DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 15(8), 1217-1230 (1989)

PROLONGED RELEASE OF PENTAZOCINE FROM MULTIPLE O/W/O EMULSIONS

B. Mishra* and J.K. Pandit**

Departments of Pharmacology* and Pharmaceutics**
Banaras Hindu University, Varanasi-221005, India

ABSTRACT

Multiple O/W/O emulsions containing pentazocine were prepared and tested in vitro and in vivo. The effect of two different concentrations of three additives, viz. sodium chloride, glucose and glycerol, location of the drug either in one or any two phases of the O/W/O emulsions and pH of the receptor fluid on in vitro release characteristics of the drug was studied. All the parameters influenced the drug release. Multiple O/W/O emulsions gave higher extent of drug release than the simple O/W emulsions. The results of in vivo studies in mice showed prolonged tissue levels of pentazocine from the multiple O/W/O emulsions in comparison to aqueous drug solution and simple O/W emulsion.

INTRODUCTION

Recently, the potential of using multiple emulsions for the controlled and prolonged release of drugs has been reported by various authors (1-8). Mimaki et al (9) observed an increase in survival time of mice bearing Ehrlich solid tumor after administration of W/O/W emulsion containing adriamycin.

Pentazocine is a widely used analgesic in man(10) and is usually administered through parenteral and oral routes. The conventional dosage form requires frequent administration to the patient and is inconvenient. It is expected that a prolonged release dosage form of pentazocine would reduce this problem to a great extent.

An O/W/O emulsion is likely to be the more effective dosage form since the extra partitioning step with the drug initially in the internal oil phase would be expected to affect the rate of drug release. The survey of literature shows that no substantial work has been done on drug release characteristics of O/W/O emulsions, particularly under the influence of g.i. pH, various concentrations of different additives included in the aqueous phase and the presence of drug in either one or any two phases of the O/W/O emulsions. Therefore, these parameters were undertaken in our study. No reports on in vivo studies of O/W/O emulsions prompted us to investigate the in vivo tissue levels of pentazocine in mice after oral administration of O/W/O emulsions in comparison to aqueous drug solution and simple emulsion.

MATERIALS

Pentazocine, sodium chloride, glucose, glycerol, Tween 40, Span 80, liquid paraffin, sodium carbonate, sodium bicarbonate and all other chemicals were obtained commercially and were of either pharmacopoeal or analytical reagent grade. Benzene was washed with 0.1 volume of 1N hydrochloric acid solution, 1N sodium hydroxide solution and twice with distilled water.

METHODS

Preparations: 50 ml of each formulation was prepared at a time and each contained a total of 40 mg and 100 mg of drug for in vitro and in vivo studies, respectively. Suffix 'd' used in notation of the emulsions indicates the

presence of drug in that particular phase of an emulsion. All the formulations were prepared freshly before their evaluation.

For the preparation of an aqueous drug solution, the drug was dissolved in a minimum volume of 0.1N hydrochloric acid solution, pH was adjusted to 5.0 with 0.1N sodium hydroxide solution and diluted with water to 50 ml.

The simple O_d/W emulsion was prepared by first making a suspension of the drug in 20 ml of liquid paraffin by stirring at 4000 r.p.m., the 50 ml of O/W emulsion was then made by dispersing this suspension in 30 ml of distilled water containing 2% v/v Tween 40 by stirring at 4000 r.p.m. with a stirrer.

The multiple O/W/O emulsions were prepared by an earlier reported (2) two-step emulsification procedure with modification. In the first step, the drug was suspended in 4 ml of liquid paraffin by stirring and this suspension was emulsified with 6 ml of distilled water containing 2% v/v Tween 40 by stirring at 4000 r.p.m. for 5 minutes. This gives the primary O/W emulsion. In the second step, final 50 ml of $O_d/W/O$ emulsion was made by emulsifying 10 ml of the primary emulsion with 40 ml of liquid paraffin containing 1% v/v Span 80 by stirring steadily at 200 r.p.m. for 5 minutes.

