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STUDY OF RELEASE KINETICS OF DEXAMETHASONE FROM BIODEGRADABLE PLGA *IN-SITU* IMPLANTS

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ABSTRACT

The primary objective of this study is to evaluate the effect of drug loading and the effect of excipients on the release pattern of Dexamethasone Sodium Phosphate from in situ PLGA implants formed in vitro in gelatin gel. This system is prepared by dissolving a biodegradable polymer (DL-PLGA 70K) in dimethyl sulfoxide (DMSO). Then the drug with excipients or without excipients was added to it. When the drug solution poured into the hallow of gelatin gel, the solvent dissipates into the surrounding gelatin base through diffusion leading phase separation & subsequent coagulation of the polymer and the drug to form a rod like implant in situ. Two types of implants were prepared such as implants containing Dexamethasone Sodium Phosphate and implants containing Dexamethasone Sodium Phosphate with biocompatible excipients such as Tween 20, Tween 60, Span 20, Span 80, Chremophore EL, Chremophore RH40, Stearyl alcohol, Cetyl alcohol, PEG 6000, Stearic acid, GMS, Benzyl Benzoate, Magnesium stearate, Dextrose, Lactose and Arachis oil were used. In vitro dissolution studies were performed in static condition using phosphate buffer (pH 7.4) to observe the release of drugs from these implants. Formulation containing only Dexamethasone Sodium Phosphate showed that drug loading was 86.82%, 90.60%, 87.47%, 91.79% and 92.78% against the actual drug content of 9.09%, 13.04%, 16.67%, 20% and 23.08% respectively. The release rate was 64.51%, 70.64%, 74.08%, 76.12% and 80.05 % respectively. It can be said that telease rate of drug increase with the increase in concentration of drug and Dexamethasone releases faster due to its hydrophilicity. On the other hand formulation containing Dexamethasone with excipients the release rate was 62.21%, 73.06%, 77.42%, 79.95% and 81.52%. For implants containing Dexamethasone with excipients the release rate were different due to their characteristics. It can be concluded from the experiment that although excipient lowers the release rate of drug, these can prolong the activity and the overall release kinetics is increased than that can be obtained from implants without excipients.

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. Drug delivery can offer a number of significant benefits to patients and physicians, as well as to the Pharmaceutical and Biotech Industries [1-3]. Most people think of drug delivery systems as the added ingredients that go into oral pills that people take for all-day headache pain relief, a patch that helps people stop

smoking or an inhaler to help a child with asthma breathe easier. It is this and more. It is an interesting technology that is transforming ordinary drugs into drugs optimized for their targeted applications.

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. Other companies are adding special drug delivery features to products to extend the marketing life of product lines [4-6].

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 [7-8].

MATERIALS AND METHODS:

Materials: Chemicals- Dexamethasone Sodium Phosphate, Dimethyl Sulfoxide (DMSO), DL-PLGA MW 70,000, Tween 60 (Polyoxyethylene sorbitan mono-stearate), Tween 20 (Polyoxyethylene 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, glycerol and purified water to prepare gelatin gel in vial. A rod like stainless steel rod was inserted in to each vial through cork containing gelatin gel to make a rod shaped hollow in the gelatin gel. Then the drug solution was prepared by drug, polymer, solvent and sometimes drug with excipients (Tween 20, Tween 60, Span 20, Span 80, Chremophore RH 40 and Chremophore EL).

The drug content solution (0.75 ml for each implant) was poured into the hollow of gelatin gel using 1ml micropipette. The vials were then left in room temperature overnight (for 12 hrs) for the formation of implant and then the implants were kept in an incubator for 36 hours at 37^{o}C. In this way total 24 implants (four implants from individual formulation) were prepared from six different formulations containing same dexamethasone sodium phosphate concentration but different excipients with same concentration.

Then, 40% sodium sulfate solution was prepared used for quantitative analysis of drug from gelatin gel after recovery of implants in order to minimize the loss of drug and finally, buffer solution was used for dissolution study of the implants. The gelatin solution was analyzed at 239nm for dexamethasone sodium phosphate. A schematic diagram of formation of *in-situ* implant *in vitro* has been shown in **figure 1**.

