

Prediction of drug loading in the gelatin matrix using computational methods

Rania M. Hathout ^{1*}, AbdelKader A. Metwally ^{1,2}, Timothy J. Woodman ³
and John G. Hardy ^{4,5*}

¹ Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Ain Shams University, Cairo, 11566, Egypt.; rania.hathout@pharma.asu.edu.eg (R.M.H.); AbdelKader74@yahoo.com (A.A.M).

² Department of Pharmaceutics, Faculty of Pharmacy, Health Sciences Center, Kuwait University, Kuwait; AbdelKader74@yahoo.com (A.A.M).

³ Department of Pharmacy and Pharmacology, University of Bath, Bath, BA2 7AY, UK.; T.Woodman@bath.ac.uk (T.J.W.).

⁴ Department of Chemistry, Lancaster University, Lancaster, Lancashire, LA1 4YB, UK.; j.g.hardy@lancaster.ac.uk (J.G.H.).

⁵ Materials Science Institute, Lancaster University, Lancaster, Lancashire, LA1 4YB, UK.

* Correspondence: rania.hathout@pharma.asu.edu.eg (R.M.H.); j.g.hardy@lancaster.ac.uk (J.G.H.).

25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51

Abstract

The delivery of drugs is a topic of intense research activity in both academia and industry with potential for positive economic, health, and societal impacts. The selection of the appropriate formulation (carrier and drug) with optimal delivery is a challenge investigated by researchers in academia and industry, in which millions of dollars are invested annually. Experiments involving different carriers and determination of their capacity for drug loading is very time consuming, and therefore expensive; consequently, approaches that employ computational/theoretical chemistry to speed have the potential to make hugely beneficial economic, environmental and health impacts through savings in costs associated with chemicals (and their safe disposal) and time. Here we report the use of computational tools (data mining of available literature, principal component analysis, hierarchical clustering analysis, partial least squares regression, autocovariance calculations, molecular dynamic simulations and molecular docking) to successfully predict drug loading into model drug delivery systems (gelatin nanospheres). We believe that this methodology has the potential to lead to significant change in drug formulation studies across the world.

Keywords: drug delivery; computational pharmaceuticals; machine learning; molecular dynamics simulations; docking; formulations; nanoparticles; gelatin.

52

53 1. Introduction

54 The global market for drug-delivery systems is a multibillion-dollar industry, demand
55 for which is growing in both developed and emerging economies (in part, driven by aging
56 societies and rapid urbanization)¹⁻⁹. Drug-delivery systems deliver drugs at rates controlled
57 by specific features of the systems, particularly their chemical composition (e.g.,
58 inorganic/organic components, molecular weights of their constituents, crosslinking density
59 of polymers, etc.)¹⁰⁻¹².

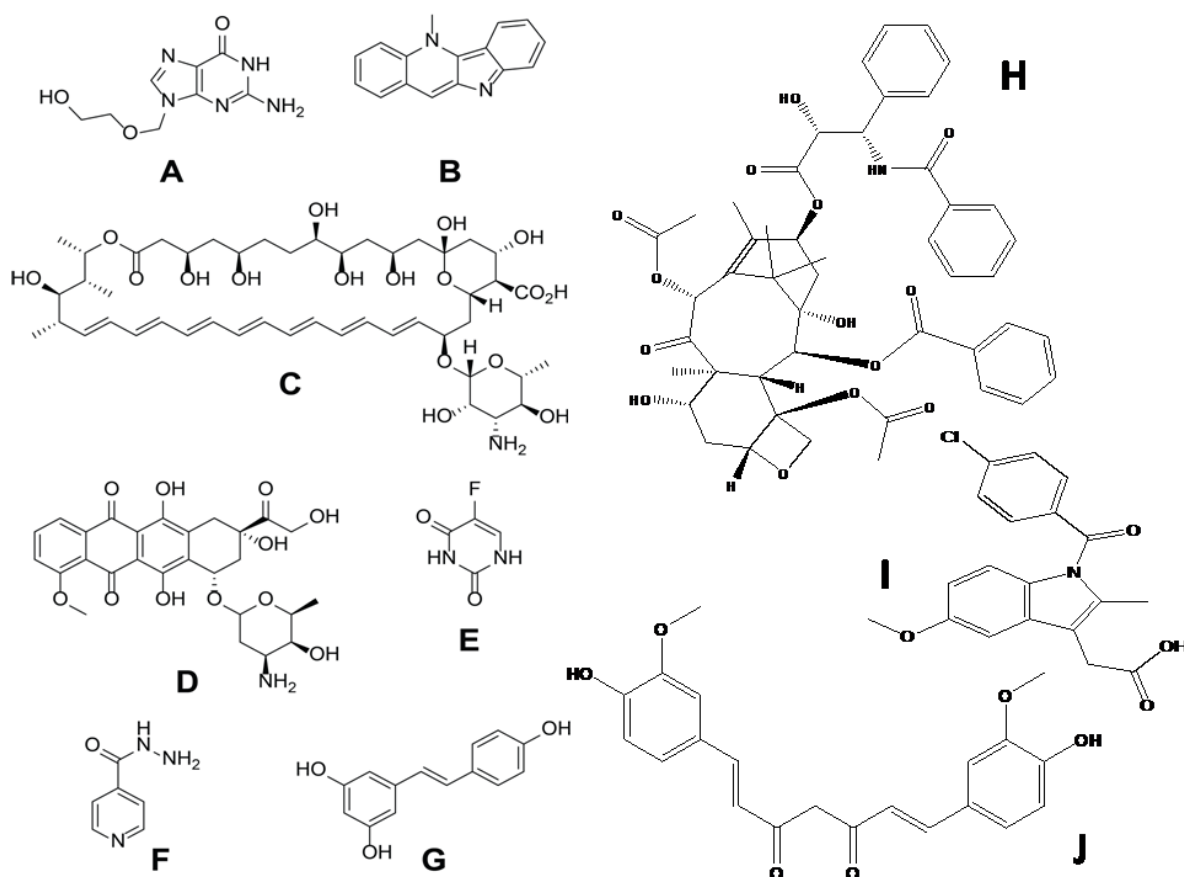
60 The selection of the appropriate system (carrier and drug) to obtain optimal delivery is a
61 challenge investigated by researchers in academia and industry, in which millions of dollars
62 are invested annually¹³. Experiments involving different carriers and determination of their
63 capacity for drug loading is very time consuming, and therefore expensive. Consequently,
64 approaches that exploit multivariate statistical methods, molecular simulations, docking
65 methods, and mining the data in the literature¹⁴⁻¹⁹, have the potential to make hugely
66 beneficial economic, environmental and health impacts through savings in costs associated
67 with chemicals (and their safe disposal) and time.

68 Computational/theoretical chemists/biochemists, biomedical/chemical engineers and
69 pharmacists have developed a variety of methodologies that can be applied to understand
70 drug formulations. Principal component analysis (PCA) and hierarchical clustering analysis
71 (HCA) are considered exploratory data analysis and unsupervised machine learning
72 methods, where these techniques extract patterns from the independent factors (x-variables)
73 only and irrelevant to the y-outcomes. Partial least squares (PLS) is a supervised pattern
74 recognition method correlating the inputs with outputs and subsequently leads to the
75 generation of a model²⁰. This data mining approach (through a retrospective analysis)
76 combined with computer-aided analysis and simulation extracts knowledge from complex
77 variables and responses obtained from historical records. The significant advantage of this

78 approach is the possibility of uncovering interactions and linear relationships that might not
79 be easily detectable with conventional experimental designs ²¹. Although not yet fully
80 explored in drug formulation/delivery, multivariate statistical methods such as PCA and
81 agglomerative HCA were previously used to develop drug delivery formulations. For
82 example, PCA was utilized to generate a quantitative composition-permeability relationship
83 for microemulsion formulations used to deliver testosterone transdermally, with a linear
84 relationship between the lower-dimensionality data generated from the main principal
85 component and the permeability coefficients of the different formulations ²². PCA and HCA
86 were used to extract stable SMEDDS (Self Microemulsifying Drug Delivery Systems) and
87 SNEDDS (Self Nanoemulsifying Drug Delivery Systems) formulations of Lovastatin and
88 Glibenclamide, respectively ^{23,24}; and PCA and PLS analysis were used to assess the
89 qualitative and quantitative effects of different variables such as lipid/surfactant type and
90 their concentrations on parameters related to storage stability ²⁵. Furthermore, PLS was
91 successfully employed to predict the sizes and polydispersity index (PDI) for lipid
92 nanocapsules based on the quantitative mixture composition ²⁶.

