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RESEARCH ARTICLE

DEVELOPMENT, CHARACTERIZATION, AND OPTIMIZATION OF REPAGLINIDE LOADED SPANLASTICS ALONG WITH INVESTIGATION OF DRUG SOLUBILITY IN VARIOUS MEDIA

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Abstract

Objective: This study seeks to explore the solubility of an antidiabetic drug with poor water solubility, Repaglinide (RPG), in various media, and develop and optimize RPG-loaded spanlastic formulations.
Methods: A 72-hours solubility study was conducted for RPG in different media, followed by UV spectrum analysis. Spanlastics containing RPG were prepared through the thin-film hydration (TFH) method combined with ultrasonication, incorporating varying concentrations ofthe lipophilic, non-ionic surfactant agents, Span 60 and Tween 80. Characterization was performed To assess particle size (PS), polydispersity index (PDI), zeta potential (ZP), and entrapment efficiency (EE%). Optimization was achieved by analysing characterization results and using a mixture design of Design Expert software which predicted the optimized formula.
Results: RPG exhibited the highest solubility in phosphate buffer saline (PBS, pH 6.8) incorporating 0.5% Tween 20 (168.595 µg/mL), followed by PBS with 0.1% Tween 80 (80.355 µg/mL), PBS alone (56.163 µg/mL), and PBS with 0.1% sodium lauryl sulphate (SLS) (53.706 µg/mL). UV scanning in methanol revealed three characteristic absorption bands for RPG, with peak selection optimized for molar absorptivity in each medium. The RPG-loaded spanlastic formulations demonstrated nano-sized vesicles with uniform size distribution, high stability, and efficient drug encapsulation. Optimized spanlastics predicted a particle size 126.162 nm, PDI 0.416, ZP-43.258 mV, and EE% 77.753%.
Conclusions: This research emphasizes the potential of optimized RPG-loaded spanlastics, developed through the thin-film hydration method, as a promising approach for improving the solubility and stability of poorly water-soluble drugs. RPG showed the highest solubility in PBS (pH 6.8) containing 0.5% Tween 20, and the formulation achieved desirable nanosize, uniformity, and high encapsulation efficiency.
Keywords: Optimization, physicochemical characterization, repaglinide, solubility enhancement, spanlastics, thin-film hydration technique.

INTRODUCTION

Repaglinide, an FDA-approved anti-diabetic medication since 1998, lowers fasting BGL by stimulating insulin secretion from pancreatic beta cells. It is characterized by its unique structure, binding properties, and excretion profile. Despite significant first-pass metabolism reducing its oral bioavailability to 56%, it extensively binds to plasma proteins (>98%) and has a short half-life of about 1 hour. While it carries a risk of hypoglycemia, this risk is generally lower than that of sulfonylureas[1]. Repaglinide undergoes extensive liver metabolism and is mainly excreted via bile, with its metabolites lacking significant hypoglycemic effects. Following a single oral dose, approximately 90% is excreted in feces, and 8% is eliminated through urine[2].

Repaglinide, a BCS Class II which has low aqueous solubility and also high permeability, with ampholytic properties, allowing pH-dependent interactions in aqueous solutions[3]. Repaglinide (RPG) is rapidly absorbed due to its lipophilicity but has low and inconsistent bioavailability (~50%) due to poor water solubility (34 µg/ml), variable dissolution in gastro-intestinal fluids, hepatic first-pass metabolism, and P-glycoprotein affinity[4]. Repaglinide, an insulin secretagogue, stimulates insulin release through the interaction with ATP-sensitive potassium channels on pancreatic beta cells. Due to its similar mechanism to

sulfonylureas, combining the two treatments is contraindicated[5].

Kakkar and Kaur developed spanlastic systems, innovative nano vesicular drug carriers using non-ionic surfactants. Designed for ocular and dermal delivery of ketoconazole, these systems incorporated Span 60, a lipophilic surfactant, along with hydrophilic edge activators to enhance flexibility by creating pores and destabilizing lipid membranes[6]. Spanlastics, structurally similar to liposomes, resemble trans-ferosomes in their exceptional elasticity and flexibility, making them highly adaptable and efficient for various applications[7]. Spanlastics are versatile vesicular carriers designed for targeted drug delivery, with applications in ocular, oral, topical, nasal, otopical, and transungual therapies[8].

