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To establish the consistency of a synthetic route developed for targeted drug delivery through a simplified Process Analytical Technology method.

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ABSTRACT

Targeted drug delivery has become one of the important criteria to treat cancer efficiently without affecting the systemic route and only targeting the cancer cell. It is also important in terms of the amount of drug administered by efficiently staging the cancer. The synthetic route developed for formulating such a product should be precise, reproducible, accurate and consistent with minimum or rather negligible deviations. We have validated the method which determines the consistency of the synthetic route for a nanoformulation containing pegylated nanosilver as the carrier and pemetrexed as the targeted drug. The study we carried out shows the usefulness of this tool, and proving the concept.

Keywords: Chemometrics, Targeted drug delivery, Cancer, Method, Validation

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INTRODUCTION

Process Analytical Technology (PAT) has become a dominant issue in pharmaceutical manufacturing. Tremendous effort is being put in method development and implementation issues in PAT. Despite this it is an early stage of development in the pharmaceutical manufacturing industry. The literature has mainly focused on topics such as the capabilities of various potential technologies, specific aspects of method development (e.g., chemometrics) or regulatory aspects. Critical issues that must be considered during PAT method development include:

- Risk analysis of the process;
- Feasibility studies;
- Experimental design;
- Sensors and technology selection;
- Model development and transfer;
- Process sampling; and
- Information management.

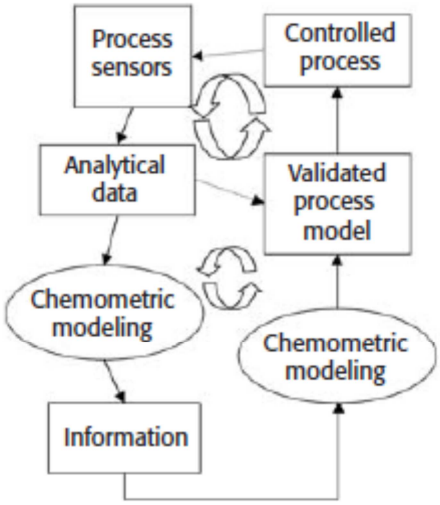
Depending on the nature of the PAT application, each issue could correspond to a number of development tasks. Model development and transfer procedures for a typical instrument, for example, might include:

- Database development and experimental design;
- Calibration model optimization;
- Predictive model validation;
- Development of calibration monitoring and reporting tools; and
- Determination of suitable methods for calibration transfer.

PAT provides a basis for monitoring the manufacturing process, determining control parameters for that process, and modeling these parameters to produce optimized production. If during a manufacturing process the measured parameters indicate a process disruption or the product is moving towards non-conformance, corrective actions can be initiated.

The analytical measurement must provide information to other systems in order for PAT to be useful. Raw data from sensors are processed into information used to control the process as illustrated by the below mentioned flow diagram, a new or existing process is measured for one or more key parameters by one or more real time sensors.

The sensor information is supplied to a computational system, which converts the raw data into useful process information.



This information is processed further into deterministic process models, which define the mathematical relationship between specific manufacturing control parameters. Once, the process model is derived and validated it is included in a process information management system as part of a broader informatics control system. This process model is used to control the process during production while the process is measured continuously using the installed sensor network. So, the analyzed sensor data provides information inputs for control of measurement processes in real-time.

Both the internal model and the process are improved as new process and information is gathered by the sensors, incorporated into both sensor calibration models and process control models. The sensor data mining approach to modeling using chemometrics and pattern recognition techniques provides insight the relationships between physical and chemical properties of products and their intermediate or final performance characteristics. After collecting the appropriate sensor data from a process and applying essential computational tools, multiple objectives can be accomplished.

MATERIALS AND METHODS

Synthetic route of the formulation and the relevant data is submitted for publication by the same authors and the same was accepted and published. The synthetic route is developed to synthesize a nanoformulation containing,

a. Nanocarrier
b. Conjugation of Nano particles with PEG (Pegylation favor the pharmacokinetics of the these nano formulations prolonging the circulation periods that grant a proper time frame for the nano particles to be accumulated in the tumor micro environment although PEG is the most effective method to reduce protein adsorption invivo and to avoid the RES system clearance).
c. Cancer specific receptor or general receptor.
d. Sensing material to identify if the cell is normal, malignant or benign.
e. Substance to indicate the amount of uptake of the nano material by the cell for staging the cancer.
f. A drug, pemetrexed which is an FDA approved drug in the treatment of malignant Pleural Mesothelioma, a type of tumor of the lining of the lung. In combination with other drugs whose disease is unrespectable or who are otherwise candidates for curative surgery.
g. A stabilizing and binding agent.

