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Comparative Dissolution Study of Metoprolol Tartrate Loaded PLGA (50:50) and PLGA (75:25) Microparticles

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To prepare and evaluate comparatively the dissolution behaviour of metoprolol tartrate loaded PLGA (50:50) and PLGA (75:25) microparticles. Metoprolol tartrate loaded PLGA microparticles were prepared using non-solvent addition phase separation technique with drug to polymer ratio 1:1, 1:2 and 1:3. Metoprolol tartrate contents were determined spectrophotometrically at 273 nm. Drug-polymer compatibility was determined by FTIR, XRD and thermal analysis. Microparticle morphology was characterized by SEM. *In vitro* dissolution studies for microparticles were performed in phosphate buffer pH 7.2 and the samples were analyzed by HPLC with UV detector operated at 273 nm. Mean particle size range was 15-65 µm for both grades of PLGA. The encapsulation efficiency range was 60-88 % in various formulations. Biphasic release phenomenon was observed with an initial fats release phase and subsequently a continuous and slower release thereafter. The non-solvent addition process led to formation of porous, spherical and discrete metoprolol tartrate microparticles with PLGA. The microparticles with PLGA 75:25 exhibited more sustained release of drug than those of with PLGA 50:50. Similarly *in vitro* release of drug was also affected by polymer concentration. FTIR, XRD and DSC results showed no drug-polymer chemical interaction during the microencapsulation except slight modification of drug particle behaviour from crystalline to amorphous behaviour.

Key Words: Dissolution, Metoprolol tartarate, Poly lactic co-glycolic acid.

INTRODUCTION

Metoprolol tartrate (MT) is widely used as a drug of choice in the treatment of hypertension, angina pectoris and arrhythmias[1]. Chemically it is 1-(isopropylamino)-3-*p*-(2-methoxyethyl)phenoxy-2-propanol (2:1) dextro-tartrate. It acts as β_1-selective adrenergic blocking agent at low doses but at high doses it loses its selectivity and blocks β_2-adrenergic receptors also. So it is necessary that an optimum level of drug must be maintained in the body to obtain required therapeutic effect. As the half life of drug is about 3-4 h and administered at a dose of 100-200 mg daily so multiple doses of the drug are required to maintain a constant plasma concentration for a good therapeutic response and improved patient compliance[2]. It has been reported that administration of immediate release tablets of metoprolol tartrate exhibit fluctuations in plasma drug levels resulting either in manifestation of side effects or reduction in drug concentrations at the receptor sites[2].

Hence, an attempt is made to formulate a sustained and controlled release formulation of MT by developing microparticles using biodegradable poly lactic-co-glycolic acid (PLGA). Poly lactic-co-glycolic acid (the copolymer of lactic and glycolic acids) has shown to be biocompatible as well as bioabsorbable and it degrades to toxicologically acceptable lactic and glycolic acids[3]. Also, PLGAs have been approved by FDA as controlled drug release microspheres[4].

As several sustained or extended release metoprolol formulations such as mucoadhesive buccal tablets[5], extended release matrix tablets[6], oros controlled release delivery system[7] and transdermal patches[8] have been previously developed by using different polymers such as carbopol-934, hydroxy-propylmethylcellulose, hydroxyethylcellulose, sodium carboxy-methylcellulose, polyvinylpyrrolidone, cellulose acetate phthalate, hydroxypropylmethylcellulose phthalate and ethyl cellulose, however there is no example of microencapsulation of MT with PLGA[9].

In the present study, multiparticulate system was preferred as a formulation over conventional tablet or capsule formulations as it has several advantages like, it increases the surface area of the formulation exposed to the absorption site thus increasing the absorption of drug and providing extended clinical effect as well as reducing dosing frequency. The experiments were designed to study the influence of PLGA grades (75:25 and 50:50) at three levels 1:1, 1:2 and 1:3 drug polymer ratio, on the release behaviour of MT from microparticles.

