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Response surface optimization and *in vitro* study of nasal solusomes nanovesicles for the bioavailability improvement and brain targetting of sumatriptan

Ahmed H Salman[1] Hussein K. Alkufi[2,*] Salam Shanta Taher[3] Sumayah Al-Mahmood[4] Mahmood A. Haiss[3]

[1]Department of Pharmaceutics, College of Pharmacy, Al-bayan university, Iraq
[2]Department of Pharmacognosy, College of Pharmacy, University of Thi-Qar, Iraq
[3]Department of Pharmaceutics, College of Pharmacy, University of Baghdad, Iraq
[4]Department of Basic Sciences, College of Dentistry, Al-Iraqia University, Iraq

***Corresponding author:** Hussein K. Alkufi, Department of pharmacognosy, College of Pharmacy, University of Thi-Qar, Iraq; Tel.: +964-7830905105; e-mail: husseinalkufi21@utq.edu.iq

ABSTRACT

Background: One effective second-generation triptan for migraine attacks is sumatriptan. Following oral use, it has a 40% restricted bioavailability because of the first-pass metabolism. *Aim*: To develop the best intranasal Solusomes formula as a substitute that delivers into the brain directly, improving its bioavailability, and removing the first-pass outcome was the aim of this effort. *Methodology*: We developed solute formulations based on the Box-Behnken design and subsequently produced them via thin-film hydration. The quality by design technique was used to establish a correlation between the formulation parameters (Soluplus® and phosphatidylcholine (PC) concentrations) and significant quality powers (entrapment efficiency (EE%), vesicle size (VS), and polydispersity index (PDI)). Fourier transform infrared spectroscopy (FTIR), optical microscopy, and an *in vitro* diffusion study were performed on the revised formula. *Results*: The enhanced formulation exhibited a VS of 93.76 nm, an EE% of 83.65%, and PDI 0.3362 with the least amount of error between the projected and observed values. *Conclusion*: This study offered a feasible and efficient intranasal formulation appropriate for further brain delivery research.

KEYWORDS

sumatriptan succinate, nasal bioavailability, pharmacokinetics, Soluplus®, solusomes

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1. INTRODUCTION

A neurovascular condition called migraines is typified by excruciating headache pain and activation of the trigeminovascular system [1]. It is characterized by unilateral tenderness and is typically accompanied by light, sound feeling, and nausea [2]. It is still unclear exactly what pathophysiology causes migraine headaches. The vascular origin of migraines was accepted for a long time, linking the headache to an extended dilation of the cerebral blood vessels [2]. Nonetheless, a

wealth of data from recent decades points to an integrated hypothesis involving both vascular and neural components. According to the neurogenic inflammation theory, inflammatory tissue responses are triggered by the production of vasoactive neuropeptides such as neurokinin A, CGRP, and substance P (SP) [3]. NSAIDs, ergot alkaloids, and triptan, a 5-HT receptor agonist, are frequently used in treatment [4]. Triptans, which include sumatriptan succinate, are thought to be the most advanced treatment for migraines since they are $5HT_{1B/1D}$ serotonin receptor agonists [2,5]. When the trigeminal ganglion and superior sagittal sinus are electrically stimulated, triptans reduce the levels of CGRP that are elicited [6]. Antagonism against $5HT_{1B/1D}$ receptors prevents nociceptive neurotransmission, the release of vasoactive neuropeptides by trigeminal neurons, and the closure of painfully dilated cerebral blood vessels [7]. Since sumatriptan effectively lessens migraine symptoms, it is regarded as a great treatment [8, 9]. Nevertheless, the main disadvantages of sumatriptan oral formulations are their delayed onset of action and limited bioavailability (40%) as a result of hepatic first-pass metabolism [8]. The half-life of sumatriptan is 1-2 hours [10]. Furthermore, vomiting and stomach stasis are associated with migraines, which can lead to irregular or delayed medication absorption [11]. For sumatriptan distribution, a route that increases brain targeting, boosts bioavailability, and circumvents first-pass metabolism would be ideal. The blood-cerebrospinal fluid barrier and the blood-brain barrier provide strong protection for the central nervous system. Both a physical and a biological barrier make up the blood-brain barrier. P-glycoprotein efflux and enzyme expression make it the rate-limiting mechanism that controls drug entry into the brain [12,13]. Drug distribution to the brain is therefore difficult [14]. The central nervous system may be directly targeted by the intranasal route [15]. It involves delivering drugs to the brain through various channels [16]. Additionally, by skipping the hepatic first-pass metabolism, the intranasal route can lessen potential side effects and boost medicine absorption [17]. Since 1995, there has been an increase in the systemic administration of nanoparticle treatments. The three main physical characteristics that affect how well nanoparticles penetrate and move across the blood-brain barrier are size, shape, and flexibility [18]. Soluplus® is added to liposomes to create soluosomes, which are surfactant-based nanovesicular structures [19]. PC and Soluplus® (surface active agent) combine to generate soluosomes. Both hydrophilic and hydrophobic medications, which are encased in the outer lipid layer and the inside hydrophilic compartment, can be delivered by solutes [1]. Therefore, the purpose of this study is to enhance the nose-to-brain administration of sumatriptan for the treatment of migraines by combining the advantages of sulosome distribution, the capabilities of nanotechnology, and the leverage offered by nose-to-brain delivery.

