IN VITRO STUDY ON TAMSULOSIN RELEASE KINETICS FROM BIODEGRADABLE PLGA *IN SITU* IMPLANTS

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ABSTRACT

The objective of this study was to evaluate the effect of drug loading and the effect of excipients on the release pattern of tamsulosin tydrochloride from *in situ* PLGA implants formed *in vitro* in gelatin gel. This system is prepared by dissolving a biodegradable polymer (DL-PLGA 70K) in biocompatible solvent, dimethyl sulfoxide (DMSO). Then either the drug or drug with excipients was added to it. The drug solution was poured into the hollow of gelatin gel, the solvent dissipated into the surrounding gelatin base through diffusion leading phase separation and subsequent coagulation of the polymer. The drug formed a rod like implant *in situ*. Two types of implants were prepared such as implants containing tamsulosin hydrochloride and implants containing tamsulosin hydrochloride with biocompatible excipients such as Tween 20, Tween 60, Span 20, Span 80, Chremophore EL, or Chremophore RH 40. *In vitro* dissolution studies were performed in static condition using phosphate buffer (pH 7.4) to observe the release of drugs from these implants for 10 days. Formulation containing only tamsulosin hydrochloride showed that drug loading was 83.54%, 90.23%, 86.72%, 89.17% and 94.08% against the actual drug content of 9.09%, 13.04%, 16.67%, 20% and 23.08% respectively. The release rate of drug was 64.51%, 70.64%, 74.08%, 76.12% and 80.05% accordingly. It can be concluded that the release rate of drug increases with increasing drug concentrations. The other formulation containing tamsulosin with excipients showed that the release rate was 74.70%, 75.14%, 60.03%, 63.83%, 70.82% and 76.43% against same conc. of drug (8.7% of drug) but different excipients such as tween 20, tween 60, span 20, span 80, chremophore EL and cremophore RH 40 respectively. The loading efficiency was 79.33%, 87.34%, 91.91%, 94.19%, 88.48% and 95.34% respectively. It can be concluded that excipient lowers the release rate of the drug and may prolong the activity and overall release kinetics.

Keywords: *In situ* implant, drug delivery, aprotic solvent, biodegradable, tamsulosin.

INTRODUCTION

Drug delivery is an application of biochemical engineering with technologies aimed at the improvement of safety and efficacy, better compliance and life extension of products. (Shalini Shahani, 2003). Drug delivery is an enabling technology that is helping to expand other pharmaceutical industry sectors such as generic drugs and specialty pharmaceuticals. The technology is being used by some pharmaceutical firms to differentiate their products so that new opportunities can be created. The industry definition has expanded to include new, targeted therapies as well as new drug containing implants that were invented by emerging companies. Monoclonal antibodies, gene delivery, MEMS (Micro Electro Mechanical Systems) implants and drug-coated stents are examples of emerging drug delivery innovations (U.S. Drug Delivery Systems Market, Emerging Technologies, Strategic Alliances, Patent Disputes, Market Size & Forecasts and R&D Activities, April, 2003).

Parenteral depot systems (PDS) have been the subject of intensive research efforts over the past two decades. These new drug delivery systems are injected or implanted into the muscle and subcutaneous tissue and release the incorporated drug in a controlled manner, allowing the adjustment of release rates over extended periods of time, ranging from several days up to one year. PDS can be classified into implants or micro particles. (Kissel T., Parenteral Depot systems from biodegradable polymers).

PDS allow the control and modulation of drug release using biodegradable polymers. Biodegradable polymer may be defined as synthetic or natural polymer which is biodegradable *in vivo*. These can be further metabolized or excreted via normal physiological pathways (Jalil & Nixon, 1989). These polymers have become increasingly important in the development of controlled release systems. Currently a number of biodegradable polymers are being evaluated as carriers for the controlled release of low molecular weight drugs. Polymers of these kinds have both active and passive roles (Lewis, 1990; Pitt, 1990). Features such as biocompatibility, predictability of biodegradation kinetics, ease of fabrication, and regulatory approval in commercial sutures applications have attracted investigators to lactic/glycolic polymers (Hillary *et al*,. 2001; Shah *et al*., 1992). Biodegradable

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polymeric implant material provides substantially continuous release of bioactive agent during *in vivo* use (De Luca *et al*., 1994). The rates of polymer degradation especially from biodegradable polymers of lactide/ glycolides follow after the parenteral administration (DeLuca *et al.*, 1993). Parenteral delivery systems based upon these biodegradable materials having various block structures, the release from certain polymer configurations is continuous and molecular mass-dependent (Kissel *et al.*, 1993 & 1996). The microparticles prepared from poly (lactide-co-glycolide) with an entrapped protein using phase separation techniques showed to follow zero-order release kinetics (McGee *et al.*, 1995).

