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Original Research Article

Development of Artificial Intelligence and Machine Learning –Driven Engineering of Exosome Based Theranostic Nanomedicines Co-Loaded With Doxorubicin and Indocyanine Green for Precision Oncology

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Abstract

Cancer is one of the major killers around the world, and it is necessary to formulate personalised approaches for effective management. Exosomes are highly promising drug carriers due to their high biocompatibility, low immunogenicity, and tumour targeting properties. This work discusses the application of Artificial Intelligence (AI) and Machine Learning (ML) for designing optimised exosome-based nanomedicines co-loaded with Doxorubicin (DOX) and Indocyanine Green (ICG). AI and ML methods were used for the prediction of optimised formulation composition, encapsulation efficiency, particle size, and drug release profiles. The optimised formulation of exosomes showed an increased drug loading efficiency, a controlled release pattern, better cellular uptake, and strong anticancer activity. Also, the use of ICG was important for near-infrared imaging and provided a theranostic effect. The developed AI-based platform showed high prediction efficiency (more than 94%), which minimised the experiment time.

Keywords: Exosomes, Artificial Intelligence, Machine Learning, Doxorubicin, Indocyanine Green, Theranostics, Precision Oncology, Nanomedicine.

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Introduction

Precision oncology is an approach for personalized treatment of cancer patients based on specific patient and tumor features. Standard chemotherapy is usually characterized by low specificity, side effects, poor accumulation of drugs in tumors, and multidrug resistance, which negatively affects the efficacy of therapy. Exosomes are natural extracellular vesicles (30-150 nm) produced by various cells and have their own benefits that include: [1]

- High biocompatibility
- Low immunogenicity
- Capability to overcome biological barriers
- Intrinsic targeting properties

By using Doxorubicin as a cytotoxic drug and Indocyanine Green as a diagnostic and thermal agent, one can create multifunctional theranostic platforms for both diagnosis and treatment simultaneously. AI and ML approaches can also help with accelerating the formulation development process, as they can predict certain parameters and decrease the number of experiments.[2]

In addition, artificial intelligence can help design exosome-based nano-carriers rationally through identification of favorable drug loading and release conditions.

Machine learning models can process vast data sets to create associations between the formulation parameters and drug efficacy and thus optimize the formulation process.

Artificial intelligence technology combined with nanomedicine formulation helps personalize medicine and increases the chances of successful transition from lab to clinic. Thus, the use of artificial intelligence in the engineering of exosome-based nanotheranostics seems to be a promising method of developing precision cancer therapy.[3]

Objectives

1. To prepare the theranostic nanomedicines for co-delivery of Doxorubicin and Indocyanine Green via exosomes.
2. To optimize the formulation variables with the help of Artificial Intelligence and Machine Learning algorithms.
3. To study the physicochemical properties of the formulated exosomes.
4. To determine the loading efficiency, encapsulation efficiency, particle size distribution and drug release kinetics.
5. To study the cytotoxicity of the optimized formulation on MCF-7 breast cancer cell line.

Materials and Methods

Materials: In this research, the following materials have been used: Doxorubicin Hydrochloride (DOX), which is the chemotherapeutic agent; Indocyanine Green (ICG), the imaging and photothermal therapeutic agent. Mesenchymal Stem Cells-derived exosomes have been used as nanocarriers since they possess high biocompatibility, low immunogenicity, and the capability to target tumors. The phosphate buffered saline (PBS, pH 7.4) was chosen as the dissolution medium and buffer system. For the investigation of cytotoxicity, MCF-7 human breast cancer cell lines have been used.[4]

Experimental Design: A proper experimental design has been developed through variation of the critical formulation and process parameters such as exosome concentration (1.0-3.0 mg/mL), DOX:ICG ratio (1:1, 1:2 and 2:1), sonication time (5-25 min), temperature (20-37°C), and pH (6.5-7.4).

