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SOLUBILITY ENHANCEMENT OF POORLY WATER SOLUBLE DRUG USING NATURAL CARRIER

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ABSTRACT

Atorvastatin calcium is a synthetic lipid-lowering agent. Atorvastatin (ATR) is an inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase. This enzyme catalyzes the conversion of HMG-CoA to mevalonate, an early and rate-limiting step in cholesterol biosynthesis. According to the biopharmaceutical classification, ATR comes under Class II (low solubility and high permeability). Because of the limited aqueous solubility, it exhibits dissolution rate limited oral absorption. The objective of this investigation was to improve the solubility of the poorly water soluble drug atorvastatin, using solid dispersion (SD) techniques, with Aegel marmelos Gum (AMG) as a hydrophilic carrier. The effect of two variables related to solid dispersions preparation (drug to carrier ratio and method of preparation) were investigated. All the SDs prepared by Microwave induced fusion and Lyophilisation techniques showed remarkable increase in the solubility compared to the pure ATR.The solubility analysis demonstrated highest increase in the solubility of drug observed with ATR-AMG ratio 1:1 by lyophilisation technique. During In Vitro study result obtained that the SD prepared using the Lyohilisation method containing 1:1 ATR-AMG ratio displays faster dissolution rates compared with those prepared using the other that is 98.8±0.09% drug release within 90 min. The SD was characterized using DSC and XRD technique.

Keywords: Atorvastatin Calcium**,** Lyophilisation, Microwave, Solid Dispersion

INTRODUCTION

In recent years, the formulation of poorly soluble compounds presented interesting challenges for formulation scientists in the pharmaceutical industry. Up to 40% of new chemical entities discovered by the pharmaceutical industry are poorly soluble or lipophilic compounds, which lead to poor oral bioavailability The enhancement of oral bioavailability of poorly water soluble drugs remains one of the most challenging aspects of drug development. Atorvastatin (ATR) is a synthetic lipid-lowering agent.[1-3] Atorvastatin is an inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase. This enzyme catalyzes the conversion of HMG-CoA to mevalonate, an early and rate-limiting step in cholesterol biosynthesis. According to the biopharmaceutical classification, ATR comes under Class II (low solubility and high permeability). Because of the limited aqueous solubility, it exhibits dissolution rate limited oral absorption.[10, 11, 12] Solid dispersion (SD) techniques have been used to enhance the dissolution and oral bioavailability of many poorly water soluble drugs.[4, 5] To overcome the solubility problem, many authors formulated solid dispersions using number of various polymers and methods. In spite of tremendous research activity on solid dispersions since 1961, their commercial application is limited. Only a few products have been marketed so far.[6, 7, 8, 9] One aspect of solid dispersion technology on which most workers in the field would agree is that the number of marketed products arising from this approach has been disappointing. Research for alternative carriers has been increasing to suit for the industrial applications as well as to reduce the production cost and toxic effects. Recently, many natural polymers have been evaluated for their uses in formulation of solid dispersion. Cost effective pharmaceutical excipients are always desirable.[13,14] Pharmaceutical excipients developed from natural sources are economic. Present day consumers look for natural ingredients in food, drugs and cosmetics as they believe that anything natural will be more safe and devoid of side effects.[15,16] Natural excipients show lack of toxicity, easy availability and economic considerations in pharmaceutical industry as compared to their synthetic counterparts. Naturally, derived excipients have shown promising results in the modification of drug release from the formulations.[17,18] Aegel marmelos Gum (AMG) is a natural polymer that is obtained from fruits of Aegle marmelos belonging to family Rutaceae is indigenous to India. AMG has been investigated for use as a tablet binder and mucoadhesive agent. AMG is widely used because of its high swelling index, high water retention capacity, digestible nature, binding ability, and easy availability. [19-20] The objective of this investigation was to improve the solubility of the poorly water soluble drug atorvastatin, using solid dispersion techniques, with AMG as a hydrophilic carrier. The effect of two variables related to solid dispersions preparation (drug to carrier ratio and method of preparation) were investigated. The apparent solubility was investigated, and in vitro dissolution studies were performed.

MATERIALS AND METHODS

Atorvastatin calcium gift sample was provided by Zydus Cadila Healthcare, Ahmedabad, India. Aegel marmelos fruits were collected from nearby locality of Pune region of Maharashtra, India.