According to US PAT No.4,938,763 the biodegradable polymer is introduced in a body as a flowable formulation. The method (The thermoplastic system) may be employed for the *in situ* formation of implants by the injection of a solution containing a water–immiscible biodegradable polymer and a water-miscible biologically compatible (non toxic) solvent into an animal. The solvent is quickly carried away from the injection site and the polymer left behind in the aqueous environment of the body quickly coagulates or solidifies into a solid matrix structure. If the implant is meant to serve as a drug delivery system, then the drug is incorporated into the solution prior to injection and is trapped in the solid matrix formed upon coagulation of the polymer (Eliaz *et al*., US Patent 6,206,920).

Drug delivery for extended duration of action is still a long sought desire for the pharmaceutical scientists. Although the silicon implants may serve as a sustained release of drugs for a longer period of time, it has also some limitations. As the silicone implant is not biodegradable it requires a surgical intervention for the removal of the implant once the drug supply has been depleted. With the development of biodegradable drug delivery system, the follow-up surgical procedure has been eliminated which provides an additional advantage.

MATERIALS AND METHODS

Materials

Tamsulosin hydrochloride, dimethyl sulfoxide (DMSO), DL-PLGA 50:50 MW 70,000, Tween 60 (Poly-oxyethylene sorbitan monostearate), Tween 20 (Poly-oxyethylene sorbitan monolaurate), Span 80 (Sorbitan monooleate), Span 20 (Sorbitan monolaurate), Cremophor RH 40 (Polyoxy hydrogenated castor oil), Cremophor EL (Polyoxyethylated castor oil), Gelatin, Glycerin, Sodium Dihydrogen Phosphate 2-Hydrate Cryst. (Pure), Sodium Phosphate dibasic GR Dihydrate, 40% Sodium Sulfate Solution.

Methods

Preparation of implants

At first the gelatin solution was prepared in a clean beaker using gelatin (10%), glycerol (10%) and purified water. Then the solution was turned into solidified gel by taking 16 ml solution in each vial and keeping them into refrigerator for 3 hours. A stainless steel rod was inserted in to each vial through cork containing gelatin soluition to make a rod shaped hollow in the gelatin gel. Then the drug solution for different formulations was prepared according to tables 1 and 2. The drug solution (0.75 ml for each implant) was poured into the hollow of gelatin gel. The vials were left in room temperature overnight (for 12 hrs) for the formation of implant and then the vials containing the implants were kept in an incubator for 36 hours at 37^{o}C. In this way implants from different formulations were prepared, collected, dried and stored for further use. A schematic diagram of formation of *in situ* implant in vitro has been shown in fig. 1.

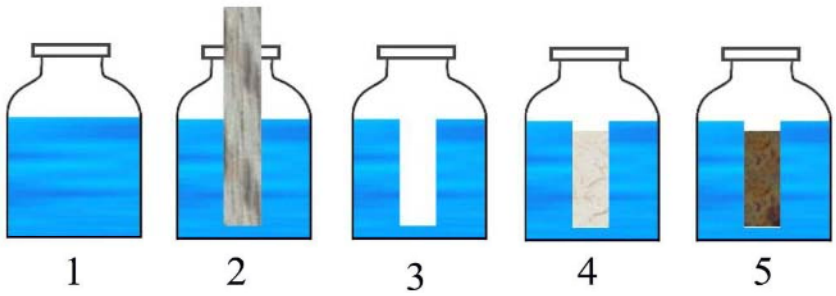

Fig. 1: *In situ* implant formation *in vitro* in gelatin gel.

1. Gelatin gel in vial in melted condition.
2. Stainless steel rod inserted into the gelatin gel through the hole in to the cap and kept under refrigeration for solidification of gelatin gel.
3. The steel rod was withdrawn and a rod like cavity was formed into the solidified gelatin
4. Drug solution prepared from drug and PLGA (with or without excipient) in DMSO was poured into the cavity of solidified gel.
5. The vials were kept in room temperature overnight and then kept in incubation for 36 hrs. Ultimately solid *in situ* implant was formed.

