

# In vitro characterization of methotrexate loaded poly(lactic-co-glycolic) acid microspheres and antitumor efficacy in Sarcoma-180 mice bearing tumor

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## Abstract

Methotrexate (MTX) loaded poly(lactic-co-glycolic) acid (PLGA) microspheres were prepared by emulsion solvent evaporation technique. The mean diameter of the microspheres was affected by the type of emulsion stabilizer, polymer concentration, aqueous and organic phase volume and stirring speed. The in vitro release was triphasic and was dependent on copolymer composition and molecular weight of the polymer. Antitumor efficacy in Sarcoma-180 tumor bearing mice exhibited increased volume doubling time ( $18 \pm 2.7$  days) compared to plain subcutaneous injection of methotrexate ( $8 \pm 0.7$  days). Preliminary pharmacokinetic studies following subcutaneous administration of MTX loaded PLGA microspheres illustrated the controlled release of the drug. The studies demonstrated the feasibility of employing PLGA as an effective carrier for antineoplastic drug like methotrexate.

*Keywords:* Methotrexate; PLGA; In vitro release; Antitumor efficacy

## 1. Introduction

Controlled drug delivery systems have received tremendous attention over the last two to three decades and the significant research interest in the long term maintenance of therapeutic drug levels coincides with the increased medical and public acceptance of such systems (Langer, 1981; Tice, 1985).

The biodegradability and biocompatibility of polyesters such as poly(glycolide) and poly(lactide) and their copolymers poly(lactic-co-glycolic) acid have been established from their uses in surgical grafts, implants and various prosthetic devices (Chu, 1982; Fredericks and Melveger, 1984; Zimmerman et al., 1987; Hay et al., 1988) and they do not cause adverse tissue reaction (Thies, 1981; Leung et al., 1987). Biodegradable carriers are being widely used because they offer advantages of being reabsorbed by the

body, alleviating the need for the surgical removal, etc. (Peppas, 1995). It is the biocompatible, biodegradable synthetic polymer which degrades at a rate dependent on properties such as molecular weight, crystallinity and lactide-glycolide ratio (Cutright et al., 1974; Hutchinson and Furr, 1986). Drug incorporation into the microspheres makes drug administration possible by injection (Aftabroushad and Doelkar, 1994; Morris et al., 1994). The various properties of the polymeric drug carrier can be used to control the drug release. Techniques such as film casting (Jackanicz et al., 1973), microencapsulation (Beck et al., 1979), molding (Schwope et al., 1975), and spray drying (Deligiannis et al., 1987) have been applied to the preparation of biodegradable dosage forms for the delivery of drugs such as anaesthetics (Corre et al., 1994), anti-cancer drugs (Sanchez et al., 1993; Singh et al., 1996), hormones and peptides (Heya et al., 1994), anti-inflammatory agents (Chandrashekhara and Udupa, 1996), etc. Employing these drug delivery systems the therapeutic efficacy of anticancer drugs may improve by possible localization of the drug at the site of action. Peptides and proteins

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may be protected against chemical and enzymatic degradation (Tice, 1985; Morris et al., 1994; Service, 1994).

Methotrexate (MTX) is widely used in the treatment of neoplastic disorders. MTX when given orally has a short elimination half life and it can cause diarrhea and ulcerative stomatis. On intravenous administration the drug disappears in triphasic fashion, the first phase has a half life of 45 min, the second phase 2.5 h and the final phase of 7 h. Macromolecular carriers for MTX include microsphere preparation using MTX–albumin conjugate (Kim and Oh, 1988), immunoglobulin–MTX conjugate (Ghosh et al., 1988), Polylysine–MTX conjugate (Shen and Ryser, 1979), Depo–MTX using cyclodextrin (Chatelut et al., 1994), gelatin–MTX conjugate (Narayani and Rao, 1993, 1996). Other carriers and formulations include niosomes (Azmin et al., 1985), polyanhydride microspheres (Bhagat et al., 1994) and multiple emulsions (Law and Lin, 1991).

MTX is chosen as the antineoplastic drug because it is cell-cycle phase specific whereby prolonged exposure of the drug to the cancer cells is necessary for optimal efficacy and since these polymers release the drugs for a long period of time they could be effectively utilised for such drugs. Furthermore, encapsulation of MTX in PLGA microspheres may eliminate the inherent drawbacks experienced with oral and intravenous administration of MTX and also to increase the half life of the drug by administering the delivery system by other parenteral routes such as subcutaneous route. The present study is undertaken to explore the feasibility of employing PLGA for encapsulation of MTX, to characterise microspheres for different physico–chemical properties using PLGA of different copolymer composition and molecular weight and the utility of MTX encapsulated PLGA microspheres in tumor treatment.