Isolation and Purification of Aegle marmelos Gum

Fresh pulpy parts of edible fruits of Aegle marmelos were soaked in distilled water and boiled for 2-3 hours in a water bath until slurry was formed. The slurry was cooled and kept in refrigerator overnight so that most of the undissolved portion was settled out. The upper clear supernatant solution was decanted off and concentrated at 60ºC on a water bath until the volume reduced to its one third. Solution was cooled down to the room temperature and was poured into thrice the volume of acetone by continuous stirring. The precipitate was washed repeatedly with acetone and dried at 50ºC. The dried gum was powdered and stored in tightly closed container for further use.[19, 20]

Characterization of Aegel marmelos Gum [20,25]

Macroscopic Property

Color, Taste, Odor

Solubility

Small amount of AMG powder added into 5ml solvent and solubility checked visually.

Swelling Index

AMG (1 g) was weighed accurately and transferred to a 100mL measuring cylinder. The initial volume of the powder was noted. Then the gum was dispersed thoroughly in distilled water by vigorous shaking. The measuring cylinder was maintained for 24 hours at ambient temperature and humidity. The volume occupied by the AMG sediment after 24 hours was noted. The swelling index, expressed as a percentage, was calculated according to the following equation:

$$SI = (X1 - X0/X0) \times 100$$

Where X0 is the initial height of the powder in the graduated cylinder and X1 is the height of the swollen gum after 24 hours.

Water Retention Capacity

After the swelling index study was carried out, the content of the measuring cylinder was filtered using a muslin cloth, and the water was allowed to drain completely into a dry 100mL graduated cylinder. The volume of the water collected was noted. The water retained by the sample was determined as the difference between the original volume of the mucilage and the volume of the drained water. The amount of water retained per unit volume of a polysaccharide is referred to as its water retention capacity or water absorption capacity.

Viscosity Measurement

The viscosity of a 1% aqueous AMG solution was measured according to USP specifications using a Brookfield DV-E viscometer.

Angle of Repose

The angle of repose was measured using the fixed funnel method. An accurately weighed quantity of powdered gum was poured through a funnel. The height of the funnel was adjusted such that its tip just touched the top of the heap of powder below it. The powder was allowed to flow through the funnel freely on to the heap of powder, and the angle of repose was calculated using the following equation: $\tan\theta = h/r$ .Where h is the height of the heap of powder and r is the radius of the heap of powder.

Density

The loose bulk density (LBD) and tapped bulk density (TBD) of the AMG powder were determined. Powdered gum (5 g) was poured into a calibrated measuring cylinder (10mL capacity), and the initial volume was noted. Then the cylinder was dropped onto a hard surface from a height of 2.5 cm at 2-second intervals. The tapping was continued until no further change in volume was noted. The LBD and TBD were calculated using the following equation

LBD = Weight of the powder /Initial volume of the packing

TBD = Weight of the powder /Tapped volume of the packing

Compressibility index

The compressibility index (Carr's index) was determined using the following equation:

$$\text{Carr's index (\%)} = [(\text{TBD} - \text{LBD} / \text{TBD})] \times 100$$

Methods of Preparation of Solid Dispersion (SD)

A physical mixture (PM) of AMG and ATR was prepared by simple blending using a spatula. The PM was passed through a 100# sieve.

Microwave Induced Fusion Method

Solid dispersion was prepared by thoroughly grinding accurately weighed quantities of ATC and AMG in various ratios (1:1 and 1:2) for 20-30 minutes in glass mortar individually. The dispersions were then sifted through sieve no.80 and stored in desiccators till further use.[21, 22]

Lyophilisation Technique

The earlier prepared 1:1 and 1:2 physical mixtures of the ATR and AMG were wetted with 1:1 water: methanol mixture and this was kneaded to form a homogeneous suspension. This was then frozen and subjected to lyophilisation for 24hrs at -54°C. The final product was then pulverized and shifted through sieve no. 80. [23, 24]

Characterization of SDs

Drug Content

Quantities of the physical mixtures (Equivalent to 20 mg of ATR) produced using the Microwave induced fusion and lyophillisation methods were dissolved in 100mL of phosphate buffer solution of pH 6.8.The samples were filtered through a Whatman filter paper (No. 41). It was then diluted appropriately with the solvent and the drug content of each was determined spectrophotometrically at 241 nm. Phosphate buffer solution (pH 6.8) was used as the blank.

