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Artificial Intelligence and Machine Learning Applied Interpretation and Analysis of Optimization Study and In-Vitro Release of Formulated Curcumin Loaded Nanoparticles

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Keywords

Nanoparticles, Curcumin, Piperine, Artificial Intelligence,Jupyter Notebook

Abstract

Artificial Intelligence and Machine Learning studies of data collected during the research and review studies has been done using Jupyter Notebook (Anaconda) and MATLAB R2016a. Pie chart along with exclusive graphs like tree map, donut graph and others are used to make a comprehensive study of the data. Similar to Box Behenken the prediction model developed are exclusive models to predict the particle size and percentage entrapment efficiency of nanoparticles in any desired combination of working parameters as chosen. MATLAB R2016a which is a programming and numeric computing platform has been used for the graphical representations of Box behenken studies. The technique is very fast and accurate clearly depicting the analysis between the dependant and independent variables. Also MATLAB helps in simulation studies that has been incorporated in the introduction depicting the effect of curcumin that blocks Pet and EspC cytotoxicity on epithelial cells by preventing their release from the outer membrane. Curcumin-piperine loaded nanoparticles are prepared successfully using Fessi method. In vitro release data showed that Cu-Pi NPs showed Non-Fickian diffusion and sustained release mechanism for the release.

1. Introduction

Artificial Intelligence is one of a wide field of computer science which emphasizes on organizing intelligent machines which are capable of completing various tasks, which requires human intelligence. Artificial intelligence is also considered as a technique or a technology that allows machines to replicate human behavior. Machine learning is a subspace of Artificial Intelligence, which allows the machine to learn from the past given data without any obvious planning accordingly. Artificial Intelligence has been used in the field of medicine for discovery and drug designing. Curcumin is one of the important source of *Curcuma longa* L.(Zingiberaceae family). The yellow colour of the herb is due to curcumin. It is mostly cultivated in tropical and subtropical regions. It is also used to flavour food and dye clothes. It also enhances the effects of antibacterial, antifungal, antioxidant, and,

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anticancer activities. Curcumin is less to nil toxic at active doses. Curcumin has a variety of specific characterizations which makes it an interest of study for scientists in recent years. [1,2,3]

A simulation model has been developed during this research using MatLAbs 2016.

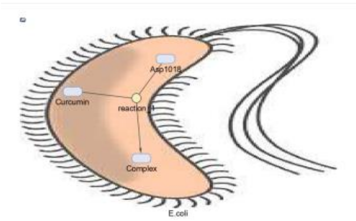

Diagram of simulation model CPK

2. Material And Methods

Procurement of curcumin ,piperine, Eudragit , Dimethyl sulphoxide (DMSO) & acetone and downloading the Artificial intelligence and machine learning tools like python 3 and Anaconda (Jupyter notebook) .

Optimization of the Process Variables:- The design and analysis of 15 experimental runs were developed using Design-Expert-7®. The independent variables were Concentration of Curcumin, Concentration of piperine, and Concentration of Surfactant. 1, 0, and +1, respectively were used to depict low, medium and high levels. Their actual values as depicted in Table 2 below. The dependent variables in the study included Entrapment Efficiency percentage as Y1 & Particle Size as Y2.

Characterization of Cu-Pi Nanoparticles

Entrapment Efficiency Percent (EE%):- The amount of curcumin entrapped within the nanoparticles was determined by indirect method, through calculating the amount of unentrapped drug. EE% for each formulation was calculated using the following equation:

On the addition of water to a 100ml solution of curcumin (100mg), piperine (100mg) and Eudragit 100 RS (2gm) in 100ml acetone nanoparticles were formed and turned the solution slightly turbid.

The responses obtained from each formulation were suited in different models and ANOVA studies were done further.

The composition of 15 experimental trials with three repetitions of the center point generated by the BBD are illustrated in Table 3. Selection of the most fitted model to each parameter and its significance was performed using analysis of variance (ANOVA),expressed as p-value<0.05.The coefficient of determination (R^2) was employed for the selection of the best fit model.

$$EE\ \% = \frac{\text{Total curcumin added - Free curcumin in supernatant}}{\text{Total curcumin added}} \times 100$$

Particle size(PS):- Measurements of the Particle Size was done on the samples prepared and diluted in deionised water after which measured at 25°C.