For the preparation of O/W/O emulsions containing equal half of the total drug either in internal oily and intermediate aqueous phase ($O_d/W_d/O$) or in internal and external oily phases ($O_d/W/O_d$), the drug was first added to corresponding phases and then the two-step emulsification was commenced. The drug level in each phase of the $O_d/W_d/O$ and $O_d/W/O_d$ emulsions was thus 20 mg for in vitro and 50 mg for in vivo studies.

For the preparation of all the simple and multiple emulsions containing additive at 0.6% and 3% concentra-

tions, either 300 mg or 1500mg of the additive was first dissolved in the aqueous phase and then the emulsification was commenced.

In Vitro Evaluation: The in vitro drug release studies were done upto 7.5 hours, in triplicate, in a well designed and standardized glass diffusion apparatus (11). It consists of a small donor compartment containing emulsion (10ml) and a larger stirred (100 r.p.m.) compartment as a sink containing 250 ml of the receptor fluid. The donor compartment was separated from the sink by a pretreated cellophane membrane (11) with a mean thickness of 0.025 mm. The whole apparatus was placed on a magnetic stirrer and maintained at $37 \pm 0.2^{\circ}$ C by circulating thermostated water. The receptor fluids used were buffer solutions of pH 1.4, 4.5, 5.8, 7.0 and 7.4 and were changed periodically in the sink as follows: During first hour- pH 1.4, second- pH 4.5, third- pH 5.8, fourth- pH 5.8, fifth- pH 7.0, sixth- pH 7.0, seventh- pH 7.4 and till 7.5 hours- pH 7.4. The emulsions, however, in the donor compartment remain undisturbed. An one ml aliquot sampled at the end of each run was diluted suitably with 0.1N hydrochloric acid solution and the absorbances were read at 278 nm in a U.V. Spectrophotometer (Carl Zeiss, Jena, DDR). The actual amount of released drug was computed from a calibration curve.

In Vivo Evaluation: Unused male albino mice, weighing between 35-40 gms, were used and maintained on pellet diet. 18 mice were used for the in vivo testing of each formulation. When tissue drug levels were high even upto 6 hours, the sampling was done till 8 hours and 21 mice were then used. Overnight fasted mice (with water ad libilum) were fed with 1 ml of the

formulation containing 2 mg of pentazocine. Three mice were used for the collection of tissue samples at each subsequent time interval of 0, 0.5, 1, 2, 4, 6 and 8 hours. Three unmedicated mice used at zero hour served as the control. The blood samples were collected from the jugular vein in heparinized tubes and stored frozen until assayed. Liver, lung and kidney samples from each mouse were also removed as quickly as possible, blotted with filter paper, quickly weighed on a precision balance and stored frozen until assayed.

The liver, lung and kidney were homogenized in a 25 ml ground glass tissue homogenizer and diluted with distilled water in the following ratios: blood 1:4, liver 1:5, lung and kidney 1:10.

The Spectrophotofluorometric method of El-Mazati and Way (12) was used with modification for the analysis of pentazocine in biological samples which, in short, is as follows: The diluted biological sample (1ml) was mixed with 100 mg of 1:1 mixture of sodium carbonate-sodium bicarbonate and shaken with 5 ml of benzene for 10 minutes. A 4 ml portion of the organic phase was separated by centrifugation at 5000 xg and further shaken with 3 ml of 0.2 N HCl solution, which had previously been saturated with benzene, for 10 minutes. After centrifuging, the aqueous layer was separated and its fluorescene was measured in an Aminco Bowman Spectrophotofluorometer at the excitation and emission wavelength of 278 and 310 nm, respectively. The level of pentazocine was read from a standard curve derived from known concentrations of pentazocine added to each blood sample or tissue homogenate from unmedicated mice.

The tissue and blood concentrations of pentazocine so obtained were corrected by deducting from it the value obtained in blank samples (at zero hour), which

were always less than 0.2 mcg/ml or per g. The lowest detectable limit of tissue and blood drug concentrations obtained by this method was 0.1 mcg per ml or per g. More than 90 percent recoveries were found of added pentazocine to the biologic media at a level of 1 mcg/ml or per g.

