Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 4, 2014

Original Article

FORMULATION AND CHARACTERISATION OF TIZANIDINE HYDROCHLORIDE LOADED ETHOSOMES PATCH

BANTUPALLI NAGADEVI, KONDA SHRAVAN KUMAR, PASHAM VENKANNA, DONTHA PRABHAKAR*

Department of Pharmaceutics, Trinity College of Pharmaceutical Sciences, Peddapalli, Karimnagar-Dist, A.P, India.

Email: prabhakardontha@gmail.com

Received: 01 Feb 2014 Revised and Accepted: 16 Feb 2014

ABSTRACT

Objective: The objective of study is to development and characterisation of Tizanidine hydrochloride loaded ethosomes transdermal patches.

Methods: In this studies first developed ethosomes in suspension from using different ratios of soya lecithin, ethanol and cholesterol by following cold method. The best ethosomes prepared in transdermal patch form by adding suitable quantity of HPMC E15 as film forming agent and triethyl citrate as plasticizer. All these formulations are characterized by performing different evaluation studies e.g. Drug-excipients compatibility studies, entrapment efficiency, vesicle size, zeta potential, *in-vitro* drug release and stability.

Results: FT-IR studies were showed no incompatibility between drug and excipients. The size of the ethosomes was found in the range of 217.0 nm to 472.7 nm and the entrapment efficient found in the range of 33.98±0.68 to 65.69±0.39%. Zeta potential of optimized formulation ET5 and ET10 was found to be -39.7 and -42.1 respectively; high zeta potential prevents the aggregation between vesicles and hence, enhances its physical stability. *In-vitro* drug release of ethosomal suspensions were shown in the range of 60.12±0.23 to 95.61±2.59% at 24h. The *in-vitro* drug release of ethosomes patches were shown in the range of 45.23±0.59 to 89.17±0.45% at 24h. During stability studies the formulations ET5 and ET10 was shown no significant change in entrapment efficiency and *In Vitro* drug diffusion pattern.

Conclusion: The optimized formulations were suggested that used as transdermal drug delivery system.

Keywords: Cholesterol, Diffusion, Ethosomes, Patch, Tizanidine Hydrochloride.

INTRODUCTION

Tizanidine Hydrochloride is a muscle relaxant drug used to treat muscle spasms [1]. Muscle spasm is a sudden, involuntary contraction of a muscle, a group of muscles, or a hollow organ such as a heart. Muscle spasms, which can affect any part of the body, are an involuntary contraction in the muscle tissue. Tizanidine hydrochloride can reduce stiffness and spasms and may be particularly useful to treat painful night-time spasms, because its effects last for only 3-6 hours. So by formulating it as transdermal preparation we can increase its duration of action and can reduce side-effects. Transdermal route is a good alternative to oral route and transdermal route of administration has a high patient compliance, which derives from it being non-invasive and the long interval between applications. Transdermal administration also provides a means to obtain constant systemic drug levels. Transdermal drug delivery offers many advantages as compared to traditional drug delivery systems, including oral and parenteral drug delivery system. Avoidance of gastrointestinal disturbances and first pass metabolism of the drug stratum corneum is the most formidable barrier to the passage of most of the drugs, except for highly lipophilic, low molecular weight drugs [2]. However, penetration through transdermal route always remained an area of concern. In order to deliver drugs through the skin, most compounds require various degrees of permeation enhancers. More recent search makes use of innovative vesicular carriers, electrically assisted delivery. The best approach to improve drug penetration and/or localization is to manipulate the vehicle or to utilize a drug carrier concept [3].

The vesicles have been well known for their importance in cellular communication and particle transportation from many years. One of the major advances in vesicle research was the finding a vesicle derivative, known as an ethosomes. Ethosomes are novel lipid carriers developed by Elka Touitou which are soft, malleable vesicles and made up of phospholipids, propylene glycol, water and higher concentration of ethanol. Due to the interdigitation effect of ethanol on lipid bilayer, it was believed that high concentration of ethanol is detrimental to liposomal formulations [4]. One reported characteristic of Ethosomes is their smaller vesicle size (tens of nm to few microns) than liposomes, when both are obtained by preparation methods not involving any size reduction steps. Ethanol confers a surface negative net charge to liposome which causes the size of the vesicles to decreases [5]. Contrary to deformable liposomes, Ethosomes are able to improve skin delivery of drugs both under occlusive and non-occlusive conditions [6]. Ethosomes are more useful in delivering drugs to/through the skin in terms of both quantity and depth when compared to liposomes, and traditional dermal and transdermal system.

