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## A Simulation Based Screening on Different Coloring Agents used in Cosmetics and Pharmaceutical Industry for their Safety Profile

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#### ABSTRACT

Coloring agents are inseparable part of cosmetic, pharmaceutical Industry and food industry. A study conducted by Food Dyes Health Effects Assessment OEHHA in 2020 reported The percentage of US children and adolescents diagnosed with Attention Deficit Hyperactivity Disorder (ADHD) increased from an estimated 6.1% to 10.2% in the past 20 years. Several studies revealed that Attention Deficit Hyperactivity Disorder could be caused by Artificial Food dyes In This study an attempt was made to perform the in silico safety analysis of Ferric ferrocyanide, caramel, Carmine, Betanine, Erythrosine, Tartrazine, Indigotine, Allura red, sunset yellow, Brilliant blue, Curcumin, lawsone and Juglone coded in Python 2.7. The properties generated by the software were analyzed were molecular Weight (MW) Volume, TPSA, LogS, LogP, LogD, Caco-2 permeability, HIA, Protein binding, BBB permeability, skin sensitivity, carcinogenicity, eye irritation, respiratory toxicity, genotoxicity, non biodegradability and aquatic toxicity. The result of this study revealed a totally new dimension of the safety of these dyes and pigment which are widely used in the cosmetic, Food and Pharmaceutical Industry.

**Key words:** in silico, prediction, Pigment, toxicity

### 1. INTRODUCTION

Colorants provide the desired color and thus make it possible to obtain satisfying products for consumers in a wide variety of domains, such as the textile, food or cosmetic industries.<sup>1</sup>

Colouring agents can be classified in two main groups: colorants (or dyes) and pigments. Colorants are generally soluble synthetic organic colouring agents. They are majorly used to colour cosmetic products, Pigments are insoluble colouring agents, which when utilized, remain in crystals or particles form. They are mineral and organic pigments, Make-up Toothpaste, soaps and beauty masks are the products which majorly have mineral and organic pigments and important as coloring agents.

There are wide variety of coloring agents used in Cosmetic and pharmaceutical Industry minority of them are Ferric ferrocyanide<sup>3</sup> which is a dark blue pigment and produced by oxidation of ferrous ferrocyanide salts. Another is Caramel<sup>4</sup> is a dark-orange color product. It is made by heating a variety of sugars, Carmine<sup>6</sup> or cochineal or carmine a cochineal extract, which is natural bright-red color.

Betaine or phytol accamin a water soluble red pigment occurs in present in the roots of beet root, *Beta vulgaris*<sup>7</sup>, Erythrosine is a pink dye, an organoiodine compound, and a derivative of fluorone<sup>8</sup>, Tartrazine is a synthetic azo dye which is lemon yellow in color and used in cosmetic<sup>9</sup>, Indigotine is a blue color dye, soluble in water .It is organic salt derived from indigo by aromatic sulfonation. Allura red is a red azo water soluble dye<sup>11</sup>. Sunset yellow is used as an orange or yellow-orange dye<sup>12</sup>. Brilliant blue is blue color synthetic organic compound<sup>13</sup>, Curcumin is a yellow chemical produced by plants *Curcuma longa*.<sup>14</sup> Lawsone hennotannic acid, is a red-orange dye present in the leaves of the henna plant (*Lawsonia inermis*)<sup>15</sup>, Juglone or 5-hydroxy-1,4-naphthalenedione (IUPAC) is an organic compound also known as C.I. Natural Brown 7 and C.I. 75500<sup>16</sup>.

The latest software-based methods are powerful tools for scanning and predicting new agents<sup>17</sup> depends on their potential and properties and this led to screen the best and safe coloring agent by using Biopharmaceutical attributes, Drug likeliness properties, Skin sensitivity and toxicity study.

