Formulation Design and Evaluation of Self Micro Emulsifying Drug Delivery System (SMEDDS) of Valproic acid

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ABSTRACT

The present investigation was aimed at developing a stable self micro emulsifying drug delivery system (SMEDDS) of valproic acid (VPA) and evaluating its in vitro potential. The solubility of VPA was determined in various vehicles. Pseudoternary phase diagrams were used to evaluate the micro emulsification existence area and the release rate of VPA was investigated using a dissolution method. SMEDDS were characterized for clarity, precipitation and particle size distribution. Formulation development and screening was done based on results of solubility from phase diagram. The optimized formulation used for in vitro dissolution was composed of castor oil (38.4 %), Cremaphor RH 40 (42.4 %), PEG 400 (14.4 %). The SMEDDS formulation showed complete release in 15 min. as compared with the plain drug and conventional marketed formulation which showed a limited dissolution rate. VPA loaded SMEDDS were subjected to various conditions of storage as per ICH guidelines for 3 months. VPA SMEDDS successfully withstood the stability testing. It has been found that dissolution profile of valproic acid from SMEDDS was much improved than valproic acid.

Keywords: SMEDDS; Valproic acid; Pseudo-ternary Diagram; Poorly Water Soluble Drug, Formulation.

INTRODUCTION

Valproic acid (VPA) is an anticonvulsant drug structurally unrelated to other currently marketed anti epileptic agent[1]. It remains in clinical use as the favored anticonvulsant for controlling myoclonic seizures. VPA occurs as colorless to pale yellow, slightly viscous liquid, sparingly soluble in water. The pharmaceutical problems associated with VPA are manipulation of liquid materials as well as side effects such as gastric irritation, hepatotoxicity and teratogenicity. Apart from this VPA shows low solubility to cross blood-brain-barrier. This effect is mediated by efflux transport systems involving P-glycoprotein[2]. As a result high dose is required for effective concentration in brain tissues. It is classified under biopharmaceutical classification system class-II drug[3, 4] as a result it has low bioavailability. This needs to increase the solubility for better dissolution. Researchers have tried various methods (e.g. Cyclodextrin complexation, solid dispersion) to overcome these limitation[5]. Furthermore, VPA is a small molecule fatty acid. Thus formulating a lipid based system of VPA can be viewed as option for improving solubility as well as oral bioavailability. VPA is available in dose strength of 250 mg in US market as a brand name Depakene[6]. For our study 250 mg selected as working dose.

The main objective of the study were to develop and evaluation an optimal SMEDDS formulation containing VPA and compared with sodium valproate marketed formulation.

MATERIALS AND METHODS

Materials:

Valproic acid was a gift sample from Sanofi

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Synthelabo (Ind.) Ltd. Cremaphor RH 40, Softisan F 68, Softisan F 127, Plurol oleique CC 497, (FDC, Mumbai, India), Gelucire 44/14, Gelucire 50/13, (Sun Pharma, Ahmadabad, India), Polyethylene Glycol 400,

Polyethylene Glycol 200, Polypropylene Glycol 200, 400, Tween 20, 80, Span 20, 80 Castor oil, Corn oil, (Loba chem. Pvt, Ltd), All these excipients and reagents were used as received. All other chemicals and reagents used were of AR and HPLC grade.

Methods:

Solubility Studies:

The solubility of VPA in various components (oils, surfactants, cosurfactants) were determined as follows: briefly an excess amount of VPA was added to each ependroff tube containing 1 ml of the selected vehicles. After sealing the mixture was vortexed using a cyclomixer for 10 min. in order to facilitate proper mixing of VPA with the vehicle. Mixtures were than shaken for 24 h in incubator shaker maintained at 37±1 ^{o}C. Mixtures were centrifuged at 5000 rpm for 5 min. followed by filtration through membrane filter (# 0.22 µm). The concentration of VPA was then determined by HPLC method. No interference was observed from the excipients used to solubilize VPA.