Table 1: Formulation for the preparation of DL-PLGA (mw 70,000) Implants using tamsulosin hydrochloride (T) in gelatin gel

Ingredients	T10	T15	T20	T25	T30
Tamsulosin Hydrochloride (T)	100 mg	150 mg	200 mg	250 mg	300 mg
DL-PLGA 70,000	1 g	1 g	1 g	1 g	1 g
Dimethyl Sulfoxide (DMSO)	3 g	3 g	3 g	3 g	3 g

Table 2: Formulation for the preparation of DL-PLGA (mw 70,000) implants using Tamsulosin Hydrochloride (T) and Excipients in gelatin gel

Ingredients/Implants	TTW20	TTW60	TSP20	TSP80	TCPEL	TCPRH
Tamsulosin Hydrochloride (T)	100 mg	100 mg	100 mg	100 mg	100 mg	100 mg
DL-PLGA 70,000	1 g	1 g	1 g	1 g	1 g	1 g
Dimethyl Sulfoxide (DMSO)	3 g	3 g	3 g	3 g	3 g	3 g
Tween 20 (TW20) (Polyoxyethylene sorbitan monolaurate)	50 mg					
Tween 60 (TW60) (Polyoxyethylene sorbitan monostearate)		50 mg				
Span 20 (SP20) (Sorbitan monolaurate)			50 mg			
Span 80 (SP80) (Sorbitan monooleate)				50 mg		
Cremophor EL (CPEL) (Polyoxyethylated castor oil)					50 mg	
Cremophor RH 40 (CPRH) (Polyoxy hydrogenated castor oil)						50 mg

Table 3: Quantitative analysis of implants containing tamsulosin before dissolution

Formulation Code	Weight / Implant (mg)	Theoretical Drug Content (%)	Actual Drug Content (%)	Loading Efficiency (%)
T10	283	9.09	7.59	83.54
T15	313	13.04	11.77	90.23
T20	322	16.67	14.45	86.72
T25	327	20.00	17.83	89.17
T30	339	23.08	21.71	94.08

Dissolution study of implants

After formation of implants, *in vitro* dissolution studies of those implants were carried out in static condition in order to observe the drug release profile. For tamsulosin hydrochloride implants, two from each individual formulation i.e. 10 implants from only tamsulosin hydrochloride implants and 12 implants from tamsulosin with excipients implants were placed in 22 different 125 ml dissolution devices. Then 50 ml of phosphate buffer (pH 7.4) was added in each dissolution device. The devices were kept at room temperature. Then 5 ml phosphate buffer samples were withdrawn at a predetermined rate using 5 ml syringe and it was replaced with fresh phosphate buffer (pH 7.4) at the same time. The withdrawn sample was analyzed for drug content with the help of UV spectrophotometer at 226 nm. The dissolution was carried out for 6 and 10 days for implants containing tamsulosin hydrochloride and tamsulosin hydrochloride with excipient respectively.

Quantitative analysis of drug in implant

Quantitative analysis of implants for drug content was carried out before the dissolution started and after the dissolution was over. For analysis a small portion (20 mg) of implant was weighed accurately and then it was dissolved in 100 ml water taking in a 100 ml volumetric flask and thoroughly shaken in ultrasonic bath at 37^{o}C for 2 hrs. Then the solution was filtered through whatman filter paper and analyzed spectrophotometrically at 226nm for the drug concentration.

Quantitative analysis of drug in the gel media

For analysis of drug in the gelatin media, the media was precipitated using 40% sodium sulphate solution. 5 ml of

Table 4: Quantitative analysis of implants containing tamsulosin hydrochloride and surfactant before dissolution

Formulation Code	Weight / implant (mg)	Theoretical drug content (%)	Actual drug content (%)	Loading efficiency (%)
TTW20	332	8.7	6.9	79.33
TTW60	347	8.7	7.6	87.34
TSP20	320	8.7	8.0	91.91
TSP80	325	8.7	8.2	94.19
TCPEL	347	8.7	7.7	88.48
TCPRH	330	8.7	8.3	95.34

Table 5: Release rate vs concentration of tamsulosin hydrochloride at different phase

