

Multiple Emulsions of Flurbiprofen – Formulation Development and Evaluation

¹B.Mishra*, D.Thennarasu and C.Sankar

¹Department of Pharmaceutics, IT, BHU, Varanasi - 221005, INDIA

Abstract

Multiple w/o/w and o/w/o emulsions of flurbiprofen were prepared and studied to see the effect of certain parameters like; gastro-intestinal pH of diffusion media, presence of polyvinylpyrrolidone in either internal or external phase, type of oil phase and presence of drug either internal or in both (external & internal) phases, on *in vitro* drug release. *In vivo* anti-inflammatory studies were also conducted in adult albino rats by measuring the decrease in carrageenin induced pedal oedema, through Plethismography. The release of Flurbiprofen was observed to be emulsion-type dependent. The various pH of the diffusion media reflected different release characteristics of drug from multiple emulsions. Incorporation of additive like polyvinylpyrrolidone, presence of drug either in internal phase or in both of the internal and external phases and the type of oil used in the preparation of multiple w/o/w and o/w/o emulsions significantly affected the drug release. Multiple w/o/w emulsion could provide maximum and controlled inhibition of pedal oedema in rats for prolonged periods in comparison to drug solution.

Keywords: Flurbiprofen, multiple emulsions, additives, controlled drug release.

* Corresponding author: e-mail: bmishra@bhu.ac.in, mrcsankar@yahoo.com.

Introduction

Controlled drug delivery systems are important means of achieving sustained, relatively constant effective drug levels, coupled with predictable and reproducible drug release kinetics (Welling., 1983; Mallinowski., 1983). Among the newer emerging systems, multiple emulsion systems have the potential for unlimited use, ranging from controlled and prolonged delivery of drugs (Brodin *et al.*, 1978; Benoy *et al.*, 1972; Mishra and Pandit., 1990a), cosmetic (Fukada., 1978) and food applications (Takada *et al.*, 1985) and protection of labile drugs (Schichri *et al.*, 1974). Their spherical vesicular structure and the selective permeability of the interfacial barriers make them useful in controlled and prolonged delivery of drugs. The greatest advantage with multiple emulsions is the ability to modify the rate and the extent of drug release using various methodologies. We had earlier made a series of investigations (Mishra and Pandit., 1990a;1990b Tandon and Mishra., 1999a; 1999b; Mishra *et al.*, 1999a, 1999b) to study the prolonged and controlled release potentials of multiple emulsions using different drugs. Flurbiprofen (Fb) is a NSAID used in painful and inflammatory rheumatic and certain non-rheumatic conditions. This drug has relatively shorter half-life (~3.5 hours) and requires thrice administrations to patients, if given in conventional formulations, which is inconvenient (Adam., 1977). Based on the above facts, w/o/w and o/w/o types of multiple emulsions of Fb were prepared using different formulation variables with an objective of achieving controlled and prolonged release formulations of Fb. All the prepared emulsions were evaluated for *in vitro* drug release characteristics and anti-inflammatory activities.

Materials and Methods

Materials

Flurbiprofen(Fb) was a gift sample from Knoll Pharmaceuticals Pvt Ltd, India. All other materials of analytical grade were used as received.

Preparation of Multiple Emulsions:

All the multiple emulsions were prepared by following well-established two-step emulsification technique. Multiple w/o/w emulsion was prepared by emulsifying 8 ml of drug solution (prepared by dissolving in phosphate buffer pH 7.4) with 12 ml of liquid paraffin containing 5 % v/v Span 80 at 4000 rpm for five minutes. The resultant 20 ml w/o emulsion was further emulsified in the second step with 30 ml distilled water (DW) containing 1 % v/v Tween 40 at 2000 rpm for three minutes. This gave 50 ml of control w/o/w emulsion (**MW**) containing 200 mg of drug in innermost aqueous phase. This emulsion is referred as control emulsion. Following above method, other two w/o/w emulsions were prepared using soya bean oil (**MW_s**) and arachis oil (**MW_a**) replacing liquid paraffin. Some more multiple emulsions were also prepared similarly as control except that 2% w/v PVP (in reference to total volume of **MW**) was added either in innermost aqueous phase (**MWP_i**) or in outer most aqueous phase (**MWP_e**), prior to first and second step emulsification. To study the effect of drug location, another w/o/w emulsion (**MWD_{ie}**) containing 100 mg drug in each innermost and outermost aqueous phase was prepared by incorporating 100 mg drug in aqueous phase of the first step and another 100 mg in aqueous phase of the second step emulsification. Rest of the emulsification method was same as for control w/o/w (**MW**) emulsion.

