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Research Article

INFLUENCE OF PELLETIZATION TECHNIQUE AND EROSION ENHANCERS ON CEPHALEXIN RELEASE PATTERN FROM NON-POLYMERIC BIODEGRADABLE IMPLANT

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ABSTRACT

Non-polymeric biodegradable implants for post operative site delivery were developed and evaluated in vitro. The glyceryl monostearate (GMS) based cephalexin pellets were formulated using compression and molding technique with different percentage of erosion enhancers like polyethylene glycol (PEG 6000 and PEG 400) and propylene glycol and the effect of these parameters on drug release pattern from non polymeric matrix were studied. These formulations were subjected to *in vitro* drug release by USP dissolution method. The pellets without PEG 400 showed about 52.0% drug release in 30 h while pellets containing 5.0% PEG showed 66.0% drug release (prepared by compression technique) in same time; while same formulation prepared by molding technique showed 81.0% drug release. In case of propylene glycol in similar concentration (5.0%) showed 75.0% drug release by compression technique and 83.0% drug release by molding technique in 30 h. The formulations containing maximum amount of erosion enhancer which can be compressed to form pellets i.e.5.0% PEG 400 or propylene glycol, were further subjected to in vitro drug release by agar gel method and stability studies. It was observed that glyceryl monostearate forms hydrophobic matrix and had delayed the drug delivery. It was observed that the release profile was dependent on the combination and percentage of erosion enhancers and preparation technique. The formulation prepared by compression method showed more delayed release compared to formulations prepared by molding method.

Key words: Controlled release biodegradable cephalexin implants, Glyceryl monostearate.

INTRODUCTION

The vascular system distributes the drug uniformly throughout the entire body when administered systemically and only fraction of given dose reaches the desired site.[1] Direct application and local infusion of antibiotics to the surgical wound has been found to be more effective. However, the rapid absorption of antibiotics from the wound site reduces the duration of protection against infection, and local infusion is cumbersome and requires continuous medical attention.[2,3]. A biodegradable dosage form, which could be implanted at the site of the surgical incision to release a drug over an extended period of time, could be one such method. Extensive work has been done to provide such dosage

form using poly(DL-lactide-co-glycolide) [PLGA] and its derivatives as biodegradable polymer matrix. Chengji et al.[4] reported that immunogenicity of a contraceptive peptide device can be enhanced by injectable polymer microsphere containing PLGA. PLGA was also used as biodegradable matrix in urological injection therapies by Kang et al.[5] Zaghloul AA[6]. developed beta-estradiol biodegradable controlled delivery system using PLGA. *In vivo* studies of Paclitexel and Etanidazole combined biodegradable implant using PLGA for glioma was studied by Kumar et al.[7] The implants containing poly(DL-lactide-co-glycolide), Tween or Span and tomsulosin hydrochloride were prepared and release was observed upto 10 days by Mamun et al.[8] The study of *in vitro* and *in vivo* for localized treatment of osteomylitis, for 6 weeks release of ciprofloxacin from implants using poly(DL-lactide-co-glycolide) 50:50 was reported by Ramchandani et al.[9] A biodegradable long-acting contraceptive capsule-type implant was developed using biodegradable polyester, polycaprolactone as the principle matrix and a water-leachable polyether, Pluronic F68 as drug releasing enhancer.[10] The levonorgestrel (LNG) containing capsules showed an average release rate of 7.0 µg/d/cm length for a period of 2 years in rats. Baxter Healthcare Corp. (USA) has recently introduced a gentamicin implant using biodegradable polymers which may reduce incidences of surgical site infections and postoperative cast and complications.[11]

Very little work has been conducted using non-polymeric materials as biodegradable matrix. Guse et al.[12] studied mass transport mechanism involved in the control drug release from lipid-based implant using different types of triglyceride (glyceryl trilaurate, glyceryl trimyristate, glyceryl tripalmitate and glyceryl tristearate) based cylinders were prepared by compression technique. Granules of triglyceride and erosion enhancers were mixed with the drug and compressed to form pellets, then dry coated or compressed with glyceryl monostearate to form an implant.[13] After initial delay these multilayered implants disintegrate and the compressed mixture of granules containing glyceryl monostearate, triglyceride, erosion enhancers and powdered drug are exposed to the dissolution media. This may lead to a pulsatile drug delivery system. To provide a sustained release of the from the drug delivery system; the drug might be dispersed in the molten mass of glyceryl monostearate containing release rate modifier before compression.