Tissue-to-Blood Partition Coefficient (Kp): Kp values were calculated (13) by using the following formula.

$$Kp = \frac{\text{AUC for Tissue}}{\text{AUC for Blood}}$$

Where, AUC is the area under concentration-time curve.

RESULTS AND DISCUSSION

All the emulsions without additive are expressed as control emulsions.

In Vitro Release of Pentazocine: The cumulative percent release of pentazocine from different emulsions are shown in table 1. The drug release from O/W/O emulsions are much higher than the simple O/W emulsions. The lower drug release from O/W emulsions confirms the finding of Brodin et al (2) and is attributed to one or more reasons: Pentazocine, a hydrophobic drug, was present in the oily phase and may not partition well with the immediate surrounding of aqueous phase. While in O/W/O emulsions the external oily phase exerts a drawing force on the drug present in the internal oily phase, the aqueous phase of the O/W emulsion could not impart any such drawing force on the drug. It is also possible that the higher volume percent of oil and water in O/W emulsion, as compared to the primary O/W emulsion of O/W/O emulsions, may exhibit a slower release because of the larger oil droplets in the simple O/W emulsion. However, further study may be required to explain the lower release from the simple emulsion.

TABLE 1

In Vitro Release of Pentazocine from Different Emulsions

Emulsion	Mean* Cumulative Percent Release							
	pH of the Receptor Fluid							
	1.4	4.5	5.8	5.8	7.0	7.0	7.4	7.4
	Time in Hours							
	1.0	2.0	3.0	4.0	5.0	6.0	7.0	7.5
O_d/W, WA	8.2	13.8	17.5	20.4	23.2	25.4	26.7	27.4
" 3.0%SC	6.7	13.0	18.2	22.9	25.8	27.5	28.4	28.4
" " Glu	8.2	15.7	22.8	28.3	33.1	36.8	39.3	40.6
" " Gly	7.9	14.9	20.5	25.2	29.3	33.0	34.7	35.6
" 0.6%SC	7.4	14.1	19.3	22.5	25.4	27.1	27.7	27.7
" " Glu	7.4	14.5	21.2	24.9	27.8	30.3	32.4	33.7
" " Gly	7.9	14.2	19.3	22.6	25.5	28.3	29.3	29.8
$O_d/W/O$, WA	23.1	27.9	30.7	32.9	34.6	35.9	36.8	37.3
" 3.0%SC	26.9	34.0	40.3	45.9	49.9	52.0	53.0	53.0
" " Glu	27.4	30.6	33.2	35.8	38.1	40.1	41.6	42.3
" " Gly	23.1	30.2	36.5	40.2	43.4	45.9	47.7	48.2
" 0.6%SC	26.2	30.9	35.3	37.5	39.6	41.3	42.3	42.3
" " Glu	25.8	30.6	32.3	34.0	35.8	37.1	38.4	39.0
" " Gly	23.1	27.9	31.1	33.6	36.1	37.8	39.2	39.7
$O_d/W_d/O$, WA	16.3	24.1	29.3	34.1	38.1	41.0	43.5	44.7
" 3.0%SC	16.3	27.2	36.2	42.9	46.9	49.4	50.7	51.3
" " Glu	18.2	30.7	40.0	47.1	53.4	56.7	59.2	60.4
" " Gly	17.4	29.9	41.6	51.0	60.3	65.5	69.2	71.3
" 0.6%SC	15.5	24.2	30.8	36.8	41.9	44.4	45.8	46.3
" " Glu	16.7	23.7	27.8	31.8	35.8	39.8	42.3	43.7
" " Gly	14.0	21.1	27.0	31.4	35.4	38.7	41.9	44.1
$O_d/W/O_d$, WA	29.3	35.2	38.1	40.9	43.4	45.2	46.9	47.7
" 3.0%SC	36.8	43.9	47.1	49.6	51.7	53.0	53.6	53.6
" " Glu	36.1	43.1	47.5	49.6	51.7	53.0	53.6	53.6
" " Gly	30.0	39.0	47.6	52.4	56.4	58.9	60.6	61.2
" 0.6%SC	30.6	33.9	36.2	38.5	40.8	43.1	44.5	45.6
" " Glu	31.2	35.9	38.0	39.3	40.8	42.0	43.3	43.9
" " Gly	28.5	34.0	37.8	40.6	43.4	45.6	46.9	47.5

* Mean of three observations.