Solubility Study

Solubility study was performed according to method reported by Higuchi and Connors. The solubility data of SD prepared using the Microwave induced fusion and lyophillisation methods in phosphate buffer solution (pH 6.8) were determined. Quantities of the SD equivalent to 20mg of the drug were added to 10 ml Phosphate Buffer Ph 6.8 taken in glass vials with cap and shaken for 24 hrs. The vials were kept on a orbital shaker maintained at 37±0.5°C for 24 h. After shaking, the vials were kept equilibrated at 37±0.5°C for 12 h. Then, the solution was filtered through a 0.45-μm millipore filter and the filtrate was assayed spectrophotometrically at 241nm. It was then diluted appropriately with the solvent and its absorption was observed through UV spectrophotometer at 241nm.

In Vitro Drug Release Study

The dissolution rates of the different SD were determined using 900mL of phosphate buffer solution (pH 6.8) at 37 ± 0.5°C using a type II USP dissolution test apparatus (EDT-08L-Electrolab, Mumbai, India) run at 75 rpm. 5ml aliquots of the dissolution medium were withdrawn at 10, 20, 30, 40, 50, 60,70, 80 and 90 minutes. The samples were suitably diluted and analyzed spectrophotometrically at 241 nm.

Infrared Spectroscopy

The Infrared spectroscopy of the pure ATR, AMG and SDs were carried out to ascertain identity of the drugs. The drug powder was placed on IR compartment and scanned between wave number 4000-1 - 500 cm-1 using a Shimadzu Model 8400.

X-Ray Diffraction Studies

Powder XRD patterns of pure ATR, AMG and SDs were recorded using a diffractometer. The diffractometer (Bruker D5) was run at a scanning speed of 2∘/mm and a chart speed of 2∘/2 cm per 2θ.

DSC Thermogram

DSC thermogram of pure ATR, AMG andSDs were obtained using a differential scanning calorimeter with a heating rate of 10^0C/minute from 50^0C to 200^0C in a nitrogen atmosphere using Mettle Toledo DSC-1 Thermal Analyzer.

RESULT AND DISCUSSION

Polymer Characterization

The results of the AMG characterization studies are listed in Table 1. AMG has a low viscosity and high water retention capacity. The water retention capacity of a carrier is the amount of water retained in it which indicates the hydrophilic nature of the carrier. Low viscosity of AMG makes it suitable candidate for solubility and bioavailability enhancement of poorly water soluble drugs.

Drug Content

The drug content of the SD and the PM is provided in Table 2. The results clearly suggest that the drug content of each formulation is within the theoretical range, indicating that the method used to prepare the formulations is suitable and reproducible in nature.

Solubility Study

The solubility values of different SDs containing ATR+AMG (1:1) and ATR+AMG (1:2) are reported in Table 3 and Table 4.The solubility of ATR has definitely increased in presence of AMG. The method of preparation and ratio with AMG also had definite impact on the solubility of ATR. It followed following pattern for solubility enhancement of ATR.

Method: Lyophilization Method >Microwave induced fusion > Physical Mixture

Ratio of ATR-AMG = 1:1 > 1:2

The SD prepared using the Lyophilisation method containing ATR-AMG in proportion of 1:1 ratio displays highest solubility compared with those prepared using the other. (**Table 3, 4**)

In Vitro Drug Release Study

The in vitro drug release profiles of ATR, the PM, and the SD prepared using the Microwave induced fusion and Lyophilisation methods with AMG as the hydrophilic carrier are shown in Figure 3 and 4. The SD prepared using the Lyohilization method containing 1:1 ATR: AMG ratio displays faster dissolution rates compared with those prepared using the other. On the basis of results of solubility and dissolution study SD prepared by Lyophilization method containing ATR: AMG in proportion of 1:1 ratio was used for further characterization. Results are shown in Figure 1and 2.

