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PREPARATION AND EVALUATION OF MUCOADHESIVE GELLAN GUM *IN-SITU* GELS FOR THE OCULAR DELIVERY OF CARBONIC ANHYDRASE INHIBITOR NANOVESICLES

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ABSTRACT: A novel approach was tested utilizing mucoadhesive in-situ gelling (ISG) Gellan Gum /HPMC solutions containing dispersions of methazolamide (MZA) loaded Spanlastic Vesicular systems (SVs). The tested systems were designed to combine high corneal permeability of SVs as well as ease of application and prolonged eye retention of ISGs. SVs consisted of Span 60 mixed with different ratios of edge activators (EA) (Tween 60, Tween 80, Brij 35 and Brij 58) were initially prepared and examined to select formulae that had small vesicle size and high drug entrapment. The evaluation of SVs systems included measurement of particle sizes, entrapment efficiencies (EE %) and relative deformability. SVs in ISG systems were characterized by in vitro release, viscosity, intraocular pressure (IOP) measurement after their administration to rabbit eyes and histopathological examination. It was found that SVs containing 10% Tween 60 (90:10) in ISG solutions (SG1) produced the highest reduction in IOP with the highest prolongation of MZA effect

INTRODUCTION: Eye contains several protection mechanisms which are responsible for removing foreign objects including drugs from its surface. Eye protection mechanisms represent the main obstacle which decreases the amount of absorbed drug to eye tissues and necessitate the frequent administration of drug solution to the eye and eventually decrease patient compliance. Eye protection mechanisms includes rapid tear turnover with eye blinking, limited corneal permeability and the passage of drug to GIT via nasolacrimal duct reducing the ocular bioavailability of drugs to be not more than 1-5% [1].

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In order to enhance drug ocular bioavailability, several approaches have been utilized to either enhance drug penetration by using penetration enhancers like bile salts, and surfactants or prolong the contact of drugs with eye tissues using inserts or collagen shields. However, the use of penetration enhancers was found to cause corneal damage and ocular inserts were disadvantageous being easily lost during use which eventually lead to patient incompliance [2, 3].

An approach which involve preparing dispersions of drug loaded SVs as a nanovesicular systems [4-6] that consist of a mixture of Spans together with different edge activators (e.g. Tween 20 and Tween 80) was tested aiming to deliver drugs to the posterior segments of the eye. SVs as a nanovesicular systems represent a good drug carrier that can penetrate the compact corneal barrier. Being elastic, it can easily squeeze itself between

corneal cells and penetrate tissues more efficiently, hence increase drug bioavailability in the eye tissues [7].

Gellan gum is a high molecular weight linear anionic extracellular microbial heteropolysaccharide that is secreted by *pseudomonas elodea.* The anionic nature of the polymer is due to the presence of free carboxylate groups which emerge by the deacetylation of polymer backbone. Thus, in presence of mono- and divalent cations, gellan gum undergoes sol-to-gel phase transition [8, 9] and hence it was frequently used in formulating ISG systems that increase the corneal residence time of some drugs e.g. timolol [10].

In order to increase the adherence of ocular drug delivery systems with the eye, mucoadhesive agents (e.g. HPMC) should be incorporated [11] in ISG formulations. These agents would increase the contact time of formulations with eye tissues and consequently drug bioavailability [10].

Methazolamide (MZA) is a slightly soluble weakly acidic sulfonamide derivative. It is indicated for the treatment of glaucoma by inhibiting the action of carbonic anhydrase enzyme. However, it was found to have some serious side effects when taken orally such as allergic reactions (e.g. difficulty of breathing, swollen lips, tongue or face), bleeding, and tremors in the hands or feet, hence, the topical administration of MZA is more preferred in order to reduce such side effects.

However, the poor aqueous solubility of MZA (~1.7mg/ml) [12], as well as its low corneal permeability [13] render its topical delivery inefficient. Different technique have been utilized to enhance the efficiency of MZA topical delivery e.g. MZA cyclodextrin eye drops [14], MZA calcium phosphate nanoparticles [15], MZA cationic nanostructured heterolipid matrices [16], MZA solid lipid nanoparticles [17].

Moreover, MZA ISGs have been formulated using Poloxamer 407/188 ISG solutions and this approach had enhanced drug residence time and increased drug release interval compared to control solution [15].

The aim of work in this paper is to prepare and characterize MZA loaded SVs which consist of a mixture of Span 60 together with different edge activators (Brij 35, Brij 58 Tween 60 and Tween 80) in mucoadhesive ISG Gellan Gum /HPMC solutions to combine the high penetration power of Spanlastic SVs, ease of application of ISG solutions and the prolonged residence time of mucoadhesive gels produced inside the eye.

MATERIAL AND METHODS:

Materials:

Methazolamide (MZA) was purchased from Jiaxing Taixing chemical and Pharma Co. Ltd (Jiaxing, China). Brij35, Brij58, gellan gum, hydroxypropyl methyl cellulose (HPMC), Span60, Tween60, and Tween80 were purchased from Sigma Aldrich chemical Co. (St. Louis, MO, USA). Calcium chloride dihydrate, disodium hydrogen phosphate, absolute ethanol, potassium dihydrogen phosphate, sodium bicarbonate, and sodium chloride were purchased from ADWIC, El-Nasr pharmaceutical CO. (Cairo, Egypt). Benoxinate hydrochloride eye drops 0.4 % (w/v) was purchased from Egyptian International Pharmaceutical Industries (EIPICO, Cairo, Egypt).

Preparation of MZA-loaded SVs:

Methazolamide loaded Spanlastics vesicular systems (SVs) composed of Span 60 and edge activators (EAs) (namely Brij 35, Brij 58, Tween 60 and Tween 80) were prepared using the ethanol injection technique [18] using different weight ratios of Span 60: EA (90:10; 80:20 and 70:30 w:w). Briefly, Span 60 and calculated amount of MZA were dissolved in 4ml of ethanol using different concentrations of drug and then injected into magnetically stirred aqueous solution of the EA. Ethanol was evaporated by rotary evaporator, and the formed milky vesicular dispersions were used for further investigations.

Experimental design:

A full factorial experimental design was built up to evaluate the main effects and interactions of two variables: EA type (factor A) and Span 60: EA ratio (factor B)**.** The studied responses were: particle size (PS) and entrapment efficiency (EE %). The complete setup of the full factorial design and the composition of the prepared formulae

according to the factorial design are shown in **Tables 1** and **2** respectively.

TABLE 1: FACTORIAL DESIGN INDEPENDENT VARIABLES AND THEIR LEVELS.

Variables	Level
EA type	Tween 60
	Tween 80
	Brij 35
	Brij 58
Span 60: EA ratio (w:w)	90:10
	80:20
	70:30

TABLE 2: EFFECT OF FORMULA COMPOSITION OF MZA-LOADED SVS (Span 60: EA RATIO) ON DRUG EE% AND VESICLE PARTICLE SIZE.

Formulae Code	Formulae Composition	Weight Ratio (w:w)	EE % ± SD	Mean Particle size (nm) ± SD
S1	Span 60 : Tween 60	90:10	82.80 ± 1.65	276.26 ± 2.77
S2	Span 60: Tween 60	80:20	81.99 ± 2.18	585.10 ± 8.64
S3	Span 60 : Tween 60	70:30	68.36 ± 1.24	704.93± 4.46
S4	Span 60 : Tween 80	90:10	81.74 ± 3.43	328.56 ± 3.19
S5	Span 60 : Tween 80	80:20	80.89 ± 2.20	657.53 ± 6.15
S6	Span 60 : Tween 80	70:30	78.69 ± 2.32	761.06± 6.40
S7	Span 60 : Brij 35	90:10	94.19 ± 3.13	315.53 ± 1.56
S8	Span 60 : Brij 35	80:20	93.69 ± 2.15	279.13 ± 2.45
S9	Span 60 : Brij 35	70:30	91.94 ± 1.16	995.66 ± 3.05
S10	Span 60 : Brij 58	90:10	68.76 ± 1.26	530.46 ± 7.59
S11	Span 60 : Brij 58	80:20	27.76 ± 2.04	631.63 ± 3.09
S12	Span 60 : Brij 58	70:30	23.29 ± 1.95	633.46 ± 8.30

Characterization of MZA-SVs:

Dynamic light scattering technique (DLS) was used to determine the particle size (PS), polydispersity index (PI), and zeta potential (**ζ**) of the freshly prepared SVs dispersions utilizing a Zetasizer® Nano-ZS (Malvern instruments, Malvern, UK).

