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A new evidence-based design-of-experiments approach for optimizing drug delivery systems with exemplification by emulsion-derived Vancomycin-loaded PLGA capsules

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This paper introduces an evidence-based, design-of-experiments (DoE) approach to analyze and optimize drug delivery systems, ensuring that release aligns with the therapeutic window of the medication. First, the effective factors and release data of the system are extracted from the literature and meta-analytically undergo regression modeling. Then, the interaction and correlation of the factors to each other and the release amount are quantitatively assessed. Finally, the factors are numerically and graphically optimized via linking the meta-analyzed release data and the well-documented therapeutic window of the drug, followed by verification. For a more in-depth explanation, the introduced approach is exemplified by a drug delivery, consisting of emulsion-derived poly lactic-co-glycolic acid-vancomycin (PLGA-VAN) capsules for treating *Staphylococcus Aureus*-induced osteomyelitis. Novel and validated findings for the model system, along with the thorough architecture of the introduced approach, suggest its potential applicability for any delivery systems with sufficient reliable data in the literature.

Keywords Local controlled drug delivery systems, Burst and sustained release kinetics, Pharmacokinetics

The target of local drug delivery is to infuse a minimal level of the drug directly into the target tissue for a specific duration. The performance of these delivery systems is dependent on several parameters, such as the type, release level, release rate, and release duration of the loaded drug[1,2]. Achieving the maximum efficacy and safety of the systems necessitates the thorough optimization of their independent factors. Typically, without proper optimization, issues like inadequate delivery, diminished treatment efficacy, and potential adverse effects become inevitable[3,4].

In pursuit of optimizing drug delivery systems, the most elementary and traditional option that comes to mind is the trial-and-error method. Nevertheless, this is a costly and time-consuming approach, with outputs strongly dependent on the formulator's expertise. In most cases, this method involves maintaining one or several factors at constant values, a practice that does not necessarily lead to optimal outcomes. Alternatively, design-of-experiments (DoE)-based approaches are effective options to lower the number of experimental tests and to realize the interactions and contribution of factors. These mathematical-based approaches have been frequently used in the field of delivery, for instance, for drugs[5–7], growth factors[8–10], and genes[11–13]. While the application of these approaches offers insights into the efficient optimization and design of delivery systems, they still necessitate several experiments beforehand a thorough analysis of the system's behaviors.

The most efficient scenario seems to be the development of methods that can offer reliable outcomes without the necessity for conducting new experiments. This idea brings to mind the well-established concepts of evidence-based analysis and meta-analysis. Evidence-based mathematical analysis is of great attractiveness in evidence-based medicine. Typically, it was meta-analytically pointed out that the initial burst release of small-molecule drugs loaded in nanogels is not significant in pharmacotherapy[14]. Also, Shin et al.[15]reviewed meta-

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analytic approaches introduced by various authors to eradicate intracellular infections. Another meta-analysis study evaluated the significance of polymeric nanoparticles in the bioavailability of oral drugs[16]. Walkey et al.[17] also used a meta-analytic approach for the treatment of nosocomial pneumonia caused by Methicillin-resistant *Staphylococcus aureus (S. aureus)* using two antibiotic groups, linezolids and glycopeptides. Additionally, Chen et al.[18] reviewed, systematically and meta-analytically, the efficacy of antibiotics integrated inside total joint bone cements for eradicating periprosthetic infections. Through this type of analysis, researchers could easily evaluate the results of prior experiments, requiring only a review of the target system to characterize the factors contributing to the release kinetics. However, meta-analysis alone is not capable of optimization. Our hypothesis in this work is, for the first time, the meta-analytic utilization of valid data published in the literature on any specified delivery system as the input of DoE, called the evidence-based DoE optimization approach.

To provide a comprehensive understanding of the approach and to assess the validity and novelty of its outcomes, antibacterial poly lactide-co-glycolide-vancomycin (PLGA-VAN) capsules are optimized by this approach in this paper. The PLGA-VAN system is essentially utilized to treat *S. aureus*-caused osteomyelitis. VAN, as a glycopeptide antibiotic drug, is commonly used as a prototype drug in clinics to treat severe infections caused by *S. aureus*[19,20]. Management of osteomyelitis with local drug delivery systems benefits from appropriate therapeutic antibiotic levels beyond the minimum inhibitory concentration (MIC) or minimum bactericidal concentration (MBC) at the infected site without systemic toxicity[21,22]. A wide range of biomaterials has been utilized to carry VAN for optimal release kinetics. In this regard, biodegradable polymeric carriers exhibiting a controlled release of antibiotics can be a promising choice. Among them, PLGA is the most significant polyester utilized as a local carrier of VAN to treat osteomyelitis[23–25], mainly due to its considerable entrapment capacity and controlled biodegradability. PLGA-VAN platforms in the form of hollow particles are further used due to their flexibility in performance and application in comparison to other forms. The most common method used to fabricate these formulations is double emulsion followed by solvent evaporation[26–28], owing to its high encapsulation efficacy, narrow size distribution, and small particle size.

To the authors' knowledge, no meta-analysis or DoE optimization has been published on the PLGA-VAN system, although there are a decent number of research papers on this system. Accordingly, this study exemplifies the introduced evidence-based DoE optimization approach on this illustrative emulsion-derived system, in terms of optimizing the molecular weight (MW) of PLGA, its lactic acid to glycolic acid (LA/GA) molar ratio, the polymer-to-drug mass ratio (P/D), and the particle size.

Methodology

The approach introduced in this work to optimize any delivery system is schematically illustrated in Fig. 1. After conducting a brief systematic review of the system for an understanding of its behavior, the historical data of release should be extracted, evaluated for the interaction and correlation of factors, and then regression-fitted as a critical step of meta-analysis. Also, the success status of the system in treating the target disease should be determined through relevant biological tests, yielding information about the therapeutic window of the medication. The outputs of these steps would be then linked for the DoE optimization of the system, followed by verification to ensure the reliability of the results.