## 2. METHODS

The screening of the best and safe coloring agent was done in different steps Initially majorly used pigments and dye were selected from literature in cosmetic and pharmaceutical Industry .Then their structure were generated in desired format using software. These agents were then subjected for the in silico screening on the basis of Biopharmaceutical properties like molecular weight, polarity, solubility, TPSA, Log S, LogP, Permeability, Drug likeliness property by utilizing Lipinski rule of 5 and Pfizer rule and Toxicity study using BBB permeability ,skin sensitivity ,carcinogenicity ,Eye irritation ,Respiratory toxicity ,genotoxicity , non biodegradability and aquatic toxicity. This study was conducted using online open accessible software. The software used for the *in-silico* study was mainly coded in Python 2.7.

### 2.1 Screening for Biopharmaceutical attributes

In the screening of pigments and dye biopharmaceutical attributes were generated with the help of open access software then they were analyzed on the basis of their safety and permeability profile. In the screening of pigment and dye the parameters which were analyzed were Molecular Weight (MW)Volume ,Total Polar surface area, LogS, LogP, LogD,Caco-2 Permeability, Pgp-inhibitor, Pgp-substrate, Human intestinal Absorption , Protein binding, Volume of distribution. Blood brain barrier permeability, half-life and clearance .On the basis of these properties different radar plots were generated for the analysis of best agent.

### 2.2 Drug likeliness property

The vital potential of these pigments and dyeing agent were analyzed by Lipinski Rule and Pfizer Rule.The violation counts were analyzed for individual agents in order to find best agent . the criteria for Lipinski rule of 5 :<5 hydrogen bond donors, <10 hydrogen bond acceptors, molecular weight < 500, AlogP < 5. <sup>18</sup>

### 2.3 Prediction of Toxicity study

This study was also conducted in silico in which the toxicity prediction was done using Toxicophore rule like Genotoxic Carcinogenicity Rule, Non-Genotoxic Carcinogenicity Rule, Skin Sensitization Rule, Aquatic Toxicity Rule and NonBiodegradable Rule. The main toxicity analyzed were sensitivity which were analyzed were Skin Sensitization, Carcinogenicity ,Eye Corrosion, Eye Irritation.

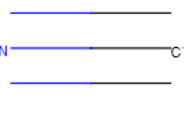
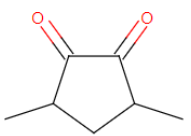
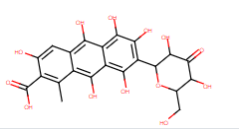
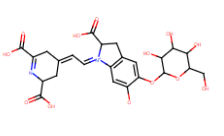
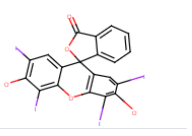
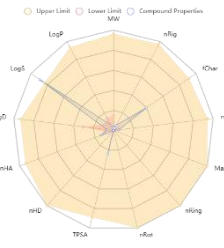
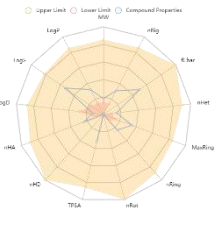
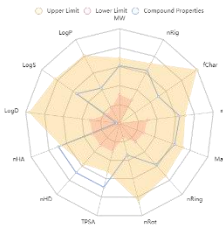
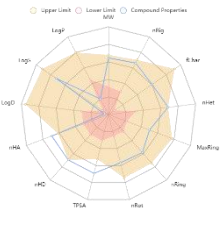
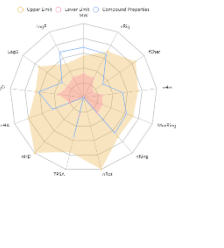
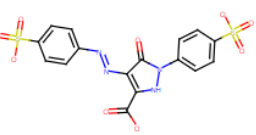
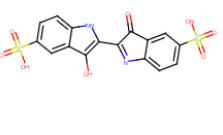
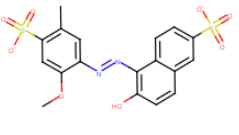
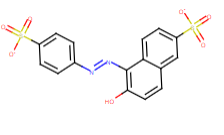
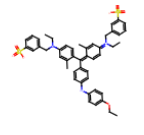
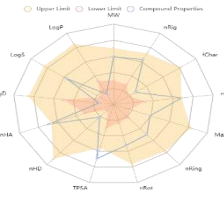
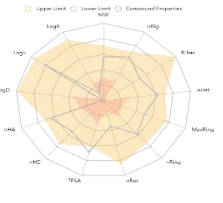
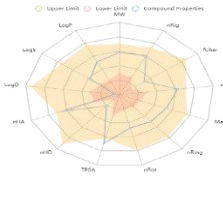
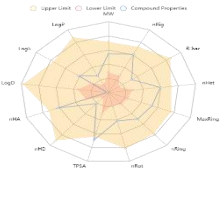
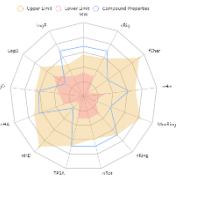
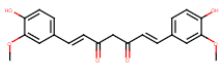
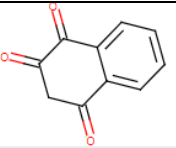
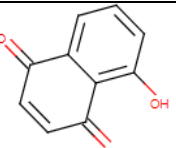
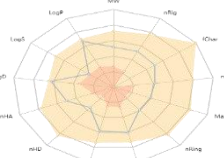
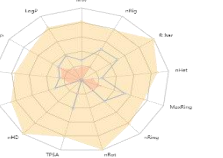
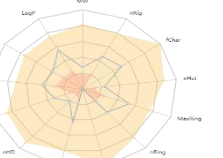
## 3. RESULT AND DISCUSSION