MZA EE% was determined by measuring the concentration of free drug in the vesicular dispersions. The non-encapsulated MZA was separated by centrifugation of SVs dispersions using Nanosep® at 5000 rpm for 15 minutes using cooling centrifuge adjusted to a temperature of 4°C (Herml Z216MK, Gosheim, Germany). The amount of free drug in the supernatant was determined by UV spectrophotometry (UV-1601 PC, Shimadzu, Kyoto, Japan). Drug EE% was calculated according to the following equation:

$$EE\% = \left[\frac{(total\ \ MZA - free\ \ MZA)}{total\ \ MZA}\right] \times 100 \qquad (1)$$

The measurement of elasticity of SVs dispersions was carried out by extrusion technique [19, 20] through a locally fabricated stainless steel pressure filter holder. The vesicles were extruded through cellulose acetate/surfactant-free membrane filters with pore size of 220nm (Minisart, Sartorius, Göttingen, Germany) at constant pressure of 2.5 bar and relative deformability as an indicator for elasticity was calculated according to the following equation [21]:

$$D = \frac{j}{t}\left(\frac{r_v}{r_p}\right)^2 \qquad (2)$$

Where D is the deformability index (ml/s),
j is the amount of dispersion extruded (ml),
t is the extrusion time (s),
r_v is the vesicle size after extrusion (nm),
r_p is the pore size of the extrusion membrane (nm).

Preparation of mucoadhesive in-situ gel (ISG):

Selected MZA- SVs formulae were mixed with 0.6 % w/v gellan gum [22], forming *in-situ* gelling formulations containing spanlastic vesicles (SGs). Mucoadhesive SGs formulations were prepared by adding HPMC to the prepared *in-situ* gelling formulations at different concentrations: 0.5, 1 and 1.5 w/v % [23] till homogenous mixtures were formed. All of the above steps were done under

aseptic conditions. All glassware were sterilized by autoclaving, and the entire procedure was carried out in a laminar flow hood [24].

Sterilization of SGs using gamma radiation was tested at an exposure dose of 5, 15 and 25 KGy and the radiation dose 5 KGy was found to be the best to produce stable and sterile formulae (data not shown).

Characterization of SGs:

Gelation time:

Gelation time was carried out by the tube inversion method [25] for selected SGs using microcentrifuge tube containing a solution which when titled sol phase will flow, however if a gel phase is formed it will not flow.

100 µl of the tested formulae were carefully placed into microcentrifuge tubes (1.6 ml), and 300 µl of artificial tear fluid (ATF) were added slowly on the side wall of each microcentrifuge tube. The tubes were then incubated in a temperature-controlled bath at 37°C. The sol-gel transition time was determined by inverting the tubes horizontally every minute. The time at which the gel did not flow in minutes was examined by visual examination and recorded as the gelation time [26].

Viscosity measurement:

The viscosities of the prepared SGs containing 0.6 w/v % gellan gum were determined using cone and plate programmable viscometer (Brookfield Engineering Laboratories Inc., Model HADV-II, USA), connected to a digital thermostatically controlled circulating water bath (Polyscience, Model 9101, USA) and experiment was conducted at 37±0.5°C.

ATF was used for gel formation during this study. It was added to SGs 10 min before the viscosity values were re-measured to mimic the physiological condition and record rheological changes that may occur after ocular administration of *in-situ* gelling systems. The same setting was used for measuring the viscosities of selected mucoadhesive SGs containing 0.6 w/v % gellan gum and different concentrations of HPMC (0.5, 1 and 1.5 w/v %) before and after addition of ATF.

***In vitro* release of MZA from SGs:**

In vitro release studies were performed by dialysis membrane diffusion technique using modified USP dissolution apparatus I (Pharma Test, Hainburg, Germany) replacing device baskets with glass cylinders (10cm in length and 2.5 cm in diameter). The cylinders were fixed in the device shafts from one end using basket clips while the other end was covered by dialysis membrane (MWCO 12,000-14,000) to retain tested SGs allowing the free drug to be released into the dissolution medium pre-equilibrated at 37±0.5°C in the dissolution tester vessels [27]; [28].

Accurately measured amounts of SGs solutions equivalent to 1mg MZA were transferred to the glass cylinders that was immersed to 1mm distance below the surface of 50 ml of ATF pH 7.4. The dissolution media was kept at 37± 0.5°C while the cylinders were rotated at 50 rpm. At specified time intervals (0, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6 and 8 hrs), 1ml samples were removed, replaced with fresh media and analyzed spectrophotometrically to determine the concentration of MZA.

Scanning Electron Microscopy (SEM):

Selected mucoadhesive SGs (SG1) was prepared and mixed with ATF then freeze dried for 48hr using Christ Alpha 1-2LD plus freeze drier (Martin Christ GmbH, Germany). Dried gel was coated with gold sputter coater and the morphology of the gel with its vesicle content morphology was imaged using scanning electron microscope (JEOL-JSM-5500LV, Japan)..

***In vivo* pharmacodynamic study:**

Animal handling:

For all animal studies, the experimental procedures conformed to the Ethical Committee of Faculty of Pharmacy, Ain-Shams University on the use of animals. Adult albino normotensive rabbits were kept in individual cages and fed a normal diet and water ad libitum in a constant temperature environment of 25ºC and a period of 7 days was allowed for acclimatization of rabbits.

IOP lowering effect of selected formulae:

The efficacy of the selected formulae SG1, SG4 and SG8 in lowering the intraocular pressure (IOP) was evaluated on normotensive albino rabbits. The

results of these formulations were compared to that of MZA dispersion [24, 27]. Concentration of the drug in all formulae was adjusted to be equivalent to 0.05 w /v % MZA. Twelve adult albino normotensive rabbits weighing between 2.5 and 3 Kg, were randomly divided into four groups, each consisting of three rabbits according to the following scheme: Group I received SG1, Group II received SG4, Group III received SG8 and Group IV received control MZA dispersion. A single 50µl dose of each preparation was administered in the lower conjunctival sac on the corneal surface of the left eye of the rabbit, and the right eye was used as a control during this study.

After instilling one drop of 0.4 (w/v %) benoxinate hydrochloride to the rabbit's eyes as local anesthetic, IOP was measured using tonometer (Riester, Germany) [16, 30] at time intervals of 0, 1, 2, 3, 4, 5, 6, 7, 8, 10 and 12 hrs. To decrease the diurnal, seasonal, and individual variations usually observed in rabbits, the ocular hypotensive activity was expressed as the average difference in IOP between the treated and control eye of the same rabbit [31]. IOP values recorded in this study were calculated according to the following equation [32]:

$$\Delta \text{ IOP} = \text{IOP treated eye} - \text{IOP controled eye} \quad (3)$$

The Institutional Animal Ethical Committee reviewed the animal protocol prior to the experiment. All rats were treated in accordance with the guideline for the care and use of laboratory animals and with the permission of Faculty of Pharmacy- Ain Shams University Animal Ethical Committee.

Assessment of ocular irritancy of mucoadhesive SGs:

Six rabbits weighing 2.5 -3 Kg were divided into two groups. Group I received mucoadhesive SG1 and group II received mucoadhesive SG8 twice daily in the left eye only for a period of 10 days. The right eye was kept as a control in all the experimental rabbits. All the rabbits were killed after 10 days and their eyes were separated, fixed, cut vertically, dehydrated, cleared, and embedded in paraffin at 56^{o}C in hot air oven for 24hr. Eyes were then sectioned and stained by hematoxylin and eosin. Corneal histological examination was completed after photographing the stained sections using light microscope [33].

Statistical analysis:

The results are expressed as mean of 3 ± standard deviation (SD). The complete setup of the full factorial design statistical and factorial analysis were performed using MINITAB (version 15.1.3) software. Comparison of the mean values was performed using either Student′s t test or ANOVA using Graph Pad Instat software setting statistical significance at p-value ≤ 0.05.