EXPERIMENTAL

Metoprolol tartrate was gifted by Sun Pharmaceuticals, Pakistan. Poly (d,l-lactide-co-glycolide), PLGA (50:50) (RG 502; Mw 100,000) and PLGA (75:25) (RG 506; Mw 107,000) were obtained from Sigma-Aldrich, USA. Dichloromethane and *n*-hexane were supplied by BDH, UK and Merck, Germany, respectively. Mineral oil was obtained from Acros Organics, USA. Magnesium trisilicate was obtained by Alfa Aesar, China.

Preparation of microparticles: Metoprolol tartrate was encapsulated by a non solvent addition-phase separation technique. Dichloromethane and liquid paraffin were used as solvent and non solvent, respectively. PLGA was dissolved in 25 mL dichloromethane in a closed beaker with magnetic stirring (Velp, Europe) at 500 rpm for 1 h subsequently with the incorporation of MT with continuous stirring. The coacervation was induced by the addition of 50 mL liquid paraffin (non-solvent) containing 0.2 g magnesium trisilicate which would inhibit the microparticles from sticking with each other. The stirring was continued throughout the procedure. Finally, the microparticles were washed with *n*-hexane in triplicate and dried in air for 2 h followed by drying in oven (Memmert, Germany) at 40 ºC for 6 h. Two forms of biodegradable PLGA such as PLGA (50:50) and PLGA (75:25) were employed as a coating polymer. The drug to polymer ratios employed were 1:1, 1:2 and 1:3 for MT to PLGA (50:50) and these microparticulate formulations were named as M_1, M_2 and M_3. Similarly M_4, M_5 and M_6 abbreviations were used when 1:1, 1:2 and 1:3 MT to PLGA (75:25), respectively were employed.

Physico-chemical evaluation of microparticles: The encapsulation efficiency and microparticle yield were calculated according to previously described techniques[10]. Light microscope method was used to determine the particle size[10]. The microparticles from all formulations were examined to study their surface morphology using scanning electron microscope (Hitachi S-3400N, Japan)[10].

To elaborate drug-polymer compatibility analysis in complementary studies, metoprolol tartrate and metoprolol loaded microparticles were evaluated by FTIR (M 2000 MIDAC, USA) by KBr disc method[10]. X-Ray powder diffractometric (XRD) analysis of metoprolol tartrate, PLGA and metoprolol loaded microparticles was carried out by using D8 Discover (Bruker, Germany) to find out any change in the crystallinity of drug during microencapsulation[10]. Differential scanning calorimetric (DSC) analysis of metoprolol tartrate, PLGA and metoprolol loaded microparticles was conducted by using TA Instruments (Model SDQ 600, USA)[10].

The microparticles were tested for release behaviour in 900 mL of phosphate buffer of pH 7.2 using USP apparatus 1 at 50 rpm and 37 ºC. Dissolution samples (5 mL) were collected at scheduled times (0, 0.5, 1, 2, 4, 6, 8, 10, 12, 16, 18, 20, 22 and 24 h) using a filter pipette and replaced with 5 mL of fresh medium. The samples were analyzed by using high performance liquid chromatography. All experiments were run in triplicate.

A reverse phase system was used for chromatographic separation consisting of a Hypersil ODS-C_{18} column (250 mm × 4.6 mm, 5 µm). The mobile phase consisted of distilled water, acetonitrile and triethylamine (850:150:1.4, v/v/v). Its pH was adjusted at 3 with orthophosphoric acid. Mobile phase was filtered under vacuum pressure of 150-200 torr using 0.45 µm membrane filters and degassed by flushing it with nitrogen for 2-3 min until complete degassing of the mobile phase was ensured. Analysis was performed at room temperature using HPLC (Perkin Elmer, 200 Series) and absorbance of metoprolol was detected at a wavelength of 273 nm using UV detector. The flow rate was 1.0 mL/min and the sample injection volume was 20 µL[9].