Spanlastics consist of two key components: a non-ionic surfactant and an edge activator[9]. Vesicles made from non-ionic surfactants like Span 80 and Span 40 tend to be more prone to disruption, aggregation, and instability, whereas those derived from Span 60, with its lipophilic properties, form stable lamellar matrix vesicles, ensuring greater stability[10,11]. The saturated alkyl chains in Span 60 provide a lipophilic character, Facilitating the formation of unilamellar or multilamellar vesicles with a matrix structure. Its surface activity boosts the effect of the edge activator, lowering interfacial tension and promoting the formation of stable, small Spanlastic dispersions, leading to a more sustainable vesicular formulation[12]. Edge activators, with high HLB values, reduce interfacial tension, increasing the flexibility of bilayer vesicle membranes[13]. Ethanol enhances nano-vesicular carriers by improving drug entrapment, reducing membrane thickness, and forming smaller vesicles. It also adjusts the Overall charge of the system to a Zeta potential with a negative value, providing stability due to steric effects and preventing aggregation, thereby improving the vesicles' performance in drug delivery[14].

MATERIALS AND METHODS

Repaglinide gift samples from Egyptian International Pharmaceutical Industrial Company (EIPICO),Tween® 80 (polyoxyethylene (20) sorbitan monooleate), acquired from Sigma Chemical Co. (St. Louis, USA), Sorbitan monostearate Span 60, obtained from Sigma Chemical Co. (St. Louis, USA), 0.45 µm membrane filter, sourced from Millipore Iberica S.A.U., Madrid (Spain), Sodium hydroxide, potassium dihydrogen orthophosphate, methanol, ethanol provided by El-Nasr Pharm. Chem. Company, Cairo (Egypt), sodium lauryl sulphate obtained from BASF (Germany) and all other chemicals were of analytical grade .

Solubility study of RPG

A solubility study was performed as described in a previous study with some modifications to determine the solubility of pure Repaglinide in different media after 72 hours[15]. Solubility is studied in (PBS pH 6.8, PBS pH 6.8 containing 0.5% Tween 20, PBS pH 6.8 containing 0.1% Tween 80 and PBS at pH 6.8 with 0.1% sodium lauryl sulfate) to mimic physiological conditions in small intestine and based on previous release experiments[15-18].

Weighed excess of RPG (25 mg) was added to the vials containing the candidate media (50 mL each) to create saturated solutions. The vials were sealed and shaked for 24 hours at 37±0.5ºC/100 rpm using water bath shaker. The vials were maintained at a controlled temperature 37±0.5 ºC/100 rpm using a Shaking water bath to ensure maximum solubility. The overall experiment was conducted for 72 hours. After the incubation period, the solutions were doubled filtered using a Grade 1 filter paper then syringe filter (0.45 µm) to remove undissolved particles. The collected filtrates were measured, the spectrum of the drug in each solvent was recorded and the highest absorbance peak was selected then the concentration of the dissolved drug was determined at the selected λ_{max} using UV-Vis Spectrophotometer (Shimadzu, the model UV- 1800 PC, Kyoto, Japan). After selection of the media with highest dissolved Repaglinide concentration, a calibration curve was constructed measuring absorbance versus concentration at maximum wavelength (λ_{max}) and the solubility was calculated in term of µg/mL.

UV Scanning of RPG

Before each measurement of RPG in these media a UV spectrum of the drug was recorded to ensure accurate, reliable and reproducible results by determining $\lambda_{max,}$ verifying drug identity, assess purity and determining solvent compatibility.

Preparation of Repaglinide loaded spanlastics

Repaglinide-loaded spanlastics were formulated using the thin-film hydration (TFH) method[19] with some modifications. In short, Span® 60 was dissolved in 10 mL of ethanol within a 250 mL round-bottom flask and then evaporated under vacuum using a rotary evaporator (Heidolph WB 2000) at 60±0.5ºC and 90 rpm. This process resulted in the formation of a thin, dry film, which was subsequently hydrated through the addition of the hydration solution comprising 10 mL of phosphate-buffered saline (PBS) at pH 7.4 + 2 ml Ethanol + Tween 80 + Repaglinide 5 mg) at 60±0.5 ºC and 150 rpm for 15 minutes then Magnetic stirring (Wise-stir hot plate stirrer, Model MSH-20D, from Korea) for another 15 minutes with the same previous conditions to create the spanlastic dispersion.