HPLC Analysis Method:

The solubility of VPA in vehicles was determined by a validated RP-HPLC method. The apparatus consisted of Jasco PU-2080 Plus intelligent UV-VIS detector, a Rheodyne 7725 injector (Rheodyne, USA), Jasco Borwin chromatography software (version 3.5), integrator software and a C18 column, 5µ particle size. The mobile phase consist of mixture of acetonitrile: water (1 % TFA) = 60:40 at a flow rate of 1.0 ml/min[7, 8, 9, 10] that lead to retention time 6 min when detection was carried out at 213 nm. The assay was linear (r^2) = 0.998 in the concentration range 10-100 µg/ml with lowest detection of 1 µg/ml of VPA. The method was validated with respect to accuracy and inter and intra day precision as per ICH guidelines and the RSD was less than 2 % in both the cases.

Preparation of SMEDDS

A series of SMEDDS was prepared (Table-1) with varying ratio of oil to surfactant + cosurfactant mixtures (67 % Cremaphor RH 40 + 33 % PEG 400) was varied from 9:1 to 1:9. The ratio of surfactant to cosurfactant kept constant at 3:1 and the concentration of VPA was also kept constant in all formulation and area for microemulsion was shown using pseudo ternary phase diagram (fig 1). Briefly, VPA was dissolved in surfactants in ependroff tube. Then, the components were mixed by gentle stirring and vortex mixer and heated at 37^{o}C in an incubator to obtain a homogeneous isotropic mixture. The SMEDDS formulations were optimized by pseudo ternary diagram and stored at room temperature until used[11, 12, 13].

Globule Size Analysis:

SMEDDS formulation, 0.1 ml was diluted to 20 ml with distilled water. Visual observation was made immediately after dilution for assessment of self-emulsifying efficiency, phase separation and precipitation of drug[14]. The mean globule size and polydispersity index of the resulting emulsion was determined by photon correlation spectroscopy (malvensizer). The resulting emulsions were also allowed to stand for 24 h for stability assessment.

***In vitro* Dissolution Test:**

Formulation C 5 (Table I; SMEDDS of VPA containing 250 mg VPA) was adsorbed on to suitable adsorbent i. e. aerosil and filled into capsules. *In-vitro* release profiles of SMEDDS of VPA were studied using USP apparatus I (Lab India) at 37±1^{o}C with a rotating speed of 100 rpm in pH 1.2 and FaSSIF pH 7.4 as the media (900 ml) so as to evaluate the effect of pH on *in vitro* release rate. During the study, 2 ml of aliquots were removed at predetermined time intervals of 10, 20, 30, 40, 50 and 60 min. and maintained sink condition. The amount of VPA released in the dissolution medium was determined by RP- HPLC method using UV detector at λmax 213 nm. [15, 16]

Stability Studies:

Chemical and physical stability of VPA loaded SMEDDS (formulation C 5) was assessed under various storage conditions like room temperature, 30±2°C/65±5%RH, 40±2 °C/75±5% RH as per ICH guide lines. SMEDDS equivalent to 200 mg of VPA was filled in size "2" hard gelatin capsules and stored at various aforementioned storage conditions for 3 months. Samples were removed at 0, 60, 90 days of interval and checked for VPA content and dissolution efficiency at 15 min ($DE_{15\%}$). The % dissolution efficiency at 15 min was calculated as follows[17]

$$DE_{15\%} = \frac{(\text{AUDC upto 15 min})}{\text{Area under 100 \% dissolution curve}} \times 100$$

Where AUDC = Area under dissolution curve

Content Uniformity Test:

Content of VPA in various microemulsion preconcentrate was determined in triplicate by developed HPLC method. Microemulsion preconcentrate, **200 mg** (equivalent to 100 mg) was accurately transferred to 100 ml volumetric flask and dissolved in acetonitrile**.** The volume was made up with methanol to 100 ml so as to obtain a stock solution of concentration 1000 µg/ml. Stock solution was then diluted suitably to obtain solution of conc. 100 µg/ml. The solution was then injected in HPLC system so as to determine drug content. All experiments were carried out in triplicate for all the VPA formulations.

Freeze- thaw cycling test:

The optimized VPA microemulsion was subjected to freeze-thaw cycling. One freeze-thaw cycle consisted of storing of microemulsion at -20°C for 24 h. after this they were stored at room temperature for another 24 h. Three such freeze thaw cycles were carried out and then the physical stability of the microemulsion was observed.