WA - Without Additive, SC - Sodium Chloride, Glu - Glucose, Gly - Glycerol.

Initially at one hour O/W/O emulsions exhibited higher drug release which later slowed down with the increase in pH of the receptor fluid. The drug release from the $O_d/W/O_d$ emulsions was higher than the $O_d/W/O$ and $O_d/W_d/O$ emulsions. The maximum extent of drug release from the $O_d/W/O_d$ emulsions is due to rapid diffusion of drug from the external oil phase and the subsequent controlled slow release from the internal oil phase. The lower drug release from the $O_d/W/O$ than the $O_d/W/O_d$ emulsions is because of the presence of the drug only in the internal oil phase.

In case of $O_d/W_d/O$ emulsions drug release was initially lower than the $O_d/W/O$ emulsions but was higher in latter hours. The initial low drug release is probably due to less migration of the ionised drug from the aqueous phase to the external oil phase, while in latter hours, the drug from the internal oil phase migrated under the influence of a concentration gradient between the two oil phases and in combination with the small amount of drug release from the aqueous phase, resulted in higher drug release in comparison to $O_d/W/O$ emulsions.

Effect of Additives on In Vitro Release of Pentazocine: The effect of 3% and 0.6% w/v concentrations of sodium chloride, glucose and glycerol on drug release from simple and multiple emulsions are also shown in Table 1. All the additives (at both concentrations) produced an enhancing effect on drug release from the O_d/W emulsions because of their destabilizing action on the interfacial film of the hydrophilic emulsifier (Tween 40).

In presence of additives, at both concentrations, $O_d/W/O$ emulsions also gave higher drug release than the control emulsion, in the following order: sodium chloride > glycerol > glucose. The increase in drug release

is explained as : additives weaken and thus destabilize either one or both of the interfacial films of O/W/O emulsions, and this destabilizing effect is of greater extent at 3% than at 0.6% concentration of individual additive.

In $O_d/W_d/O$ emulsions, 3% glycerol produced maximum increase in drug release (71.3% in 7.5 hrs.) followed by 3% of both glucose (60.4%) and sodium chloride (51.3%) and 0.6% sodium chloride (46.3%), while 0.6% of both glucose and glycerol gave an extent of drug release almost similar to the control (44% in 7.5 hrs.) emulsion. These results indicate that when half of the total drug was present in the aqueous phase, 0.6% of both glucose and glycerol were not effective as destabilizer of the interfacial film and this might be the possible reason for drug release being similar to the control emulsion. The maximum destabilization of the one or both interfacial film is caused by 3% glycerol followed by glucose and sodium chloride.

In $O_d/W/O_d$ emulsions, only 3% of the additives enhanced the drug release in comparison to the control emulsion, and is explained as above.

In Vivo Results: Fig. 1 and Table 2 represent pentazocine lavels in blood and liver, lung, kidney, respectively, at different time intervals after oral administration of different formulations to mice. The peak concentrations of pentazocine from all the formulations were maximum in lung and/or kidney, followed by liver and blood. Drug solution showed the highest peak in all the tissues at half hour, but drug levels declined very rapidly. O_d/W emulsion also exhibited a faster decline in drug levels in all the tissues, although peak drug levels were lower than the solution but peak times were the same, i.e. half hour.

