Real-Time Analysis and Investigation of the Mechanism of the Palladium on Carbon Catalyzed Isomerization of Megestrolacetate

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REAL-TIME ANALYSIS AND INVESTIGATION OF THE MECHANISM OF THE PALLADIUM ON CARBON CATALYZED ISOMERIZATION OF MEGESTROL ACETATE

by
Charlene K. Wiglesworth

A Thesis
Submitted to the
Faculty of The Graduate College
in partial fulfillment of the
requirements for the
Degree of Master of Science
Department of Chemistry

Western Michigan University
Kalamazoo, Michigan
April 2008
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Charlene K. Wiglesworth
2008
Megestrol Acetate is a semi-synthetic steroid hormone of the progestin family and currently used as a drug therapy in many areas including treatments of cancers and AIDS. The final step to form the bulk drug is an isomerization using palladium on carbon with cyclohexene as an activator. This step is sensitive to catalyst quality and is hazardous to sample because of the reactivity of the catalyst. Physical and catalytic properties of the catalyst were evaluated, as were the effects of solid support and different washing strategies. Response of the reaction mixture to catalyst poisons was studied as a function of catalyst type. Different catalysts were evaluated in an effort to identify a more consistent catalyst for the reaction. The reaction mechanism was explored as well as the paths which lead to higher impurity levels. A simple on-line UV method was developed to monitor reaction progress in real time to avoid unnecessary sampling.
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INTRODUCTION

The term "Process Analytical Technology," or "PAT," is typically used to describe a system for design and control of manufacturing. This is done through timely in-situ measurements (i.e. during processing) of critical quality and performance attributes for processes with the goal of ensuring final product quality. The PAT initiative focuses on building quality into the product and the manufacturing processes, as well as on continuous process improvement. The term "analytical" in PAT is generally agreed to include chemical, physical, microbiological, mathematical, and risk analysis conducted in an integrated manner. A major benefit of PAT is that of using multivariate process data to provide more information and a better understanding of manufacturing processes than conventional approaches, such as univariate statistical process control (SPC).

PAT includes much more than just mechanical sensing technology; it is an overall system for designing, measuring, controlling, analyzing and predicting a manufacturing process in real or near real time via measurements that are on-line (i.e. probe in the tank) or at-line (i.e. probe at the tank). The ultimate goal of PAT is to provide a thorough process understanding such that variability is understood and controlled. This ensures that quality attributes, such as impurity levels, can be accurately predicted. With feedback control, the outcome of this is a process that can
adjust in real time to consistently deliver a product that meets more stringent quality specifications in a varying environment. PAT has been widely available for years and has been applied extensively in the chemical and petrochemical industries. While the technology and its application is not novel, the acceptance and guidance by the Food and Drug Administration (FDA) is new. The goal of the FDA guidance document from September 2004 is to promote a more scientific, risk-based approach to manufacturing drugs and to encourage innovation. This is a major departure from 21 CFR Part 11, which was viewed by many manufacturers as having the potential to stifle innovation. If process understanding is demonstrated through the use of PAT tools, the FDA may become amenable to less restrictive regulatory approaches in managing change.

By applying PAT a company could realize gains in quality, safety, and/or efficiency that would all result ultimately in cost savings. Quality improvements might be derived from the identification and understanding of parameters that are critical to quality, and reduction or elimination of deviations. Safety compliance can also be improved by on-line analysis, as operator exposure to hazardous chemicals would be minimized. Improvements in capacity utilization to reduce inventory levels and reduce cycle times generate savings from efficiency.
Using PAT tools to identify important parameters in a process during the development phase, and use of those tools during the scaleup and production phases allows an in-depth understanding of the process. A process more thoroughly understood has a reduced occurrence of rejections, deviations and reprocessing. A reduction in variability is also likely. Direct feedback control may then be used to control or remove the sources of unwanted variation to result in a robust and predictable process. Multivariate analysis tools can be used to monitor and understand complex variable interactions.

In many processes that involve sampling for off-line analysis (i.e. sampling for pH, for reaction completion by HPLC, etc), the operator is potentially exposed to many hazardous chemicals or on-going chemical reactions. While the worker would most certainly utilize personal protection equipment to minimize the risk of exposure, it is generally agreed that elimination of the exposure scenario is preferred. Use of on-line analysis, where a probe is in the reactor, and no sampling is needed, allows the safest form of PAT control.

All product cost has an element of labor and overhead (L&O) pertaining to the equipment being used. L&O is a fixed cost and will be absorbed by the products produced in a given workcenter. It is therefore important that the material process through the equipment as efficiently as possible to minimize waste. PAT can assist
this by reducing cycle time, as in-line measurements are real time, as opposed to the delay of several hours often seen in off-line measurements (delay due to delivery of the sample to the lab, preparation of the sample, analysis of the sample, and evaluation of the results of the analysis). Use of PAT to make a more robust process also allows more reliability in the prediction of inventory levels and allows a company to reduce work-in-progress costs.

Many chemical processes were developed and implemented years before the PAT initiative and before the analytical tools existed to explore complete process understanding. One such process is the one used by Pfizer Inc. to manufacture the bulk drug megestrol acetate (MA). The final step in the synthesis, isomerization of the 6-exo double bond (6-methylene-17α-acetoxyprogesterone, "4MPO") to the 6(7) position within the B ring of the steroid nucleus, is accomplished by treatment of the starting steroid with a catalytic amount of 5% palladium on carbon in methanol and THF; sodium acetate is present as a buffer to limit impurity formation and a small amount of cyclohexene is added to activate the palladium (see the figure below).
The reaction is completed in a few hours at reflux temperature (~60°C). The process has in recent years been plagued by slow and stalled reactions and elevated impurities. The bulk of the problems have been traced back to variation between different lots of the palladium catalyst.

The existing analytical techniques used to monitor the isomerization require the collection of samples of the hazardous reaction mixture followed by GC analysis. As the catalyst palladium on carbon is pyrophoric, it is desirable to limit sampling as much as possible for safety reasons. Additionally, the only current way to determine if a reaction is slow or has stalled is by sampling and analysis. Such reactions require multiple samples to determine that the reaction has reached its endpoint. This adds substantially to the cost via labor and overhead since the sampling by this method slows down the overall processing; quality is also impacted in that impurities are
known to increase with extended processing times. Therefore an on-line method to monitor the reaction which can tolerate the presence of the solid catalyst is needed. Large differences in the solubility and UV spectra of the starting material and product MA enable the use of on-line UV spectroscopy to follow the rate of reaction (see figure 2 below). The spectroscopic measurement is facilitated by the use of an attenuated total reflectance (ATR) probe for the application due to the high concentration of steroid present and the presence of the particulate catalyst substrate. The absorbance at 292 nm provides a measure of the concentration of MA, which can be used to accurately predict the reaction endpoint. This allows the determination of the best point to take a sample for the registered endpoint assay or when to take remedial action if the reaction has stalled. This method is very simple and has the potential to eliminate a great deal of unnecessary sampling of a highly hazardous reaction mixture and can reduce labor and overhead due to shorter reaction cycles. It will be invaluable as a troubleshooting tool in the case of problematic reactions.
The pharmaceutical company Pfizer is a major producer of megestrol acetate (MA) bulk drug; this active pharmaceutical ingredient (API) is then sold to other companies for formulation. MA is a marketed product and the process is registered as a Drug Master File (DMF) which is referenced by the formulating companies with the FDA. Any major change to the MA process would require a costly and time consuming change process that would have to be approved and filed with the FDA by every company that references Pfizer’s DMF. It is therefore undesirable to make a substantial change in the process. The process, as performed by Pfizer is covered by a patent.3
MA is a semi-synthetic steroid hormone of the progestin family; it also has some glucocorticoid properties. It was initially developed as a contraceptive but much research has since been done to explore other potential uses of this drug as a therapy in many areas including treatments of AIDS and cancer (cancers include endometrial, breast, and prostate cancers). Weight gain is a frequent side effect of treatment with MA, although weight gain is associated with increased appetite rather than fluid retention. This side effect is the basis for MA’s most popular current use, as an appetite enhancer for AIDS patients. Many AIDS patients suffer from cachexia, the loss of more than 5% of the baseline body weight over a span of a few months, and use of an appetite enhancer can help these patients maintain higher quality of life as well as increase their tolerance to other therapies, as it is essential that AIDS patients maintain a minimum weight. Clearly, MA is an important therapy in the treatment of these devastating illnesses.\textsuperscript{4,5}

The PAT tool described in this document offers a means to monitor the process in real time for relatively little expense, thereby offering faster cycle times and quick identification of problematic reactions without unnecessarily exposing workers to hazardous materials. Ultimately UV-ATR was chosen for the development work, although several off line options still exist. On line options are limited due to the presence of the solid palladium catalyst; this catalyst does not interfere in any way with the use of the ATR technology with UV. Several process variables were also
evaluated to better understand the chemistry and process dynamics to improve the process further.