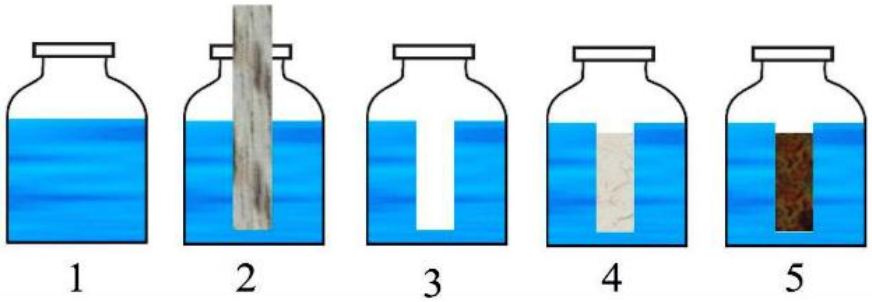

FIG. 1: *IN-SITU* IMPLANT FORMATION *IN- VITRO* IN GELATIN GEL

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.

Dissolution Methods for 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 dexamethasone sodium phosphate implants, two from each individual formulation i.e., 10 implants from only dexamethasone sodium phospahte implants and 12 implants from dexamethasone 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 & 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 239 nm.

The absorbance values were recorded. By using these values, the drug content of each implant was determined from the standard curve of dexamethasone sodium phosphate. The dissolution was carried out for 7, 12, 6 and 10 days for implants containing dexamethasone sodium phosphate and dexamethasone sodium phosphate with excipient respectively.

Quantitative Analysis of Implant: Quantitative analysis of implants for drug content was done before the dissolution started and after the dissolution was finished. The dissolution was carried out for 7, 12, 6 and 10 days for implants containing dexamethasone sodium phosphate and dexamethasone sodium phosphate with excipient respectively. 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 Whatmann filter paper and analyzed for the drug spectrophotometrically at 239nm for dexamethasone sodium phosphate.

Quantitative Analysis of Drug in the Gel Media: After the removal of implants, the gelatin gel was preserved in the refrigerator for quantitative analysis. For the analysis of the drug being released in the gelatin media at first the gelatin was precipitated using 40% sodium sulphate solution. 5 ml of 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. Finally, the absorbance of the filtered solution was taken spectrophotometrically using distilled water as blank. The gelatin solution was analyzed at 239nm for dexamethasone sodium phosphate.

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 drug loading efficiency can be calculated from the analysis also. From **table 1** it was observed that the loading efficiency was 86.82% for 9.09% drug concentration whereas it was found 92.78% loading efficiency when the drug concentration was increased to 23.08%. So, it can be concluded that with the increase in concentration of dexamethasone sodium phosphate, the loading efficiency was increased.

TABLE 3: QUANTITATIVE ANALYSIS OF IMPLANTS CONTAINING DEXAMETHASONE BEFORE DISSOLUTION

Formulation Code	Weight / Implant (mg)	Theoretical Drug Content (%)	Actual Drug Content (%)	Loading Efficiency (%)
D10	315	9.09	7.89	86.82
D15	332	13.04	11.82	90.60
D20	346	16.67	14.58	87.47
D25	361	20.00	18.36	91.79
D30	373	23.08	21.41	92.78

According to **table 2 and 3** it has shown that the implants of dexamethasone sodium phosphate was prepared by using solvent (DMSO), drug (Dexamethasone Sodium Phosphate), polymer (DL-PLGA) and excipients (Tween 20, Tween 60, Span 20, Span 80, Cremophor RH 40 and Cremophor EL).