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Formulation Optimization

Optimization of the Cu-Pi nanoparticles was developed using Design Expert® software (RSM-BBD) after applying specific constraints on the chosen dependent variables. [4]

Preparation and Characterization of Curcumin loaded Nanoparticles:- Curcumin, Piperine and Eudragit E 100 were dissolved in 20 ml of acetone under sonication. In 50 mL of ultra pure water this organic phase was mixed containing sodium lauryl sulphate (SLS) under constant stirring at 500 or 1000 rpm for a time period of atleast 1 hour. Nanoparticles were spontaneously formed turning the solution slightly turbid. Overnight stirring was done to remove the organic solvent.Centrifugation was done to remove the free drug at 3000rpm for 10minutes at 4°C .The resultant formulation was used for further characterization. [5]

***In-Vitro* release study**

The release data demonstrated that both Cu-Pi Nanoparticles followed biphasic release design with an initial fast release in the initial 8 h followed by slower and sustained release. The *in vitro* release data was also suited to zero-order, first-order, Korsmeyer-Peppas and Higuchi's matrix as shown in (Table 9). Korsmeyer- Peppas was the best suited model.Values of ‘n’ for Cu-Pi Nanoparticles indicated that curcumin and piperine were released from Cu-Pi-Nanoparticles through Non-Fickian diffusion (0.5 < ‘n’ value < 1) where drugs were released through diffusion and erosion of Eudragit matrix.[6]

Characterization of Curcumin loaded Nanoparticles

Table 1: Concentrations Independent variables

Independent variables	Level		
	-1	0	+1
A Concentration of Curcumin (mg)	50	100	150
B Concentration of Piperine (mg)	50	100	150
C Concentration of Surfactant (mg)	1000	2000	3000

Table 2: Dependent variables

Dependent variables	Constraints
R1 (%EE)	Maximum
R2 (Particle size)	Minimum

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Table 3. Actual Values of the factors

Actual values of the factors			
Formulations	**Curcumin concentration (%)**	**Piperine Concentration (%)**	**SurfacantConcentration(%)**
F1	0.10	0.10	2
F2	0.05	0.05	2
F3	0.15	0.10	3
F4	0.15	0.10	1
F5	0.1	0.15	3
F6	0.15	0.05	2
F7	0.10	0.05	1
F8	0.10	0.15	1
F9	0.15	0.15	2
F10	0.05	0.10	3
F11	0.05	0.10	1
F12	0.10	0.05	3
F13	0.10	0.10	2
F14	0.05	0.15	2
F15	0.10	0.10	2

Characterization of Cu-Pi Nanoparticles

Table 4. Particle size and Entrapment Efficiency

Formulations	PS ± SD (nm)	Entrapment Efficiency (%)
F1	259±1.8	48±0.25
F2	250±2.6	44±0.43
F3	229±1.4	57±0.52
F4	276±1.3	65±0.32
F5	278±2.1	70±0.62
F6	247±2.6	65±0.53
F7	260±1.5	57±0.72
F8	235±1.4	56±0.24
F9	256±1.3	45±0.32
F10	263±1.5	54±0.26
F11	357±2.1	53±0.23
F12	262±1.6	55±0.83
F13	244±1.7	63±0.62
F14	357±1.4	51±0.33
F15	281±1.9	68±0.62

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Values are expressed as Mean ± SD

Table 5. ANOVA analysis of the investigated responses (% Entrapment efficiency)

Model	R^2	R^2 Adjusted	R^2 Predicted	Adequate precision	Remarks
Linear	0.9715	0.9665	0.9607	54.126	Suggested
2FI	0.9715	0.9577	0.9347	33.802	
Quadratic	0.9826	0.9691	0.9736	37.155	

Table 6. ANOVA analysis of the investigated responses(Particle size)

Model	R^2	R^2 Adjusted	R^2 predicted	Adequate precision	Remarks
Linear	0.8259	0.7559	0.7557	21.765	
2FI	0.9119	0.8377	0.8268	33.244	
Quadratic	0.9857	0.9866	0.9784	48.271	Suggested

Formulation Optimization

Design Expert® was used for the optimization. A formulation with a calculated desirability of 0.889 was selected when maximum EE% and minimum particle size was taken. The optimized level for each factor was 0.13 % for the concentration of curcumin , 0.09 % for concentration of piperine and 2.3 % for concentration of Surfactant.