Strategy for identifying eligible datasets

To facilitate the understanding of the optimization approach introduced in this paper, binary PLGA-VAN carrier-drug capsules produced by the emulsion method were considered as a model drug delivery system. Using proper combinations of two or more indexes from "PLGA, poly lactic-co-glycolic acid, osteomyelitis, vancomycin, antibiotics, drug delivery, burst release, sustained release, *Staphylococcus Aureus (or S. Aureus)*, and bone infection", 624 research papers were identified from Scopus and Google Scholar. After a meticulous assessment of the articles' title, abstract, and conclusion, 36 papers were recognized to be within the scope of osteomyelitis eradication using binary PLGA-vancomycin platforms. Among these studies, 17 contained actionable data for a meta-analytic assessment. These data included production procedures, PLGA characteristics such as MW and LA/GA, P/D, structure and size, drug loading method and capacity, encapsulation efficiency, cumulative release curves, and in some cases, drug concentration.

Data extraction

The historical release data of the system in the literature, in terms of the values of independent factors (MW, LA/GA, P/D, and particle size) vs. released drug concentration, were extracted utilizing the GetData graph digitizer software. It was followed by normalization to have cumulative release percentages for all extracted data sets. Also, a hypothetical concentration of 500 µg/ml for PLGA-VAN particles was considered across all studies, since the majority of published data on the system's in vitro antibacterial behavior has been conducted at this specific concentration.

Interaction and correlation analyses

The extracted data were input into the Design-Expert experimental design and optimization software. Generally, factors are considered interactive when the influence of one on a dependent variable (response) depends on the magnitude of the others. In other words, interaction refers to the simultaneous influence of the examined factors on the response. Graphical representation, for instance through scatter plots or line graphs, enables a visual examination of the relationship between two specific factors at various levels. If the lines representing the various levels of these factors intersect or diverge, it signifies the presence of interaction between the factors. Correlation is also a statistical measure that indicates the extent to which two factors change together, but it does not imply causation. Indeed, the term "correlation" is used to show synergistic, summative, or antagonistic effects and the dependency of the factors on each other. This characteristic is built in accordance with the degrees of freedom

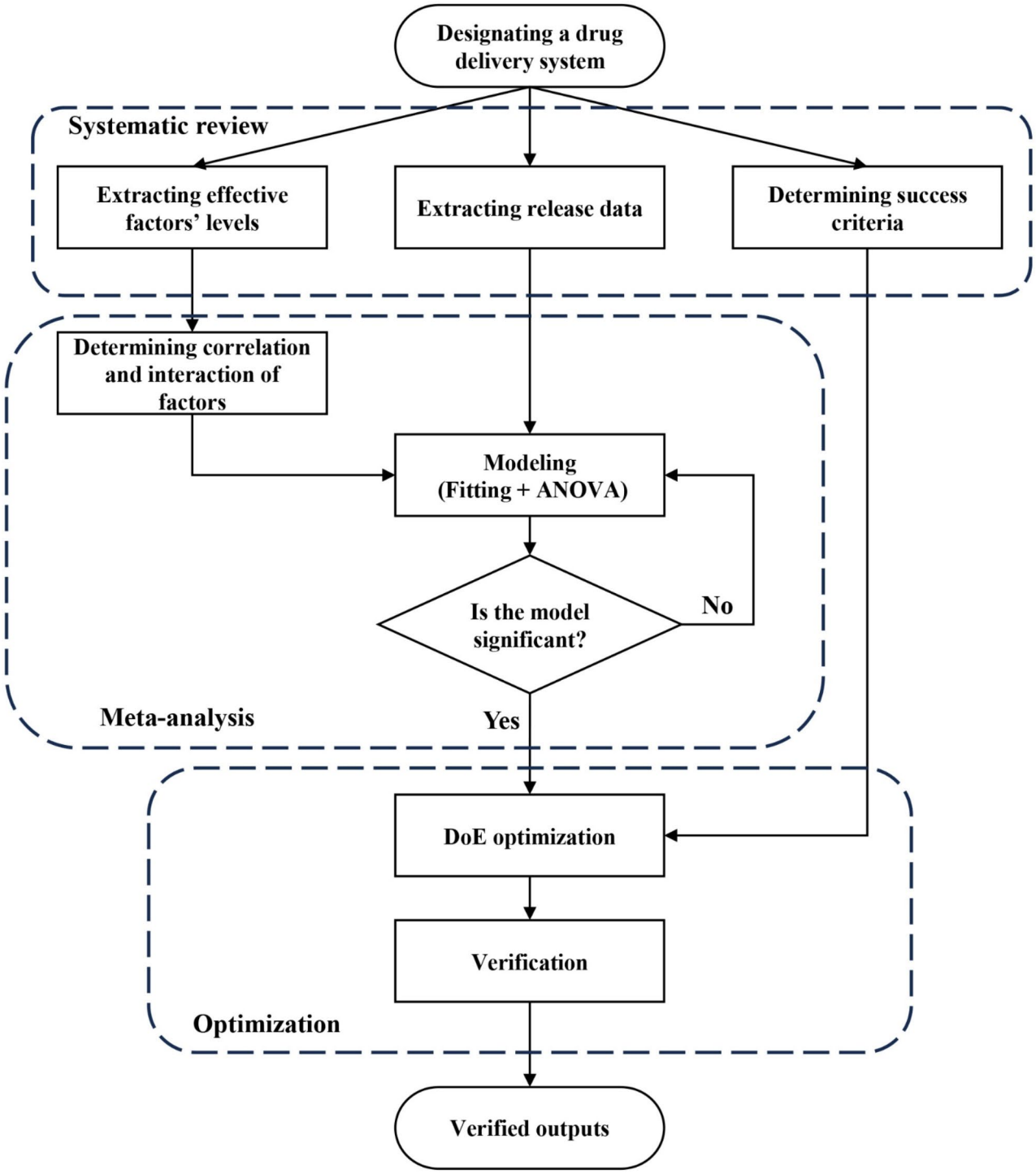

Fig. 1. Flowchart of the evidence-based DoE optimization approach introduced in this study.

for each factor using the Pearson correlation coefficient (*r*) (Eq. 1), where the freedom of factors is hindered if they are correlative:

$$r = \frac{\sum (x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum (x_i - \bar{x})^2 \sum (y_i - \bar{y})^2}} \quad (1)$$

where x_i and y_i are the values of the two variables which are being evaluated against each other, and $\bar{x}$ and $\bar{y}$ are the mean values of these variables to make *r*. The correlation coefficient ranges from -1 to $+1$; -1 for total

antagonism, 0 for no correlation, and + 1 for complete synergy. Overall, these statistical assessments give a basic view on the significance of each factor in governing the performance of the system, as well as examining the design space to provide a suitable distribution of design points.