This work aims to create sumatriptan-loaded solusomes (SLS) by using PC and Soluplus® in the thin-film hydration process. A Box-Behnken design was used to optimize the developed formulation. PC, Soluplus®, and sonication time were used as independent factors, and their effects were evaluated on the dependent variables (VS, EE%, and PDI).

2. METHODOLOGY

2.1. Materials

Sumtriptan was kindly provided by Hyperchem Pharmaceuticals, China. PC was kindly provided by Hyperchem Pharmaceuticals, China. Soluplus® was gifted from BASF Pharma. Dichloromethane (HPLC grade) was purchased from Merck, USA. All other materials were of analytical grade.

2.2. Preparations of SLS formulations

Thin film hydration was used to create SLS. 75 mg of PC was dissolved in 5 mL of dichloromethane in a round-bottomed flask. Using a rotary evaporator, the organic blend was evaporated under vacuum to create a thin, uniform coating of lipid that was placed around the walls of the RBF. For a full day, the RBF was kept in a desiccator. After the dry film was soaked for two hours in a nasal saline buffer (pH 6.5) containing a solution of 10 mg of sumatriptan and 175 mg of Soluplus®, it was refrigerated to achieve the desired swelling. To create a fine solusome dispersion and stop aggregates from developing, the produced dispersion was sonicated using a bath sonicator at various times. Ultimately, the mixtures were refrigerated for further examination [20].

2.3. Sulosome optimisation using Box-Behnken design

Initial showing studies were conducted to identify possible factors influencing sulosomes' advantageous characteristics for intranasal administration. After identifying suitable criteria, Design Expert version 11 software was used to build a three-factor Box-Behnken design (State-ease, Minneapolis, MN, USA). The effects of lipid concentration,

Soluplus® concentration, and sonication time on the response variables—PDI, EE%, and VS—were investigated using Box-Behnken design. There were 32 experimental runs in the design (Table 2).

Table 1 shows that the dependent variables were VS, PDI and EE, and the selected independent factors were PC concentration, sonication time and Soluplus® concentration.

Table 1. The dependent variables were VS, PDI and EE, and the selected independent factors were PC concentration, sonication time and Soluplus® concentration

Variables	**Levels**		
	(-1)	(0)	(+1)
Independent variables			
PC	25	75	125
Soluplus®	125	175	225
sonication time(min)	0	15	30
Dependent Variable			
PDI			
VS			
EE%			

2.3.1. Entrapment efficiency (EE)

The EE% of SLS was evaluated using the ultracentrifugation method [20]. After being stored at 4°C for the entire night, the samples were centrifuged for 1 hour at 4°C using a centrifuge set to 15,000 rpm (REMI, cooling centrifuge machine, Mumbai, India). After separating and diluting the filtrate, which contained free sumatriptan, with the suitable medium, the sumatriptan content was measured spectrophotometrically at λ_{max}. The following formula was used to calculate the EE%:

$$\text{EE\%} = \frac{\text{Total sumatriptan} - \text{sumatriptan in supernatant}}{\text{Total sumatriptan}} \times 100$$

2.3.2. VS and PDI analysis

A magnetic stirrer was used to mix amounts of SLS equal to 10 mg of sumtriptan in 10 mL of water at 500 rpm for up to one hour. In [21]. The generated sumtriptan formulations were subjected to VS and polydispersity index (PDI) measurements utilizing a Zetasizer from Malvern Panalytical Ltd. A single-use quartz cuvette containing 1 milliliter of samples was used to quantify the hydrodynamic diameter of a particle undergoing Brownian motion in the dispersion at 25 °C [22]. Based on EE, VS, and PDI, we selected the optimal SLS formula, as well as a few other formulations to examine and test further.