Table 7. Predicted and observed values

Response	Predicted value	Observed value	Low confidence interval	High confidence interval
EE %	58.05	56.01± 1.36	46	63
PS(nm)	229	233± 3.28	218	248

Table 8 *In-vitro* drug release from Cu-Pi Nanoparticles (pH 7.4)

Time	Release
2	0
4	25
6	46
8	56
12	59
18	62
24	62

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In-Vitro release study

Table 9

Release model	Release of curcumin from Cu-Pi NPs	
	R^2-value	n-value
Zero order	0.9414	-
First order	0.8242	0.7885
Higuchi	0.9130	0.8828
Korsmeyer-peppas	0.9442	0.9088

Artificial Intelligence Analysis of Box-Behenken Using Jupyter Notebook

In [14]:
```
# preprocessing
import pandas as pd
import numpy as np
from sklearn.preprocessing import LabelEncoder
from sklearn.preprocessing import StandardScaler
import pandas as pd
import seaborn as sns
import matplotlib.pyplot as plt
import matplotlib.dates as mdates
import plotly.express as aware

# visualisation
import matplotlib.pyplot as plt
import seaborn as sns
import warnings
warnings.filterwarnings('ignore')
```

In [15]:
```
df = pd.read_csv('BB 1.csv')
df
```

Out[15]:

	Formulations	PS (nm)	Entrapment Efficiency (%)
0	F1	259	48
1	F2	250	44
2	F3	229	57
3	F4	276	65
4	F5	278	70
5	F6	247	65
6	F7	260	57
7	F8	235	56
8	F9	256	45
9	F10	263	54
10	F11	357	53
11	F12	262	55
12	F13	244	63
13	F14	357	51
14	F15	281	68

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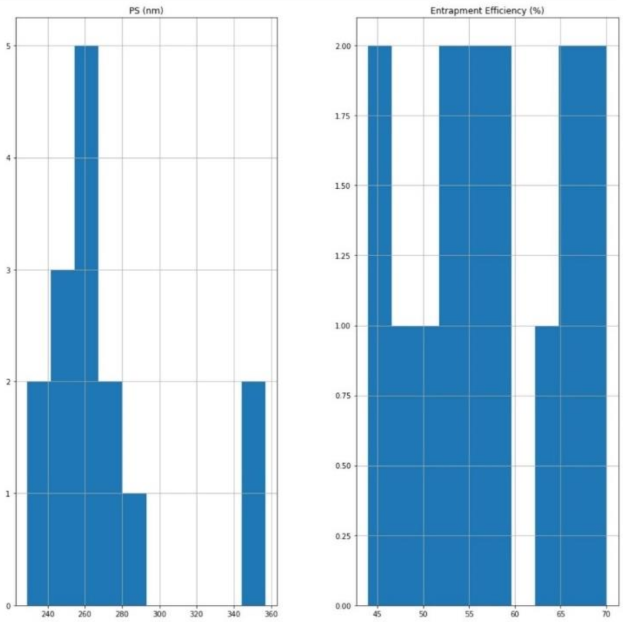
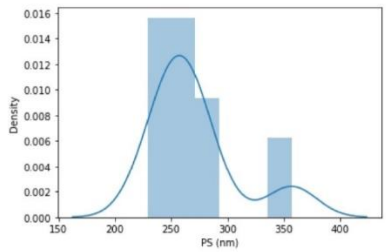
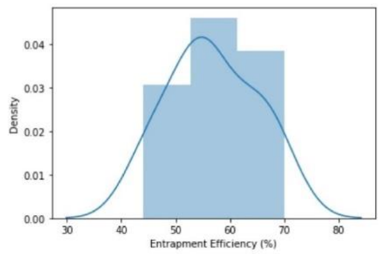
```
In [16]: ## Plotting histogram
         fig = plt.figure(figsize = (15,15))
         ax=fig.gca()
         g=df.hist(ax=ax)
```




```
In [17]: sns.distplot(df['PS (nm)'])
Out[17]: <AxesSubplot:xlabel='PS (nm)', ylabel='Density'>
```




```
In [18]: sns.distplot(df['Entrapment Efficiency (%)'])
Out[18]: <AxesSubplot:xlabel='Entrapment Efficiency (%)', ylabel='Density'>
```



