DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 14(10), 1351-1357 (1988)

CONTROLLED RELEASE OF CYCLOSPORINE FROM MICROSPHERES

Martin J. D'Souza, Ph.D.
Department of Pharmaceutical Sciences
School of Pharmacy, Mercer University
345 Boulevard, N.E., Atlanta, Georgia 30312

ABSTRACT

A controlled release microsphere formulation of cyclosporine is reported for the first time. Ethylene-vinyl acetate copolymer was utilized to prepare microspheres containing cyclosporine. The polymer was dissolved in methylene chloride (10% w/v) to which different quantities of cyclosporine was added to give drug loadings of 5, 25 and 50 % in the microspheres. The polymer-drug solution was extruded into ethanol, where gelling of the polymer was achieved at -78°C in an ethanol-liquid nitrogen bath. Microspheres containing 25% of cyclosporine retained their shape and gave optimum results. Lower drug loadings (5 % cyclosporine) resulted in deformed microspheres, whereas high drug loadings (50 % cyclosporine) produced tear drop shaped microspheres. This method used to prepare microspheres is simple, quick and inexpensive and can be used for drugs that are unstable to heat. Furthermore, the polymer used is bio-compatible and can be used to design formulations such as films containing cyclosporine which can then be used as subcutaneous implants.

INTRODUCTION

There are several methods used to formulate controlled release products of which the retardation of drug release by the use of substances that serve as barriers to dissolution is the most widely used method for tablets, granules and matrix systems (1-3). However, the matrix system seems to be the most common approach to achieve sustained release. Several polymers such as waxes, acrylic resins, cellulose derivatives, high molecular alcohols, glycols and hydrogels have been reviewed as possible controlled release retardant matrices (4-6).

Cyclosporine, an immunosuppressive drug, is used chronically post organ transplant to prevent organ rejection. While effective dosages can be given by injections, the need for long term administration makes the intravenous route rather impractical. At the present time it is marketed in an oily solution (olive oil based - Sandimmune ^{R} - Sandoz Inc., NJ.) which must be administered once or twice a day, after mixing it with milk or orange juice just before administration. The bioavailability of cyclosporine in the marketed form (Sandimmune) is extremely poor and variable and ranges between 15-25% (7). The aim of the present experiment was to prepare controlled release ethylene-vinyl acetate copolymer microspheres containing cyclosporine.

METHODS AND MATERIALS

Preparation of Microspheres:

Ethylene-vinyl acetate copolymer [Elvax 40, Dupont Chemical Co., DE] was dissolved in methylene chloride to give a 10% (w/v) solution. Cyclosporine was added to the polymer solution in a 20 ml glass vial and the mixture was vortexed to solubilize cyclosporine in the polymer solution. The mixture was drawn into a 3 ml glass syringe and extruded quickly via a 26 gauge needle (Becton, Dickinson & Co., NJ) drop by drop in a liquid nitrogen-ethanol bath (-78^{o}C). The mixture gelled virtually immediately on contact with the cold ethanol in near spherical

shape, and the hard, gelled spheres sank to the bottom of the beaker. Microspheres were prepared with loadings of 5, 25 and 50% cyclosporine by weight. After 20 minutes, the beaker containing the microspheres was removed from the dry ice bath and allowed to warm to room temperature. The microspheres turned white as the methylene chloride was extracted into the ethanol. After about half an hour, the ethanol was replaced with about 20 ml of fresh ethanol and set aside for 2 hours. The ethanol was then decanted and the microspheres were dried overnight in a vacuum desiccator.

Determination of Cyclosporine Content in Microspheres:

An accurately weighed 50 mg quantity of microsphere sample was transfered to a 50 ml volumetric flask, to which 25 ml of methylene chloride was added to solubilize the polymer. The volume was then adjusted to 50 ml with ethanol. The cyclosporine conent in the mixture was determined in a 100-150 microliter aliquot by an high performance liquid chromatographic procedure (8).