Different kinetic models such as zero order, first order, Higuchi and Korsmeyer-Peppas model were used for kinetic analysis of *in vitro* dissolution data to determine the mechanism of MT release from the prepared microparticles[10].

Analysis of variance (ANOVA) was used to determine the differences between the calculated parameters using SPSS, version 12.0. The level of significance was set at 0.05.

RESULTS AND DISCUSSION

Physico-chemical evaluation of microparticles: Poly (lactic-co-glycolic acid) or PLGA is a biocompatible and biodegradable copolymer which is increasingly used in the development of drug delivery systems. PLGA synthesis is accomplished by the co-polymerization of two different monomers, the cyclic dimers (1,4-dioxane-2,5-diones) of glycolic acid and lactic acid by ester linkages (Fig. 1). Based on the ratio of lactide to glycolide units, various grades of PLGA are obtained such as PLGA 75:25 represent a copolymer containing 75 % lactic acid and 25 % glycolic acid. Moreover, PLGA undergoes hydrolytic degradation of its ester linkages in aquatic conditions and produce the parent monomers, lactic acid and glycolic acid. Under normal physiological situations, lactic acid and glycolic acid are the by-products of various metabolic cycles in the body. Thus PLGA is responsible for very low systemic toxicity when used for the development of drug delivery systems[12].

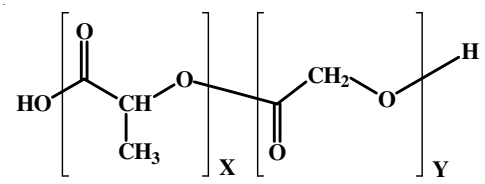


Fig. 1 Chemical structure of PLGA (X indicates number of the units of lactic acid and Y shows number of the units of glycolic acid)

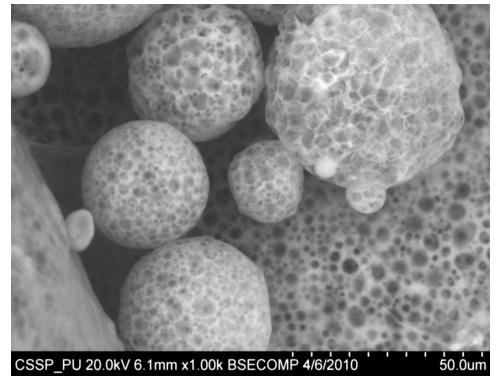
Different formulations of metoprolol-loaded PLGA microparticulate systems having different drug to polymer ratio were fabricated. The encapsulation efficiency range was 60-88 % in various formulations. The percentage encapsulation efficiency of microparticles prepared from high polymer (PLGA 50:50 and PLGA 75:25) concentration was found to be higher than that of prepared from low polymer contents. As by increasing polymer content, more amount of coating material was available to encapsulate the drug particles properly leading to less partitioning of drug into non-solvent phase. In addition, it was considered that the higher concentration of PLGA, results in a higher viscosity of system, which makes it difficult for small polymer droplets to form and

TABLE-1

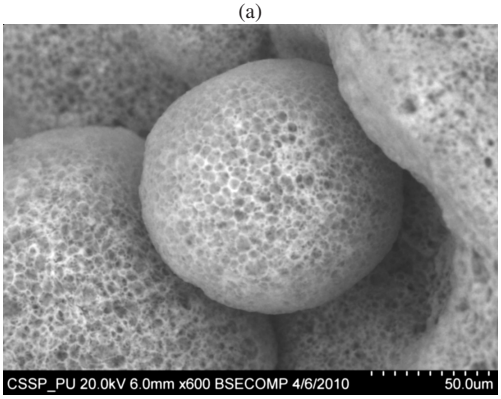
Form.	Zero Order		First Order		Higuchi		Korsmeyer-Peppas	
	Y-Equation	R^2	Y-Equation	R^2	Y-Equation	R^2	Y-Equation	n
M_1	3.196x + 4.238	0.992	18.86x + 3.349	0.814	16.41x - 9.120	0.925	0.751x + 1.9	0.920
M_2	3.017x + 3.784	0.994	17.74x + 3.057	0.810	15.46x - 8.734	0.922	0.744x + 1.85	0.924
M_3	2.877x + 1.576	0.992	16.70x + 1.271	0.788	14.64x - 10.07	0.908	0.802x + 1.575	0.905
M_4	2.922x + 0.469	0.986	16.71x + 0.607	0.760	14.75x - 11.02	0.887	0.806x + 1.527	0.897
M_5	2.736x - 0.124	0.979	15.40x + 0.450	0.731	13.70x - 10.57	0.867	0.781x + 1.486	0.866
M_6	2.426x - 0.942	0.977	13.62x - 0.369	0.725	12.13x - 10.15	0.863	0.857x + 1.132	0.854