RESULT AND DISCUSSION

Solubility studies:

Solubility studies were performed to identify oil, surfactants, cosurfactant that possesses good solubiliing capacity for VPA. Identifying the suitable oil, surfactants, and co surfactants having maximal solubilising potential for drug under investigation is very important to achieve optimum drug loading and also to minimize the final volume of SMEDDS [11, 21]. Solubility of VPA in various oils, surfactants and cosurfactants were conducted as per the procedure. The drug exhibited highest solubility in castor oil whereas least solubility was observed in Span 20 (fig. 02(a)). The dramatic difference in the solubility can be contributed to the chemical nature and HLB of the oily phases. Castor oil was shown maximum solubility among other oils (fig 2(b)).

However, it is noteworthy that there was considerable difference in the solubilising potential of castor oil. Castor oil was selected as an oil phase for further investigation due solubility and compatibility for VPA. Amongst various surfactants that were screened (Cremaphor RH 40, Softisan 168, Tween 80, Span 80). Cremaphor RH 40 exhibited highest solubilising potential for VPA. Whereas, among cosurfactants PPG 400, PEG 200, PEG 400, Plurol oleique CC 497, PEG 400 shown highest solubility.

Evaluation of VPA Loaded SMEDDS:

***In vitro* dissolution test:**

The dissolution profile of VPA loaded SMEDDS in FaSSIF pH 7.4 and 0.1N HCl is shown in fig 3 and fig 4. The SMEDDS was found to release approximately 60 % of VPA within 10 min. in FaSSIF and maximum of drug release 40 % in 0.1N HCl (pH 1.2). Hence, it may be concluded that VPA disperses immediately into both the medium due to its globule size.

Stability Studies and Dissolution Efficiency:

In formulation development research, the chemical stability of the active ingredient in the developed

formulation and the physical stability of the developed formulation are criteria that govern the acceptance or rejection of developed formulation. The physical instability could be due to interaction of the drug with the excipients used in the formulation. The degradation of the drug may occur due to its inherent instability or due to its interaction with excipients used in the formulation given Table 2 and 3. The formulations under investigation were shown good physical and chemical stability during the 90 days stability studies.

Globule Size Analysis:

The effect of Km on the globule size of various VPA microemulsions is shown in the fig 4 and that on particle size in fig 5. All the VPA microemulsions under investigation remained stable after centrifugation at 500 rpm for 20 minutes. No creaming or phase separation was observed with optimized batch. Thus microemulsion under investigation can be considered to have good chemical and physical stability and long shelf life.

Freeze- thaw cycling test:

The freeze-thaw cycling test was performed to test the robustness of the formulation. One freeze-thaw cycle consisted of storing of microemulsion at -20^{o}C for 24 h. after this they were stored at room temperature for another 24 h. The drug content was determined as 95.73 %.

CONCLUSION

SMEDDS appeared to be an interesting approach to improve problems associated with oral delivery of VPA using pseudo ternary diagram for optimization. VPA SMEDDS formulation was superior to commercial formulation with respect to *in vitro* dissolution profile. Thus SMEDDS can be considered as novel and commercially feasible alternative to current marketed sodium valproate.

Table 1: Composition of Various SMEDDS

	Formulation								
Ingredients	C0	C1	C2	C3	C4	C5	C6	C7	C8
Cremaphor RH 40 + PEG 400, Km=3.0	42	84	126	168	210	252	294	336	378
Castor oil	378	336	294	525	210	168	126	84	42
VPA	250	250	250	250	250	250	250	250	250

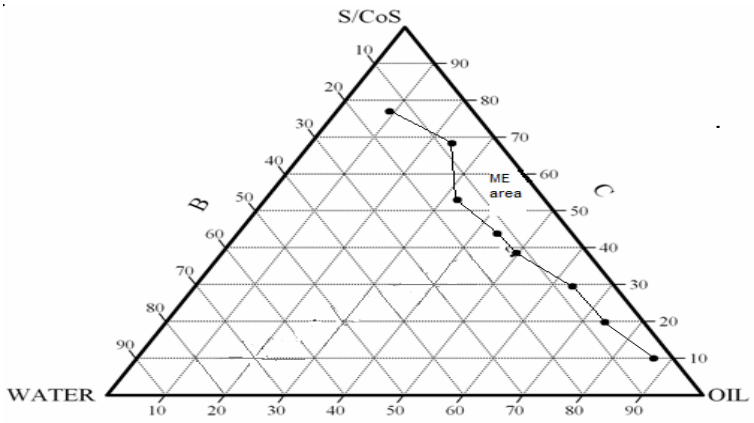

Fig 1 Pseudo ternary phase diagram (Km=3.0)