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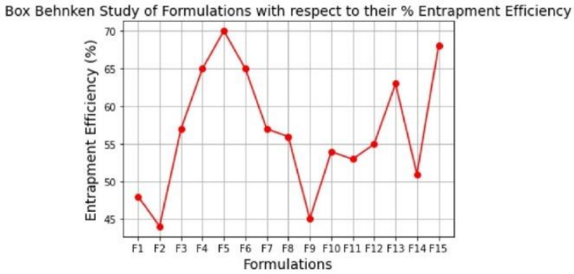
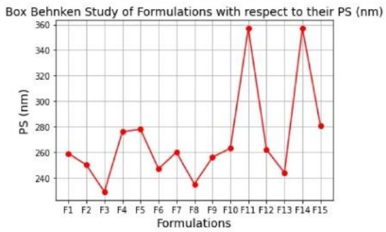
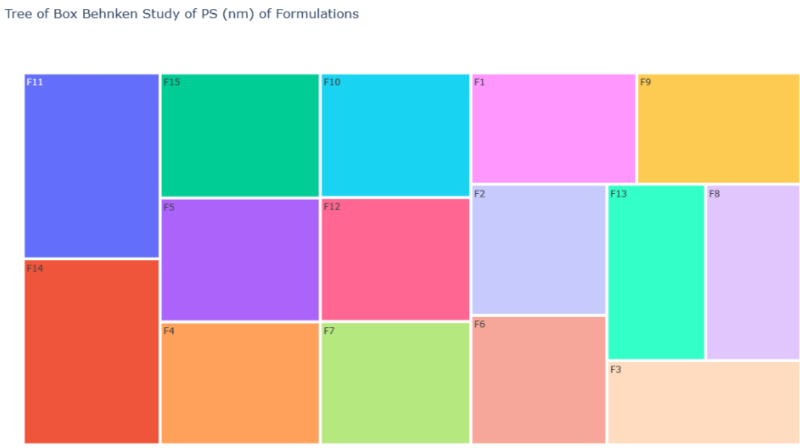
```
In [19]: plt.plot(df['Formulations'], df['Entrapment Efficiency (%)'], color='red', marker='o')
         plt.title('Box Behnken Study of Formulations with respect to their % Entrapment Efficiency', fontsize=14)
         plt.xlabel('Formulations', fontsize=14)
         plt.ylabel('Entrapment Efficiency (%)', fontsize=14)
         plt.grid(True)
         plt.show()
```




```
In [20]: plt.plot(df['Formulations'], df['PS (nm)'], color='red', marker='o')
         plt.title('Box Behnken Study of Formulations with respect to their PS (nm)', fontsize=14)
         plt.xlabel('Formulations', fontsize=14)
         plt.ylabel('PS (nm)', fontsize=14)
         plt.grid(True)
         plt.show()
```




```
In [27]: fig = aware.treemap(df, path=['Formulations'], values='PS (nm)',
                title='Tree of Box Behnken Study of PS (nm) of Formulations', width=1200, height=700)
         fig.show()
```




```
In [22]: fig = aware.treemap(df, path=['Formulations'], values='Entrapment Efficiency (%)',
                title='Tree of Box Behnken Study of Entrapment Efficiency (%) of Formulations',width=1200, height=700)
         fig.show()
```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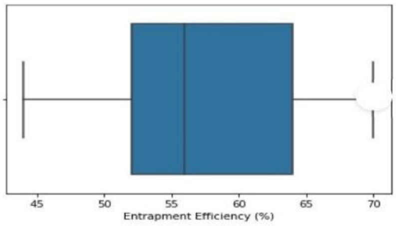
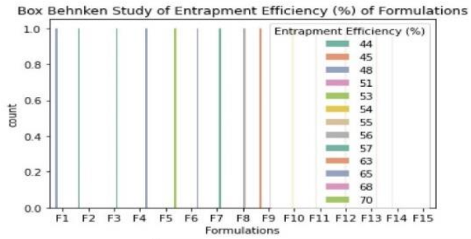
Tree of Box Behnken Study of Entrapment Efficiency (%) of Formulations