93 Here we extend these exciting studies by combining PCA, HCA and PLS with molecular
94 dynamics and docking analysis ²⁷ to give valuable insight into drug loading in a polymer
95 matrix. As a model polymer matrix we use protein-based nanoparticulate drug delivery
96 systems (i.e. nanospheres composed of collagen-derived gelatin). Gelatin is an abundant and
97 inexpensive protein ²⁸, which is amphiphilic in nature due to its amino acid contents (ca. 12%
98 anionic glutamic and aspartic acid; ca. 13% cationic lysine and arginine amino acids, and ca.
99 11% hydrophobic leucine, isoleucine, methionine and valine) ²⁹, and gelatin-based matrices
100 can in principle be used to deliver both small molecules and macromolecules ³⁰⁻³⁶. In this
101 study we focus on a selection of low molecular weight drugs used in the clinic as depicted in
102 **Figure 1**.

103



104

105

106 **Figure 1.** The chemical structures of the substances studied herein: A) Acyclovir,

107 B) Cryptolepine, C) Amphotericin B, D) Doxorubicin, E) 5-Fluorouracil (5FU), F)

108 Isoniazid, G) Resveratrol, H) Paclitaxel, I) Indomethacin and J) Curcumin.

109

110 **2. Materials and Methods**111 *2.1. Data set*

112 The data set contained 4 input variables (descriptors) and one output response (mass of
 113 drug loaded per 100 mg gelatin nanospheres determined experimentally) for different drugs.

114 Data mining was performed through different databases such as: Pubmed and Web of
 115 Science® to obtain the output response for ten drugs: Acyclovir ³⁷, Amphotericin B ³⁸,

116 Cryptolepine ³⁹, Doxorubicin ⁴⁰, 5 Fluorouracil (5FU) ⁴¹, Isoniazid ⁴², Resveratrol ⁴³,

117 curcumin ⁴⁴, paclitaxel ⁴⁵ and indomethacin ⁴⁶.

118

119 *2.2. Calculation of molecular descriptors*

120 The drugs were analyzed using Bioclipse® version 2.6 (Bioclipse project, Uppsala
121 University, Sweden) [39]. The four descriptors chosen were constitutional (molecular
122 weight), electronic (number of hydrogen bond donors and number of hydrogen bond
123 acceptors) and physico-chemical (xLog P).

124

125 *2.3. Hierarchical clustering analysis (HCA).*

126 The molecular descriptors generated using Bioclipse® version 2.6 were subjected to
127 Hierarchical Clustering Analysis using JMP® 7.0 (SAS, Cary, NC, USA). Ward's minimum
128 variance method was adopted to join the clusters and generate a dendrogram. Ward's method
129 is considered an agglomerative hierarchical technique where the merging in the dendrogram
130 starts at the final clusters (leaves) and merging occurs stepwise until it reaches the trunk.
131 Ward's minimum variance criterion minimizes the total within-cluster variance. At each step,
132 the pair of clusters possessing minimum between-cluster distance is merged (i.e. the pair of
133 clusters that leads to the minimum increase in the total within-cluster variance after merging
134 is selected) ⁴⁷.

135

136 *2.4. Principal component analysis (PCA).*

137 PCA was used to extract patterns using an exploratory data analysis method that deals
138 with the variances in sample observations. PCA was performed using JMP® 7.0. Four
139 principal components were calculated by taking a linear combination of an eigenvector of the
140 correlation matrix built up from standardized original variables. The dimensionality of the
141 data was reduced by extracting two main principal components possessing the two highest
142 Eigen values and plotting the data with respect to these two new orthogonal axes.

143

144 *2.5. Partial least squares analysis (PLS) for model generation and validation of the model.*

145 PLS was used to study correlations between the molecular descriptors and the output
146 response. PLS was performed using JMP[®] 7.0 using 4 latent vectors. The PLS generated
147 model was validated by checking the differences between the mean actual and predicted
148 response values using t-test statistical analysis at $P < 0.05$ using GraphPad Prism[®] v.5.0
149 (GraphPad software Inc., San Diego, CA, USA) and by performing a k-fold (5-folds)
150 cross-validation (leave-two-out) to check the predictability of the model and its ability to
151 navigate the experimental space. The value of Q^2 (Predicted R-squared) was calculated as
152 follows:

153
$$Q^2 = \frac{PRESS}{ISS}$$

154 Where PRESS represents the predicted residual error sum of squares while ISS stands for the
155 total initial sum of squares. Moreover, a predicted versus actual correlation was obtained.

156

157 *2.6. Molecular dynamics simulations (MDS) of the gelatin matrix.*

158 Molecular dynamics simulations (MDS) were carried-out using the GROMACS⁴⁸ v.
159 4.6.5 freeware (<http://www.gromacs.org/>). To prepare the gelatin system, 48 peptide
160 molecules were constructed, with 18 amino acids in each molecule. The primary sequence of
161 the peptides was AGPRGQ(Hyp)GPAGPDGQ(Hyp)GP. Six hypothetical probe molecules
162 (with calculated molecular weight of 767.13) were added at random positions to the system.
163 The force field parameters were obtained from CgenFF⁴⁹ (<https://cgenff.paramchem.org/>).
164 The system was energy minimized by the steepest descent method. Molecular dynamics was
165 subsequently carried-out, with a time step of 2 fs, full periodic boundary conditions, and a
166 cut-off distance of 1.2 nm for Van der Waal's and electrostatic interactions⁵⁰. PME was
167 chosen to handle long-range electrostatic interactions. All bonds were constrained by the
168 LINCS algorithm. The MDS were carried-out for 3 ns, at 373K and 1 bar using a v-rescale
169 thermostat and a Berendsen barostat respectively⁵¹.

170

171 *2.7. Drug docking in simulated gelatin nanospheres.*

172 The chemical structures of the studied drugs were drawn using ChemDraw[®] Ultra
173 version 10 (Cambridgesoft, Waltham, MA, USA). The corresponding ‘.mol2’ files needed
174 for docking experiments were obtained using Chem3D[®] Ultra version 10 (Cambridgesoft,
175 Waltham, MA, USA) after energy minimization using the MM2 force field of the same
176 program. Docking analysis was generated by Argus Lab version 4.0.1. (Mark Thompson and
177 Planaria Software LLC, Seattle, WA, USA). The hypothetical probe molecules were utilized
178 to construct corresponding binding sites on the carrier (gelatin-probe), the AScore was
179 utilized for calculating the scoring function. The size of the display box in the x, y and z
180 dimensions were 15 x 15 x15 Å as these dimensions were suitable to the size of the docked
181 molecules and ensured a central position for them inside the gelatin matrix. Additionally, the
182 genetic algorithm was used as the docking engine with 150 maximum poses. The type of
183 calculation and ligand (as chosen using the software options) were Dock and Flexible,
184 respectively; and the binding energies (ΔG , kcal/mole) reflecting the docking efficiencies
185 were calculated.