Hence the aim of present study was to develop controlled topical ethosomal patch of Tizanidine hydrochloride and to evaluate with respect to various *In Vitro* evaluation tests. Presently no scientific reports are available on the formulation of topical drug delivery system of ethosomal patch of Tizanidine hydrochloride. Hence, in this study an attempt has been made to prepare and evaluate a topical drug delivery system of ethosomal patch of Tizanidine hydrochloride.

MATERIALS AND METHODS

Materials

Tizanidine hydrochloride was obtained as a gift sample from Apotex Pvt ltd, Bangalore and Soya lecithin received as gift from Ozone Chemicals, Mumbai and Cholesterol was obtained from Qualikems Fine Chemicals Pvt.Ltd, New Delhi, HPMC E15 purchased from Molychem Chemicals, Mumbai. All chemicals were used in analytical grade.

Preparation of ethosomal suspension

Ethosomes of Tizanidine hydrochloride were prepared by cold method. The composition of Tizanidine hydrochloride ethosomes

given in table 1. Ethosomes suspension was prepared by dissolving Tizanidine hydrochloride, phospholipids (soybean lecithin) and cholesterol in ethanol. Propylene glycol was added during mixing. This ethanolic mixture was heated at 30°C in a water bath. In another beaker, distilled water also heated at 30°C in a water bath. Then, this water was slowly added to ethanolic mixture while stirred at 700 rpm. The solution was continuously stirred for 15 min to allow the formation of vesicles [6-9].

Preparation of Ethosomal Patch:

The formulation chart was given in table 2. Ethosomal patch was prepared by dissolving the weighed amount of HPMC E15 in solvent mixture of dichloromethane (DCM) and methanol(M) (1:1) on magnetic stirrer, then 3.75ml of ethosomal suspension is added to polymer solution during stirring to get uniform mixing of suspension with polymer solution, after formation of homogenous mixture incorporated triethyl citrate as plasticizer then again continuously mixing to obtain uniform mixture, obtained solution is poured into moulds and allow it to dry for 24h at room temperature and the obtained patches were stored in desiccators to remove excess moisture in them [10].

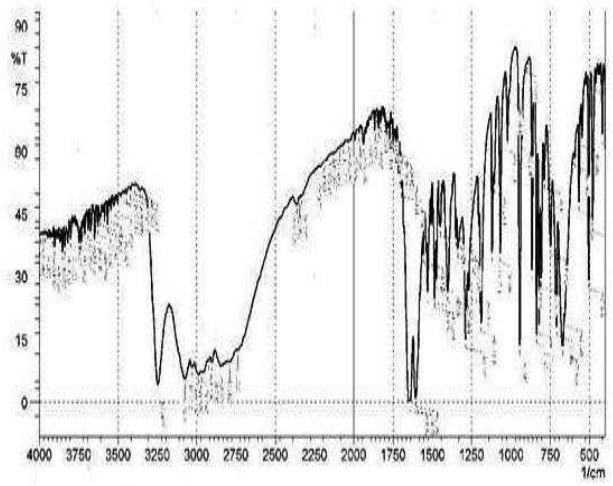

Fig. 1: FTIR of Tizanidine HCl.

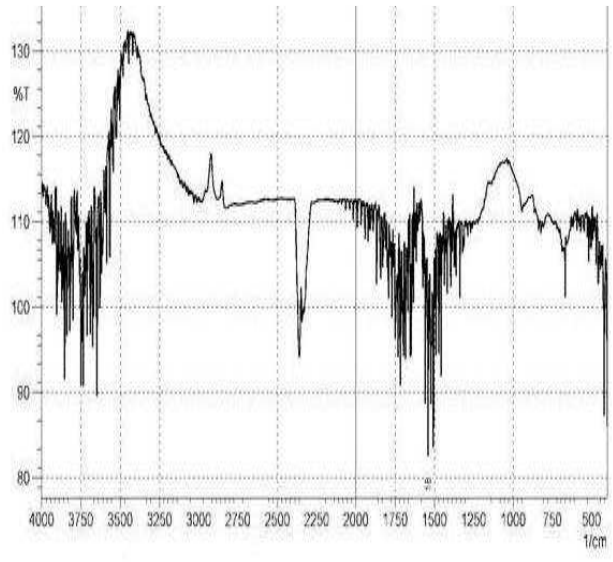

Fig. 2: FTIR of Formulation ET5.