In summary, this work pursues the development of a Process Analytical Technology (PAT) tool for the on-line monitoring of the hazardous megestrol acetate isomerization reaction mixture. The PAT UV-ATR method outlined in chapter 1 should permit the determination of the reaction endpoint without any sampling or exposure of the operator to the reaction. Since the isomerization reaction is sensitive to minor differences in the activity of the palladium on carbon catalyst, several candidate catalysts and other variables will be studied in depth to provide more robust reaction conditions for optimum performance on a production scale. This is discussed in chapter 2. Throughout the work, insights into the reaction mechanism will be evaluated (i.e. catalyst, solvent, and hydrogen source) and will be discussed in Section 3.
METHODS

Physical and general properties of megestrol acetate (MA) are presented in table 1 below.  

Table 1. General information on MA

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical formula:</td>
<td>C_{24}H_{32}O_{4}.</td>
</tr>
<tr>
<td>Formal name:</td>
<td>17α-acetoxy-6-methylpregna-4,6-diene-3,20-dione</td>
</tr>
<tr>
<td>CAS registry number:</td>
<td>595-33-5</td>
</tr>
<tr>
<td>Physical appearance:</td>
<td>White or nearly white crystalline powder</td>
</tr>
<tr>
<td>Solubility:</td>
<td>insoluble in water, sparingly soluble in alcohol, slightly soluble in ether, soluble in acetone, very soluble in chloroform.</td>
</tr>
<tr>
<td>Molecular weight:</td>
<td>384.51 g/mole</td>
</tr>
<tr>
<td>Melting range:</td>
<td>is 213-219°C</td>
</tr>
<tr>
<td>UV λ_{max}:</td>
<td>292 nm</td>
</tr>
</tbody>
</table>

Organic synthesis

Lab scale reactions were typically run in a 250 mL round bottom three-neck flask that had been previously treated with nitric acid. Most reactions used solid 4MPO starting
material from the Pfizer Inc plant, solid sodium acetate from EMD Chemicals Inc, 5% palladium catalyst from Engelhard, and solvents from EMD. Some recovered solvents (methanol and THF) from the Pfizer Kalamazoo manufacturing plant were also utilized.

General lab procedure for reaction flask pre-treatment

The 250 mL three neck round bottom flask was submerged in a bath containing concentrated nitric acid and was allowed to soak for at least 15 minutes. Following the soak period, the flask was rinsed multiple times with reverse osmosis (R.O.) water then acetone. The flask was blown dry with nitrogen before use.

General lab procedure for making megestrol acetate (MA)

Starting material 4MPO (78 mmoles) was combined with 5% palladium on carbon (0.28 mmoles), sodium acetate (3.7 mmoles), methanol (2 mL/g) and THF (2 mL/g). The slurry was heated to reflux and cyclohexene (2.7 mmoles) was added to activate the catalyst. The reaction was stirred at reflux until complete, as determined by GC, HPLC and/or UV. The reaction was then filtered to remove the catalyst. The product was isolated by crystallization from methanol. The slurry was cooled, filtered, and washed with cold methanol. Product thus isolated is referred to as “MA” or as “MA UNF,” where the “UNF” refers to the material being an unfinished, or non-
micronized, grade.

*Off line analytical methods*

Three off line methods were used to monitor reactions and are described in detail below.

1. Conditions for the Capillary GC-FID protocol for registered endpoint determination and impurity evaluation are presented in table 2 below.

Table 2. GC conditions

<table>
<thead>
<tr>
<th>Stationary Phase: DB-1, Rtx-1 (or equivalent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column Dimensions: 15m / 0.25mm I.D. / 0.25µm film</td>
</tr>
<tr>
<td>Injection Port Type: Split/Splitless; helium as carrier gas</td>
</tr>
<tr>
<td>Detector Type: flame ionization detector (FID)</td>
</tr>
<tr>
<td>Oven Temperature: 240°C</td>
</tr>
<tr>
<td>Injection Port Temperature: 250°C</td>
</tr>
<tr>
<td>Detector Temperature: 250°C</td>
</tr>
<tr>
<td>Carrier Gas Head Pressure: 15 psig</td>
</tr>
<tr>
<td>Split Flow: 60 mL/min</td>
</tr>
<tr>
<td>Sensitivity (Range x Attn): 1 x 4</td>
</tr>
</tbody>
</table>
Sample preparation for MA Reaction Mixture: Filter 5 mL of the reaction mixture into a 20-mL screw-cap vial. Pipet 200 µL of filtered sample into another vial containing 1 mL of toluene. Mix well and inject 1 µL. A typical chromatogram is shown below in figure 3.

Figure 3. Sample capillary GC-FID chromatogram of MA reaction mixture
2. Conditions for the HPLC assay with ultraviolet light detection protocol for endpoint determination are described in table 3 below.

Table 3. HPLC conditions

<table>
<thead>
<tr>
<th>Column: 25 cm x 4.6 µm Nucleosil C18 Luna</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flowrate: 1.5 mL/minute</td>
</tr>
<tr>
<td>Mobile phase: 50% acetonitrile/ 50% water (isocratic)</td>
</tr>
<tr>
<td>Injection volume: 5 µL</td>
</tr>
<tr>
<td>Analysis time: 25 minutes</td>
</tr>
<tr>
<td>Wavelength: 254 nm</td>
</tr>
<tr>
<td>Reaction endpoint: no more than 1.0% starting material</td>
</tr>
</tbody>
</table>

Sample preparation: samples were prepared by diluting a filtered aliquot of the reaction mixture with mobile phase at a concentration at or below 2 mg/mL. Sample filtration was done via a syringe filter using a Pall 0.45 micron PTFE membrane disk filter (Acrodisc® CR13 mm).

A typical chromatogram is shown in the figure below.
3. Conditions for monitoring the uptake of the catalyst activator cyclohexene and formation of byproduct benzene by GC-FID are shown in the table below.

Table 4. GC-FID conditions

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Stationary Phase:</strong></td>
<td>DB-1 or equivalent</td>
</tr>
<tr>
<td><strong>Column Dimensions:</strong></td>
<td>30 m/0.25 mm I.D./ 1 micron film</td>
</tr>
<tr>
<td><strong>Detector:</strong></td>
<td>flame ionization detector (FID)</td>
</tr>
<tr>
<td><strong>Injection volume:</strong></td>
<td>2 µL</td>
</tr>
</tbody>
</table>

Figure 4. Sample HPLC-UV chromatogram of MA reaction

![HPLC-UV chromatogram](image)

Elapsed Time (minutes)
Sample preparation: reaction samples were injected directly with no additional preparation.

On line UV methods

Spectroscopic measurements were performed by using a SI Photonics Model 440 UV-VIS spectrometer, a fiber optic based instrument with a charge coupled device (CCD) array detector. A trans-reflectance dip-probe with a path length of 10 mm was used for determination of extinction coefficients.