The dispersion was then further sonicated using Ultrasonic processor (probe sonicator, GE130, probe CV18, USA) for 2 minutes (amp 70%, pulse 10/10) to reduce the particle size. The formulations were then centrifuged using (Stratos centrifuge, maximum 23300 rpm, Germany) at 20,000 RPM, 4°C for 2 hours. The supernatant was used for measuring the EE% while the pellets were rehydrated with distilled water, stirred by the mean of magnetic stirrer (1000 RPM, 60±0.5ºC For 10 minutes), followed by probe sonication for 2 minutes (amp 70%, pulse 10/10) to disrupt the aggregation of the pellets that resulted from the cooling centrifugation then measured for PS, PDI and ZP.

Determination of particle size and polydispersity index (PDI)

The mean particle size was determined using DLS at 25°C, with light scattering detected at 90° using a 633

nm helium-neon laser. Samples, diluted 200x with Milli-Q water, were analyzed via photon correlation spectroscopy and the cumulants method, assuming spherical particles. Results included the effective diameter (Z-Ave) and polydispersity index (PDI), reflecting size distribution and uniformity[20].

Determination of Zeta potential

The zeta potential of RPG-loaded spanlastics was measured using a calibrated Zetasizer (Malvern, UK) at 25±2°C. Samples were diluted 200 times dilution with Milli-Q water (18.2 MΩ·cm) after calibrating the instrument to 50 mS/cm using a 0.9% (w/v) sodium chloride solution. This process ensured accurate readings and followed an established procedure[21].

Measurement of entrapment efficiency

Samples of Repaglinide loaded spanlastics placed in Eppendorf tubes and centrifuged at 20000 rpm for 2 hours at 4°C. The supernatant was then separated each time from nanovesicles pellets and passed through a Millipore® membrane with a pore size of 0.220 µm subsequently, it was prepared for the free drug assay. Each result represents the mean of three measurements (± SD). The drug content was quantified using UV spectroscopy against a phosphate buffer saline mixed with an appropriate quantity of methanol as blank[22].

The % entrapped of Repaglinide was calculated by the following equation:

$$(\mathbf{EE\%}) = \frac{\textit{Total drug amount} - \textit{free unentrapped drug}}{\textit{Total drug amount}} \times \mathbf{100}$$

Statistical analysis

The measurements were conducted at least three times, and the resulting data were expressed as mean ±standard deviation (SD). To analyse the data, a one-way analysis of variance (ANOVA) was employed using Microsoft Excel 365 software and IBM SPSS Statistics 27. A *p*-value of less than 0.05 was deemed to indicate a statistically significant difference among the groups.

RESULTS AND DISCUSSION

Solubility study of RPG

RPG has been shown to have very low solubility in distilled water, with a value of (31.15±1.15) µg/ml, as reported in a previous study[23]. However, when dissolved in a phosphate buffer saline with a pH of 6.8, its solubility moderately increased to (56.163±0.337) µg/ml. This increase in solubility highlights RPG's dependence on pH due to its ampholytic properties, as previously mentioned. The solubility showed no significant increase with adding 0.1% w/v SLS (53.706±2.2) µg/ml while there was an observed improvement using media containing 0.1% (w/v) Tween 80 (80.355±0.355). The solubility of RPG reached its highest value, (168.595±8.9975) µg/ml, in phosphate-buffered saline (pH 6.8) containing 0.5% Tween 20 at a temperature of 37±0.5°C.

Table 1: Solubility of Repaglinide in different media.

Media	Solubility after 72 hours; Average µg/ml
PBS pH 6.8	56.163 ±0.337
PBS pH 6.8 + 0.1% Tween 80	80.355 ±0.355
PBS pH 6.8 + 0.1% SLS	53.706 ±2.2
PBS pH 6.8 + 0.5% Tween 20	168.595± 8.9975

UV Scanning of RPG

UV Spectrum of these media was recorded (Figure 1 to Figure 5).

Formulation of RPG-loaded spanlastics

The nanovesicles were successfully formulated using thin film hydration technique (TFH). To reduce the surface tension between two liquids, such as an aqueous and an oily phase, surfactants are employed Non-ionic surfactants like Spans (sorbitan alkyl esters) form concentric bilayers that define spanlastics' vesicular structure[26]. The fatty acid type determines the Span variant (e.g., Span 80: monooleate; Span 60: monostearate). Stability varies by Span type, with Span 60's saturated alkyl chains offering superior stability for unilamellar or multilamellar vesicles, while Span 80 and Span 40 often result in instability. The surfactant's surface activity enhances edge activation, reduces interfacial tension, and supports smaller spanlastic dispersions[12].