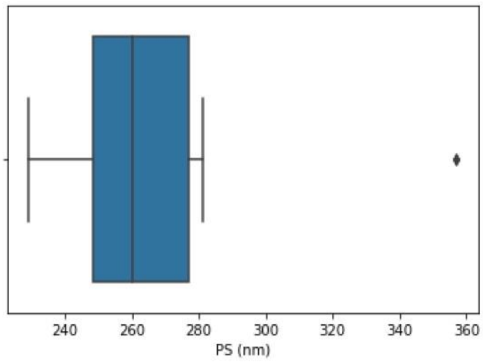

```
In [24]: sns.boxplot(df['Entrapment Efficiency (%)'])
Out[24]: <AxesSubplot:xlabel='Entrapment Efficiency (%)'>
```




```
In [25]: sns.countplot(x ='Formulations', hue = "Entrapment Efficiency (%)", data = df, palette = "Set2")
         plt.title('Box Behnken Study of Entrapment Efficiency (%) of Formulations')
         plt.show()
```




```
In [23]: sns.boxplot(df['PS (nm)'])
Out[23]: <AxesSubplot:xlabel='PS (nm)'>
```



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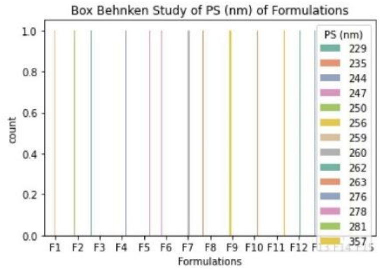
In [26]:
```
sns.countplot(x ='Formulations', hue = "PS (nm)", data = df, palette = "Set2")
plt.title('Box Behnken Study of PS (nm) of Formulations')
plt.show()
```



Prediction Model to predict Size ≤ 281(nm)

In [5]:
```
import pandas as pd
df = pd.read_csv('Curcumin Size Prediction.csv')
df
X = df.drop(columns=('Size'))
y = df['Size']
model = DecisionTreeClassifier()
model.fit(X, y)
predictions = model.predict([ [61, 0.25, 100, 55] ])
predictions
```

```
C:\ProgramData\Anaconda3\lib\site-packages\sklearn\base.py:450: UserWarning:
X does not have valid feature names, but DecisionTreeClassifier was fitted w
ith feature names
          warnings.warn(
```

Out[5]:
```
array(['Y'], dtype=object)
```

Prediction Model to predict %EE ≥ 57%

In[6]:
```
import pandas as pd
df = pd.read_csv('Curcumin %EE prediction.csv')
df
X = df.drop(columns=('%EE'))
y = df['%EE']
model = DecisionTreeClassifier()
model.fit(X, y)
predictions = model.predict([ [61, 0.25, 100, 259] ])
predictions
```


-

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```
C:\ProgramData\Anaconda3\lib\site-packages\sklearn\base.py:450: UserWarning:
X does not have valid feature names, but DecisionTreeClassifier was fitted w
ith feature names
  warnings.warn(
```

Out[6]:

```
array(['Y'], dtype=object)
```

Analysis of the results of *In-vitro* release Kinetics obtained using Artificial Intelligence & Machine Learning

In [1]:

```
# preprocessing
import pandas as pd
import numpy as np
from sklearn.preprocessing import LabelEncoder
from sklearn.preprocessing import StandardScaler
import pandas as pd
import seaborn as sns
import matplotlib.pyplot as plt
import matplotlib.dates as mdates
import plotly.express as aware

# visualisation
import matplotlib.pyplot as plt
import seaborn as sns

import warnings
warnings.filterwarnings('ignore')
```

In [2]:

```
df = pd.read_csv('kinetics.csv')
df
```

Out[2]:

	Time (hrs)	Release
0	2	0
1	4	25
2	6	46
3	8	56
4	12	59
5	18	62
6	24	62

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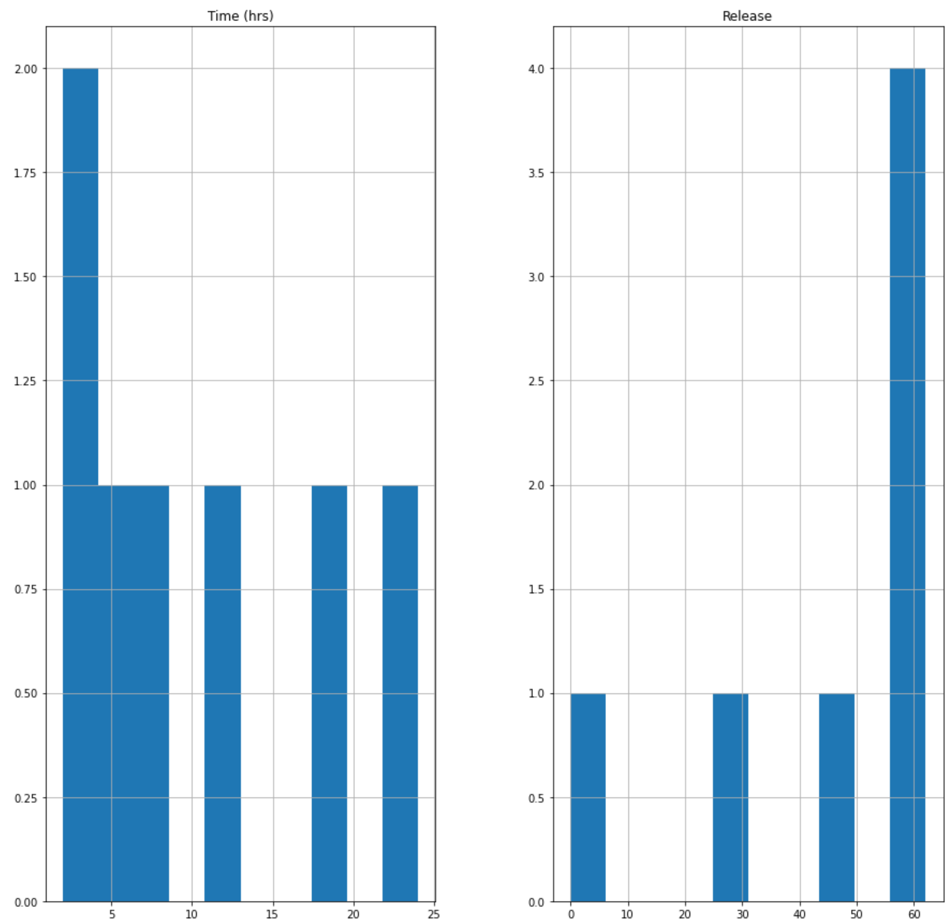
In [3]:
```
## Plotting histogram
fig = plt.figure(figsize = (15,15))
ax=fig.gca()
g=df.hist(ax=ax)
```



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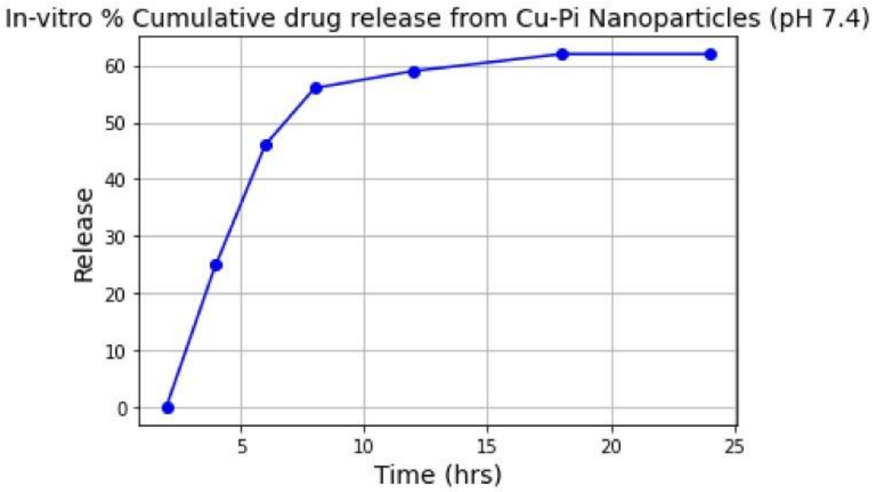
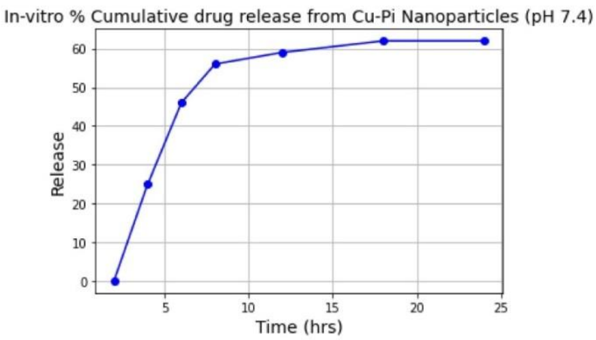
```
In [5]: plt.plot(df['Time (hrs)'], df['Release'], color='blue', marker='o')
        plt.title('In-vitro % Cumulative drug release from Cu-Pi Nanoparticles (pH 7.4)',
                  fontsize=14)
        plt.xlabel('Time (hrs)', fontsize=14)
        plt.ylabel('Release', fontsize=14)
        plt.grid(True)
        plt.show()
```