2.3.3. *In vitro* dissolution rate studies

The drug release dialysis membrane technique was used to assess SLS and sumatriptan solution release *in vitro* (control). Both formulations were packed on a 12,000–14,000 Da preactivated dialysis bag (Hi Media, Mumbai, Maharashtra, India). The membrane was attached with the shafts positioned in a 500-mL beaker containing phosphate buffered saline (pH 6.5) as a release medium, which was controlled at 37°C and 100 rpm of uniform stirring. Samples were taken at predetermined intervals of 0.5, 1, 2, 4, 8, and 24 hours, and a new release medium was added. A graph was created by graphing the time (hours) and percentage of cumulative drug release, and the amount of sumatriptan was determined using the spectrophotometric technique [23].

2.3.4. Fourier transform infrared spectroscopy

Sumatriptan, Soluplus®, PC, and a few SLS were subjected to IR spectroscopy using an FTIR spectrophotometer (Shimadzu Europe FTIR-8400S). Using the potassium bromide (KBr) pellet technique, the spectra were produced. After combining about 2-4 mg of the material with dry KBr, the spectra were scanned at a resolution of 4 cm^{-1} over a wave number range of 4,000–200 cm^{-1} [23].

2.3.5. Morphology (optical microscopy)

A phase contrast optical microscope (Medilux, Kyowa Opticals Co. Ltd., Hashimoto, Japan) was used to characterize the produced vesicles for surface morphological investigation at an appropriate magnification [24].

2.3.6. Statistical analysis

The VS, PDI, and EE% data were analyzed using the one-way analysis of variance (ANOVA) to determine significant results ($p<0.05$) or non-significant findings ($p>0.05$) by using the Box-Behnken design [25].