RESULTS AND DISCUSSION:

MZA loaded SVs (MZA-SVs) were successfully prepared using ethanol injection technique [18]. Preliminary studies (data not shown) were carried out to determine the effect of initial MZA amount on particle size (PS) and entrapment efficiency (EE %). Different initial MZA amounts were investigated for testing drug incorporation into SVs formulations, and we found that SVs prepared using10 mg found to be the most appropriate, MZA to be the most appropriate for further studies, i.e. they have the lowest particle size andthe highest attainable EE%. This amount was used in all the prepared formulations.

In situ gelling (ISG) formulations were also prepared to achieve a prolonged ocular residence time of SVs formulations. Gellan gum was chosen, being capable of forming a clear gel in the presence of mono and divalent electrolytes in the tear fluids. 0.6 w/v % gellan gum was found to be the most appropriate concentration to be mixed with MZA-SVs, forming SVs-in-ISGs formulations.

Furthermore, the influence of different concentrations of HPMC, as a mucoadhesive polymer, on the mucoadhesive potential of the prepared SVs-in-ISGs (SG) formulations was tested.

Effect of variables of the factorial design on MZA-SVs:

EE% response: Table 2 reveals the effect of Span 60: EA ratio and EA type on EE% of MZA-SVs. It is obvious that the EE% of the prepared MZA-SVs were in the range of 23.29 to 94.19%. The high values of EE % were noticed with all edge activators except Brij 58 could be due to the high

transition temperature (Tc) of Span 60 which represents most of vesicle composition [34]. It was previously reported that Span 60 showed high EE% compared to the unsaturated Span 80 when tested for the entrapment of various drugs [35-38]. **Table 3** shows that both EA type and Span 60: EA ratio and their two way interaction had a significant effect on MZA entrapment into SVs ($p< 0.05$).

TABLE 3: ANOVA STATISTICAL ANALYSIS FOR EE% RESPONSE OF MZA-SVS.

Source of Variation	DF	Sum of Squares	Mean Squares	F Value
Surfactant type	3	3670.9	1223.6	15.11
Span 60: EA ratio	2	2309.0	1154.5	14.26
Surfactant type*Span 60: EA ratio	6	4413.6	735.6	9.09

DF: Degrees of freedom

All the experimental formulations are significantly different ($P < 0.05$)

The effect of EA type on EE % was studied as shown in **Fig. 1.** The mean EE % values for different edge activators were found to be 93.27, 39.93, 77.71 and 80.44% for formulations containing Brij 35, Brij 58, Tween 60 and Tween 80 respectively. There is a significant difference in EE % between Brij 58 and other types of edge activators ($p< 0.05$). This could be due to the fact that Brij 58 which havelow hydrocarbon chain volume compared hydrophilic surface area. Thus, it may be unable to form intact vesicles that encapsulate MZA efficiently [23]. Furthermore, significantly reduced EE % values were revealed with the increase in Span 60: EA ratio ($p< 0.05$). The initial increase in the proportion of EA for forming a shield that prevent vesicles results in pore formation of vesicular bilayer until the concentration reaches a certain threshold. When such threshold concentration is reached, vesicles based on micelles or mixed micelles start to form leading to a decrease in EE % [19].

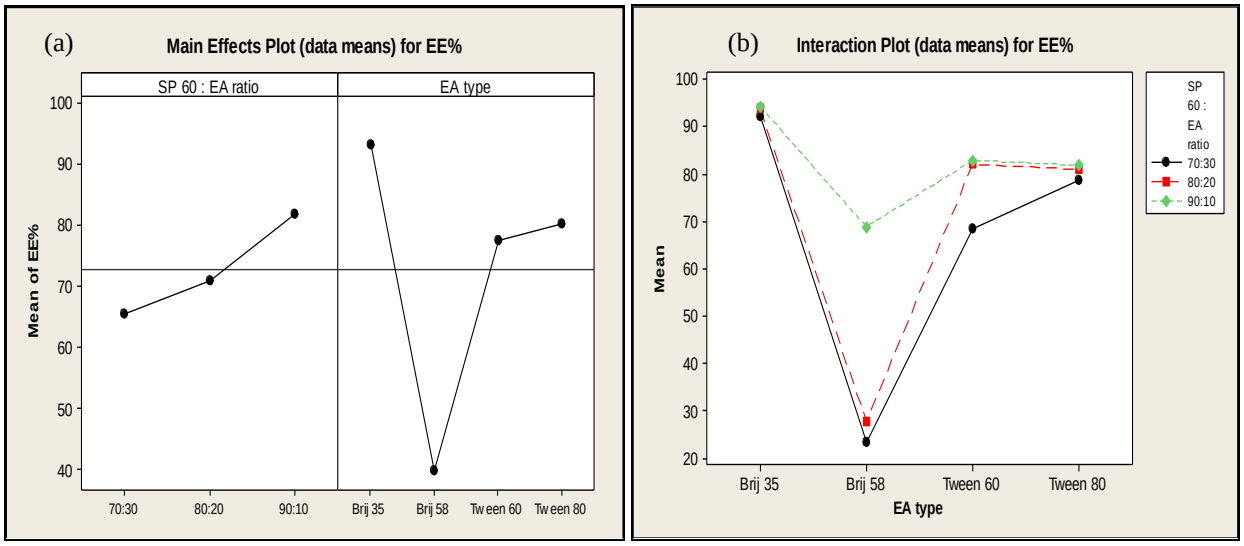


FIG. 1: MAIN EFFECT (a) AND TWO WAY INTERACTION PLOT (b) FOR MZA EE% IN SVs.

PS response:

As shown in **Table 2**, the PS values of the freshly prepared MZA-SVs ranged between 276 and 995 nm. Likewise their effect on EE%, both EA type and Span 60: EA ratio and their two way interaction significantly affected PS ($p< 0.05$), see **Table 4**.

TABLE 4: ANOVA STATISTICAL ANALYSIS FOR PS RESPONSE OF MZA-SVs.

Source of Variation	DF	Sum of Squares	Mean Squares	F- Value
surfactant type	3	3245996	1081999	43.63
Span 60: EA ratio	2	572148	286074	11.54
surfactant type*Span 60: EA ratio	6	2361074	393512	15.87

DF: Degrees of freedom

All the experimental formulations are significantly different ($P < 0.05$)

Data analysis of PS revealed that the mean particle size for formulations prepared using Brij 35, Brij 58, Tween 60 and Tween 80 were 530.10, 565.18, 522.09 and 582.38 nm respectively. Upon studying the effect of HLB value of surfactants on PS for conventional vesicles, it was found that the use of surfactants with increased hydrophobicity resulted in decrease in surface energy leading to the formation of vesicle with smaller size. Hydrophilic surfactants with high aqueous solubility do not facilitate the formation of compact vesicular structures resulting in coalesced lamellar aggregates. However, in case of elastic vesicles, the selection of surfactant with specific HLB value to achieve smaller size vesicle may be irrelevant, as they are ultra deformable [39]. Furthermore**,** a significant increase in the PS of the formulations containing Span 60: EA ratio 70:30 was noticed (**Fig.2**) when compared to those containing other Span 60: EA ratios, namely 90:10 and 80:20 ($p<0.05$). The increase in the content of EA in the vesicles might lead to incorporation of EA in vesicles which initially form small sized vesicles followed by vesicle coalescence resulting in the eventual increase the particle size [40]**.**

Compiling the effect of Span 60: EA ratio and EA type on EE% and PS of the prepared vesicles, S1, S4, S7 and S8 showed the smallest particle size and highest EE % and hence, these formulae were selected for further studies.