A three bounce attenuated total reflectance (ATR) probe, manufactured by Hellma GmbH, was used for analysis of the reaction mixtures. An ATR probe intrinsically has a very short path length, which permits direct spectral measurements in the concentrated reaction mixture. Transmittance measurements using longer path length probes or cuvettes would require dilution of the reaction mixture sample. Due to the probe design, the presence of solid catalyst had no effect on the measurements made. The ATR probe was encased in a ¾ inch stainless steel housing and was interfaced with the spectrometer by using two 4 meter, 400 µm core diameter fiber optic cables.

Absorbance and concentration are related by Beer’s Law, $A = \varepsilon b c$, where $A$ is absorbance, $\varepsilon$ is the extinction coefficient of the material (i.e. molar absorptivity), $b$ is the cell thickness (path length) and $c$ is the concentration. $A$ is, of course, measured
in the experiments. $C$ may be known from what is charged into the reactor. The $e$ is a constant, but was previously unknown for 4MPO and MA. The constant $b$ for the ATR probe was not precisely known, so it was calculated at the wavelengths of interest. Due to the nature of the probe design it is possible that the effective path length will change depending on conditions (solvent, substrate, temperature, etc.). Therefore the transmittance values presented apply only to the conditions stated.

The extinction coefficient, $e$, was determined for 4MPO using a trans-reflectance dip probe with a known path length (10 mm). Solutions of 4MPO of known concentrations were made and the absorbance was measured. The results gave a straight line plot and the slope of the line was the product of the extinction coefficient and the probe’s path length. In the case of 4MPO, $e$ was found to be $1.137 \times 10^3 \text{M}^{-1}\text{mm}^{-1}$ at 260 nm in ambient temperature 1:1 THF:Methanol. See figure 5 below.
Figure 5. Beer’s law calibration curve for 4MPO, 10 mm trans-reflectance probe, $\lambda=260$nm

![Beer's Law Calibration Curve for 4MPO](image)

The extinction coefficient for MA was determined similarly, and was found to be $2.47 \times 10^3 \text{ M}^{-1} \cdot \text{mm}^{-1}$ at 292 nm in ambient temperature 1:1 THF:Methanol. See the figure below.
The path length for the ATR probe was determined with the known extinction coefficient for MA. A known amount of MA was dissolved in 1:1 THF/methanol and several dilutions were made with a spectrum taken each time using the ATR probe. The results yielded a straight line as shown below, with the slope of the line being $eb$. The effective path length for the ATR was then calculated to be 0.424 $\mu$m using the extinction coefficient determined earlier. See figure 7 below.
Figure 7. Beer's law calibration curve for MA, ATR probe, $\lambda=292$ nm

UV Spectra of the product MA and the starting material 4MPO were taken and overlapped as shown in figure 8 below. MA exhibited a $\lambda_{\text{max}}$ at 292 nm and at that wavelength the absorbance of starting material 4MPO was negligible. The $\lambda_{\text{max}}$ of 4MPO 260 nm.
Figure 8. UV spectra of 4MPO and MA

For the isomerization of MA using UV-ATR to monitor the reaction, the absorbance at 292 nm increased as the reaction progressed and ultimately leveled off when the reaction was complete. The rate of reaction could thus be monitored directly and much could be learned about the behavior of the catalyst. The profile of a typical reaction course, as viewed only at 292 nm, is shown in the figure below.
Using reaction profiles such as the one shown above, catalysts could be directly compared and considerable information could be collected to better follow the course of the reaction.

**Statistical methods**

Design of Experiments (DOE) methodology was used to evaluate the interactions between catalyst type (fast/slow) and the temperature of cyclohexene addition. DOE involves a series of experiments in which purposeful changes are made to several independent variables to determine the most important experimental factors using the fewest number of experiments. Each factor to be studied is evaluated at two levels (high/low) and several different factors may be studied at once. Using this approach
fewer experiments are done than if the researcher did the traditional one-factor-at-a-time approach. This method also allows study of the interactions between variables, which is not possible with traditional methods. A simple DOE was performed for work described in chapter 3 in which two factors (catalyst type and temperature of cyclohexene addition) were studied simultaneously. The four experiment DOE was performed in triplicate and in random order using slow catalyst 5665U and fast catalyst 5666U for the catalyst levels and room temperature vs. reflux for the cyclohexene addition temperature. Responses were evaluated for the individual reduced impurities, the total of reduced impurities (as monitored during the reaction), isolated yields of the product, and impurities measured in the isolated crystals. The Main Effects charts of these responses and statistical analyses were generated using the DOE options within Minitab software.

Other relevant statistics presented in this report were calculated with Minitab software.
USE OF ATR-UV TO MONITOR THE ISOMERIZATION REACTION TO FORM MEGESTROL ACETATE

Theory of UV

Organic molecules absorb ultraviolet light at different wavelengths. The wavelength of strongest absorbance, \( \lambda_{\text{max}} \), is dependent on the functional groups of the molecule. When an organic molecule absorbs energy, valence electrons are promoted from their ground state to an excited state. Organics that contain unstaturation may undergo \( n \rightarrow \pi^* \) and \( \pi \rightarrow \pi^* \) transitions; \( \pi^* \) electrons absorb light in the experimentally useful region of 200-700 nm. 7

The solvent used for studying a molecule by UV may impact the absorbance. Peaks from \( n \rightarrow \pi^* \) transitions shift to lower wavelengths with increasing solvent polarity; this is termed a blue shift. The lone pair on polar solvents lowers the energy of the \( n \) orbital due to solvation effects. Conversely \( \pi \rightarrow \pi^* \) transitions result in a shift to higher wavelength with increasing solvent polarity; this is termed a red shift and is due to the attractive polarization forces between the solvent and the absorbing molecule, which lowers the energy levels in both the excited and unexcited states. As this effect is greater for the excited state, the energy difference between the excited and unexcited states is slightly reduced, and this gives the small red shift. This occurs
also in $n \rightarrow \pi^*$ transitions, but is so weak that it is overshadowed by the blue shift from the solvation of lone pairs.\textsuperscript{8}

Spectroscopic fiber optic cables consist of a silica fiber core, cladding, buffer coating and a sheath. The core is generally fused silica and can vary in diameter, with the larger core fibers able to carry more light than a smaller diameter core. The fiber is optimized to carry near IR, IR or UV/vis light. The cladding is a thin coating of glass, plastic or polymer with an index of refraction lower than the core of the fiber. The cladding reflects light back into the core as it moves down the fiber via total internal reflection. The buffer coating is plastic or polymer and protects the core and cladding and adds strength to the fiber. The sheath is made of metal, plastic or polymer and provides additional strength, protection and resistance to stray light and moisture. Fibers with low moisture levels (i.e. 2 ppm or less) are used for near IR and IR spectroscopy while fibers with high moisture levels (~800 ppm) can be used for UV/vis spectroscopy.\textsuperscript{9}

\textit{Feasibility studies: On line measurement of reactions}

Direct measure of the amount of starting material in the MA reaction is not feasible by using the ATR probe, as the spectral overlap around 4MPO’s $\lambda_{\text{max}}$ at 260 nm is expected to be too high to accurately determine the amount of 4MPO present in a reaction; see the figure below.
Figure 10. UV spectra of 4MPO and MA

![Absorbance of 4MPO and MA UNF](image)

Figure 11 below shows a three dimensional view of a typical MA reaction. Indeed, the area around 260 nm is difficult to interpret.
However the reaction profile at the λmax of MA at 292 nm can be very useful. The figure below shows a typical reaction profile with reaction time plotted against the absorbance at 292 nm.
Testing has shown that the amount of starting material "4MPO" present at the point just after the curve levels out is below the no more than 0.5% limit by GC. Detail of this is shown in the figure below.
The raw absorbance data may be used to determine when sampling is necessary. Alternatively the change in absorbance may be trended and the sampling done when the reaction rate drops below a set level, as shown in the figure below.
Applications of the PAT method