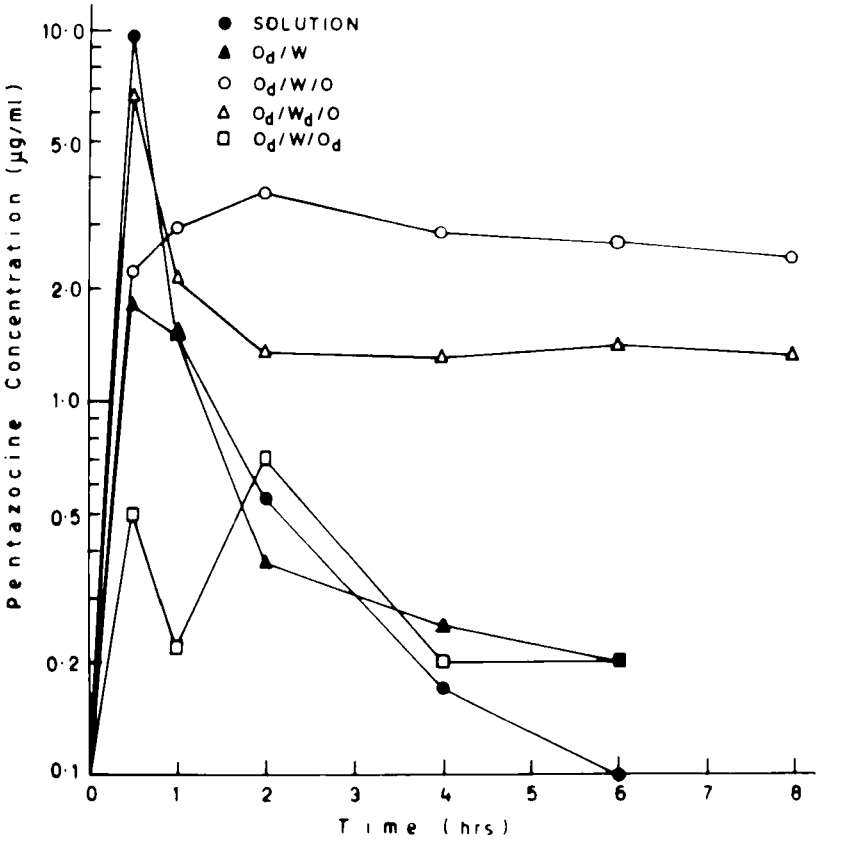

FIG 1 - PROFILES OF AVERAGE BLOOD CONCENTRATIONS OF PENTAZOCINE AFTER 2 mg/ml ORAL ADMINISTRATION OF FIVE DIFFERENT FORMULATIONS TO MICE (n = 3)

Multiple $O_d/W/O_d$ emulsion exhibited two peaks, one at half hour and the other at two hours, in all tissues. Inspite of low drug levels in blood, the levels of pentazocine in other three tissues were appreciably high probably due to rapid drug uptake by tissues (12). Since the total amount of drug was divided into two equel half in the internal and external oil phases, it seems probable that the first peak at half hour is due to drug released from the external oil phase only. Later on, the dr-

TABLE 2

Average Tissue Levels (mcg/g) of Pentazocine after Oral Administration of Different Formulations to Mice (n=3)