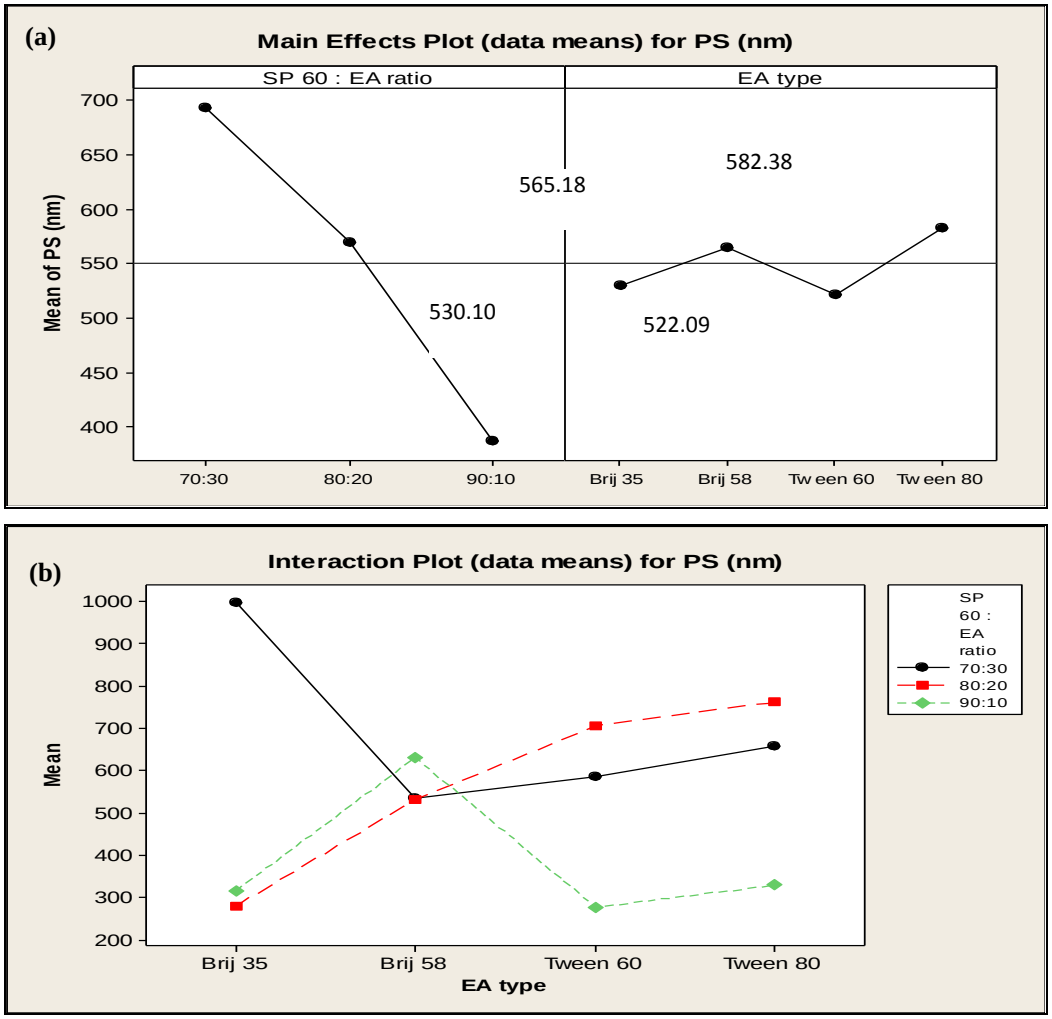


FIG 2: (a) MAIN EFFECT AND (b) TWO WAY INTERACTION PLOTS FOR PS OF MZA-SVs.

Characterization of the prepared MZA-SVs:

Selected MZA-SVs (formulae S1, S4, S7 and S8) carried a negative charge ranging from -16.30 to -27.70 mV (see **Table 5**). The negative charge on the surface of SVs is responsible for forming a hield that prevent vesicles from aggregation and impart colloidal stability [41, 42].

TABLE 5: ZETA POTENTIAL VALUES OF MZA SOLUTION AND SELECTED MZA-SVs FORMULAE.

Formulae Code	Formulae Composition	Weight Ratio	Zeta potential (mV) ± SD
S1	Span 60 : Tween 60	90:10	-16.30 ± 1.70
S4	Span 60 : Tween 80	90:10	-27.70 ± 1.15
S7	Span 60 : Brij 35	90:10	-19.73 ± 1.37
S8	Span 60 : Brij 35	80:20	-23.03 ± 1.68
MZA	MZA solution	0.05%	-25.20± 2.26

Elasticity is an important attribute of elastic vesicular formulations, that should be able to squeeze or pass through pores of biological membranes (e.g. corneal cells), decreasing the risk of vesicle disruption [20]. For effective ocular drug delivery, the drug as well as its carrying vesicles must penetrate through the corneal tissues in order to concentrate in eye tissues and produce therapeutic effect.

SVs penetration through corneal tissues is a function of vesicle deformability and elasticity. Selected formulae (S1, S4, S7 and S8) were subjected to deformability study using extrusion technique and were compared with the deformability of niosomal control formula (N) which composed of Span 60: cholesterol (90:10 w:w).

The elasticity results were expressed as deformability index and illustrated in Fig. 3. Significant differences were found between tested formulae and the niosomal control formula ($p < 0.05$). This could be explained by the presence of cholesterol in niosomal control formula which added rigidity and orientational order to the non-ionic surfactants in the niosomal vesicle bilayer [43].

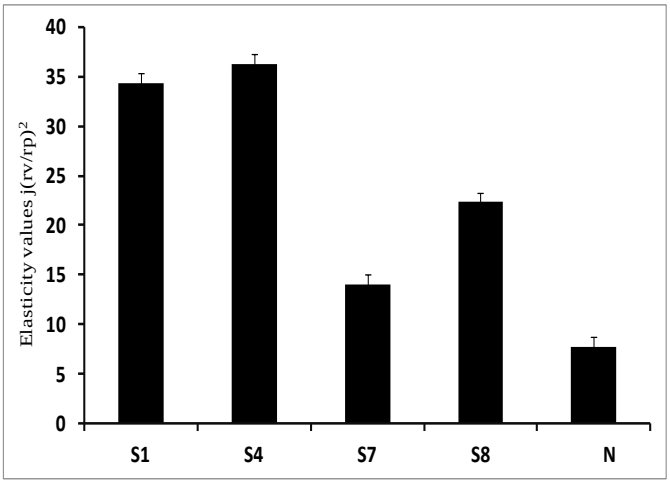


FIG. 3: ELASTICITY VALUES OF MZA-SVs COMPARED TO NIOSOMAL CONTROL FORMULA (N).

MZA-SVs formulation containing Tween 80 (S4) showed higher elasticity values when compared to those containing Brij35 (S7 and S8) ($P < 0.05$).

However, no significant difference ($P > 0.05$) was found between the elasticity values for S1 containing Tween 60 and S4 containing Tween 80. The highly flexible and non-bulky hydrocarbon chains of Tween 80 might be the cause of increased vesicles elasticity value [20].

In addition, the unsaturated alkyl chain of Tween 80 could render it more membrane permeable, enhancing vesicle elasticity [38]. The differences between elasticity values for selected SVs formulation were narrow (ranging from 14-36). This could be due to the membrane softening effect of Span 60, which is the main component of SVs. Similar finding was reported for niosomal formulations containing Span 85 that was found to have higher elasticity compared to liposomal formulation. [25]

Characterization of SGs:

Gelation time:

The time required for gelation of the prepared SGs formulations is a critical parameter in our study. The shorter the time required for gel formation, the lower the amount drained from the formulae and the higher the amount of drug retained near the cornea for subsequent absorption and the higher the drug bioavailability [44].

The selected SG formulations were examined to measure the time required for gelation using tube inversion method. It was found that SG1 and SG4 gelled rapidly, requiring about 1 min to form non-flowing gel, whereas SG8 required about 5 min to become non-pourable (**Table 6**).

TABLE 6: TIME REQUIRED FOR GELATION OF SELECTED FORMULAE IN ATF.