A reaction with a slow activation can be easily monitored and corrected if necessary. In the profile shown below no MA was formed until a second dose of cyclohexene was added. It can be easily seen without hazardous sampling that no reaction occurred until the additional cyclohexene was added, at which point the reaction initiated and proceeded very quickly to completion.
To determine how useful the UV method would be to identify a stalled reaction, several reactions were intentionally poisoned with low levels of dimethylsulfide. The charts below show the difference between a normal reaction and one poisoned late in the reaction and the second chart shows two cases of poisoning— one early and one late in the reaction.
In looking at the chart for the poisoned catalyst an experienced operator would recognize that the reaction had most likely stalled, as the absorbance was well below the normal level, and the absorbance was not increasing with time. This could be easily confirmed by analyzing the reaction mixture with the standard GC assay and appropriate measures could be taken to quickly restart the reaction.
From the early absorbance values, shown in figure 17 of the poisoned reaction mixture, it is obvious from the lack of increase in absorbance that little product was being generated. In a case like this, poisoning could be recognized within a half hour of starting the reaction and investigation could begin; without PAT a stalled reaction would not be recognized as such for at least 12 hours.

It must be recognized that this UV method works because MA UNF does not crystallize in the reaction solvents. If it did crystallize, the absorbance growth rate would not be a reliable indicator of reaction completion. Crystallization during the reaction has never been observed in production and is unlikely to occur due to the
solubility of MA in the reaction solvents. In the lab, one reaction followed by UV was allowed to go 21 hours to look for crystallization issues, but the reaction continued in solution and the absorbance remained relatively constant. It was not until the solvent ratio was significantly modified to determine the ruggedness of the method that crystallization was observed. As expected, crystallization was very easy to see by UV as a sudden decrease in absorbance, as shown in the figure below. In this case, the endpoint could be determined because crystallization occurred after reaction completion.

Figure 18. Crystallization during the reaction

The chart above shows three reactions, with one of them normal (104947-52) and two crystallized. In one case (104947-116) crystallization occurred significantly after the endpoint was reached and in the other (104947-127) crystallization occurred
simultaneously with reaction completion. The actual final absorbances are different between the three reactions due to solvent effects which will be discussed later in this thesis.

*Additional evaluation of the PAT method*

The solvent ratio was modified to determine the ruggedness of the method and surprising results were found. Rates of reaction were expected to be different for differing amounts of THF and methanol, but absorbance differences were unexpected. While solvent can impact the absorbance of a given material, it was not expected in this case since the solvents remained the same and only the ratio changed. The normal ratio is 1:1 THF to methanol. The 3:1 THF:methanol reaction gave a higher absorbance at the endpoint and the 1:3 THF:methanol reaction gave a lower absorbance than normal, as shown in figure 19 below. This issue was investigated and is explained below.
To investigate the method’s tolerance for smaller solvent overcharges, the solvent composition was modified by adding 21% more THF to one reaction (ckw-69 in figure 20 below) and 21% more methanol to another reaction (ckw-79). Differences again were seen in the total absorbances, but the profiles were all similar and the endpoint was easily determined in each case.
As shown in figure 19 previously, significant changes in the solvent ratio lead to changes in the absorption of MA UNF, with more methanol leading to lower total absorption. This phenomenon was explored further using both the ATR and trans-reflectance probes.

The charts below show the variation in absorbance for MA in different solvents and solvent ratios, as measured with the trans-reflectance probe. The maxima shifts slightly with solvent changes, but it appears that there is little difference in the extinction coefficients of MA in the THF/Methanol solvent blends, although there is difference between the individual solvents.
Figure 21. Absorbance of MA in different solvents (data normalized for concentration) using the trans-reflectance probe

![Graph showing absorbance of MA in different solvents](image)

Figure 22. Absorbance (292 nm) of MA in different solvents using the 1 cm pathlength trans-reflectance probe

![Graph showing absorbance (292 nm) of MA in different solvents](image)
The differences in absorbances for MA in the reaction plots must therefore be due to changes in the refractive index for the different solvent blends. Different refractive indices would cause an effective difference in the pathlength for the ATR probe since each mixture bends light a bit differently. The chart below shows the UV activity of MA in each of the solvent blends, as measured by the ATR probe. As the extinction coefficient is a constant, the changing absorption must be due to changing pathlength.

Figure 23. Impact of solvent composition on absorbance using the ATR probe

Each time the instrument was used it was blanked on either solvent or the reaction mixture. In the current MA process all of the ingredients are combined at room temperature; the cyclohexene which starts the reaction is added and the reaction mixture is heated to reflux, where it is held several hours to complete the reaction. The instrument could be either blanked on virgin solvent (1:1 THF:Methanol) or
could be blanked on the reaction mixture. The small amount of 4MPO in solution had no impact on the subsequent measurements, as 292 nm is the wavelength of interest and 4MPO has an insignificant absorbance at that wavelength. Process improvements studied at the same time this PAT project was in progress involved the addition of cyclohexene at reflux temperatures and the method proved useful for this approach as well. In these reactions the probe was installed in the reaction flask once all ingredients except cyclohexene had been added. The mixture was then heated to reflux. The instrument was blanked on the refluxing reaction mixture and cyclohexene was added to initiate the reaction.

To explore the impact that blanking on room temperature vs. refluxing solvent had on the overall measurement, a 1:1 solution of THF and methanol was heated to reflux while being monitored by the ATR probe and spectrometer. The figure below shows the changes that occurred with the heating process. The change is considered to be trivial, so it does not appear to matter what the temperature of the blanking solvent is.
The absorbance that is lost by blanking the instrument on the reaction before cyclohexene is added is shown in the figure below. Using Beer’s law, the amount of 4MPO in solution is calculated to be 0.04% of the 4MPO charge. The absorbance at 292 nm is only 0.0163 absorbance units, which is trivial when compared to the 0.65 that will be achieved during the reaction. At any rate, blanking at this point has no impact on the shape of the reaction curve, which is the primary focal point of this PAT method. The choice of which method of blanking to use should be determined by the needs of the production environment. It is expected that the experimental results described here cover the most likely needs of production.
The solvent used in all of the lab experiments was bottle stock and the production process uses a different grade metered in through piping. One reaction was done using the solvents from production and the reaction went normally. Prior to the reaction, a spectrum of the bottle solvent and production solvent was taken for comparison purposes and the results are shown in figure 26 below. The differences between the two solvent sources were judged to be minor.
In summary we have demonstrated a useful application of UV-Vis spectroscopy using an ATR probe to monitor in real time a hazardous reaction mixture. Use of this PAT tool reduces the need for sampling the pyrophoric MA reaction mixture, thus reducing the potential for injury to the operators involved in running the process. Real-time analysis identifies the point at which the reaction is complete so that workup may begin. This shortens the cycle time of the process, saving dollars in labor and overhead, and also improves the quality of the product by limiting overreaction.
STUDIES WITH PALLADIUM ON CARBON CATALYST ACTIVITY

Early laboratory work on the megestrol acetate process was stymied by inconsistency, as the isomerization reaction would not consistently go to completion in laboratory glassware. No amount of repetitive use, additional reagents, or alternative glassware sources alleviated the problem. It was suggested that the flasks in which the reaction did work had been oxidized by previous unrelated work, which removed some unknown substance. If this is the case, then a pre-treatment by some oxidant might solve the problem. Indeed, pre-treatment with 50% H$_2$O$_2$ helped, as did soaking glassware in nitric acid. No additional treatments were tried, as the nitric acid immersion was convenient and effective. A protocol by which each flask was soaked for at least 15 minutes in concentrated nitric acid was followed for every reaction after that point and the reaction then worked reliably. The flasks were always thoroughly rinsed with water followed by acetone and were dried before use in the reaction. Inquiries with the glassware manufacturer failed to identify a reason for the problems with the glassware. This glassware issue is a laboratory problem only, as the production reactor material of construction is 316 stainless steel.
Once the reaction could be run consistently in the lab, it was demonstrated that
different lots of catalyst behaved differently in the isomerization. Batches of the same
grade/type of catalyst could be classified as either “fast” (causing the MA reaction to
go to completion in two or three hours) or “slow” (MA reactions done in about six
hours). The number of batches of catalyst available for use testing was limited, but
eight different lots were obtained for the lab work. Of these, five were experimentally
classified as “fast” and three were “slow” catalysts. In general lots 5666U (fast) and
5665U (slow) were used for the comparison studies.

