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ORIGINAL RESEARCH

Screening of Adapalene Microsponges Fabrication Parameters with Insight on the In vitro Biological Effectiveness

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Purpose: The objective of the present study was to scrutinize the microsponges (MS) as a carrier system using Adapalene (ADA) as a model drug.

Methods: Data modelling was implemented using Plackett-Burman design to identify the main variables affecting the formulation of ADA-MS. The adopted method of preparation for MS was quasi-emulsion solvent diffusion method. The nominated independent variables were volume of organic phase, sonication time, stirring speed, drug percent, polymer type, emulsifier concentration, and method of organic phase addition. As for the dependent variables, they included entrapment efficiency (E.E.%), production yield (P.Y.%), particle size (P.S.) and morphology. Furthermore, selected ADA loaded microsponges (ADA-MS) were in vitro assayed for their biological activities via cytotoxicity, UVA irradiation and cell viability, and antimicrobial activity.

Results: The study indicated that the drug percent, polymer type and surfactant concentration have the key significant effect on E.E.% and P.Y.%, while, the drug percent, stirring speed and volume of organic phase have had a significant effect on P.S. and their morphology. Furthermore, ADA-MS had a momentous cytotoxic effect on A431 and M10 cell-lines with exceptional enrichment when the polymer Eudragit RS100 was used. Also, the ADA-MS increased the cell viability after UVA irradiation on HFB-4 cell-line by 14% to 43%, especially when using Ethyl Cellulose as a polymer. Lastly, the antimicrobial activity of ADA against *Propionibacterium acnes* was boosted when incorporated into MS.

Conclusion: The Plackett−Burman design proved its impact in discerning preparation variables affecting the quality of ADA-MS formulation, with heightening of the in vitro biological activities of ADA. Thus, MS was presumed to be an auspicious carrier system for ADA.

Keywords: Plackett-Burman, anticancer, antibacterial, UVA irradiation

Introduction

Microsponges have been widely exploited for their usage in drug delivery pertaining to their numerous merits. Microsponges are polymeric system that highly resembles the sponge structure being extremely porous, and spherical in shape. The particle size of microsponges' varies between 5 and 300 µm in diameter.[1] The internal pore structure of a MS particle is approximately equivalent to 3 meters, owing to the presence of large number of interconnected pores (a 25 µm sphere can have up to 25,000 pores).[1,2] Accordingly, the fundamental advantage of this system is its ability of entrapping a wide range of active ingredients, on its surface and/or loaded into the matrix of the particle. Together with the delivering of active moiety in a controlled, site-specific manner, with a minimal dose, which thereby minimize the side effects. MS can be prepared by liquid–liquid suspension method or emulsion solvent diffusion method, which are considered easy and cost-effective using different polymers.[3] The most commonly used polymers for the fabrication of

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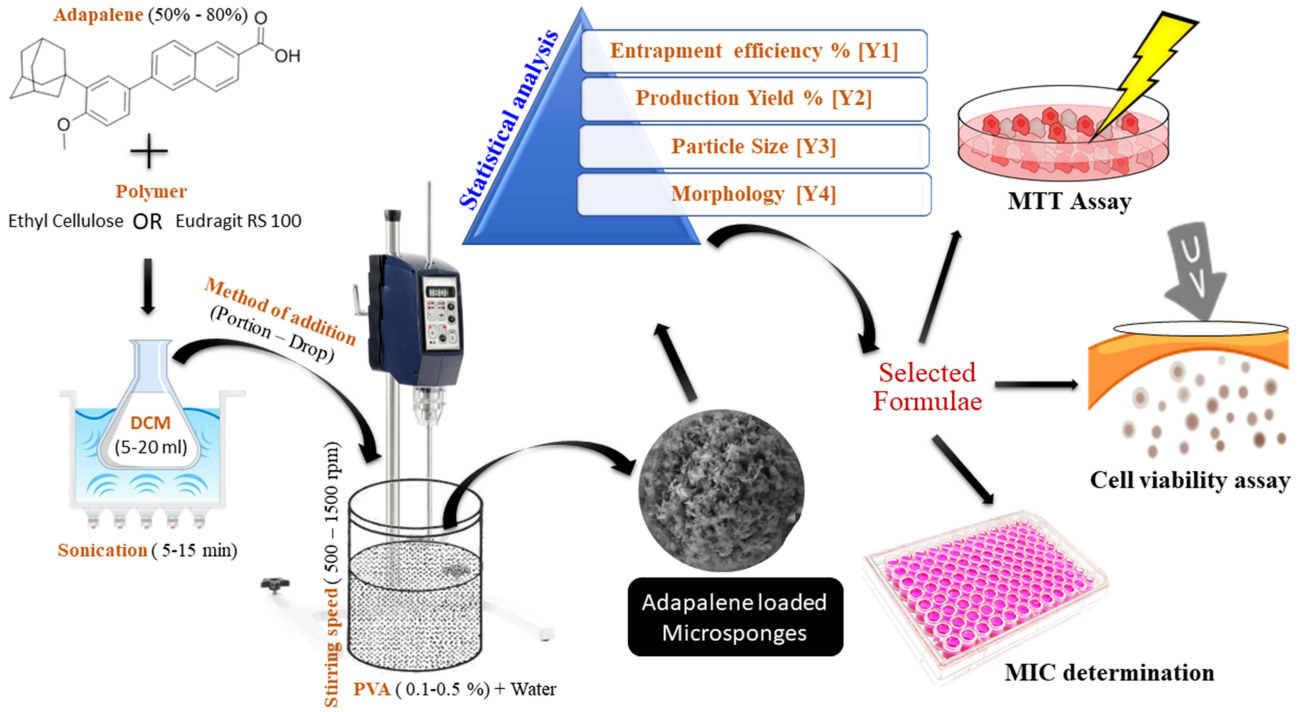
Graphical Abstract



MS are Ethyl cellulose (EC) and Eudragit RS100 (EUD). Both polymers have the nature of being hydrophobic, besides, EUD exhibits minor hydrophilic properties due to the existence of structural quaternary ammonium groups. They are capable of matrix formation and controlled release characteristics which are reliant on their swelling capacities and permeabilities.[4] Nevertheless, multiple parameters influence the MS formulation process and thus affect their characteristics and ability to efficiently deliver active moieties. Therefore, a screening process will be useful in minimizing the variables affecting the preparation of microsponges.[1–3,5,6]

Screening multiple parameters that influence formulation development is a wearisome study, particularly if it is carried out by a trial-and-error approach. This consumes a lot of materials and by this means increases the cost of the whole process, not to mention the time and effort wasted.[7] Utilizing statistical designs of experiment (DOE) is an efficacious approach for multifactorial analysis of an experiment with indication of the attributes factors influences on the dependent responses.[8] The main benefit of this type of design is the small number of experimentations required, which shortens the time needed to complete the study together with providing statistically verified data.[9] Several statistical DOE are used. The most commonly used DOE for screening a large number of parameters is Plackett–Burman, which is a highly efficient screening design tool.[10] It can screen up to eleven factors, at two levels and be evaluated by means of twelve runs of experiment. Such a test simplifies the optimization process by identifying the root causes of variability that require further investigation.[11,12] The Plackett Burman design has been very successful in screening numerous parameters and has also been considered a fitting tool for early investigations.[13]

The selected model drug to be used to access the formulation process and effect of encapsulation into microsponges is Adapalene (ADA); 6-[3-(1-adamantyl)-4-methoxyphenyl] naphthalene-2-carboxylic acid. It is a third-generation retinoid derivative. It is extremely hydrophobic, exhibits higher stability to light and oxidation compared to other retinoids.[14] ADA mechanism of action mainly involves selective binding to retinoic acid receptor (RAR). ADA has great affinity to RARs: RAR-ɣ and RAR-β, the first is mainly located in the epidermis while the latter mainly in the fibroblasts,[15] then, RAR-ADA complex binds to retinoid X receptor (RXR) and this modulates gene transcription by binding to specific

DNA sites, therefore it inhibits cell proliferation and modulates cell differentiation and inflammatory process.[16,17] As a result, it attains several dermatological effects.

The biological activities of ADA are beneficial in the treatment of various dermatological conditions.[18] Firstly, it has been used topically as a first line treatment of mild to moderate acne vulgaris for years. Also, it attains a positive impact on differentiation and maintenance of hair follicles, together with, the recently reported depigmentation[19] and anti-photo aging activities.[20] Newly, some studies shed the light on the anticancer activity of ADA, yet the antiproliferative effect and mechanism of ADA is not fully exploited. ADA displayed an anticancer activity in human colorectal cancer cells, hepatoma cells, ovarian cancer ES-2 cells, cervical intraepithelial neoplasia, immortalized human keratinocytes (HaCat cells), and lastly in the treatment of melanoma.[18,21]

Despite the topical route being favourable for the delivery of ADA to avoid the reported systemic toxicities,[22] yet it causes momentous local side effects including photosensitivity, redness, erythema, dryness, skin discomfort, pruritus, desquamation, stinging and burning.[18] Therefore, a sophisticated carrier system for ADA delivery was crucially needed to enhance its tolerability while minimizing systemic side effects. Accordingly, ADA is an optimum candidate to be used for the investigation of microsponges' characteristics as a carrier system for efficient drug delivery.

The focus of the study was to statistically analyse the key parameters affecting the process of Adapalene loaded microsponges (ADA-MS) fabrication utilizing Plackett-Burman approach. Furthermore, our golden objective was to study the contribution of the MS system on the in vitro biological activities of ADA via studying the cytotoxicity, UVA irradiation and cell viability, and antimicrobial effects.

Materials and Methods

Materials

Adapalene (ADA) was generously supplied by Borg Pharmaceutical Industries (Alexandria, Egypt). Eudragit RS100 (EUD) was gifted by Evonik Operations GmbH (Essen, Germany). Ethyl Cellulose (EC) and Polyvinyl alcohol (PVA) were kindly supplied by Egyptian International Pharmaceutical Industries Co., EIPICO (10th of Ramadan City, Egypt). Dichloromethane was purchased from El-Nasr Pharmaceutical Chemicals Co. (Cairo, Egypt). Tetrahydrofuran was purchased from Fisher Scientific (Massachusetts, USA). All aqueous solutions were prepared using distilled water produced in-house (Aquatron Water Still, A4000D, UK).