Formulae Code	Time /min
SG1	1
SG4	1
SG7	No gelation
SG8	5

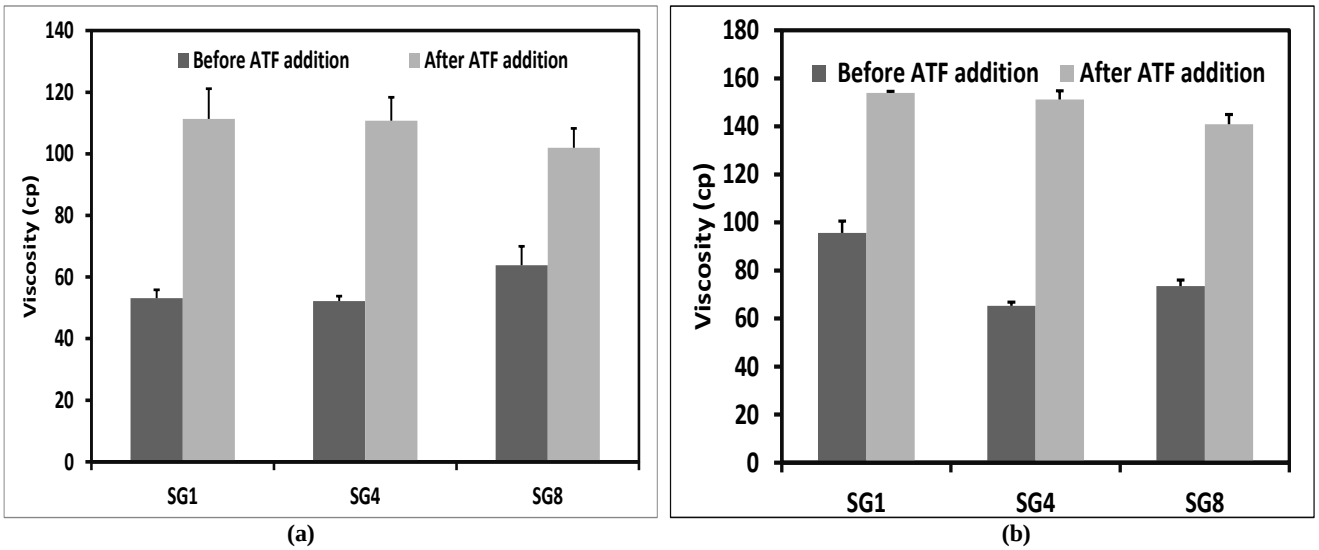
However, SG7 failed to form a gel after the addition of ATF even after 24 hr from the starting time of the experiment, thus, it was excluded from any further characterization experiments. The failure of gelation of SG7 could be due to the high HLB of Brij 35 which can interact with polysaccharide polymer chains and coat the hydrophobic region responsible for entanglement and network formation. Upon the increase of concentration of Brij 35, the EA might be more associated with Span 60 in the vesicle structure and small amount of the EA was left to interact with polysaccharide chains, thus, allowing gel formation [45]. Furthermore, the gelation time results were in agreement with those obtained from viscosity measurement of gellan gum containing formulae.

Viscosity measurement:

It is very important for an ocular *in situ* gelling system to possess suitable viscosity values that allow easy installation of the formulation as a liquid from a dropping device, which change rapidly to gel by ionic interaction once introduced to the eye. Furthermore, the gelled formulation should maintain its viscosity even at eye blinking shear rates to allow drug sustained release to the eye tissues. **Fig. 4** show the viscosity results of selected SGs which were prepared by mixing MZA-SVs with gellan gum (0.6 w/v %) before and after the addition of ATF, measured at fixed low shear rate 1 sec^{-1} [46]. The viscosity values recorded for the prepared formulations SG1, SG4 and SG8 mixed with 0.6 w/v % gellan gum were found to be 111.33, 110.76, and 101.93 cp respectively after the addition of ATF. The viscosity values of SG1, SG4 and SG8 were also recorded after mixing with different concentrations of HPMC (0.5, 1 and 1.5 w/v %) before and after the addition of ATF. This was done in order to select the most appropriate HPMC concentration.

It was found that the viscosity values increased with the increase in HPMC concentration from 0.5 to 1.5 w/v %. The gel formed after mixing with ATF had viscosity values of 150.90, 151.23 and 140.90 cp respectively for 0.5 w/v % HPMC. However, the viscosity values for selected formulations containing 1 w/v % HPMC were 445.36, 451.66 and 455.70 cp respectively and the viscosity values for ISG formulations containing 1.5 w/v % HPMC were 1132.40, 885.20 and 889.20 cp respectively.

Thus, 1 and 1.5 w/v % HPMC concentrations were difficult to be applied to the eye. It is well known that the optimum viscosity for ophthalmic preparations at a low shear rate (1 sec^{-1}) should be within a preferred range not exceeding about 150 cp so that when such formulations are applied to the human eye, no discomfort can be experienced [46], therefore, 0.5 w/v % HPMC was chosen for further studies.



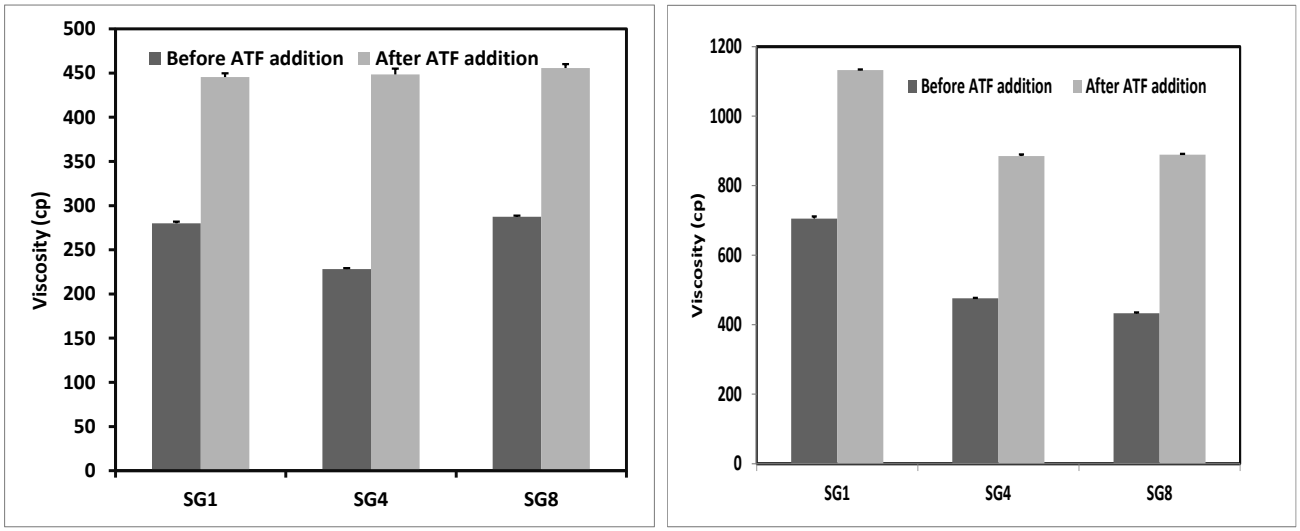


FIG 4: VISCOSITY OF SG1, SG4 and SG8 PREPARED USING 0.6 W/V % GELLAN GUM WITHOUT HPMC (a) AND WITH 0.5 W/V % HPMC (b), 1% HPMC (c), 1.5 % HPMC (% W/V) (d) BEFORE AND AFTER ATF ADDITION.

In vitro drug release studies:

The results of release study of SG1, SG4 and SG8 compared to methazolamide control solution are illustrated in **Fig. 5**. The release of MZA from control solution was significantly higher than any other tested formulae ($p < 0.05$). On the other hand, MZA release was prolonged from all selected formulae reaching 86.18, 91.25 and 84.48 % after 8h for SG1, SG4 and SG8 respectively. This could be attributed to the influence of viscosity on drug diffusion from mucoadhesive ISG formulae as previously reported [47]. The increase in formulation viscosity might lead to slow drug diffusion from gel matrix into the surrounding media [48]. Kinetic analysis of the release data revealed that MZA release from SVs mucoadhesive SGs formulae followed diffusion mechanism (data not shown).

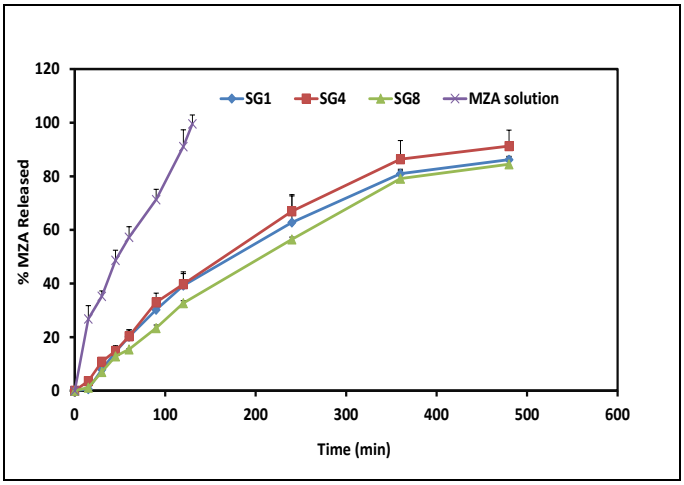


FIG. 5: RELEASE PROFILES OF MZA FROM ISG FORMULATIONS COMPARED TO MZA CONTROL IN ATF AT 37°C.

