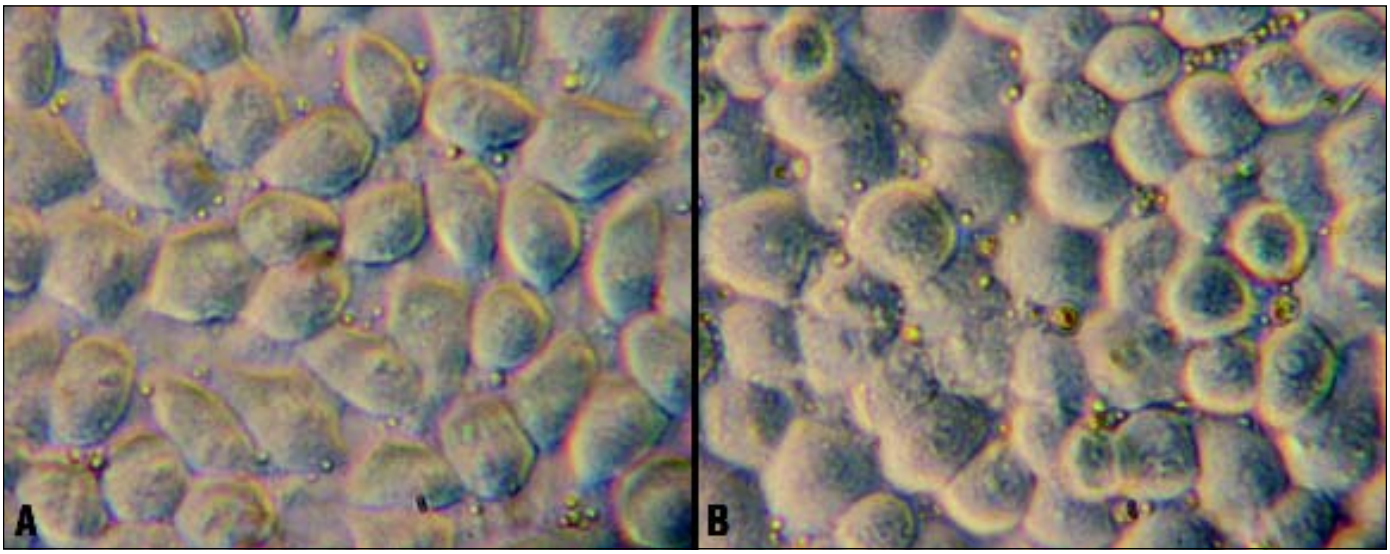

Figure 5. NB2a cells at 1 hour after incubation with PLGA microcapsules, A) unmodified, B) modified. Size bar represents 50μm (0.002 in).