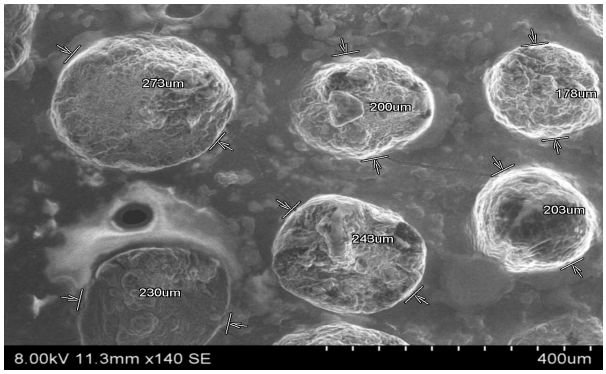

Fig. 3: SEM Photographs of Sonicated Ethosomes (ET10)

Containing Tizanidine HCl

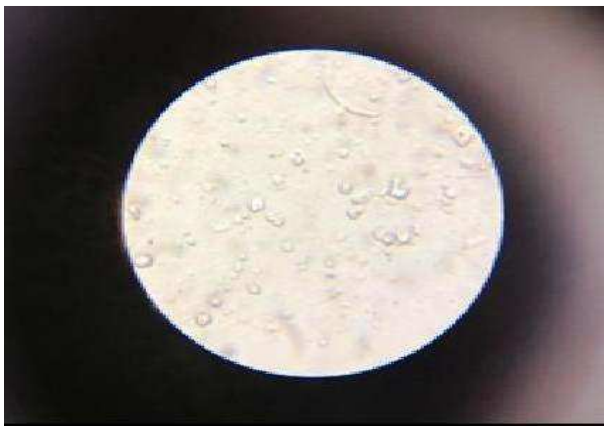

Fig. 4: Microscopic Photograph of Sonicated Ethosomes (ET10)

Containing Tizanidine HCl at 100x.

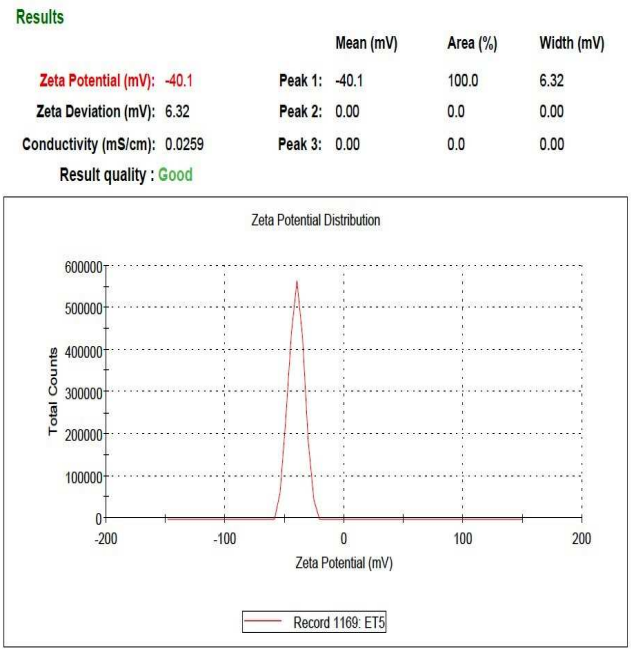

Fig. 5: Graph of Zeta Potential of Formulation ET5.

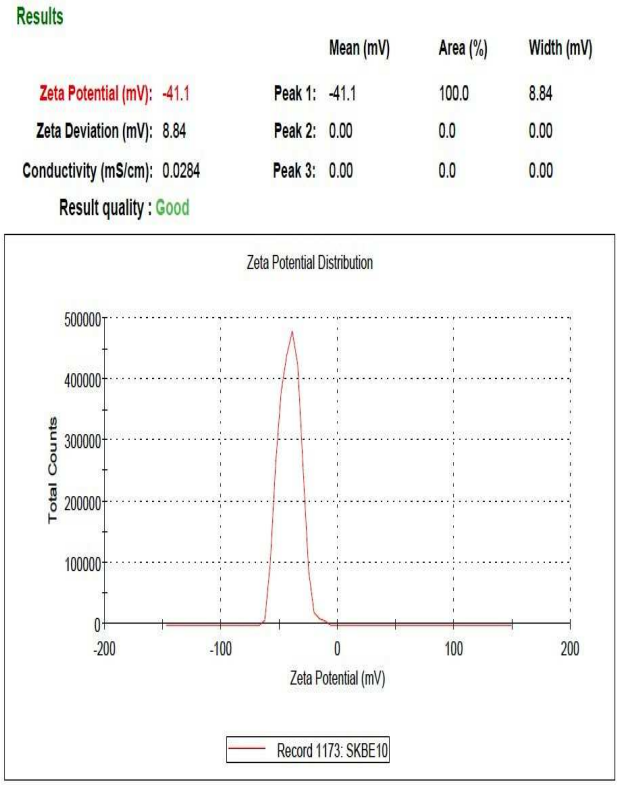

Fig. 6: Graph of Zeta Potential of Formulation ET10.