Multiple o/w/o emulsion (**MO**) was prepared by emulsifying 8 ml of drug dispersed in liquid paraffin containing 2 % v/v Span 80 with 12 ml of DW containing 5 % v/v Tween 40 at 4000 rpm for five minutes. The resultant 20 ml o/w emulsion was further emulsified with 30 ml liquid paraffin containing 2 % v/v Span 80 at 2000 rpm for three minutes. This gave 50 ml of o/w/o emulsion (**MO**) containing 200 mg of drug in innermost oil phase.

For comparative evaluation, simple w/o emulsion (**SW**) containing drug in aqueous phase and simple o/w emulsion (**SO**) containing drug in oily phase were also prepared by following first step emulsification technique of control **MW** and **MO** emulsions, respectively.

The formation of emulsions was confirmed by microscopic observations and through the microphotographs taken with the help of a polarised-light microscope.

***In vitro* drug release studies**

All the above emulsions were evaluated *in vitro* in triplicate, till seven hours using a diffusion cell developed in our laboratory (Mishra and Pandit., 1990a). Ten ml of freshly prepared emulsion was placed in the donor compartment, which separates the receptor compartment with a pretreated diffusion membrane (Sigma, USA) (thickness 0.025 mm). The receptor compartment was placed on a magnetic stirrer with an energy controlled hot plate which maintained the temperature of the diffusion media at $37 \pm 0.1^\circ\text{C}$. A teflon-coated iron rod (3.3 x 0.4 cm) placed at the bottom of the receptor compartment was rotated at 100 rpm to equilibrate the diffused drug in the diffusion medium. Pre-warmed ($37 \pm 0.1^\circ\text{C}$) buffer (250 ml) solutions of increasing pH i.e., pH 1.9, 4.5, 6.0, 7.0 and 7.4 were used as diffusion media and were changed periodically as follows: during the first hour, pH 1.9; second, pH 4.5; third, pH 6.0; fourth, pH 6.0; fifth, pH 7.0; sixth, pH 7.0 and seventh, pH 7.4, in the receptor compartment (Mishra and Pandit., 1990a). Samples were collected at each hour and were analysed spectrophotometrically at 247 nm. Released drug content was calculated from a calibration curve of Fb.

***In vivo* anti-inflammatory studies**

In vivo anti-inflammatory studies were conducted by following slightly modified procedure (Winter *et al.*, 1962) in adult male albino rats weighing between 200 - 250 mg. The animals were obtained from animal house of Institute of Medical Sciences, BHU, Varanasi and were housed in colony cages under identical conditions in the departmental animal room at an ambient temperature of $25 \pm 0.1^\circ\text{C}$. They were fed on with the standard Hind lever diet and were acclimatized for a week prior to experiment. For *in vivo* evaluation, two groups containing five rats (each overnight fasted with water *ad libitum*), were used. One group received formulation containing drug and the other group received the same formulation without drug (control group). However, the food was given to them four hours after the administration of the drug formulation. A homogenous suspension of 1 % w/v carrageenin powder in 0.9 % w/v sodium chloride solution was prepared. Before 30 min of formulations

administration, experimental inflammation was induced by injection of a volume of 0.1ml of carrageenin into plantar surface of the rats left hind paw below the plantar aponeurosis. The percentage inhibition of oedema was measured till 24 hours-after oral intubation of drug solution, MW, MWP₁ and MO emulsions. The volume of the hind paw of the rats upto ankle joint was measured plethysmographically, by the mercury displacement method, before and at one hour interval upto 6 hours, two hours interval upto 12 hours and finally at 24 hours after the injection of carrageenin. The difference in the paw volume before and after carrageenin administration was taken as a measure of the pedal oedema.

Results and Discussion

In vitro release profiles of Fb from various multiple emulsions are shown in Figs. (1- 4). Drug release from simple w/o (SW) and o/w (SO) emulsions were highly affected by the pH of the diffusion media (Fig.1). They gave lesser quantum of drug release initially upto 2 hours, when pH of the diffusion media were 1.9 and 4.5, followed by a rapid release of drug in later hours when pH of the diffusion media were higher towards alkaline. This is attributed to acidic nature of the drug Fb and its least solubility in acidic diffusion media in first two hours, however, more solubility of Fb in diffusion media of higher pH in later hours causes higher drug release.