Methods

Development of Experimental Screening Design

Plackett Burman design (PBD) (Design-Expert® v.13.0.0 (Stat-Ease, Inc., Minneapolis, MN, USA)) was used to design this study by screening seven independent variables at two levels to achieve the constraints presented in Table 1. The

Table 1 Investigated Variables in Plackett-Burman Experimental Design

Investigated Variables	**Levels**	
	−1	**1**
Volume of organic phase (mL) (A)	5	20
Sonication time (minutes) (B)	5	15
Stirring speed (rpm) (C)	500	1500
Drug percent (%) (D)	50	80
Polymer type (E)	Eudragit RS100	Ethyl Cellulose
Emulsifier concentration (%) (F)	0.1	0.5
Method of organic phase addition (G)	Portionwise	Dropwise
Dependant Variables	**Constrains**	
Entrapment efficiency (%) (Y1)	Maximize	
Production yield (%) (Y2)	Maximize	
Particle size (μm) (Y3)	Minimize	
Morphology (Y4)	Maximize	

established design included twelve runs of ADA loaded microsponges (ADA-MS) formulations as shown in Table 2. The chosen independent variables were volume of organic phase (A), sonication time (B), stirring speed (C), drug percent (D), polymer type (E), emulsifier concentration (F), and lastly method of organic phase addition (G). The independent variables were screened and studied for their effects on the dependent variables; entrapment efficiency (Y1), yield percentage (Y2), particle size (Y3) and morphology (Y4).

Preparation of ADA Loaded Microsponges

According to Table 2 twelve microsponges formulae were prepared using the quasi-emulsion solvent diffusion method.[24] The ADA and polymer in ratios 1:1 and 4:1, respectively, were dispersed in dichloromethane (DCM), followed by sonication using bath sonicator (Ultrasonic cleaning unit Elmasonic S60H (Germany)) to form the internal organic phase. The external aqueous phase was prepared by dissolving the amount of the emulsifying agent PVA in 100 mL distilled water and warmed to 70°C using hotplate - magnetic stirrer (DAIHAN Scientific, Korea) and then left to cool. Afterwards, the internal phase was added to the external phase under constant stirring using overhead stirrer (HS-30D, DAIHAN Scientific, Korea) at room temperature for two hours. The obtained microsponges were filtered with 0.45 µm Whatman filter paper, washed with distilled water and further dried using an oven at 50°C for few hours till complete dryness. Then, the ADA-MS were collected and stored in a tightly closed container at room temperature for further investigations.

Measurement of Responses of the Prepared ADA-MS

The twelve prepared formulae were investigated for entrapment efficiency (Y1), yield percentage (Y2), particle size (Y3) and morphology (Y4).

Entrapment Efficiency (EE%) (Y1)

Entrapment efficiency % was determined by dissolving a certain weight of the prepared ADA-MS in 10 mL tetrahydrofuran to disrupt the microparticles and then sonicated using a bath sonicator for 10 min, followed by filtration and dilution. The samples were then analysed using a UV spectrophotometer (V-630, Jasco, Japan) at wavelength (λ) 237 nm. The data were analysed by using the calibration curve obtained from the spectrophotometric method. This experiment was triplicate for each sample, and the percentage of entrapped drug was calculated according to the following equation.[25]

$$EE(\%) = \frac{M_{act}}{M_{the}} \times 100 \quad (1)$$

Where M_{act} is the actual ADA content in the microsponges, and the M_{the} is the theoretical ADA content in the microsponges.

Table 2 Composition of Adapalene Loaded Microsponges and the Observed Values of the Dependent Responses

Formulae	A (mL)	B (min)	C (rpm)	D (%)	E	F (%)	G	Y1 (%) ± S.D.	Y2 (%) ± S.D.	Y3 (µm) ± S.D.	Y4
F1	5	15	1500	50	EC	0.5	DW	50.9 ± 3.7	54.4 ± 2	47.2 ± 16.7	2
F2	20	15	1500	50	EUD	0.1	DW	86.3 ± 2.3	62.3 ± 0.4	14.97 ± 4	3
F3	20	5	500	50	EC	0.1	DW	85 ± 3.3	85 ± 1.2	43.3 ± 4.65	1
F4	20	5	1500	80	EC	0.1	PW	96.6 ± 1.2	88 ± 0.8	30.27 ± 10.2	2
F5	5	15	1500	80	EUD	0.1	PW	92 ± 1.7	82.3 ± 1.3	31.32 ± 11.3	1
F6	5	5	500	50	EUD	0.1	PW	80.9 ± 2.1	68.8 ± 0.3	64.76 ± 8.25	3
F7	20	5	1500	80	EUD	0.5	DW	90.9 ± 1.9	86 ± 1	29.3 ± 6.7	3
F8	5	15	500	80	EC	0.1	DW	95.4 ± 1.1	81.3 ± 0.8	106.1 ± 23.1	1
F9	5	5	1500	50	EC	0.5	PW	50.9 ± 2.2	43.3 ± 2.2	29.4 ± 7.7	2
F10	5	5	500	80	EUD	0.5	DW	90.8 ± 1.5	87.3 ± 0.7	162.6 ± 37.4	1
F11	20	15	500	80	EC	0.5	PW	99.8 ± 0.5	93.1 ± 0.6	35.5 ± 11.2	1
F12	20	15	500	50	EUD	0.5	PW	38.5 ± 3.5	33.2 ± 1.3	26.3 ± 4.5	3

Notes: A: volume of organic phase, B: sonication time, C: stirring speed, D: drug percent E: polymer type, F: emulsifier concentration, G: method of organic phase addition. Y1: entrapment efficiency, Y2: yield percentage, Y3: particle size, Y4: morphology.
Abbreviations: EC, Ethyl Cellulose; EUD, Eudragit RS100; DW, dropwise; PW, portionwise.

Production Yield % (P.Y.%) (Y2)

Percentage production yield was obtained by determining the initial weight of raw materials used and final weight of microsponges and estimated by using this formula.[26]

$$Production\ Yield(\%) = \frac{Practical\ mass\ of\ microspones}{Theoretical\ mass\ (polymer\ +\ adapalene)} \times 100 \quad (2)$$

Particle Size (Y3) and Morphology (Y4)

The Particle size and surface morphology were determined using Scanning Electron Microscope SEM (Thermo Scientific FEG SEM Quattro model, USA). All samples were spread over the studs without coating. The images were taken at a high voltage 10 KV using a low vacuum detector. The morphological reproducibility was assessed by means of replicate analysis of each sample.[27] Images were captured at multiple magnifications. A scale was given to assess the morphology of the microsponges where: 1 indicates the presence of a low number of regular spherical microsponges, 2 average number of spherical microsponges, and 3 a high number of regular spherical homogenous microsponges present in the field.

Statistical Analysis of the Data

The assessed effects of parameters on responses were displayed by standardized pareto charts and response surface plots, which permitted the inspection of the statistical significance of the PBD. Analysis of variance (ANOVA) was executed on experimental data to address the statistical significance of the model. A p value less than 0.05 was considered significant for the model terms. The statistical software Design-Expert® v.13.0.0 (Stat-Ease, Inc., Minneapolis, MN, USA), was used for the regression analysis and the graphical presentation.

In vitro Biological Activity Assays for Selected Prepared ADA-MS

The biological effects of ADA loaded into selected microsponges formulae; using either Ethyl cellulose as polymer (ADA-MS-EC) or using Eudragit RS100 as polymer (ADA-MS-EUD) were investigated in comparison with ADA in free form. The biological tests used were the cytotoxic effects on epidermoid carcinoma and melanoma cell lines (A431 and M10 respectively), the photoprotection effect on normal cell line (HFB-4), and the antimicrobial activity against *Propionibacterium acnes (P. acnes)*.

Cytotoxicity Assay

The human epidermoid carcinoma cell line (A431) (squamous carcinoma) and the human melanoma cell line (M10) were obtained from American Type Culture Collection (ATCC, Rockville, MD). All cells were cultured using DMEM (Invitrogen/Life Technologies) supplemented with 10% fetal bovine serum FBS (Hyclone), 10 ug/mL of insulin (Sigma), and 1% penicillin-streptomycin. Cells were incubated in a humidified 5% CO_2 incubator at 37°C. The growing cells from both tumour cell lines (A431 and M10) were incubated in a 96-well plate for 24 hours to allow cell adhesion to occur. Afterwards, the cells were treated with serial concentrations (µg/mL) of the tested samples and incubated for 48 hours at 37°C. The tested samples were ADA, ADA-MS-EC and ADA-MS-EUD. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was then added into each well and incubated for 4 hours at 37°C. The resulting formazan crystals were dissolved by adding DMSO. The number of viable cells was determined from the optical density measured at 570 nm with microplate reader (SunRise, TECAN, Inc, USA) and the percentage of viability was calculated. Furthermore, the relation between surviving cells and drug concentration was plotted to construct the survival curve of each cancerous cell line after treatment with the stated compounds. The concentration that produced 50% inhibition of cell growth with a 95% CL was obtained from a non-linear regression using GraphPad Prism 8 software (San Diego, CA, USA).[21,28]