Fast catalysts and slow catalysts generate different reaction UV profiles, as
determined by the PAT method discussed in chapter 1. The endpoint remained the
same: when the absorbance stops increasing. In the case of slow catalysts the reaction
was complete within 15 minutes after the reaction rate slowed significantly. For a fast
catalyst the reaction was complete when the growth of MA stopped. The figure below
shows this graphically.
To see if the reaction rate issues were a general problem with this grade of catalyst, samples of one “fast” and two “slow” catalysts were tested both in a hydrogenation reaction and in a reduction of a nitro compound. In the hydrogenation experiments one of the “slow” catalysts gave a very fast reaction, while the “fast” and other “slow” catalyst were slower, but all three reactions were done in an acceptable period of time with no significant differences in impurities or yield. All three catalysts gave identical rates, impurity levels and yields in the reduction of the nitro compound. An observed reaction rate for MA (a double bond migration) was not necessarily a predictor of how a particular lot of catalyst will behave in other reactions.
The physical properties of the catalyst would intuitively seem to be the source of the differences in reaction rate. Therefore samples of fast and slow catalysts, as well as one lot of fast catalyst previously used in an MA reaction, were submitted to Quantachrome (Boynton Beach, FL) for surface characterization. Results of their analytical determinations are shown in the table below.

Table 5. Properties of fast and slow 5% palladium on carbon catalysts

<table>
<thead>
<tr>
<th>Property</th>
<th>6769T (fast)</th>
<th>5666U (fast)</th>
<th>5666U* (*spent fast catalyst)</th>
<th>5667U (slow)</th>
<th>5665U (slow)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dispersion (%): Combined A</td>
<td>22.12</td>
<td>18.29</td>
<td>8.70</td>
<td>13.89</td>
<td>11.0</td>
</tr>
<tr>
<td>Surface Area (m^2/g): Combined</td>
<td>4.93</td>
<td>4.07</td>
<td>1.96</td>
<td>3.09</td>
<td>2.45</td>
</tr>
<tr>
<td></td>
<td>Strong</td>
<td>3.42</td>
<td>2.89</td>
<td>1.37</td>
<td>0.89</td>
</tr>
<tr>
<td>Crystallite size (Å): Combined</td>
<td>50.65</td>
<td>61.24</td>
<td>127.40</td>
<td>80.64</td>
<td>101.80</td>
</tr>
<tr>
<td></td>
<td>Strong</td>
<td>72.88</td>
<td>86.27</td>
<td>242.70</td>
<td>182.70</td>
</tr>
<tr>
<td>Monolayer uptake (µmole/g): Combined</td>
<td>103.90</td>
<td>85.93</td>
<td>41.31</td>
<td>65.26</td>
<td>51.68</td>
</tr>
<tr>
<td></td>
<td>strong</td>
<td>72.20</td>
<td>61.00</td>
<td>21.69</td>
<td>28.81</td>
</tr>
</tbody>
</table>

^a Combined = reversible + irreversible chemisorption
^b Strong = irreversible chemisorption of CO

The fast catalyst lost activity after use in the MA reaction. This change is most likely due to sintering, as evidenced by the larger crystallite size in the spent catalyst as compared to the virgin catalyst. Sintering increases the size of the palladium crystallite, which results in decreased activity.
The results for the virgin catalysts indicate that there are some differences in metal dispersion and active metal surface area between the fast and slow catalysts.

Unfortunately two additional catalyst samples (one fast and one slow) submitted for testing failed to follow the trend implied above.

The production data indicated that there was a clear step change in the reaction completion time in 2001. Laboratory studies with the catalysts made it clear that the reaction rate issue is primarily due to the catalyst itself. The catalyst supplier (Engelhard) was therefore consulted and they indicated that there had been no manufacturing changes made that could account for the inconsistency or sudden change in catalyst activity. However, they did agree to do a washing study to evaluate how additional water washing impacts the activity. The unwashed and washed lots thus produced were use-tested into the MA reaction and a difference was indeed found, with the washed catalyst giving a significantly faster reaction than the parent catalyst. The chart below shows the difference graphically.
The good effects of additional water washing could only be reproduced in the lab when ultra pure water (Milli-Q) was used and only with one of the slow catalyst lots. Trituration in reverse osmosis (RO) water failed to significantly improve the reaction rate, and all efforts to wash another slow lot failed to give any good effect at all, despite the loss of 3% of the weight from the washing. While drying is known to cause thermal sintering, which can deactivate the catalyst, these catalysts were dried carefully in a low temperature vacuum oven with a nitrogen bleed; thus thermal sintering is not thought to be relevant in the lab studies.

The material washed off with the water was analyzed and consisted mostly of sodium (strong signal) and chloride (moderate signal) as determined by X ray fluorescence
(XRF), but spiking a reaction with NaCl did not negatively impact the reaction rate. The pH of the residue was tested and was found to be ~10, but the wash residue of a fast lot had the same pH with lesser amounts of the same elements found in the slow lot. Engelhard has thus far been unwilling to disclose the identity of the high pH material. Results have been communicated to Engelhard, and it is expected that improved water washing on their part should eliminate at least part of the inconsistency problem. Clearly another reason for the discrepancies in catalyst activity remains, as evidenced by the relative inactivity of one of the slow lots.

A literature reference indicates that pre-treatment of the catalyst with dilute hydrogen peroxide could improve the activity of the catalyst in the hydrogenation of cinnamic acid to dihydrocinnamic acid. In this reference the peroxide is thought to remove traces of surface impurities such as iron, aluminum and magnesium. In theory these same elements can negatively impact an isomerization reaction as well. The MA reaction rates with a slow catalyst treated with hydrogen peroxide did not improve however. Triturations of the catalyst in dilute acetic acid and dilute nitric acid were also tried, with no improvement in reaction rate and a minor increase in impurity formation.

Use of a spent fast catalyst resulted in a 50% slower rate than the parent catalyst (about the same rate as with a slow catalyst). A literature reference was found that
indicated a trituration in the reaction solvents can improve the activity of used catalysts. This trituration yielded a 90% recovery of the catalyst. When used in the MA reaction there was no significant change in the reaction rate as it was roughly the same rate as crude spent catalyst.\textsuperscript{13} See the figure below.

Figure 29. HPLC monitoring (UV detection) of reaction rates with spent catalysts

Given the change in physical attributes outlined in table 5 it appears that the change in catalyst activity between fresh and spent catalyst is due to an increase in crystallite size that occurred over the course of the first reaction. The results imply that sintering occurs to some extent during the reaction.
Reaction rate is not the only factor impacted by the differences in the catalysts, as the tolerance of a MA reaction to catalyst poisons was also dissimilar between different lots of catalyst. A very small amount of sulfur can theoretically poison a significant quantity of catalyst by blocking several catalytic sites. Sulfur decreases the potential chemisorption sites, thus blocking the effective metal surface area\(^\text{14}\). Sulfur in the form of dimethyl sulfide was used for the spiking experiments. It was found that a slow catalyst became dramatically slower with a 2 ppm spike, while the fast catalyst could tolerate twice that amount with no rate impact. A spike of 9 ppm was enough to stall the fast catalyst, but that reaction could be restarted by the addition of more cyclohexene. Adding more cyclohexene to a stalled reaction with slow catalyst had no effect.