become larger particles. The increased drug encapsulation efficiency can also be attributed to the increase in viscosity and the larger size of polymer droplets, which prevents leakage of the drug through the polymer membrane. Moreover, non-significant ($p > 0.05$) difference was observed between the encapsulation efficiency of the complementary formulations prepared from both grades.

Fig. 2 (A and B) shows the SEM pictures of M_1 and M_5 microparticles respectively to elaborate the surface morphology. The microparticles of all formulations were discrete, monodispersed, spherical in shape with porous but relatively smooth surface.



(a)



(b)

Fig. 2. Scanning electron microscopic pictures of M_2 (A) and M_5 (B) microparticles

Mean particle size range was 15-65 μm for both grades of PLGA. The particle size from both grades was increased with the increase in polymer concentration. This escalating trend was because of the increase in the thickness of polymer wall around the drug particles which is in agreement with the results obtained previously[10]. However, non-significant ($p > 0.05$) difference was observed between the particle size of the complementary formulations prepared from both grades.

***In vitro* release study:** The cumulative *in vitro* release profiles of MT from microparticles, prepared with different ratios and grades of PLGA, in phosphate buffer of pH 7.2 are shown in Fig. 3. All formulations showed a slight initial burst effect due to the release of water soluble nature of MT adsorbed on the particle surface due to which the release of drug was biphasic. After an initial burst, a sustained release phenomenon was observed in which drug diffuses through the specific channels in the polymer. It was observed that increase in the concentration of PLGA resulted in more sustained release profile and low initial burst effect. The microparticles with PLGA 75:25 released drug in more delayed and controlled manner than those encapsulated with PLGA 50:50. This is may be due to the difference in the monomeric contents of both forms of PLGA and as the first form contains 75 % lactic acid and 25 % glycolic acid so it takes more time in degradation as compared to the latter because the higher the content of glycolide units, the lower the time required for degradation with higher hydrophilicity of this copolymer[12]. During first 6 h, *ca.* 23, 20, 18, 17, 13 and 10 % of drug was released from M_1, M_2, M_3, M_4, M_5 and M_6 formulations, respectively. On comparison between the quantities of drug released in first 6 h from the complementary formulations with both grades of PLGA, there was significant difference between the respective compared values such as M_1 (23) *versus* M_4 (17), M_1 (20) *versus* M_4 (13) and M_1 (18) *versus* M_4 (10). Increase in the concentration of PLGA in the microparticles may reduce the penetration of water molecules into the polymer, thus reducing the extent of swelling of microparticles resulting in slower release of MT. As the concentration of polymer increased, it took greater time for polymer degradation and subsequent release of the drug. In addition, the solidification of microparticles is more rapid at high PLGA concentration, which may result in viscous polymer