UVA Irradiation and Cell Viability Assay

The normal human melanocytes (HFB-4) cells were incubated in a 96-well plate for 24 hours to allow cell adhesion to occur. Afterwards, the cells were treated with varying concentrations (3–12 µg/mL) from ADA either free or in formulations (ADA-MS-EUD and ADA-MS-EUC) for 24 hours.[29] In order to test the effect of ADA and ADA microsponges formulations on UVA-induced cytotoxicity on normal melanocytes, HFB-4 cells treated with the indicated doses of ADA and ADA-MS were irradiated with 64.8 J/cm^2 of UVA radiation; using UV lamp (Biotech, Vl-L.C., Spain)

at 365 nm for 6 hours.[30] Afterwards, the cells were washed and cell viability was determined using a regular MTT assay. The relative cell viability was determined by the amount of formazan salt formed. The number of viable cells was determined by measuring the optical density at 570 nm using a microplate reader (BioTek ELX800, USA). The quantification of cell viability was through using the percentage viable in comparison with control. The data were expressed as mean from at least triplicate independent experiments. The results were analysed using one-way and two-way ANOVA and $P < 0.05$ was considered significant, utilizing GraphPad Prism 8 software (San Diego, CA, USA).[31]

Antimicrobial Activity Assay

The antibacterial activity of pure ADA either free or in MS formulae (ADA-MS-EUD and ADA-MS-EUC) against *P. acnes* (ATCC 6919) were examined using various concentrations of the ADA (6–500 µg/mL) against respective control. The bacteria were cultivated on reinforced clostridial medium (RCM) agar for 48–72 h at 37°C. In 96-well plate, 100 µL of RCM broth was added into each well. A 100 µL of each formulation dispersed in RCM broth was added to the first well and the drug suspension was serially diluted with RCM (2-fold dilution). Following that, the culture was diluted to 100-fold. Finally, the *P. acnes* suspension adjusted to half McFarland (1×10^8 cfu/mL) was added to each well. Further, the plates were incubated at 37°C under anaerobic condition. Each investigation was performed in triplicates. After 48 hours incubation, the minimal inhibitory concentration (MIC) was determined from the absorption measured spectrophotometry at 570 nm using a microplate reader (BioTek ELX800, USA).[32,33] The statistical analysis of linear regression was performed and 95% CL was obtained using the GraphPad Prism 8 software (San Diego, CA, USA).

Results and Discussion

Data Modelling

Using experimental design (DOE) such as Plackett Burman design (PBD) is an effectual statistical technique for screening to pinpoint the significant variables amongst a vast number of factors that influence a procedure using a low number of experimental runs.[23]

PBD was performed in the current study for the most frequently used independent parameters in the MS formulation process with the aim of achieving the maximum entrapment efficiency and production yield percentage, together with minimizing the particle size, and electing the best particles morphology. The design generated 12 runs of different combinations for formulation process.

The effect of independent factors on the dependent variables is shown in Table 2 and Figure 1. It is obvious that the characterized microsponges entrapment efficiency varied between 38.48% and 99.76%, also the yield percentage oscillated from 33.2% to 93.14%. As for particle size, it ranged from 14.97 µm to 162.6 µm and lastly the morphology mixt between the three categories of low (1), average (2) and high homogeneity (3).

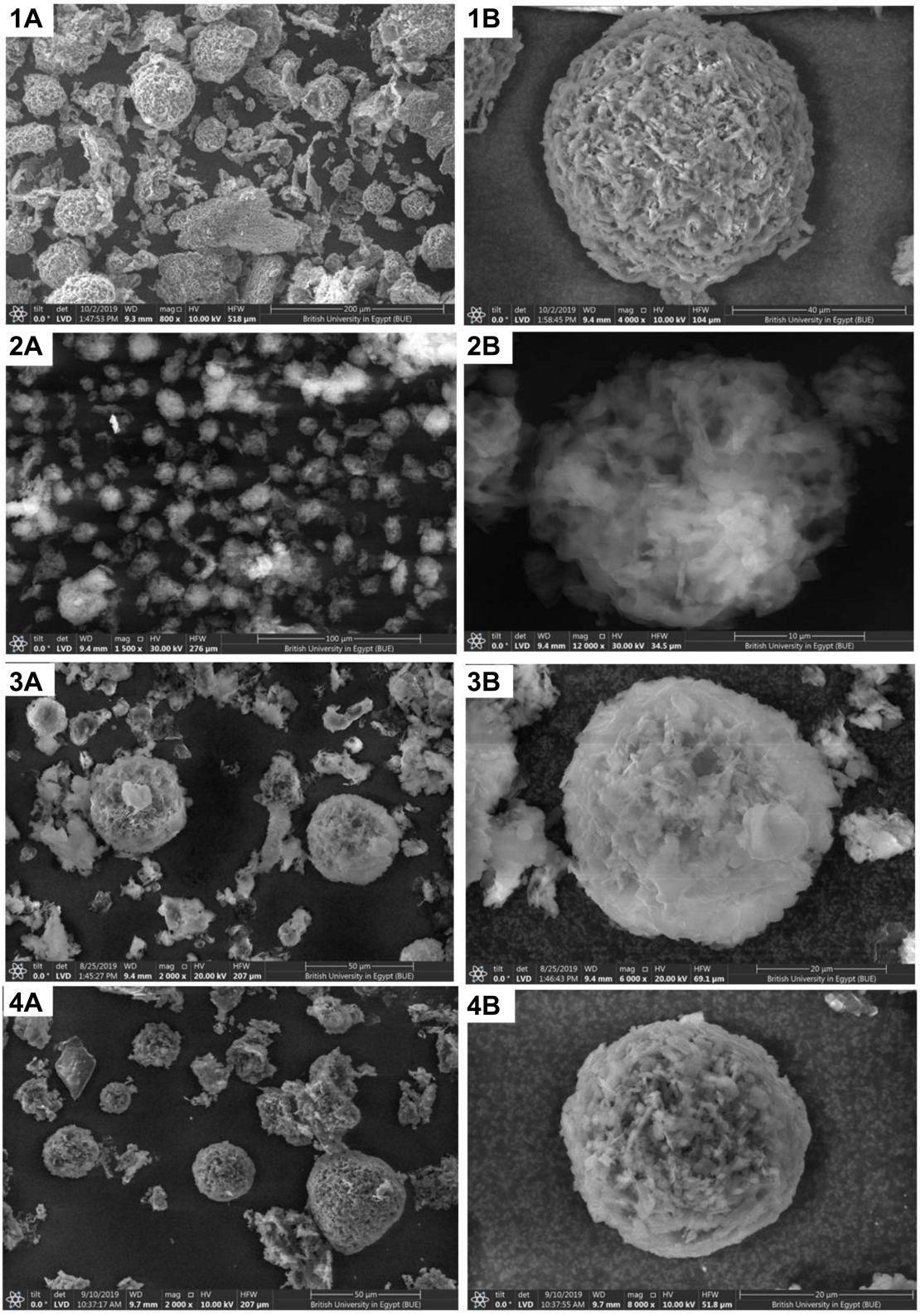
Statistical analyses of variance (ANOVA) of the responses are presented in Table 3, accordingly all the four models were considered appropriate to navigate the design space and from the design, polynomial equations were generated. The equations of the fitted model are given in coded variable as follows:

$$Entrapment\ Efficiency\ \%\ (Y_1) = 79.81 - 1.05A + 1.39B + 2.18C + 14.41D + 4.00E - 9.54F - 0.69G + 12.23DF \quad (3)$$

$$ProductionYield\%(Y_2) = 72.08 - 0.82A + 0.57B + 0.64C + 11.35D + 8.4E - 7.28F + 0.56G - 4.27BG + 10.22DF + 4.44FG \quad (4)$$

$$Particle\ Size\ (Y_3) = 51.75 - 21.81A + 0.47B - 27.22C + 17.19D - 6.21E + 6.08F + 6.83G + 8.36AC - 17.62AF \quad (5)$$

$$Morphology\ (Y_4) = 1.92 + 0.25A + 0B + 0.37C - -0.75D - -0.083E + 0.29F - -0.17G + 0.62AC + 0.37AF \quad (6)$$



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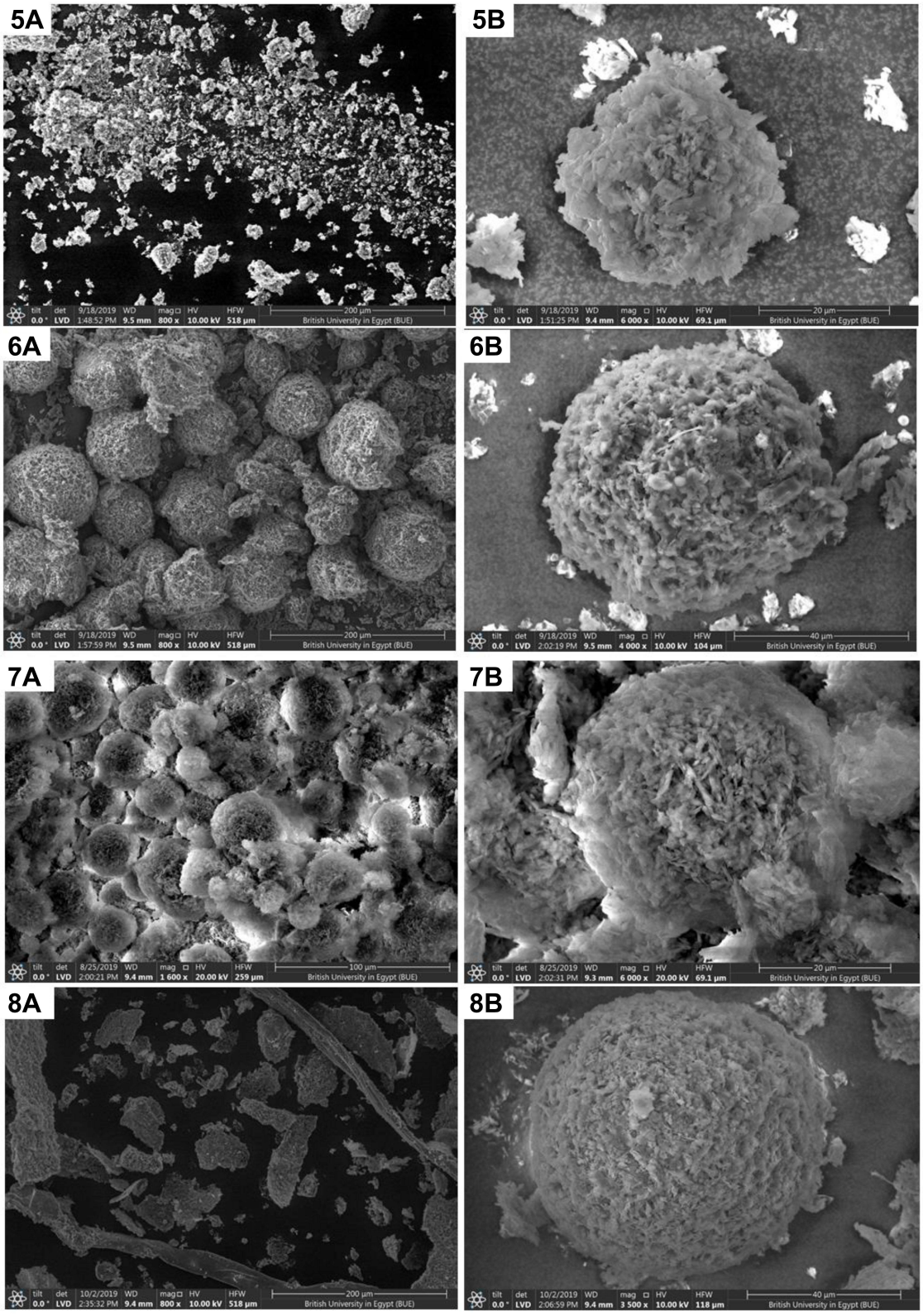