Scanning Electron Microscopy (SEM:

SEM imaging was done for SG systems to examine the effect of mixing MZA-SVs with gellan gum and HPMC on SVs PS. Since it was not possible to separate the vesicles from the mucoadhesive ISG formulations, the only alternative was to freeze samples then dry them to check the size changes that may happen during formulation process.

The electron photomicrograph of freeze dried SG1 (**Fig.6**) shows the spherical vesicles of S1 with average diameter of 240.12 nm embedded in the gel matrix of gellan gum and HPMC. SVs of S1 were attached to the surface individually (white arrow) or in groups. Although some SVs appeared rounded and spherical, others showed good spreading while adhering to the gel surface.

SEM image also shows continuous space filling SVs networks and sometimes patches were noticed in the gel matrix. The images also revealed the macroporosity of dried gels with most pores being in the range of 0.1- 0.5 μm in diameter (black arrow).

This finding indicates that mixing process of MZA-SVs with mucoadhesive ISG systems did not destroy SVs structure and only caused minimal decrease in PS from (276.26 to 240.12 nm) which may be due to the effect of freeze drying utilized in sample preparation.

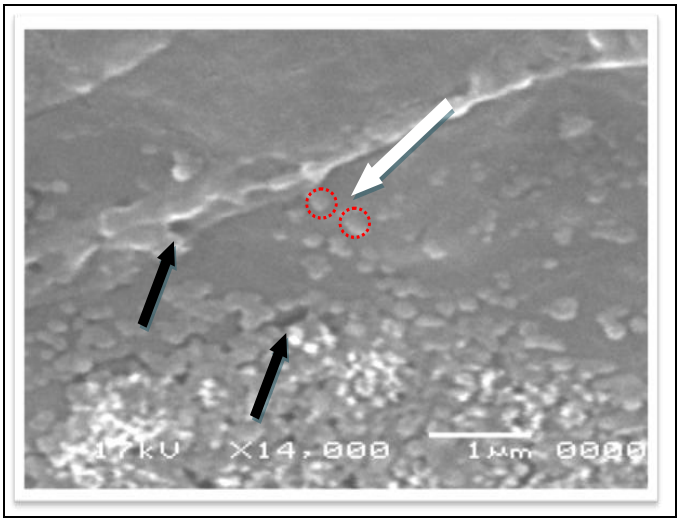


FIG. 6: SCANNING ELECTRON MICROGRAPH OF MZA-SVs MUCOADHESIVE IN SITU GEL (SG1). SPHERICAL VESICLES (RED CIRCLES), SVS ATTACHED TO GEL SURFACE (WHITE ARROW) AND MACROPORES IN GELS (BLACK ARROW).

The mucoadhesive force of SG formulations:

The mucoadhesive potential of ocular ISG formulations is an important physiochemical parameter that prevent the rapid drainage of the formulation from ocular cavity and subsequently increase the precorneal residence time leading to an increase in drug bioavailability. Mucoadhesive polymers are capable of forming strong non-covalent bonds with mucin layer coating the eye cornea and remain in place as long as the mucin layer is present [49]. Using polysaccharide based mucoadhesive polymers could increase both viscosity and mucoadhesive properties of the formulations, increasing the retention time of drug in ocular globe.

Fig.7 show values of mucoadhesive force for selected SG formulae before and after the addition of 0.5% w/v HPMC. The measured mucoadhesive force for formulae SG1, SG4 and SG8 were found to increase significantly by 1.5, 1.7 and 1.7 folds respectively upon the addition of 0.5 w/v % HPMC ($p < 0.05$). The presence of HPMC with the presence of many hydroxyl group in the backbone could enhance the mucoadhesive property of the tested formulations compared to those without HPMC [50].

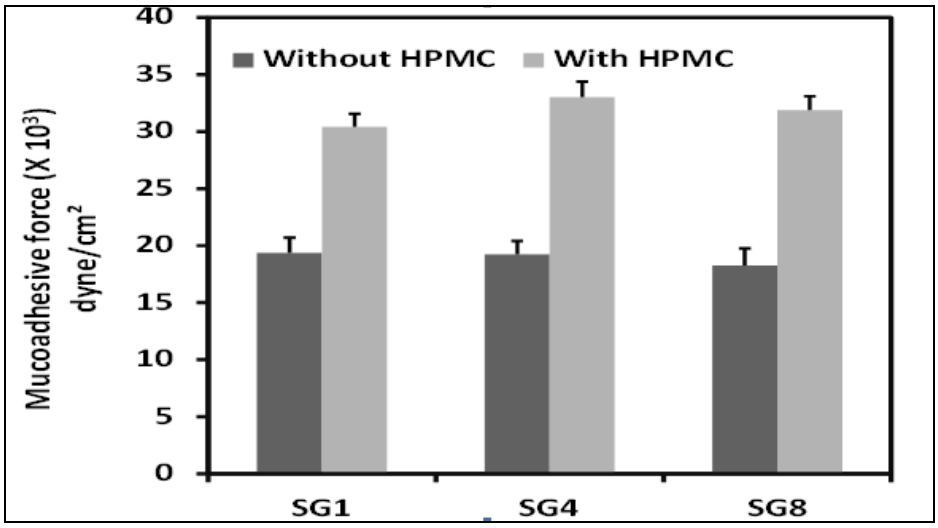


FIG. 7: THE EFFECT OF ADDING HPMC (0.5 W/V %) ON THE MEASURED MUCOADHESIVE FORCE FOR SG1, SG4 AND SG8.

***In vivo* Studies:**

IOP lowering effect: **Fig. 8** show the reduction in the IOP of SG1, SG4 and SG8 compared to MZA control solution. It was found that MZA control solution caused reduction in IOP with a recorded value of -4.26 mmHg after 3 hrs reaching the value of -1.33 mm Hg after 4 hrs. Compared to MZA control solution, all selected SG formulae showed a significant decrease in IOP ($p < 0.05$).

The increase in ocular bioavailability could be explained by several mechanisms. First, the presence of surfactants in the formulations, being penetration enhancers, reduce the thickness of mucus layer on the cornea and break junctional complexes between cells in corneal tissues increasing the system penetration ability [51]. Second, incorporating MZA-SVs in gellan gum ISGs

resulted in the formation of gels upon ocular administration. The formed gel acted as drug release controlling matrix that decrease drug drainage from the eye. The IOP lowering effect of SG1, SG4 and SG8 reached its peak after 3, 5 and 4 hrs with values of -8.2, -7.3 and -8.0 mm Hg, and lasted for 12, 10 and 10 hrs respectively. The fastest onset of action of SG1 might be due to its small particle size which is expected to increase the vesicle penetration capability across corneal membrane and consequently, improve drug bioavailability. Thus, the particle size represents the rate limiting step for the process of lowering IOP [52].