Formulation code	Concentration of drug (%)	Burst phase		Phase II	
		Release rate (% release/hr)	r^2	Release rate (% release/hr)	r^2
T10	9.09	2.3197	0.9199	0.6036	0.8994
T15	13.04	2.3698	0.8920	0.6855	0.9049
T20	16.67	2.5113	0.8906	0.723	0.8785
T25	20.00	2.6718	0.9018	0.7281	0.8791
T30	23.08	3.1343	0.9378	0.7308	0.8945

Table 6: Statistical data for release rate of different implants

Formulation Code	Concentration of Drug (%)	Average Release of Drug (%) after 72 hr	Mean ± SD
T10	9.09	64.51	64.51 ± 0.09
T15	13.04	70.64	70.64 ± 0.18
T20	16.67	74.08	74.08 ± 0.75
T25	20.00	76.12	76.12 ± 1.29
T30	23.08	80.05	80.05 ± 2.02

40% sodium sulphate solution was used in each vial containing 16 ml of gelatin gel. Then the clear solution was poured and filtered through filter paper. The optical density of the sample was measured spectrophotometrically.

RESULTS AND DISCUSSION

After the preparation of implants, the implants were analyzed quantitatively to observe the actual drug content against the theoretical drug content. The implants were analyzed quantitatively before the dissolution started to determine the amount of drug entrapped in the implant. This was shown in the table 3. The data in the table shows that the loading efficiency was 83.54% for 9.09% of the drug concentration whereas loading efficiency was 94.08% when the drug concentration was increased to 23.08%. So, it can be concluded that with the increase in concentration of tamsulosin hydrochloride, the loading efficiency was increased.

Besides the implants, prepared from tamsulosin hydrochloride with excipients, were analyzed in the same way. The table 4 indicated that the loading efficiency of tamsulosin was increased without excipients. This is probably due to more hydrophilic behavior of tamsulosin. As excipient has property to retain the drug in implants rather than diffuse in to the gel media, therefore, the loss of drug was smaller.

Effects of tamsulosin loading on the release from PLGA in situ implants

When the implants were prepared from drug tamsulosin with different concentration, the actual drug loading was determined to correlate the efficiency of drug loading with the concentration of drug and also the effect of drug loading on the release of drug from the PLGA *in situ* implants. The theoretical drug content was 9.09%, 13.04%, 16.67%, 20.00% and 23.08% for the formulation code T10, T15, T20, T25 and T30 respectively. The actual drug content of implants has also been determined through analysis after recovery of implants. From the analysis it was found that the loading efficiency was 83.54%, 90.23%, 86.72%, 89.17%, and 94.08% and the release rate was 64.51%, 70.64%, 74.08%, 76.12% and 80.05% after 72 hrs for the formulation code T10, T15, T20, T25 and T30 respectively. The tamsulosin release rate was plotted against time in the fig. 2. It was observed

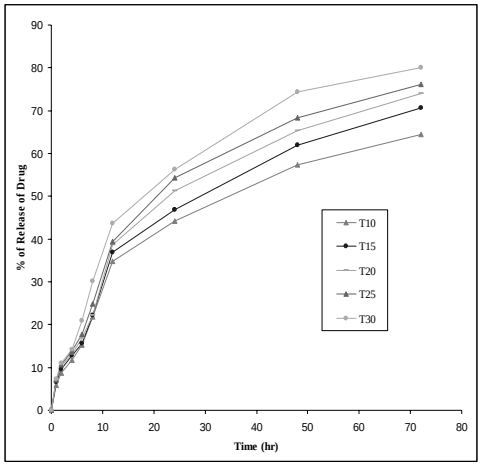

Fig. 2: Zero order release of tamsulosin hydrochloride from different concentration of drug loaded PLGA *in situ* implants (buffer P^H 7.4, temp 37^0 C)

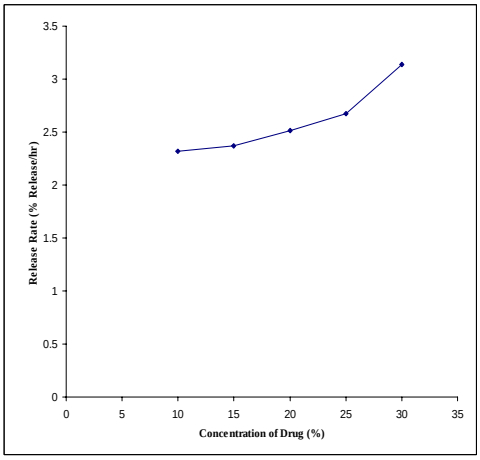

Fig. 3: Effect of drug (tamsulosin hydrochloride) loading on the burst phase release rate