Characterization of RPG-loaded spanlastics

Particle size

Particle size (PS) and Polydispersity Index (PDI) are critical parameters in the development of nano-medicines for therapeutic use. They impact the *in vivo* distribution, toxicity, and targeting potential of nanovesicles[27].

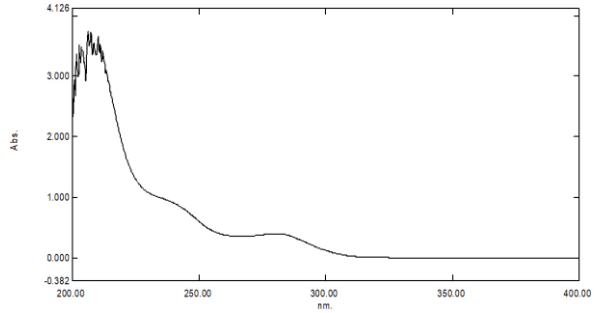

Figure 1: UV Spectrum of RPG in PBS pH 6.8.

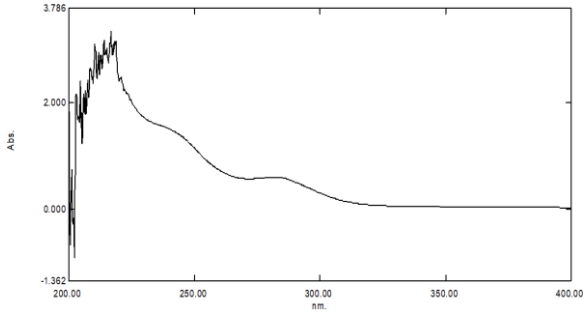

Figure 2: UV Spectrum of RPG in PBS pH 6.8 + 0.1% Tween 80.

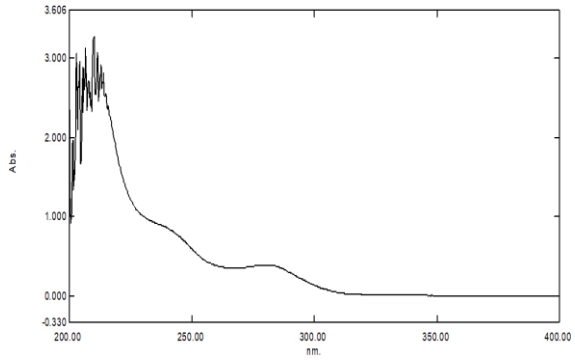

Figure 3: UV Spectrum of RPG in PBS pH 6.8 + 0.1% SLS.

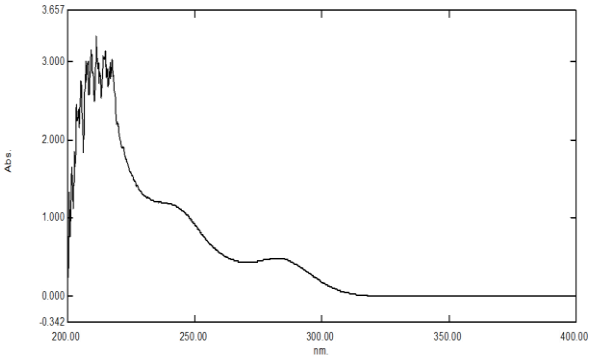

Figure 4: UV Spectrum of RPG in PBS pH 6.8 + 0.5 % Tween 20.

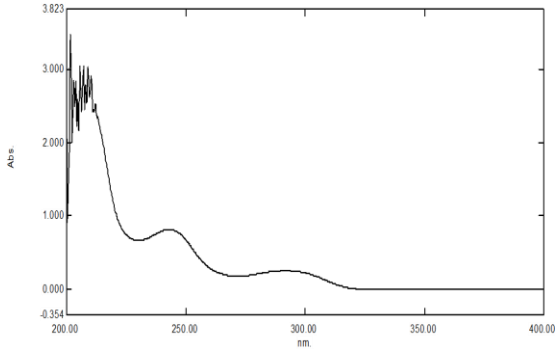

Figure 5: UV Spectrum of RPG in Methanol.