## 2. Experimental

### 2.1. Materials

Methotrexate was a gift sample from American Cyanamide, Pearl River, New York, USA. Poly (DL-lactide)-co-glycolide 50:50 (molecular weight 54 000), 80:20 (molecular weight 70 000), 70:30 (molecular weight 62 000) were procured from Polysciences, Warrington, PA, USA. All other ingredients and reagents were of analytical grade.

### 2.2. Animals

For tumor studies, six to eight weeks old BALB/c mice weighing 20–25 g were used from an inbred colony

maintained in our animal house under controlled conditions of temperature ( $23 \pm 2^\circ\text{C}$ ), humidity ( $50 \pm 5\%$ ) and light and darkness (10 and 14 h respectively). The animals were given sterile food prepared in the laboratory as per the standard formulation (wheat 70%, bengalgram 20%, fish meal 5%, yeast powder 4%, sesame oil 0.75% and shark liver oil 0.25%) and water ad libitum. Throughout the experiment 5–6 animals were housed in polypropylene cage containing sterile paddy husk as bedding material.

### 2.3. Methods

#### 2.3.1. Microspheres preparation

Methotrexate loaded PLGA microspheres were prepared by solvent evaporation method (Beck et al., 1979; Arshady, 1991). PLGA was dissolved in dichloromethane (DCM) and MTX was added to this solution and sonicated at 100 W, 55000 Hz. The organic phase was slowly added to the aqueous phase containing emulsion stabilizer and stirred using a stirrer with a stainless steel propeller having a 50 mm diameter (Remi Instruments, Bombay). The resulting oil in water emulsion was stirred till DCM evaporated. The microspheres were collected by filtration, washed with deionised water and dried. The effect of various parameters viz, nature and concentration of emulsifier, stirring speed and polymer concentration on size distribution, were also studied by preparing placebo microspheres using PLGA (50:50 ratio).

#### 2.3.2. Surface morphology and size distribution

The shape and surface characteristics of the microsphere were studied by scanning electron microscopy (SEM) (S240 Cambridge Instruments UK). The mean diameter of the microspheres were measured by Droplet and particle size analyser using Malvern Laser analyser 2600 D.

#### 2.3.3. Entrapment efficiency and yield

A known amount of MTX loaded PLGA microspheres were dissolved in DCM. Methyl alcohol was added to precipitate PLGA. The drug was extracted with phosphate buffered saline pH 7.4 (PBS) and then analysed at 303 nm spectrophotometrically for the drug content. The microsphere recovery yield was determined according to

$$\% \text{ Yield} = \frac{\text{weight of microspheres}}{\text{weight of drug} + \text{weight of polymer}}$$

#### 2.3.4. In vitro release studies

Weighted MTX loaded microspheres containing known quantity of drug were suspended in phosphate buffered

saline pH 7.4 in stoppered flasks. The flasks were placed in an incubator shaker bath thermostated at  $37 \pm 1^\circ\text{C}$  at a speed setting of 25 cycles/min. Aliquots were withdrawn at various time intervals, filtered and analysed for MTX content at 303 nm spectrophotometrically against appropriate blank (placebo microspheres). To maintain constant volume, an amount of medium equal to the volume of the withdrawn sample was added immediately.

### 2.3.5. Tumor propagation (Uma Devi et al., 1994)

The tumor Sarcoma-180 obtained from the Cancer Research Institute, Bombay, India, was propagated in adult inbred BALB/c mice by serial transplantation of  $10^6$  viable tumor cells into the intraperitoneal cavity of female mice. Solid tumors were produced by intradermal injection of  $5 \times 10^5$  viable cells on the dorsal skin after dilution with Dolbecco's Modified Eagle's Medium so as to get  $10^6$  cells in 100  $\mu\text{l}$ . Tumor cells were injected (0.05 ml) on the dorsal skin of the mouse and the tumor growth was monitored.

The tumor volume ( $V$ ) was calculated using the formula

$$V = \frac{\pi}{6} (D_1 \cdot D_2 \cdot D_3)$$

where  $D_1$ ,  $D_2$ ,  $D_3$  are the diameters of each tumor in three perpendicular planes.