Multiple w/o/w (MW) and o/w/o (MO) emulsions exhibited (Fig.1) higher and controlled release than simple emulsions is attributed to diffusion of drug from the internal dispersed phase to the external continuous phase under the influence of concentration gradient across the intermediate dispersed drop. Also, in case of w/o/w (MW) emulsion the external aqueous phase provides an attractive force to the solubilized drug molecules, present in the internal aqueous phase and results in higher drug release. Similarly, in case of o/w/o (MO) emulsion, the drug is in the form of fine suspension in the internal dispersed oil phase and the influence of pulling force exerted by the external oil phase resulted in higher drug release. Such an attractive force is absent in simple SW and SO emulsions. Slightly lower drug release from o/w/o (MO) emulsion in comparison to w/o/w emulsion is attributed to higher viscosity of external oil phase and also due to dissolution dependent release of drug present as suspension in the internal oil phase of o/w/o (MO) emulsion (Fig.1).

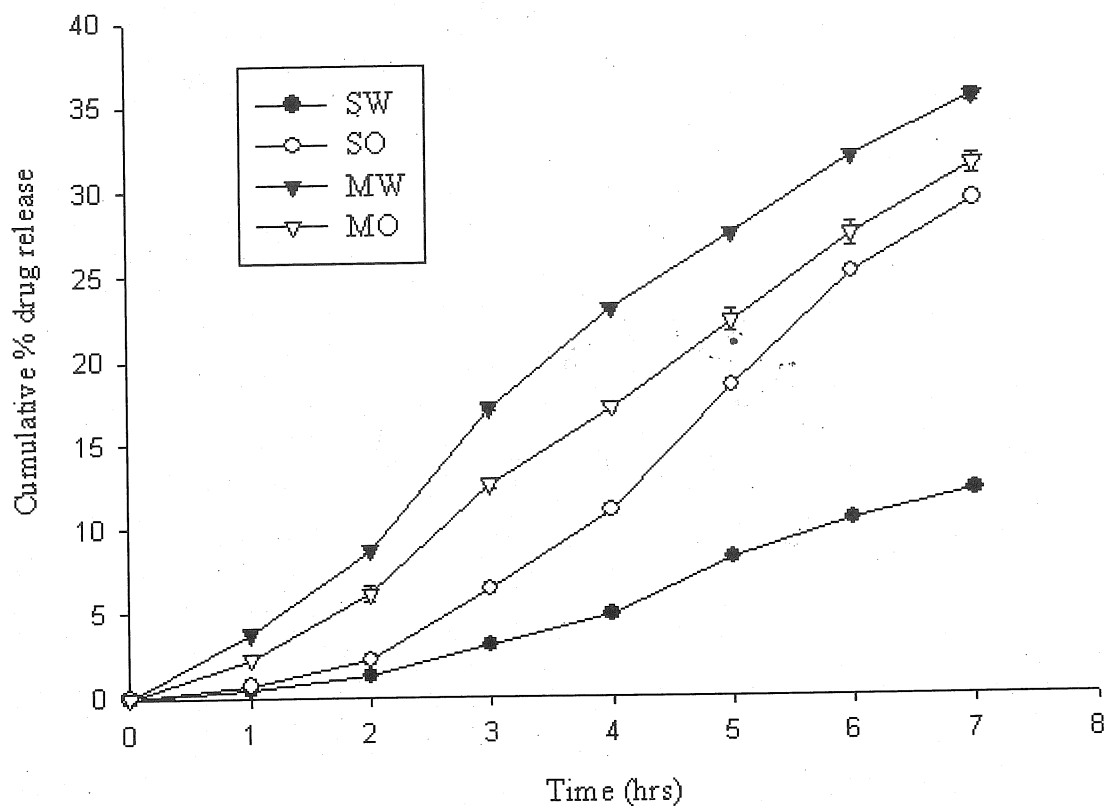


Fig. 1. *In vitro* release profiles showing effect of different types of emulsions on drug release. (Mean \pm S.D) (n = 3).