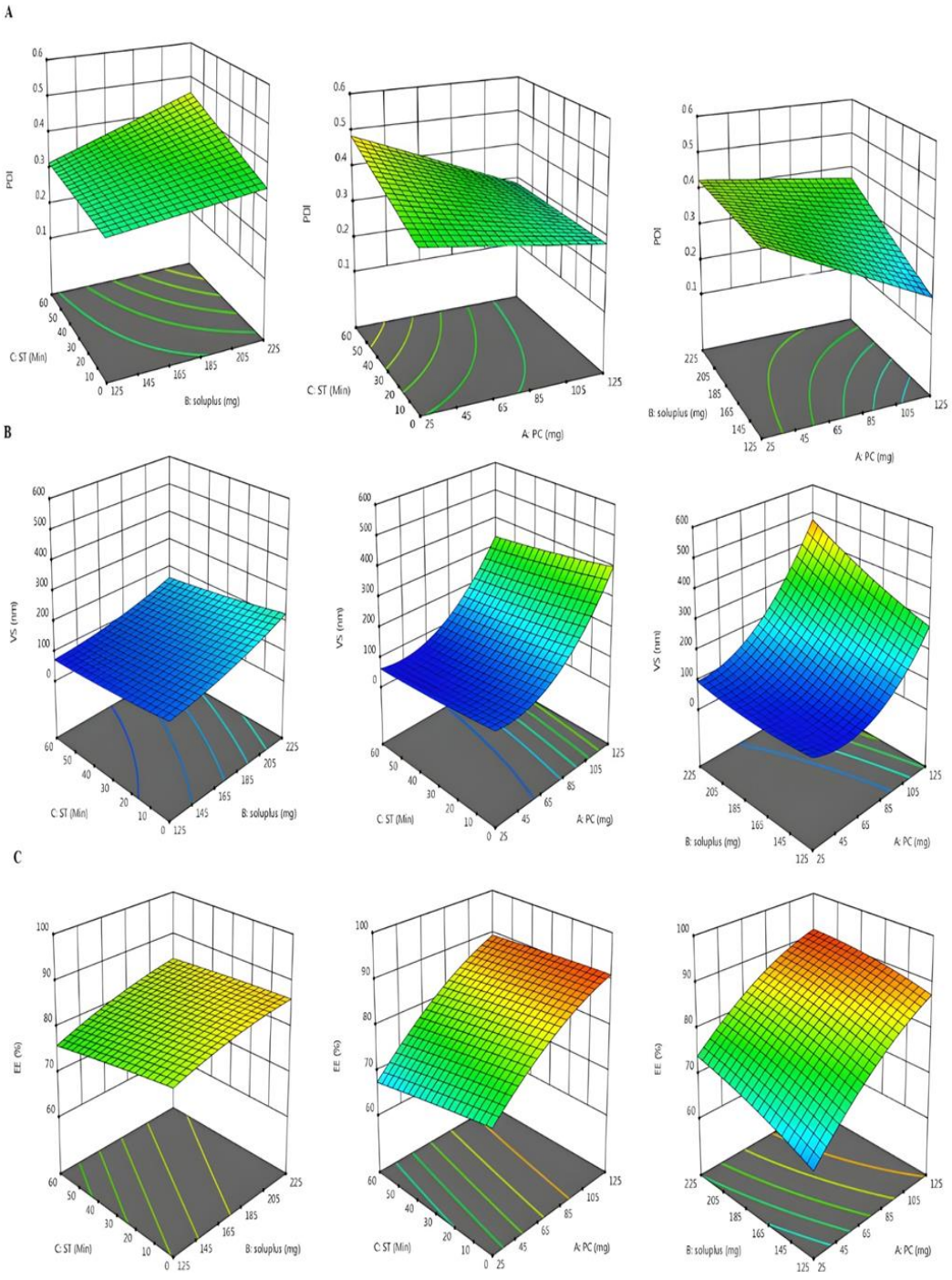

Figure 1. 3D-response charts expressive the effect of independent variables on (A) PDI, (B) VS, and (C) EE%.

3. RESULTS

3.1. Optimisation of SLS by Box-Behnken design

The 3D-response diagrams in Figure 1 show the effects of the adopted parameters (PC, Soluplus®, and sonication time) on PDI, EE%, and VS of SLS.

3.2. Effect of independent variables on PDI, VS and EE%

The PDI of all 27 runs was reckoned to be between 0.0792 and 0.2789 (Table 2). The effect of Soluplus® on PDI is shown in Table 3. The enhanced VS formulation in SLS9 (Table 4) and VS distribution are depicted in Figure 2. The effect of independent variables on EE% is shown in Table 5.

Table 2. Box-Behnken experimental design with measured responses. Study three factors: PC, soluplus, and ST (sonication time) on (EE%), (VS), and (PDI).

	Factor 1	**Factor 2**	**Factor 3**	**Response 1**	**Response 2**	**Response 3**
Run	A:PC	B:soluplus	C:ST	PDI	VS	EE
	mg	mg	Min		nm	%
1	125	125	0	0.2234	300.78	88.7
2	75	125	30	0.3214	112.33	77.21
3	25	175	60	0.4567	70.97	65.32
4	125	225	0	0.3121	550.87	93.34
5	75	125	60	0.4773	90.65	75.21
6	125	225	30	0.4567	480.54	91.22
7	125	125	60	0.1786	250.23	87.05
8	75	175	30	0.3212	120.54	85.22
9	75	175	60	0.3362	93.67	83.65
10	25	125	60	0.4456	66.65	60.21
11	25	125	0	0.3421	99.21	70.21
12	125	225	60	0.4421	450.32	90.21
13	25	175	30	0.4321	90.99	70.21
14	25	225	0	0.3125	130	75.11
15	125	125	30	0.1324	280.34	87.21
16	75	225	60	0.4321	150.88	83.22
17	125	175	0	0.2984	390.33	90.21
18	25	225	60	0.5783	80.44	73.99
19	25	125	30	0.3125	70.36	66.32
20	25	225	30	0.4125	99.21	74.01
21	125	175	60	0.2154	340.97	89.11
22	75	225	30	0.3124	180.32	84.31
23	75	125	0	0.3344	130.21	80.21
24	75	225	0	0.3334	200.22	86.21
25	125	175	30	0.2589	350.98	90.01
26	75	175	0	0.3567	155.19	85.1
27	25	175	0	0.4356	110.68	71.55

Table 3. Summary of ANOVA for the PDI response parameters.

Source	Sum of squares	df	Mean square	*F*-value	*p*-value	
Model	0.1784	6	0.0297	7.00	0.0004	significant
A-PC	0.0813	1	0.0813	19.14	0.0003	significant
B-soluplus	0.0378	1	0.0378	8.89	0.0074	significant
C-ST	0.0209	1	0.0209	4.92	0.0382	significant
AB	0.0187	1	0.0187	4.39	0.0490	significant
AC	0.0126	1	0.0126	2.96	0.1010	
BC	0.0071	1	0.0071	1.68	0.2094	
Residual	0.0850	20	0.0042			
Cor Total	0.2634	26				

Table 4. Summary of ANOVA for the VS response parameters.