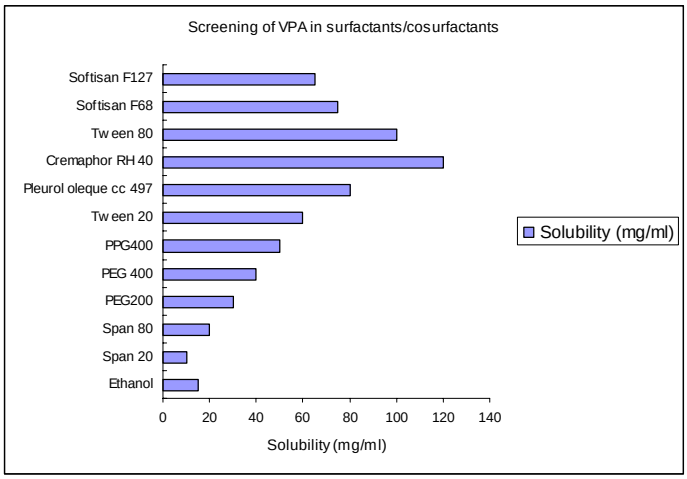

Fig 2: (a) Solubility screening of surfactants and cosurfactants.

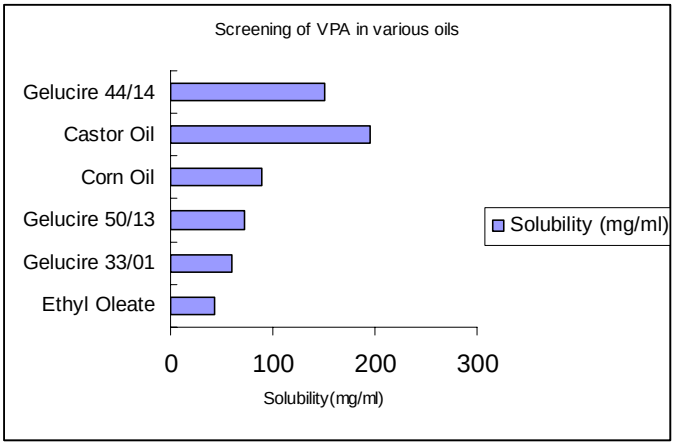

Fig 2:(b) Solubility screening of various oils.

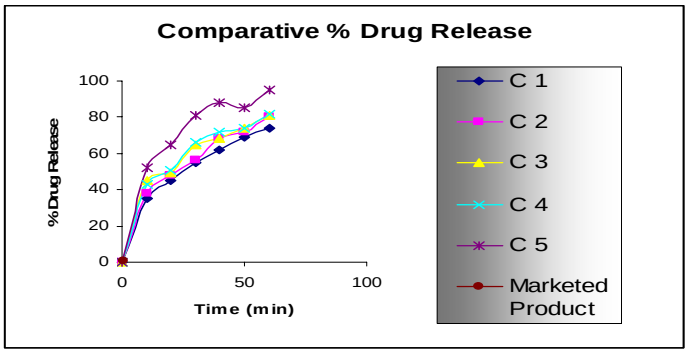

Fig 3: Dissolution Profile of SMEDDS in FaSSIF (pH 7.4)

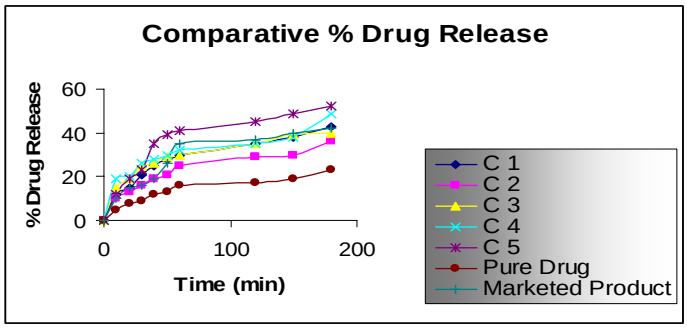

Fig 4: Dissolution Profile of SMEDDS in 0.1N HCl

Table 2: Effect of Storage Condition on VPA content SMEDDS

Storage condition	**Time (days)**		
	0	**60**	**90**
Room Temperature	100.4 ± 0.85	100.08 ± 0.62	99.85 ± 0.97
30 ± 2°C /65±5% RH	100.10 ± 0.5	100.01± 0.81	101.01 ± 0.41
40±2°C/75±5% RH	100.5±0.5	99.77± 0.75	99.12 ± 0.25

n = 3

Table 3: Effect of Storage Condition on Dissolution Efficiency (DE $_{15\%}$) VPA content SMEDDS