Formulation	Tissue	Time in Hours 0.5	1.0	2.0	4.0	6.0	8.0
Solution	Li	37.23	8.65	3.10	1.10	0.10	-
	Lu	56.45	22.05	6.18	1.40	1.00	-
	Ki	62.57	23.96	5.78	1.58	1.00	-
O_d/W	Li	12.00	11.47	4.35	1.80	0.65	-
	Lu	13.98	10.60	4.40	1.07	0.86	-
	Ki	10.47	9.87	2.60	1.03	0.65	-
$O_d/W/O$	Li	7.18	1.92	1.18	1.10	1.15	0.85
	Lu	13.02	4.38	4.68	5.00	5.10	2.60
	Ki	13.22	4.70	3.20	3.35	3.20	1.15
$O_d/W_d/O$	Li	28.57	8.17	1.50	1.05	0.55	0.35
	Lu	31.85	15.60	5.83	2.30	1.35	1.25
	Ki	29.17	14.00	3.05	1.85	1.85	1.80
$O_d/W/O_d$	Li	6.80	1.43	2.97	0.70	0.20	-
	Lu	20.55	7.95	22.25	3.43	2.60	-
	Ki	20.05	7.70	21.55	1.73	0.68	-
$O_d/W/O$ 3.0%SC	Li	50.32	4.92	3.72	0.86	0.84	-
	Lu	13.22	10.23	8.53	5.75	5.75	-
	Ki	22.28	8.75	5.67	2.20	2.15	-
$O_d/W/O$ 3.0%Glu	Li	32.25	4.02	3.33	0.77	0.68	-
	Lu	11.00	7.80	6.68	5.30	4.93	-
	Ki	10.65	3.78	2.98	1.85	1.85	-
$O_d/W/O$ 3.0%Gly	Li	11.53	5.48	1.05	1.05	1.00	-
	Lu	12.38	10.70	5.60	4.20	3.10	-
	Ki	18.37	13.78	3.03	2.78	2.20	-
$O_d/W_d/O$ 3.0%Gly	Li	6.65	3.22	2.02	1.60	1.60	-
	Lu	14.38	13.60	9.58	6.23	5.20	-
	Ki	13.10	10.22	9.08	6.52	2.50	-
$O_d/W/O_d$ 3.0%Gly	Li	24.45	20.95	2.82	0.95	0.60	-
	Lu	17.18	10.78	3.87	1.43	1.40	-
	Ki	8.80	7.47	3.25	3.05	3.05	-

- Not Determined.

Li - Liver, Lu - Lung, Ki - Kidney.

SC - Sodium Chloride, Glu - Glucose, Gly - Glycerol.

ug from the internal oil phase also becomes available for absorption and in combination with the small amount of drug released earlier from the external phase, it gives a second peak at 2nd hour which is slightly higher than the first peak. Thereafter the drug level continues to decline due to reduced concentration of the drug in the internal oil phase.

Multiple $O_d/W_d/O$ emulsion exhibited prolonged blood levels of pentazocine till 8 hours. In the other three tissues, though the drug levels were not as constant as in the blood, but were constantly maintained in kidney between 4 and 8 hours.

Similarly, $O_d/W/O$ emulsion also exhibited a good prolongation in tissue levels of pentazocine and drug levels were constantly maintained between 2 to 6 and/or 8 hours in the different tissues.

The blood and tissue levels of pentazocine after oral administration of various O/W/O emulsions containing 3% w/v additives to mice are also shown in Table 2 and in Fig.2. The $O_d/W_d/O$ emulsion containing 3% glycerol showed appreciably high and prolonged levels of pentazocine in all the tissues. Unlike the control $O_d/W/O_d$ emulsion, the same emulsion containing 3% glycerol does not produce two peaks. For the control $O_d/W/O_d$ emulsion, the Kp values were: 5.11 for liver, 27.22 for lung and 25.37 for kidney, whereas for the same emulsion containing 3% glycerol, Kp values were: 4.04 for liver, 3.09 for lung and 2.86 for kidney. These values indicate that there is very high accumulation of drug in the tissues, particularly in lung and kidney, in the case of control emulsion, while the drug accumulation was negligible in the tissues when glycerol was present in the $O_d/W/O_d$ emulsion. This clearly shows that glycerol has modified the interfacial properties and controlled the drug release from the $O_d/W/O_d$ emulsion.