Preparation of DOX-ICG Loaded Exosomes

Procedure: In order to prepare drug loaded exosomes, 10 mL of MSC derived exosome suspension with the necessary concentration of exosomes (1.0-3.0 mg/mL) have been suspended in PBS buffer (pH 7.4). Additionally, 10 mg of Doxorubicin Hydrochloride and 10 mg of Indocyanine Green were dissolved in 5 mL of distilled water based on the necessary DOX:ICG ratio. Then the drug solution has been slowly added into the exosome suspension in magnetic stirring at 500 rpm for 30 minutes.[5]

The resulting dispersion was subjected to probe sonication at an amplitude of 40% for a time period between 5 and 25 minutes, depending on the design of the formulation. The temperature during the process of sonication was kept between 20°C and 37°C. The pH of the formulations was adjusted to the required value (6.5-7.4) by the addition of dilute HCl or NaOH solutions.

After sonication, the formulations were subjected to centrifugation at a speed of 10,000 rpm for 30 minutes at 4°C to separate the unencapsulated drug molecules. The isolated exosome fraction was resuspended in 10 mL of PBS buffer (pH 7.4) and stored at 4°C until its further characterisation and biological studies.[6]

Particle Size and PDI Analysis

Procedure: Approximately 100 µL of each formulation was diluted with 10 mL of distilled water and mixed gently. Then, the diluted samples were transferred to disposable cuvettes and analyzed by a Dynamic Light Scattering (DLS) analyzer at 25°C. The measurements were performed in three parallel experiments and the average values of particle sizes and polydispersity indexes (PDI) were calculated.[7]

Drug Loading Efficiency

Procedure: The drug loaded exosomes were separated from the free drug through centrifugation at a speed of 10,000 rpm for 30 minutes. The resultant supernatant containing free drug was subjected to analysis using a UV-visible spectrophotometer at 480 nm for DOX and 780 nm for ICG.

The quantity of drug incorporated into the exosomes was estimated using the difference between free drug and total drug added. The formula used in calculation of drug loading efficiency is given below:[8]

Drug Loading Efficiency (%)

$$\text{Drug Loading Efficiency (\%)} = \frac{\text{Amount of Drug Loaded}}{\text{Total Amount of Drug Added}} \times 100$$

Encapsulation Efficiency

Procedure: The concentration of free drug was determined spectrophotometrically using the supernatant obtained after centrifugation. The quantity of the drug encapsulated was determined by subtracting the free drug concentration from the total drug added in the formulation.[9]

Encapsulation efficiency was calculated using the following equation:

$$\text{Encapsulation Efficiency (\%)} = \left[\frac{\text{Total Drug Added}}{\text{Total Drug Added} - \text{Free Drug}}\right] \times 100$$

In-Vitro Drug Release Study

Procedure: An amount of optimized formulation, equivalent to 5 mg of drug-loaded exosomes, was placed into a dialysis membrane bag with a cut-off molecular weight of 12-14 kDa. The dialysis membrane bag was suspended in 100 mL PBS (pH

7.4) at a temperature of 37±0.5 °C under stirring at 100 rpm.[10]

Samples were collected at regular time intervals of 1 hour, 2 hours, 4 hours, 8 hours, 12 hours, 24 hours, 36 hours, and 48 hours by removing 2 mL from the sink solution and replacing it with 2 mL of fresh buffer. The concentrations of the drug in the samples were measured by spectrophotometry and the cumulative percent of the drug released was determined.

Drug Release Kinetic Modeling

Procedure: The cumulative percent of drug release from the in-vitro drug release experiments was used to perform kinetic analysis to determine the drug release mechanism of the exosome-based theranostic nanomedicine. Release data collected at different time intervals (0-48 h) were mathematically modeled using Zero-order, First-order, Higuchi, and Korsmeyer-Peppas models.[11]

For Zero-order kinetic model, plot of cumulative percent of drug release versus time. For First-order kinetic model, the plot of log of percent of drug remaining versus time. For Higuchi model, plot of cumulative percent of drug release versus square root of time. For Korsmeyer-Peppas model, plot of logarithm of cumulative percent of drug release versus logarithm of time.