Infrared Spectroscopy

IR spectrum was taken by ATR technique and graph is shown in Figure 3, 4, 5 and 6. The IR spectra of mixture of ATR + AMG (1:1) SD prepared using lyophilisation shows peaks at 1317 cm-1 for C-N (stretching), 1647cm-1 for C=O, 1581cm-1 for N-H (bend), 1055.06cm-1 for C-O (stretching), 3410.5cm-1 O-H (stretching), 2933.7cm-1 for –CH3 and other important peaks are seen. (Figure 5). The IR spectra of mixture of ATR + AMG (1:1) SD prepared using Microwave induced fusion method shows peaks at 1317cm-1 for C-N (stretching), 1651.07cm-1 for C=O, 1059.99cm-1 for C-O (stretching) CH3 and other important peaks are seen.(Figure 6)The spectra indicated intact peaks for pure ATR as well as pure AMG. In all cases however, there was reduction in intensity of ATR peak which is indicating formation of SDs. This shows that there is no difference in the absorption band position.

X Ray Diffraction Studies

The X-ray diffractogram of ATR shows sharp and intense peaks at diffraction angles (2θ) of 16.23∘, 20.91∘, 22.6∘, 28.56∘, 36.32∘ suggesting a typical crystalline pattern. The X-ray diffractogram of AMG shows broad peaks suggesting its amorphous nature. Few characteristic crystalline peaks appear in the diffractograms of the SDs but at low intensity. This proves that the crystallinity of ATR decreases as most of the drug gets converted to an amorphous form. (Figure: 7, 8, 9 and 10).

DSC Thermogram

The DSC thermograms of ATR, AMG and SDs are shown in Figure 11, 12, 13 and 14. The thermograms of ATR exhibit an endothermic peak at 163.6°C, corresponding to its melting point, while AMG exhibits a broad endothermic peak owing to its amorphous nature. The broad endothermic peak of ATR was observed at 141^0C and 161^0C in the thermo gram of SDs prepared using Lyophilisation and Microwave induced fusion method respectively, suggesting that the crystalline form of ATR was converted to the amorphous one.

Table 1
AMG characterization

Parameters		Aegel marmelos Gum
Macroscopic property	Color	Yellowish color
	Taste	Sweet
	Odour	Characteristic sweetish
Solubility	Water	Soluble
	Acetone	Insoluble
	Ethanol	Insoluble
	Chloroform	Insoluble
Swelling index (%)		27.5 ± 2.5
Water retention capacity (mL)		7.71 ± 0.175
Viscosity (cps)		303 ± 2.645
Angle of repose		32.46±1.17
Density (gm/cm^3)		
(1) Bulk density		0.58 ± 0.02
(2) Tapped density		0.66± 0.02
Carr's index (%)		12.73±5.35

***All values are expressed as mean ±SD, n=3*

Table 2
Drug content

RATIO (ATR+AMG)	PM	MICRO	LYO
1:1	98.40± 0.8	98.46 ±0.94063	99.11 ±1.165
1:2	99.45±1.36	99.12±1.06	99.97± 0.657

****All values are expressed as mean ±SD, n=3;***
ATR: atorvastatin; PM: physical mixture; MICRO:Microwave induced fusion method; LYO: Lyophilisation method

Table 3
Solubility values for various SDs with ATR + AMG (1:1) in phosphate buffer (pH=6.8)

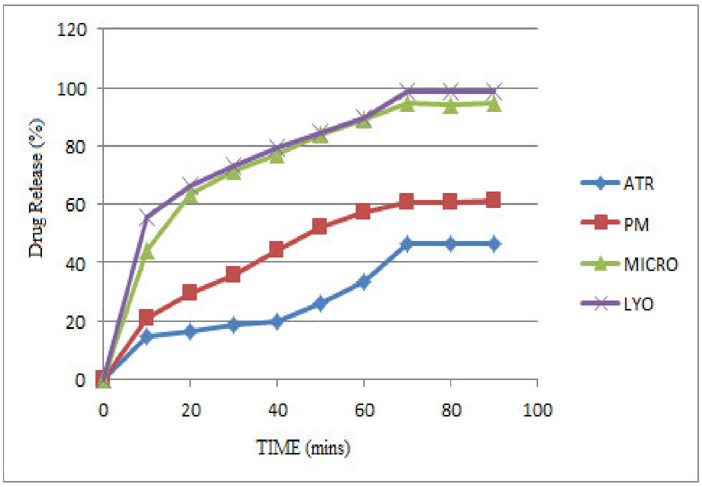
Sr. No	Method	Solubility (mg/ml)
1	Physical Mixture (PM)	0.348791±0.0124
2	Microwave Induced Fusion Method (MICRO)	0.563 ±0.012
3	Lyophilization Method(LYO)	0.59301±0.015004