Prussian blue, Caramel, Tartrazine and Juglone were observed as best coloring agents as per the biopharmaceutical properties indicated in table - I out of Prussian blue , Carmine, Betanine, Erythrosine, Indigotine, Allura red, sunset yellow, brilliant blue, lawsone Curcumin.

Least molecular weight was exhibited by the Prussian blue among Caramel, Carmine, Betanine, Erythrosine. Highest polarity was exhibited by Betanine. Two coloring agent were showed no violation from drug likeness property Lipinski rule and Pfizer rule. Carmine, Betanine and Erythrosine showed higher human intestinal absorption. Least protein drug binding was exhibited by Prussian blue and that's why it had high volume of distribution .Skin sensitization was exhibited by Prussian blue, Carmine, Betanine and Erythrosine, Highest Carcinogenicity revealed by Prussian blue, Eye Corrosion and Eye Irritation shown by Prussian blue, Caramel and Erythrosine. Notoxicophore rules was violated by Prussian blue but rest of the coloring agent Caramel, Carmine, Betanine, Erythrosine showed violation in toxicophore rules as shown in table-2.

The least molecular weight was exhibited by the sunset yellow as compared to Tartrazine, Indigotine and Allura red. Highest polarity was revealed by the Tartrazine. All these coloring agent accepted Lipinski Rule and Pfizer rule. The maximum human intestinal absorption and minimum protein binding exhibited by the Indigotine. Sunset yellow only showed skin sensitivity but Eye Corrosion and Eye Irritation showed by almost all of them.

**Table 1: Different coloring agents along with structure and vital properties**

<p><b>Ferric ferrocyanide(Prussian blue)</b></p>	<p><b>caramel</b></p>	<p><b>Carmin</b></p>	<p><b>Betanine</b></p>	<p><b>Erythrosine</b></p>
				
				
<p><b>Tartrazine</b></p>	<p><b>Indigotine</b></p>	<p><b>Allura red</b></p>	<p><b>sunset yellow</b></p>	<p><b>brillant blue</b></p>
				