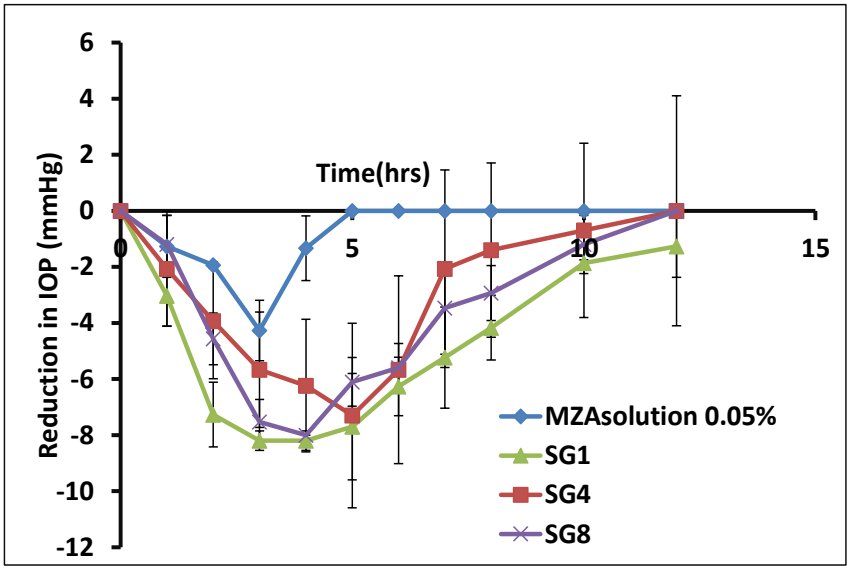


FIG. 8: IOP LOWERING EFFECT OF MZA-SVs AFTER ADMINISTRATION OF TOPICAL MZA-LOADED MUCOADHESIVE ISG FORMULATIONS (SG1, SG4 AND SG8) COMPARED TO MZA SOLUTION.

Assessment of ocular irritancy of mucoadhesive SGs:

Fig. 9 show the cross sections of control rabbit corneal tissue, rabbit cornea treated with SG1 and SG8 twice daily for 10 days respectively. Normal cornea showing no sign of edema, inflammation or histological changes. Also no losses were observed in the epithelial layer of cornea after the administration of SG1 and SG8.

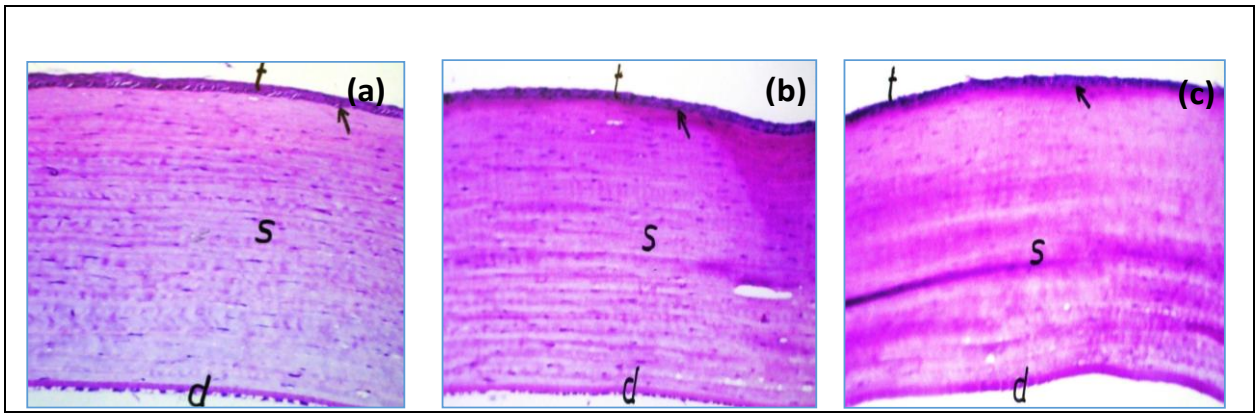


FIG 9: HISTOLOGICAL EXAMINATION OF (A) CONTROL RABBIT'S CORNEAL TISSUE AND RABBIT'S CORNEAL TISSUE TREATED WITH (B)SG1 (C)SG8 WHERE t, s, and d ARE EPITHELIUM, STROMA AND ENDOTHELIUM RESPECTIVELY.

In the stroma, no keratocyte loss was observed after the continuous exposure to both formulae for 10 days. Moreover, no neovascularization that may be associated with the anterior stroma was revealed. Finally, no endothelial cell losses were noticed in the eyes of individual treated rabbits. The absence of any morphological changes after administration of mucoadhesive SGs is expected due to the lack of

any membrane disrupting materials in their content. The non-ionic nature of span 60, edge activators as well as the biodegradability of gellan gum and HPMC would impart a good biocompatibility to our tested formulation, and could be considered safe for short and long term treatment. It has been previously reported that the irritating power of surfactants is minimum for non-ionic surfactants when compared to ionic surfactants [53].

CONCLUSION: MZA was encapsulated successfully in the elastic SVs using different ratios of Span 60: edge activators. The best formulae were selected based on their PS and MZA EE% to be incorporated in mucoadhesive in-situ gel containing gellan gum/HPMC mixtures. The prepared formulae showed more prolonged lowering in IOP when compared to MZA control solution and were found to be safe and well tolerated. Thus, this suggests the potential usefulness of SGs as controlled ocular delivery system to improve ocular bioavailability of MZA and decrease frequency of drug administration, superseding MZA control solution.

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REFERENCES:

1. Jarvinen K, Jarvinen T, and Urtti A, Ocular absorption following topical delivery. Advanced Drug Delivery Reviews, 1995; 16 (1): 3 – 19.
2. Green K, The effects of preservatives on corneal permeability of drugs. In: Edman, P. (Ed.), Biopharmaceutics of Ocular Drug Delivery. CRC Press, Boca Raton, 1993; 43–49.
3. Merkus F, Schipper N, and Hermeus W, Absorption enhancers in nasal drug delivery. efficacy and safety, Journal of Controled Release, 1993; 24: 201–208.
4. Shilpa K, and Kaur IP, Spanlastics—A novel nanovesicular carrier system for ocular delivery, International Journal of Pharmaceutics, 2011; 413: 202-210.
5. El Meshad AN, and Mohsen AM, Enhanced corneal permeation and antimycotic activity of itraconazole against Candida albicans via a novel nanosystem vesicle. Drug Delivery, 2014; 0, 1-9.
6. Kaur IP, Rana C, Singh M, Bhushan S, Singh H, and Kakkar S, Development and Evaluation of Novel Surfactant-Based Elastic Vesicular System for Ocular Delivery of Fluconazole, Journal of Ocular Pharmacology and Therapeutics, 2012; 28(5): p. 484-496.
7. Williams KA, Irani YD, and Klebe S, Novel Therapeutic Approaches for Corneal Disease, Discovery Medicine, 2013; 15(84):291-299.
8. Sanzgiri YD, Maschi S, Crescenzi V, Callegaro L, Topp EM, and Stella VJ, Gellan-based systems for ophthalmic sustained delivery of methylprednisolone, Journal of Controled Release, 1993; 26: 195–201.
9. Rozier A, Mazuel C, Grove J, and Plazonnet B, Functionality testing of gellan gum, a polymeric excipient material for ophthalmic dosage forms, International Journal of Pharmaceutics, 1997; 153: 191–198.
10. Ludwig A, The use of mucoadhesive polymers in ocular drug delivery. Advanced Drug Delivery Reviews, 2005; 57: 1595– 1639.
11. Rahamatullah S, Singh R, Raghu T, James G, David W, and Ryan D, Mucoadhesive drug delivery systems, 2011; 3(1): 89-100.
12. Maren TH, Haywood JR, Chapman SK, and Zimmerman TJ, The pharmacology of methazolamide in relation to the treatment of glaucoma, Invest Ophthalmol Vis Sci, 1977; 16(8): 730–742.
13. Edelhauser HF, and Maren TH, Permeability of human cornea and sclera to sulfonamide carbonic anhydrase inhibitors. Arch Ophthalmol, 1988; 106(8): 1110–1115.
14. Fridriksdóttir H, Loftsson T, and Stefánsson E, Formulation and testing of methazolamide cyclodextrin eye drop solutions, Journal of Controled Release, 1997; 44(1): 95–99.
15. Chen R, Qian Y, and Li R, Methazolamide calcium phosphate nanoparticles in an ocular delivery system, Yakugaku Zasshi, 2010; 130(3): 419–424.
16. Youshia J, Kamel AO, El Shamy A, and Mansour S, Design of cationic nanostructured heterolipid matrices for ocular delivery of methazolamide, International Journal of Nanomedicine, 2012; (7): 2483-2496.
17. Li R, Jiang S, and Liu D, A potential new therapeutic system for glaucoma: solid lipid nanoparticles containing methazolamide. Journal of Microencapsulation, 2011; 28(2): 134–141.
18. Kakkar S, and Kaur IP, Spanlastics A novel nanovesicular carrier system for ocular delivery. International Journal of Pharmaceutics, 2011; 413: 202– 210.
19. Van den Bergh BAI, Wertz PW, Junginger HE, and Bouwstra JA, Elasticity of vesicles assessed by electron spin resonance, electron microscopy and extrusion measurements, International Journal of Pharmaceutics, 2001; 217: 13–24.
20. El Zaafarany GM, Awad GAS, Holayel SM, and Mortada ND, Role of edge activators and surface charge in developing ultradeformable vesicles with enhanced skin delivery, International Journal of Pharmaceutics, 2010; 397: 164–172.
21. Salama HA, Mahmoud AA, Kamel AO, Abdel Hady M, and Awad GAS, Phospholipid based colloidal poloxamer–nanocubic vesicles for brain targeting via the nasal route, Colloids and Surfaces B: Biointerfaces, 2012; 100: 146-154.
22. Gan L, Gan Y, Zhu C, Zhang X, and Zhu J, Novel microemulsion in situ electrolyte-triggered gelling system for ophthalmic delivery of lipophilic cyclosporine A: in vitro and in vivo results, International Journal of Pharmaceutics, 2009; 365(1-2): 143-149.
23. Mansour M, Mansour S, Mortada ND, and Abd Elhady SS, Ocular poloxamer-based ciprofloxacin hydrochloride in situ forming gels. Drug Development and Industrial Pharmacy, 2008; 34(7): 744-752.
24. Guinedi AS, Mortada ND, Mansour S, and Hathout RM, Preparation and evaluation of reverse-phase evaporation and multilamellar niosomes as ophthalmic carriers of acetazolamide. International Journal of Pharmaceutics, 2005; 306(1): 71-82.

