International Journal of Drug Development & Research
| July-Sept 2010 | Vol. 2 | Issue 3 | ISSN 0975-9344 |
Available online http://www.ijddr.in
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INFLUENCE OF EXPERIMENTAL PARAMETERS ON CHARACTERISTICS OF GLYCEROL MONOOLEATE DISPERSION

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ABSTRACT

First part of work was focused on effect of equipments on stability and dimension characteristics of glycerol monooleate dispersion, while second part was focused on to study effect of formulation parameters on the characteristics of dispersion by applying 4²-factorial design at fixed ratio (1:5) of dispersed phase to dispersion medium. In this work lipid was dispersed in previously prepared PVA solution with the help of different equipments. Rest of batches was prepared by using selected equipment. Stability study was conducted using Thermo lab humidity chamber. Finally selected batches were subjected for transmission electron microscopy and X-ray diffraction study to investigate the structure. Propeller stirrer followed by homogenizer (volume mean diameter 1.42 µm and no phase separation were found), was the best equipment. Concentration of GMO was significant for %fraction of large particles, %lost on paddle, % water lost and volume mean diameter, but not for % recovery while concentration of PVA was significant for % recovery , volume mean diameter, % water lost , %lost on paddle, but not for %fraction of large particles.

1. Introduction

Fatty acids and monoglycerides possess antiviral and antibacterial activities. It has been demonstrated that enveloped viruses, such as herpes simplex virus type 1 (HSV-1), vesicular stomatitis virus (VSV), and visna virus are inactivated by long-chain unsaturated and medium-chain saturated fatty acids. Microbicidal hydrogel containing monoglyceride as active ingredient was developed against herpes simplex virus type I. In this view, monoglyceride based formulations can be proposed for intra vaginal use as microbicides against sexually transmitted diseases [1].

Beside ability of GMO to disturb viruses (e.g. HSV, HIV) structure it has ability to form different structures. Aqueous dispersions of monoglycerides such as monoolein forms the structures like reverse micelles, reverse hexagonal, bicontinuous cubic and sponge phase. Structure formation depends on the water content and temperature [2]. These structures can be used as carrier to load the active pharmaceutical ingredients and also has ability to control their release. Several examples of drug studied for their sustain release from GMO based liquid crystalline dispersion are cefazolin, cefuroxime, indomethacin, lidocaine hydrochloride etc [3, 4].High pressure homogenization and sonication method was used for preparation of dispersion [5]. During this technique there are chances for degradation of some desire phase (cubic phase) which is more applicable for pharmaceutical point of view. To over come this difficulty author Patrick spicer utilize ethanol as hydrotropic agent for preparation of liquid precursor that form cubic

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phase spontaneously when come in contact with excess body fluid [6].

In this article we restricted our self for preparation of GMO dispersion by using different equipments like propeller stirrer, magnetic stirrer, homogenization and combination of these equipments. Finally selected equipment was adopted for preparation of further batches. Some preformulatory parameters were evaluated like % water lost, % Lost on Paddle, % Recovery, % fraction of large particles and volume mean diameter. Accelerated stability study was conducted and finally selected batch was subjected for TEM and XRD for structural investigation.

Materials and Methods

Materials

Glycerol monooleate (pale yellow liquid) and PVA (80% hydrolyzed, white colored granular powder) were purchased from National chemicals, Vadodara, India.

Preparation of PVA Solution

Heat the water at 70 ^{0}C, add PVA in required quantity at a time with constant stirring by using the magnetic stirrer at 1200 RPM (Remi Equipments, Mumbai).

Equipments study

In first step of process Polar lipid like Glycerol Monooleate (GMO) was heated at 40^{0}C after that it was emulsified in prepared PVA solution with the help of different equipments as mentioned in table 1. Same composition was used for all the equipments (GMO: 0.625 g, PVA: 0.375 g, Distilled Water: Quantity sufficient to 50 ml). All the calculation was carried out on weight basis. Prepared batches were evaluated for particle size distribution study and physical stability.