TABLE 2: FORMULATION FOR THE PREPARATION OF DL-PLGA IMPLANTS USING DEXAMETHASONE SODIUM PHOSPHATE (D) IN GELATIN GEL

Ingredients/Implants	D10	D15	D20	D25	D30
Dexamethasone Sodium Phosphate (D)	100 mg	150 mg	200 mg	250 mg	300 mg
DL-PLGA	1 g	1 g	1 g	1 g	1 g
Dimethyl Sulfoxide (DMSO)	3 g	3 g	3 g	3 g	3 g

TABLE 3: FORMULATION FOR THE PREPARATION OF DL-PLGA IMPLANTS USING DEXAMETHASONE SODIUM PHOSPHATE (D) AND EXCIPIENTS IN GELATIN GEL

Ingredients/Implants	DTW20	DTW60	DSP20	DSP80	DCPEL	DCPRH
Dexamethasone Sodium Phosphate (D)	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

Percent Release and effect of Dexamethasone with or without excipient like Polysorbates (D10, DTW20. DTW60), Sorbitan monolaurate and monooleate (D10, DSP20, DSP80), Polyoxyethylated and polyoxyhydrogenated Castor oil (D10, DCPEL, DCPRH) from PLGA *in-situ* Implants has shown in **figure 2, 3 and 4** respectively. This is probably due to less hydrophilic behavior of dexamethasone. But the loss in the gel media was somewhat smaller than that of Dexamethasone without excipient only. As excipient has property to retain the drug in implants rather than diffuse in to the gel media.