186

187 **3. Results**

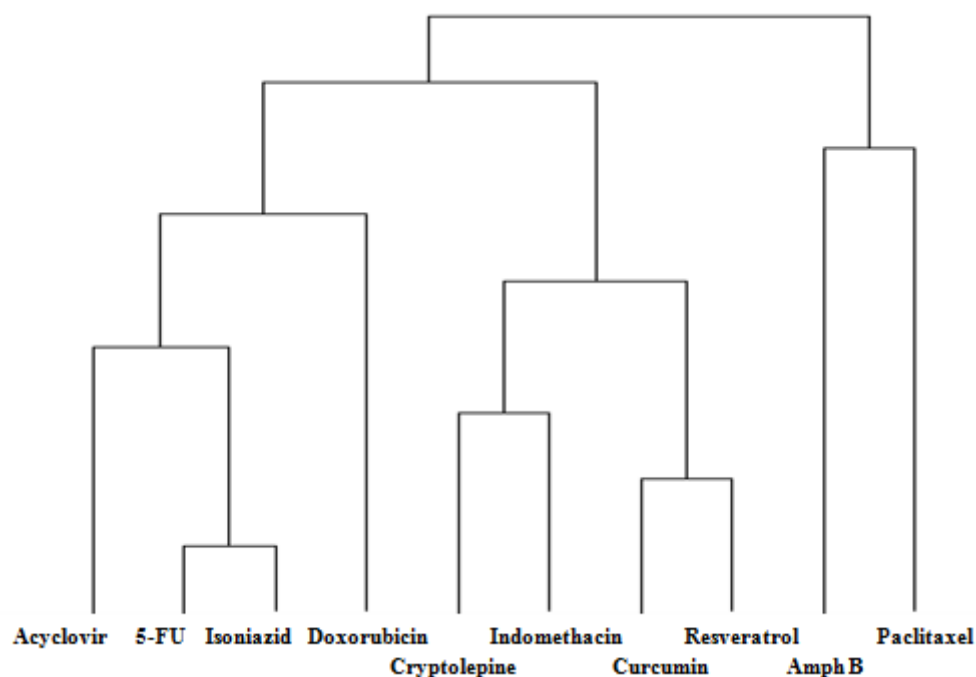
188 **Table 1** reports the molecular descriptors (number of hydrogen bond donors, number of
189 hydrogen bond acceptors, xLogP and molecular weight) for the investigated drugs. The
190 dendrogram classifying these drugs according to HCA using Ward’s minimum variance
191 method (an agglomerative type of analysis) is displayed in **Figure 2**. Isoniazid and 5FU were
192 clustered together according to their 4 descriptors, Resveratrol and Cryptolepine clustered
193 together, whereas Doxorubicin, Acyclovir and Amphotericin B constituted separate clusters.
194 Importantly, the loading pattern followed this classification (see Table 1) where 5FU and
195 Isoniazid scored the highest loading masses followed by Acyclovir which is closest to the

196 aforementioned drugs in the dendrogram. Cryptolepine and Reseveratrol were very close,
 197 with Doxorubicin near to them. Amphotericin B had the lowest mass loaded into the
 198 nanospheres which was clear from its separate branch (furthest distance) in the dendrogram.

199 **Table 1.** The descriptors of the drugs, amounts of loaded drug, and the obtained
 200 binding energies from docking of the drugs on a simulated gelatin matrix.

Drug	xLogP	No. H-bond donors	No. H-bond acceptors	Molecular Weight (g/mol)	Actual Amount of Drug Loaded (mg/100mg gelatin)	Lamarckian Genetic Algorithm ΔG (kcal/mole)
Acyclovir	-1.650	3	8	225.21	8.74	-3.94
Amphotericin B	2.068	12	18	923.49	1.16	144.4
Cryptolepine	2.180	0	2	233.30	2.00	-3.81
Doxorubicin	-1.900	6	9	543.52	2.10	58.29
5-Fluorouracil	-0.760	2	4	130.00	25.07	-4.19
Isoniazid	-0.683	3	4	137.14	22.00	-4.16
Resveratrol	2.050	3	3	228.24	1.96	-3.74
Curcumin	1.95	2	6	368.13	3.50	-2.59
Paclitaxel	6.15	4	14	853.33	0.52	173.5
Indomethacin	3.78	1	4	338.14	1.91	-1.99

201



202

203

204

205

206

207

208

209

210

211

212

213

214

215

216

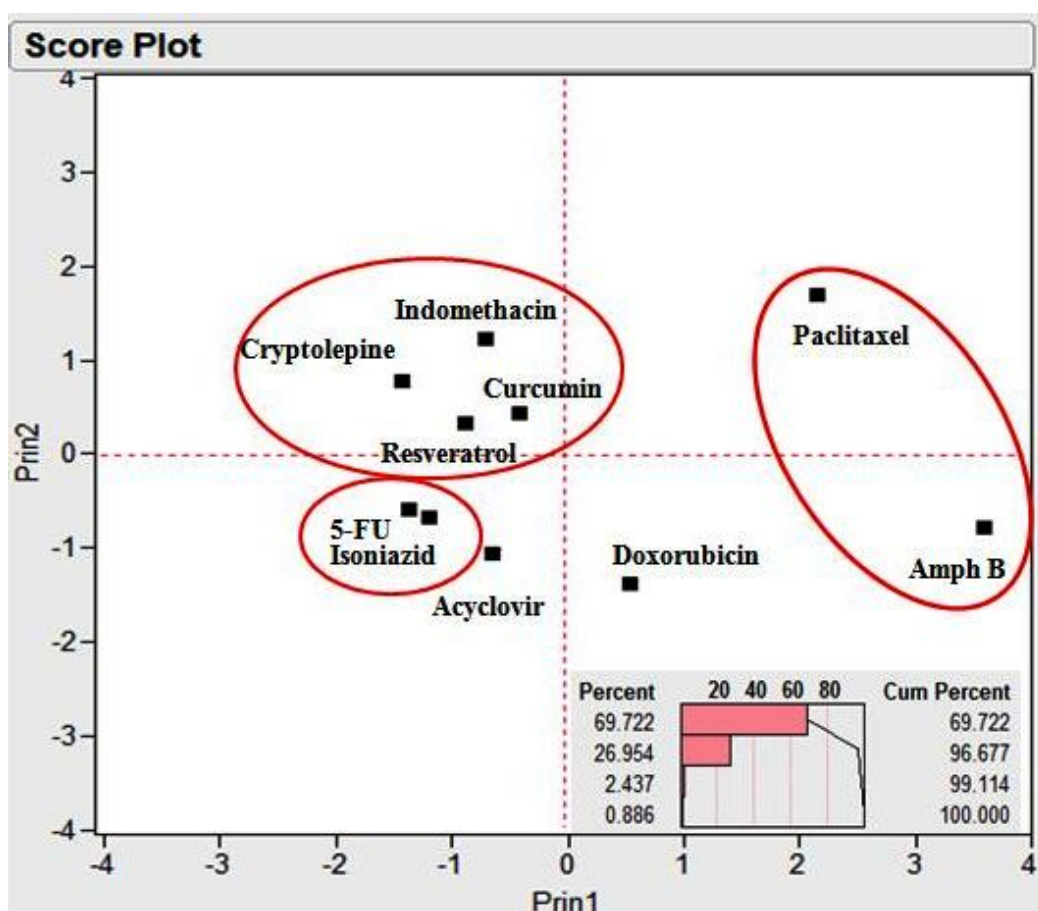
217

218

Figure 2. Hierarchical Clustering Analysis (HCA) of the investigated drugs with respect to 4 constitutional, electronic and physico-chemical descriptors: number of hydrogen bond donors, number of hydrogen bond acceptors, xLogP and molecular weight.