Equipment used	Time (minutes)	RPM
Magnetic Stirrer	60	2500
Propeller Stirrer	60	2500
Homogenizer	3	4000
Magnetic Stirrer + Homogenizer	60 (propeller) + 3 (homogenizer)	2500 4000
Propeller Stirrer + Homogenizer	60 (propeller) + 3 (homogenizer)	2500 4000

Table 1: Process Parameters

Formulation parameters

Once the equipments study was overed, remaining batches were prepared by process described bellow. In the first step of the process, GMO was heated at 40^{0}C than added drop wise in PVA solution during constant stirring with the help of propeller stirrer at 70^{0}C, 2500 RPM and stirring was continued for 1 hr. After that the dispersion was subjected for homogenization (kalweka, Mumbai) for 3 minutes at 4000 RPM. Filter the dispersion. Particle size distribution study was conducted by using particle size analyzer (Sympatec, Germany); for structure investigation TEM (Philips, Netherlands) and XRD (Philips, Holland) were used. Accelerated stability study was conducted using thermo lab humidity chamber (Mumbai). A 4^2 factorial design was used to study the effect of concentration of GMO and concentration of PVA on dispersion characteristics at four different levels of GMO (5%, 7.5%, 10%, and 12.5%) and PVA (1%, 2%, 3%, and 4%). Compositions of various batches prepared were given in table 2.

Code	GMO %W/W (g)	PVA %W/W (g)
C_1	[5%] 0.416	[1%] 0.416
C_2	0.416	[2%] 0.833
C_3	0.416	[3%] 1.25
C_4	0.416	[4%] 1.66
C_5	[7.5%] 0.624	0.416
C_6	0.624	0.833
C_7	0.624	1.25
C_8	0.624	1.666
C_9	[10%] 0.832	0.416
C_{10}	0.832	0.833
C_{11}	0.832	1.25
C_{12}	0.832	1.666
C_{13}	[12.5%]1.041	0.416

C_{14}	1.041	0.833
C_{15}	1.041	1.25
C_{16}	1.041	1.666

Table 2: Composition of prepared batches (Distilled water quantity sufficient to 50 g)

Evaluation parameters (For formulation parameter study)

Prepared dispersions were evaluated for % water lost, %lost on paddle, % recovery, % fraction of large particles and volume mean diameter.

Water lost: Due to high speed of propeller stirrer (2500 rpm) and temperature (70^0C) % water lost was calculated with respect to quantity of water added. In calculation water lost on paddle was not considered.

% water loss = **(**weight of water added + weight of GMO + weight of PVA – {weight of dispersion after mixing with propeller stirrer+ weight of disperse phase lost on paddle}**)** ×100/ weight of water added

%lost on paddle: Lost on paddle was calculated with respect to total weight of dispersion before production. To find out lost on paddle, weight the filter paper and collect the material form paddle and than re weight the filter paper.

% lost on paddle = **(**weight filter paper after collecting material form paddle - initial weight of filter paper**)** × 100/ weight of dispersion before production

% fraction of large particles: Fraction of large particles after filtration of dispersion was calculated with respect to the weight of the disperse phase before dispersion production. To calculate the % fraction of large particles dispersion was filtered through filter paper. Before filtration note the initial weight of filter paper. The filter paper was then left to desiccate for 24 hrs in order to eliminate as much as possible of water taken up by the particles. Determine the weight of filter paper after desiccation. And finally find the %fraction of large particles.

% fraction of large particles = **(**weight of filter paper after desiccation- initial weight filter paper**)** × 100/weight of disperse phase

% recovery: Recovery was calculated on the basis of weight of dispersion after production and initial weight of dispersion.

% recovery: weight of dispersion after production ×100/ initial weight of dispersion

Characterization of Dispersions

Dispersions prepared by different equipments were stored in glass vial for 7 days and observed for phase separation phenomena and also subjected for Particle size measurement. GMO-based dispersions were characterized by particle size analyzer, TEM and XRD.

Particle size analysis was performed on filtered dispersions using a particle size analyzer (sympatec, Germany) Model: laser diffraction particle size analyzer, Measuring range 0.1 to 35 µm, measuring duration 5 seconds, cycle time 1000 ms.