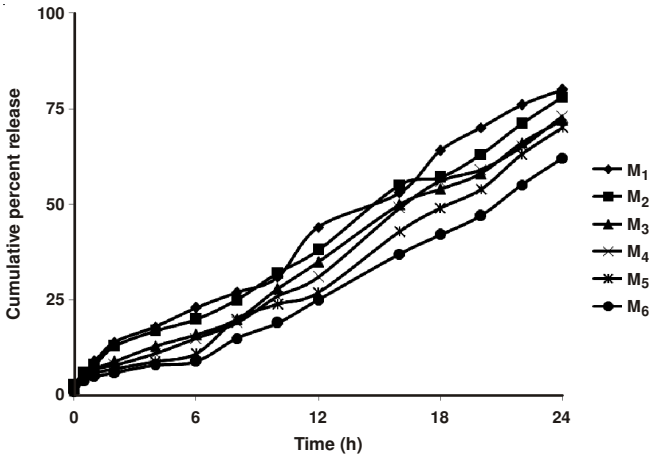


Fig. 3. *In vitro* dissolution profiles of all formulations

layer around the microparticles, resulting in smaller pores and a more tortuous structure. It was evident that the co-polymer composition was a very crucial parameter in controlling the release profile of metoprolol from microparticles.

Based on successful application of various model dependent approaches[10] to the dissolution data, the drug release pattern was best explained by zero order model (due to high values of coefficient of determination) followed by the Higuchi model. Zero order model elaborates the release of drug independent of the initial concentration in formulation which is an ultimate goal of an optimum and ideal formulation. Anomalous diffusion (50:50) microparticles. To elaborate the mode of release pattern, the *in vitro* dissolution data was fitted to Korsmeyer-Peppas model. Based on this model, the value of release exponent "n" indicates the mode of drug release such as Fickian diffusion, anomalous transport or case II transport. In the case of the Fickian release mode, drug release is mainly dependent on the diffusion through the polymer matrix. In the anomalous transport, drug release occurs combinely by the diffusion and polymer erosion processes. Values of "n" between 0.45 and 0.89 can be considered as an indication of anomalous transport. Case II transport commonly refers to the polymer erosion and refers generally to the initial concentration independent release phenomenon[13]. In the present study, the limits considered were n = 0.45-0.89 that indicated anomalous transport of drug and n > 0.89 that indicated a case II erosion based transport, also considered as zero-order release).

FTIR spectral study: It is evident from FTIR analytical tests that slight shift in some of the peak characteristics of drug occurred in the spectrum of metoprolol loaded microparticles as compared to that of pure drug. The other difference between both spectra is the difference in the sharpness of peaks in metoprolol tartrate spectrum while microparticulate spectrum exhibits peaks at the same values but no sharpness. This decrease in sharpness may be attributed to the overlapping of metoprolol tartrate and PLGA peaks. No new bands were detected in the spectra of microparticles indicating no interaction between MT and PLGA. These observations suggest that the embedment of drug into polymer with this technique takes place through conjugation, not chemically due to which the drug was chemically stable even after encapsulation.

X-Ray diffractometric analysis: The X-ray powder diffraction was conducted for MT, PLGA and metoprolol loaded microparticles to determine the physical state of drug (Fig. 4). The XRD patterns indicated that pure MT possesses crystalline characteristics, while PLGA diffractogram showed its amorphous nature. In case of XRD for microparticles, the peaks of drug are denser/lesser sharp possibly due to the re-crystallization of drug on the surface of microparticles. It also indicates polymorphic modifications (from crystalline to amorphous form). On the other hand, the intensity of the peaks of drug is different in both diffractograms. This is probably due to the different crystal habits. However, the positions of peaks of drug are identical in both states, alone and encapsulated.

DSC analysis: The results of DSC for MT and microparticles (M_2) exhibiting identical endotherm at about 130 ºC that indicates the inertness of drug in the microparticles. However, the endotherm for MT in M_2 shows reduced peak sharpness indicating slight reduction in the crystallinity of drug encapsulated in the microparticles.