Regression modeling

Based on the experimental design and optimization software's suggestions, various regression models were tested to determine the best fit of the extracted data. Subsequently, the analysis of variance (ANOVA) was done to assess the significance of the model and the factors using p- and F-values. Essentially, in the case of an improper model, ANOVA suggests more suitable regression-fitting models in conjugation with other analyses like R^2 and lack-of-fit. The lack-of-fit values of ANOVA acknowledge whether the system is accurately fitted or not, which puts these results up with R^2 for the decency assessment of fitting. It is desirable for the lack-of-fit p- and F-values to be insignificant. The significance of each factor in the drug release kinetics was also examined using the p-value (referring to the presence probability of unacceptable responses with extreme values) and F-value (indicating the significance of differences between the factors).

Optimization criteria assignment

To effectively prevent osteomyelitis using the PLGA-VAN system, both initial burst and long-term sustained medication release stages must be successful. An effective initial burst release is characterized by the prevention of biofilm formation during the critical time window of one day[29,30]. An initial burst release of the drug was in vitro considered effective when the concentration of the bactericidal agent surpasses MIC, or when it prevents bacterial growth in inhibition zone tests. However, the high limit of release must be opted on MBC to control the amount of the drug released, preventing its toxicity. Another approach to assessing the antibacterial success of the system was through in vivo evaluations. In this scenario, if any signs or symptoms of infection or toxicity were observed, the bactericidal effectiveness of the PLGA-VAN system was compromised. To achieve success in the sustained release phase, the drug concentration must be maintained between the MIC and the toxicity limit for at least 28 days[31,32]. Despite the fact that the antibacterial behavior is the principal mission of the aforementioned capsules, high doses of initial burst release, high total drug dosage, and low tolerance against the drug can reduce the biocompatibility of the system. Thus, the in vitro cell viability above 90% was regarded as the optimal minimum to determine whether the capsules are cytocompatible or not.

Based on the literature, the therapeutic concentration of VAN should be above 2 µg/ml (MIC) for the inhibition of strain growth and 8 µg/ml (MBC) for killing strains[32,33]. Also, the drug concentration should not exceed the cytocompatibility limit of 20 µg/ml for prolonged release[34]. Accordingly, the VAN release concentrations of 2–8 µg/ml for initial burst release and 2–20 µg/ml for long-term sustained release were established as the optimization criteria in this illustrative work.

Optimization practice

As the significant novelty of the approach introduced in this work, numerical and graphical optimizations were carried out by linking the meta-analyzed release data with the well-established therapeutic window of the medication regarded as the optimization criteria. From an experimental design perspective, optimization involves treating all historical data as distinct experiments, with responses that have been analyzed prior to the optimization process. This approach enhances comprehensibility and expedites the optimization process. For this purpose, the extracted historical data of initial burst release on the first day and prolonged release on the 28th day were employed as the optimization parameters of the PLGA-VAN system. In this paper, they are called initial burst release yield (BRY) and cumulative total release yield (TRY), respectively. The influential factors (LA/GA, P/D, MW, and size) were first treated as numerics and gathered based on the information present in the literature. Then, to simulate the commercially-available LA/GA values, discrete levels of 50:50, 70:30, 75:25, 85:15, and 90:10 were considered for optimization.

The desirability (D) analysis was also conducted to indicate whether a set of factors is optimal and if so, to what degree they are optimal (zero being the lowest and one being the most), as calculated by Eq. 2:

$$D = \left(\prod {}^{n}_{i=1} d_i\right)^{1/n} \tag{2}$$

where d_i are the responses of each data set, and n is the number of the sets.

Results and discussion

Extracted data and design space

The extracted data of BRY and TRY for the model PLGA-VAN system with the levels of their constituting variables are tabulated in Table 1, serving as the optimization design space.

Interaction and correlation of factors

To evaluate the interaction of the factors impacting the release concentration, interaction plots were drawn on the basis of the difference of the responses for various sets of the factors. Typically, Fig. 2 represents the interaction graphs of LA/GA and P/D at different MWs for BRY and TRY within the PLGA-VAN system. It is observed that by increasing MW, the difference of the drug release concentrations at various LA/GAs and P/Ds becomes less notable for both BRY and TRY. For instance, at P/D = 8, the difference in BRY for the LA contents of 90 and 50% is 271.5, 129.67, 44.14, and 5.05 µg/ml for the MWs of 20, 30, 51, and 136 kDa, respectively. That is, by increasing MWs, the impact of LA/GA and P/D on the release kinetics is less significant. During the double emulsion method used to fabricate the PLGA-VAN capsules, the drug is first dissolved in a water medium

System	Factors				Responses		Ref.
	LA in PLGA (%)	P/D	MW (kDa)	Size* (nm)	BRY (μg/ml)	TRY (μg/ml)	
1	50	1	20	200	3.6	13.9	[27]
2	50	2	20	260	6.0	23.3	
3	50	3	20	330	4.9	19.2	
4	50	1	20	180	4.2	16.5	
5	50	2	20	210	7.1	27.8	
6	50	3	20	290	5.1	20.0	
7	50	1	20	240	21.5	47.9	[36]
8	70	1.5	26	5,000	21.4	47.6	[37]
9	70	3	26	5,000	17.3	53.9	
10	75	2	30	67,500	8.9	34.4	[38]
11	75	4	30	64,300	10.7	49.6	
12	75	8	30	61,600	11.7	61.4	
13	75	2	51	79,000	9.7	23.1	[26]
14	75	4	51	61,000	14.7	35.0	
15	75	8	51	70,000	24.3	69.5	
16	75	5	136	77,000	3.5	14.3	[39]
17	85	0.1	100	260	7.9	18.7	[40]
18	85	0.1	100	5,200	8.3	30.6	[41]
19	90	1.5	26	5,000	87.3	168.9	[37]
20	90	3	26	5,000	60.2	145.3	