Release Kinetics:

A 100 mg aliquot of microspheres was placed in a vial containing 20 ml of saline (0.9% NaCl) at room temperature. The liquid was sampled periodically and the concentration of cyclosporine was determined spectrophotometrically [Perkin Elmer] at 215 nm. Control batches of pure ethylene-vinyl acetate copolymer microspheres showed no material exhibiting spectrophotometric absorbance in the saline solution. Drug release was monitored for a period of 144 hours.

RESULTS AND DISCUSSION

This study reports for the first time the preparation of a controlled release formulation of cyclosporine. The % ($\pm$ SD) recovery of cyclosporine from the microspheres were 82 ($\pm$ 2.2), 79 ($\pm$ 3.1) and 76 ($\pm$ 4.4) percent from the 5, 25 and 50% cyclosporine loaded microspheres respectively. The higher loss

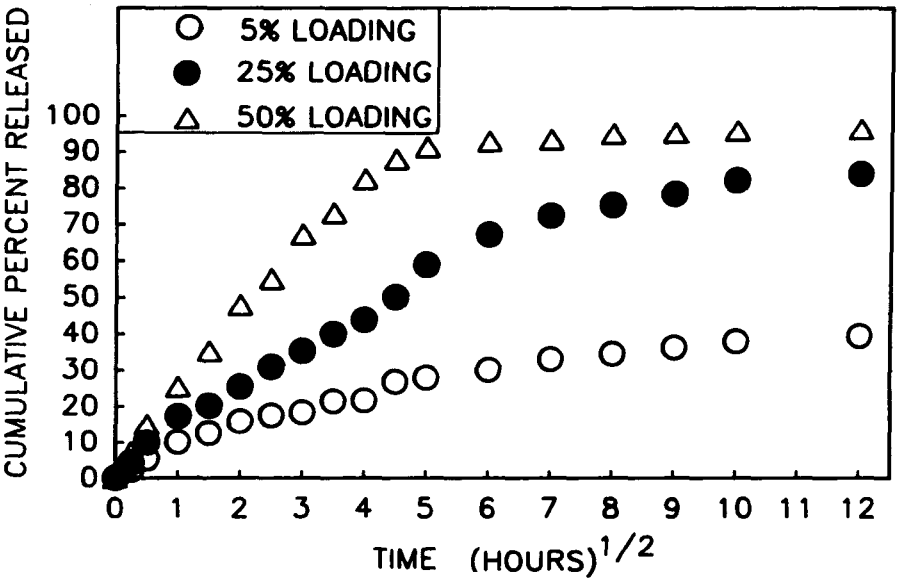

FIGURE 1.

Cumulative percent release of cyclosporine vs square root of time from microspheres prepared with different drug loadings. Values are the mean of four measurements.

O,-- 5 % cyclosporine; ●,-- 25 % cyclosporine; Δ,-- 50 % cyclosporine

of cyclosporine from the microspheres with higher drug loading during manufacture could be due to greater porosity of the polymer matrix at higher drug loading. The release kinetics of 5, 25 and 50% cyclosporine loaded microspheres are shown in Figure 1 as a plot of cumulative release rate vs square root of time. Almost 96.3 percent of cyclosporine was released from the 50% drug loaded microspheres within 144 hours, whereas about 83.9% of the drug was released during the same time from the 25% cyclosporine loaded microspheres. The drug release from the 5% cyclosporine loaded microspheres was the slowest and only 39.4% was released in 144 hours. The higher rate of drug release from microspheres with higher drug loadings is probably due to greater porosity of the polymer matrix at higher drug loadings.