The effect of type of oil phases on *in vitro* release of Fb from the w/o/w emulsions are shown in Fig.2. It has been observed that w/o/w emulsion formulated with liquid paraffin (MW) gave significantly ($p < 0.05$) higher rate and extent of drug release than the w/o/w emulsions formulated with soya (MW_s) and arachis oil (MW_a). However, emulsions MW_s and MW_a gave almost same rate and extent of drug release at almost each pH of the diffusion media, probably because both are of vegetable origin and have no appreciable difference in their viscosity.

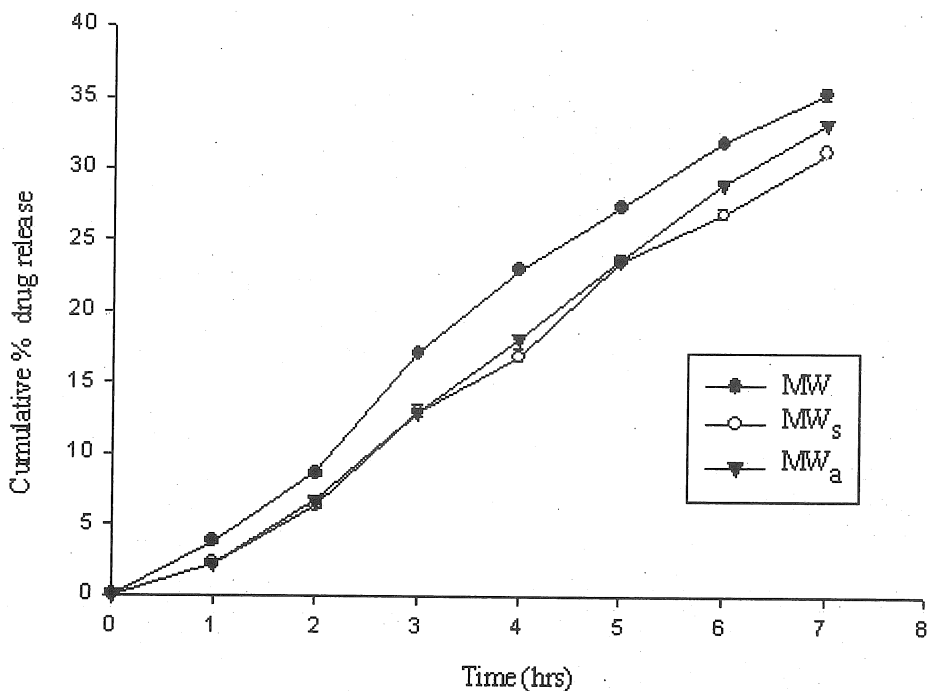


Fig. 2. *In vitro* release profiles showing effect of different types of oils on drug release. (Mean \pm S.D) (n = 3).

The w/o/w emulsion containing drug in internal phase (MW) and 2 % w/v PVP in either internal (MWP_i) or external (MWP_e) aqueous phases were studied to see the influence of presence of additive (PVP) on drug release characteristics from w/o/w emulsion (Fig.3). The emulsion without any additive (MW) was treated here as control. The PVP based emulsions exhibited much slower rate and extent of drug release during initial hours followed by rapid rate of drug release, in comparison to controlled drug release provided by MW control emulsion. It is attributed that PVP might have been involved in such interaction with interfacial components that it caused strengthening of the interfacial film causing less drug release during initial hours, but alkaline favourable pH of diffusion media for the drug in later hours might have resulted into more diffusion and thus faster drug release.