Source	Sum of squares	df	Mean square	*F*-value	*p*-value	
Model	4.573E+05	6	76214.74	24.32	< 0.0001	significant
A-PC	3.689E+05	1	3.689E+05	117.71	< 0.0001	significant
B-soluplus	47230.99	1	47230.99	15.07	0.0009	significant
C-ST	12414.15	1	12414.15	3.96	0.0604	significant
AB	27739.28	1	27739.28	8.85	0.0075	significant
AC	515.22	1	515.22	0.1644	0.6894	
BC	491.26	1	491.26	0.1568	0.6964	
Residual	62679.07	20	3133.95			
Cor Total	5.200E+05	26				

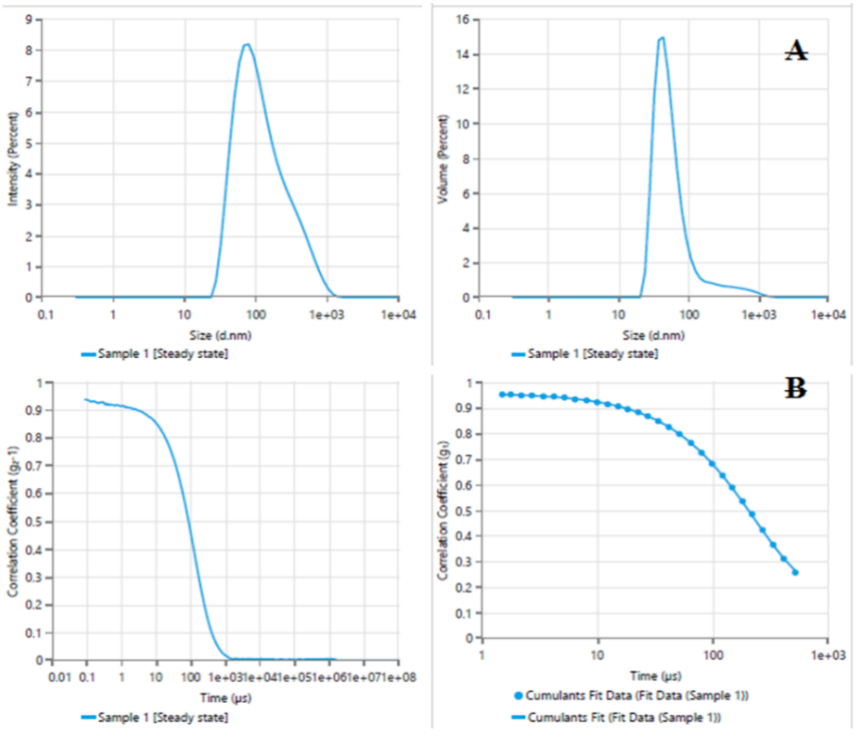

Figure 2. Average VS for formula F9 by Malven Zeta Seizer. (**A**) intensity of VS and (**B**) cumulative data.

Table 5: Summary of ANOVA for the EE% response parameters.

Source	Sum of squares	df	Mean square	*F*-value	*p*-value	
Model	2093.20	6	348.87	77.31	< 0.0001	significant
A-PC	1802.60	1	1802.60	399.47	< 0.0001	significant
B-soluplus	195.29	1	195.29	43.28	< 0.0001	significant
C-ST	59.30	1	59.30	13.14	0.0017	significant
AB	17.67	1	17.67	3.91	0.0618	
AC	10.96	1	10.96	2.43	0.1347	
BC	7.38	1	7.38	1.64	0.2156	
Residual	90.25	20	4.51			
Cor Total	2183.45	26				

3.3. Solusome morphology

The optical microscopic inspection of the SLS9 (Figure 3).

3.4. *In vitro* drug release study

The improved SLS9 formulation demonstrated an 88.21% release of sumatriptan across the dialysis membrane, whereas the *in vitro* release of pure sumatriptan was estimated to be 99.55% at 2 hr. (Figure 4). The graph indicates that the drug is released quickly in SLS9 during the first four hours and then more slowly for the remaining twenty-four hours. At every point, there was a noticeable medication release.