Table 1. Historical data of the PLGA-VAN system, used in the design space of this work. *The size values have been rounded for uniformity in presentation.

and then encapsulated within the polymeric matrix. Higher MW polymers form more complex and denser networks, which slow down water diffusivity into the polymeric matrix. This slower diffusion impacts the drug encapsulation process, leading to a predominant accumulation of VAN molecules in the surface layers of the capsules rather than within the interior[41,42]. As a result, the impact of the other factors (LA/GA and P/D), which are more related to the bulk characteristics of the system, becomes less significant in determining the release profile. Moreover, at higher LA/GAs, the effect of P/D on the response is more pronounced. This is because the increase of LA/GA increases the hydrophobicity of PLGA, reduces the water-solubility and distribution of VAN in PLGA, and promotes the encapsulation of the drug within water-filled pores at the center of the capsules, due to the inherent hydrophilic of VAN[39,43]. Therefore, the amounts of both the further hydrophobized polymer and the loaded drug, as indicated by P/D, play a more critical role in determining the success status of the setting, given the decreased degradability of PLGA at higher LA/GAs.

In good agreement with the interaction analysis outcomes, the correlation assay on the PLGA-VAN system suggests that the increase of the LA content is proportional to the rise of P/D, with a correlation value of +0.872. This implies that when LA/GA increases, the impact of P/D on the release kinetics becomes more noteworthy. The LA content is also negatively correlated with MW with a correlation value of −0.976. This indicates that when one of them increases, the influence of the other one in governing the release kinetics becomes less significant. The corresponding correlation value of MW to P/D is −0.879, signifying an antagonistic effect on the release kinetics. These findings underscore the high interrelations among the factors, so that their contributions to the release behavior differ at their different values.

Modeling formulation

The choice of the best-fitting model relies on the significance of ANOVA and fitting parameters. According to ANOVA, the linear model of an inverse square root transform for both BRY and TRY in the PLGA-VAN system is significant, based on F- and p-values (Table 2). Small lack-of-fit values are other indicatives of proper fitting for BRY and TRY models. It is also found that the size of these emulsion-derived PLGA-VAN capsules, which ranges from a few hundred nanometers to tens of micrometers (Table 1), is less significant in the initial burst and sustained release models in comparison to the other factors, based on the p-value (>0.05) and F-value (<4) of this factor. Thus, to avoid unnecessary complications, the size was not included in Table 2 and following calculations are after this refinement in modeling. It is also observed that MW and LA/GA are significant, whereas P/D is less significant due to its high p-value (0.8187 for BRY and 0.1516 for TRY, which are above the level of significance with p-value = 0.05), and low F-value (0.05 for BRY and 2.27 for TRY, which are above the level of significance with F-value = 4.00). These findings were concluded by the interaction and correlation analyses above from different perspectives.

Using the fitting results of the proper models for BRY and TRY (Eqs. 3 and 4) with $R^2 = 0.6261$ and 0.7109, respectively, fitting contours were plotted in terms of color-coded surfaces that link calculated BRY and TRY with the impacting factors (Fig. 3). It is observed that by increasing MW, the influence of P/D on release becomes less significant, which is in agreement with the results obtained from the correlation and interaction evaluation