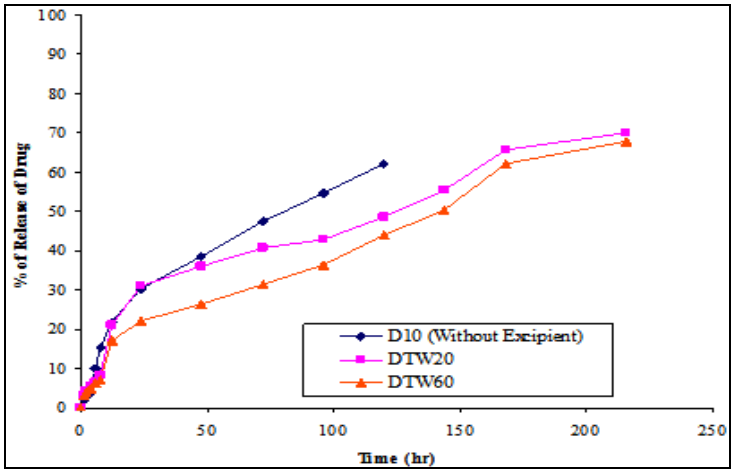

FIG. 2: EFFECT OF POLYSORBATES ON THE RELEASE RATE OF DEXAMETHASONE FROM PLGA *IN-SITU* IMPLANTS

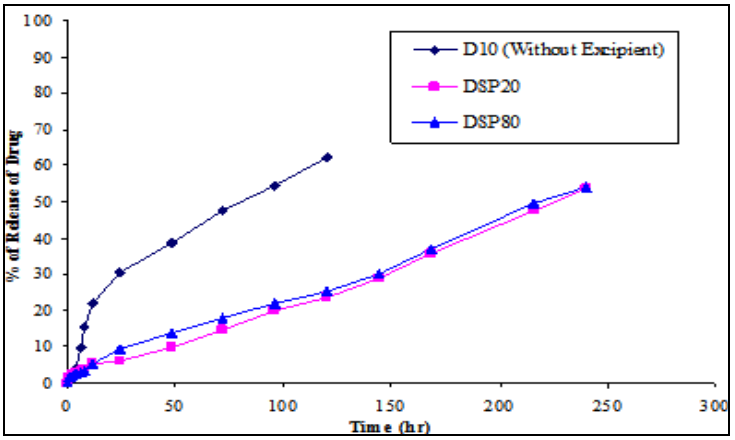

FIG. 3: EFFECT OF SORBITAN MONOLAURATE AND MONOOLEATE ON THE RELEASE RATE OF DEXAMETHASONE FROM PLGA *IN-SITU* IMPLANTS

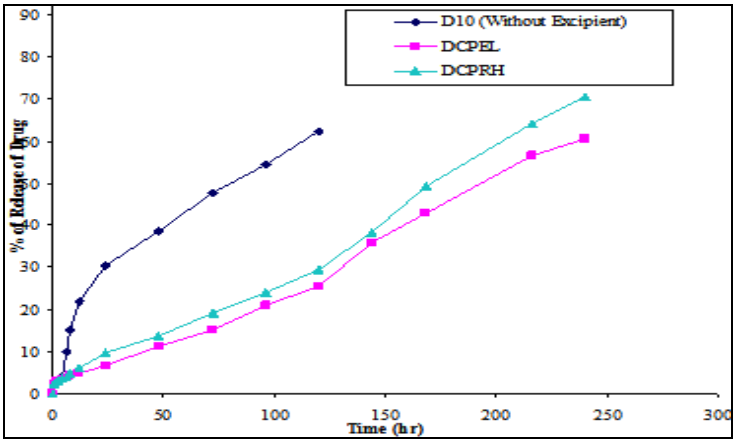

FIG. 4: EFFECT OF POLYOXYETHYLATE AND POLYOXYHYDROGENATED CASTOR OIL ON THE RELEASE RATE OF DEXAMETHASONE FROM PLGA *IN-SITU* IMPLANTS

From **table 4** it was found that the loading efficiency of dexamethasone implants were 90.60%, 87.47%, 91.79%, and 92.78% for the formulation code D10, D15, D20, D25 and D30 respectively. During the dissolution studies, the release rate of dexamethasone from the implants were 62.21%, 73.06%, 77.42%, 79.95% and 81.52% after 120 hrs for the formulation code D10, D15, D20, D25 and D30 respectively. The dexamethasone release rate was plotted against time with release rate (%) in the Y-axis and time (hr) in the X-axis that was shown in **Figure 5.**

TABLE 4: QUANTITATIVE ANALYSIS OF IMPLANTS CONTAINING DEXAMETHASONE SODIUM PHOSPHATE AND SURFACTANT BEFORE DISSOLUTION

Formulation Code	Weight / Implant (mg)	Theoretical Drug Content (%)	Actual Drug Content (%)	Loading Efficiency (%)
DTW20	356	8.7	6.3	72.38
DTW60	323	8.7	6.9	79.06
DSP20	311	8.7	7.3	84.08
DSP80	340	8.7	7.2	82.41
DCPEL	325	8.7	7.5	85.75
DCPRH	346	8.7	6.3	72.38

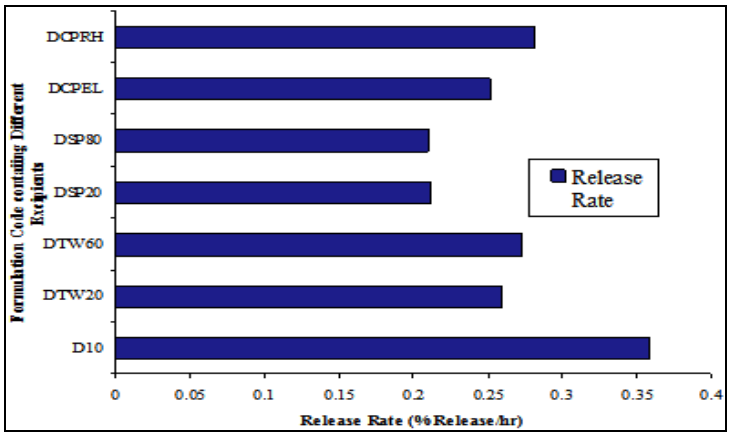

FIG. 5: EFFECT OF DIFFERENT EXCEPIENTS ON THE RELEASE RATE OF DEXAMETHASONE FROM PLGA *IN-SITU* IMPLANTS

It was observed from **figure 6** that the release of dexamethasone increased with the increase of concentration of drug. That means the implants containing highest dexamethasone content 23.08% for D30 showed the highest release than the other four formulations. The figure also showed the biphasic that there was a huge release of dexamethasone almost 30% in the burst phase than the phase II release of dexamethasone. The correlation coefficient values of the trendlines of the graph showed that all five formulations (D10, D15, D20, D25 and D30) 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 details the actual mechanism of release since, the polymer degradation starts during dissolution period. The release rate of Dexamethasone salt 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 was shown in **table 5**.