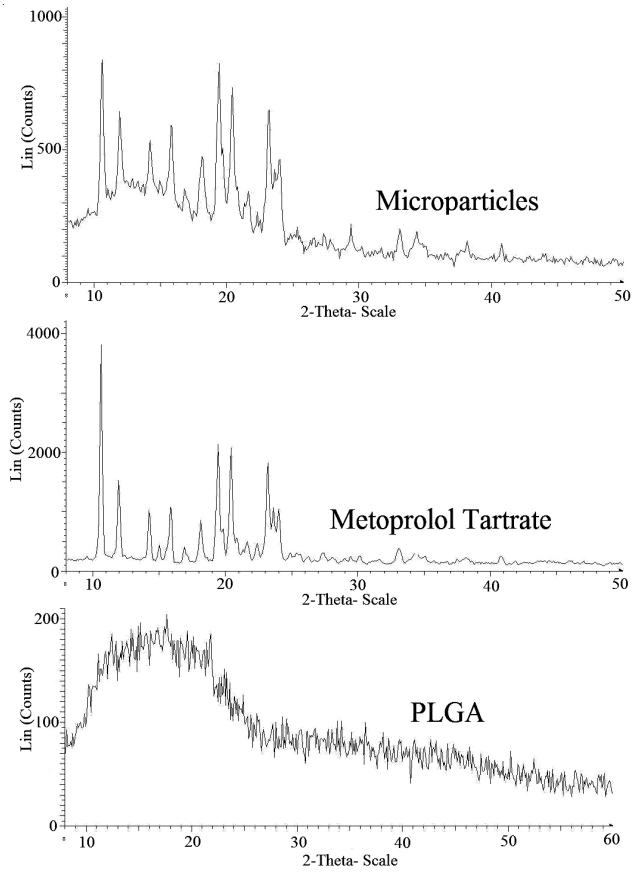


Fig. 4. XRD profiles of PLGA, MT and MT-loaded PLGA microparticles

In conclusion, the influence of polymer properties should be considered in microparticle formulation.

REFERENCES

1. B.B. Hoffman, In eds.: J.G. Hardman and L.E. Limbird, Catecholamines, Sympathomimetics Drugs and Adrenergic Receptor Antagonists. Goodman and Gilman's The Pharmacological Basis of Therapeutics, edn. 10, New York, NY: McGraw-Hill; pp. 255-6 (2001).
2. C. Narendra, M.S. Srinath and A. Moin, *Chem. Ind. Chem. Eng. Quart.*, **14**, 17 (2008).
3. J. Herrman and R. Bodmeier, *Int. J. Pharm.*, **126**, 129 (1995).
4. R. Langer, *Acc. Chem. Res.*, **33**, 94 (2000).
5. M.V. Ramana, C. Nagda and M. Himaja, *Indian J. Pharm. Sci.*, **69**, 515 (2007).
6. R.V. Nellore, G.S. Rekhi, A.S. Hussain, L.G. Tillman and L.L. Augsburger, *J. Control Rel.*, **50**, 247 (1998).
7. W. Good, L.J. Leeson, S.L. Zak, W.E. Wagner, J.B. Meeker and J.D. Arnold, *Br J. Clin. Pharm.*, **19**, 231 (1985).
8. S.S. Agrawal and P. Munjal, *Indian J. Pharm. Sci.*, **69**, 535 (2007).
9. F. Rasool, M. Ahmad, G. Murtaza, H.M.S. Khan, S.A. Khan, S. Khiljee and M. Qamar-Uz-Zaman, *Proceed. World Acad. Sci. Inform. Technol.*, **63**, 140 (2010).
10. G. Murtaza, M. Ahmad and G. Shahnaz, *Trop. J. Pharm. Res.*, **09**, 187 (2010).
11. C.E. Astete and C.M. Sabliov, *J. Biomater. Sci.-Polym. Ed.*, **17**, 247 (2006).
12. F. Cui, D.M. Cun, A.J. Tao, M.S. Yang, K. Shi, M. Zhao and Y. Guan, *J. Control. Rel.*, **107**, 310 (2005).
13. R.M. Kotadia, V.A. Patel and H.V. Patel, *Res. J. Pharm. Biol. Chem. Sci.*, **1**, 19 (2010).