TABLE 1. Microsphere Diameter

	Diameter, mm		
	5% Drug Loading	10% Drug Loading	50% Drug Loading
	0.60	0.71	0.78
	0.52	0.72	0.81
	0.55	0.65	0.79
	0.55	0.68	0.75
Mean(±SD)	0.56(0.03)	0.69(0.03)	0.78(0.03)

The diameters of microspheres prepared with different drug loadings are listed in Table 1. The microspheres prepared from 10% ethylene-vinyl acetate copolymer in methylene chloride were almost perfectly spherical, as compared to microspheres prepared from 5% ethylene-vinyl acetate copolymer. The cyclosporine content of the microspheres was a very critical factor in obtaining well characterized spherical microspheres. Microspheres with lower than 25% cyclosporine were easily deformed whereas microspheres with 25% cyclosporine were almost spherical. Microspheres with 50% cyclosporine were generally oblong or tear drop shaped.

The release rate of macromolecules such as proteins and high molecular weight drugs from polymer matrices can be varied by as much as 2000-fold by manipulating fabrication parameters such as drug loadings, aggregate sizes and polymer properties (9). The mechanism of release of macromolecules from polymeric matrices occurs via diffusion through interconnecting pores (10). A technique similar to the one used in this study has also been effectively used for albumin which unlike cyclosporine is insoluble in the polymer-methylene chloride mixture (11). These studies demonstrate that polymer matrices such as ethylene-vinyl acetate copolymer can be used as constant release polymer matrices for high molecular weight compounds such as

cyclosporine. The fabrication procedures for these systems are relatively simple and can be performed without expensive apparatus. The ethylene-vinyl acetate copolymer system used in this study has been shown to be highly bio-compatible (12). The low temperature manufacturing procedure is very useful for thermolabile drugs. Further work is being conducted towards the development of ethylene-vinyl acetate copolymer films containing cyclosporine, which can be used as subcutaneous implants. The ultimate goal is to formulate various formulations of cyclosporine with good bioavailability as well as sustained release properties so as to preclude the present necessity for daily oral administration of cyclosporine.

ACKNOWLEDGEMENTS

The authors are grateful to Sandoz Inc., East Hanover, NJ for the gift of cyclosporine, and to Dupont Chemical Co., DE. for the gift of the ethylene-vinyl acetate copolymer. Special thanks are also due to Mr. Yeshwant Sanzgiri for the assistance in plotting the figure and to Ms. Lynn Hampton for typing the manuscript.

REFERENCES

1. S.C. Khanna, and P. Speiser, J. Pharm. Sci., 59, 1398 (1970).
2. S. Dincer, and S. Ozdurmus, J. Pharm. Sci., 66, 1070 (1977).
3. R. Korsmeyer, R. Gurny, E. Doelker, P. Buri, and N. Peppas, J. Pharm. Sci., 72, 1189 (1983).
4. C.G. Cameron, and J.W. McGinity, Drug Dev. Ind. Pharm., 13, 1409 (1987).
5. V.H. Lee, and J.R. Robinson, In "Sustained and Controlled Release Drug Delivery Systems," J.R. Robinson, ed., Marcel Dekker, New York, NY, 1978, p. 156.
6. J.W. McGinity, C.G. Cameron, and G.W. Cuff, Drug Dev. Ind. Pharm., 9, 57 (1983).
7 C.T. Ueda, M. Lemaire, G. Gsell, and K. Nussbaumer, Biopharm. Drug Disp., 4, 113 (1983).

8. R. J. Sawchuck, and L. L. Cartier, Clin. Chem., 17, 1368 (1981).
9. W. Rhine, D.S.T. Hsieh, and R. Langer, J. Pharm. Sci., 69, 265 (1980).
10. R. Langer, W. Rhine, D.S.T. Hsieh, and R. Bawa, In "Controlled Release of Bioactive Materials," R. Baker, Ed., Academic, New York, N.Y., 1980, p. 83.
11. M.V. Sefton, L.R. Brown, and R.S. Langer, J. Pharm. Sci., 73, 1859 (1984).
12. R. langer, H. Brem, and D. Tapper. J. Biomed. Mat. Res., 15, 267 (1981).