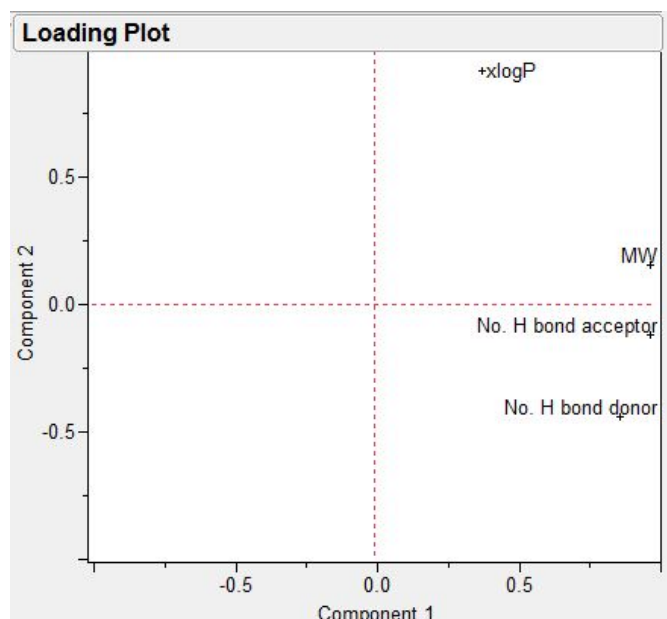
A score plot of the drugs with respect to their descriptors after projecting the data into two main principal components is displayed in **Figure 3**, where principal component 1 and principal component 2 reflect 69.72% and 26.95 % of the data variation, respectively (corresponding to 96.68 % of total variance, **Figure 3**, top right panel) and 5FU and Isoniazid are clustered together with Acyclovir having the nearest score, and Amphotericin B the furthest score. Figure 4 depicts the loading plots of the two main principal components. It is obvious that principal component 1 is mainly composed of the descriptors; the molecular weight, the number of the H-bond donors and the number of the number of H-bond acceptors while principal component 2 mainly depends on the remaining descriptor; xLogP. These results confirm the presentation of the 4 investigated variables in the two generated principal components.

219



220

221 **Figure 3.** Principal Component Analysis (PCA) score plot of the investigated drugs
 222 with respect to 4 constitutional, electronic and physico-chemical descriptors:
 223 number of hydrogen bond donors, number of hydrogen bond acceptors, xLogP and
 224 molecular weight, displaying only two main combined components. The upper
 225 panel depicts the scree plot revealing the percentage variation of each extracted
 226 component (combined from the four descriptors).



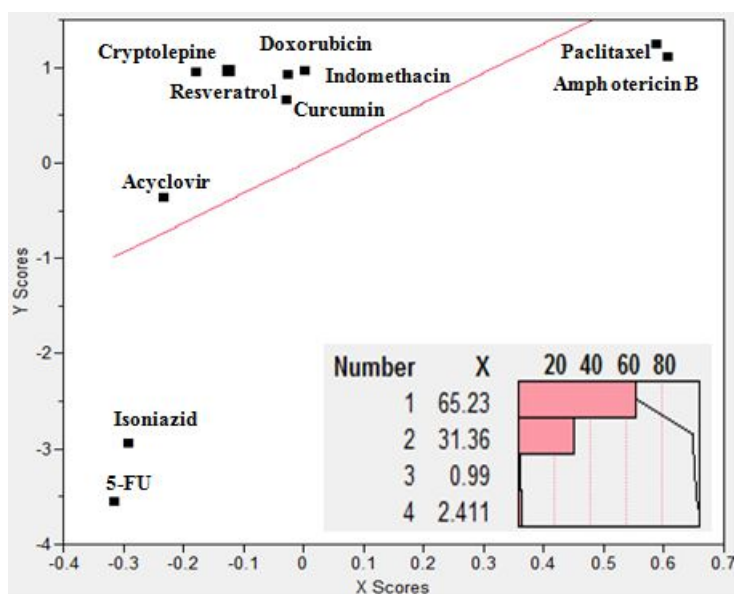
227

228 **Figure 4.** Principal Component Analysis (PCA) loading plot of the two main principal
 229 components.

230

231 The relationship between the obtained combined x-scores (combining the contribution
 232 from the 4 x-variables *viz.* descriptors) and y-scores is displayed in **Figure 5**, and the scree
 233 plot (**Figure 5**, bottom right) depicts the contribution of each individual latent factor to the
 234 combined x-scores with the first two factors accounting for 96.64% of the obtained scores.

235



236

237 **Figure 5.** Partial Least Squares Regression Analysis (PLS) of the investigated drugs
 238 with 4 constitutional, electronic and physico-chemical descriptors: number of
 239 hydrogen bond donors, number of hydrogen bond acceptors, xLogP and molecular
 240 weight as the x-factors and the mass of loaded drug per 100 mg gelatin nanoparticles
 241 as the y-factor. The lower panel depicts the contribution of each latent x-factor
 242 (combined factor) to the x-scores representing the combined x-dimension.

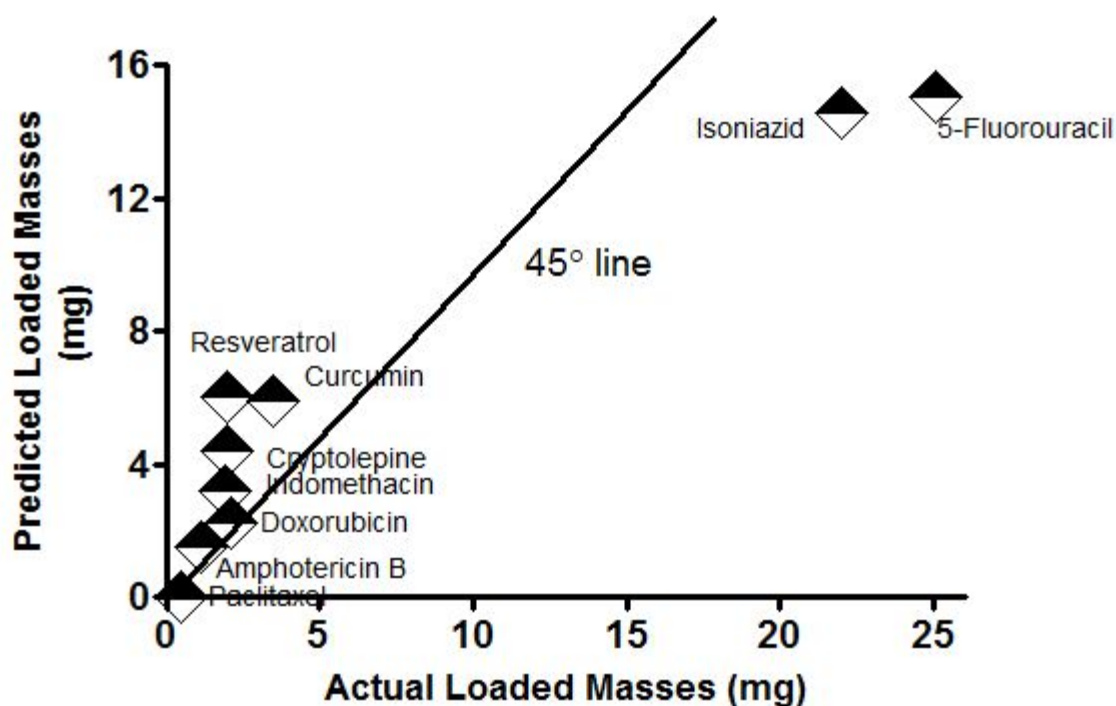
243

244 It is noteworthy that the generated x- and y- scores represent the distances of the points
 245 in space of all the dimensions to the main vector summarizing the final dimension (in the
 246 current case there is a principal component or vector for the x-dimension comprising all the
 247 descriptors, and another for the y-dimension representing the loaded mass). Therefore, the
 248 aforementioned scores can be negative numbers. Consequently, a generated model was
 249 developed where:

$$\begin{aligned}
 Y \text{ (mass of drug loaded per 100 mg of gelatin nanoparticles)} &= 13.175 + 0.115 \times \\
 &\text{xLogP} + 0.001 \times \text{number of hydrogen bond donors} + 2.346 \times \text{number of} \quad (1) \\
 &\text{hydrogen acceptors} - 0.059 \times \text{molecular weight.}
 \end{aligned}$$

250 The values and the signs of the coefficients of the x-factors in the equation were
 251 indicative of the importance of increasing the number H- bond acceptors in the drugs
 252 chemical structure in the presence of a balanced xlogP and low molecular weight to increase
 253 the loading of the drug. The model was validated by performing a t-test statistical analysis
 254 between the actual experimental results for drug loading and the predicted drug loading using
 255 the model where no significant difference was obtained between the means at $P < 0.05$. The
 256 calculated Q^2 or the predicted r-squared after 5-folds cross-validation scored a value of 0.721
 257 (a highly acceptable value) ⁵². Figure 6 further demonstrates the predicted versus actual
 258 relationship where it is observed that most of the points are scattered around the 45° line.
 259 Proximity of the points to this line usually indicates the favorable similarity of the results.