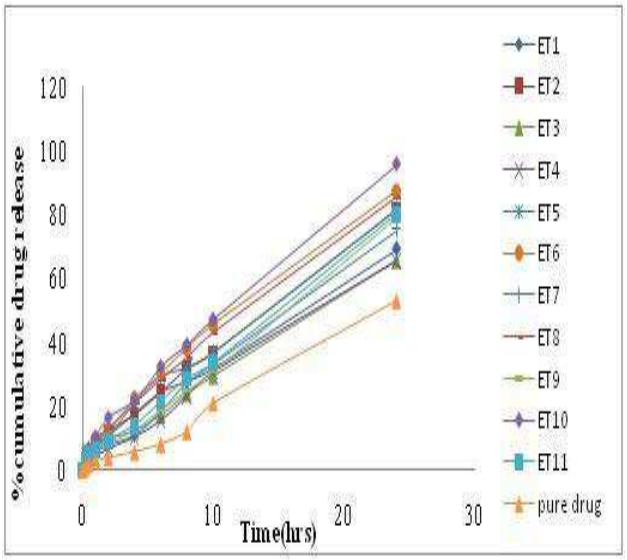

Fig. 7: *In-Vitro* Drug Release of Ethosomal Suspension through Goat Ear Skin.

Characterisation Of Ethosomes Of Tizanidine Hydrocholride

Drug-Excipients compatibility studies

Drug-excipients compatibility studies were carried out using FT-IR. Infrared spectrum of pure drug was seen in between 600 to 3800 cm-1. The study was carried out on individual pure drug and its physical mixture of excipients used in the study [11].

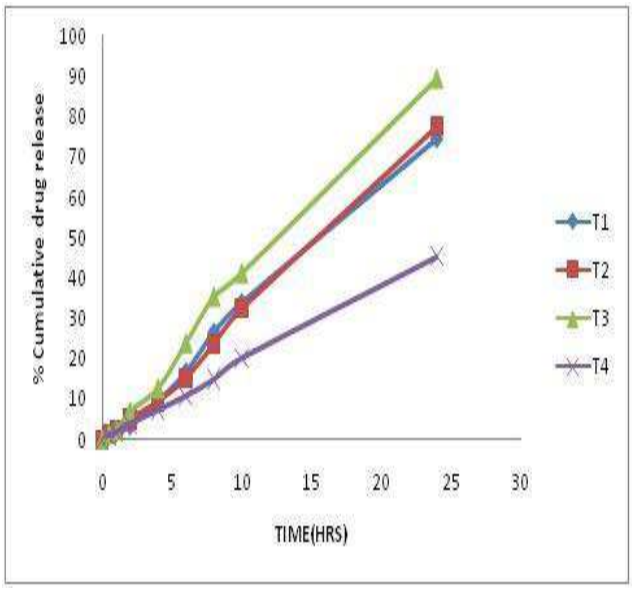

Fig. 8: *In-Vitro* Drug Release of Ethosomal Transdermal Patches through Goat Ear Skin.

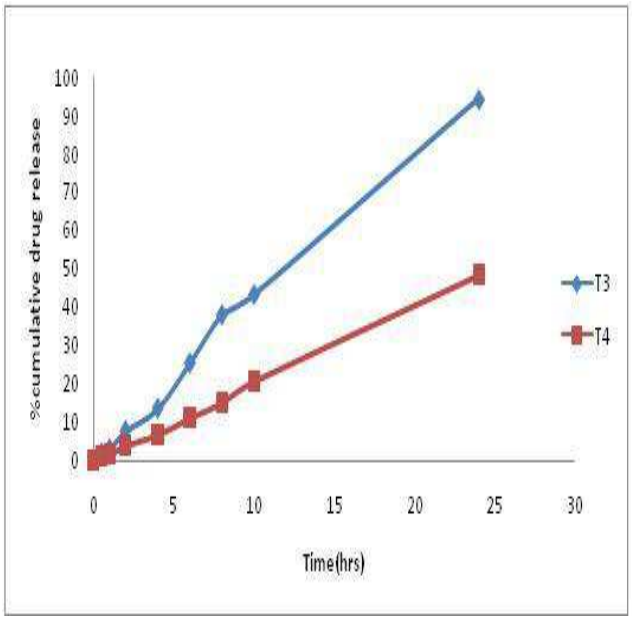

Fig. 9: *In-Vitro* Drug Release Comparison of Ethosomal Transdermal Patches and Transdermal Patch of Tizanidine Hcl through Rat Abdominal Skin.