For transmission electron microscopy studies, a drop of the sample was put on an untreated pure copper transmission electron microscopy (TEM) grid (300 mesh, Philips, netherland ,Teenai-20), where most of the liquid was removed with blotting paper leaving a thin film stretched over the grid holes. The TEM was operated at an acceleration voltage of 80 kV. Magnification approximates 30000.

X-Ray Diffraction Measurements, Diffraction measurements were carried out using a Philips, Holland Model: X' Pert (Specification: Source: Cu target X-Ray tube,X-Ray power: 2KW, Detector: Xe-filled Counteract or propotional detector ,Software: JCPD software).

Stability study

After production and elimination of large particles by filtration, dispersions were stored in a glass vial

at accelerated conditions (Temp $45^{O}C$+/-$2^{0}C$; humidity 75 +/-5 %RH) for 45 days in thermo lab stability chamber. In order to asses the physical stability of dispersions, morphological aspect such as phase separation and volume mean diameter were investigated as function of time.

Results and discussion

Equipment study

Selection of equipment was carried out on the basis of dispersion characteristic, here the morphological aspect such as phase separation (measured after 7 days of preparation) and volume mean diameter (measured immediately after preparation) were evaluated. Dispersion having lowest particle size and free form phase separation phenomena was selected. From above result magnetic stirrer was not able to form the stable dispersion. Propeller stirrer, Homogenizer, Propeller stirrer+ homogenizer and magnetic stirrer+ homogenizer gave the stable dispersion.

Formulation parameters

All the obtained data were subjected for multivariate test by applying full factorial design using SPSS software. Data were shown in table 5.

Equipments used	VMD (µm)	Phase separation (after 7 days)
Magnetic Stirrer	7.23 µm	Present
Propeller Stirrer	4.33 µm	Absent
Homogenizer	2.01 µm	Absent
Magnetic Stirrer + Homogenizer	1.79 µm	Absent
Propeller Stirrer + Homogenizer	1.42 µm	Absent

Table 3: Results of Equipments Study (VMD= Volume mean diameter)

Code	% w/w water loss	%w/w lost on paddle	Volume mean diameter (µm)	% w/w fraction of large particles	% w/w Recovery
C1	29.92	1.12	2.7	0.014	55.82
C2	33.8	1.2	2.24	0.024	53.7
C3	34.38	1.3	1.64	0.054	45.52
C4	31.91	1.4	1.36	0.046	44.34
C5	25.04	1.18	2.86	0.014	56.38
C6	30.27	1.12	2.28	0.024	56.52
C7	38.89	1.38	1.8	0.054	50.48
C8	30.93	1.46	1.38	0.046	42.82
C9	28.22	1.18	2.69	0.08	60.36
C10	33.07	1.34	2.21	0.096	55.16
C11	39.41	1.34	1.65	0.088	38.98
C12	40.39	1.36	1.09	0.092	38.62
C13	30.19	1.38	2.65	0.085	57.12
C14	35.66	1.4	2.22	0.099	49.68
C15	37.28	1.78	1.64	0.093	40.36
C16	44.69	1.82	0.923	0.065	34.78
Results of stability study					
Batch	VMD (µm)			VMD after 45 days (µm)	
C2	2.24			2.931	
C8	1.38			1.96	
C_{16}	0.923 µm			1.49 µm	

Table 4: Results of prepared batches (all the calculation were carried out on weight basis)

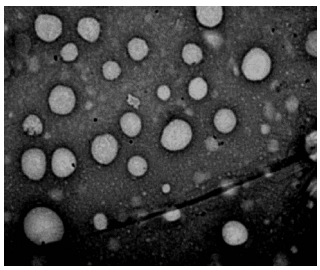
From table 5, concentration of PVA is significant for %water lost, %lost on paddle, VMD and % recovery but not for % fraction of large particles. Again the B value indicates the magnitude of effect. Here the B value was positive for % water lost and % lost on paddle means increase in concentration of PVA increase in measure responses (% water lost and % lost on paddle). For VMD and % recovery B value was negative it indicates that increase in concentration of PVA decrease in measure responses (VMD and % recovery). Concentration of GMO is significant for %water lost, %lost on paddle, VMD and % fraction of large particles but not for % recovery. Here the B value was positive for % water lost and % lost on paddle and % fraction of large particles it indicates that increase in concentration of GMO increase in measure responses (% water loss and % lost on paddle and % fraction of large particles). For VMD and % recovery B value was negative it indicates that increase in concentration of GMO decrease in measure responses (VMD and % recovery).