In the present study, non-polymeric biodegradable sustained release implants for post operative drug delivery were prepared by dispersing the cephalexin in molten mass of glyceryl monostearate as biodegradable hydrophobic matrix having different concentrations of erosion enhancers like polyethylene glycol (PEG 6000 & PEG 400) and propylene glycol. The pellets were formed by compression and molding technique. To observe the variation in drug release pattern, the release profile of the compressed formulation was compared with the molded formulation having the same composition and size.

MATERIALS AND METHODS

Materials

Cephalexin was received as a gift sample from Zim Pvt. Ltd. (Nagpur). Polyethylene glycol (PEG 6000 & PEG 400), propylene glycol, agar and potassium dihydrogen phosphate were purchased from Adroit Pharmaceuticals Pvt. Ltd. (Nagpur). Glyceryl monostearate was supplied by Suyog Chemicals Pvt Ltd. (Nagpur) and all other chemicals used were of Laboratory reagents / Analytical reagents grade.

Preparation of antibiotic loaded implants by compression technique

Glyceryl monostearate, polyethylene glycol (PEG 6000 & PEG 400) or Propylene glycol in the specified quantities (**Table 1**) were heated to 70°C on water bath under stirring with glass rod. The weighed quantity of drug was dispersed uniformly just before the solidification of the mass. The solidified blend was stored in a freezer for 1 h, the hard mass thus obtained was ground to fine powder and passed through #200. These granules were compressed by compression machine punch size 8 mm (flat) to form tablet shaped pellets. Different formulations were prepared by using various

concentrations of polyethylene glycol (PEG 6000 & PEG 400) and propylene glycol.

Preparation of antibiotic loaded implants by molding technique

The molten mass and the various formulations were prepared **(Table 1)** as above, except that the molten mass was drawn up into 10 ml syringe and injected into cylindrical stainless steel mould of 8 mm inner diameter and length about 2 cm. The mold was allowed to cool at 2-8°C and each cylindrical pellet was cut into 3 mm long, equivalent to the thickness of pellets prepared by compression technique, to make the same size pellets by both techniques.

***In vitro* release studies**

The release of cephalexin was studied by USP[14] (Disso 2000, paddle method at 50 rpm) at 37±1°C in 900 ml of pH 7.4 (0.1 mol L^{-1}) phosphate buffer. Samples (5 ml) were filtered and analyzed UV-spectrophotometrically at 262 nm[15] (Shimadzu 1601).

Gel simulating *in vitro* implantation (gel method)

In vitro release was followed by placing the pellet/implant in agar gel simulating subcutaneous tissues condition with respect to viscosity and water content[16]. The agar crystals were dissolved in boiling pH 7.4, 0.1 mol L^{-1} phosphate buffer to prepare 1.5% agar solution, which was poured into Petri dish and left to congeal. A hole (8 mm) in the center of agar plate was drilled with cork borer and the pellet/implant was placed in the hole. Sufficient quantity of hot (50-60^{0}C) agar solution was poured on the top to cover the implants and left to congeal. The plate was covered and placed in oven (37^{0}C). Several agar plates implanted with cephalexin devices were prepared at the same time and the samples were collected at 6, 24, 48, 72, 96 h. At each sampling time, one plate was removed from the oven. The plate was divided into four sampling zones and three samples were removed from each zone using a cork borer size 4 (8 mm in diameter). The samples were accurately weighed and dissolved in boiling buffer containing 25% NaCl. The solution was cooled in an ice bath to precipitate the agar. The resultant suspension was weighed, sonicated, and then centrifuged to obtain a clear supernatant containing cephalexin. The supernatant was analyzed by UV assay to determine the concentration of cephalexin.