Entrapment efficiency

Ethosomal formulation was subjected to 15000 rpm for 2 h. The unentrapped drug concentration was determined spectrophotometrically at 318nm. The drug entrapment percentage was calculated using the given equation [8].

Entrapment efficiency % = [⟨Q t − Q s ⟩ ÷ Q t] × 100

Where, Q t = Total amount of drug added

Q s = Amount of drug obtained in supernatant

Vesicle shape

Shape of the ethosomal vesicle was determined by using Scanning Electron Microscope. In this method, sample was fixed on carbon tape and fine gold sputtering was applied in a high vacuum evaporator. The acceleration voltage was set at 20 kV during scanning [12].

Vesicle size

Particle size of the ethosomes can be determined by dynamic light scattering (DLS) and photon correlation spectroscopy (PCS) [13].

Zeta potential

Zeta potential of the formulation can be measured by Zeta meter. A value of 25 mV (positive or negative) can be taken as the arbitrary value that separates low-charged surfaces from highly charged surfaces ((table 3). The significance of zeta potential is that its value can be related to the stability of colloidal dispersions.

***In-vitro* drug permeation through goat ear membrane from ethosomal suspension**

In-vitro diffusion study of ethosomal suspension was carried out by using two side opening boiling tube and beaker assembly with goat ear membrane. The goat ear membrane was tied to one end of boiling tube and this was assembled in to the beaker containing 30 ml of phosphate buffer pH7.4 and the medium was mixed with magnetic stirrer. 1ml of ethosomal suspension was poured on the goat membrane. The study was carried out at 37 ± 1°C for 24 h. 5ml of samples were withdrawn from reservoir compartment at predetermined time interval and absorbance was measured spectrophotometrically at 318nm. Each time the reservoir compartment was replenished with the same quantity of fresh phosphate buffer pH 7.4 [15].

***In-vitro* drug release studies of ethosomal transdermal patch through goat ear skin**

The best ethosomal suspensions were prepared in transdermal patches form and conducted drug release studies through goat ear skin using Franz diffusion cell. The treated goat ear membrane was mounted on the Franz diffusion cell. Place 2×1cm^2 size patch on the membrane in donor compartment.

Reservoir compartment was filled with 20 ml phosphate buffer of pH 7.4 and the diffusion medium was maintained uniformly by magnetic stirring. The study was carried out at 37 ± 1°C for 24 h. 5ml of samples were withdrawn from reservoir compartment at predetermined time interval and absorbance was measured spectrophotometrically at 318nm. Each time the reservoir compartment was replenished with the same quantity of fresh 7.4 pH phosphate buffer.

***In-vitro* drug permeation through rat abdominal skin from ethosomal Transdermal Patches**

In-vitro diffusion study for ethosomal Patch was carried out in a Franz diffusion cell using rat skin membrane. The rat skin membrane was mounted on the Franz diffusion cell. Place 2×1cm^2 size patch on the membrane in donor compartment. Reservoir compartment was filled with 20 ml phosphate buffer of pH 7.4 and the medium was maintained uniformly by magnetic stirring. The study was carried out at 37 ± 1°C for 24 h. 5ml of samples were withdrawn from reservoir compartment at predetermined time interval and absorbance was measured spectrophotometrically at 318nm. Each time the reservoir compartment was replenished with the same quantity of fresh 7.4 pH phosphate buffer [16].

Stability studies

Stability testing of drug products begins as a part of drug discovery and ends with the commercial product. To assess the drug and formulation stability, stability studies were done. The stability studies were carried out for the most satisfactory formulation (ET5 and ET10) and without cholesterol using formulations ET8 and ET10. The formulation was sealed in a glass vial and kept at 4 ± 2°C and at R.T (room temperature) for 2 months. At the end of 2 months, the sample was analyzed for the entrapment efficiency and *In Vitro* skin diffusion study.

Drug release kinetics

The data of Tizanidine hydrochloride from different ethosomal formulations was processed to understand the linear relationship. The data are processed for regression analysis, using MS excel statistical functions.