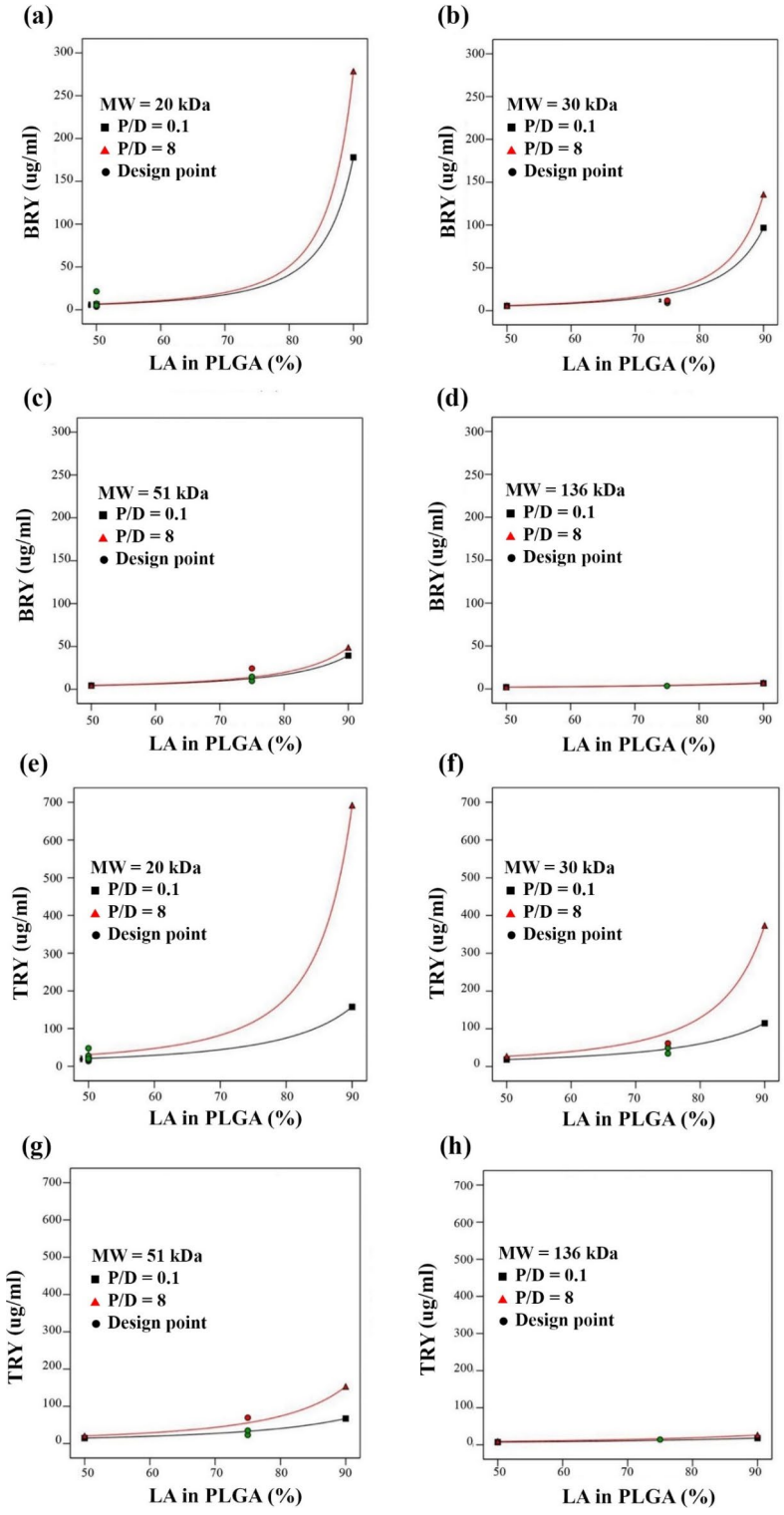

Fig. 2. Demonstrative interaction plots of LA/GA and P/D for BRY (**a**-**d**) and TRY (**e**-**h**) at MW of 20 (**a**, **e**), 30 (**b**, **f**), 51 (**c**, **g**), and 136 (**d**, **h**) kDa in the PLGA-VAN system.

Terms	BRY		TRY	
	F-value	*p*-value	F-value	*p*-value
Model	11.61	0.0003	16.57	< 0.0001
LA content	31.04	< 0.0001	37.52	< 0.0001
MW	17.50	0.0007	27.65	< 0.0001
P/D	0.05	0.8187	2.27	0.1516
Lack of fit	0.25	0.9730	0.20	0.9873

Table 2. ANOVA results for the linear model of BRY and TRY within the PLGA-VAN system.

of the factors. Moreover, lower LA/GA results in faster release, as understood from green-blue colors in the contours.

$$\frac{1}{\sqrt{BRY}} = -0.007603 \times (LA\,\%\,in\,PLGA) - 0.001860 \times \left(\frac{P}{D}\right) + 0.002536 \times (MW) + 0.002536 \quad (3)$$

$$\frac{1}{\sqrt{SRY}} = -0.003342 \times (LA\,\%\,in\,PLGA) - 0.004806 \times \left(\frac{P}{D}\right) + 0.001275 \times (MW) + 0.367056 \quad (4)$$

The direct relationship of MW with BRY and TRY is inferred from the dominance of intense red-colored regions in the contours. As discussed in the interaction analysis, this is due to the enhanced accumulation of VAN molecules in the surface layer of the spheres caused by increasing MW, which heightens BRY. The similar susceptibility of TRY to MW suggests the domination of initial burst release in the total release process. This is supported by the fact that the sustained release, occurring after the release of surface-accumulated VAN molecules, is retarded by increasing MW due to the reduced degradability of PLGA[41,42].

Optimization

Numerical representation of optimization

The meta-analyzed data were linked to the defined optimization criteria of the model system to determine successful LA/GA, P/D, and MW settings. Due to the correlated nature of the factors, there are infinite sets of the factors that serve as optimal solutions. However, the numerical approach may not represent all optimal conditions of the system due to the limitations of the software. In the case of infinite results, Design-Expert essentially presents a maximum of 100 solutions, whereas preferred outcomes are a continuous range of the factors. Despite this issue, some valuable conclusions can be extracted from the numerical representation of optimization.

Typically, about 88% of the optimal solutions are composed of LA/GA of 50:50. In contrast, only 2% of the solutions are related to 70:30 and 7% to 75:25. The rest is correlative to 85:15 and 90:10. The mean optimal P/D is 4.03 for LA/GA of 50:50, whereas it is approximately 2.43 for the other LA/GAs. This confirms the correlative and interactive nature of the PLGA-VAN system. Despite P/D being deemed less significant according to ANOVA, its effect cannot be neglected. At higher LA/GAs, an increase in the VAN content may lead to a drastic increase in the release rate. Apparently, at higher levels of LA/GA, higher MWs serve as optimal. This is because higher LA/GAs lead to more efficient drug encapsulation and thus enhanced sustained release. In such cases, higher MWs are needed to facilitate effective initial burst release against bacteria attacks via the predominant surface accumulation of the drug. Additionally, since water diffusion is more challenging in this scenario, sustainability is achieved through higher encapsulation efficiencies.

Graphical representation of optimization

Figure 4 depicts the surface plots of desirability for several demonstrative MWs of the illustrative system. It appears that at low MWs, lower LA/GAs are optimal. By increasing MW, higher P/D and LA/GA become optimal. Interestingly, at the highest MW, LA/GA of 50:50 and P/Ds less than 3 become undesirable because both high MW and GA contents contribute to higher initial burst release rates that may exceed the safe limits. Additionally, it indicates that most of the desirable solutions occur at higher MWs, lower LA/GA, and lower P/Ds. These findings confirm the highly correlated and interactive nature of the factors of the PLGA-VAN system, in good agreement with the numerical optimization results.

Graphical frameworks are another representation style of optimization, for instance, at selected MWs, as shown in Fig. 5a-d. In these graphs which were drawn based on the modeling (Sect. 3.3) and optimization (Sect. 2.5, 2.6, and 3.4.1) criteria, the values of optimal P/D and LA/GA settings across multiple MWs are shown by yellow-colored areas. As can be observed in Fig. 5a, at low MWs, a small window of values is optimal, where the increase of MW expands this window. Interestingly, at MW of 136 kDa, there is a small area of values that is not satisfactory (Fig. 5d). Moreover, at a particular MW, optimal conditions occur either at higher LA/GAs and lower P/Ds or lower LA/GAs and higher P/Ds. Therefore, if both factors are increased, achieving a proper BRY would be challenging. In other words, when one factor is increased, the other one should be decreased to maintain all the predefined optimization criteria.