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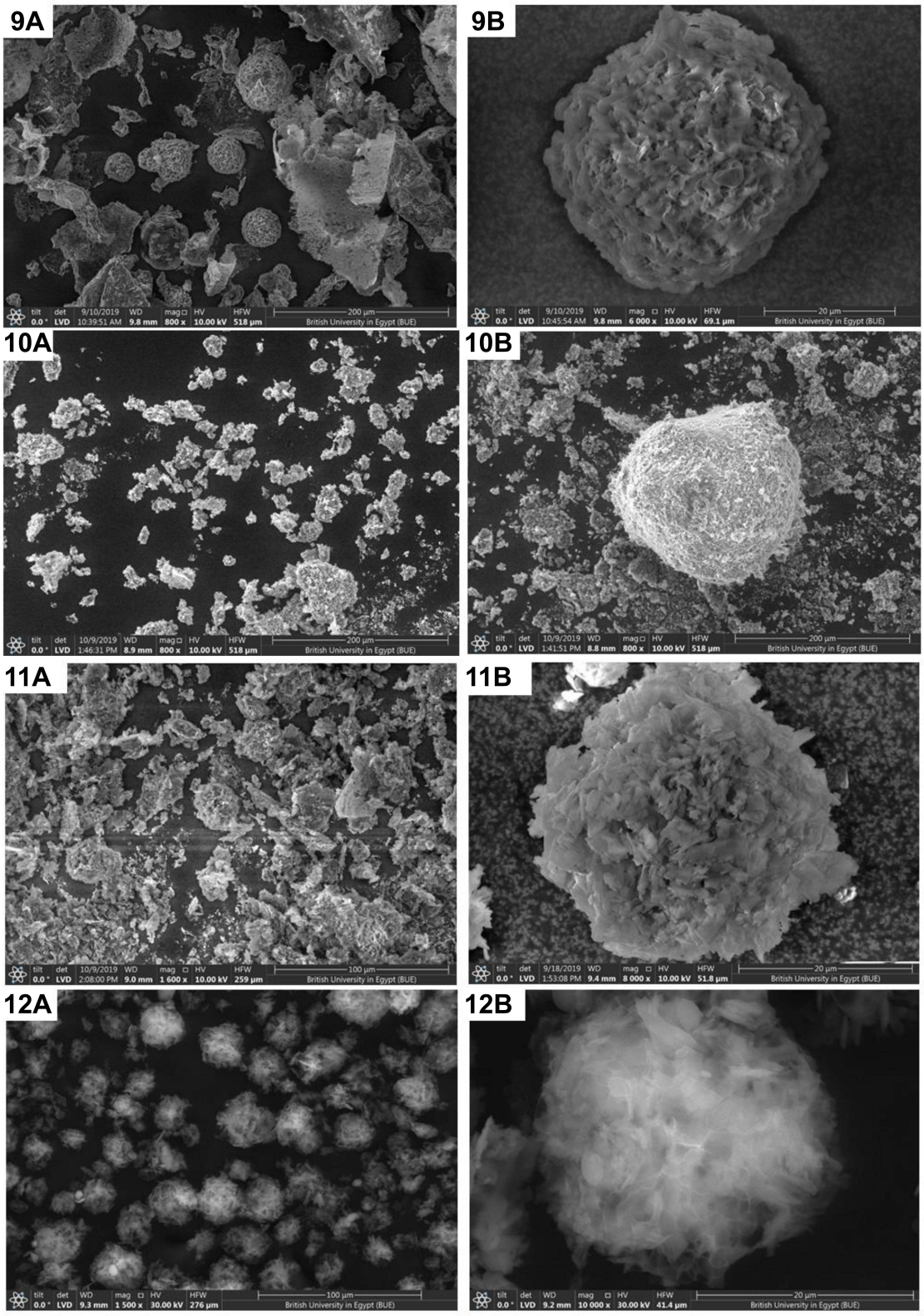

Figure 1 The SEM images of F1 to F12, where 1A to 12A showing images of the field and 1B to 12B showing images of a single microsponge particle.

Table 3 Statistical Analysis of Variance (ANOVA) of the Responses (Y1−Y4)

Independent Variable	Y1		Y2		Y3		Y4	
	Coefficient Estimate	P-value	Coefficient Estimate	P-value	Coefficient Estimate	P-value	Coefficient Estimate	P-value
Model		0.0004*		0.0314*		0.0293*		0.0814
A	−1.05	0.1211	−0.82	0.2356	−21.87	0.0112*	0.25	0.0955
B	1.39	0.0659	0.57	0.3039	−2.3	0.8831	0	1
C	2.18	0.0211*	0.64	0.2938	−27.22	0.009*	0.37	0.0565
D	14.41	<0.0001*	11.35	0.0165*	19.97	0.0263*	−0.75	0.018*
E	4	0.0039*	8.41	0.024*	−9	0.1607	−0.083	0.5
F	−9.54	0.0002*	−7.28	0.0234*	3.3	0.1439	0.29	0.0887
G	−0.69	0.2534	0.56	0.2826	9.62	0.1383	−0.17	0.2441
	(**DF**) 12.23	0.0003*	(**DF**) 10.22	0.0224*	(**AF**) −7.62	0.0369*	(**AC**) 0.62	0.0377*
R^2	0.9985		0.9998		0.9934		0.9813	
Adi. R^2	0.9947		0.9982		0.9638		0.8972	
Adeq. Percision	46.524		75.302		19.557		8.222	
SEE	1.52		0.83		12.94		0.29	
MAE	78.81		72.08		51.75		1.92	

Notes: *Significant value with $p < 0.05$. A: volume of organic phase, B: sonication time, C: stirring speed, D: drug percent E: polymer type, F: emulsifier concentration, G: method of organic phase addition. Y1: entrapment efficiency, Y2: yield percentage, Y3: particle size, Y4: morphology.

Influence of Investigated Parameters on Entrapment Efficiency (Y_1)

As shown in Table 2, the entrapment efficiency of the prepared ADA-MS formulations varied from 38.48% in F12 to 99.76% in F11. The significant positive effect of drug % on E.E.% shown in Table 3 may be explained by the fact that increasing drug amount concurrently decreases the amount of polymer used leading to decrease the viscosity of the medium, which allows easier diffusion of drug moiety and the formation of a more flexible polymer coat.[34] Also, the favoured polymer was Ethyl cellulose because of the differences in the polymers' structures, swelling capacities and hence the loading capacities between the used polymers.[35–37]

Additionally, the emulsifier concentration (F) significant antagonist effect on the E.E.% was probably because the increase in the PVA concentration has been reported to increase the pore size of the MS leading to an increase in leaching of drug moiety.[38,39] Together with, the fact that ADA being a hydrophobic drug and PVA being a non-ionic emulsifying agent, so in high concentration of PVA hydrophobic regions may be formed, which would be able to dissolve amounts of the ADA leading to decreased drug content.[40] A lesser amount of PVA led to a decreased drug solubility in the external phase, which contributed in minimizing the total drug loss.[24,40] Subsequently, the interaction between the drug percentage and the emulsifier concentration (DF) (Figure 2A) was also found to have a positive significant effect.

High-speed rates have been reported to increase the mechanical shear strength, which, as a result, uniformly and rapidly disperse droplets, facilitating MS particles formation;[24,41] therefore, in our study it had a direct significant effect on E.E.%.