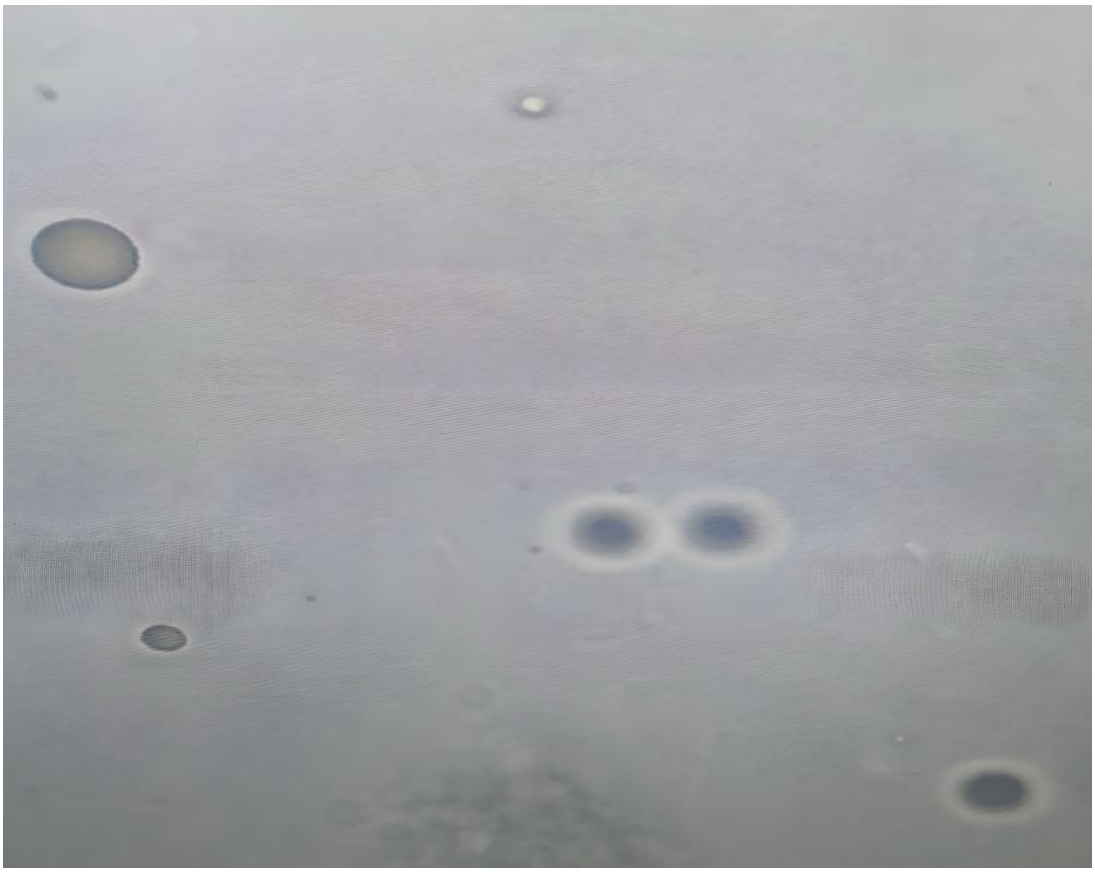

Figure 3. An optical microscopy image with magnification of × 100 obtained with polarized light from the selected SLS9 formulation.

3.5. Drug-excipients compatibility studies by FTIR

The reference FTIR spectra were compared with the FTIR of sumatriptan (Figure 5) to identify distinctive peaks at 3371.57 cm^{-1}, 1338.60 cm^{-1}, 1205.51 cm^{-1}, 1143.79 cm^{-1}, and 638.44 cm^{-1}. Peaks for O-H stretching, aliphatic C-H stretching, and C-O stretching in the C-O-C group were seen at 3483.44, 2856.01, and 1238.30 cm^{-1} in the FTIR spectra of PC and Soluplus®, individually. The N-H and C-N peaks were measured at 3415.93 cm^{-1}, 1234.44 cm^{-1}, 1375.29 cm^{-1} and 1082.07 cm^{-1} for S=O and 605.65 cm^{-1} for C-S, respectively, which are the characteristic peaks of SLS 9 [26].

