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Research Article

FORMULATION AND EVALUATION OF SUSTAINED RELEASE MULTIPLE EMULSION OF HYDROXYPROGESTERONE

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ABSTRACT

Parenteral emulsions are special O/W emulsions used to feed patients whose medical condition makes them unable to eat normally. One is that the maximum droplet size must be below 5µm in order to avoid the risk of a pulmonary embolism. The hydrodynamic and physicochemical formulation parameters were manipulated to reduce energy input and equipment requirements. The 'Fa' preparation shows the significant result and it was selected as an optimized formulation on the basis of low viscosity and excellent syringability. *In-vitro* diffusion study reveled that multiple emulsion sustained the release of drug more than the marketed preparation. It can be predict that the formulation sustained the release up to seven days. The drug release data shows that the drug release of all formulations in the following order Fe > Fd > Fc > Fb > Fa > Marketed formulation. The amount of the drug diffused out was relatively small. The resulting emulsions by this simplified and less energy intensive processes complies with droplet size requirements and are stable over several months.

Keywords: Parenteral emulsions, Formulation, Hydroxyprogesterone caproate, Multiple emulsions.

INTRODUCTION

The influence of route of administration on drug availability is generally in the following order parenteral > oral > rectal > topical with few exceptions. Oral route is the most convenient route for access to the systematic circulation. But it does not always give rise to sufficiently high plasma concentration to be effective because of two reasons, decreased absorption and first pass/ Presystemic metabolism.[1] To overcome bioavailability and the first pass metabolism of the drugs, the parenteral route is suitable for the administration of the drug. This route increases the bioavailability and prevents the drugs from first pass metabolism. Routes such as intravenous, subcutaneous, intradermal, intramuscular, and intrathecal may administer the injectables.[2]

Parenteral emulsion were utilized as carrier of drug substances due to their ability to incorporate drug within innermost phase, thus allowing for solubility and stability constrains of the drug to be minimized or by passed altogether. As the drugs were not in contact with the body fluid, the partitioning of drug from the internal phase to the external phase may contribute to sustained release. The water based emulsions i.e., O/W or W/O/W given by intravenous route and oil based or oily emulsions i.e., W/O or O/W/O emulsions can be given by intramuscular route as a depot preparation.[3] Double or Multiple emulsions, provide a more prolonged delivery of the drug by introducing an extra partitioning step before release to the body. The potential of these systems for application in pharmacy, as parenteral prolong drug delivery system and separation technology. [4-7]

The Hydroxyprogesterone caproate ($C_{27}H_{40}O_4$) is used for the several uterine diseases and as a contraceptive. It has low aqueous solubility and low bioavailability though it is lipophilic drug. It is given orally at a dose of 500-1000 mg/day, IM in the dose of 250 mg/ml, which sustained the action up to three days. [8-11] Multiple emulsions are undoubtebally more complex than their two-phase counterparts from the standpoint of formulation, stability, and drug release. They are useful tool in achieving sustained release drug delivery for intramuscular route. The present study aims towards formulation of multiple emulsions, which contain an additional reservoir that is an extra step for partioning of the drug, which can effectively retard the release rate of the drug and decrease the dose frequency.

MATERIALS AND METHODS

Materials

Hydroxyprogesterone caproate as a gifted sample by Jagsonpal Pharm. Ltd., Delhi and Alpha–tocopherol as a gifted sample by RPG Life Sciences, Mumbai. All the chemicals and reagents were of analytical grade and purchased from Bombay Research Lab., Pune and Research Lab., Mumbai.

Method

a) Preparation of the multiple emulsions:

Multiple emulsions (W/O/W or O/W/O) were prepared using a two-step procedure. Primary emulsion (O/W emulsion) prepared by using oil and high HLB surfactant solution in water. In first step for the preparation of the primary emulsion, oil and aqueous phase heat up to 70-80^0 C and blending usually carried out in a high shear device to produce very fine droplets. The second emulsification step used to prepare O/W/O emulsion by using low HLB surfactant in oil for dispersion of primary emulsion and this phase also heat up to the desire temperature[12]. The second emulsification step is carried out in a low shear device to avoid the rupturing of the multiple droplets at different phase volume ratio of oil and primary emulsion[13] as shown in table 1.