This study demonstrates that the particle size (PS) of the formulations ranges from 119.9 to 160.1 nm, falling within the desirable nano range. The statistical analysis of raw data using one way ANOVA showed significant difference between groups (T10, T20, T30) with *p*-value 0.00024 < 0.05.

Polydispersity index (PDI)

The Polydispersity Index (PDI) measures particle size (PS) distribution uniformity in nano systems. Low PDI values near zero indicate a homogeneous and uniform PS distribution, while higher values (around 1) signify a highly polydisperse system with significant size variability[27]. The obtained PDI values for the developed nanovesicles ranged from 0.407to 0.428. This range indicates that the nanovesicle dispersions are highly homogeneous. The statistical analysis of raw data using one way ANOVA showed significant difference between groups (T10, T20, T30) with *p*-value 0.0128< 0.05.

Table 2: Composition of various RPG loaded spanlastics formulations.

Spanlastics formulations	Non-ionic Surfactant			Edge Activator			Total W (mg)	RPG Content (mg)
	Type	%	W (mg)	Type	%	W (mg)		
T10	Span 60	90	360	Tween 80	10	40	400	5
T20	Span 60	80	320	Tween 80	20	80	400	5
T30	Span 60	70	280	Tween 80	30	120	400	5

Zeta potential (ZP)

The surface charge responsible for forming an electrical barrier and serving as a repulsive force in colloid stabilization is known as Zeta-potential[28]. Zeta potential (ZP) values, whether they are positive or negative, should be kept at a high level to effectively ensure the stability of the colloid. High ZP values are crucial for preventing the particles from clumping together or aggregating, thus maintaining the uniform dispersion of the particles within the colloidal system[29]. The data presented clearly demonstrated that all the developed nanovesicles displayed negative zeta potential (ZP) values, which ranged between -36.7 mV and -77.5 mV. These negative ZP values are generally expected due to the ability of ethanol to condense the membrane or the formation of a phase with interpenetrating hydrocarbons[30].

Table 3: Characterization of RPG loaded spanlastics formulations.

Formulations	Mean particle size (nm)	Mean PDI	Mean ZP (mV)	% EE
T10	122.2±0.2	0.407±0.008	-36.7±3.1	85.247±3.2
T20	119.9±0.3	0.428±0.004	- 41.1±2.9	72.835±1.4
T30	160.1±10.05	0.414±0.005	-77.5±7.3	56.9±2.6

EE= Entrapment efficiency

Table 4: Responses and it's optimization goal.

Responses	Optimization goal
Particle size	Minimize
Polydispersity index	Minimize
Zeta Potential	Maximize (absolute value)
Entrapment Efficiency %	Maximize

The statistical analysis of raw data using one way ANOVA showed significant difference between groups (T10, T20, T30) with *p*-value 0.00009< 0.05.

Entrapment efficiency percentage (EE %)

A high encapsulation efficiency (EE%) significantly enhances the probability that nanovesicles will successfully deliver a sufficient quantity of the drug to the target site. This is especially true when the nanovesicles are equipped with an effective induced release mechanism that maintains an appropriate drug release rate. Together, these factors work synergistically to ensure that the drug reaches its intended destination in the right amount, enhancing the overall effectiveness of the treatment[31]. The entrapment efficiency (EE%) of RPG-loaded nanovesicle dispersion exhibited a wide range of variation, Spanning from a high of 85.247% in sample T10 to a low of 56.9% in sample T30.The statistical analysis of raw data using one way ANOVA showed significant difference between groups (T10, T20, T30) with *p*-value 0.000027< 0.05.

Optimization of RPG-loaded spanlastics formulations

The mixture design was conducted on all RPG Loaded Spanlastic formulations (T10 to T30) by using Design Expert 13 software based on measured characteristics such as Particle size (PS), polydispersity index (PDI), zeta potential (ZP), and Entrapment efficiency % and the optimized formula was selected due to achieving the optimum desired characteristics; minimal (PS and PDI) and maximal (EE% and ZP absolute value).

The optimized formula was chosen on the basis of highest desirability noting that particle size and entrapment efficiency % was given a higher priority above other responses. This study employed a thin-film hydration technique with specific modifications to develop spanlastic formulations. However, these modifications may significantly impact the resulting characteristics of the vesicles. A comparison with another study that utilized the same lipophilic surfactant, EA, concentrations, and technique (with some variations)[32] reveals a notable difference in the results.