The study was started when the tumor volume reached  $100 \pm 10 \text{ mm}^3$  and the time taken to reach  $200 \pm 10 \text{ mm}^3$  was considered as the volume doubling time and was calculated as follows

$$\text{VDT} = \log 2 \times (T_1 - T_0) / \log V_1 - \log V_0$$

where  $V_0$  is the volume of the tumor at time  $T_0$  and  $V_1$  is the volume at time  $T_1$  (Shilong et al., 1989). The animal survival was followed up to 120 days which was roughly comparable to 5 years survival in man (Nias, 1990).

### 2.3.6. Volume doubling time (VDT) and animal survival

The time required to double the treatment volume (VDT) from  $100 \text{ mm}^3$  to  $200 \pm 10 \text{ mm}^3$  was taken as criterion to assess the antitumor efficacy. The mice were divided into different groups, each group consisting of 10 mice. The first group, control group, was s.c. injected PBS pH 7.4, the second group placebo microspheres, the third group s.c. injection of plain MTX in PBS (5 mg/kg body weight) and fourth group formulation A2 (10 mg/kg body weight) dispersed in 0.5% CMC in PBS. The tumor volume was monitored till it reached  $200 \pm 10 \text{ mm}^3$ . The animal survival was monitored at different time intervals.

### 2.3.7. Pharmacokinetic studies

MTX concentration in plasma was determined in 40 BALB/c mice (2 groups) bearing S-180 tumor. Formulation A2 (10 mg/kg body weight) was s.c. injected after dispersing in 0.5% CMC in PBS pH 7.4. To the second group s.c. injection of plain MTX (5 mg/kg body weight) in PBS pH 7.4 was administered. Blood was collected at predetermined time intervals from cubital vein. The plasma was separated and analysed for MTX content by the method reported by Chakrabarthy and Bernstein (1969) spectrofluorometrically. In brief, to the plasma, trichloroacetic acid was added to precipitate the proteins. The contents were filtered and sodium hydroxide and 5 M acetate buffer pH 5 was added and the contents mixed thoroughly. The pH of the contents was adjusted to pH 5 by addition of sodium hydroxide or acetic acid. Potassium permanganate was added for oxidation and after 5 min hydrogen peroxide was added to destroy the excess potassium permanganate. The fluorescence was measured: the excitation and emission wavelength were set to 360 and 450 nm, respectively.

The pharmacokinetic parameters were calculated using non compartmental analysis (Gibaldi and Perrier, 1982).

Prior to the in vivo studies the formulations were sterilised by gamma irradiation (1.8 Mrad) (Ruiz and Benoit, 1991) from  $^{60}\text{Co}$  source (Gammtron-R, Siemens, Germany).

## 3. Results and discussion

### 3.1. Influence of nature and concentration of emulsion stabilizer

Hydroxypropyl methylcellulose and polyvinyl alcohol (PVA) of different concentrations were used to see the effect on mean diameter of the microspheres. At all the concentrations tried with the stabilizers, microspheres were obtained successfully (Table 1). Increased surfactant con-

Table 1  
The effect of stabilizer type and concentration on mean diameter

HPMC Conc. (%)	PVA Conc. (%)	Mean diameter ( $\mu\text{m} \pm \text{SD}$ )
0.5	—	219.79 $\pm$ 9.53
1.0	—	207.79 $\pm$ 3.32
1.5	—	179.93 $\pm$ 7.90
—	0.5	59.22 $\pm$ 2.40
—	1.0	45.38 $\pm$ 1.69
—	1.5	40.31 $\pm$ 1.75

Microspheres were prepared as follows: 250 mg of PLGA (50:50) was dissolved in 20 ml of DCM. This was emulsified with 100 ml stabilizer at 1200 rpm.

Table 2  
The effect of the stirring speed on the size of microspheres

Stirring speed (rpm)	Mean diameter ( $\mu\text{m} \pm \text{SD}$ )
600	67.38 $\pm$ 7.03
1200	45.15 $\pm$ 4.57
1800	27.86 $\pm$ 5.82

Microspheres were prepared as follows: 250 mg of PLGA (50:50) was dissolved in 20 ml DCM, emulsified with 100 ml of 1% PVA at various stirring speed.

centration decreased the mean diameter of the microspheres ( $P < 0.05$ ). 1% PVA was selected as a stabilizer of choice for further studies because it resulted in preparation of microspheres of desired mean diameter for subcutaneous injection. It is reported that when microspheres are administered subcutaneously, depending on their size and hydrophobicity, they can either be phagocytosed by various macrophage populations or remain in the subcutaneous tissues (Tabata and Ikada, 1988; Sah and Chien, 1996). PVA has also been used by other workers (Benita et al., 1984; Jeffery et al., 1991). The microspheres reported in this study remain localized at the site of injection since they are too big to be engulfed and migrated in the draining lymph nodes by macrophages.