Stability studies

Formulations C3 and M3 (both containing 5.0 % of PEG 400) six of each were wrapped in aluminum foil, sealed and kept for stability studies as per ICH guidelines[17], under conditions 40 ± 2°C/75 ± 5 % RH. Every month, one sample from each formulation was withdrawn and estimated for drug concentration. Weighted formulation was crushed and dissolved in 0.1 mmol L^{-1} HCl under stirring for 2 h and the drug concentration was calculated.

RESULTS AND DISCUSSION

Formulation prepared by compression technique C1 (containing 40.0% PEG 6000) showed to 59.5% of drug release in 36 h while the formulation C6 containing 5.0% propylene glycol showed 82.0% of drug release compared to 72.0% in 36 h from C3 containing the same concentration of PEG 400. Initial a higher drug release from implants containing erosion enhancers may be due to the solubility of erosion enhancers leading to the generation of higher porosity in the formulation, agitation also helps the formulation to disintegrate and provide larger surface area to enhance drug release. In the case of formulations containing propylene glycol, higher percentage of drug release was observed because of the higher hydrophilicity of propylene glycol as compared to the formulations containing PEG 400 **(Figure 1)**. Increasing the concentration of PEG 400 (C4) or propylene glycol (C7) from 5.0% to 10.0% leads to the soft and sticky nature of formulation causing difficulty in compression; hence were not used in further studies.

The uncompressed formulations prepared by molding technique showed faster dissolution rate due to higher erosion during dissolution. Formulation M1 (containing 40.0% polyethylene glycol

6000) showed 72.0% drug release in 36 h. The formulation M6 (containing 2.5% of propylene glycol) showed higher drug release as 83.0% while M2 containing same concentration of PEG 400 showed comparatively slow drug release as 80.5 in 36 h. The higher drug release (87.5%) was observed in formulation M7 (containing 5.0% of propylene glycol) as compared to formulation M3 (containing 5.0% PEG 400) showed 85.5% in 36 h. Increasing the concentration of propylene glycol from 5.0% to 10.0% (M8) and further 15.0% (M9), the drug release were found as 89.5% and 94.5% respectively when same concentration enhancement of PEG 400, the formulation M4 and M5 showed the drug release as 88.5% and 92.5% respectively in 36 h **(Figure 2)**.

Delayed drug release from compressed formulations was observed compared to the molded formulations which could be due to the compactness in the dosage form leading to the reduction in porosity, consequently, the lower erosion and penetration of dissolution fluid in the dosage form.

In vitro drug release kinetics of the formulations prepared by the compression technique and molding technique having average concentration of PEG 400 i.e. C3 and M3 respectively were analyzed by PCP Disso V-3 software.

Considering the release pattern from the formulations C3 (containing 5.0% of PEG 400) prepared by the compression technique and similar formulation M3 (containing identical percentage of PEG 400) prepared by the molding technique were selected for evaluation by the gel simulation method.

Release of cephalexin from glyceryl monostearate matrixes in the gel method was conducted in order to stimulate the *in vivo* implantation conditions, under which the implanted matrixes are surrounded by tissues rather than by aqueous solution. Drug release from the insoluble matrix is generally achieved by penetration of the release medium into the matrix and dissolution of the drug, followed by diffusion of the drug solution through the channels and pores of the matrix.

The drug release from formulations C3 and M3 (both containing 5.0 % PEG 200, but prepared by using different pelletezation technique) in agar gel simulating subcutaneous tissue resulted in significant difference in the drug release pattern compared to the release pattern in dissolution studies. Pellets prepared by the molding technique using 5.0 % PEG 400 (M3) showed 68.5% higher drug release as compared to the pellets prepared by the compression technique, 33.0 % in 96 h **(Figure 3)**. In the agar gel method, drug release taken place by diffusion; a layer close to the implant has higher drug concentration and is inversely proportional to the distance. Percentage diffusion of the drug in the absence of any agitation is also dependent on the solubility of the drug in the aqueous phase.