Stability study

Table 4

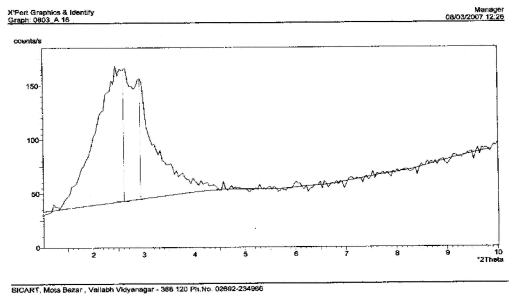
No phase separation was found after 45 days. To determine the particle size randomly three batches were selected. It was found that volume mean diameter were increase after 45 days.

Fig.1 TEM photograph



Batches having the lowest particle size were subjected for TEM.TEM images show the dispersion characterized by spherical particles no clear internal structure was observed.

Fig.2:X-ray diffraction graph



Peak present in X-Ray diffraction graph confirm the existent of liquid crystalline phase though for determination of type of shape (cubosomes or hexosomes) detail characterization with XRD or NMR is required.

Dependent variable	parameter	B (magnitude of effect)	Std. Error	t	Sig. (p)	95% confidence interval	
						Lower bound	Upper bound
%Water loss	Intercept	20.387	3.398	6.000	.000	13.046	27.728
	Con-GMO	8.314	3.675	2.263	.041	.376	16.252
	Con-PVA	7.265	1.839	3.951	.002	3.292	11.237
%Lost on paddle	Intercept	.729	.112	6.490	.000	.486	.972
	Con-GMO	.499	.121	4.108	.001	.237	.762
	Con-PVA	.257	.061	4.228	.001	.126	.388
VMD	Intercept	3.441	.101	34.054	.000	3.222	3.659
	Con-GMO	-.262	.109	-2.394	.032	-.498	-.026
	Con-PVA	-1.241	0.55	-22.699	.000	-1.359	-1.123
% fraction of Large particles	Intercept	-.643	.381	-1.688	.122	-1.493	.206
	Con-GMO	.210	.022	9.369	.002	.160	.260
	Con-PVA	-.306	.033	-1.111	.293	-.109	.036
%Recovery	Intercept	69.937	3.421	20.443	.000	62.546	77.327
	Con-GMO	-7.821	3.700	-2.114	.054	-15.814	0.171
	Con-PVA	-14.844	1.851	-8.018	.000	-18.844	-10.84

Table 5: Statistical analysis (Multivariate test) for obtained results of formulation parameters. (Con= concentration)

Conclusions

In conclusion, propeller stirrer followed by homogenizer was the best equipment that gave the stable dispersion, having the lowest particle size with the limitation of increase in mean diameter with respect to time. The work performed here enabled an assessment of the standard condition for the preparation of dispersions containing micro particles characterized by spheroid shape and few aggregates. Further the GMO was key ingredient for controlling fraction of large particles and lost on paddle (may be because of sticky nature) while increase in concentration of PVA decrease the recovery because of increase in viscosity of dispersion that makes the process more complicated and also decrease in volume mean diameter but it increase the lost on paddle.

Acknowledgement

Our sincere thanks to SICART, for providing support during XRD and TEM investigation .We wish to thank Dr. Jolly R Parikh, Dr V.A. Patel and Dr N.P. Chotai for their support in different parts of this work.

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Article History:----------

Date of Submission: 20-02-10

Date of Acceptance: 22-04-10

Conflict of Interest: None

Source of Support: Nil