### 3.2. Influence of stirring speed on microsphere size

When the stirring speed increased, the mean diameter decreased ( $P < 0.05$ ) (Table 2).

The nature and concentration of emulsion stabilizer and stirring speed are parameters of vital importance in the microsphere preparation step. The stirring speed provides the energy and the emulsion stabilizer decreases the inter-

Table 3  
Influence of polymer concentration on particle size

Wt. of polymer (mg)	PLGA (50:50) (%)	Mean diameter ( $\mu\text{m} \pm \text{SD}$ )
300	1.5	42.89 $\pm$ 1.5
400	2.0	43.17 $\pm$ 2.1
500	2.5	45.72 $\pm$ 2.9
Wt. of polymer (mg)	PLGA (80:20) (%)	Particle size ( $\mu\text{m}$ )
300	1.5	41.12 $\pm$ 3.1
400	2.0	43.03 $\pm$ 2.7
500	2.5	45.25 $\pm$ 1.3
Wt. of polymer (mg)	PLGA (70:30) (%)	Particle size ( $\mu\text{m}$ )
300	1.5	43.58 $\pm$ 1.5
400	2.0	45.05 $\pm$ 3.3
500	2.5	46.19 $\pm$ 1.1

Microspheres were prepared as follows: PLGA was dissolved in 20 ml of DCM and emulsified in 100 ml of 1% PVA at 1200 rpm.

Table 4  
Influence of aqueous phase volume

Volume of aqueous phase (ml)	Mean diameter ( $\mu\text{m} \pm \text{SD}$ )
100	110.62 $\pm$ 6.52
300	124.81 $\pm$ 1.79
500	134.87 $\pm$ 1.05
700	144.03 $\pm$ 2.11

Microspheres were prepared as follows: 250 mg of PLGA (50:50) was dissolved in 20 ml DCM. This was emulsified in 1% PVA at 600 rpm.

facial tension between the organic droplets and aqueous medium. For higher stirring speed and PVA concentration, the energetic conditions are appropriate for the maximum separation of organic phase, so that small mean particle diameter and narrow particle size distribution are obtained.

### 3.3. Influence of polymer concentration

The particle size increased with increasing concentration of PLGA dissolved in a fixed volume of DCM although the increase was nonsignificant ( $P > 0.05$ ) (Table 3). Slight fusion of semi formed particles may have resulted due to increased frequency of collision when the PLGA concentration was increased. The stirring efficiency may have been reduced due to increased viscosity with increased polymer concentration. The influence of polymer

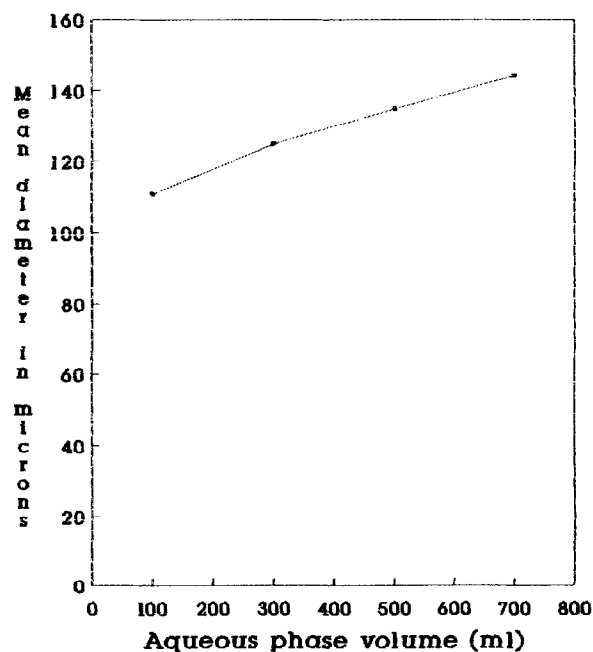


Fig. 1. The relationship between mean diameter and aqueous phase volume (refer to Table 4).