Although being insignificant the increase in sonication time (B) had a positive effect on the encapsulation efficiency. This may be due to the fact that high sonication time decreases the particle size of the drug moiety and, hereafter, contributes to enhancement of the drug loading in the microsponges.[34]

Influence of Investigated Parameters on Production Yield (Y_2)

The P.Y.% refers to the amount, expressed as percentage, of dry powder of microparticles compared to the amount of drug and polymer added.[26] The production yield percent is highly correlated with the entrapment efficiency. Therefore, the increase in E.E.% usually was accompanied by increase in P.Y.%; that is why it was found in this study that the best

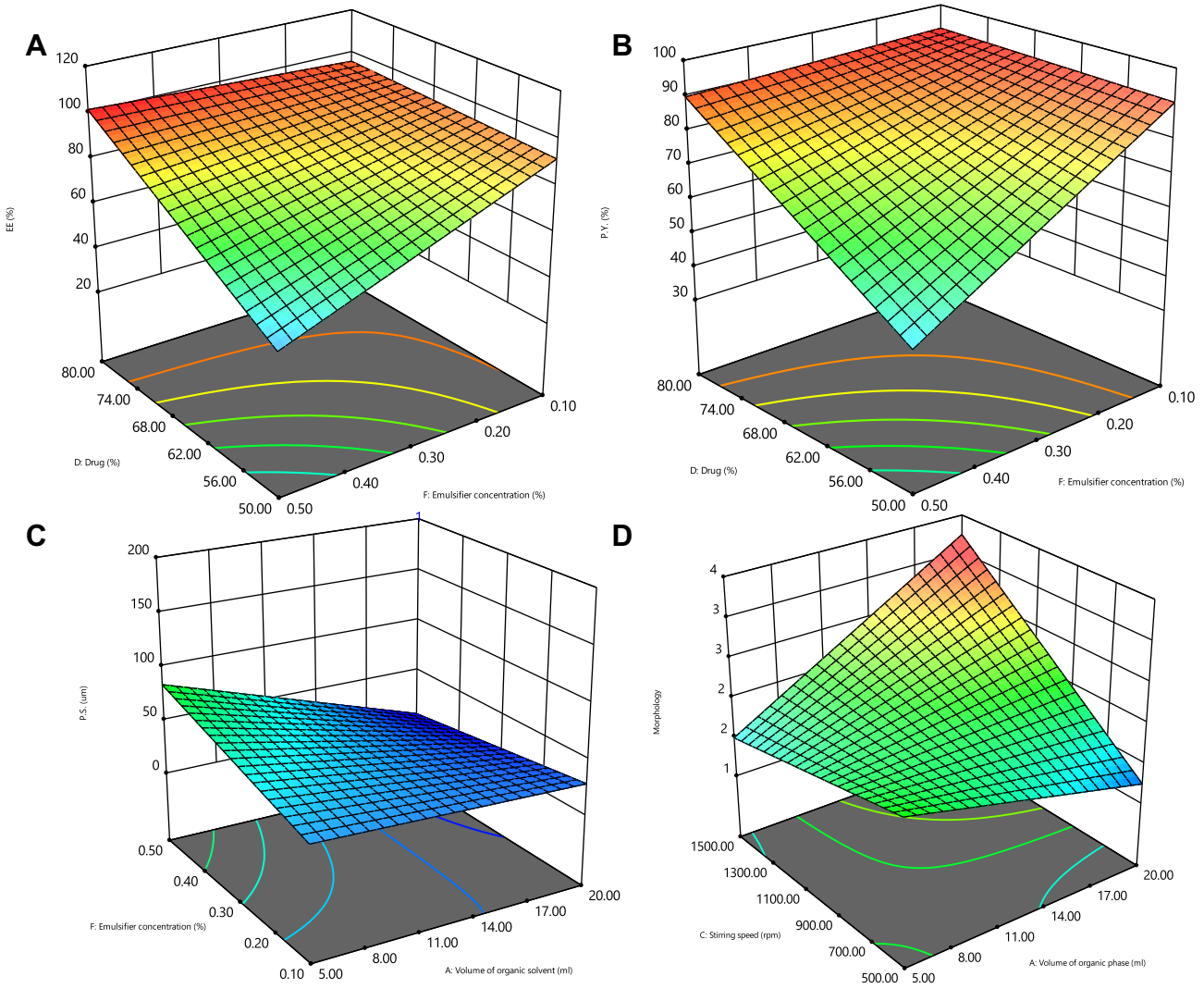

Figure 2 Three-dimensional (3D) of Plackett Burman design generated response surface plots of: (**A**) entrapment efficiency percentages in terms of DF, (**B**) production yield percentage in terms of DF, (**C**) particle size in terms of AF, and (**D**) morphology in terms of AC. The change in colour from blue-green-red indicates increase in response value.

P.Y.% in the prepared ADA-MS formulations was also in F11 (93.14%), while the lowest was in F12 (33.2%). The independent variables affecting P.Y.% were highly similar to those of the E.E.%.

The independent variable that exhibits the highest direct significant action on P.Y.% was also the drug percentage (D). This may possibly be due to the lowered diffusion rate of the organic solvent (DCM) from concentrated solutions into the aqueous phase, by the effect of polymer viscosity, which provides additional time for the droplet formation and as a result an improvement in the yield of microparticles occur.[24,42] Also, the increase in entrapment efficiency means an increase in the amount of drug entrapped in MS and thereby the overall yield will increase. These results are consistent with the findings found in various studies.[34,35,43] Additionally, the effect of polymer type (E) was found significant. This is most likely due to the connection between the E.E.% and P.Y%, so with the increased E.E.% when using EC as a polymer it subsequently caused better yield percentage of the formulation containing it.

Likewise, the emulsifier concentration (F) factor had an antagonist significant effect on the P.Y.%. These results are in agreement with the findings of Zaenglein et al 2016; Nokhodchi et al 2007; Kumari et al 2018; and Ivanova et al 2019. This may be attributed to the abridged DCM diffusion rate from the concentrated organic phase to the aqueous one, which provides extra time for droplet formation eventually leading to enhanced yield percent.[35,36] However, further increase in the PVA concentrations lead to an inverse effect. This is pertaining to the formation of hydrophobic regions

which hinders the drug loading process causing a reduction in the total drug content and hence a lowered P.Y.% within MS formulae.[24] Correspondingly, the interaction between the drug percentage and the emulsifier concentration (DF) (Figure 2B) was also found significant. An increase in this interaction brought a desirable significant increase in P.Y. %.

Nevertheless, the effect of volume (A) had an antagonistic insignificant effect. Yet, the decrease of the volume had a positive effect on the yielded percentage. This agrees with the findings of Junqueira et al 2020, where lower volumes of DCM yielded greater amounts of MS.[43]

Influence of Investigated Parameters on Particle Size (Y_3)

One of the momentous features of microsponges is its particles size as it influences most of its characteristics. The particle size (P.S.) of the prepared ADA-MS formulations ranged between 14.97 ± 4 µm in F2 and 162.6 ± 37.4 µm in F10.

The factor with the upmost effect on particle size was the stirring speed (C) showing high antagonistic significance. Where, the increase in stirring rate from 500 to 1500 rpm decreases the particle size. This was owing to occurrence of rapid dispersion of the droplets by the effect of speed, which would probably cause a lesser incidence of coalescing into larger droplets. Also, the high-speed rates resulted in higher kinetic energy and agitation forces, which break down the droplets into smaller ones.[24] These results consent with the conclusions of the researchers who investigated the same parameter, where the increase in rpm resulted in a significant decrease in the P.S.[26,34,41,44] Conversely, an increased exposure to high agitation force by the effect of elevated rpm for prolonged periods (4 to 6 hours) has been reported to increase the tendency of both small and large particles to bind together forming larger P.S.[35,45] Yet, this was not the case in our study as the stirring time was fixed to a reasonable period of time (2 hours).

The effect of drug percentage (D) also had a positive significant influence on P.S. This may be elucidated by the effect of intermolecular cohesive forces between drug and polymer moieties,[46] especially that ADA being hydrophobic in nature will be more likely to bind with the polymer than to migrate into the aqueous phase. This agrees with the findings of Salah et al 2018.[25]

Furthermore, the volume of organic phase (A) had an inverse significant effect on P.S. The effect of microenvironment viscosity could be the reason behind this, where the higher the volume of DCM, the lower the viscosity of the internal phase, and as result smaller globules of emulsion are formed producing MS with low diameter.[24,46] Opposite to the higher viscous media, where large globules are formed requiring high energy to divide, resulting in increased size of particles.[25,34,37,47]

In conjunction with this, the interaction between the volume of organic phase and the emulsifier concentration (AF) (Figure 2C) was found significant, despite the fact that the emulsifier concentration (F) alone was found insignificant. In addition, an increase in this interaction resulted in an inverse effect on the particle size. In other words, when the organic phase (DCM) volume is increased and the emulsifier agent (PVA) concentration increases, the combination has a tremendous effect of minimizing the particle size. This interaction has been previously reported by Patel et al 2016,[26] although it had a minor effect on the P.S. in their study. This could be interpreted by the surface tension phenomenon,[25,36] where the increase of the PVA concentration resulted in lowering of the surface tension of the water phase, accompanied by the formation of smaller globules of emulsion by the effect of PVA and lowered viscosity of the organic phase, ultimately resulting in decreased particle size.

Influence of Investigated Parameters on Morphology (Y_4)

As shown in Figure 1, four out of the twelve prepared ADA-MS formulations showed the best morphology (scale 3) due to the presence of a large sum of homogenous spherical microsponges particles (F2, F6, F7 and F12), and only three formulations showed average quality (scale 2) of spherical MS (F1, F4 and F9). While the rest of the formulation showed a much lower amount of regular MS with high number of irregular ones and fragmentations (scale 1). The surface of the MS showed a highly porous structure as an effect of DCM evaporation. The ADA crystals were found in both the core and surface of MS indicating efficient drug loading.

It was revealed that the most significant antagonist factor affecting the morphology of microsponges (Y_4) was the drug percentage (D). The increase in the drug concentration resulted in a less elegant morphology of the microsponges. This could be because at higher drug concentration, the amount of polymer available to surround the drug moiety is lowered and consequently less homogenous microsponges are formed.

The interaction between the organic phase volume and the stirring speed (AC) (Figure 2D) was found significant, although each factor on its own was found insignificant. The additive effect of increasing both the organic phase volume to 20 mL and stirring speed to 1500 rpm caused a noteworthy effect on the enhancement of particles morphology. This could be clarified by the effect of these parameters on particle size as higher volume of DCM with a high rotation speed result in fine droplets of emulsion which is later transformed into microsponges particles with regular homogenous shape.[24,48,49]

On the other hand, the polymer type (E) had an insignificant effect on the morphology of particles. Nonetheless, it is noticeable that the formulations with the finest morphology were all prepared using Eudragit RS100 as a polymer. This might be as a result of the advanced technologies used in the synthesis of the polymer, tailoring its functional groups probably helped in forming elegant MS.[50]

With the purpose of achieving the constraints of this study, we reached the preferable parameters which were: a sonication time of 10 minutes, a stirring speed of 1500 rpm, the method of organic phase addition to be portionwise, as well as an emulsifier (PVA) concentration of 0.1% for the development of ADA-MS formulation. On the other hand, the polymer type, volume of organic phase and drug percent can be subsequently examined in a further optimization study.