Storage conditions	Time (days)		
	0	60	90
Room Temperature	96.4 ± 0.78	96.08 ± 0.72	95.85 ± 0.79
30 ± 2°C /65±5% RH	94.10 ± 0.05	95.01± 0.08	93.01 ± 0.81
40±2°C/75±5% RH	92.5±0.58	91.77± 0.45	90.12 ± 0.25

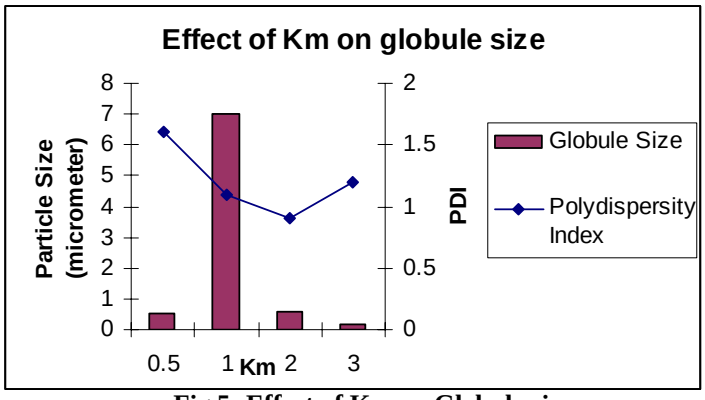

Fig 5: Effect of Km on Globule size

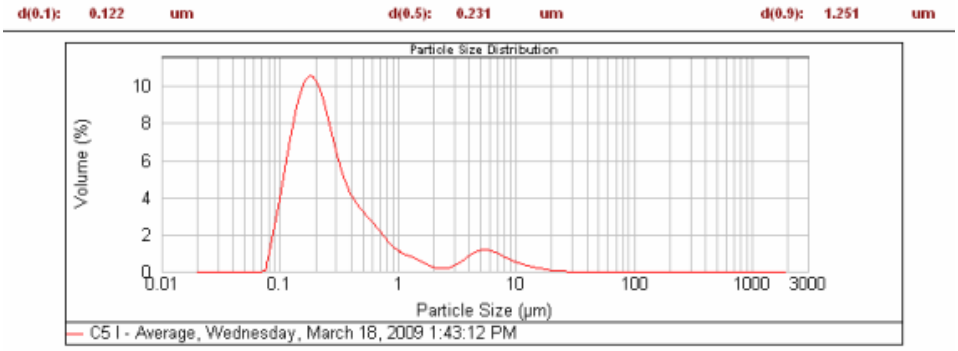

Fig 6: Particle size analysis of SMEDDS

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تولیف وتقییم نظام لایصال دواء valproic acid عن طریق الاستحلاب الذاتي المتناهي الصغر

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ملخـص

یهدف هذا البحث لتطویر نظام ایصال valproic acid عن طریق الاستحلاب الذاتي المتناهي الصغر. کما یهدف إلى تقییم فاعلیته خارج الجسم کي تم تحدید ذائبیة دواء valproic acid في سوائل مختلفة وتم استعمال رسومات مرحلة التواجد الثلاثي لتقییم وجود مساحة المستحلب وتم حساب سرعة انفلات الدواء عن طریق فحص الذائبیة.
یتمتع هذا النظام بخاصیة النقاء والتوریع لحجم الجزیئات المکونة له.
عملیة التولیف والفحص تم عملها بالاعتماد على نتائج الذائبیة والرسم الثلاثي المستحلب.
وقد کانت التولیفة المتالیة تتکون bcasbroil 384% و Cremaplor RH(42.4%) و (14.4%) وأظهرت التولیفة تحرزاً کاملاً خلال 15 دقیقة ونجحت في جمیع اختبارات الثبات.

الکلمـات الدالـة: الاستحلاب الذاتي، حمض فالبرویك، الرسم الثلاثي، الأدویة قلیلة الذائبیة في الماء، تولیف.

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