Table 5  
Influence of organic phase volume

Volume of organic phase (ml)	Mean diameter ( $\mu\text{m} \pm \text{SD}$ )
20	43.52 $\pm$ 4.72
10	64.18 $\pm$ 5.11
5	87.70 $\pm$ 4.96

Microspheres were prepared as follows: 250 mg of PLGA (50:50) was dissolved in DCM. This was emulsified in 1% PVA (100 ml) at stirring speed of 1200 rpm.

concentration on mean diameter was studied using all co-polymer ratios and the mean diameter slightly increased although it was nonsignificant ( $P > 0.05$ ) with increase in concentration of polymer irrespective of PLGA co-polymer ratios. There was no apparent relationship between particle size and co-polymer composition or molecular weights. Similar observations were also reported by Jeffery et al. (1991).

### 3.4. Influence of the aqueous and organic phase volume

Increasing the volume of aqueous phase from 100 to 700 ml influenced the mean diameter (Table 4). The mean diameter increased with increase in aqueous phase volume ( $P < 0.05$ ). A linear relationship was established between mean diameter and aqueous phase volume (Fig. 1). Increasing the volume of organic phase, while keeping the volume of aqueous phase constant resulted in increase in mean diameter of microspheres with decrease in organic phase volume ( $P < 0.05$ ) (Table 5). This may be due to the increasing viscosity of internal phase of emulsion with decreasing volume of organic phase, PLGA remaining constant.

### 3.5. Surface morphology and physico-chemical parameters

The microspheres were smooth spherical (Plate 1) without aggregation. The mean diameter and entrapment effi-

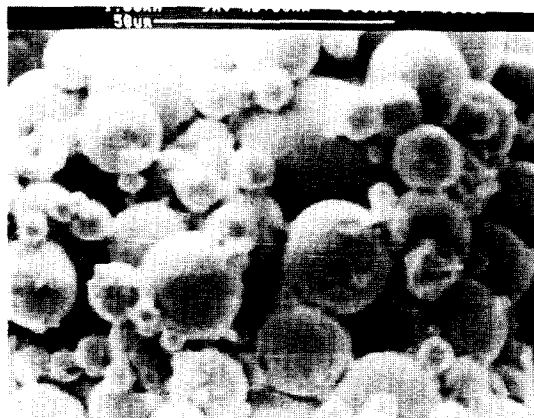


Plate 1. Scanning electron microscope of PLGA loaded methotrexate microspheres (formulation A2).

ciency of MTX loaded PLGA microspheres prepared using three different drug loadings and different co-polymer ratios are shown in Table 6. The entrapment efficiency ranged from 65 to 73% and was independent of different co-polymer ratios. As the percentage drug loading increased there was insignificant increase in entrapment ( $P > 0.05$ ). Since methotrexate is practically insoluble in water, it was preferentially partitioned in the organic phase of the emulsion and the loss of drug in the aqueous phase was much less. The mean diameter slightly influenced the entrapment efficiency and can be explained on the basis that larger surface area of microspheres results in greater drug loss during microencapsulation and washings. The yield ranged from 79% to 86% and was independent of co-polymer ratios. The % CV for yield, % entrapment efficiency and homogeneity of the drug content within batches was less than 7% indicating the suitability of the fabrication process.

### 3.6. In vitro release

MTX was released in a triphasic manner from the microspheres, characterised by burst effect followed by a

Table 6  
Physico-chemical data of microspheres

Abb.	Co-polymer ratio PLGA	Drug loading (%)	Polymer (%)	Yield (% $\pm$ SD)	Drug entrapped (% $\pm$ SD)	Mean diameter ( $\mu\text{m} \pm \text{SD}$ )
A1	50:50	16.6	83.4	85.50 $\pm$ 1.20	66.20 $\pm$ 2.05	45.72 $\pm$ 2.51
A2	50:50	20.0	80.0	82.30 $\pm$ 1.75	69.54 $\pm$ 1.77	41.17 $\pm$ 1.78
A3	50:50	25.0	75.0	84.75 $\pm$ 2.43	71.77 $\pm$ 0.97	42.89 $\pm$ 1.40
B1	80:20	16.6	83.4	80.80 $\pm$ 3.41	67.14 $\pm$ 2.25	45.25 $\pm$ 2.11
B2	80:20	20.0	80.0	81.20 $\pm$ 4.20	69.93 $\pm$ 3.11	43.03 $\pm$ 3.05
B3	80:20	25.0	75.0	78.59 $\pm$ 3.67	72.42 $\pm$ 1.63	41.12 $\pm$ 2.92
C1	70:30	16.6	83.4	86.52 $\pm$ 5.20	66.32 $\pm$ 3.21	46.19 $\pm$ 1.64
C2	70:30	20.0	80.0	79.21 $\pm$ 2.40	67.51 $\pm$ 4.73	45.05 $\pm$ 1.81
C3	70:30	25.0	75.0	81.72 $\pm$ 2.91	71.45 $\pm$ 2.11	43.58 $\pm$ 1.93

Microspheres were prepared as follows: PLGA was dissolved in 20 ml of DCM, MTX added to it. This was emulsified with 1% PVA (100 ml) at 1200 rpm.