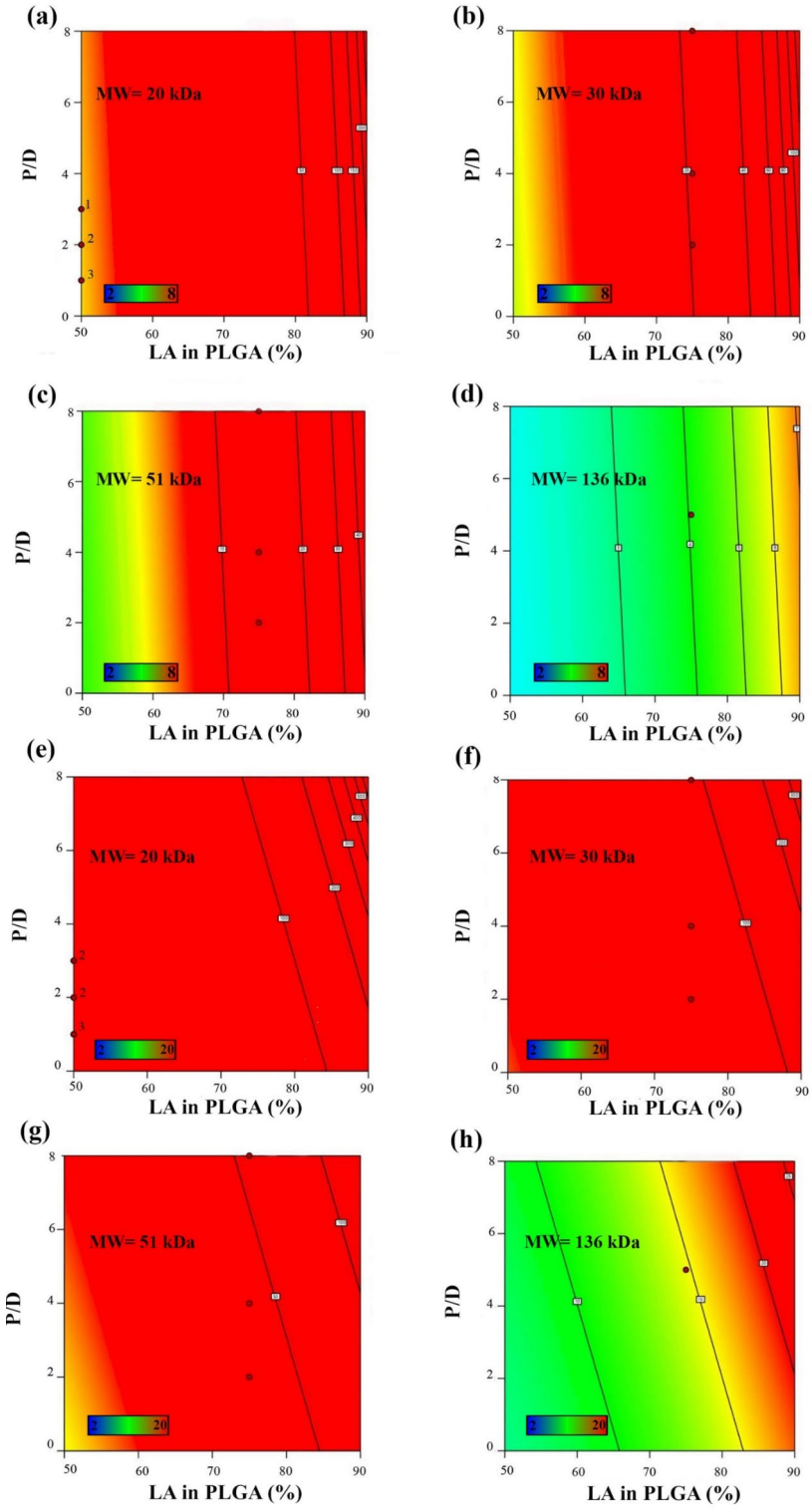

Fig. 3. Demonstrative contours of P/D vs. LA in PLGA (LA/GA) for BRY (**a**-**d**) and TRY (**e**-**h**) of the model system at MW of 20 (**a**, **e**), 30 (**b**, **f**), 51 (**c**, **g**), and 136 (**d**, **h**) kDa. λ is used to show the design points and color-coded terms are in μg/ml.

Optimization verification

For optimization verification, several experimental successful and unsuccessful settings were checked with the optimization frameworks, where these settings had not been used to establish the frameworks. A new model was established using 19 of the 20 settings listed in Table 1 and that one remaining system was put aside for verification. Then, graphical optimization contours like Fig. 5 were plotted. If that aside-put system is successful