25. Gupta PN, Mishra V, Rawat A, Dubey P, Mahor S, Jain S, Chatterji D, and Vyas SP, Non-invasive vaccine delivery in transfersomes, niosomes and liposomes: a comparative study. International Journal of Pharmaceutics, 2005; 293(1): 73-82.
26. Kumar R, Jaya K, and Selvadurai M, Formulation and In vitro Evalution of Gellan Gum/Carbopol and Sodium Alginate based Solution to Gel Depot of Ketotifen Fumarate System. Journal of Pharmaceutical Sciences & Research, 2012; 4: 1973-1977.
27. Nasr M, Mansour S, Mortada ND, and El Shamy AA, Lipospheres as Carriers for Topical Delivery of Aceclofenac: Preparation, Characterization and In Vivo Evaluation. AAPS PharmSciTech, 2008; 9(1): 154–162.
28. Hathout RM, Mansour S, Mortada ND, and Guinedi AS, Liposomes as an ocular delivery system for acetazolamide: in vitro and in vivo studies. AAPS Pharm Sci Tech, 2007; 8(1):1.
29. Attama AA, Reichlm S, and Müller- Goymann CC, Diclofenac sodium delivery to the eye: in vitro evaluation of novel solid lipid nanoparticle formulation using human cornea construct. International Journal of Pharmaceutics, 2008; 355(1–2): 307–313.
30. Monem AS, Ali FM, and Ismail MW, Prolonged effect of liposomes encapsulating pilocarpine HCl in normal and glaucomatous rabbits. International Journal of Pharmaceutics, 2000; 198(1): 29–38.
31. Winum J, Casini A, and Mincione F, Carbonic anhydrase inhibitors: N-(p-sulfamoylphenyl)-alpha-D-glycopyranosylamines as topically acting antiglaucoma agents in hypertensive rabbits. Bioorganic & Medicinal Chemistry, 2004; 14(1): 225–229.
32. Kaur IP, Singh M, and Kanwar M, Formulation and evaluation of ophthalmic preparation of acetazolamide. International Journal of Pharmaceutics, 2000; 199: 119–127.
33. Banchroft JD, Stevens A, and Turner DR, Theory and practice of histological techniques. Vol. Fourth Ed. 1996.
34. Manosroi A, Wongtrakul P, Manosroi J, Sakai H, Sugawara F, Yuasa M, and Abe M, Characterization of vesicles prepared with various non-ionic surfactants mixed with cholesterol. Colloids and Surfaces B: Biointerfaces, 2003; 30(1): 129-138.
35. Akhilesh D, Bini K, and Kamath J, Review on Span-60 Based Non-Ionic Surfactant vesicles (Niosomes) as Novel Drug Delivery. International Journal of Research in Pharmaceutical and Biomedical Sciences, 2012; 3 (1): 6-12.
36. Yoshioka T, Sternberg B, and Florence A, Preparation and properties of vesicles (niosomes) of sorbitan monoesters (Span 20, 40, 60, 80) and sorbitan triesters (Span 85). International Journal of Pharmaceutics, 1994; 105: 1-6.
37. Lingan M, Sathali A, Kumar M, and Gokila A, Formulation and evaluation of topical drug delivery System containing clobetasol propionate niosomes. Scientific Reviews & Chemical Communications, 2011; 1(1): 7-17.
38. Abdallah M, Sammour O, EL-Ghamry H, and Abu-Selem M, Preparation and in-vitro evaluation of diclofenac sodium niosomal formulations. International Journal of Pharmaceutical Sciences and Research, 2013; 4(5): 1757-1765.
39. Jacob L, and Anoop K, A review on surfactants as edge activators in ultradeformable vesicles for enhanced skin delivery, International Journal of Pharma & Bio Sciences, 2013; 4(3):337-344.
40. Nir S, and Nieva JL, Interactions of peptides with liposomes: pore formation and fusion, Progress in lipid research, 2000; 39(2): 181-206.
41. Wilson B, Samanta M, and Santhi K, Poly(n-butylcyanoacrylate) nanoparticles coated with polysorbate 80 for the targeted delivery of rivastigmine into the brain to treat Alzheimer's disease, Brain Res, 2008; 1200: 159–168.
42. Huang Y, Tsai M, and Wu P, Elastic liposomes as carriers for oral delivery and the brain distribution of (+)-catechin, J Drug Target, 2011; 19: 709–718.
43. Girigoswami A, Das S, and De S, Fluorescence and dynamic light scattering studies of niosomes-membrane mimetic systems. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 2006; 64(4): 859-866.
44. Tangri P, Khurana S, Basics of ocular drug delivery systems. International Journal of Research in Pharmaceutical and Biomedical Sciences, 2011; 2(4): 1541-1552.
45. Ganji F, Abdekhodaie M, and Ramazani S, Gelation time and degradation rate of chitosan-based injectable hydrogel. Journal of Sol-Gel Science and Technology, 2007; 42(1): 47-53.
46. Dikstein S, Eyedrops having non-newtonian rheological properties. 1992; Google Patents.
47. Boddupalli BM, Mohammed ZNK, Nath RA, and Banji D, Mucoadhesive drug delivery system: An overview, Journal of Advanced Pharmaceutical Technology & Research. 2010; 1(4): 381-387.
48. Rupenthal ID, Green CR, and Alany RG, Comparison of ion-activated in situ gelling systems for ocular drug delivery. Part 1: physicochemical characterisation and in vitro release. International Journal of Pharmaceutics, 2011. 411(1-2): 69-77.
49. Almeida H, Amaral MH, Lobao P, and Sousa Lobo JM, Applications of poloxamers in ophthalmic pharmaceutical formulations: an overview, Expert Opin Drug Deliv, 2013; 10(9): 1223-1237.
50. Harish NM, Prabhu P, Charyulu RN, Gulzar MA, and Subrahmanyam EVS, Formulation and Evaluation of in situ Gels Containing Clotrimazole for Oral Candidiasis. Indian J Pharm Sci., 2009; 71(4): 421–427.
51. Kaur, Garg A, Singla A, and Aggarwel D, Vesicular systems in ocular drug deliver. International Journal of Pharmaceutics, 2004; 269: 1-14.
52. Kassem MA, Abdel Rahman AA, Ghorab MM, Ahmed MB, and Khalil RM, Nanosuspension as an ophthalmic delivery system for certain glucocorticoid drugs. International Journal of Pharmaceutics, 2007; 340(1-2): 126-133.
53. Van Abbe N, Eye irritation: studies related to responses in man and lab animals. Journal of the Society of Cosmetic Chemists, 1973; 24: 685–687.

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