260 Accordingly, the developed model can be exploited in predicting the loaded mass of any new
 261 physically loaded or entrapped investigated drug molecule in a gelatin matrix after projecting
 262 its structure to the aforementioned four descriptors (**Table 1**).



263

264 **Figure 6.** Predicted versus actual drug loading in gelatin nanospheres.

265

266 4. Discussion

267 In the HCA utilized and studied method (Ward's method), the distance between two
 268 clusters is the ANOVA sum of squares between the two clusters added up over all the
 269 variables. At each generation, the within-cluster sum of squares is minimized over all
 270 partitions obtainable by merging two clusters from the previous generation. The sums of
 271 squares are usually easily interpreted when they are divided by the total sum of squares to
 272 give the proportions of variance (squared semi-partial correlations). Ward's method works
 273 under the assumptions of spherical covariance matrices and the condition of equal sampling
 274 probabilities. Distances between clusters in Ward's method are calculated according to the

275 squared Euclidean distance. It is considered very useful in joining clusters with a small
276 number of observations and it is very accurate though sensitive to outliers ⁵³.

277 PCA was used to confirm the hierarchical clustering analysis results. This type of
278 multivariate analysis deals with the x-factors (descriptors) to reduce their dimensionality by
279 projecting the data into new orthogonal axes that display the directions (vectors) of the
280 highest variation. These results confirmed the HCA results and correlate the x-factors (drug
281 descriptors) with the y-outputs (mass of drug loaded per 100 mg of gelatin) where clustered
282 points (Especially in the same quadrants) represents high similarity between them regarding
283 their projected descriptors ⁵⁴.

284 Accordingly, a supervised learning tool (PLS) was used to generate an accurate and
285 sensitive model that would correlate the x-factors with the y-outputs quantitatively. The
286 techniques implemented in the PLS platform work by extracting successive linear
287 combinations of the predictors, called factors (also called components or latent vectors),
288 which optimally address the combined goals of explaining both response and predictor
289 variation. In particular, the method of PLS balances the two objectives and maximizes their
290 correlation ⁵⁵.

291 The obtained results can be explained by the fact that gelatin is a protein carrier with a
292 relatively balanced hydrophilic/hydrophobic character displaying several hydrogen bond
293 donor and acceptor groups with a repetitive sequence of amino acids
294 -Ala-Gly-Pro-Arg-Gly-Glu-4Hyp-Gly-Pro- along its backbone ⁵⁶. This structure can be
295 transformed to some numerical values that are generated of each amino acid. Among which
296 are the highly condensed variables “z-scale descriptors” ⁵⁷ that are derived from a PCA
297 analysis of several experimental and physicochemical properties of the 20 natural amino
298 acids; z1, z2, and z3 and which represent the amino acids hydrophobicity, steric properties,
299 and polarity, respectively. Additionally, they are useful in QSAR analysis of peptides where
300 they have proven effective in predicting different physiological activities ⁵⁸⁻⁶⁰. Herein, we

301 used an extended scale (including 67 more artificial and derivatized amino acids)⁶¹ due to the
 302 presence of 4-hydroxyproline in the gelatin structure.

303 In this study, we expand the use of the first descriptor (z1) to predict the drug loading
 304 properties of nanoparticles. The first scale (z1) was chosen as it represents a lipophilicity
 305 scale that encompasses several variables (amino acid descriptors) such as: the thin layer
 306 chromatography (TLC) variables, log P, nonpolar surface area (Snp) and polar surface area
 307 (Spol) in combination with the number of proton accepting electrons in the side chain
 308 (HACCR)⁶². In this scale, a large negative value of z1 corresponds to a lipophilic amino acid,
 309 while a large positive z1 value corresponds to a polar, hydrophilic amino acid. Therefore, the
 310 gelatin typical structure amino acids (-Ala-Gly-Pro-Arg-Gly-Glu-4Hyp-Gly-Pro-) can be
 311 represented by their z1 values as follows: (0.24), (2.05), (-1.66), (3.52), (2.05), (3.11),
 312 (-0.24), (2.05) and (-1.66). Furthermore, an overall topological description of the repetitive
 313 sequence was accounted for by encoding the z1 descriptors of each amino acid into one auto
 314 covariance variable [49] that was first introduced by Wold et al.⁶³. The auto covariance value

315 (AC) was calculated as follows: $AC_{z.lag} = \sum_{i=1}^{N-lag} \frac{V_{z,i} \times V_{z,i+lag}}{N-lag}$ (2)

$$AC_{z.lag} = \sum_{i=1}^{N-lag} \frac{V_{z,i} * V_{z,i+lag}}{N-lag} \quad (2)$$

316 where AC represents autocovariances of the same property (z-scale); i = 1, 2, 3,...; N is the
 317 number of amino acids; lag = 1, 2, 3, ... L (where L is the maximum lag which is the longest
 318 sequence used and V is the scale value).

319 Therefore, the AC value for the gelatin typical structure sequence was calculated with
 320 lag 1 scoring a value approaching zero (0.028) indicating a balanced
 321 hydrophobicity/hydrophilicity structure. In light of the above, the high loading of 5FU and
 322 Isoniazid can be ascribed to their amphiphilic nature with LogP values approaching 0, and to

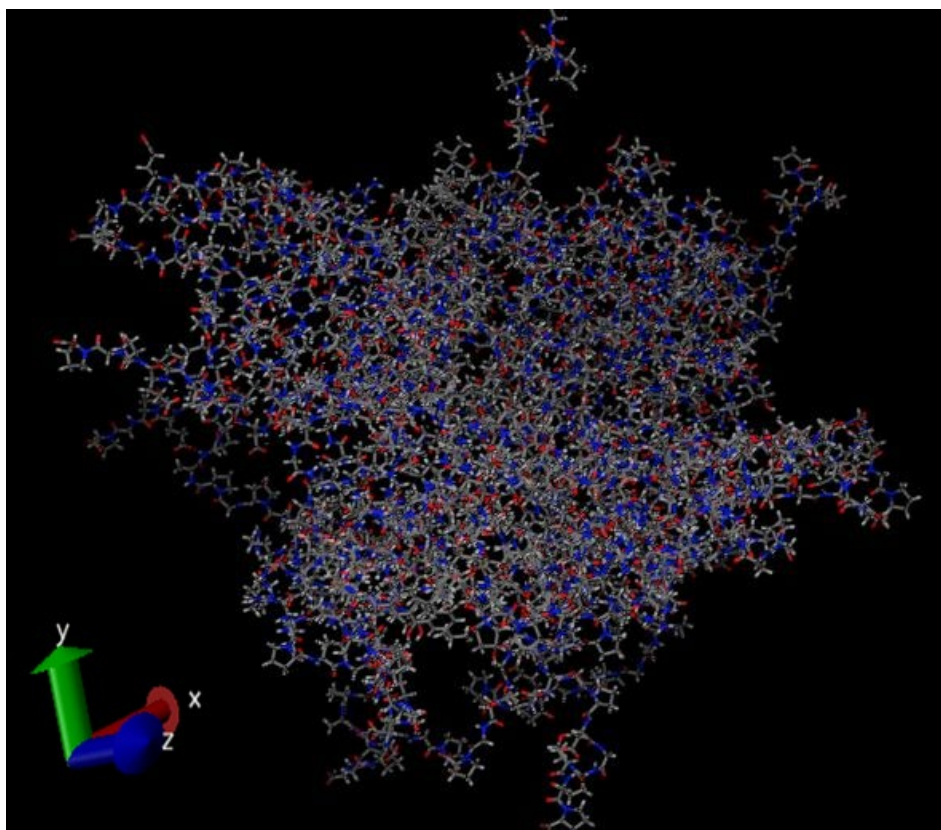
323 the presence of several hydrogen bond donors and acceptors groups relative to their low
 324 molecular weight that is favorable in both diffusion through and entrapment in a protein
 325 matrix like that of gelatin nanospheres. Since there was a recorded deviation between the
 326 actual and the predicted values regarding Isoniazid and 5FU (may be attributed to their small
 327 molecular weight that helps their non-stoichiometric physical entrapment in the gelatin
 328 matrix, therefore, the results were further confirmed by molecular dynamics and docking
 329 experiments, where the drugs were docked on the gelatin matrix simulated structure. **Figure**
 330 **7** shows the molecular simulation of the gelatin nanosphere matrix. Interestingly, the best
 331 binding energy values ΔG (- 4.19 and -4.16 kcal/mol) corresponded to the highest loaded
 332 drugs 5FU and Isoniazid, respectively, followed by Acyclovir (see **Figure 8**). In the same
 333 context, Amphotericin B scored a highly positive ΔG value which explains its low loading
 334 values. The confirmation of the docking results with their experimental counterparts can be
 335 attributed to the inclusive scoring function of the Arguslab® software. This scoring function
 336 is based on the XScore calculated according to the following equation ⁶⁴:

$$\Delta G_{\text{bind}} = \Delta G_{\text{vdw}} + \Delta G_{\text{hydrophobic}} + \Delta G_{\text{H-bond}} + \Delta G_{\text{H-bond (chg)}} + \Delta G_{\text{deformation}} + \Delta G_0 \quad (3)$$

337 where ΔG_{bind} is the total calculated binding energy, ΔG_{vdw} is the binding energy due to Van
 338 der Waal's forces, $\Delta G_{\text{hydrophobic}}$ is the binding energy due to hydrophobic forces, $\Delta G_{\text{H-bond}}$ is
 339 the binding energy due to H-bonding, $\Delta G_{\text{H-bond (chg)}}$ is the binding energy due to H-bonding
 340 due to charged molecules, $\Delta G_{\text{deformation}}$ is the energy due to rotational bonds and atoms
 341 involved in torsions (rotors) that were frozen due to binding, and finally, ΔG_0 represents the
 342 regression-obtained binding energy. As can be inferred, the equation terms encompass nearly
 343 all the possible interactions that can occur between the drug and its carrier that may lead to
 344 drug entrapment which explains the high correlation obtained between the real experimental
 345 values and the docking results.

346 An exponential model was generated correlating the actual experimental molar masses of the
 347 loaded drugs and their corresponding docking binding energies. This model was highly

348 fitting with an obtained r-squared value of 0.95. This relationship can highly estimate the
349 molar masses of physically loaded drugs through docking the investigated molecule on the
350 simulated gelatin matrix. The only limitation of the model was the number of the
351 experimental studies that are involved in it (10 studies) which we recommend to increase in
352 further similar studies.

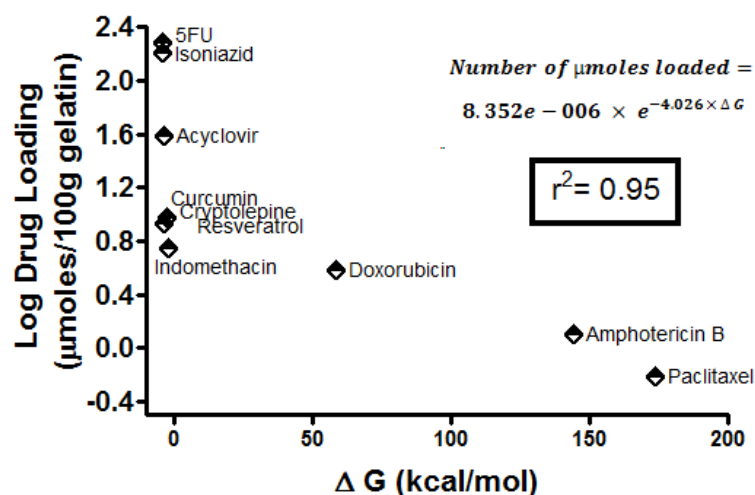


353

354

355 **Figure 7.** Molecular dynamics simulation of the gelatin nanosphere matrix.

356



357

358 **Figure 8.** Drug loading versus the obtained binding energies plot of the investigated
 359 drugs after docking on a simulated gelatin matrix built up using molecular dynamics
 360 simulation displaying an exponential relationship.

361

362 5. Conclusions

363 The current study introduces new approaches of interpreting and predicting drugs
 364 loading on protein carriers, such as gelatin nanospheres. These approaches comprise
 365 multivariate statistical methods such as: hierarchical clustering analysis, principal
 366 component analysis, partial least squares regression, molecular dynamics and docking.
 367 Moreover, the utilization of the amino acids z-scales descriptors represents a new and
 368 important asset in interpreting drug loading in protein-based carriers. We believe that this
 369 methodology has the potential to lead to significant change in drug formulation studies across
 370 the world.

371

372 **Funding:** There are no funding sources to report.

373

374 **Conflicts of Interest:** The authors declare no conflict of interest .

375

376

377

References

- 378 1. Bae, Y. H. Stimuli-Sensitive Drug Delivery. In *Controlled Drug Delivery: Challenge*
379 *and Strategies*, Park, K., Ed.; American chemical Society: Washington, 1997; pp
380 147-160.
- 381 2. Hoffman, A. S. Intelligent Polymers. In *Controlled Drug Delivery: Challenge and*
382 *Strategies*, Park, K., Ed.; American chemical Society: Washington, 1997; pp
383 485-497.
- 384 3. Kanjickal, D. G.; Lopina, S. T. Modeling of drug release from polymeric delivery
385 systems--a review. *Crit Rev. Ther. Drug Carrier Syst.* 2004, 21 (5), 345-386.
- 386 4. Kumar, M.; Curtis, A.; Hoskins, C. Application of Nanoparticle Technologies in
387 the Combat against Anti-Microbial Resistance. *Pharmaceutics* 2018, 10 (1).
- 388 5. Liechty, W. B.; Kryscio, D. R.; Slaughter, B. V.; Peppas, N. A. Polymers for drug
389 delivery systems. *Annu. Rev. Chem. Biomol. Eng* 2010, 1, 149-173.
- 390 6. Manzur, A.; Oluwasanmi, A.; Moss, D.; Curtis, A.; Hoskins, C. Nanotechnologies
391 in Pancreatic Cancer Therapy. *Pharmaceutics* 2017, 9 (4).
- 392 7. Patel, S.; Bhirde, A. A.; Rusling, J. F.; Chen, X.; Gutkind, J. S.; Patel, V. Nano
393 delivers big: designing molecular missiles for cancer therapeutics.
394 *Pharmaceutics* 2011, 3 (1), 34-52.
- 395 8. Spizzirri, U. G.; Curcio, M.; Cirillo, G.; Spataro, T.; Vittorio, O.; Picci, N.;
396 Hampel, S.; Iemma, F.; Nicoletta, F. P. Recent Advances in the Synthesis and
397 Biomedical Applications of Nanocomposite Hydrogels. *Pharmaceutics* 2015, 7
398 (4), 413-437.
- 399 9. Zhang, N.; Wardwell, P. R.; Bader, R. A. Polysaccharide-based micelles for drug
400 delivery. *Pharmaceutics* 2013, 5 (2), 329-352.
- 401 10. Farid, M. M.; Hathout, R. M.; Fawzy, M.; bou-Aisha, K. Silencing of the scavenger
402 receptor (Class B - Type 1) gene using siRNA-loaded chitosan nanoparticles in
403 a HepG2 cell model. *Colloids Surf. B Biointerfaces* 2014, 123, 930-937.
- 404 11. Mehanny, M.; Hathout, R. M.; Geneidi, A. S.; Mansour, S. Exploring the use of
405 nanocarrier systems to deliver the magical molecule; Curcumin and its
406 derivatives. *J Control Release* 2016, 225, 1-30.
- 407 12. Mehanny, M.; Hathout, R. M.; Geneidi, A. S.; Mansour, S. Studying the effect of
408 physically-adsorbed coating polymers on the cytotoxic activity of optimized
409 bisdemethoxycurcumin loaded-PLGA nanoparticles. *J Biomed. Mater. Res. A*
410 2017, 105 (5), 1433-1445.
- 411 13. Soltani, S.; Sardari, S.; Soror, S. A. Computer simulation of a novel
412 pharmaceutical silicon nanocarrier. *Nanotechnol. Sci Appl.* 2010, 3, 149-157.
- 413 14. Fagir, W.; Hathout, R. M.; Sammour, O. A.; ElShafeey, A. H.
414 Self-microemulsifying systems of Finasteride with enhanced oral
415 bioavailability: multivariate statistical evaluation, characterization,
416 spray-drying and in vivo studies in human volunteers. *Nanomedicine (Lond)*
417 2015, 10 (22), 3373-3389.