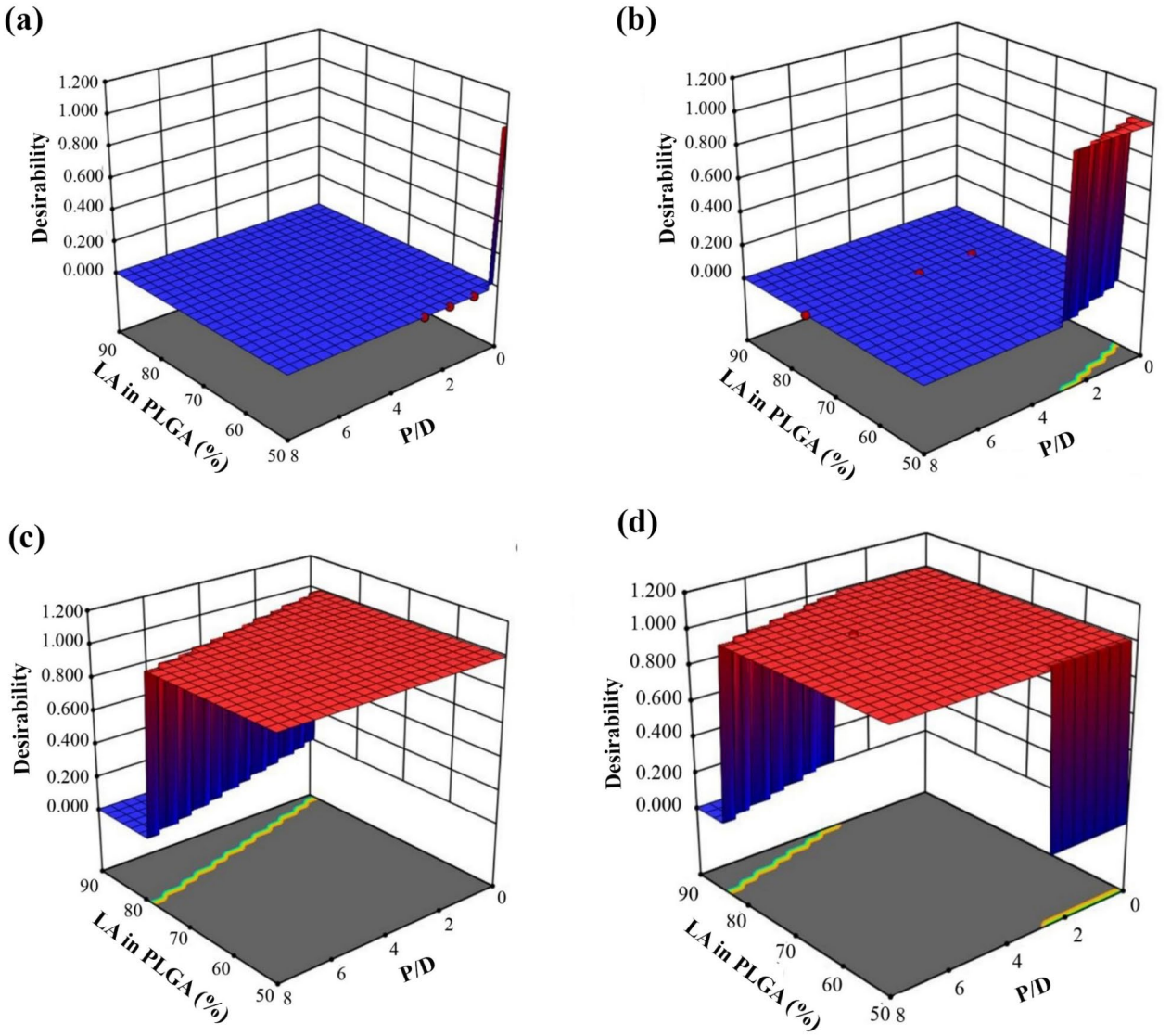

Fig. 4. Demonstrative surfaces of the model desirability at MW of 20 (**a**), 51 (**b**), 120 (**c**), and 136 (**d**) kDa for the illustrative PLGA-VAN system. λ is used to show the design points.

and is placed inside the yellow parts of the contour, or it is unsuccessful and is located outside these areas, the model is verified. This cycle was repeated 20 times to check the comprehensive validity of the model for predicting all of the settings. Table 3 summarizes the experimental results of the antibacterial and cytocompatibility assays of the settings, where the setting numbers in this table correspond to those of Table 1. Comparing the last two columns of Table 3 clearly suggests that all of the successful and unsuccessful settings verify the validity of the optimization approach used for the illustrative system.

Conclusions

This paper introduced a novel approach that originally combines meta-analysis with DoE to optimize drug delivery systems, providing accurate and reliable results without the need for additional experiments. The approach essentially relies on linking the meta-analyzed release data with the well-documented therapeutic window of the drug. For a better understanding, the introduced optimization approach was implemented on an illustrative delivery system, emulsion-derived PLGA-VAN capsules with PLGA's MW, LA/GA, P/D, and size as independent factors. According to this evidence-based study, the particle size of the emulsion-derived capsules, ranging from a few hundred nanometers to tens of micrometers, had a less significant impact on the drug release kinetics compared to the other factors. Thus, the size was not further considered in the meta-analysis and optimization to avoid unnecessary complications. It was found that increasing MW reduced the impact of LA/GA and P/D on the drug release kinetics, along with higher initial burst and total release yields. These consequences were associated with the increased tendency of VAN molecules to accumulate in the surface layers of the capsules with MW increase during the drug loading process (double emulsion). The significance of P/D