Table 5: Design table of RPG loaded spanlastics formulations.

Spanlastics formulations	Independent variables		Responses			
	X1	X2	Y1	Y2	Y3	Y4
	Span 60 %	Tween 80 %	PS	PDI	ZP	EE%
T 10	90	10	122.2	0.407	-36.7	85.247
T 20	80	20	119.9	0.428	- 41.1	72.835
T 30	70	30	160.1	0.414	-77.5	56.9

Table 6: The selected optimized formula and it's responses.

Span 60 %	Tween 80 %	PS (nm)	PDI	ZP (mV)	EE%	Desirability
84.171	15.829	126.162	0.416	-43.257	77.573	0.599

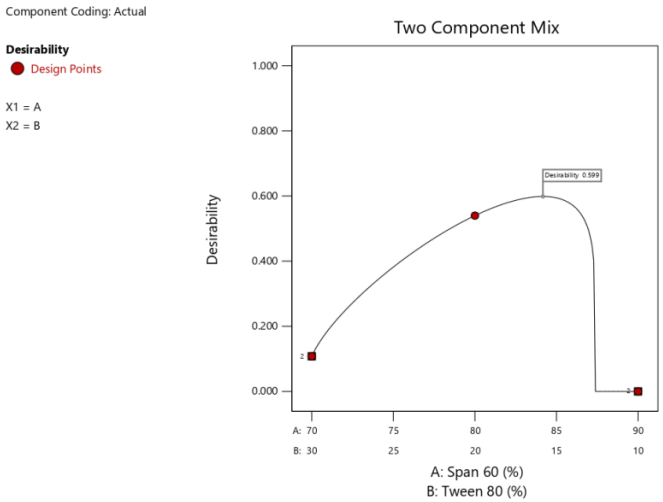

Figure 6: Effect of independent variables X1 and X2 on desirability.

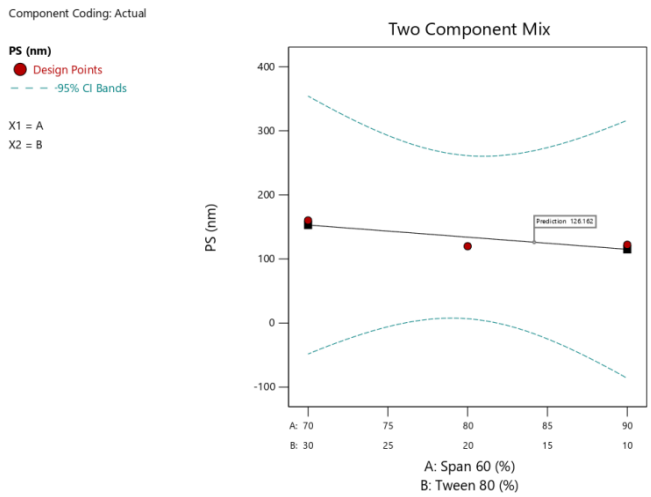

Figure 7: Effect of independent variables X1 and X2 on PS.

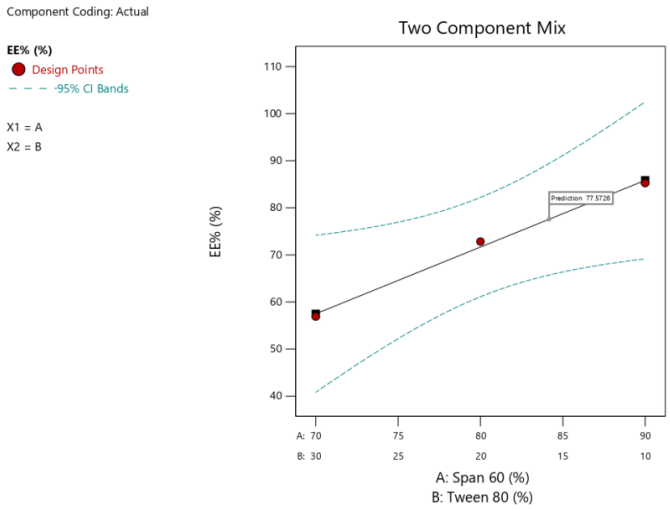

Figure 8: Effect of independent variables X1 and X2 on % EE.