***All values are expressed as mean ±SD, n=3**

Table 4
Solubility values for various SDs with ATR + AMG (1:2) in phosphate buffer (pH=6.8)

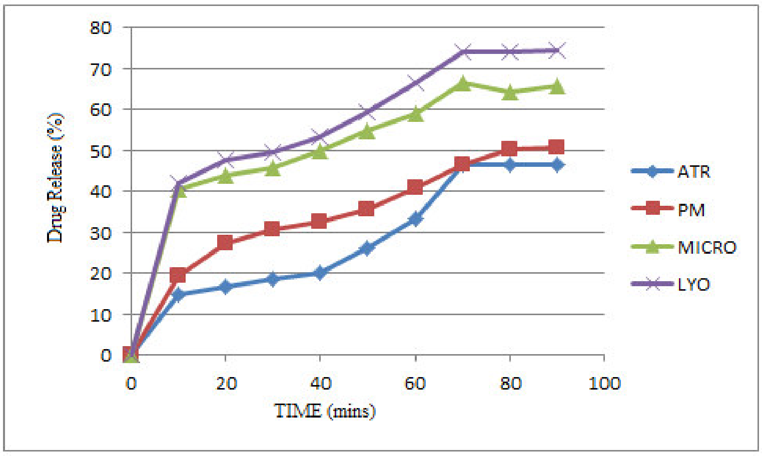
Sr .No	Method	Solubility (mg/ml)
1	Physical Mixture(PM)	0.265±0.004242
2	Microwave Induced Fusion Method (MICRO)	0.4467±0.011
3	Lyophilization Method (LYO)	0.521±0.0395

***All values are expressed as mean ±SD, n=3**



** ATR: Atorvastatin Calcium; PM: Physical mixture; MICRO: Microwave induced Fusion Method; LYO: Lyophilisation*

Figure 1
Dissolution data for various SDs with ATR + AMG (1:1) in phosphate buffer (pH=6.8)



** ATR: Atorvastatin Calcium; PM: Physical mixture; MICRO: Microwave induced Fusion Method; LYO: Lyophilisation*

Figure 2
Dissolution data for various SDs with ATR + AMG (1:2) in phosphate buffer (pH=6.8)

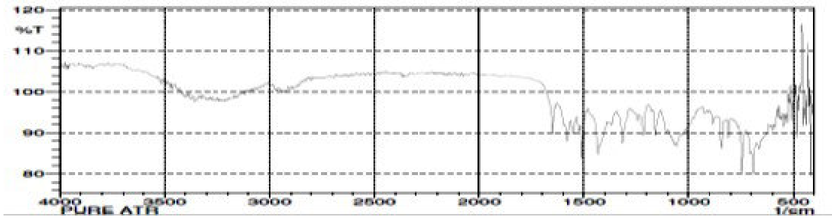


Figure 3
IR spectra of pure ATR

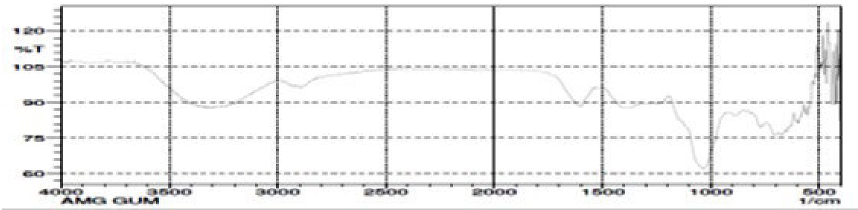


Figure 4
IR spectra of AMG

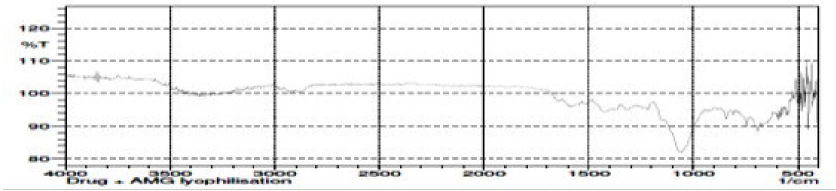


Figure 5
IR spectra of ATR+AMG (1:1) solid dispersion using lyophilization method