In vitro Biological Activity Assays for Selected Prepared ADA-MS

In order to further explore the effect of encapsulation of ADA into microsponges system utilizing the two polymers EC or EUD (ADA-MS-EC and ADA-MS-EUD, respectively), F4 and F7 were selected. As both formulae attained almost similar dependent and independent variables.

Cytotoxicity Assay

ADA has been previously reported to attain an anticancer effect on various cancer cell lines including melanoma cells; human melanoma cell lines A375 and M14.[21] Therefore, the human epidermoid carcinoma cell line A431 and the human melanoma cell line M10 were selected to investigate the topical anticancer activity of ADA, ADA-MS-EC and ADA-MS -EUD, by the determination of their IC_{50} (95%, CI).

As shown, Figure 3 and Table 4 shows the results of the MTT cell proliferation assay. The ADA alone was highly effective against cancerous cells by an IC_{50} of 14.54 µg/mL and 38.05 µg/mL on A431 and M10 cell lines, respectively. Even though the effect on A431 cell seems better than that of M10, yet the difference is insignificant. An interesting finding to this variation is that unlike melanoma, the squamous cell carcinoma is occasionally associated with chronic immuno-inflammatory processes[51] and ADA has been reported to exhibit an immunomodulatory potential.[18]

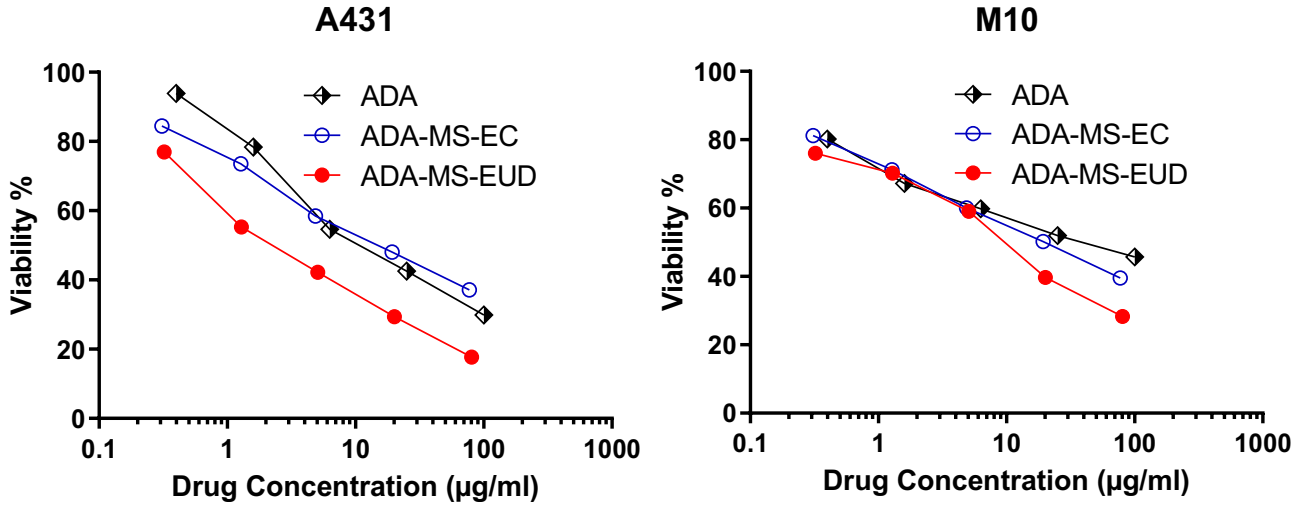

Figure 3 Cytotoxicity profile of the free ADA, ADA-MS-EC and ADA-MS-EUD against A431 and M10 cell lines.

Table 4 In vitro Cytotoxicity Results on Different Cell Lines

Cell Line	Treatment	IC_{50} µg/mL ± S.D.	95% CL
A431	**Adapalene**	14.54 ± 0.86	6.761–31.28
	ADA- MS - EC	16.4 ±1.05	10.54–25.52
	ADA- MS - EUD	2.765 ± 0.22	1.768–4.316*
M10	**Adapalene**	38.05 ± 1.91	15.6–92.77
	ADA- MS - EC	19.72 ± 1.26	15.36–25.32
	ADA- MS - EUD	8.811 ± 0.52	5.257–14.77*

Note: *Significant difference compared with both Adapalene and ADA-MS-EC.

On the other hand, the ADA-MS-EC had an IC_{50} of 16.4 µg/mL on A431 cell line, although it has a higher value than that of ADA alone, but the difference is insignificant. Also, on the M10 cell line, the IC_{50} was 19.72 µg/mL, despite being lower than that of pure drug, the variation was also found insignificant. This may be because of the encapsulation effect, in which the Ethyl cellulose polymer, which is biologically inert in nature, formed the matrix that releases the drug in a controlled manner.[52]

While encapsulation of ADA into MS using EUD had a remarkably significant effect on the viability of A431 cells compared to the both ADA and ADA-MS-EC with an IC_{50} of 2.765 µg/mL (5.26, 5.93 folds decrease in IC_{50}, respectively). This reduction in cell viability and enhancement of the cytotoxic effect of the ADA loaded microsponges using EUD provoked further testing of Eudragit RS100 alone on A431 cell line. It was found that the EUD achieved a moderate cytotoxic effect with an IC_{50} of 86 ±4.38 µg/mL. In a similar manner, the ADA-MS-EUD had a highly significant effect on cancerous cell viability of the M10 cell line with an IC_{50} of 8.811 µg/mL (4.3, 2.24 folds reduction in IC_{50}). The revealed EUD cytotoxic activity together with, the knowledge of MS attaining high lipophilicity that increases their contact time with cells,[6] eventually lead to an augmented action on the cancerous cells.

UVA Irradiation and Cell Viability Assay

Adapalene was reported to produce serious irritation in the skin cells when exposed to sunlight.[53] The photoprotection process is the main function of melanocytes, which are located in the last epidermal skin layer "basal layer".[54] The UVA radiation unlike UVB is capable of deeply penetrating the skin layers reaching the level of the dermal-epidermal junction of the skin.[55]

The quantification of cell viability on HFB-4 cells after UVA irradiation was through using the percentage in comparison with control. Based on a preliminary study, the cytotoxic dose of ADA on HFB-4 cells was determined, and the HFB-4 cells were treated with various sub-cytotoxic concentrations of ADA (3, 6, 12 µg/mL), in free form and in MS forms (ADA-MS-EUD and ADA-MS-EC). Statistical analysis of the data was performed using both two-way ANOVA for the whole model and one-way ANOVA for each concentration. Two-way ANOVA revealed that there was an exceedingly significant difference between all treatments ($p < 0.0001$) and between the various used concentrations ($p < 0.0001$), as well as the interaction between treatment and concentrations ($p = 0.005$). This indicated that the increase in ADA concentration led to a significant reduction in cell viability, which is consistent with the reported irritation effect upon UV light exposure.[56]

In order to study the individual effect of each treatment, one-way ANOVA followed by Tukey's multiple comparisons test was conducted for each concentration. As demonstrated in Figure 4, the ADA-MS-EUD, presented a highly significant enhancement in relative cell viability percent in all tested concentrations (90.5 ± 4.59%, 86.5 ± 4.04% and 80.5 ± 4.91%) compared with that of their respective ADA relative cell viability percent (76% ± 3.18, 68.9% ± 3.33 and 64% ± 4.79) ($p < 0.0005$). While the ADA-MS-EC accomplished an exceptionally significant difference in relative cell viability percent (110 ± 3.76%, 112.2 ± 4.62% and 94.6 ± 5.87%) compared with both the ADA and ADA-MS-EUD in all concentrations ($p < 0.0001$), achieving the upmost cell protection effect.

These interesting findings could be interpreted by the fact that encapsulation of the drug moiety into the MS formulation reduces the direct contact with the whole dose of ADA and as a result the irritation effect upon exposure

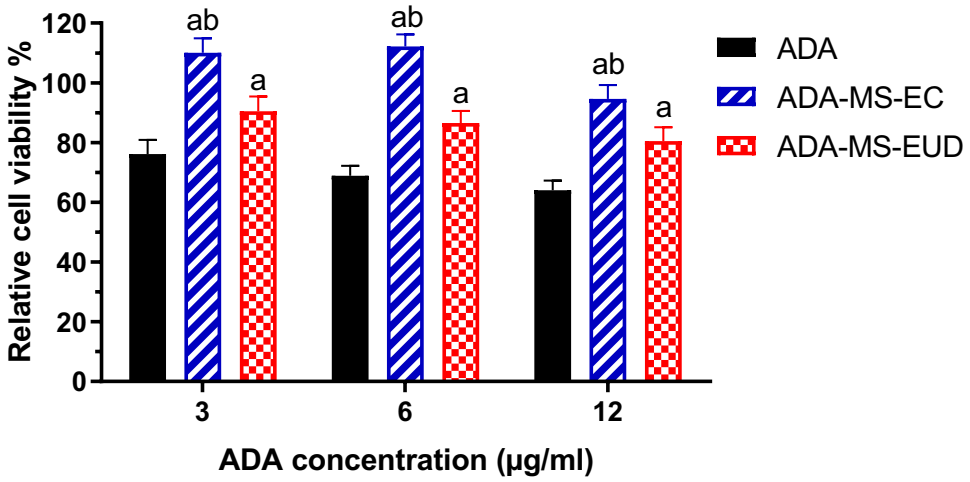

Figure 4 Cell viability assay after UVA irradiation and exposure to different concentrations of Adapalene in form of the free ADA, ADA-MS-EC and ADA-MS-EUD against HFB-4 cell line. Results were compared for each concentration using one-way ANOVA followed by Tukey's post hoc test. [a]$p < 0.0005$ compared with respective ADA relative cell viability percent, [b]$p < 0.0001$ compared with respective ADA-MS-EUD relative cell viability percent.

to UVA radiation on the normal skin cells is significantly minimized[2,57] and thereby the overall cell viability was heightened. On the other hand, the EUD was found in the previously mentioned cytotoxicity study to have a slightly cytotoxic effect on cells, while the Ethyl cellulose is biologically inert, this would explain the substantial difference in relative cell viability percent between MS formulations using EUD and EC.