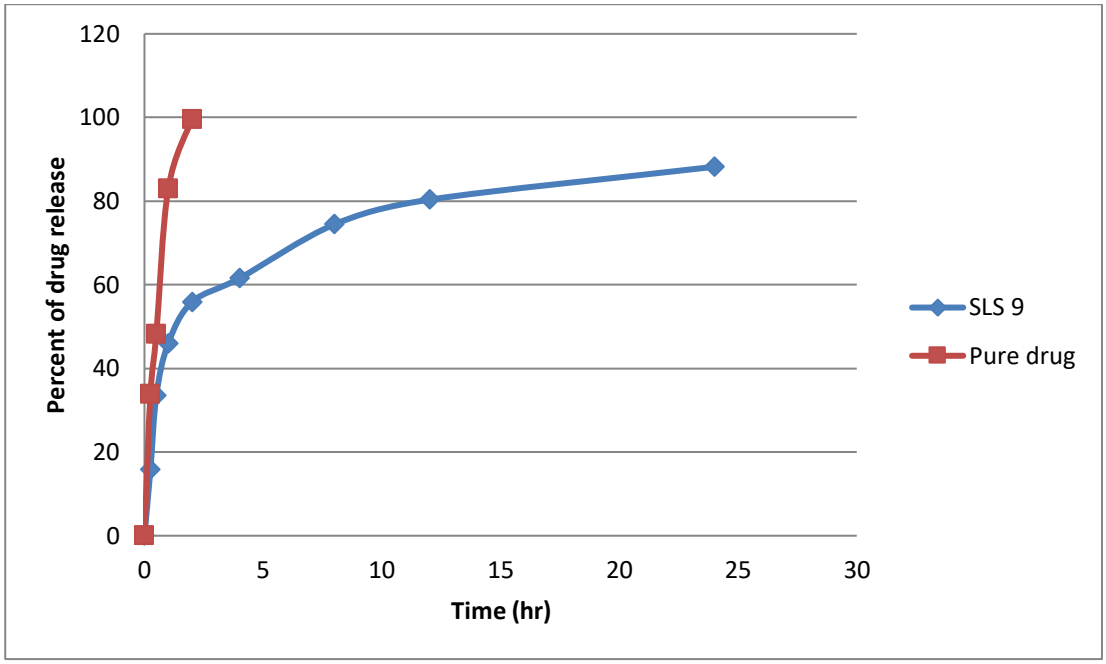

Figure 4. Comparative *in vitro* drug release profile of sumatriptan solution and selected SLS9 formulation.

4. DISCUSSION

It was determined using the aforementioned polynomial equation that the PC positively affects PDI. When PC concentration rose from 25 to 125 mg, the PDI increased. On the other hand, Soluplus® improves PDI. PDI decreases when Soluplus® concentration is raised from 125 to 225 mg (Table 3).

Nanoscale VS is seen in formulations with PC and soluplus, and all ratios utilized show that Soluplus can produce sumatriptan with an SLS. For Soluplus with PC, the ideal drug-carrier ratio is 7:3.

It may be inferred from the preceding polynomial equation that PC increases EE% and that Soluplus® increases EE%. An increase in PC concentration (from 25 to 125 mg) was found to increase EE%. This might be the result of more vesicles forming, which increases the bilayer domain dimension and gives sumatriptan more room to get trapped in SLS vesicles (Table 4). The experimental data indicates that a rise in Soluplus® concentration (125–225 mg) leads to an increase in sumatriptan EE% in vesicles. When the concentration of Soluplus® increases, more micelles form. As a result, there are more "containers" available to store the drug molecules, increasing the effectiveness of entrapment. The micelles absorb additional medication molecules [27]. During sonication, sound waves cause high-pressure and low-pressure zones in the liquid to form. Tiny vapor bubbles grow in a low-pressure zone and then abruptly collapse as the pressure decreases rapidly. The bubbles collapsing so violently cause the liquid to experience strong shock waves and shear stresses. The particles suspended in the liquid are broken up into smaller fragments by these forces [28].

Using PC (75 mg), Soluplus® (175 mg), and a 60-minute sonication time—as per the formula produced by the rotary evaporator method—an optimal formulation was created based on the results.