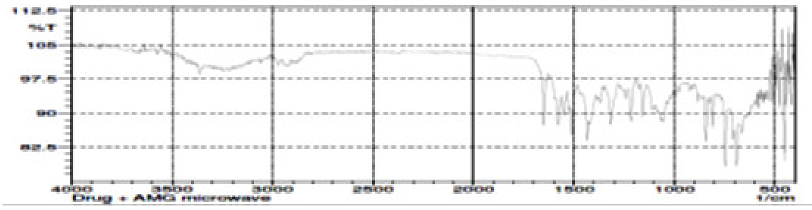


Figure 6
IR Spectra ATR+AMG (1:1) Solid dispersion using Microwave induced fusion method

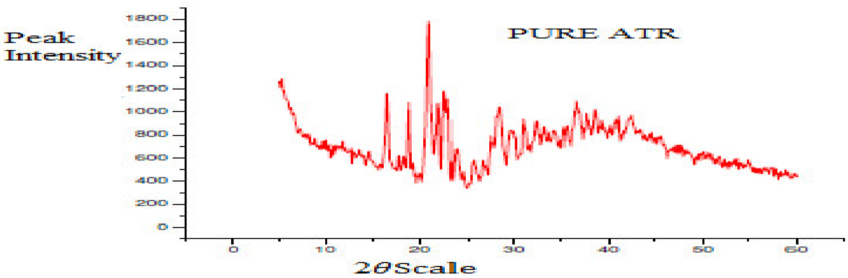


Figure 7
XRD of pure ATR

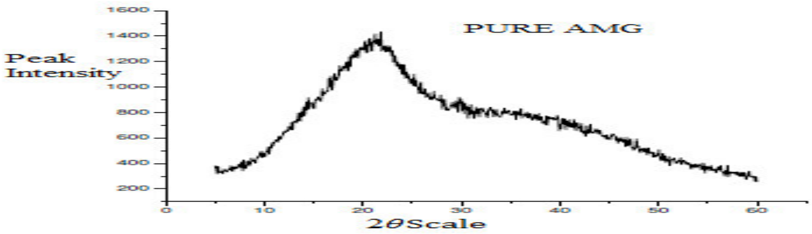


Figure 8
XRD of Aegel Marmelos Gum

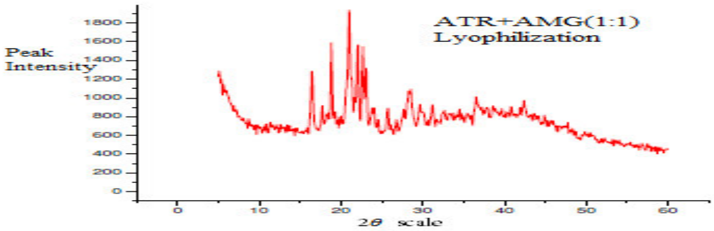


Figure 9
XRD of ATR+AMG (1:1) solid dispersion using lyophilization method

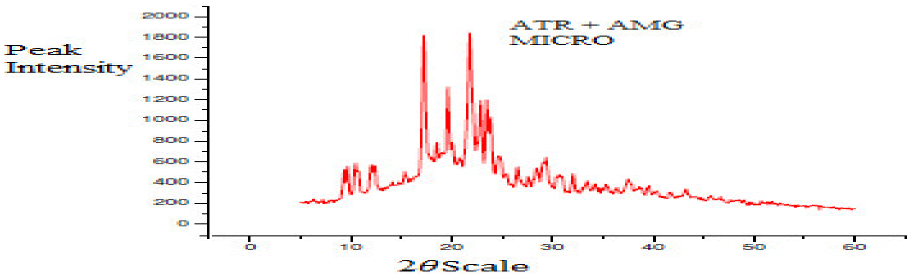


Figure 10
XRD of ATR+AMG (1:1) Solid dispersion using Microwave induced fusion method