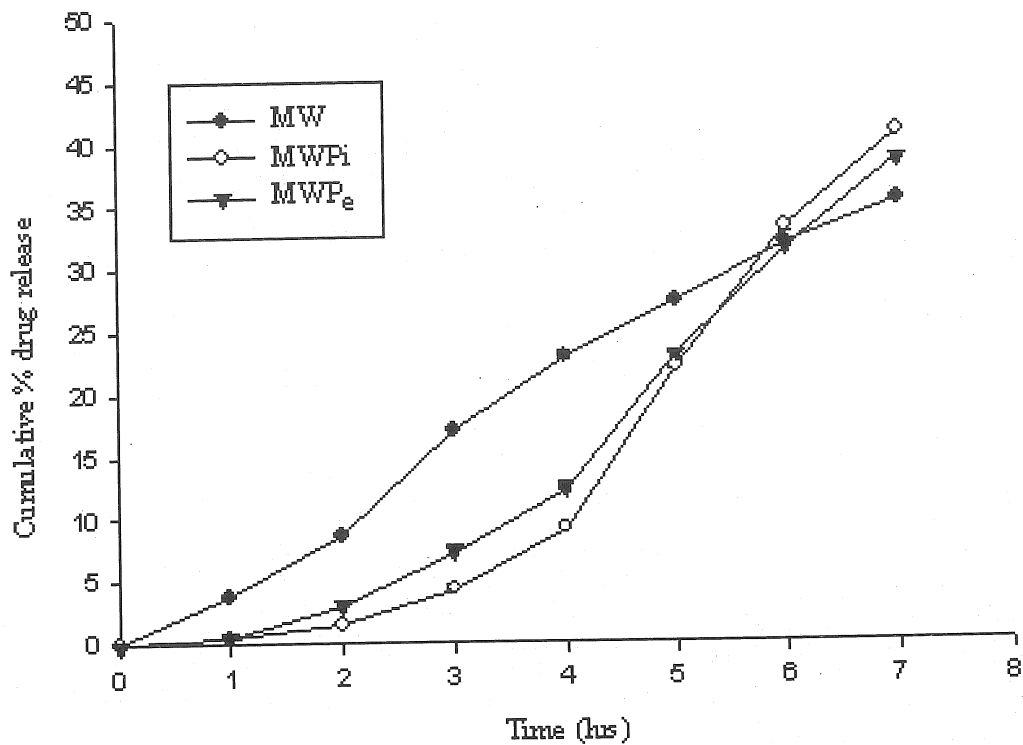


Fig. 3. *In vitro* release profiles showing effect of 2 % w/v PVP present in internal or external aqueous phase on drug release from w/o/w emulsions. (Mean \pm S.D) (n = 3).

The results of *in vitro* release of Fb observed from the multiple w/o/w emulsion containing whole amount (200 mg) of drug in internal aqueous phase only (emulsion MW) and equal amount of drug (100 mg) in both internal and external aqueous phase (emulsion MW_{ie}) are shown in Fig.4.

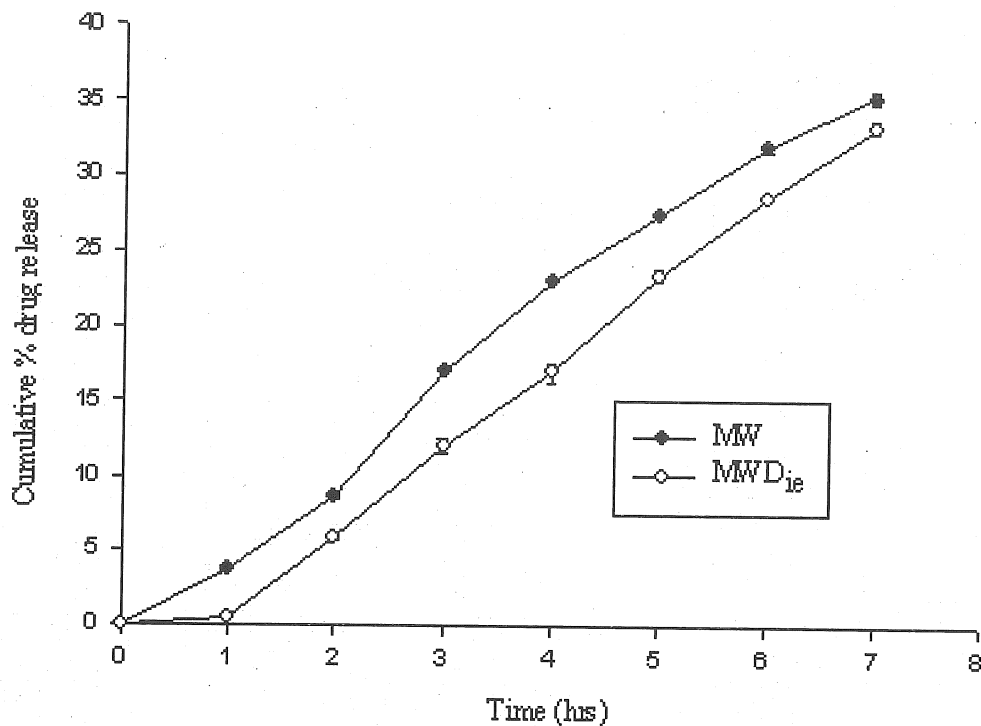


Fig. 4. *In vitro* release profiles showing effect of location of drug from w/o/w emulsions. (Mean \pm S.D) (n = 3).