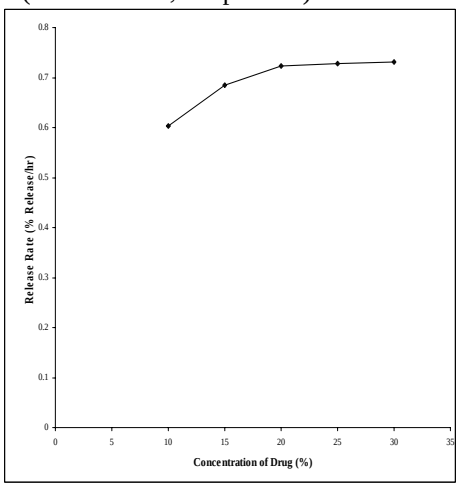

Fig. 4: Effect of drug (tamsulosin hydrochloride) loading on the phase II release rate

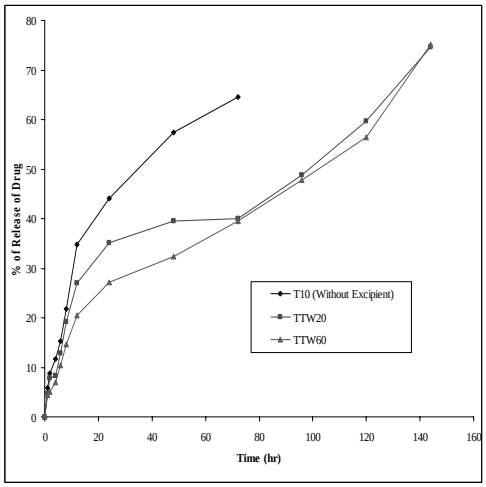

Fig. 5: Effect of polysorbates on the release rate of tamsulosin from PLGA i*n situ* implants

that the release of tamsulosin increases with the increase of concentration of drug. That means the implants containing highest tamsulosin content 23.08% for T30 showed the highest release than the other four formulations. The release pattern of tamsulosin was biphasic, projecting initial 40% burst phase followed by the phase II release of tamsulosin. The standard deviation of the release rate of different implants from the table 6 was found statisticallty insignificant.

The correlation coefficient values of the trendlines of the graph showed that all five formulations best fits in zero order pattern. It may be due to the huge burst phase. However it is very difficult at this stage to explain in the actual mechanism of release since, the polymer degradation starts during dissolution period. The release rate of tamsulosin at burst phase and phase II was also calculated from the trendlines of the graphs for all five formulations. The values of the correlation coefficients and release rate at burst phase and phase II are shown in table 5. While plotted the release rate against concentration of drug at burst phase and phase II in the figs. 3 and 4, it was clearly observed that T30 having drug loading 23.08% (theoretical) of tamsulosin showed higher release rate than four other formulations those have lower drug loading of 20.00%, 16.67%, 13.04% and 9.09% for T25, T20, T15 and T10 respectively. This is obvious phenomenon that the higher loaded matrices release faster due to higher pore formation and high flux due to faster saturation at the diffusion layer.

Effect of biocompatible excipient on release of tamsulosin from* PLGA *in situ implant

Biocompatible excipients play an important role in the release of drug from matrices. As surfactants have the property of lowering interfacial tension and providing a stabilizing emulsifying layer, so the solubility of these surfactants in the continuous phase produces the right

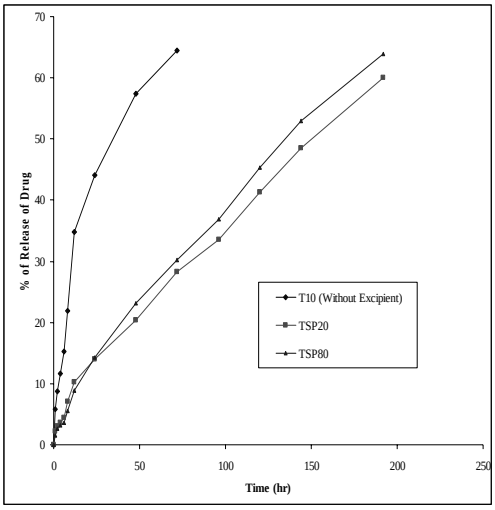

Fig. 6: Effect of sorbitan monolaurate and monooleate on the release rate of tamsulosin from PLGA *in situ* implants