Antimicrobial Activity Assay

To the extent of our knowledge, the ADA antibacterial activity solely has not been thoroughly investigated.[18] But it has been reported to enhance the antimicrobial activity when used in combination with other active ingredients in formulations.[32] In the present study, the antimicrobial potential of ADA alone and in MS formulation was investigated. The minimum inhibitory concentrations (MIC) of ADA alone, ADA-MS-EC and ADA-MS-EUD were evaluated against *P. acne*. Perceiving that the main mechanism of action of ADA in treating acne is through targeting the RAR receptors

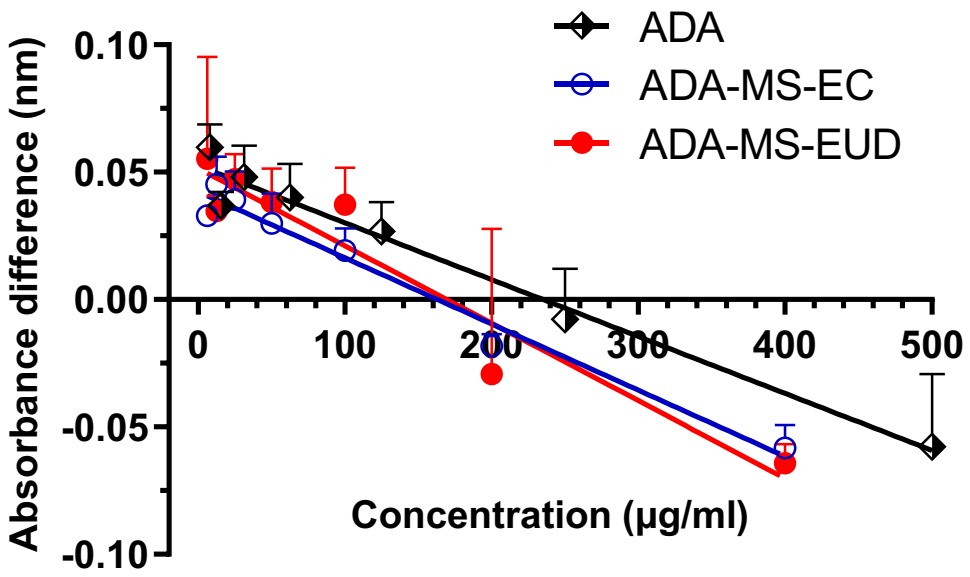

Figure 5 Minimum inhibitory concentration determination after exposure to different concentrations of ADA, ADA-MS-EC and ADA-MS-EUD against *P. acne*.

not via antimicrobial activity. As shown in Figure 5, the linear regression analysis indicated that free ADA exhibits an antimicrobial activity with a MIC of 235 µg/mL (CL 201.2: 275.7). As for the microsponges formulations whether using EUD or EC, it presented a reduction in the MIC 169.4 µg/mL (CL 126.0: 226.2), and 163 µg/mL (CL 138.6: 190.8) respectively. The results revealed that there was no significant difference between the ADA and ADA-MS-EUD, and a significant difference between ADA and ADA-MS-EC. Nevertheless, the variation in results could be explained by the MS being composed of hydrophobic polymers, interacts with the microbial membrane and destabilizes it, which thereby causes an improvement in the antibacterial activity. Also, the difference in the hydrophobic power of each polymer led to variability in antimicrobial efficacy.[58]

Conclusion

In the current study, Adapalene loaded microsponges were successfully prepared using either Ethyl cellulose or Eudragit RS100 as polymers. Also, the study revealed that indeed multiple processing factors significantly affect the formulation of ADA-MS, and that they could be tailored accordingly. Interestingly, the MS system showed promising significant effect on the advance of ADA cytotoxic activity on cancerous cell line A431 and M10, with an enhanced protection of normal cell line HFB-4, together with an augmented antibacterial activity against *P. acnes*. Henceforth, microsponges could be considered as propitious carrier system for ADA delivery achieving rewarding effects on in vitro biological activities for ADA, encouraging further optimization studies.

Data Sharing Statement

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

The authors declare that they have no competing interests.

References

1. Jadhav N, Patel V, Mungekar S, Bhamare G, Karpe M, Kadams V. Microsponge delivery system: an updated review, current status and future prospects. *J Sci Innov Res*. 2013;2(6):1097–1110.
2. Kappor D, Patel M, Vyas RB, Lad C, Tyagi BL. A review on microsponge drug delivery system. *J Drug Deliv Therap*. 2014;4(5):29–35. doi:10.22270/jddt.v4i5.978
3. Patel EK, Oswal RJ. Nanosponge and micro sponges: a novel drug delivery system. *Int J Res Pharm Chem*. 2012;2(2):2281–2781.
4. Akhgari A, Tavakol A. Prediction of optimum combination of Eudragit RS/Eudragit RL/ethyl cellulose polymeric free films based on experimental design for using as a coating system for sustained release theophylline pellets. *Adv Pharm Bull*. 2016;6(2):219. doi:10.15171/apb.2016.030
5. Singhvi G, Manchanda P, Hans N, Dubey SK, Gupta G. Microsponge: an emerging drug delivery strategy. *Drug Dev Res*. 2019;80(2):200–208. doi:10.1002/ddr.21492
6. Mahant S, Kumar S, Nanda S, Rao R. Microsponges for dermatological applications: perspectives and challenges. *Asian J Pharm Sci*. 2020;15(3):273–291. doi:10.1016/j.ajps.2019.05.004
7. Lewis GA, Mathieu D, Phan-Tan-Luu R. Mixtures in a constrained region of interest. In: *Pharmaceutical Experimental Design*. New York: Marcel Dekker; 1999:413–454.