In our present study on the Synthesis of a nanoformulation for targeted drug delivery in cancer, the analytical instruments such as UV-Visible spectrophotometer, Fourier Transform infrared spectrophotometer, Raman spectrophotometer, Particle size and Zeta Potential analyzer were found to be very useful online methods for characterizing the nano formulation. The details of the measurement techniques and the sample preparation are presented below.

UV-Vis Spectrophotometer: The instrument is from Shimadzu, double–beam spectrophotometer, UV-3600 with spectral range of 165 nm to 1000 nm with a spectral resolution of ±0.2 nm. The stray light of the instrument is 0.00005% at 340 nm.

Fourier Transform infrared (FTIR) spectrophotometer: For FTIR measurements, the nanoformulation solution was centrifuged at 10,000 rpm for 30 min. The samples were dried and grinded with KBr pellets and analyzed on a JASCO FT/IR-5300 model in the diffuse reflectance mode operating at a resolution of 4 cm^{-1}.

Raman Spectrophotometer: The Raman spectra were carried out on Jobin-Yvon U1000 Raman spectrometer with a Spectra- Physics model 165 argon-ion laser. The 90° scattering and back-scattering configurations were adopted for silver colloid system, the spectral resolution of Raman spectrometer was about 4 cm^{-1}.

Particle size and Zeta Potential analyzer: Zetasizer Nano ZS of Malvern is used for the studies. It can measure Maximum particle size range (diameter) of 0.3nm - 10µm, Zeta potential measurement of 3.8nm - 100µm and Molecular weight range of 342 - $2x10^7$ Da. Zetasizer Nano series can measure three of the most important parameters for the colloid and polymer chemistry.

Particle size - of particles and molecules from a maximum size range 0.3nm to 10 microns using NIBS technology and Dynamic Light Scattering.

Zeta potential - in aqueous and non-aqueous dispersions using M3-PALS technology.
Molecular weight - An absolute measurement using Static Light Scattering and the sensitivity from an avalanche-photodiode detector and fibre detection optics.

RESULTS AND DISCUSSION

UV-VIS absorbance at the maximum peak intensity, FT-IR characteristic spectral data, Raman Characteristic spectral data, average particle size of the formulation, zeta potential of the formulation over a 10 batch synthetic samples were used to evaluate the quality and authenticity of the synthesis of the formulation. The chemometric method through Process Analytical technology (PAT) consists of a number of statistical, mathematical and graphic techniques that analyze the variables simultaneously.

The consistency of the formation of the final nanoformulation is confirmed for ten synthetic batches of the product. As seen in the data from the Fig. 1 & table -1 and Fig.2 and Table-2 (UV-VIS spectral data of the final nanoformulation at its characteristics absorption peaks (410 nm and 540 nm respectively), the formation of the product is consistently same for the pegylated nanosilver embedded with curcumin.

Table-1: UV-VIS spectral data of the final nanoformulation at its characteristic absorption peak at around 410nm.

Wavelength (nm)	Absorbance
410	0.234
408	0.236
407	0.235
410	0.235
412	0.236
410	0.236
406	0.234
409	0.237
408	0.238
410	0.235
SD	0.001265
AVG	0.2356
RSD	0.536889

Figure-1: UV-VIS spectral graph of the final nanoformulation at its characteristic absorption peak at around 410nm.

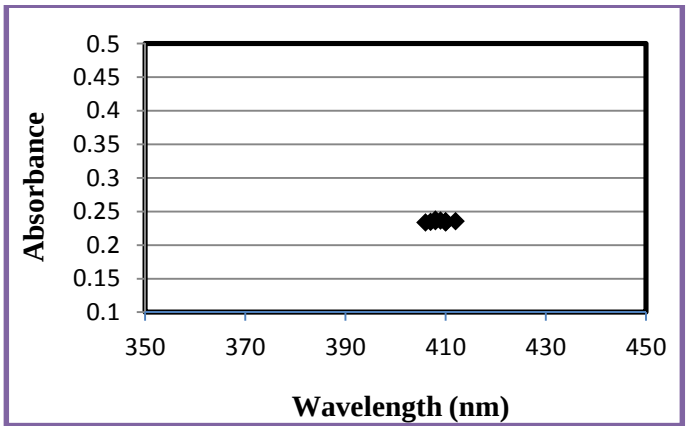


Table-2: UV-VIS spectral data of the final nanoformulation at its characteristic absorption peak at around 540nm.