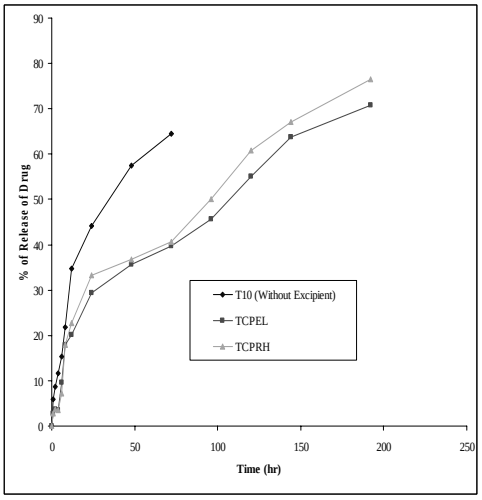

Fig. 7: Effect of polyoxyethylated and poly-oxyhydrogenated castor oil on the release rate of tamsulosin from PLGA *in situ* implants

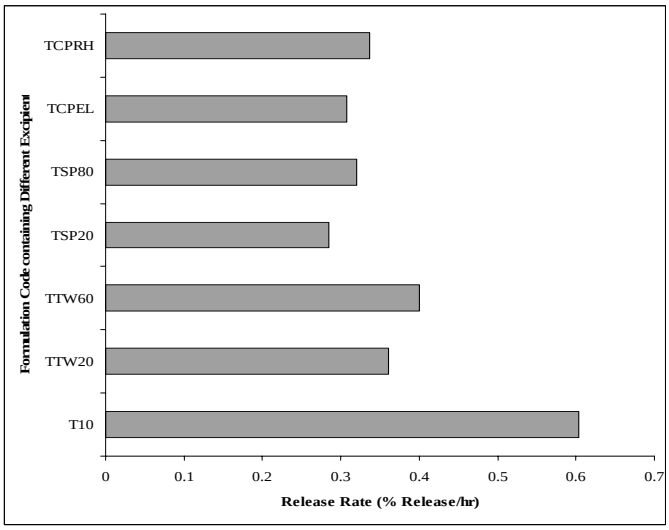

Fig. 8: Effect of different excipients on the release rate of tamsulosin from PLGA *in situ* implant

condition for stabilization. The surfactants used are Tween 20 for formulation code TTW20, Tween 60 for TTW60, Span 20 for TSP20, Span 80 for TSP80, Chremophore EL for TCPEL and Chremophore RH 40 for TCPRH. From table 4 the theoretical and actual drug content were found & the loading efficiency was 79.33%, 87.34%, 91.91%, 94.19%, 88.48% and 95.34% for formulation code TTW20, TTW60, TSP20, TSP80, TCPEL and TCPRH respectively. The effects of surfactants were evaluated by *in vitro* tamsulosin release kinetics and were compared with the release kinetics of implants containing same amount of same drug without surfactant.

The release data for all six formulations (surfactants incorporated) were plotted in graph against the release data of tamsulosin hydrochloride without surfactant (T10) as shown in figs. 5, 6 and 7. The graphical interpretation of data showed that about 74.70% (For TTW20), 75.14% (For TTW60), 60.03% (For TSP20), 63.83% (For TSP80), 70.82% (For TCPEL), 76.43% (For TCPRH) drug was released after 8 days from the implants incorporated surfactants whereas about 64.51% (T10) drug was released from the implants without surfactants after 3 days. It predicts that surfactant except Span series enhances the release rate of tamsulosin tydrochloride from *in situ* implant. This is due to the fact that the physicochemical characteristics of tamsulosin hydro-chloride incorporated with various surfactants were significantly improved compared with tamsulosin hydrochloride without any surfactant, probably by decreasing particle size as well as by increasing the zeta