The commonly adopted model for understanding the release behaviour of the drug from an implant is the Korsmeyer-Peppas equation[18]. The release exponent *n* is related to the drug release mechanism; the values of 0.4054 and 0.4243 for C3 and M3 respectively indicate anomalous transport (diffusion coupled with the erosion mechanism). R^2 values of linear regression for the Higuchi plot were 0.9914 and 0.9660 for C3 and M3, respectively, indicating that the data fit best the Higuchi model**.**

The values of *n* in the agar gel method were 0.2468 and 0.4289 for C3 and M3, respectively, indicating Fickian transport (diffusion mechanism). The R^2 values for the Higuchi plot were 0.9624 and 0.9890 for C3 and M3, respectively. This system was also best presented by the Higuchi model (Table 4 and 5).

In accelerated stability studies, both formulations C3 and M3, having the same composition but differing in the preparation technique, remained stable enough over the designated period under accelerated conditions (40 ± 2°C/75 ± 5 % RH). As drug concentration remained constant for a month, after 3 and 6 months, the drug content was found to be 99.1 and 98.2 respectively for C3; 99.4 and 98.5 respectively for M3 respectively.

CONCLUSION

According to the results, it can be concluded that the glyceryl monostearate forms

hydrophobic matrix for controlled drug delivery system. The formulations prepared by compression and molding technique can be implanted subcutaneously at the site of surgery to prevent postoperative infection. Dissolution profile of the formulations was not dependent only on solubility of drug but also on the concentration of erosion enhancer and preparation technique.

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Table 1: Formulations ratio of compressed and molded implants using erosion enhancers

S. No	Formulation	Ingredients used (%)				
		Cephalexin	GMS	PEG 6000	PEG 400	PG
	Compressed					
1	C1	20.0	40.0	40.0	0.0	0.0
2	C2	20.0	40.0	37.5	2.5	0.0
3	C3	20.0	40.0	35.0	5.0	0.0
4	C4	20.0	40.0	30.0	10.0	0.0
5	C5	20.0	40.0	37.5	0.0	2.5
6	C6	20.0	40.0	35.0	0.0	5.0
7	C7	20.0	40.0	30	0.0	10
	Molded					
8	M1	20.0	40.0	40.0	0.0	0.0
9	M2	20.0	40.0	37.5	2.5	0.0
10	M3	20.0	40.0	35.0	5.0	0.0
11	M4	20.0	40.0	30.0	10.0	0.0
12	M5	20.0	40.0	25.0	15.0	0.0
13	M6	20.0	40.0	37.5	0.0	2.5
14	M7	20.0	40.0	35.0	0.0	5.0
15	M8	20.0	40.0	30.0	0.0	10.0
16	M9	20.0	40.0	25.0	0.0	15.0

GMS: Glyceryl monostearate
PEG: Polyethylene glycol (PEG 6000 & PEG 400)
PG: Propylene glycol