Wavelength (nm)	Absorbance
540	0.421
542	0.425
541	0.423
543	0.424
541	0.423
539	0.422
542	0.423
540	0.421
542	0.425
538	0.422
SD	0.001449
AVG	0.4229
RSD	0.342667

Figure 2: UV-VIS spectral graph of the final nanoformulation at its characteristic absorption peak at around 540nm

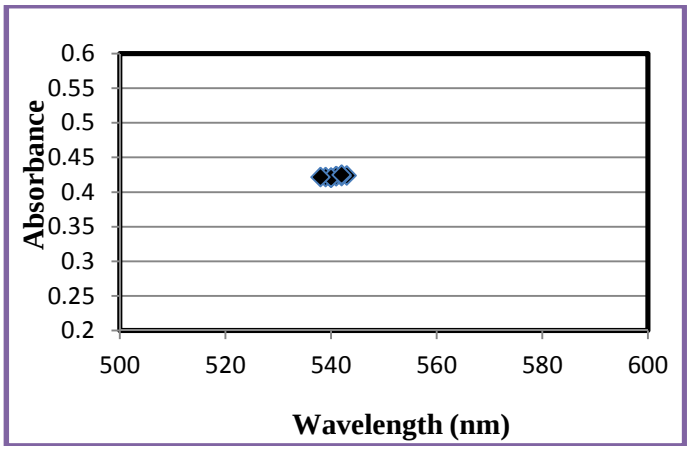


Table-3: FT-IR peaks of the final nanoformulation

Sample batch number	Wave number (Cm^{-1})					
Batch 1	3443.25	2920.49	2849.12	1635.78	1022.36	590.27
Batch 2	3445.87	2916.56	2851.78	1632.58	1023.57	591.57
Batch 3	3449.78	2917.67	2846.34	1634.56	1024.58	593.48
Batch 4	3441.12	2923.49	2853.56	1637.59	1017.47	589.48
Batch 5	3448.67	2921.89	2852.91	1636.38	1021.46	588.29
Batch 6	3440.56	2924.34	2847.12	1631.57	1022.89	592.47
Batch 7	3451.91	2917.17	2848.95	1635.89	1019.48	586.49
Batch 8	3443.89	2925.32	2850.39	1636.73	1020.39	593.68
Batch 9	3452.67	2916.35	2853.48	1632.79	1021.49	588.69

Batch 10	3430.89	2919.98	2852.59	1636.48	1023.36	590.32
SD	6.511637	3.344346	2.644782	2.05164	2.135349	2.342312
AVG	3444.861	2920.326	2850.624	1635.035	1021.705	590.474
RSD	0.189025	0.114533	0.092779	0.12548	0.208999	0.396683

Figure-3: FT-IR graph of the final nanoformulation

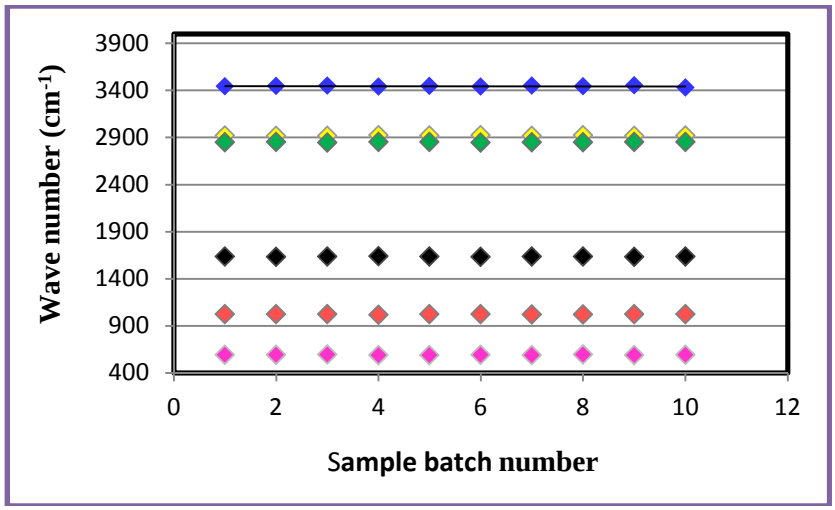


Table-4: Raman peak data of the final nanoformulation

Sample Batch number	Wave number (Cm^{-1})					
batch 1	217	302	379	429	467	487
batch 2	213	298	382	430	466	486
batch 3	218	297	373	428	468	489
batch 4	219	300	378	431	469	488
batch 5	220	301	377	432	465	485
batch 6	215	299	380	427	464	489
batch 7	216	296	376	429	470	487
batch 8	219	305	383	433	468	484
batch 9	216	302	375	428	465	490
batch 10	220	304	381	430	469	489
SD	2.311805	2.951459	3.204164	1.888562	2.024846	1.95505
AVG	217.3	300.4	378.4	429.7	467.1	487.4
RSD	1.063877	0.98251	0.846766	0.439507	0.433493	0.401118