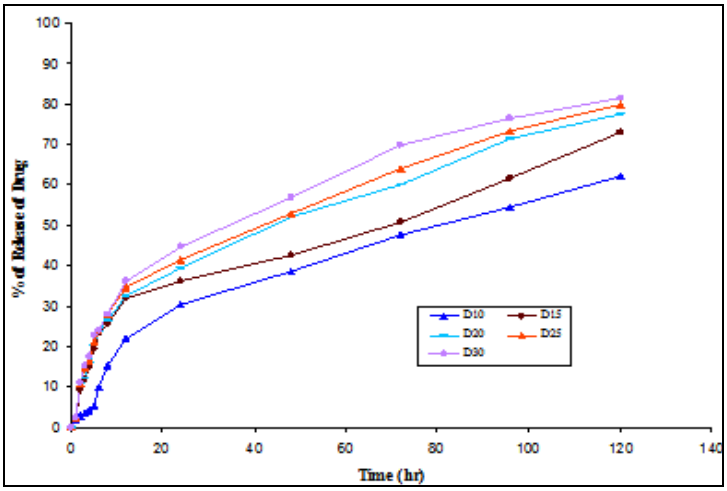

FIG. 6: ZERO ORDER RELEASE OF DEXAMETHASONE SODIUM PHOSPHATE FROM DIFFERENT CONCENTRATION OF DRUG LOADED PLGA IN-SITU IMPLANTS (BUFFER Ph 7.4, TEMP.- 37°C)

TABLE 5: RELEASE RATE VS CONCENTRATION OF DEXAMETHASON SODIUM PHOSPHATE AT DIFFERENT PHASE

Formulation Code	Concentration of Drug (%)	Burst Phase		Phase II	
		Release Rate (% Release/hr)	R^2	Release Rate (% Release/hr)	R^2
D10	9.09	1.7389	0.8916	0.359	0.9871
D15	13.04	3.4269	0.9645	0.3734	0.9883
D20	16.67	3.517	0.9582	0.4187	0.9876
D25	20.00	3.6282	0.9567	0.4243	0.9896
D30	23.08	3.6849	0.9446	0.4259	0.9667

While plotted the release rate against concentration of drug at burst phase and phase II in the **figure 7 and 8**, it was clearly observed that D30 having drug loading 23.08% (theoretical) of Dexamethasone salt showed higher release rate than four other formulations those have lower drug loading of 20.00%, 16.67%, 13.04% and 9.09% for D25, D20, D15 and D10 respectively.