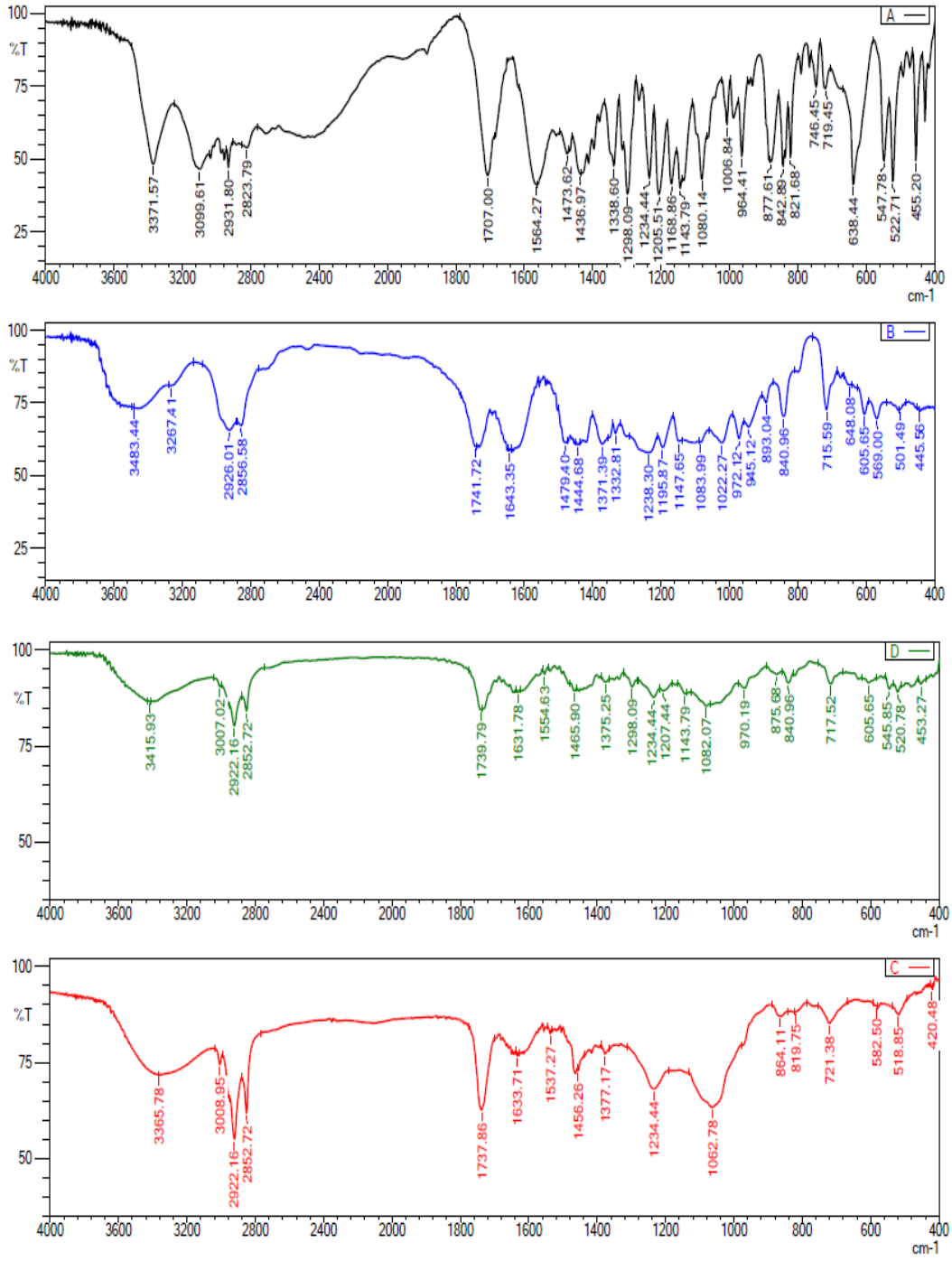

Figure 5. FTIR of (A) sumatriptan, (B) PC, (C) Soluplus®, and (D) selected SLS9 formulation.

It was then assessed for PDI, EE%, and VS. Using the Box-Behnken design, the SLS showed a VS of 93.67±1.32 nm, an EE% of 83.65± 2.59%, and a PDI of 0.3362±0.33.

The resulting vesicles had a homogeneous size distribution, a well-defined sealed structure, and a spherical shape, as revealed by optical microscopic inspection of the SLS formulation (Figure 3).

The SLS formulation showed a delayed medication release when compared to pure drug. Because of sumatriptan's slow diffusion and requirement to cross the brain's lipid bilayer, sulosomes have the ability to control medication release. The graph indicates that the drug is released quickly during the first four hours and then more slowly for the remaining twenty-four hours. Releasing oneself in this way is the best way to increase treatment efficacy. Extended slow release enhances therapeutic efficacy, while initial quick release helps achieve therapeutic concentration.

Drugs and other additives in SLS did not interact, as evidenced by the lack of substantial variations in the drug's primary characteristic bands.

5. CONCLUSION

Using a polymeric solubilizer (Soluplus) in a lipid matrix (PC), sumatriptan solusomes were effectively created by the thin-film hydration approach.

SLS9, consisting of PC and Solu (3:7 w/w), was able to improve the nasal bioavailability of sumatriptan and increase its permeability through the nasal channel. It also improved sumatriptan's solubility, releasing 88.21% of its total weight after 60 minutes of sonication. To validate these findings, more safety and clinical research is required.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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