- 418 15. Hathout, R. M.; Metwally, A. A. Towards better modelling of drug-loading in solid
419 lipid nanoparticles: Molecular dynamics, docking experiments and Gaussian
420 Processes machine learning. *Eur. J Pharm Biopharm.* 2016, *108*, 262-268.
- 421 16. Metwally, A. A.; Hathout, R. M. Computer-Assisted Drug Formulation Design:
422 Novel Approach in Drug Delivery. *Mol Pharm* 2015, *12* (8), 2800-2810.
- 423 17. Metwally, A. A.; El-Ahmady, S. H.; Hathout, R. M. Selecting optimum protein
424 nano-carriers for natural polyphenols using chemoinformatics tools.
425 *Phytomedicine.* 2016, *23* (14), 1764-1770.
- 426 18. Metwally, A. A.; Hathout, R. M. Replacing microemulsion formulations
427 experimental solubility studies with in-silico methods comprising molecular
428 dynamics and docking experiments. *Chemical Engineering Research and Design*
429 2015, *104*, 453-456.
- 430 19. Hathout, R. M.; El-Ahmady, S. H.; Metwally, A. A. Curcumin or
431 bisdemethoxycurcumin for nose-to-brain treatment of Alzheimer disease? A
432 bio/chemo-informatics case study. *Nat. Prod. Res.* 2017, 1-10.
- 433 20. Gad, H. A.; El-Ahmady, S. H.; bou-Shoer, M. I.; Al-Azizi, M. M. Application of
434 chemometrics in authentication of herbal medicines: a review. *Phytochem. Anal.*
435 2013, *24* (1), 1-24.
- 436 21. Ronowicz, J.; Thommes, M.; Kleinebudde, P.; Kryszynski, J. A data mining
437 approach to optimize pellets manufacturing process based on a decision tree
438 algorithm. *Eur. J Pharm Sci* 2015, *73*, 44-48.
- 439 22. Hathout, R. M. Using principal component analysis in studying the transdermal
440 delivery of a lipophilic drug from soft nano-colloidal carriers to develop a
441 quantitative composition effect permeability relationship. *Pharm Dev. Technol.*
442 2014, *19* (5), 598-604.
- 443 23. Singh, S. K.; Verma, P. R.; Razdan, B. Development and characterization of a
444 lovastatin-loaded self-microemulsifying drug delivery system. *Pharm Dev.*
445 *Technol.* 2010, *15* (5), 469-483.
- 446 24. Singh, S. K.; Verma, P. R.; Razdan, B. Glibenclamide-loaded self-nanoemulsifying
447 drug delivery system: development and characterization. *Drug Dev. Ind. Pharm*
448 2010, *36* (8), 933-945.
- 449 25. Martins, S.; Tho, I.; Souto, E.; Ferreira, D.; Brandl, M. Multivariate design for the
450 evaluation of lipid and surfactant composition effect for optimisation of lipid
451 nanoparticles. *Eur. J Pharm Sci* 2012, *45* (5), 613-623.
- 452 26. Malzert-Freon, A.; Hennequin, D.; Rault, S. Partial least squares analysis and
453 mixture design for the study of the influence of composition variables on lipidic
454 nanoparticle characteristics. *J Pharm Sci* 2010, *99* (11), 4603-4615.
- 455 27. Ossama, M.; Hathout, R. M.; Attia, D. A.; Mortada, N. D. Enhanced Allicin
456 Cytotoxicity on HEPG-2 Cells Using Glycyrrhetic Acid Surface-Decorated
457 Gelatin Nanoparticles. *ACS Omega* 2019, *4* (6), 11293-11300.

- 458 28. Elzoghby, A. O.; Samy, W. M.; Elgindy, N. A. Protein-based nanocarriers as
459 promising drug and gene delivery systems. *J Control Release* 2012, *161* (1),
460 38-49.
- 461 29. Elzoghby, A. O. Gelatin-based nanoparticles as drug and gene delivery systems:
462 reviewing three decades of research. *J Control Release* 2013, *172* (3), 1075-1091.
- 463 30. Abozeid, S. M.; Hathout, R. M.; bou-Aisha, K. Silencing of the metastasis-linked
464 gene, AEG-1, using siRNA-loaded cholamine surface-modified gelatin
465 nanoparticles in the breast carcinoma cell line MCF-7. *Colloids Surf. B*
466 *Biointerfaces* 2016, *145*, 607-616.
- 467 31. Hathout, R. M.; Omran, M. K. Gelatin-based particulate systems in ocular drug
468 delivery. *Pharm Dev. Technol.* 2016, *21* (3), 379-386.
- 469 32. Jones, R. J.; Rajabi-Siahboomi, A.; Levina, M.; Perrie, Y.; Mohammed, A. R. The
470 influence of formulation and manufacturing process parameters on the
471 characteristics of lyophilized orally disintegrating tablets. *Pharmaceutics* 2011,
472 *3* (3), 440-457.
- 473 33. Kanth, V. R.; Kajjari, P. B.; Madalageri, P. M.; Ravindra, S.; Manjeshwar, L. S.;
474 Aminabhavi, T. M. Blend Hydrogel Microspheres of Carboxymethyl Chitosan
475 and Gelatin for the Controlled Release of 5-Fluorouracil. *Pharmaceutics* 2017, *9*
476 (2).
- 477 34. Panizzon, G. P.; Bueno, F. G.; Ueda-Nakamura, T.; Nakamura, C. V.; as Filho, B.
478 P. Preparation of Spray-Dried Soy Isoflavone-Loaded Gelatin Microspheres for
479 Enhancement of Dissolution: Formulation, Characterization and in Vitro
480 Evaluation. *Pharmaceutics* 2014, *6* (4), 599-615.
- 481 35. Taguchi, K.; Yamasaki, K.; Seo, H.; Otagiri, M. Potential Use of Biological
482 Proteins for Liver Failure Therapy. *Pharmaceutics* 2015, *7* (3), 255-274.
- 483 36. Xuan, X. Y.; Cheng, Y. L.; Acosta, E. Lecithin-linker microemulsion gelatin gels
484 for extended drug delivery. *Pharmaceutics* 2012, *4* (1), 104-129.
- 485 37. Kharia, A. A.; Singhai, A. K.; Verma, R. Formulation and evaluation of polymeric
486 nanoparticles of an antiviral drug for gastroretention. *Int. J. Pharm. Sci.*
487 *Nanotechnol.* 2012, *4* (4), 1557-1562.
- 488 38. Khatik, R.; Dwivedi, P.; Khare, P.; Kansal, S.; Dube, A.; Mishra, P. R.; Dwivedi,
489 A. K. Development of targeted 1,2-diacyl-sn-glycero-3-phospho-l-serine-coated
490 gelatin nanoparticles loaded with amphotericin B for improved in vitro and in
491 vivo effect in leishmaniasis. *Expert Opin. Drug Deliv.* 2014, *11* (5), 633-646.
- 492 39. Kuntworbe, N.; Al-Kassas, R. Design and in vitro haemolytic evaluation of
493 cryptolepine hydrochloride-loaded gelatine nanoparticles as a novel approach
494 for the treatment of malaria. *AAPS PharmSciTech* 2012, *13* (2), 568-581.
- 495 40. Leo, E.; ngela Vandelli, M.; Cameroni, R.; Forni, F. Doxorubicin-loaded gelatin
496 nanoparticles stabilized by glutaraldehyde: Involvement of the drug in the
497 cross-linking process. *International Journal of Pharmaceutics* 1997, *155* (1),
498 75-82.