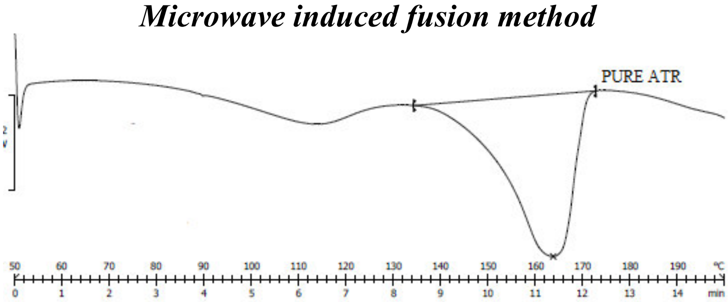


Figure 11
DSC of pure ATR

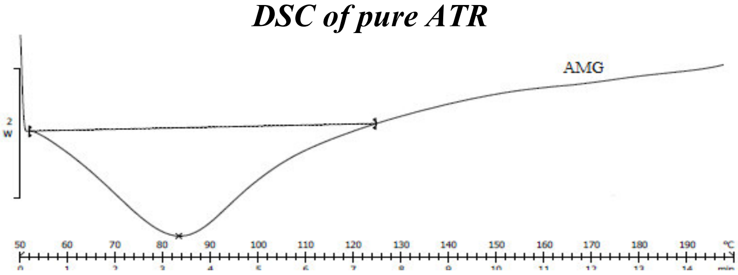


Figure 12
DSC of Aegel Marmelos Gum

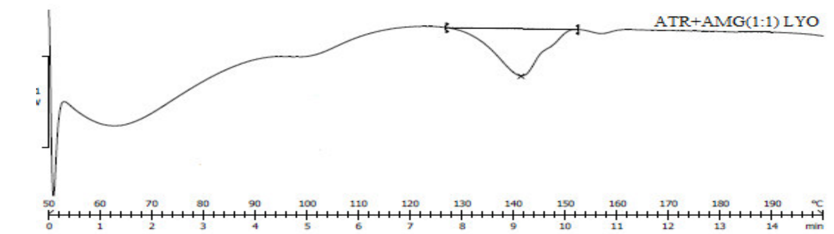


Figure 13
DSC of ATR+AMG (1:1) solid dispersion using lyophilization method

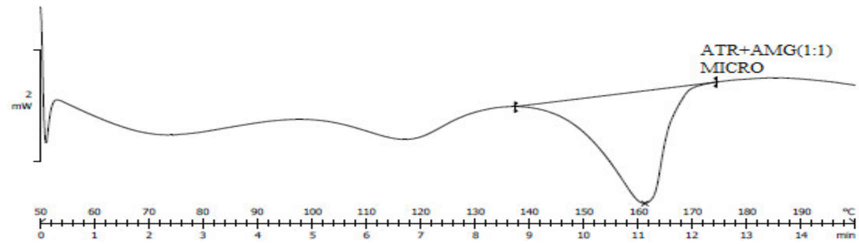


Figure 14
DSC of ATR+AMG (1:1) Solid dispersion using Microwave induced fusion method

CONCLUSION

Solid dispersions significantly improved the dissolution profiles. All the SDs prepared by Microwave induced fusion and Lyophilisation techniques showed remarkable increase in the solubility compared to the pure ATR. IR and UV spectral analysis results indicated that there was no probable interaction between drug and carrier. XRD, DSC studies showed almost inhibition of crystallinity in ATR solid dispersions with AMG. The SD prepared using the Lyohilisation method containing 1:1 ATR: AMG ratio displays faster dissolution rates compared with those prepared using the other. In conclusion, AMG could be used as a potential natural carrier to enhance the rate of dissolution of ATR.

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REFERENCES

1. Habib, M.J., Pharmaceutical solid dispersion Technology, Technomic Publishing Company, Inc. Lancaster, Pennsylvania (U.S.A.). 2001,1-36.
2. Patel DB, Patel MM. Natural Excipient in controlled Drug Delivery Systems. J Pharmacy Res 2009; 2:900-7.
3. Shirwaikar A, Prabu SL, Kumar GA. Herbal excipients in novel drug delivery systems. Indian J Pharm Sci 2008; 70: 415-22
4. Karanth H, Shenoy VS, Murthy RR. Industrially feasible alternative approaches in the manufacture of solid dispersion; A technical report, AAPS Pharmscitech. 2006; 7 : E1-E8.
5. Chiou WL, Riegelman S. Pharmaceutical application of solid dispersion. J Pharm Sci.1971; 60:1281-1302.
6. Vasconcelos T, Sarmanto B, Costa P. Solid dispersion as strategy to improve oral