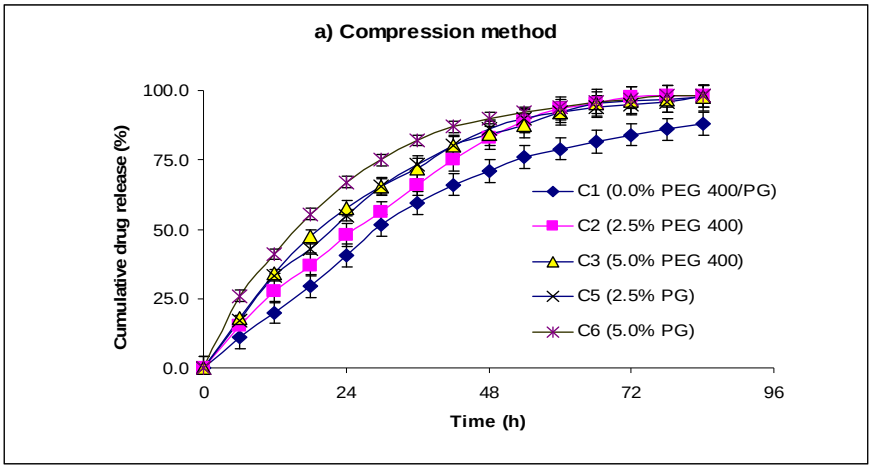

Fig.1: Comparative *in vitro* cephalexin release profiles from formulation prepared by compression method. Each point denotes mean ± SD, n = 3

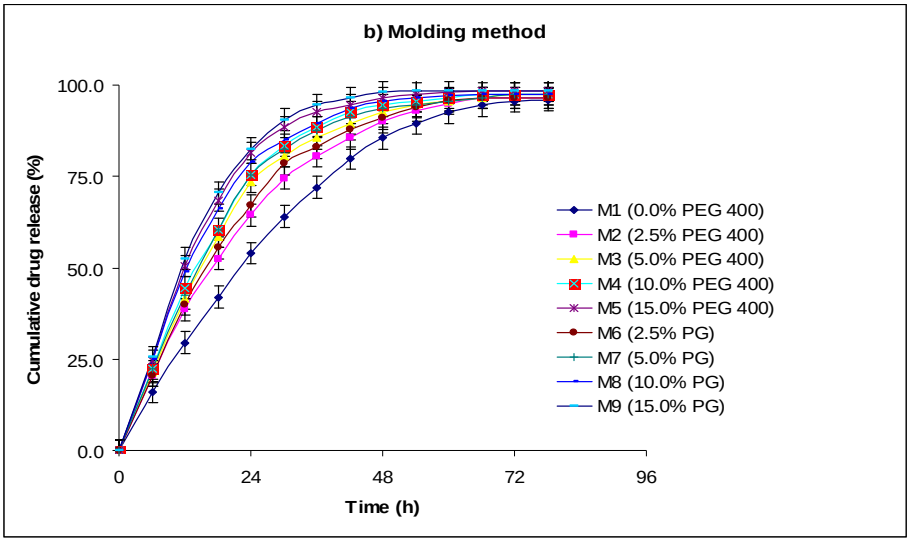

Fig. 2: Comparative *in vitro* cephalexin release profiles from formulation prepared by molding method. Each point denotes mean ± SD, n = 3

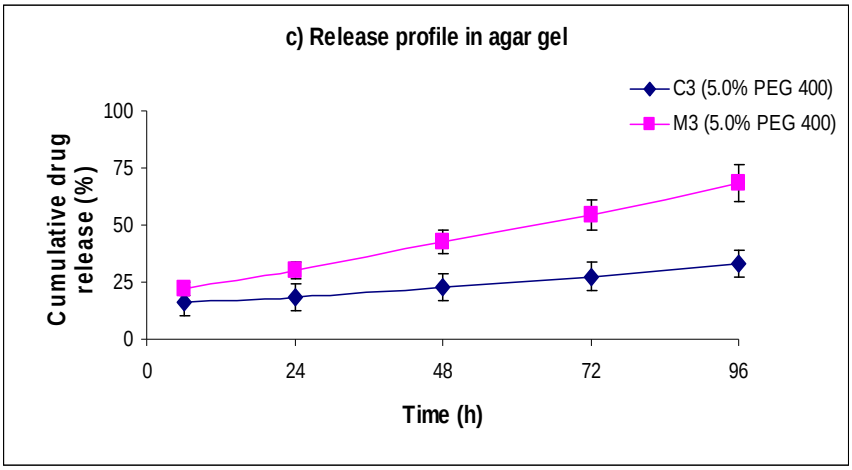

Fig. 3: Comparative *in vitro* cephalexin release profiles of C3 and M3 (both containing 5.0 % PEG 200) from agar gel. Each point denotes mean ± SD, n = 3

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