In comparison to emulsion MW, emulsion MWD_{ie} gave significantly lower rate and extent of drug release but exhibited more linear profile after one hour. Lower drug release from MWD_{ie} is attributed to less concentration gradient driving force across oil layer in case of MWD_{ie} than MW emulsion.

Formulations tested for their anti-inflammatory activity in rats at a dose level of 100 mg/kg (in each case) are shown in Fig.5. The multiple emulsions MW, MO and MWP_i decreased (inhibited) the rats paw oedema for much longer duration in comparison to decrease in oedema produced for less than 8 hours by aqueous drug solution (S). The multiple emulsions

MW, MO and MWP_i provided maximum inhibition of oedema at 4, 3 and 3 hours respectively, followed by a decline in inhibition effect, except MW emulsion that provided almost constant and controlled oedema inhibition even after 8 hours till 24 hours. This clearly indicate that multiple emulsion MW produced maximum anti-inflammatory effect even for much longer duration followed by emulsions MO and MWP_i than aqueous drug solution (S).

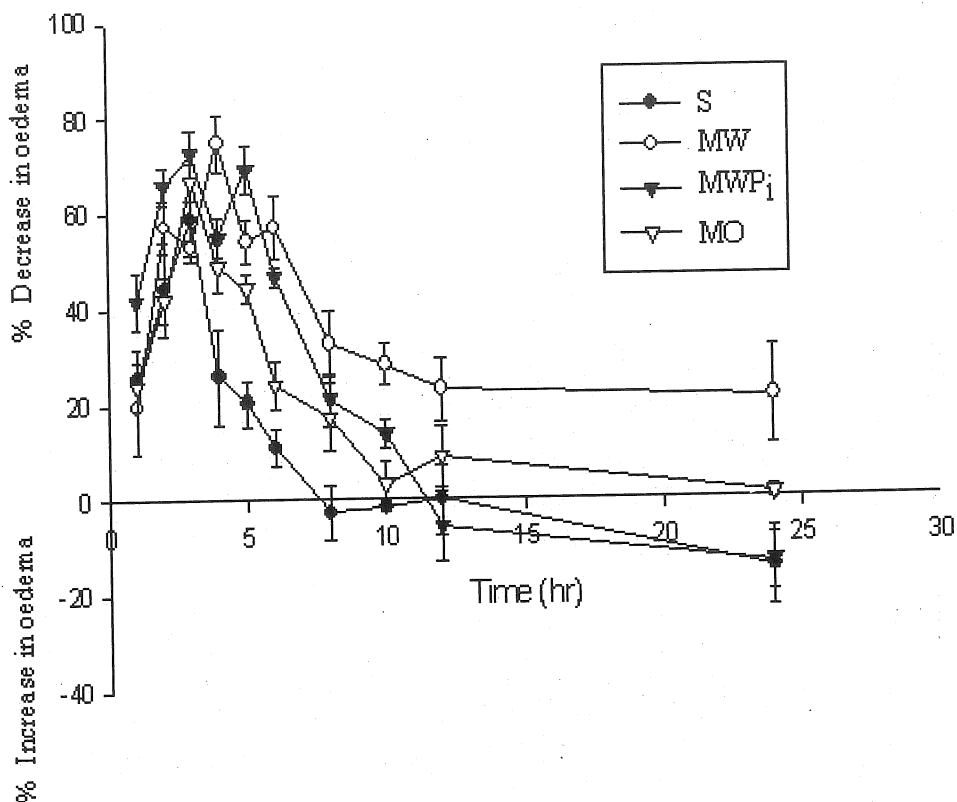


Fig. 5. Profiles showing effect of orally administered (100 mg/kg) different formulations of Fb on carrageenin induced paw oedema in rats. (Mean \pm S.E.M) (n = 5).

Based on above observations, it could be concluded that by optimizing formulation variables, multiple w/o/w and o/w/o emulsions could more effectively be utilised as potential prolonged and controlled release liquid formulation of Flurbiprofen.

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