				
<p><b>Curcumin</b></p> 	<p><b>lawsone</b></p> 	<p><b>Juglone</b></p> 		
				

**Table 2: Different properties of coloring agents**

Parameters	Prussian blue	Caramel	Carmines	Betanine	Erythrosine
Molecular Weight (MW)	26.000	126.070	492.090	550.140	833.640
Volume	30.258	133.379	445.387	501.973	427.751
TPSA	0.859	0.945	1.105	1.096	1.949
LogS	23.790	34.140	245.670	246.550	81.650
LogP	0.839	-1.542	-1.401	-0.543	-3.129
LogD	-0.291	0.746	1.121	-2.144	5.583
	0.241	0.498	0.128	0.183	2.721
<b>Medicinal Chemistry</b>					
Lipinski Rule	Accepted	Accepted	Rejected	Rejected	Rejected
Pfizer Rule	Accepted	Accepted	Accepted	Accepted	Accepted
<b>Absorption</b>					
Caco-2 Permeability	-4.312	-4.425	-6.487	-6.737	-5.035
Pgp-inhibitor	---	---	---	---	---
Pgp-substrate	---	---	---	+++	---
HIA	---	---	+++	+++	+
<b>Distribution</b>					
PPB	13.495%	81.850%	92.879%	21.953	100.402%
VD	0.969	0.509	0.747	0.485	0.390
BBB	+++	++	---	--	---
<b>Excretion</b>					
CL	5.320	9.108	1.683	1.617	2.767
T1/2	0.880	0.644	0.916	0.835	0.836
<b>Toxicity</b>					
Skin Sensitization	+	--	++	++	+++
Carcinogenicity	+++	-	---	---	---
Eye Corrosion	+++	++	---	---	---
Eye Irritation	+++	+++	-	---	+++
Respiratory Toxicity	+++	++	---	+++	+++
<b>Toxicophore rules</b>					
Acute Toxicity Rule					
Genotoxic Carcinogenicity Rule	0 alert(s)	0 alert(s)	0 alert(s)	0 alert(s)	0 alert(s)
NonGenotoxic Carcinogenicity Rule	0 alert(s)	0 alert(s)	4 alert(s)	1 alert(s)	0 alert(s)
Skin Sensitization Rule	0 alert(s)	0 alert(s)	1 alert(s) 13 alert(s)	0 alert(s)	0 alert(s)
Aquatic Toxicity Rule	0 alert(s)	2 alert(s)	3 alert(s)	2 alert(s)	1 alert(s)
NonBiodegradable Rule	0 alert(s)	0 alert(s)		0 alert(s)	2 alert(s)

**Table 3: Different properties of coloring agents**

Parameter	Tartrazine	Indigotine	Allura red	sunset yellow
Molecular Weight	464.980	421.990	450.020	405.990
TPSA	217.040	174.190	168.580	159.350
LogS	-1.287	-0.255	-2.854	-2.493
LogP	-1.069	-0.620	1.563	0.576
LogD	-1.034	0.045	0.200	-0.071
Medicinal Chemistry				
Lipinski Rule	Accepted	Accepted	Accepted	Accepted
Pfizer Rule	Accepted	Accepted	Accepted	Accepted
Absorption				
Caco-2 Permeability	-5.211	-6.403	-4.655	-4.760
Pgp-inhibitor	-	---	---	---
Pgp-substrate	---	---	---	---
HIA	--	++	---	---
Distribution				
PPB	100.047%	98.064%	100.044%	100.096%
VD	0.169	0.351	0.378	0.460
BBB Penetration	---	---	---	---
Excretion				
CL	0.817	0.924	0.819	0.456
T <sub>1/2</sub>	0.087	0.046	0.056	0.026
Toxicity				
Skin Sensitization	---	---	-	+++
Carcinogenicity	---	---	---	---
Eye Corrosion	---	++	+	+
Eye Irritation	++	+++	+	+
<b>Toxicophore Rules</b>				
Acute Toxicity Rule	0 alert(s)	0 alert(s)	0 alert(s)	0 alert(s)
Genotoxic Carcinogenicity Rule	5 alert(s)	0 alert(s)	6 alert(s)	6 alert(s)
NonGenotoxic Carcinogenicity Rule	0 alert(s)	0 alert(s)	0 alert(s)	0 alert(s)
Skin Sensitization Rule	0 alert(s)	0 alert(s)	2 alert(s)	2 alert(s)
Aquatic Toxicity Rule	1 alert(s)	0 alert(s)	1 alert(s)	1 alert(s)
NonBiodegradable Rule	2 alert(s)	1 alert(s)	2 alert(s)	2 alert(s)