In the lab there are other factors that can slow or stall a reaction, including exposure to air and the use of rubber septa to seal addition ports on the flasks. Air injected under the surface of the reaction mixture appeared to slow the reaction somewhat, but the reaction still went to completion in a reasonable period of time. Rubber septa contain sulfur species and exposure of the reaction to this type of rubber will quickly stall a reaction so severely that it cannot be restarted. The action of the refluxing solvent on a rubber septum sealing an addition port (in the vapor space) is evidently fast enough to prevent some reactions from even starting. These issues are not relevant in the production setting, as the reactor is well purged with nitrogen and any
sampling will be done such that oxygen exposure is avoided. Exposure to rubber is unlikely as the production workcenter is constructed of stainless steel with some Teflon used as seal materials; rubber is seldom used as a gasket material in pharmaceutical bulk drug manufacture.

The inconsistent catalyst issue prompted a screening of several alternative palladium catalysts. The focus was on catalysts on a carbon support but a few others were also tested to gain additional information. The current catalyst is made by Engelhard and is type C3632 and is an “MRD” catalyst on a CP97 carbon support, with “M” meaning mixed (the way the palladium is dispersed on the support; mixed implies that some is on the surface and some is buried deeper in the carbon), “R” meaning reduced (reduced with hydrogen after the precipitation on the support), and “D” meaning dry (as opposed to water wet). Other common catalyst types are “E” (edged or “eggshell” dispersion, meaning the palladium is mostly on the outside of the support), “U” (unreduced) and “W” (wet, generally about 50% wet with water). Multiple catalysts made by Engelhard and Johnson-Matthey were screened in the MA reaction and several promising alternatives were identified: Johnson-Matthey type A503038-5 EUW on carbon 38, A503032-5 EUW on carbon 32, and A503023-5 ERW on carbon 23, and Engelhard types 3699 MRWS on carbon CP97 and C5068 MRW on carbon CP87 plus one promising catalyst on alumina support, type C4365.
The “S” in the C3699 catalyst means sulfided- the catalyst contains a certain amount of sulfide, but no information on how much is present has been made available by the manufacturer. This C3699 catalyst is very similar to the current catalyst, with the exception being the sulfide and the state of drying. This catalyst gave excellent yields with very low levels of impurities in MA reactions.

For the most part the mixed vs. edged dispersion made no difference in reactivity for MA. With the exception of the two catalysts listed above, most of the unreduced catalysts were very slow or inactive in the reaction. However, not all of the reduced catalysts were active. Water had no negative impact on the reaction. Wet catalysts are actually favored for two reasons: they are safer to handle (less flammable) and they have not been through the harsh drying conditions that can damage the activity of the catalyst through the process of sintering (formation of a larger palladium crystallite, which is less active).

Engelhard was contacted about sending some additional lots of the two most promising carbon supported catalysts and they sent samples of each lot that was available in inventory, which was only one other lot of the MRWS type and two lots of the MRW type (commonly referred to as ESCAT 160). The ESCAT 160 lots were use tested to evaluate the consistency of the catalyst activity. Variability was observed with this catalyst as well. One lot gave a fast reaction while the second lot
was classified as slow. To determine if the slow lot was slow because of inadequate washing, which is an easily solved problem, the catalyst was triturated in Milli-Q water and was evaluated in the MA reaction. This also resulted in a slow reaction, indicating that the issues with ESCAT 160 are similar to those of its dried counterpart. As only one other lot of MRWS catalyst was available no further testing was done, as a meaningful statistical comparison cannot be done with only two data points.

The most promising catalyst by far was the type C4365 on alumina AP30 support, although palladium on the more readily available alumina type AP8 was no better than the currently used carbon supported catalyst. The AP30 catalyst consistently gave very low impurity results with excellent yields (highest ever seen). Unfortunately the catalyst proved difficult to remove, generally bleeding through the filter no matter what filter aid or filter pore size was used to assist filtration. The catalyst was easily removed by filtration over magnesol, but only if the solvent was methylene chloride. Despite this problem, the promise of significantly higher yields prompted further investigation, so additional lots of the catalyst were requested from Engelhard. It was hoped that the activity of the catalyst would be more reliable since alumina quality is more consistent than carbon quality. This is due in part to the fact that carbon is a natural product from many varied sources while alumina is more predictable. Two additional samples of C4365 were received and neither gave impurity results lower
than the standard. One of the two was relatively inactive, as evidenced by a slow reaction. Evidently the inconsistency found in standard MA catalyst applies to alumina supported catalyst as well. Work on the AP30 catalyst was therefore abandoned.

Catalysts that were slow in a laboratory setting were much slower in a production setting, as the reaction in production is often forced to go a long time (12+ hours) due to time delays in waiting for GC results to verify reaction completion. Multiple samples are needed to determine the endpoint of a slow reaction and each sample adds many more hours to the total reaction time. The GC is a registered endpoint test so production must wait until the endpoint has been confirmed by this method. Extended reaction times can cause increases in impurity levels, as MA may react further under the reaction conditions. This was confirmed in the series of experiments shown in the table below.
Table 6. Extended reaction times and resulting impurity levels for four different MA reactions (impurity levels measured by GC)

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>reaction time (minutes)</th>
<th>MA</th>
<th>4MPO</th>
<th>6β MPB</th>
<th>Δ5</th>
<th>6-OH</th>
<th>MPB</th>
<th>total reduced impurities</th>
</tr>
</thead>
<tbody>
<tr>
<td>115-7</td>
<td>288</td>
<td>96.34</td>
<td>0.23</td>
<td>0.57</td>
<td>0.79</td>
<td>0.6</td>
<td>0.38</td>
<td>1.74</td>
</tr>
<tr>
<td>115-8</td>
<td>686</td>
<td>95.92</td>
<td>0.17</td>
<td>0.67</td>
<td>1.19</td>
<td>0.62</td>
<td>0.57</td>
<td>2.43</td>
</tr>
<tr>
<td>115-9</td>
<td>1437</td>
<td>95.05</td>
<td>0.13</td>
<td>0.75</td>
<td>1.36</td>
<td>0.55</td>
<td>0.96</td>
<td>3.07</td>
</tr>
<tr>
<td>116-4</td>
<td>290</td>
<td>96.29</td>
<td>0.23</td>
<td>0.59</td>
<td>0.77</td>
<td>0.67</td>
<td>0.36</td>
<td>1.72</td>
</tr>
<tr>
<td>116-7</td>
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<td>95.86</td>
<td>0.18</td>
<td>0.67</td>
<td>1.21</td>
<td>0.63</td>
<td>0.57</td>
<td>2.45</td>
</tr>
<tr>
<td>116-8</td>
<td>1438</td>
<td>95.21</td>
<td>0.12</td>
<td>0.69</td>
<td>1.23</td>
<td>0.57</td>
<td>0.89</td>
<td>2.81</td>
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<tr>
<td>117-2</td>
<td>174</td>
<td>95.38</td>
<td>0.28</td>
<td>0.96</td>
<td>0.86</td>
<td>0.82</td>
<td>0.4</td>
<td>2.22</td>
</tr>
<tr>
<td>117-5</td>
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<td>94.63</td>
<td>0.2</td>
<td>1.17</td>
<td>1.43</td>
<td>0.84</td>
<td>0.82</td>
<td>3.42</td>
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<tr>
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<td>93.74</td>
<td>0.14</td>
<td>1.23</td>
<td>1.33</td>
<td>0.82</td>
<td>1.28</td>
<td>3.84</td>
</tr>
<tr>
<td>118-2</td>
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<td>0.28</td>
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<td>0.92</td>
<td>0.7</td>
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<tr>
<td>118-5</td>
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<td>94.9</td>
<td>0.19</td>
<td>0.94</td>
<td>1.25</td>
<td>1.01</td>
<td>0.69</td>
<td>2.88</td>
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<tr>
<td>118-6</td>
<td>1435</td>
<td>93.99</td>
<td>0.14</td>
<td>1.09</td>
<td>1.29</td>
<td>0.9</td>
<td>1.09</td>
<td>3.47</td>
</tr>
</tbody>
</table>