8. Rahman Z, Zidan AS, Habib MJ, Khan MA. Understanding the quality of protein loaded PLGA nanoparticles variability by Plackett–Burman design. *Int J Pharm*. 2010;389(1–2):186–194. doi:10.1016/j.ijpharm.2009.12.040
9. Singh B, Ahuja N. Pharmaceutical experimental design (Drugs and the Pharmaceutical Sciences, Vol. 92), Edited by GA Lewis, D. Mathieu and R. Phan-Tan-Luu, Marcel Dekker, New York, 1999. vi+ 498 pp., 23.5 x15. 5 cm., 1.9 lb., Hardcover, ISBN 0-8247-9860-0, Price $175.00. *Int J Pharm*. 2000;195(1–2):247–248. doi:10.1016/S0378-5173(99)00384-1
10. Plackett RL, Burman JP. The design of optimum multifactorial experiments. *Biometrika*. 1946;33(4):305–325. doi:10.1093/biomet/33.4.305
11. El-Say KM, Abdelaziz AE, Samy AM, Kassem AA. Screening study for formulation variables in preparation of diclofenac sodium niosomes using Plackett – Burman design. *Azhar J Pharma Sci*. 2008;38:36–53.
12. Badawi N, El-Say K, Attia D, El-Nabarawi M, Elmazar M, Teaima M. Development of pomegranate extract-loaded solid lipid nanoparticles: quality by design approach to screen the variables affecting the quality attributes and characterization. *ACS Omega*. 2020;5(34):21712–21721. doi:10.1021/acsomega.0c02618
13. Jayakumar M. When and how to use Plackett-Burman experimental design. iSixSigma; 2013. Available from: https://www.isixsigma.com/tools-templates/design-of-experiments-doe/when-and-how-to-use-plackett-burman-experimental-design/. Accessed September 10, 2022.
14. Irby CE, Yentzer BA, Feldman SR. A review of adapalene in the treatment of acne vulgaris. *J Adolesc Health*. 2008;43(5):421–424. doi:10.1016/j.jadohealth.2008.06.005
15. Waugh J, Noble S, Scott LJ. Adapalene. *Drugs*. 2004;64(13):1465–1478. doi:10.2165/00003495-200464130-00005
16. Czernielewski J, Michel S, Bouclier M, Baker M, Hensby C. Adapalene biochemistry and the evolution of a new topical retinoid for treatment of acne. *J Eur Acad Dermatol Venereol*. 2001;15(SUPPL. 3):5–12. doi:10.1046/j.0926-9959.2001.00006.x
17. Piskin S, Uzunali E. A review of the use of Adapalene for the treatment of acne vulgaris. *Ther Clin Risk Manag*. 2007;3(4):621.
18. Rusu A, Tanase C, Pascu GA, Todoran N. Recent advances regarding the therapeutic potential of Adapalene. *Pharmaceuticals*. 2020;13(9):217. doi:10.3390/ph13090217
19. Treesirichod A, Chaithirayanon S, Wongjitrat N, Wattanapan P. The efficacy of topical 0.1% Adapalene gel for use in the treatment of childhood acanthosis nigricans: a pilot study. *Indian J Dermatol*. 2015;60(1):103. doi:10.4103/0019-5154.147838
20. Herane MI, Orlandi C, Zegpi E, Valdés P, Ancić X. Clinical efficacy of Adapalene (Differin®) 0.3% gel in Chilean women with cutaneous photoaging. *J Dermatol Treat*. 2012;23(1):57–64. doi:10.3109/09546634.2011.631981
21. Li H, Wang C, Li L, et al. Adapalene suppressed the proliferation of melanoma cells by S-phase arrest and subsequent apoptosis via induction of DNA damage. *Eur J Pharmacol*. 2019;851:174–185. doi:10.1016/j.ejphar.2019.03.004
22. Lauterbach A, Mueller-Goymann CC. Development, formulation, and characterization of an Adapalene-loaded solid lipid microparticle dispersion for follicular penetration. *Int J Pharm*. 2014;466(1–2):122–132. doi:10.1016/j.ijpharm.2014.02.050
23. Thiruchelvi R, Venkataraghavan R, Sharmila D. Optimization of environmental parameters by Plackett-Burman design and response surface methodology for the adsorption of Malachite green onto Gracilaria edulis. *Mater Today Proc*. 2020;37(Part 2):1859–1864. doi:10.1016/j.matpr.2020.07.448
24. Jelvehgari M, Siahi-Shadbad MR, Azarmi S, Martin GP, Nokhodchi A. The microsponge delivery system of benzoyl peroxide: preparation, characterization and release studies. *Int J Pharm*. 2006;308(1–2):124–132. doi:10.1016/j.ijpharm.2005.11.001
25. Salah S, Awad GEA, Makhlouf AIA. Improved vaginal retention and enhanced antifungal activity of miconazole microsponges gel: formulation development and in vivo therapeutic efficacy in rats. *Eur J Pharma Sci*. 2018;114:255–266. doi:10.1016/j.ejps.2017.12.023
26. Patel N, Padia N, Vadgama N, Raval M, Sheth N. Formulation and evaluation of microsponge gel for topical delivery of fluconazole for fungal therapy. *J Pharm Investig*. 2016;46(3):221–238. doi:10.1007/s40005-016-0230-7
27. Ivanova NA, Trapani A, Di Franco C, et al. In vitro and ex vivo studies on diltiazem hydrochloride-loaded microsponges in rectal gels for chronic anal fissures treatment. *Int J Pharm*. 2019;557:53–65. doi:10.1016/j.ijpharm.2018.12.039
28. Badawi NM, Teaima MH, El-Say KM, Attia DA, El-Nabarawi MA, Elmazar MM. Pomegranate extract-loaded solid lipid nanoparticles: design, optimization, and in vitro cytotoxicity study. *Int J Nanomedicine*. 2018;13:1313. doi:10.2147/IJN.S154033
29. Park JG, Son YJ, Aravinthan A, Kim JH, Cho JY. Korean Red Ginseng water extract arrests growth of xenografted lymphoma cells. *J Ginseng Res*. 2016;40(4):431–436. doi:10.1016/j.jgr.2016.07.006
30. Chen SJ, Hseu YC, Gowrisankar YV, et al. The anti-melanogenic effects of 3-O-ethyl ascorbic acid via Nrf2-mediated α-MSH inhibition in UVA-irradiated keratinocytes and autophagy induction in melanocytes. *Free Radic Biol Med*. 2021;173:151–169. doi:10.1016/j.freeradbiomed.2021.07.030
31. Ryu BM, Qian ZJ, Kim MM, Nam KW, Kim SK. Anti-photoaging activity and inhibition of matrix metalloproteinase (MMP) by marine red alga, Corallina pilulifera methanol extract. *Radiat Phys Chem*. 2009;78(2):98–105. doi:10.1016/j.radphyschem.2008.09.001
32. Najafi-Taher R, Ghaemi B, Amani A. Delivery of Adapalene using a novel topical gel based on tea tree oil nano-emulsion: permeation, antibacterial and safety assessments. *Eur J Pharma Sci*. 2018;120:142–151. doi:10.1016/j.ejps.2018.04.029
33. Shamma RN, Ad-din IS, Abdeltawab NF. Dapsone-gel as a novel platform for acne treatment: in vitro evaluation and In vivo performance and histopathological studies in acne infected mice. *J Drug Deliv Sci Technol*. 2019;54:101238. doi:10.1016/j.jddst.2019.101238
34. Obiedallah MM, Abdel-Mageed AM, Elfaham TH. Ocular administration of Acetazolamide microsponges in situ gel formulations. *Saudi Pharm J*. 2018;26(7):909–920. doi:10.1016/j.jsps.2018.01.005
35. Moin A, Deb T, Osmani RM, Bhosale R, Hani U. Fabrication, characterization, and evaluation of microsponge delivery system for facilitated fungal therapy. *J Basic Clin Pharm*. 2016;7(2):39. doi:10.4103/0976-0105.177705
36. Devi N, Kumar S, Prasad M, Rao R. Eudragit RS100 based microsponges for dermal delivery of clobetasol propionate in psoriasis management. *J Drug Deliv Sci Technol*. 2020;55:101347. doi:10.1016/j.jddst.2019.101347
37. Pandit AP, Patel SA, Bhanushali VP, Kulkarni VS, Kakad VD. Nebivolol-loaded microsponge gel for healing of diabetic wound. *AAPS PharmSciTech*. 2017;18(3):846–854. doi:10.1208/s12249-016-0574-3
38. Shahzad Y, Saeed S, Ghori MU, et al. Influence of polymer ratio and surfactants on controlled drug release from cellulosic microsponges. *Int J Biol Macromol*. 2018;109:963–970. doi:10.1016/j.ijbiomac.2017.11.089
39. Orlu M, Cevher E, Araman A. Design and evaluation of colon specific drug delivery system containing flurbiprofen microsponges. *Int J Pharm*. 2006;318(1–2):103–117. doi:10.1016/j.ijpharm.2006.03.025
40. Deshmukh K, Poddar SS. Tyrosinase inhibitor-loaded microsponge drug delivery system: new approach for hyperpigmentation disorders. *J Microencapsul*. 2012;29(6):559–568. doi:10.3109/02652048.2012.668955

41. Jain V, Singh R. Dicyclomine-loaded eudragit®-based microsponge with potential for colonic delivery: preparation and characterization. *Trop J Pharma Res*. 2010;9(1):67–72. doi:10.4314/tjpr.v9i1.52039
42. Lee JH, Park TG, Choi HK. Development of oral drug delivery system using floating microspheres. *J Microencapsul*. 1999;16(6):715–729. doi:10.1080/026520499288663
43. Junqueira MV, Calçado SC, de Castro-Hoshino LV, et al. Influence of the ethanol/dichloromethane ratio on the preparation of microsponges composed of ethylcellulose and Eudragit or HPMCphthalate for hydrophilic drug delivery. *J Mol Liq*. 2020;303:112633. doi:10.1016/j.molliq.2020.112633
44. Kumari A, Jain A, Hurkat P, Tiwari A, Jain SK. Eudragit S100 coated microsponges for Colon targeting of prednisolone. *Drug Dev Ind Pharm*. 2018;44(6):902–913. doi:10.1080/03639045.2017.1420079
45. Behan N, O'Sullivan C, Birkinshaw C. Synthesis and in-vitro drug release of insulin-loaded poly (n-butyl cyanoacrylate) nanoparticles. *Macromol Biosci*. 2002;2(7):336–340. doi:10.1002/1616-5195(200209)2:7<336::AID-MABI336>3.0.CO;2-P
46. Nagula RL, Wairkar S. Cellulose microsponges based gel of naringenin for atopic dermatitis: design, optimization, in vitro and in vivo investigation. *Int J Biol Macromol*. 2020;164:717–725. doi:10.1016/j.ijbiomac.2020.07.168
47. Othman MH, Zayed GM, El Sokkary GH, Ali UF, Abdellatif AA. Preparation and evaluation of 5-fluorouracil loaded microsponges for treatment of colon cancer. *J Cancer Sci Ther*. 2017;9(1). doi:10.4172/1948-5956.1000433
48. Mishra MK, Shikhri M, Sharma R, Goojar MP. Optimization, formulation development and characterization of Eudragit RS 100 loaded microsponges and subsequent colonic delivery. *Int J Drug Discov Herbal Res*. 2011;1(1):8–13.
49. Perumal D. Microencapsulation of ibuprofen and Eudragit® RS 100 by the emulsion solvent diffusion technique. *Int J Pharm*. 2001;218(1–2):1–11. doi:10.1016/S0378-5173(00)00686-4
50. Cilurzo F, Selmin F, Gennari CG, Montanari L, Minghetti P. Application of methyl methacrylate copolymers to the development of transdermal or loco-regional drug delivery systems. *Expert Opin Drug Deliv*. 2014;11(7):1033–1045. doi:10.1517/17425247.2014.912630
51. Feller L, Khammissa RAG, Kramer B, Altini M, Lemmer J. Basal cell carcinoma, squamous cell carcinoma and melanoma of the head and face. *Head Face Med*. 2016;12(1). doi:10.1186/s13005-016-0106-0
52. Arya P, Pathak K. Assessing the viability of microsponges as gastro retentive drug delivery system of curcumin: optimization and pharmacokinetics. *Int J Pharm*. 2014;460(1–2):1–12. doi:10.1016/j.ijpharm.2013.10.045
53. Vasanth S, Dubey A, G.s. R, et al. Development and investigation of vitamin C-enriched adapalene-loaded transfersome gel: a collegial approach for the treatment of acne vulgaris. *AAPS PharmSciTech*. 2020;21(2):1–17. doi:10.1208/s12249-019-1518-5
54. Cichorek M, Wachulska M, Stasiewicz A, Tymińska A. Skin melanocytes: biology and development. *Postepy Dermatol Alergol*. 2013;1(1):30–41. doi:10.5114/pdia.2013.33376
55. Arisi M, Zane C, Caravello S, et al. Sun exposure and melanoma, certainties and weaknesses of the present knowledge. *Front Med*. 2018;5:235. doi:10.3389/fmed.2018.00235
56. Thielitz A, Gollnick H. Topical retinoids in acne vulgaris update on efficacy and safety. *Am J Clin Dermatol*. 2008;9:369–381. doi:10.2165/0128071-200809060-00003
57. Ramezanli T, Zhang Z, Michniak-Kohn BB. Development and characterization of polymeric nanoparticle-based formulation of Adapalene for topical acne therapy. *Nanomedicine*. 2017;13(1):143–152. doi:10.1016/j.nano.2016.08.008
58. Tan J, Zhao Y, Hedrick JL, Yang YY. Effects of hydrophobicity on antimicrobial activity, selectivity, and functional mechanism of guanidinium-functionalized polymers. *Adv Healthc Mater*. 2021. doi:10.1002/adhm.202100482