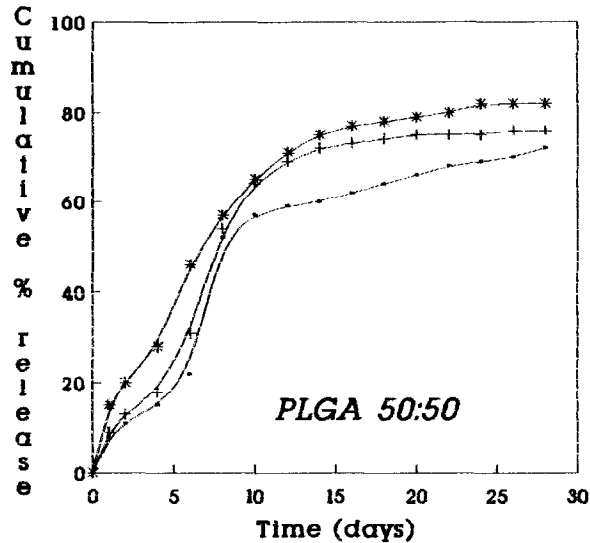


Fig. 2. The in vitro release of methotrexate from PLGA (50:50) microspheres in phosphate buffered saline pH 7.4. ■, A1 (D:P) 1:5; +, A2 (D:P) 1:4; \*, A3 (D:P) 1:3.

slow release and finally secondary burst effect (Figs. 2–4). The burst effect corresponds to the release of the drug located on or near the surface of the microspheres or release of poorly entrapped drug. The slow release period may be due to the medium being diffused into the polymer matrix, whereby degradation occurs and the drug diffuses out of the microspheres. The secondary burst effect occurs when the matrix becomes more water soluble which results

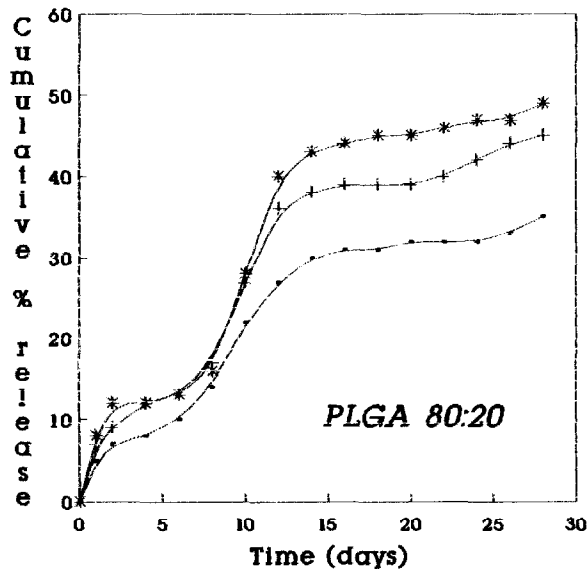


Fig. 3. The in vitro release of methotrexate from PLGA (80:20) microspheres in phosphate buffered saline pH 7.4. ■, B1 (D:P) 1:5; +, B2 (D:P) 1:4; \*, B3 (D:P) 1:3.

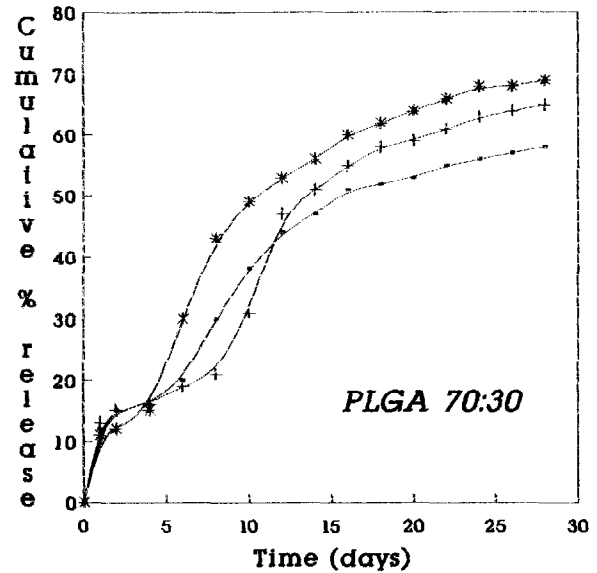


Fig. 4. The in vitro release of methotrexate from PLGA (70:30) microspheres in phosphate buffered saline pH 7.4. ■, C1 (D:P) 1:5; +, C2 (D:P) 1:4; \*, C3 (D:P) 1:3.