Figure 4: Raman graph of the final nanoformulation

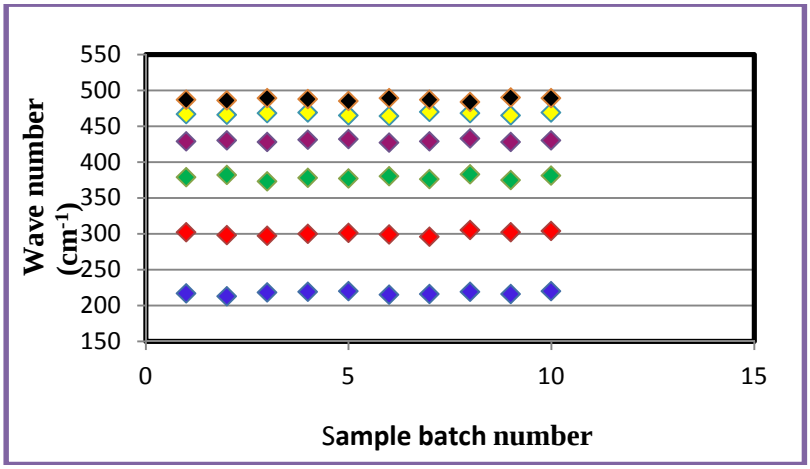


Fig.3 and table .3 for the FT-IR data and Fig. 4 and Table – 4 of the Raman Data, further confirms our observation from the UV-Visible Data.

Fig.5 and Table-5 for the particle size of the nanosilver used as the carrier consistently shows the formation of the same particle size particle through the ten batches of the synthetic route and Fig.6 and Table-6 for the particle size of the final formulation proves that consistently same particle size formulation formation through the batches.

The zeta potential data from Fig.7 and table-7 for the final formulation without the prussian blue pigment used for formulation release consistently shows the formation of the same positively charged nanoformulation, hence facilitating the addition of negatively charged Prussian blue. The Relative standard deviation through the data collected is within the tolerance region of less than 2.

Table-5: Average particle size data of the nanosilver

Sample batch number	Particle size (nm)
Batch 1	50
Batch 2	48
Batch 3	49
Batch 4	50
Batch 5	50
Batch 6	48
Batch 7	49
Batch 8	48
Batch 9	49
Batch 10	49
SD	0.816497
AVG	49
RSD	1.66632

Figure 5: Average Particle size graph of the nanosilver

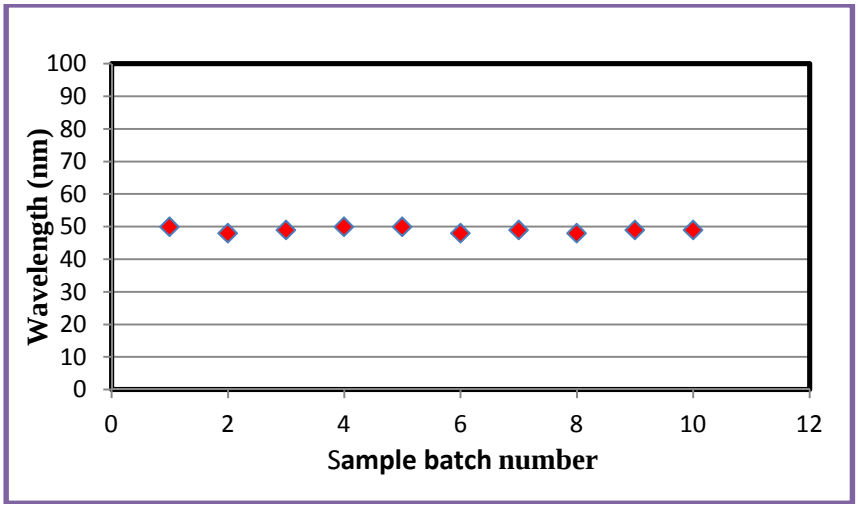


Table 6: Average Particle size data of the final formulation

Sample batch number	Particle size (nm)
Batch 1	230
Batch 2	232
Batch 3	231
Batch 4	229
Batch 5	228
Batch 6	227
Batch 7	229
Batch 8	228
Batch 9	230
Batch 10	229
SD	1.494434
AVG	229.3
RSD	0.651738