Table 1: Preparation of the final formulation of O/W/O emulsion

Ingredients	Fa	Fb	Fc	Fd	Fd	Fe
Soybean oil (ml)	30	30	30	30	30	30
Span (80%)	5	6	7	8	9	10
α-Tocopherol (mg)	0.075	0.075	0.075	0.075	0.075	0.075
Temp.(°C)	70-80	70-80	70-80	70-80	70-80	70-80

O/W emulsion (ml)	10	10	10	10	10	10
RPM	800	800	800	800	800	800

b) Sterilization of multiple emulsions

As aseptic processing is widely used in the manufacture of parenteral emulsions and emulsions were often heat sensitive which cannot tolerate terminal sterilization by heat, hence aseptic processing is preferred in the manufacture of this type of parenteral product. The materials which are soluble in aqueous or oil phases are dissolved in appropriate vehicles and then sterilized by membrane filtration method and all processing vessels, filters, ampoules, pipettes and other components were sterilised by steam sterilization, usually in steam autoclave. The mechanical stirrer sterilized by using hydrogen peroxide (15%).

Evaluation of Multiple Emulsions[14,15]

Viscosity

The flow characteristics of the low molecular weight pure substance and solutions obey Newtonian flow. Most dispersed system does not show Newtonian flow behavior but rather fallow any of the other flow behaviors, namely pseudoplastic, plastic or dialatant behavior.[16,17]

Surface tension

Measurement of the surface tension is achieved by determination of surface free energy, thus, surface tension expressed as dy/cm. There are several indirect methods for measuring the surface free energy from which the surface tension can be calculated according to the mathematical formula. Surface free energy measurements change with time, temperature, and other factors.[16,17]

Conductivity

Oil-in-water emulsions, where water is a continuous phase, show high conductivity, where as water-in-oil emulsions, where oil is a continuous phase, show little or no conductance. Measurements of emulsion conductivity are used to investigate degree of dispersion in dilution preformulaton studies and phase inversion and creaming in stability studies. [16,17]

Syringability

Syringability is the ability of a parenteral preparation to pass easily through the hypodermic needle, especially during the transfer of product from vial to the hypodermic syringe prior to injection. The various factors can affect the syringability, such as viscosity, particle or droplet size, concentration of droplet, the density of vehicle. In parenteral O/W/O emulsions, viscosity is the most difficult parameter for the formulator to control and to give the physician flexibility, the entire content of the formulation is expected to pass through the needle without difficulty. [18]

pH

The parenteral preparations subjected for the determination of pH. and must be up to the neutrality to overcome the various factors like oxidation of oil; microbial degradation or growth of microorganism .If the pH of the preparation acidic or basic it causes irritation at the site of the injection. So it is essential to determine the pH of preparations.[14]

Globule/droplet size

Fine droplets or particles are described in the terms of concentration, size, and size distribution. The droplet or the particle size, of the dispersed phase in a dispersed system is dependent on the method of manufacture and formula used. The size of the droplet can affect the product appearance. Such particles cause differences in light scattering, absorption and reflection, rheological properties and the stability of the dispersed system. Droplet of the particle size determine by microscopic method, sedimentation method, optical methods including light scattering, spectroturbidimetric, reflectance, and electrolyte displacement method. [19]

Test for sterility

To determine the sterility of the product the Sterility test direct inoculation method was used as per I.P. Fluid Thioglycolate and Soybean casein digest medium were used to carry out sterility test. A medium was prepared and sterilized by moist heat sterilization. In media 0.1% w/v of polyethoxyethanol or 1% w/v of polysorbate 80 or other suitable emulsifying agent was added in an appropriate concentration, should not have any antimicrobial properties under the condition of test.