```
In [5]: plt.plot(df['Time (hrs)'], df['Release'], color='blue', marker='o')
        plt.title('In-vitro % Cumulative drug release from Cu-Pi Nanoparticles (pH 7.4)',
                  fontsize=14)
        plt.xlabel('Time (hrs)', fontsize=14)
        plt.ylabel('Release', fontsize=14)
        plt.grid(True)
        plt.show()
```


```
In [6]: fig = aware.treemap(df, path=['Time (hrs)'], values='Release',
              title='In-vitro % Cumulative drug release from Cu-Pi Nanoparticles
                         width=1200, height=700)
        fig.show()
```

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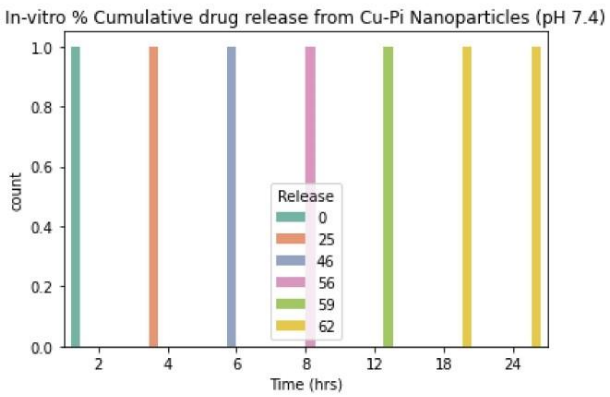
```
In [8]: sns.countplot(x ='Time (hrs)', hue = "Release", data = df, palette = "Set2")
        plt.title('In-vitro % Cumulative drug release from Cu-Pi Nanoparticles (pH 7.4)')
        plt.show()
```



Discussion

Artificial Intelligence and Machine

:

Learning studies of data collected during the research and review studies has been done using Jupyter Notebook (Anaconda) and MATLAB R2016a. With the use of algorithms exploratory data analysis (EDA) and prediction models have been designed to facilitate future researchers for most accurate and reproducible results and allowing the prediction of desired factors like particle size in nm and % Entrapment efficiency for the new formulated nanoparticles. This study will help all future researchers pursuing their work on nanoparticles with one API, one bioavailability enhancer and one surfactant to execute rapid fast data analysis may be a large data of numerous values extracted from data repositories, saving lot of time and authentic results. Different graphical representations like bar diagram, Pie chart along with exclusive graphs like tree map, donut graph and others allow to make a comprehensive study of the same data. Similar to Box Behenken the prediction model developed are exclusive models to predict the particle size and percentage EE of nanoparticles in any desired combination of working parameters as chosen. MATLAB R2016a a programming and numeric computing platform has been used for the graphical representations of

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Box behenken studies. The technique is very fast and accurate clearly depicting the analysis between the dependant and independent variables.

Using MATLAB a simulation model for the study of affect of released curcumin by nanoaprticles on microbial growth in *in-vitro* studies has been developed. Such simulation models are of great help for any further studies. Curcumin-piperine loaded nanoparticles were prepared successfully using Fessi method. The effect of Curcumin concentration, piperine concentration, and surfactant concentration on the entrapment efficiency percent & particle size were studied. Optimal formulation with a desirability of **0.889** was found using Box-behnken design. The optimized values for each factor were 0.13 % for the concentration of curcumin , 0.09 % for concentration of piperine 2.3% for the concentration of Surfactant.

Statistically satisfactory results of PS 233 nm and EE% of 56% are observed. *In vitro* release data showed that Cu-Pi Nanoparticles followed Non-Fickian diffusion and sustained release mechanism for the release.

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[6] Jinsong Liua,1, Lihua Xub,1, Chuantong Liua, Dafeng Zhanga, Siqian Wanga, Zhennan Denga,Weiwei Loua, Haihong Xua, Qing Baia, Jianfeng Maa ; Preparation and characterization of cationic curcumin nanoparticles for improvement of cellular uptake; Carbohydrate Polymers 90 (2012) 16–22