in erosion and collapse of the matrix. Plate 2 shows the SEM of microspheres during later stages of drug release during which the polymer erosion occurs. The triphasic release was also reported by Spenlehauer et al. (1988), Sanders et al. (1989), and Stuesson et al. (1993). The drug content and mean diameter were almost similar for all formulations and the difference in release pattern on this basis could be ruled out.

The difference in release behaviour can be explained on the basis of degradation rate which is dependent on co-polymer composition and molecular weights of the poly-

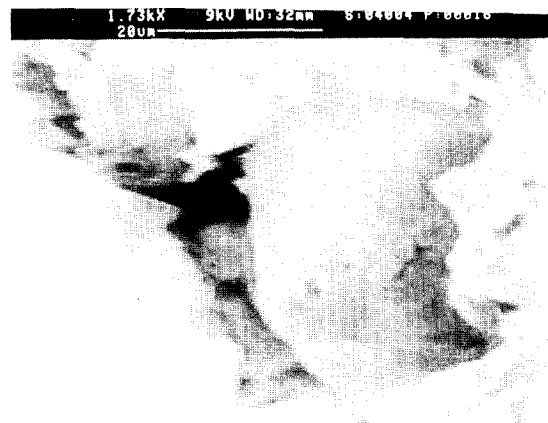


Plate 2. Scanning electron microscopy of PLGA loaded methotrexate microspheres (formulation A2) during in vitro dissolution studies. The SEM depicts erosion of the polymer matrix.

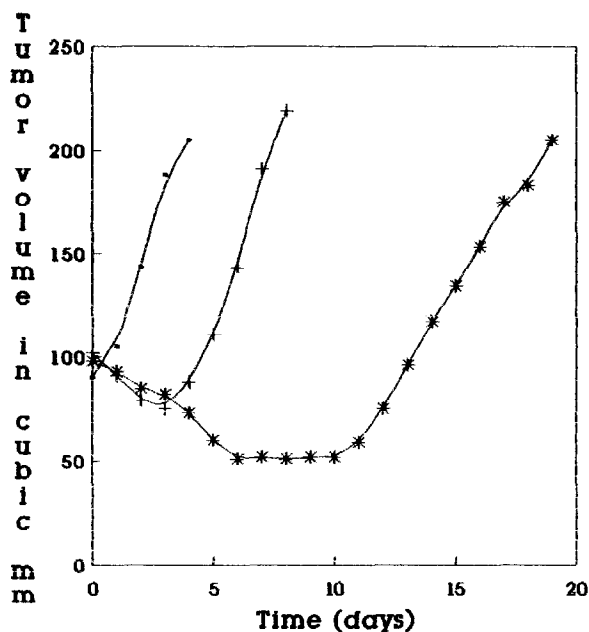


Fig. 5. The Sarcoma-180 tumor volume growth in BALB/c mice on subcutaneous administration of plain and methotrexate loaded PLGA (50:50) microspheres. ■, Control; +, Plain MTX; \*, A2 (D:P) 1:4.

mer. The microspheres prepared with polymer with higher glycolide content degrades faster than microspheres prepared with lower glycolide content, i.e. PLGA 50:50 degraded faster than PLGA 70:30 and PLGA 80:20 (Pitt et al., 1981; Jeffery et al., 1991; Vert et al., 1991). Therefore this can explain the faster release of drug from PLGA 50:50 when compared to PLGA 70:30 and PLGA 80:20. The molecular weight of the polymer decreases as degradation occurs and low molecular weight polymer degrades faster and vice versa. PLGA (50:50) molwt. 54 000 degraded faster compared to PLGA (70:30) molwt. 62 000 and PLGA (80:20) molwt. 70 000 and it was clearly indicative of the fact that degradation was faster with a polymer of higher glycolide content and lower molecular weight. The glycolide units are more hydrophilic than lactide units and promote water uptake into the polymer which encourages hydrolytic cleavage (Gilding and Reed, 1979; Dunn et al., 1988).