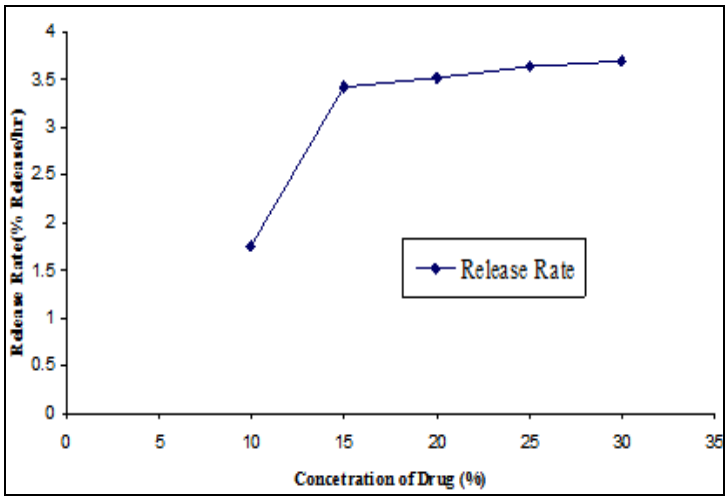

FIG. 7: EFFECT OF DRUG (DEXAMETHASONE SODIUM PHOSPHATE) LOADING ON THE BURST PHASE RELEASE STATE

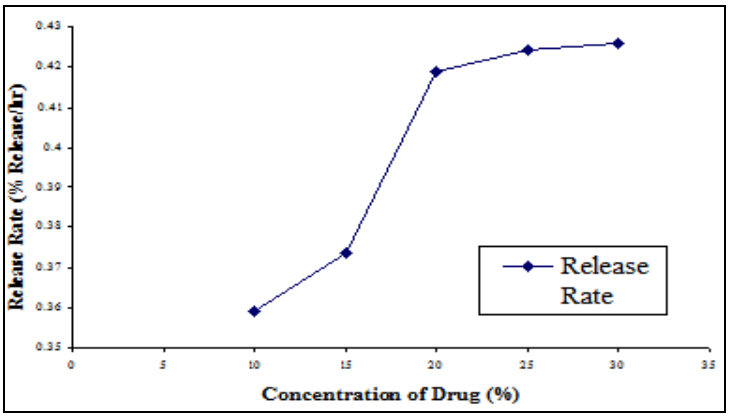

FIG. 8: EFFECT OF DRUG (DEXAMETHASONE SODIUM PHOSPHATE) LOADING ON THE BURST PHASE II RELEASE STATE

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. The implants were analyzed before and after dissolution that was shown in **figure 9** and the actual drug content in percentage in implants were determined which correlates the dissolution data along with the release rate of drug from the implant and also the drug loss in the gelatin as a small amount of dexamethasone may diffuse in to the gelatin.

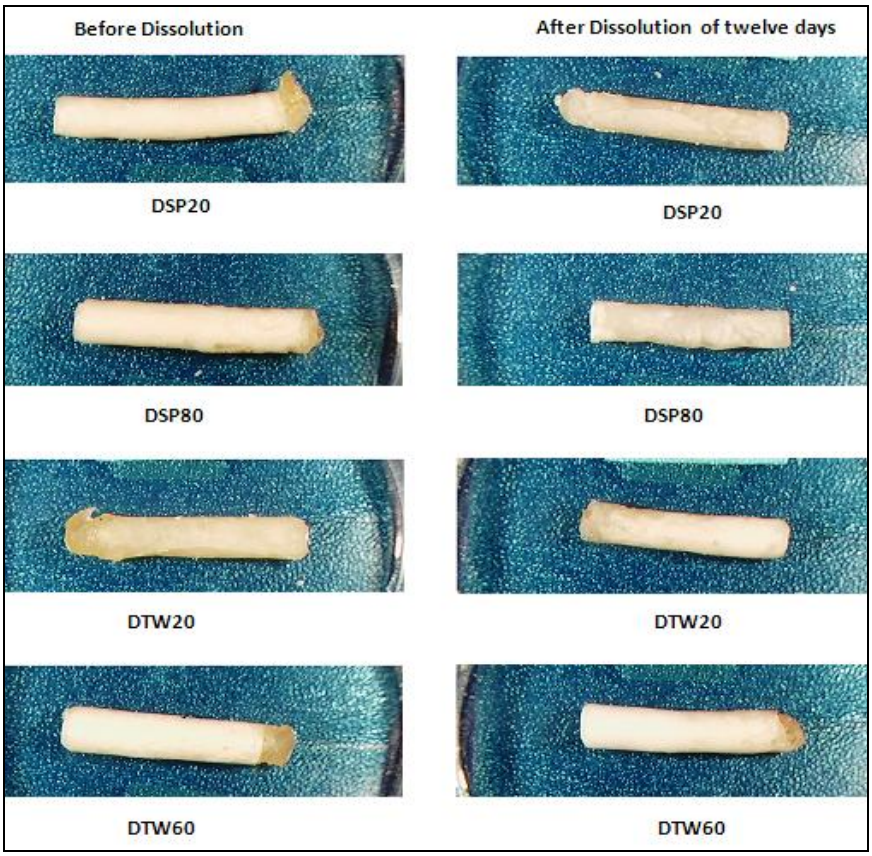

FIG. 9: PHOTOGRAPH OF *IN-SITU* IMPLANT PREPARED FROM DRUG, EXCIPIENTS AND PLGA IN DMSO

DSP20- Implants prepared from Dexamethasone (8.7%), Span 20 and PLGA in DMSO; DSP80- Implants prepared from Dexamethasone (8.7%), Span 80 and PLGA in DMSO; DTW20- Implants prepared from Dexamethasone (8.7%), Tween 20 and PLGA in DMSO; DTW60- Implants prepared from Dexamethasone (8.7%), Tween 60 and PLGA in DMSO

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 beauty 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 the 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 good shaped, implants can be obtained from *in-vitro* in gelatin gel whereas; it is difficult to obtain good 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.

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