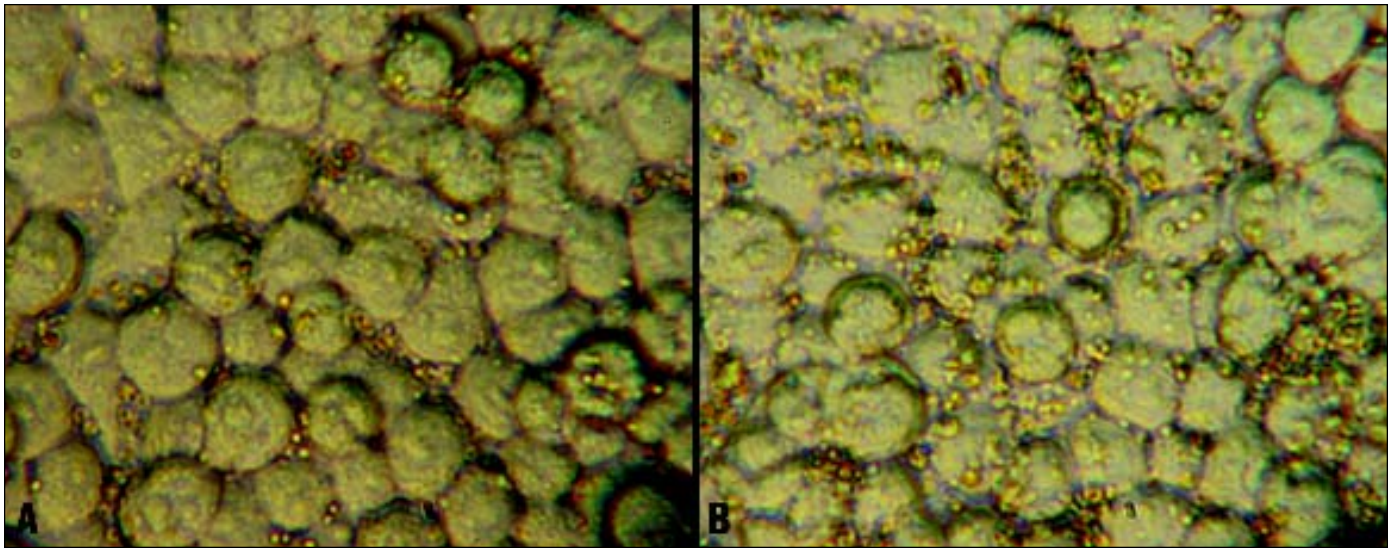

Figure 6. NB2a cells at 6 hours after incubation with PLGA microcapsules, A) unmodified, B) modified. Size bar represents 50μm (0.002 in).

Olympus DP11 digital camera interfaced with the microscope at a magnification of 1000x.

Results

At 0 hours, where the microspheres were pipetted on and immediately taken off, both the PLGA and RGD conjugated PLGA microspheres show little to no attachment - *Figure 4*. The PLGA and RGD bound PLGA microspheres showed limited attachment after one hour as seen by Figure 5. The microspheres conjugated with RGD peptide attach to a greater degree than microspheres without peptide conjugated to its surface, after 6 hours of incubation - *Figure 6*. The microspheres are contrast agent filled with a gas, air in this case, and are naturally buoyant due to their gas content. Even though they are buoyant in the medium, many still attach to the cells, suggesting the peptide coating is mediating an interaction between the cells and the microspheres that results in attachment. Furthermore, the attraction of attachment was not only greater than the buoyancy, but also withstood washing with buffered saline solution after the given time point, suggesting definitive attachment.

Another important aspect of the attachment to examine is the location of attachment of the microspheres to the cells. Definite attachment was determined by the amount of microcapsules attached to the top surface of the cells. Since there are gaps at the junctions of many cells, it could be possible that the microcapsules adhered to the proteins secreted in the gaps by the cells, and thus were not considered in determining the amount of microcapsules that attached to cell at each time point. At time 0, Figure 4, the attachment of both types of microspheres on top of cells is not present. After only one hour of incubation, Figure 5, the peptide-modified microcapsules have only 3 attached versus 0 for the unmodified microcapsules. However, after 6 hours, Figure 6, the attachment of the peptide-modified microcapsules is much greater than the unmodified microcapsules. Approximately 50 peptide-modified microcapsules attach on top of cells as compared to approximately 16 for the unmodified microcapsules. The results of this initial proof of concept study show the feasibility of using a polymer contrast agent to target cells through receptors expressed during certain pathologies, such as cancer.

Conclusions

These qualitative results suggest that RGD modified microspheres can be used to target the receptors overexpressed in cancer cells. This opens up the possibilities for targeted therapeutic imaging and drug delivery using microspheres as the vehicle.

Since the initial proof of concept study, several more studies have been conducted that have strengthened the initial hypothesis. The time of incubation for attachment has been dropped to as little as five minutes, representing a more realistic physiological situation. The binding ability of the microspheres has been tested on a human breast cancer cell line, MDA-MB-231 (courtesy of Dr. Janet Price, MDA Cancer Center). The selectivity of the adhesion was tested by first blocking the targeted receptors with the same peptide that was conjugated on the surface of the microspheres. The blocked sample set showed limited adhesion with respect to PLGA and RGD bound PLGA microspheres, indicating that the adhesion is mediated through receptors that bind RGD peptide sequences. The dynamics of cell adhesion have also been tested under flow conditions using a parallel plate flow system and the results are in line with the static attachment studies.

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