6β= 6β medroxyprogesterone acetate  
Δ5= Δ5 dihydro megestrol acetate  
6-OH= 6-hydroxy medroxyprogesterone acetate  
MPB= medroxyprogesterone acetate  
Total reduced= 6β + Δ5 + MPB

All of the hydrogenated byproducts increased significantly with time (6β MPB, Δ5, and MPB). As can be seen in the figure below all four reactions followed the same trend in increasing impurities.
When MA was resubjected to the reaction conditions it formed 1.8% Δ5, 4.0% 6β, and 0.8% MPB for a total of 6.6% reduced impurities.\textsuperscript{15}

Clearly stopping the reaction as soon as the endpoint has been reached is beneficial to the impurity level and would be expected to have a favorable impact on yield. The PAT UV method discussed in chapter 1 indicates the reaction endpoint, allowing the reaction to be stopped in a timely manner, thus avoiding overreaction. While the PAT method may not be able to completely eliminate use of the registered GC method, it does clearly indicate when the reaction completion sample should be taken, thus eliminating multiple reaction samples. Additionally, the rate of reaction is evident
early in the reaction, allowing accurate notification of the GC lab of when a sample may be expected.

Modifications to the solvent system for the isomerization reaction were briefly explored in an effort to find more robust reaction conditions that might counteract catalyst problems. Water was added in an effort to improve solubility of the steroid, and further dilutions with THF and/or methanol were tested. As indicated by no change in the initial absorbance of 4MPO, water did not appear to improve solubility, but the reaction rate was enhanced, as is shown in the chart below. Ckw-38 is the control run with no water (same catalyst), ckw- 63 had 20% water (v/v) added and ckw-57 had 11% water. A reaction done with 4.2% water was done on the first sample in four hours, so no data on the rate of the reaction was obtained, although it was clearly as fast as the other reactions with water added. The 6-hydroxy impurity appeared to be greater than normal in the reaction samples (average 0.9% vs. normal 0.5-0.7%), although the impurity level in the isolated crude product was comparable to that of the standard. Other impurities appeared to be about the same levels as were normally seen in MA lab reactions. Yields were slightly lower than normal which is consistent with increased impurity formation.
Doubling or quadrupling the THF and methanol for the reaction resulted in lower impurity levels for the reactions and products, but the overall yields were no better than standard. The ratio of THF and methanol was examined with more impurities being formed in all experiments; the reaction was also slower with high THF/low methanol.

In summary, the isomerization to form megestrol acetate is prone to slow and stalled reactions and is historically difficult to run in a laboratory. Variations in the 5% palladium on carbon catalyst have been identified as the leading cause of variability in reaction time. The catalyst variability is also a major contributor to stalled reactions, as the slow catalyst lots are substantially more prone to giving stalled reactions than
fast catalyst lots. Low levels of catalyst poisons, such as 2 ppm sulfur (i.e. dimethyl sulfide), can cause a reaction with a slow catalyst lot to stop altogether, but will only cause a slight rate inhibition with a fast catalyst lot. Many alternative catalysts were screened in an effort to find a more consistent palladium on carbon catalyst, and modifications to the existing process were examined in an effort to increase the robustness of this bond migration reaction. The PAT UV method described in chapter 1 and/or an HPLC method allowed quick and consistent evaluation of the reactions. Ultimately the reaction was found to be most reliable when the fast catalysts were used in conjunction with the PAT method to quickly identify reaction endpoint.
Reduced palladium is susceptible to attack by atmospheric oxygen to form palladium oxide. Thus the initial reaction involving cyclohexene is the reduction of palladium oxide to palladium (0) as shown below.

Figure 32. Reduction of palladium oxide

As it is only on the surface of the catalyst, the palladium oxide level should be very low and quickly removed early in the reaction, but cyclohexene continues to be consumed, indicating that it is doing more than just cleaning the surface of the catalyst. Furthermore, the MA reaction forms reduced impurities, which requires a hydrogen source; cyclohexene is the only hydrogen source available in this reaction. Formation of reduced impurities is undesirable due to co-crystallization of residual impurity. The overall lower yields correspond to higher impurity levels in the reaction mixtures. The known reduced impurities are shown in the figure below.
Figure 33. Hydrogenated impurities in MA

**Medroxoprogesterone acetate:**

![Medroxoprogesterone acetate](image)

**6β-Medroxyprogesterone acetate:**

![6β-Medroxyprogesterone acetate](image)

**Δ5-Dihydro megestrol acetate:**

![Δ5-Dihydro megestrol acetate](image)

Not unexpectedly, palladium also catalyzes the following reaction with cyclohexene which, contributes hydrogen to the reaction. This reaction appears to be necessary to
activate the catalyst for the desired isomerization reaction. No reaction takes place without the activator, even if a previously activated catalyst is used.

Figure 34. Activation of palladium

\[
\begin{align*}
\text{\text{cyclohexene}} &\quad \xrightarrow{\text{Pd}} \quad \text{benzene} + \text{H}_2
\end{align*}
\]

All efforts to remove excess hydrogen via a nitrogen gas sparge were ineffective, suggesting that the hydrogen is coordinated with the palladium. As the isomerization reaction will not proceed in the absence of cyclohexene or with a substantially lower amount of cyclohexene, the palladium appears to require reactivation during the process. The preferred amount of cyclohexene is 3.4 mole % (based on steroid charge) while the charge of palladium on carbon is 3.6 mole %.

When monitored by GC a typical reaction curve showed a decay process for the cyclohexene, as shown in the figure below.
When the rate of consumption of cyclohexene was plotted against the rate of formation of benzene the slope was nearly minus one and $R^2$ was 0.977, indicating a strong linear one-to-one correlation, as shown in the figure below.
Further experimentation revealed substantial differences in the rate of cyclohexene consumption by different catalyst lots, at different levels of cyclohexene initially charged, and with higher catalyst loading. The chart below illustrates the results, with ckw-52 being the slow catalyst (black line), ckw-58 a fast catalyst (red line), ckw-68 a double charge of slow catalyst (blue line) and ckw-70 a 15% increase in cyclohexene with slow catalyst (green line). Note that the cyclohexene value never goes to zero but more active catalysts (or more of a less potent catalyst) yield more rapid disappearance of cyclohexene. Note that a fast reaction may not be desirable, as fast cyclohexene consumption implies that there is a higher concentration of hydrogen available to form reduced impurities. Indeed, faster reactions do produce higher levels of reduced impurities.
As the results above suggest that the reaction rate is more rapid with more cyclohexene, a Design of Experiments (DOE) was run to compare 3.4 mole % to 4 mole % cyclohexene (relative to 4MPO) and to compare a fast catalyst to a slow catalyst. Overall, in terms of reaction rate, the type of catalyst (fast/slow) has a major impact while the contribution from increased cyclohexene is relatively minor.

Temperature effects on the reaction rates were also examined. In the current production process, cyclohexene is added to all of the other ingredients at ambient temperature and the mixture is heated to reflux (about 60°C). Several experiments were done to explore the impact of temperature on impurity levels. In the first experiment, the reaction was run at 50°C and the reaction formed 4.1% reduced impurities, which is more than twice the amount formed in typical lab reactions.
When the product was isolated, the resulting crude MA contained 1.18% reduced impurities, which is also more than twice the normal amount for laboratory crude MA. The yield was also lower (84.4% vs. normal 90-92%). This experimental result suggests that lower reaction temperatures favor reduction over isomerization.