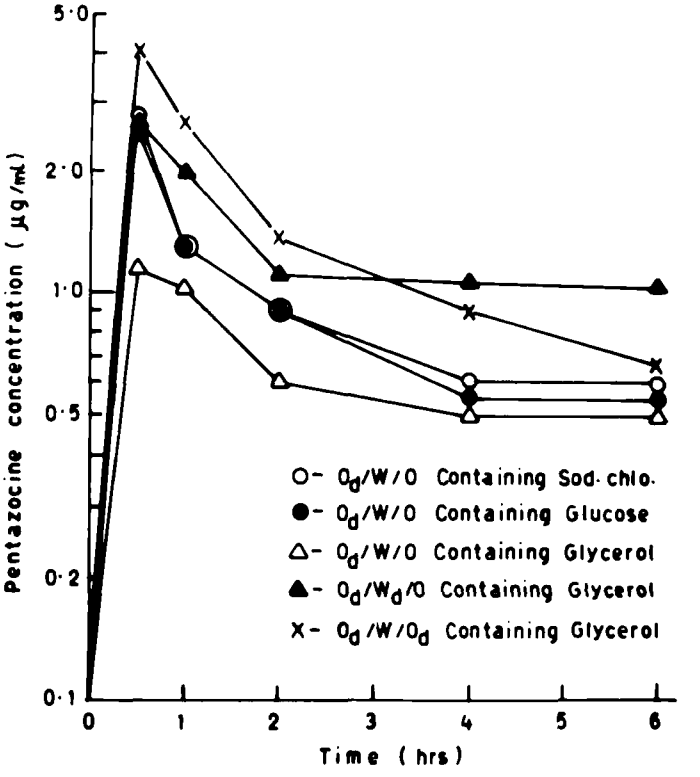

FIG.2 - PROFILES OF AVERAGE BLOOD CONCENTRATIONS OF PENTAZOCINE AFTER 2 mg/ml ORAL ADMINISTRATION OF FIVE DIFFERENT O/W/O EMULSIONS TO MICE (n=3)

The O_d/W/O emulsions containing 3% of either sodium chloride, glucose or glycerol gave almost similar (with minor exceptions) pattern of drug level profiles in blood with prolonged nature in comparison to solution and O_d/W emulsion. Drug levels in other tissues also showed similar nature of prolongation. The additives might have interacted with one or both of the interfacial coponents (components of the liquid crystal phase) and made them to act as control release barriers. However in the absence of additives also, the interfacial barr-

iers act as good controlled release membrane which resulted in controlled and prolonged pentazocine levels in the blood (c.f. Fig.1).

CONCLUSIONS

On the basis of evidence presented in this report, it can be concluded that multiple O/W/O emulsions can be utilized as a potential prolonged release dosage form. It is also recognized that further in vivo studies are needed in human volunteers to correlate our findings to human usage for this type of dosage form.

ACKNOWLEDGEMENTS

We are thankful to Ranbaxy Laboratories, New Delhi, India, for a generous gift sample of pentazocine.

REFERENCES

1. C.J. Benoy, L.A. Elson and R. Schneider, Proc. Brit. Pharmacol. Soc., 45, 135P (1972).
2. A.F. Brodin, D.R. Kavaliunas and S.G. Frank, Acta Pharm. Suec., 15, 1 (1978).
3. S. Fukushima, K. Juni and M. Nakano, Chem. Pharm. Bull., 31, 4048 (1983).
4. T. Yoshioka, K. Ikeuchi, M. Hashida, S. Muranishi and H. Sezaki, Chem. Pharm. Bull., 30, 1408 (1982).
5. J.K. Pandit, B. Mishra and B. Chand, Ind. J. Pharm. Sci., 49, 103 (1987).
6. T. Takahashi, M. Mizuno, Y. Fujita, S. Ueda, B. Nishioka and S. Mazima, Gann., 64, 345 (1973).
7. L.A. Elson, B.C. Mitchley, A.J. Collings and R. Schneider, Eur. J. Clin. Biol. Res., 15, 87 (1970).
8. J. Versteeg, U.S. Patent, 4, 083, 798 (1978).
9. Y. Mimaki, M. Shinozawa, K. Yasuda, K. Yao, Y. Etho, T. Fukuda, and Y.Yasunori, Gan to Kagakuryoho, 9, 467 (1982).
10. A.S. Keats and J. Telford, J. Pharmacol. Exp. Ther., 143, 157 (1964).
11. J.K. Pandit, B. Mishra, D.N. Mishra and P.K. Choudhary, Indian Drugs,(1988) (in press).
12. A.M. El-Mazati and E.L. Way, J. Pharmacol. Exp. Ther., 177, 332 (1971).
13. H.S.G. Chen, and J.F. Gross, J. Pharmacokin. Biopharm., 7, 117 (1979).