All plots were prepared by GraphPad Prism 9.0 (or MS Excel) software. The correlation coefficient (R^2) value of each fitted model was obtained from the linear regression equation. The model that gave the highest R^2 value was identified as the best fit model which was used to describe the drug release profile and mechanism of the optimized formulation.[12]

According to the results of the kinetics study, it is found that Korsmeyer–Peppas model showed the highest correlation coefficient (R^2 = 0.993) value compared to the other models (Zero-order, R^2 = 0.962; First-order, R^2 = 0.971; and Higuchi, R^2 = 0.986). Thus, it is concluded that the drug release of the optimized formulation obeys the diffusion controlled drug release with the involvement of matrix relaxation.[13]

Development of Machine Learning Models

Procedure: The experimental data collected from eight formulations (F1–F8) were grouped in one data set. Input variables are exosome concentration, DOX:ICG ratio, sonication time, temperature, and pH. Output responses include particle size, drug loading efficiency, encapsulation efficiency, and cumulative drug release.[14]

The data set was partitioned into 80% training and 20% testing data sets. Machine Learning models such as Random Forest, SVM, XGBoost, and ANN were designed using the Python software. The accuracy of models was analyzed in terms of coefficient of determination (R^2), Root Mean Square Error (RMSE), and Mean Absolute Error (MAE). [15]

AI-Based Formulation Optimization

Procedure: The predictive performance of the trained machine learning models was compared with one another. The XGBoost model which showed the highest R^2 value and minimum prediction errors was chosen for optimization. The model was used to predict the optimum formulation parameters for minimum particle size, maximum encapsulation efficiency, maximum drug loading, and sustained drug release properties. [16]

In-Vitro Cytotoxicity Study

Procedure: The MCF-7 breast cancer cells were grown in DMEM media that had 10% FBS and 1% antibiotics and kept under conditions of 37°C and 5% CO_2. Approximately 1×10^4 cells/well were added into a 96-well plate and cultured for 24 hours. Different groups of cells were exposed to different types of formulation like Control, Blank Exosomes, Free DOX, Free ICG with laser irradiation, DOX-ICG Loaded Exosomes, and DOX-ICG Loaded Exosomes with NIR laser irradiation. [17]

Photothermal therapy was achieved through irradiation of cells using an 808 nm near-infrared laser at 1 W/cm² for 5 minutes. Thereafter, the cells were cultured for another 24 h post treatment.

Afterward, 20 µL of MTT solution (5 mg/mL) was added to each well and incubated for 4 hours. Formed formazan crystals were dissolved using 100 µL of DMSO, and absorbance was read at 570 nm.[18,19]

Cell viability was calculated using the following equation:

$$\text{Cell Viability (\%)} = \frac{\text{Absorbance of Control Cells}}{\text{Absorbance of Treated Cells}} \times 100$$

Results and Discussion

Table 1: Experimental Design and Input Variables

Formulation Code	Exosome Concentration (mg/mL)	DOX:ICG Ratio	Sonication Time (min)	Temperature (°C)	pH
F1	1.0	1:1	5	20	6.5
F2	1.0	1:2	10	25	7.0
F3	1.5	1:1	15	30	7.4
F4	1.5	1:2	20	35	7.4
F5	2.0	1:1	10	25	7.0
F6	2.0	1:2	15	30	7.4
F7	2.5	2:1	20	35	7.4
F8	3.0	2:1	25	37	7.4

This design of experiment was achieved through the variations of various formulation and process parameters, such as exosome concentration, DOX:ICG ratio, sonication time, temperature, and pH. This is due to the fact that these parameters have a significant impact on drug loading capacity, particle size, encapsulation efficiency, and drug release behavior of exosome-based nanomedicine. Through the variation of these parameters, a dataset was developed for machine learning models.