If the impurity formation is indeed temperature dependent, then adding cyclohexene to the hot mixture would be preferable, as with the current large scale production process the undesirable side reaction would be favored during the lengthy and inconsistent heating period (heating is done over at least one hour, but it can be longer). A DOE was performed to evaluate the addition temperature of the cyclohexene (room temperature vs. reflux) and the catalyst Pd/C (fast lot 5666U vs. slow lot 5665U). Responses measured included yield, impurities in the reaction mixture and impurities in the isolated crude product. In a well designed DOE, Minitab software can be used to calculate the impact of each variable on each of the responses. The Main Effects plots in figure 38 below show a relatively small impact on each of the responses from the contribution from the catalyst type while the contribution from the addition temperature of cyclohexene is significantly larger.
The results indicate that adding cyclohexene to the hot reaction mixture can increase isolated yields as much as 2% due to lower formation of reduced impurities. Furthermore, as the impurities in the isolated crude were significantly lower than normal, it was possible to reduce the number of filter cake washes, to potentially increase the yield. Lab work that has explored this option has generated crude yields as high as 93.4%, which is substantially higher than the standard process average of 90.8%.  

Figure 38. Main effects plots from DOE
Efforts to run the reaction at higher temperatures all failed to go to completion, most likely due to the equipment, as different (pressure) reactors had to be used for these experiments. The equipment was not available for additional work to explore the temperature issue further.

In a further effort to improve the impurity profile for the isomerization, other activators besides cyclohexene were screened for the reaction. It was thought that cyclohexadiene would be a good choice, as it can only produce one mole of $\text{H}_2$ per mole of cyclohexadiene, as opposed to the two moles produced by cyclohexene. However the reaction was slow and resulted in just as many hydrogenated impurities as a normal cyclohexene reaction.

Limonene was also tried since it will give off one mole of hydrogen to form p-cymene to activate the palladium by the following reaction.$^{17}$

Figure 39. Limonene activation of palladium

![Figure 39. Limonene activation of palladium](image)

This reaction with 4MPO did not produce any megestrol acetate.
Sodium hypophosphite and sodium formate were also evaluated as activators but the hypophosphite gave no reaction at all and the formate gave high levels of side products. Formation of hydrogen from these donors is shown in the figures below.

Figure 40. Generation of hydrogen from hypophosphite salts\textsuperscript{18}

\[
[H_2PO_2]^− + H_2O \xrightarrow{\text{Pd}} [H_2PO_3]^− + H_2
\]

Figure 41. Generation of hydrogen from formate salts\textsuperscript{19}

\[
[HCO_2]^− + H_2O \xrightarrow{\text{Pd}} [HCO_3]^− + H_2
\]

In theory the methanol solvent could act as a hydrogen donor, but the reaction failed to start without another activator. The authors of the original patent on which the MA process is based suggest that the solvent ethanol does serve as a hydrogen donor, but it is much slower than cyclohexehene and is therefore not preferred.\textsuperscript{20,3}

As work on alternative activators was not fruitful, other experiments to eliminate hydrogen were explored. These options included sparging nitrogen up through the reactor’s bottom valve in an effort to strip off free hydrogen, however if the hydrogen is bound to the catalyst this will not work. All such efforts met with physical
difficulties, as the thick slurry consistently plugged the nitrogen line. Later efforts to bubble nitrogen in through a dip tube did give a good sparge through the reaction, but impurity levels were no better than normal. Therefore the hydrogen must be bound to the palladium catalyst.

A chemical scavenger of hydrogen (1,3-cyclooctadiene) was also evaluated, per the literature. This reagent adsorbs strongly on palladium and reduces to form cyclooctene, which is not strongly held by the catalyst. If the cyclooctadiene was reduced before the hydrogen could reduce the 4MPO, in theory lower impurities would be the net result. This assumes that the hydrogen generated by the cyclohexene is not a critical part of the mechanism of isomerization. Tiny amounts of cyclooctadiene were added at different times, but each time the desired isomerization reaction stopped completely when the cyclooctadiene was added. Evidently it adsorbs so strongly that no further reaction can take place, or more likely, hydrogen is necessary in the isomerization reaction mechanism.

The information presented thus far suggests a mechanism that is in theory catalyzed by palladium and hydrogen. As some hydrogen will inevitably be used for reduction rather than isomerization the actual use of hydrogen is not catalytic. An activator such as cyclohexene is therefore necessary to continue generation of hydrogen. The following sequence of reactions is proposed.
Figure 42. Reaction mechanism

I. Cleaning of the palladium surface

\[
\frac{1}{2} \text{cyclohexene} + \text{PdO} \rightarrow \frac{1}{2} \text{benzene} + \text{Pd} + \text{H}_2\text{O}
\]

II. Activation of the catalyst. The hydrogen produced from cyclohexene is complexed with the palladium.

\[
\text{cyclohexene} \xrightarrow{\text{Pd}} \text{benzene} + 2 \text{Pd-H}
\]

III. Reaction of palladium hydride with 4MPO. A complex with the double bond is formed.

IV. Rearrangement. Hydrogen from the palladium hydride is substituted on the exo-methylene and a hydrogen from the 7 position on the steroid is contributed to the palladium to regenerate the palladium hydride.
V. Reduction instead of isomerization (a competing route). Hydrogen is consumed by this route making it unavailable for further isomerization. The mechanism shown below is consistent with the observation that stoichiometric amounts of hydrogen produce Δ5 medroxyprogesterone acetate; this double bond can migrate to the 4 position with mild acid treatment, leaving either α or β MPB.
CONCLUSIONS AND SUGGESTIONS FOR FUTURE WORK

A useful application of UV-Vis spectroscopy using an ATR probe to monitor reaction progress in real time has been demonstrated. Use of this PAT tool reduced the need for sampling the pyrophoric megestrol acetate reaction mixture as well as the potential for injury to the operators involved in running the process. The real-time analysis indicates at which point the reaction is complete so that workup and isolation may begin. This shortens the cycle time of the process, saves dollars in labor and overhead, and also improves the quality of the product by limiting overreaction.

The isomerization to form megestrol acetate is prone to slow or stalled reactions and is historically difficult to run in a laboratory. Variations in the 5% palladium on carbon catalyst have been identified as the leading cause of variability in reaction time. The catalyst variability is also a major contributor to stalled reactions, as the slow catalyst lots are substantially more prone to giving stalled reactions than fast catalyst lots. Low levels of catalyst poisons, such as 2 ppm sulfur (i.e. dimethyl sulfide), can cause a reaction with a slow catalyst lot to stop altogether, but will only cause a slight rate inhibition with a fast catalyst lot. Many alternative catalysts were screened in an effort to find a more consistent palladium on carbon catalyst, and
modifications to the existing process were examined in an effort to increase the robustness of this bond migration reaction. The PAT UV method and/or an HPLC method allowed quick and consistent evaluation of the reactions. Ultimately the reaction was found to be most reliable when the fast catalysts were used in conjunction with the PAT method to quickly identify the reaction endpoint.

The catalyst activator cyclohexene has some influence on the formation of impurities in the megestrol acetate reaction, as it is the source of the hydrogen used in the reduction. Several alternative catalyst activators failed to give superior results to cyclohexene. All efforts to purge hydrogen from the reaction mixture to avoid reduction of the substrate failed to achieve the desired result. These efforts shed some light on the reaction mechanism and it is clear that a palladium hydride species is involved as a critical intermediate. The proposed mechanism for isomerization was outlined in chapter 3.

Future work on the megestrol acetate process should focus on the palladium on carbon catalyst as it is still not clear how lots of the catalyst made via the same process can produce drastically different results in the isomerization reaction.
REFERENCES


8 http://www.shu.ac.uk/schools/sci/chem./tutorials/molspec/uvvisab1.htm.


10 Email communications with Kimball-Kontes. As of 3/10/2008 no definitive answer has been received despite several requests.

11 Quantachrome “Summary Report,” issued 7/25/05.


15 Laboratory notebook 35437-ckw-60.