bioavailability of poorly water soluble drugs, J Pharm Sci. 2007; 12:1068-1075.
7. Serajuddin A, Sheen PC, Agustine MA., Improved dissolution of a poorly water soluble drug from solid dispersion in polyethylene glycol; polysorbate 80 mixture, J Pharm Sci. 1990; 79:463-464.
8. Heo MY, Piao ZP, Kim TW, Cao QR, Kim A, Lee BJ., Effect of solubilizing and microemulsifying excipient in polyethyleneglycol 6000 solid dispersion on enhanced dissolution and bioavailability of ketoconazole. Arch Pharm Res. 2005; 28:604- 611.
9. Liu R. Water-Insoluble drug formulation. New York: CRC Press. 2nd ed. 2008; 522
10. United States Pharmacopoeia, United States Pharmacopoeia Convention, 2005, USP30-NF-25.
11. Desager JP and Horsmans Y, Clinical pharmacokinetics of 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors, Clin Pharmacokinet,1996, 31: 348–371.
12. Lennernäs H, Fager G, Pharmacodynamics and pharmacokinetics of the HMG-CoA reductase inhibitors. Similarities and differences, Clinical Pharmacokinetics, 05/1997; 32(5):403-25
13. Saharan VA, Kukkar V, Kataria M, Gera M, Choudhury PK. Dissolution Enhancement of Drugs Part II: Effect of Carriers. Int J Health Res 2009; 2:207-23.
14. Dixit AK., Singh RP, and Singh S, Solid Dispersion - A Strategy for Improving the Solubility of Poorly Soluble Drugs. International Journal of Research in Pharmaceutical and Biomedical Sciences, 2012, 3(2): 960-966.
15. Kapoor B, Kaur R, Kour S, Behl H, Kour S. Solid Dispersion: An Evolutionary Approach for Solubility Enhancement of Poorly Water Soluble Drugs. Int J Recent Adv Pharm Re. 2012, 2(2): 1-16.
16. Patel M, TekadeA,Gattani S, and Surana S; Solubility Enhancement of Lovastatin by Modified Locust Bean Gum Using Solid Dispersion Technique AAPS PharmSciTech, 2008,4 (9):1262-1274.
17. Kulkarni U, RaghavendraRao NG, ,Design And Development Of Aceclofenac Fast Dissolving Tablets By Amorphous Solid Dispersion Technique Using Modified Aegle Marmelos Gum. Ijprd, 2011, 3(6): 201 – 210.
18. Reddy S A, Rangaraju A, Kant A, Shankraiah MM, Venkatesh JS, R. Nagendra Rao and C. Nagesh; Solubility And Dissolution Enhancement Of Cefixime Using Natural Polymer By Solid Dispersion Technique ijprc; 2011, 1(2): 412-423.
19. Md Tausif Alam, Nayyar Parvez, and Pramod Kumar Sharma, FDA-Approved Natural Polymers for Fast Dissolving Tablets, Journal of Pharmaceutics, 2014, 1: 1-6.
20. Yogesh Joshi, Ratendra Kumar Chaudhary, U.V.S. Teotia, Formulation and Evaluation of Diclofenac Sodium Sustained Release Matrix Tablets Using Aegle Marmelos Gum, IJCTPR, 2013, 1(3): 174-180.
21. Preeti N Sable, Swapnali R Shinde, Manisha B Desai, Vijayalaxmi A Chavan, An Approach to Enhance Solubility of Etodolac by Microwave Induced Solid Dispersion, Journal of Medical and Pharmaceutical Innovation,2014,1 (3) : 24-31.
22. Vasconcelos T, Sarmanto B, Costa P. Solid dispersion as strategy to improve oral bioavailability of poorly water soluble drugs. J Pharm Sci. 2007; 12: 1068-1075.
23. Takayama K, Nambu N, Nakai T. Factor affecting the dissolution of ketoprofen from solid disoersion in various water soluble polymers. Chem Pharm Bull. 1982; 30: 673-677.
24. Sharma DK, Joshi SB. Solubility enhancement strategies for poorly water soluble drug in solid dispersion: A Review, Asian Journal of Pharmaceutics 2007; 1:9-19.
25. Madhuri S. Rodde, Ganesh T. Divase, Tejas B. Devkar, and Avinash R. Tekade, Solubility and Bioavailability Enhancement of Poorly Aqueous Soluble Atorvastatin: In Vitro, Ex Vivo, and In Vivo Studies, BioMed Research International ,2014(10):1-10.