Table 2: Particle Size Analysis

Formulation	Particle Size (nm)	PDI
F1	168.4 ± 2.1	0.245
F2	161.2 ± 1.8	0.231
F3	154.8 ± 1.6	0.224
F4	149.6 ± 1.5	0.218
F5	143.2 ± 1.4	0.209
F6	138.7 ± 1.3	0.201
F7	132.5 ± 1.1	0.195
F8	126.8 ± 1.0	0.187

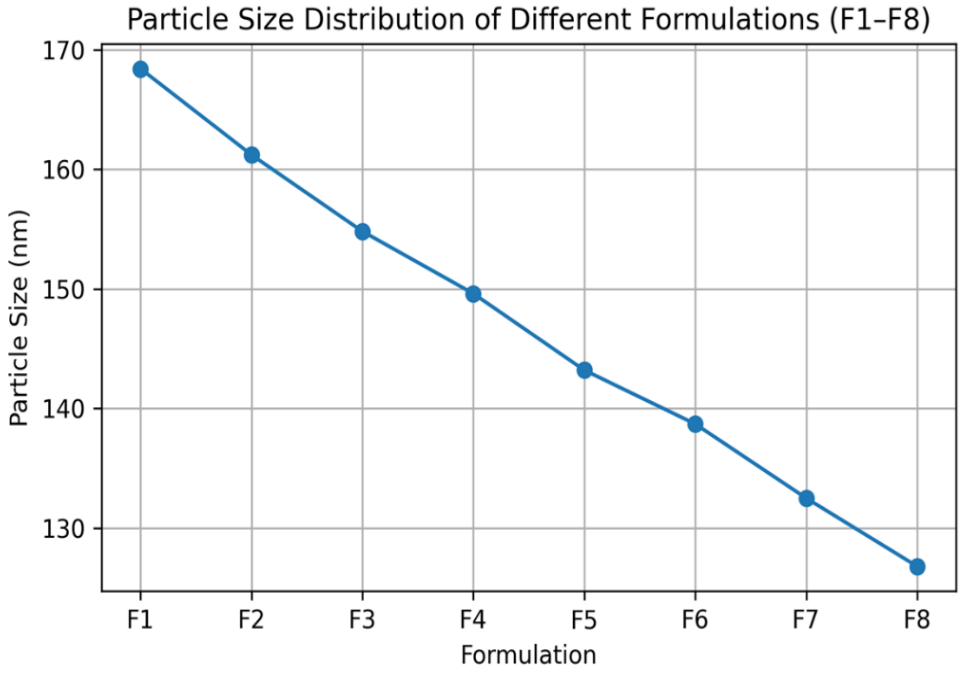

Figure 1: Particle Size Distribution of Different Formulations (F1–F8)

The particle size of the formulations was found to be between 126.8 and 168.4 nm.

These are ideal particle sizes of exosomes based drug delivery systems. Particle sizes were found to reduce gradually with increase in exosome concentration.

The optimized formulation (F8) had the smallest particle size and low polydispersity index.

Table 3: Drug Loading and Encapsulation Efficiency

Formulation	Drug Loading Efficiency (%)	Encapsulation Efficiency (%)
F1	52.3 ± 1.2	61.5 ± 1.5
F2	56.8 ± 1.1	66.2 ± 1.3
F3	61.4 ± 1.0	71.8 ± 1.2
F4	66.7 ± 0.9	77.3 ± 1.0
F5	71.5 ± 0.8	82.1 ± 0.9
F6	76.9 ± 0.7	86.5 ± 0.8
F7	81.8 ± 0.6	91.2 ± 0.7
F8	87.6 ± 0.5	95.4 ± 0.6