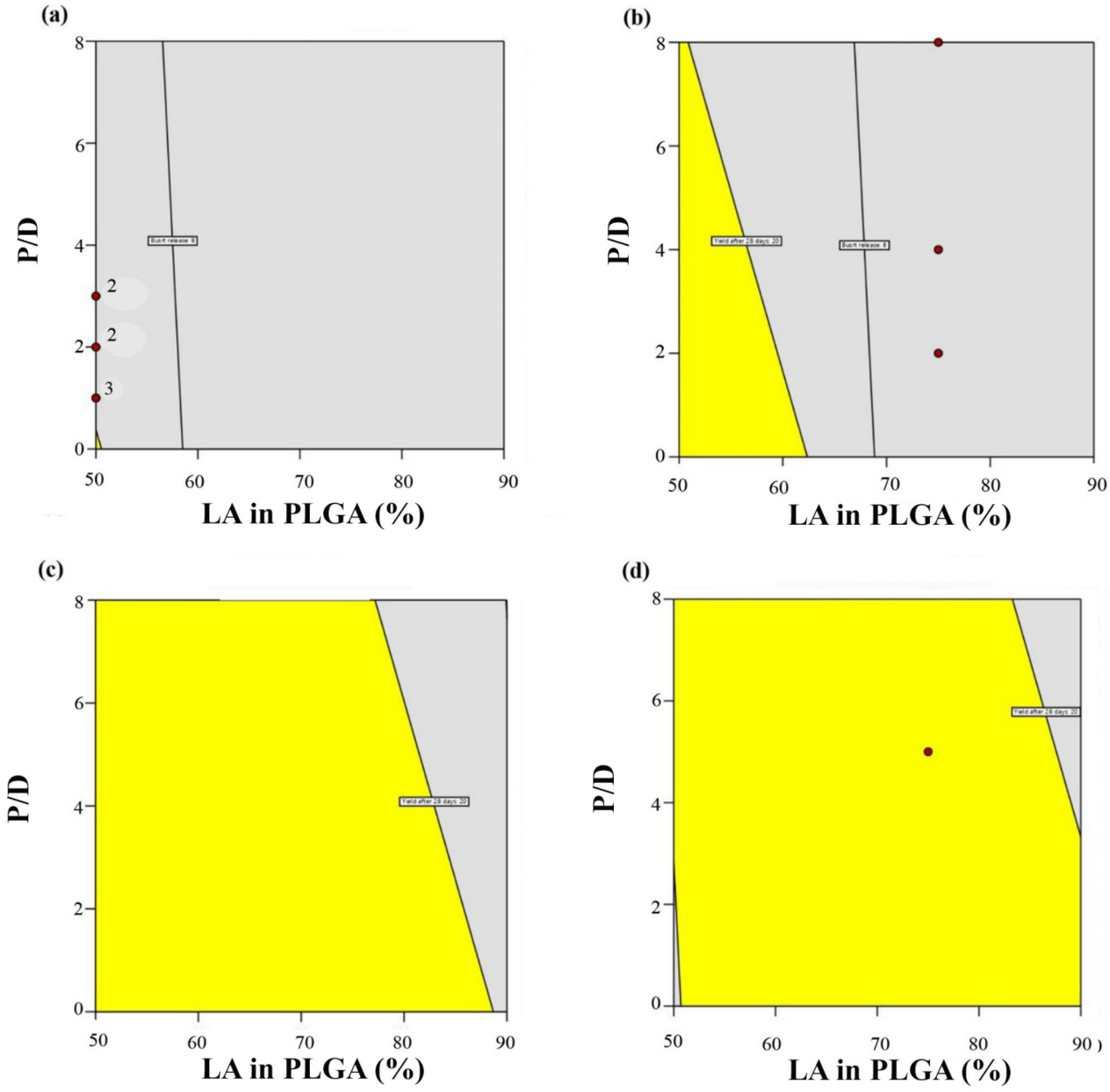

Fig. 5. Demonstrative frameworks of the optimal settings at MW of 20 (**a**), 51 (**b**), 120 (**c**), and 136 (**d**) kDa for the PLGA-VAN system. λ is used to show the design points.

in the release was also concluded to be strongly dependent on the level of the other factors, MW and LA/GA. Typically, at high LA/GAs and any predetermined MWs, P/D significantly impacted the release behavior, so that its lower amounts were favorable for a successful release to treat osteomyelitis. Overall, most of the successful settings for treating *S. aureus*-caused osteomyelitis tended to have low MW, low LA/GA, and moderate P/D or high MW, high LA/GA, and low P/D, among the commercially-available LA/GA of 50:50, 70:30, 75:25, 85:15, and 90:10. These new conclusions drawn for this illustrative drug delivery system suggest the potential applicability of the introduced analysis and optimization approach for any drug delivery systems that are supported by a sufficient level of reliable data in the literature. Although this optimization approach for the PLGA-VAN model system was based on well-established data from the literature and meta-analyses, it remains adaptable and can readily incorporate new wet lab data—whether generated through traditional experiments, DoE techniques, or a combination of these resources. Additionally, as the method is validated for optimal performance, its outcomes can confidently support future studies while significantly reducing the need for extensive experimental work.

System	Antibacterial examination method	Results of antibacterial examination	Assignment of antibacterial success	Cytotoxicity assay method	Results of cytocompatibility assay	Assignment of cytocompatibility success	Experimental success status	Success status predicted by the model
1	CFU counting (CFU/ml)	Day 1: 0 Day 7: 43.69 Day 14: 933.25 Day 21: 2.84×10^5 Day 28: 6.65×10^8	Successful	MTT assay (MG-63 cells)	88–107%	Successful	Yes	Yes
2							Yes	Yes
3							Yes	Yes
4							Yes	Yes
5							Yes	Yes
6							Yes	Yes
7	MIC consideration (μg/ml)	BRY: 21.49 TRY: 47.94	Successful	Concentration consideration (μg/ml)	BRY: 21.49 TRY: 47.94	Unsuccessful (above 20 μg/ml)	No	No
8	MIC consideration (μg/ml)	BRY: 21.43 TRY: 47.58	Successful	Concentration consideration (μg/ml)	BRY: 21.49 TRY: 47.94	Unsuccessful (above 20 μg/ml)	No	No
9		BRY: 17.32 TRY: 34.41	Successful		BRY: 17.32 TRY: 34.41	Unsuccessful (above 20 μg/ml)	No	No
10	In vivo examination & CFU counting (CFU/g tissue)	Day 30: 1.02×10^3	Successful	In vivo examination & histology	Inflammatory for all of the cases	Unsuccessful	No	No
11							No	No
12							No	No
13	MIC consideration (μg/ml)	BRY: 9.73 TRY: 23.07	Successful	Concentration consideration (μg/ml)	BRY: 9.73 TRY: 23.07	Unsuccessful (above 20 μg/ml)	No	No
14		BRY: 14.68 TRY: 34.99	Successful		BRY: 14.68 TRY: 34.99	Unsuccessful (above 20 μg/ml)	No	No
15		BRY: 24.32 TRY: 69.49	Successful		BRY: 24.32 TRY: 69.49	Unsuccessful (above 20 μg/ml)	No	No
16	In vivo examination	Two burst phases separated by a lag in between. The total release was depicted to be effective.	Successful	In vivo examination	No toxicity along the test duration (6 weeks)	Successful	Yes	Yes
17	Zone of inhibition after 18 h (mm)	29.10	Successful (above the standard of 27–28 mm)	MTT assay (MG-63 cells)	Day 1: 10.22% Day 2: 23.21%	Successful	Yes	Yes
18	Zone of inhibition after 18 h (mm)	19.40	Unsuccessful (below the standard of 27–28 mm)	MTT assay (MG-63 cells)	Day 1: 21.79% Day 6: 45.57%	Successful	No	No
19	MIC consideration (μg/ml)	BRY: 87.25 TRY: 168.89	Successful	Concentration consideration (μg/ml)	BRY: 87.25 TRY: 168.89	Unsuccessful (above 20 μg/ml)	No	No
20		BRY: 60.22 TRY: 145.26	Successful		BRY: 60.22 TRY: 145.26	Unsuccessful (above 20 μg/ml)	No	No

Table 3. Experimental and predicted success status of the PLGA-VAN system, where CFU refers to colony forming units.

Data availability

All data generated or analyzed during this study are included in this published article.

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Declarations

Competing interests

The authors declare no competing interests.

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