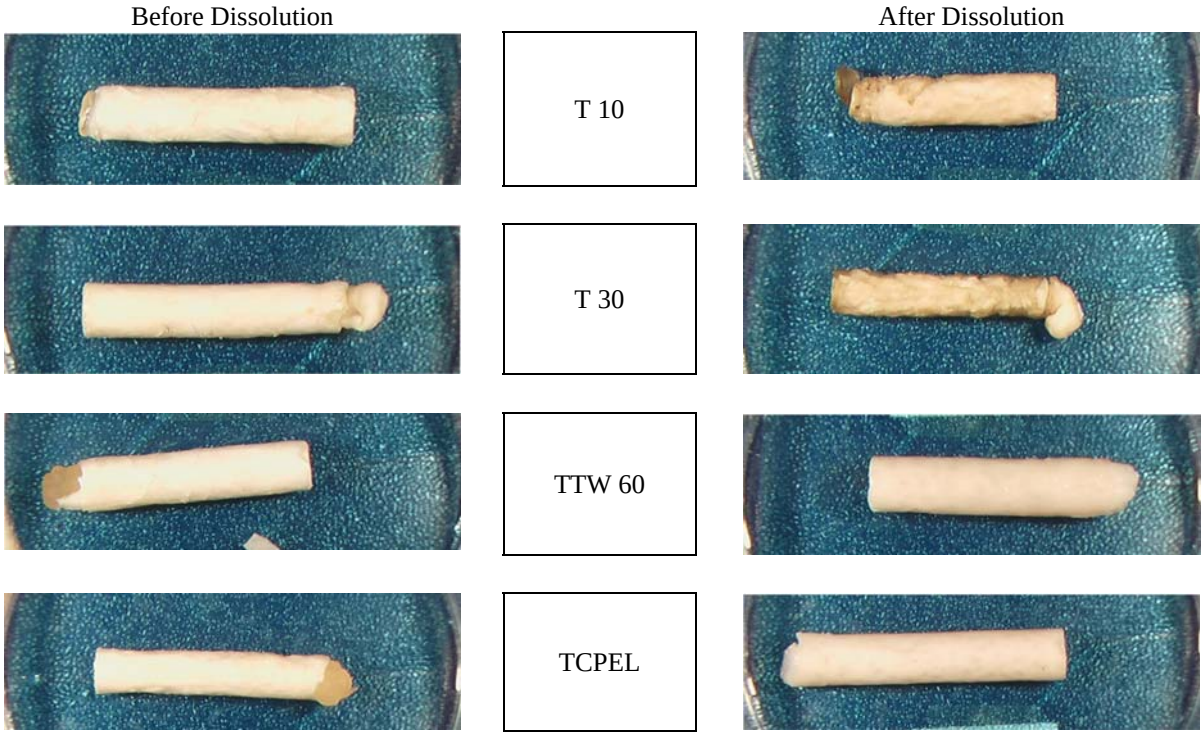

Fig. 9: Photograph of i*n situ* implants prepared from drug, excipients, and PLGA in DMSO (T10- Tamsulosin 9.09%; T 30- Tamsulosin 23.08%; TTW 60- Tamsulosin 8.7% & Tween 60; TCPEL- Tamsulosin 8.7% & Chremophore EL).

potential to minimize the agglomeration of nanoparticles. Moreover, tamsulosin incorporated with various surfactants demonstrated higher entrapment efficiency of tamsulosin hydrochloride. Results from the *in vitro* release kinetic studies indicated that Tween and Cremophore series showed faster release kinetics than the Span series due to more hydrophilic behaviour of Tween than that of the Span. While plotted the release rate against concentration of drug at phase II in the fig. 8, it was clearly observed that surfactants lower the release rate but can prolong the activity of tamsulosin. fig. 9 shows the photograph of different types of implants.

CONCLUSION

Polymeric drug delivery systems are widely used in the pharmaceutical industry for sustaining drug action. But recently the *in situ* implant drug delivery system has gain interest in the field of long-term drug delivery system. The advantage of this system is that it can be injected through traditional needle and syringe but just after injection it became a solid biodegradable implant. From this study it can be concluded that the implants can easily be formed *in vitro* rather than *in vivo*. It takes less time and easy to recover from the gelatin gel where it is very difficult to recover implants from *in vivo*. Besides properly shaped implants can be obtained from *in vitro* in gelatin gel whereas it is difficult to obtain rightly shaped implants from *in vivo* source. The present study also reveals that it is possible to design and develop *in situ* PLGA implant *in vitro* in gelatin gel as new drug delivery device for long term therapy. However, further studies have to be conducted in this aspect to produce a successful drug delivery system and to establish *in vitro* and *in vivo* correlation.

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