Microscopic method

The ordinary microscope can be used for particle size measurement in the range of 0.2 to 100 μm. According to the microscopic method, an emulsion or suspension, diluted or undiluted, is mounted on the slide or ruled cell and placed on the mechanical stage. The microscope eyepiece is fitted with a micrometer by which the size of the particle is estimated. The field can be projected on to a screen where the particles are measured more easily, photograph can be taken from which a slide is prepared and projected on the screen for measurement. [20]

Particle size distribution

When the number or weight of particle lying within a certain size ranges are plotted against the size range or mean particle size, also called frequency distribution curve is obtained. Such plots give a visible representation of particle size distribution.

Entrapment efficiency

The amount of drug entrapped is the amount of total drug minus the amount of free drug separated in the lower phase of the emulsion by centrifugation. [7, 19]

The entrapped efficiency of the drug is defined as,

$$\text{Efficiency of drug entrapped (\%)} = [(Td - Fd) / Td] \times 100$$

Where, Td = Total drug added, Fd = free drug present in the separated oil or aqueous phase.

RESULT AND DISCUSSION

Viscosity

Determination of the viscosity at fixed time of 1 minute. (Table No.2)

Viscosity (cps) = Dial reading x factor, Factor = 4M, M = 100

Table 2: Viscosities of the formulations

Sr. No.	Formulations	Viscosity (cps)
1	Aqueous phase	01
2	Oil phase	30
3	Fa	400
4	Fb	800
5	Fc	1200
6	Fd	2000
7	Fe	2800

Surface tension

Surface tension of oil phase and aqueous phase is more than that of multiple emulsions, from such value it can be conclude that surfactant reduced the tension which is present on the intermediate surface of both oil and aqueous phase. (Table No.3)

Table 3: Surface tension of the formulations

Sr. No.	Phase	Surface tension (dyne/cm)
1	Oil phase	32.00
2	Aqueous phase	70.69

3	Fa	32.00
4	Fb	29.86
5	Fc	29.00
6	Fd	28.65
7	Fe	28.12

Conductivity

The conductivity is convenient way of distinguishing between two emulsions. The conductivity study was carried out by using conductometer at room temperature. O/W emulsions pass a current of 10 mA and W/O emulsions pass current of 0.1 mA or less. From the table it can be concluded that O/W/O emulsions does not show any flow of current. (Table No.4)

Table 4: Conductance of the multiple emulsions

Formulation	Conductance (mA)
Fa	0
Fb	0
Fc	0
Fd	0
Fe	0

pH

The pH determination was carried out on digital pH meter. From the values obtained it can concluded that the multiple emulsions has a neutral pH, which was desirable for administration. Also they do not produce irritation. (Table No.5)

Table 5: The pH values of formulations

Sr. No.	Formulations	pH
1	Fa	7.2
2	Fb	7.1
3	Fc	7.0
4	Fd	7.3
5	Fe	7.2

Syringability

The formulations were subjected for the syringability test. All the formulation were tested by different size of needle under the guidance by the experters (Physicians) and the data obtained was put in tabulated form, The different sizes of needles (gauge) are used for the intramuscular route and 21G size needle is used for the delivery of oily preparation. The O/W/O multiple emulsion with a minimum concentration of surfactants showed excellent syringability. The multiple emulsion prepared with maximum concentration of surfactants and for given time period entrapped 83.14 % of Hydroxyprogesterone. (Table No.6)

Table 6: Syringability Performance of Formulations

Formulation.	Hypodermic Syringe with Needle (Gauge.)			
	21G	22	23	24
Fa	+	++	+++	++++
Fb	++	+++	++++	++++
Fc	+++	++++	++++	++++
Fd	+++	++++	++++	++++
Fe	++++	++++	++++	++++

Excellent, ++ Fair, +++ poor, ++++ very poor

Globule/droplet size determination

Droplet size was determined by the microscopy, the compound microscope was used to determine the droplet size of the formulation. The data obtained was tabulated as follows. (Table No.7) It was found that the droplets decrease in size with increase in the concentration of surfactants. The average diameters of the dispersed water droplets in O/W/O emulsions shows in figure 1.