RESULTS AND DISCUSSION

Drug excipients compatibility studies

The FTIR spectrum of pure drug and optimized formulation was shown in fig.1 and 2. Tizanidine hydrochloride characteristic peaks were observed in the range from 1068 cm^{-1} C-N stretching in amines, 1448.59 cm^{-1} C=C Stretching, 1608 cm^{-1} C=N Stretching (in ring), 2968 cm^{-1} C-H Stretching in CH_3, CH_2, 3446 cm^{-1} N-H stretching in primary amines. These indicated that there was no major change in the position of peak obtained in drug alone and in a mixture of drug and excipients, thus it was proved that there was no chemical interactions between drug and excipients.

Visualization of vesicles, size and entrapment efficiency

Ethosomal formulations were prepared by cold method were translucent and had uniform dispersion of ethosomal vesicles. The three dimensional nature of formulated ethosomal vesicles were confirmed by scanning electron microscopy shown in fig.3 and microscopic photographs shown in fig.4.

Table 1: Formulation Chart of Ethosomal Suspension.

Formulation code	Drug (mg)	Lecithin (mg)	Cholesterol (mg)	Propylene glycol(ml)	Ethanol (ml)	Water (ml)
ET1	80	200	25	1	2	7
ET2	80	200	25	1	3	6
ET3	80	200	25	1	4	5
ET4	80	200	25	1	5	4
ET5	80	300	25	1	3	6
ET6	80	400	25	1	3	6
ET7	80	500	25	1	3	6
ET8	80	400	-	1	3	6
ET9	80	200	-	1	3	6
ET10(Son)	80	400	25	1	3	6
ET11(Son)	80	200	25	1	3	6

The size of the ethosomes was found in the range of 217.0 nm to 472.7 nm. The data was shown in table 4. The concentration of ethanol an increased, the size of the vesicles decreased. However, as the phospholipids concentration an increased the size of the vesicles also increased. Furthermore, when the ethanol and phospholipids concentration were kept constant and formulations were subjected to sonication, the size of the vesicles was also reduced. Formulations ET8 and ET9 are formulated without cholesterol, these formulations

shown vesicle size of 472.7 nm and 468.2 nm respectively, this may be due to the aggregation of vesicles.

Entrapment efficiency of ethosomes containing Tizanidine hydrochloride was shown in the table 4. From these studies entrapment efficient found in the range of 33.98±0.68% to 65.69±0.39%. The entrapment efficiency percentage was found to an increased with increasing the ethanol concentration up to 30% beyond that concentration there was decreased in entrapment efficiency observed. It is also the case with phospholipids concentration, here up to 40% there was an increased in entrapment efficiency beyond that concentration was found to decrease in entrapment efficiency.

Zeta Potential

Zeta potential is an important parameter that affects the aggregation of vesicles and depicts the physical stability of vesicular systems. Zeta potential of optimized formulations ET5 and ET10 were found to be high -39.7 and -42.1 as shown in fig.5 and 6 respectively. High zeta potential prevents the aggregation between vesicles and hence, enhances its physical stability. It has been investigated that high zeta potential in ethosomes increase the inter bilayer distance owing to electrostatic repulsion. In current investigation, it was observed that there was not much difference in zeta potential of different ethosomal formulation. This signifies that there is no major influence of lecithin and ethanol concentration on zeta potential of ethosomes.

In-vitro diffusion studies of ethosomal suspension through goat ear skin

The obtained results were shown in fig.7. Among unsonicated ethosomal formulations ET5 was showed good percentage of cumulative drug release 87.23±1.04 and for sonicated ethosomes ET10 was showed good percentage of cumulative drug release of 95.61±2.59 at 24 h. It indicates that the sonicated formulation has better permeation efficiency than unsonicated formulations due to their smaller in size.

***In-vitro* drug release studies of ethosomal transdermal patch through goat ear skin**

These results were given in fig.8. Ethosomal suspension formulations ET5, ET6, ET10 were selected for transdermal patch preparation because of these exhibit good entrapment efficiency and percentage of drug release. Among the four formulations (table.2) T3 showed greater drug release 89.17±.45 at 24 h. due to the sonicated formulation(ET10) had smaller size vesicles lead to good permeation capability and T4 formulation shown 45.23±0.59 at 24h due to the patch contain non ethosomal form of drug.

Table 2: Formulation chart of Tizanidine Hcl Ethosomal Transdermal Patch.