**Table 4: Different properties of coloring agents**

Parameters	Brilliant blue	Curcumin	lawsone	Juglone
Molecular Weight (MW)	830.290	368.130	174.030	174.030
Volume	850.258	381.036	174.955	174.955
Density	0.977	0.966	0.995	0.995
TPSA	141.910	93.060	51.210	54.370
LogS	-7.856	-3.921	-3.604	-2.823
LogP	6.573	2.742	0.959	1.839
LogD	2.696	2.820	1.076	1.679
<b>Medicinal Chemistry</b>				
Lipinski Rule	Rejected	Accepted	Accepted	Accepted
Pfizer Rule	Accepted	Accepted	Accepted	Accepted
<b>Absorption</b>				
Caco-2 Permeability	-5.776	-4.834	-4.527	-4.637
Pgp-inhibitor	+++	--	---	---
Pgp-substrate	---	---	---	---
HIA	---	---	---	---
<b>Distribution</b>				
PPB	104.109%	99.799%	72.261%	94.329%
VD	0.586	0.369	0.405	0.484
BBB Penetration	---	--	++	-
<b>Excretion</b>				
CL	1.042	13.839	6.788	6.497
T <sub>1/2</sub>	0.003	0.948	0.339	0.694
<b>Toxicity</b>				
Skin Sensitization	+	+++	---	++
Carcinogenicity	+++	++	++	+++
Eye Corrosion	---	---	+	++
Eye Irritation	---	++	+	+
Respiratory Toxicity	+++	+++	+++	+++
<b>Toxicophore Rules</b>				
Acute Toxicity Rule	0 alert(s)	0 alert(s)	0 alert(s)	0 alert(s)
Genotoxic Carcinogenicity Rule	1 alert(s)	1 alert(s)	0 alert(s)	2 alert(s)
NonGenotoxic Carcinogenicity Rule	0 alert(s)	1 alert(s)	0 alert(s)	2 alert(s)
Skin Sensitization Rule	3 alert(s)	8 alert(s)	4 alert(s)	3 alert(s)
Aquatic Toxicity Rule	0 alert(s)	2 alert(s)	0 alert(s)	3 alert(s)
NonBiodegradable Rule	2 alert(s)	1 alert(s)	1 alert(s)	0 alert(s)

There was no single coloring agent which revealed no violation from toxicophore rules but Indigotine showed least violation as compared to Tartrazine, Allura red and sunset yellow as shown in table-3.

In table-4, the lowest molecular weight is exhibited by lawsone and Juglone as compared to Brilliant blue and Curcumin. Highest polarity as well as highest protein drug binding was showed by Brilliant blue. Curcumin, lawsone and Juglone revealed no violation from drug likeliness properties like Lipinski Rule and Pfizer rule. lawsone also indicated BBB permeability .In case of skin sensitization three coloring agents Brilliant blue. Curcumin, and Juglone showed sensitivity .Carcinogenicity was revealed by all four Brilliant blue. Curcumin, lawsone and Juglone coloring agents ,Except Brilliant blue all three revealed the predictability of Eye Corrosion and Eye Irritation. There was no single coloring agent which revealed no violation from toxicophore rules but lawsone exhibited least violation as compared to Brilliant blue. Curcumin, and Juglone.

#### 4. CONCLUSION

Prussian blue revealed least molecular weight as compared to Caramel, Tartrazine and Juglone but Juglone exhibited bit higher molecular weight than Prussian blue .Though all the four selected coloring agent showed accepted drug likeliness properties but only two coloring agents Caramel, Tartrazine showed no skin sensitivity .Carcinogenic was exhibited by Prussian blue and Juglone. Eye Corrosion and Eye Irritation both were exhibited by Prussian blue Caramel and Juglone excluding Tartrazine which showed only eye irritation. The toxicophore properties violation was zero in case of Prussian blue as compared to Caramel , Tartrazine and Juglone. On the basis of low molecular weight, zero violation from Toxicophore properties and drug likeliness Prussian blue can be selected as best coloring agent, but its carcinogenicity, Eye Corrosion and Eye Irritation predictability must be consider and clinically analyzed. Then only its safety profile may be accepted.

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