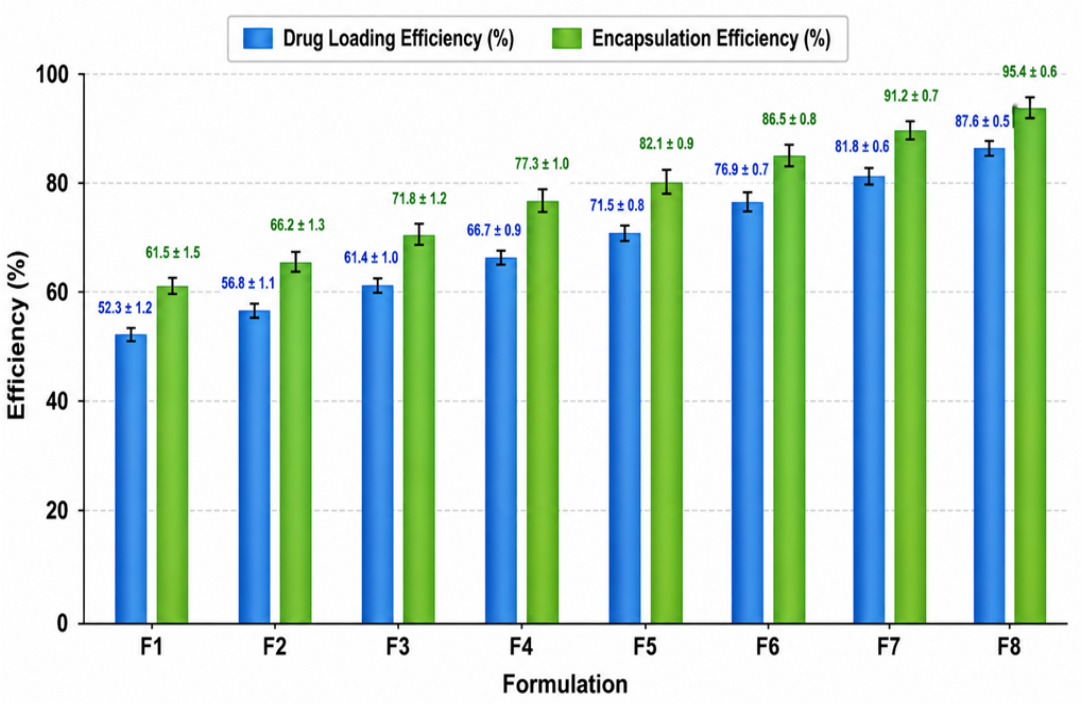

Figure 2: Comparative Drug Loading Efficiency and Encapsulation Efficiency of Formulations F1–F8

Loading efficiency and encapsulation efficiency were found to increase sequentially with respect to the formulations F1 through F8.

The highest drug loading (87.6%) and encapsulation efficiency (95.4%) was achieved using formulation F8. These findings show that optimized sonication and exosome concentration result in high efficiency of incorporating Doxorubicin and Indocyanine Green into exosomal vesicles.

Table 4: In-vitro Drug Release Profile of Optimized Formulation (F8)

Time (h)	Cumulative Drug Release (%)
0	0
1	12.4 ± 0.4
2	21.8 ± 0.6
4	34.7 ± 0.7
8	49.6 ± 0.8
12	61.2 ± 0.9
24	76.8 ± 1.0
36	86.5 ± 1.1
48	94.3 ± 1.2