Formulation code	HPMC E15 (mg)	DCM. M (1.1) (ml)	Triethyl citrate (ml)	Drug
T1	500	10	1ml	3.75ml of ET5
T2	500	10	1ml	3.75ml of ET6
T3	500	10	1ml	3.75ml of ET10
T4	500	10	1ml	60mg of drug

Table 3: Stability behavior of colloid as per zeta potential.

Zeta potential [mV]	Stability behavior of the colloid
from 0 to ±5	Rapid coagulation or flocculation
from ±10 to ±30	Incipient instability
from ±30 to ±40	Moderate stability
from ±40 to ±60	Good stability
more than ±61	Excellent stability

Table 4: Entrapment Efficiency and Average Vesicular Size of Ethosomes.

Formulation	Entrapment Efficiency ± S.D	Avg vesicular size(nm)
ET1	33.98±0.68	356.8
ET2	41.62±0.80	239.6
ET3	27.09±1.10	229.3
ET4	19.88±0.47	221.1
ET5	42.67±0.62	250.1
ET6	53.45±0.98	276.0
ET7	39.30±0.40	384.0
ET8	51.97±0.69	472.7
ET9	39.30±0.48	468.2
ET10	65.69±0.39	217.0
ET11	43.13±0.72	195.8

Table 5: Comparison of Entrapment Efficiency before and after Stability Studies.

Formulation code	Before stability studies	At R.T	At 4 ± 2°C
ET5	73.45±0.98	70.19±0.98	72.23±0.75
ET8	68.97±0.69	46.62±0.80	51.13±0.72
ET9	39.30±0.48	28.30±0.98	30.30±0.61
ET10	95.69±0.39	94.37±0.39	95.22±0.98

All the values are mean of 3 readings ± S.D

Table 6: Comparison of *In-Vitro* Drug Release before and after Stability Studies.

Formulation code	Before stability studies(24h)	After stability studies	
		At 4 ± 2°C(24h)	At R.T(24h)
ET5	87.23±1.04	86.23±1.14	85.23±1.02
ET8	85.36±0.80	71.36±0.18	63.36±0.18
ET9	79.19±1.52	68.19±1.52	59.19±1.52
ET10	95.61±2.59	93.21±2.59	92.21±1.29

All the values are mean of 3 readings ± S.D

Table 7: Drug Release Kinetics of Ethosomal Suspension.

Formulation	Zero order(R^2)	First order(R^2)	Higuchi (R^2)	Hickson Crowell(R^2)	Korsmeyer's peppas(R^2)	n-value
ET5 ET10	0.995 0.988	0.962 0.933	0.925 0.949	0.277 0.334 0.164	0.991	0.602
Pure drug	0.978	0.958	0.818		0.975	0.596
					0.975	0.556

Table 8: Drug Release Kinetics of Ethosomal Transdermal Patch.

Formulation	Zero order(R^2)	First order(R^2)	Higuchi (R^2)	Hickson Crowell(R^2)	Korsmeyer's peppas(R^2)	n-value
ET5 ET10	0.995 0.992	0.961 0.961	0.867 0.913	0.281	0.995	1.034
Pure drug	0.998	0.992	0.894	0.325	0.984	1.238
				0.144	0.998	0.946

***In-vitro* drug release studies of ethosomal transdermal patch using rat abdominal skin**

The best ethosomal transdermal patch formulation T3 and T4 selected for in-vitro drug release studies using rat abdominal skin. The percentage of drug release through rat abdominal skin found to be 94.31±0.24 and 48.44±0.23 at 24 h respectively (fig.9). When compare the both *In Vitro* studies (goat ear skin and rat abdominal skin), the percentage of drug release through rat abdominal skin found to be greater than the goat ear skin; this may be due to sensitivity and thinness of skin. In current investigation found that the ethosomal form of transdermal patch greater permeation of drug than normal transdermal patch(T4) it prove that ethosomes have greater skin permeation due to the presence of ethanol it help in skin permeation.

Stability studies

the stability studies were conducted for ethosomal suspension form formulations ET5, ET10 and ET8, ET9. Since ET5 (unsonicated) and ET10 (sonicated) were optimized best formulations and ET8 and ET9 were without cholesterol using formulations. For ET5 and ET10 found that there was no major change in evaluated parameters like entrapment efficiency and *in-vitro* drug diffusion at 4 ± 2°C and at R.T. But for ET8 and ET9 observed that drastic change in entrapment efficiency and *in-vitro* drug release profile at 4 ± 2°C and at R.T. it may due to the exception of cholesterol. It proves that cholesterol plays an important role in stability of ethosomes. The values were shown in table 5 and 6.