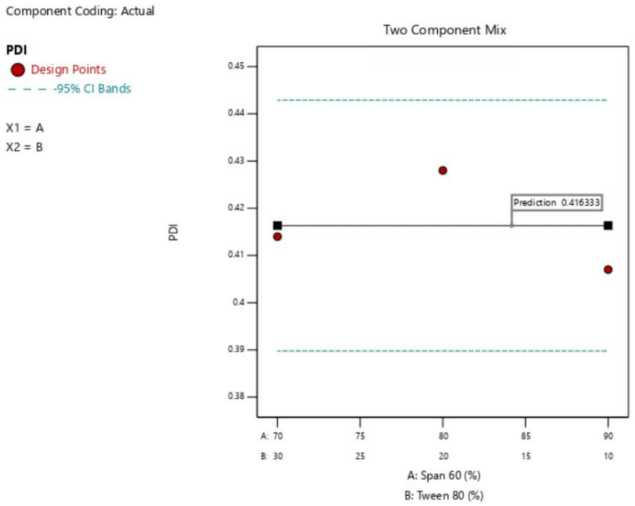

Figure 9: Effect of independent variables X1 and X2 on PDI.

Table 7: Resulting responses obtained from the compared study.

Batch	Independent variables		Responses							
	X1	X2	Y1	Y1*	Y2	Y2*	Y3	Y3*	Y4	Y4*
	% Span 60	% Tween 80	PS	PS*	PDI	PDI*	ZP	ZP*	EE%	EE%*
T 10	90	10	122.2	307.3	0.407	0.55	-36.7	-42.3	85.247	63.50
T 20	80	20	119.9	176	0.428	0.28	- 41.1	-37.3	72.835	50.10
T 30	70	30	160.1	235	0.414	0.43	-77.5	-38.1	56.9	25.30

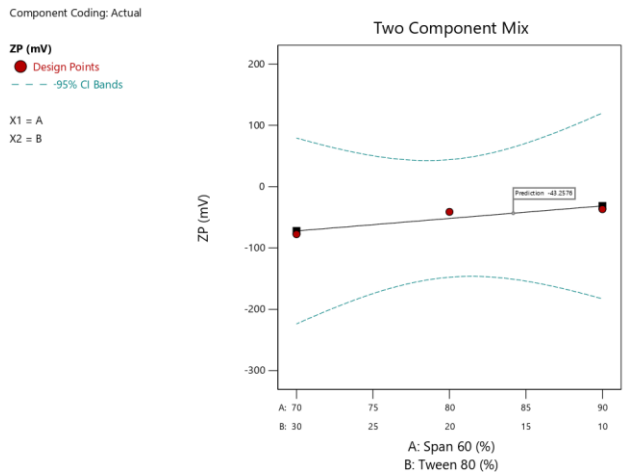

Figure 10: Effect of independent variables X1 and X2 on ZP.

Limitations of the study

The study utilized a single type of edge activator (Tween 80) in the formulation of spanlastics. While this choice demonstrated promising results, exploring other edge activators with different hydrophilic-lipophilic balance (HLB) values could potentially further optimize vesicle properties, such as stability, encapsulation efficiency, and flexibility. The optimization process using the mixture design approach is based on specific assumptions, and potential variability in real-world manufacturing conditions could influence the outcomes.

CONCLUSIONS

This study comprehensively investigated the solubility profile of Repaglinide across various media, providing valuable insights into its dissolution behaviour and addressing critical challenges related to its limited bioavailability. The successful development and optimization of Repaglinide-loaded spanlastics resulted in formulations with a favourable nanosized range, low polydispersity index, high zeta potential, and excellent encapsulation efficiency (EE%), underscoring their potential as a robust strategy for enhancing drug solubility and delivery efficiency. Detailed characterization studies confirmed the stability, uniformity, and efficacy of these spanlastic formulations, positioning them as a potential novel drug delivery system to overcome the challenges associated with poorly soluble drugs. To fully establish the clinical relevance of this innovative approach, future research should focus on *in vivo* evaluations and therapeutic efficacy studies.

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AUTHOR'S CONTRIBUTION

Seary H: Writing, methods, concept design, experiments. **Barakat EH**: Analysis, data management. **Raslan MA**: Review, revisions. **Samy AM**: Methods, analysis. Final manuscript was checked and approved by all authors.

DATA AVAILABILITY

All data and materials will be available upon request to the corresponding author.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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