$r^2$  values ranged from 0.95 to 0.98. These  $r^2$  values obtained from Higuchi (1963) square root of time model indicated that release was essentially diffusion controlled.

Formulation A2 was selected for in vivo studies due to its optimum release characteristics in vitro ( $t_{50\%} = 8$  days).

### 3.7. Antitumor efficacy and pharmacokinetic studies

Formulation A2 exhibited an increased cytotoxic effect as revealed by increased VDT ( $18 \pm 2.7$  days). The tumor

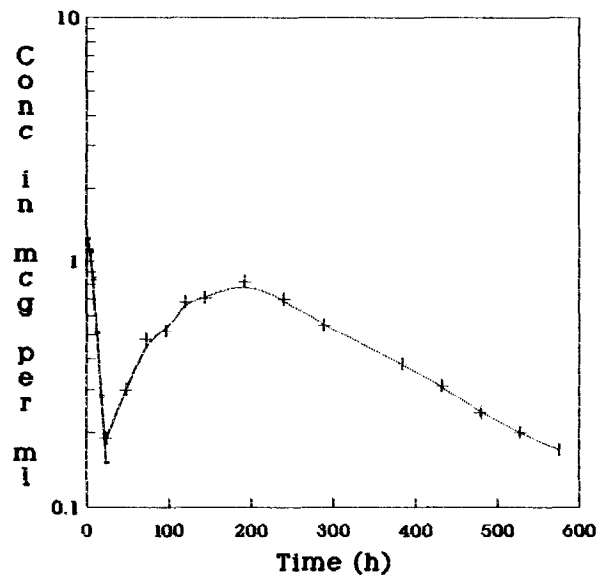


Fig. 6. The plasma drug levels of Methotrexate subsequent to subcutaneous administration of plain drug and drug loaded PLGA (50:50) microspheres. ■, Plain MTX; +, A2 (D:P) 1:4.

volume decreased considerably and the cytotoxic activity was sustained for 10 days after which the tumor volume increased sharply. Since the initial release of the drug from rigid microspheres was slow, therefore reduction in tumor volume during the initial stages was also slow. S.c. injection of plain MTX was not effective in substantially decreasing the tumor volume, although reduction in tumor volume during the first few days was higher compared to formulation A2. The control group and the second group showed similar growth curves (Fig. 5). The animal survival at the end of 40, 80 and 120 days for the control group was 10, 0, 0%, for plain MTX, 65, 15, 0% and for A2 it was 75, 20, 5%, respectively. The incorporation in the microspheres enhanced the survival time of the mice.

The plasma concentration time profile of plain MTX injection and formulation A2 are shown in Fig. 6. The AUC, MRT,  $t_{1/2}$  was higher and  $K_{el}$  was less compared to plain MTX injection (Table 7). The preliminary pharmacokinetic studies showed that the drug plasma levels were sustained.

Table 7  
Pharmacokinetic parameters of MTX subsequent to s.c. injection of formulation A2 and plain MTX

Parameters	Plain MTX $\pm$ SD	A2 $\pm$ SD
$[AUC]_0^\infty$ (mcg h/ml)	16.54 $\pm$ 1.72	339.98 $\pm$ 24.05
$[AUMC]_0^\infty$ (mcg h <sup>2</sup> /ml)	235.32 $\pm$ 17.51	187974.93 $\pm$ 1346.56
MRT (h)	14.22 $\pm$ 1.20	552.90 $\pm$ 72.45
$t_{1/2}$ (h)	10.03 $\pm$ 2.46	481.25 $\pm$ 13.32
$K_{el}$ (h <sup>-1</sup> )	0.0691 $\pm$ 0.025	1.44 $\times 10^{-3}$ $\pm$ 0.005

#### 4. Conclusion

This study has shown that the control of few manufacturing parameters for PLGA microspheres, such as nature and concentration of emulsifier, stirring speed, aqueous and organic phase volume and polymer concentration, is of vital importance in order to obtain microspheres of required size. MTX incorporation can be achieved with good yields and entrapment. The release of MTX from the microspheres was triphasic mainly dependent on co-polymer ratio and molecular weight. The antitumor efficacy as revealed by the VDT was enhanced compared to plain MTX. Thus using PLGA it is possible to design a biodegradable controlled release system for MTX which after single dose can maintain the drug at the desired concentration in plasma and may achieve an ideal mode for MTX delivery.

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