- 499 41. Naidu, B. V. K.; Paulson, A. T. A new method for the preparation of gelatin
500 nanoparticles: Encapsulation and drug release characteristics. *J. Appl. Polym.*
501 *Sci.* 2011, *121* (6), 3495-3500.
- 502 42. Saraogi, G. K.; Sharma, B.; Joshi, B.; Gupta, P.; Gupta, U. D.; Jain, N. K.;
503 Agrawal, G. P. Mannosylated gelatin nanoparticles bearing isoniazid for
504 effective management of tuberculosis. *J Drug Target* 2011, *19* (3), 219-227.
- 505 43. Karthikeyan, S.; Rajendra Prasad, N.; Ganamani, A.; Balamurugan, E.
506 Anticancer activity of resveratrol-loaded gelatin nanoparticles on NCI-H460
507 non-small cell lung cancer cells. *Biomedicine & Preventive Nutrition* 2013, *3* (1),
508 64-73.
- 509 44. Metwally, A. A.; El-Ahmady, S. H.; Hathout, R. M. Selecting optimum protein
510 nano-carriers for natural polyphenols using chemoinformatics tools.
511 *Phytomedicine.* 2016, *23* (14), 1764-1770.
- 512 45. Lu, Z.; Yeh, T. K.; Wang, J.; Chen, L.; Lyness, G.; Xin, Y.; Wientjes, M. G.;
513 Bergdall, V.; Couto, G.; varez-Berger, F.; Kosarek, C. E.; Au, J. L. Paclitaxel
514 gelatin nanoparticles for intravesical bladder cancer therapy. *J Urol.* 2011, *185*
515 (4), 1478-1483.
- 516 46. Kumar, R.; Nagarwal, R. C.; Dhanawat, M.; Pandit, J. K. In-vitro and in-vivo
517 study of indomethacin loaded gelatin nanoparticles. *J Biomed. Nanotechnol.*
518 2011, *7* (3), 325-333.
- 519 47. Kumar, R.; Nagarwal, R. C.; Dhanawat, M.; Pandit, J. K. In-vitro and in-vivo
520 study of indomethacin loaded gelatin nanoparticles. *J Biomed. Nanotechnol.*
521 2011, *7* (3), 325-333.
- 522 48. Spjuth, O.; Helmus, T.; Willighagen, E. L.; Kuhn, S.; Eklund, M.; Wagener, J.;
523 Murray-Rust, P.; Steinbeck, C.; Wikberg, J. E. Bioclipse: an open source
524 workbench for chemo- and bioinformatics. *BMC Bioinformatics* 2007, *8*, 59.
- 525 49. Milligan, G. W. An examination of the effect of six types of error perturbation on
526 fifteen clustering algorithms. *Psychometrika* 1980, *45* (3), 325-342.
- 527 50. Pronk, S.; Pall, S.; Schulz, R.; Larsson, P.; Bjelkmar, P.; Apostolov, R.; Shirts, M.
528 R.; Smith, J. C.; Kasson, P. M.; van der, S. D.; Hess, B.; Lindahl, E. GROMACS
529 4.5: a high-throughput and highly parallel open source molecular simulation
530 toolkit. *Bioinformatics* 2013, *29* (7), 845-854.
- 531 51. Vanommeslaeghe, K.; Hatcher, E.; Acharya, C.; Kundu, S.; Zhong, S.; Shim, J.;
532 Darian, E.; Guvench, O.; Lopes, P.; Vorobyov, I.; Mackerell, A. D., Jr.
533 CHARMM general force field: A force field for drug-like molecules compatible
534 with the CHARMM all-atom additive biological force fields. *J Comput Chem.*
535 2010, *31* (4), 671-690.
- 536 52. Abdel-Hafez, S. M.; Hathout, R. M.; Sasmour, O. A. Tracking the transdermal
537 penetration pathways of optimized curcumin-loaded chitosan nanoparticles via
538 confocal laser scanning microscopy. *Int. J Biol Macromol.* 2018, *108*, 753-764.

- 539 53. Spjuth, O.; Helmus, T.; Willighagen, E. L.; Kuhn, S.; Eklund, M.; Wagener, J.;
540 Murray-Rust, P.; Steinbeck, C.; Wikberg, J. E. Bioclipse: an open source
541 workbench for chemo- and bioinformatics. *BMC Bioinformatics* 2007, 8, 59.
- 542 54. Hathout, R. M.; El-Ahmady, S. H.; Metwally, A. A. Curcumin or
543 bisdemethoxycurcumin for nose-to-brain treatment of Alzheimer disease? A
544 bio/chemo-informatics case study. *Nat. Prod. Res.* 2018, 32 (24), 2873-2881.
- 545 55. Gad, H. A.; El-Ahmady, S. H.; bou-Shoer, M. I.; Al-Azizi, M. M. Application of
546 Chemometrics in Authentication of Herbal Medicines: A Review. *Phytochem.*
547 *Anal.* 2012.
- 548 56. Hathout, R. M.; Metwally, A. A. Gelatin Nanoparticles. *Methods Mol Biol* 2019,
549 2000, 71-78.
- 550 57. Hellberg, S.; Sjoström, M.; Skagerberg, B.; Wold, S. Peptide quantitative
551 structure-activity relationships, a multivariate approach. *J Med. Chem.* 1987, 30
552 (7), 1126-1135.
- 553 58. Junaid, M.; Lapins, M.; Eklund, M.; Spjuth, O.; Wikberg, J. E.
554 Proteochemometric modeling of the susceptibility of mutated variants of the
555 HIV-1 virus to reverse transcriptase inhibitors. *PLoS One* 2010, 5 (12), e14353.
- 556 59. Lapins, M.; Wikberg, J. E. Kinome-wide interaction modelling using
557 alignment-based and alignment-independent approaches for kinase description
558 and linear and non-linear data analysis techniques. *BMC Bioinformatics* 2010,
559 11, 339.
- 560 60. Strombergsson, H.; Lapins, M.; Kleywegt, G. J.; Wikberg, J. E. Towards
561 Proteome-Wide Interaction Models Using the Proteochemometrics Approach.
562 *Mol Inform.* 2010, 29 (6-7), 499-508.
- 563 61. Sandberg, M.; Eriksson, L.; Jonsson, J.; Sjoström, M.; Wold, S. New chemical
564 descriptors relevant for the design of biologically active peptides. A multivariate
565 characterization of 87 amino acids. *J Med. Chem.* 1998, 41 (14), 2481-2491.
- 566 62. Maccari, G.; Di, L. M.; Nifosi, R.; Cardarelli, F.; Signore, G.; Boccardi, C.; Bifone,
567 A. Antimicrobial peptides design by evolutionary multiobjective optimization.
568 *PLoS Comput Biol* 2013, 9 (9), e1003212.
- 569 63. Wold, S.; Jonsson, J.; Sjöström, M.; Sandberg, M.; Rönner, S. DNA and
570 peptide sequences and chemical processes multivariately modelled by principal
571 component analysis and partial least-squares projections to latent structures.
572 *Analytica Chimica Acta* 1993, 277 (2), 239-253.
- 573 64. Wang, R.; Lai, L.; Wang, S. Further development and validation of empirical
574 scoring functions for structure-based binding affinity prediction. *J Comput*
575 *Aided Mol Des* 2002, 16 (1), 11-26.
576
577