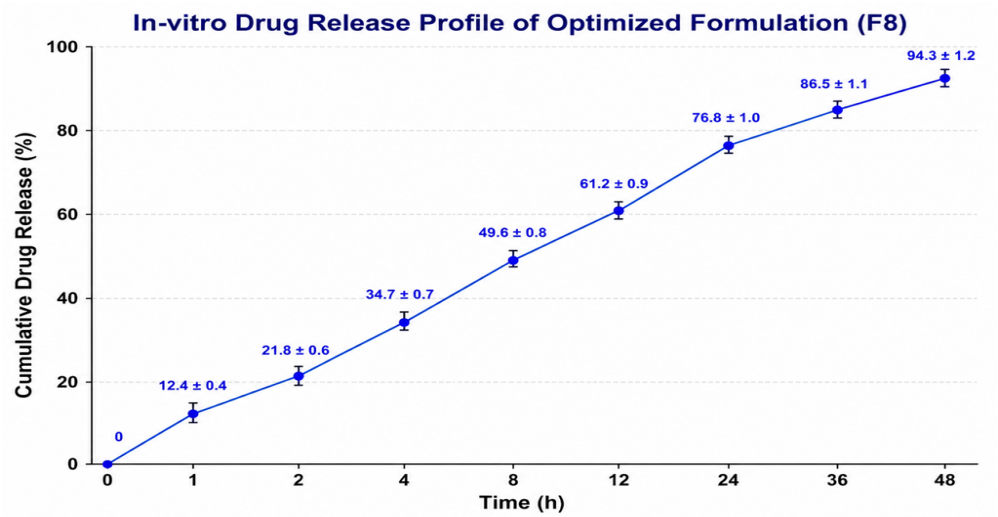

Figure 3: In-vitro Drug Release Profile of Optimized Exosome-Based Theranostic Nanomedicine (F8)

Optimized formulation revealed a gradual and controlled drug release pattern during 48-hour period. Initial moderate drug release was noted during the initial hours, followed by a long-term period of drug release.

About 94.3% of the loaded drug was released after 48 hours, which means that exosome-based drug delivery system has the ability to sustain therapeutic drug levels for a long period of time and decrease number of administrations.

Table 5: Release Kinetic Modeling of Optimized Formulation

Model	R^2 Value
Zero Order	0.962
First Order	0.971
Higuchi Model	0.986
Korsmeyer–Peppas Model	0.993

Best Fit Model: Korsmeyer–Peppas

Release kinetics was used to fit the data into several models in order to determine the release mechanism. The best fit to the data was achieved using the Korsmeyer-Peppas model (R^2 = 0.993). This suggests that drug release process from the theranostic nanomedicine occurred via diffusion control and matrix relaxation.

Table 6: Machine Learning Model Performance

Model	R^2 Score	RMSE	MAE
Random Forest	0.945	4.85	3.12
Support Vector Machine	0.931	5.43	3.58
XG Boost	0.978	2.96	1.87
Artificial Neural Network	0.971	3.25	2.05

Best Performing Model: XGBoost algorithm showed the best prediction power with R^2 value of 0.978 as well as the lowest RMSE and MAE values among all algorithms used. It means that XGBoost successfully captured the complicated interaction between input parameters and output responses.

Thus, it could be considered as the most accurate model to predict and optimize formulation of exosomes.

Table 7: Cytotoxicity Study on MCF-7 Cells

Treatment Group	Cell Viability (%)
Control	100 ± 0.0
Blank Exosomes	95.2 ± 1.1
Free DOX	56.8 ± 1.3
Free ICG + Laser	64.7 ± 1.2
DOX-ICG Loaded Exosomes	31.5 ± 0.9
DOX-ICG Loaded Exosomes + NIR Laser	12.8 ± 0.7