Figure 6: Average Particle size graph of the final formulation

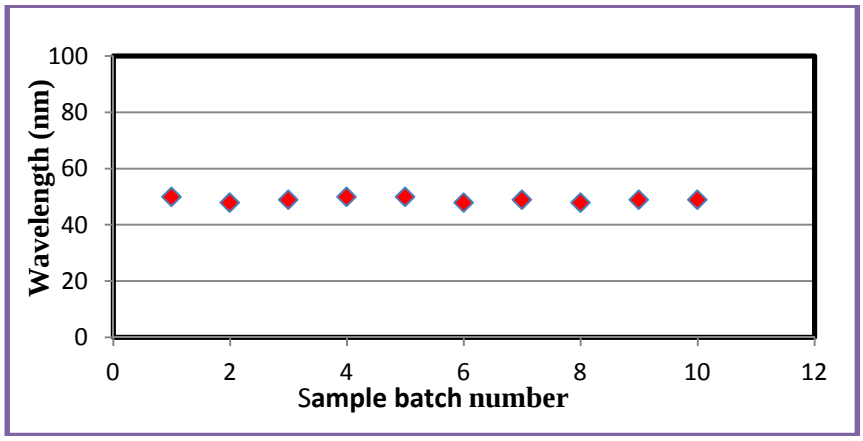
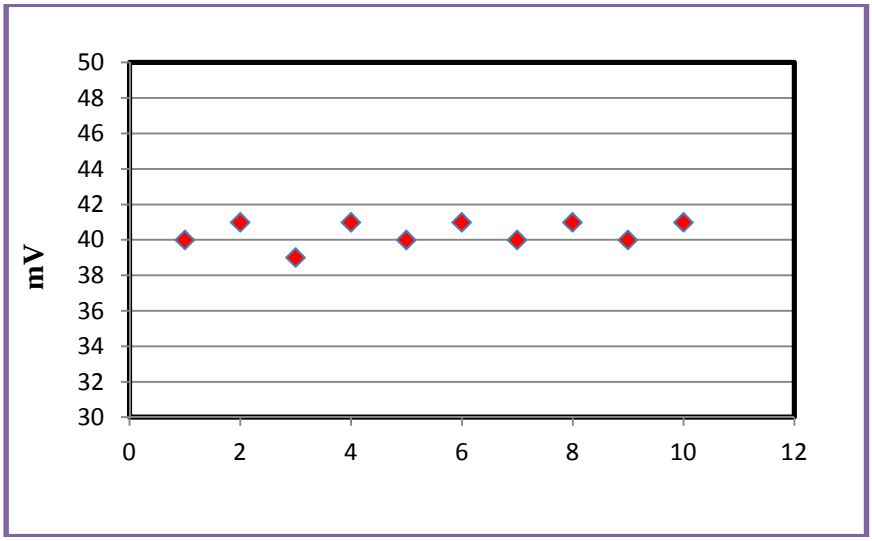


Table 7: Average zeta potential data of the nanoformulation without Prussian blue

Sample batch number	Potential (mV)
Batch 1	40
Batch 2	41
Batch 3	39
Batch 4	41
Batch 5	40
Batch 6	41
Batch 7	40
Batch 8	41
Batch 9	40
Batch 10	41
SD	0.699206
AVG	40.4
RSD	1.730708

Figure 7: Average zeta potential graph of the nanoformulation without Prussian Blue



CONCLUSION

It is necessary to obtain a consistent, reliable, highly precise, sensitive, accurate and reproducible synthetic route when designing drugs through nanoformulations, which are used to treat patients suffering from cancer through systematic route, as the drugs have high potential and should not damage the other cells. The other ingredients in the formulation should not be harmful to the systematic route and should target only the cancerous cell. The particle size of the formulation is very crucial as it should only enter the cancerous cell but not the surrounding normal cells.

Precision in designing such drugs is highly crucial and techniques like chemometrics and PCA play a crucial role in determination consistency of producing the drug over batches and in bulk. The study we carried out shows the usefulness of this tool, and proving the concept.

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