Drug release kinetics

The data of drug release kinetics was given in table 7 and 8. The release kinetics of Tizanidine hydrochloride found to be zero order and non-fickian type of transport.

CONCLUSION

The most satisfactory formulation ET10 showed better entrapment efficiency and drug release observed that 95.69±0.39 and 95.61±2.59% respectively. And it had good stability and smaller vesicle size. Therefore, it was selected as the best formulation. When it was formulated in transdermal patch form (T3) also has shown good percentage of drug release. Thus it concluded that ethosomes is a very promising carrier for transdermal drug delivery and it creating a new opportunities for topical application of Tizanidine hydrochloride in the treatment of muscle spasms.

ACKNOWLEDGEMENT

The authors are thankful to D.Manohar Reddy, Chairman, Trinity College of Pharmaceutical Sciences for their kind help and providing all necessary facilities.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

REFENCES

1. Riaz Ahmed Sheikh, Khurram Siddique,Shirin Mirza, Syeda Fizza Tauqir: Role of
2. muscle relaxant (Tizanidine) in painful muscle spasm. Ann Pak Inst Med Sci 2010; 6(1): 20-23.
3. Roge Ashish.
   B, Sakhare Ram,S, Bakal RL, Channawar MA, Bakde BV, Gawand e SR,
4. Chandewar AV: Ethosomes: Novel approach in transdermal drug delivery system. RJPD FT 2010; 2(1): 23-27.
5. ElkaTouitou: Drug delivery across the skin. Expert Opin Biol Ther 2002; 2(7): 723-33.
6. Aujla Manvir, Rana A.C, Bala Rajni, Seth Nimrata: Comparative potential of vesicular carriers for transdermal drug delivery: A review. RJPT 2012; 5(3): 302-306.
7. Ambekar Abdul Wahid,, Nagaraju Ravouru, Sawant Ramesh Lakshman: Ethosomes: A tool for transdermal drug delivery. Current Trends in Biotechnology and Pharmacy 2011; 5 (1): 972-981.
8. Rakesh. R, Anoop.K.R: Ethosomes for transdermal and topical drug delivery. Int J Pharm Pharm Sci 2012; 4(3): 17-24.
9. Anju Dhiman and Deepika Singh: Development, characterization & *In Vitro* skin permeation of Rutin ethosomes as a novel vesicular carrier. IJBR 2013; 4 (10): 559-566.
10. K. Barupal, Vandana Gupta, and Suman Ramteke: Preparation and characterization of ethosomes for topical delivery of Aceclofenac.Indian J Pharm Sci 2010; 72(5): 582–586.
13. Bhasin Vivek,, Yadav Himanshi, Markandeywar Tanmay, Murthy R.S.R: Ethosomes: The novel vesicles for transdermal drug delivery. IJPI's Journal of Pharmaceutics and Cosmetology 2011; 2(7): 68-80.
14. Xingyan Liu, Hong Liu, Jianqiang Liu, Zhiwei He, Congcong Ding, Guoliang Huang, Weihua Zhou, and Leshan Zhou. Preparation of a Ligustrazine ethosome patch and its evaluation *In Vitro* and in vivo. Int J Nanomedicine 2011; 6: 241–247.

17. Radhika Gangineni, Prabhakar Dontha, Shravan Kumar Konda: Formulation, characterization and evaluation of self emulsifying drug delivery system of Repaglinide. JPR 2013; 1(7): 665-672.
18. Soni M.L, Kumar. M and Namdeo.K.P: Sodium alginate microspheres for extending drug release: Formulation and *In Vitro* evaluation. Int J Drug Deli 2010; 2: 64-68
19. Seema M. Jadhav, Pournima Morey, Manisha Karpe, Vilasrao Kadam: Novel vesicular system: An overview. JAPS 2012; 2 (1): 193-202.
20. Yuefeng Rao, Feiyue Zheng, Xingguo Zhang, Jianqing Gao, and Wenquan Liang: *In Vitro* percutaneous permeation and skin accumulation of Finesteride using vesicular ethosomal carrier. AAPS PharmSciTech 2008; 9(3): 860–865.
21. Jadupati Malakar, Suma Oomen Sen, Amit Kumar Nayak, and Kalyan Kumar Sen: Development and evaluation of microemulsions for transdermal delivery of Insulin. ISRN Pharmaceutics 2011; Article ID 780150: 1-7.
22. Prabhakar D, Sreekanth J, Jayaveera K.N: Development and evaluation of transdermal patches of Azelnidipine. IJPPS 2013; 5(3): 805-810.