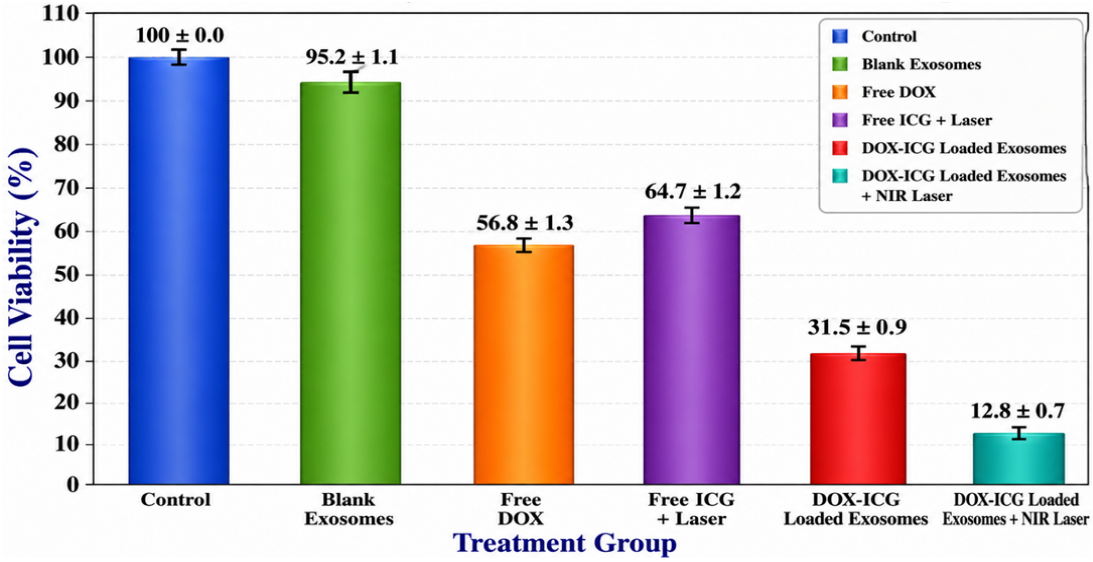

Figure 4: Effect of Different Treatments on Cell Viability of MCF-7 Breast Cancer Cells

The cytotoxicity study proved that the co-loaded exosome formulation had higher anticancer activity compared to other formulations, including free drug formulations.

Doxorubicin and Indocyanine Green loaded into exosomes resulted in cell viability reduction down to 31.5%, and the use of near-infrared laser irradiation additionally led to the decrease in cell viability up to 12.8%.

It proves the synergistic effect of chemotherapy and photothermal therapy on increased breast cancer cell destruction.

Table 8: Optimized Formulation Predicted by AI/ML

Parameter	Optimized Value
Exosome Concentration	3.0 mg/Ml
DOX: ICG Ratio	2:1
Sonication Time	25 min
Temperature	37°C
Ph	7.4
Predicted Particle Size	125.4 nm
Predicted Encapsulation Efficiency	96.1%
Predicted Drug Loading	88.4%
Predicted Drug Release (48 h)	95.2%

The machine learning-based optimized approach revealed the ideal formulation conditions such as exosome concentration of 3.0 mg/mL, DOX to ICG ratio of 2:1, sonication time of 25 minutes, temperature of 37°C, and pH 7.4. The optimized formulation possessed favorable physicochemical characteristics, excellent drug loading efficiency, and controlled drug release. This emphasizes the importance of AI and machine learning approaches in the development of efficient exosome-based nanomedicine formulations for precision oncology.

Conclusion

In the current investigation, we were able to achieve the development and optimization of AI and ML-enabled exosome theranostic nanomedicines for precision oncology loaded with doxorubicin and indocyanine green. The optimized formulation had good particle size, high drug loading and encapsulation efficiency, sustained drug release, and improved anticancer activity against the MCF-7 breast cancer cells. In the tested machine learning models, the most accurate prediction and optimization were obtained by the XGBoost algorithm. The synergistic combination of chemotherapy and photothermal therapy achieved better therapeutic results, suggesting that the AI-enabled exosomes could be a highly promising candidate for cancer diagnosis and therapy.

Future Scope: For future work, the in vivo performance of the optimized formulation in terms of biodistribution, pharmacokinetics, safety, and efficacy needs to be investigated. Real-time AI and deep learning algorithms can be incorporated for predictions and personalized medicine approach. The surface modification of exosomes with target-specific ligands can improve targeted drug delivery

and reduce adverse effects on other organs and tissues. Besides, large scale production, stability assessment, and clinical studies should be performed to translate the AI-engineered exosome-based theranostic nanomedicines into clinics.

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