Entrapment efficiency

The entrapment efficiency is the capacity of the multiple emulsions that how much quantity of drug is entrapped in the internal phase. It was calculated in %. All the formulations were centrifuged at high speed and the separated phase was evaluated for drug content. (Table No.8)

Sterility Test

All the formulations were subjected for the sterility test by direct inoculation method (I.P.). The sterility test was carried out for aerobic and anaerobic microorganism, fluid thioglycollate media and soybean casein digest media was used for sterility test. (Table No.9)

Stability study

The stability study was carried out of optimized formulation Fa. Formulation was stored in amber colored ampoule at 40^{o}C for three months. It was evaluated for physical characteristics. Stability study data was compared with the data obtained for zero time at ambient temperature. (Table No.10)

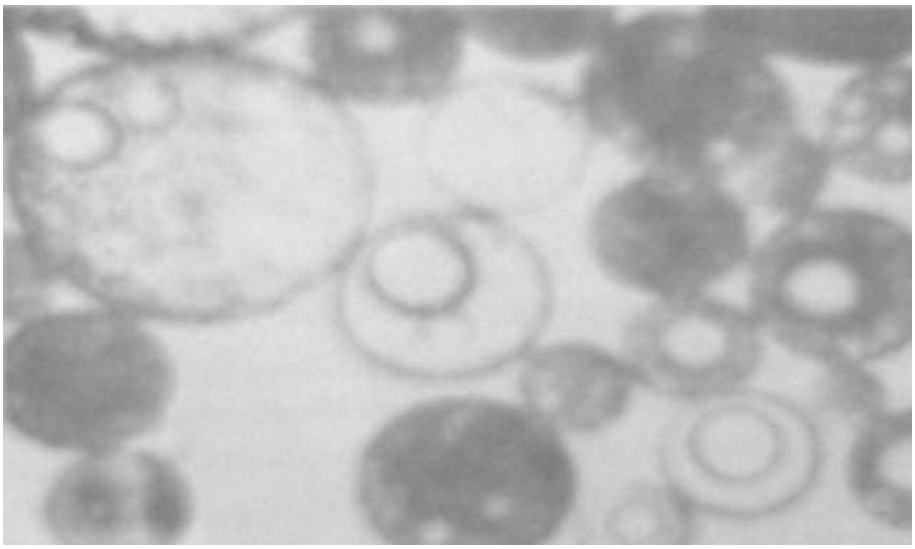

Fig. 1: Photomicrograph of multiple emulsion

Table 7: Determination of droplet size by microscopy

Droplet size by microscopy	Fa	Fb	Fc	Fd	Fe
Average Diameter = Σ(nd)/ Σn	15.44 µ	14.64 µ	14.36 µ	14.52 µ	14.52 µ
Geometric Diameter log d_{geo} = Σ (n log d)/ Σ	20.89 µ	21.37 µ	20.89 µ	20.82 µ	19.99 µ
Mean surface = $(\Sigma nd^2/\Sigma n)^{1/2}$	16.19 µ	15.47 µ	15.21 µ	14.15 µ	15.43µ

Table 8: Entrapment efficiency of formulations

Sr. No.	Formulation	Entrapment efficiency (%)
1	Fa	82.00
2	Fb	82.42
3	Fc	82.95
4	Fd	83.00
5	Fe	83.14

Table 9: Study of sterility test

Sr. No.	Formulations	Media used	Observations	Results
1	Fa	Fluid thioglycollate	_	Passed
		Soybean-casein digest	_	Passed
2	Fb	Fluid thioglycollate	_	Passed
		Soybean-casein digest	_	Passed
3	Fc	Fluid thioglycollate	_	Passed
		Soybean-casein digest	_	Passed
4	Fd	Fluid thioglycollate	_	Passed
		Soybean-casein digest	_	Passed
5	Fe	Fluid thioglycollate	_	Passed
		Soybean-casein digest	_	Passed

Indicate: '+' Growth, '**-**' No Growth

Table 10: The stability data of formulation Fa, after 0, 1, 2 and 3 months

Parameters	0 Month	1 Month	2 Month	3 Month
Drug content	98.60	98.55	98.59	98.59
Droplet size	15.44	15.46	15.40	15.49
Viscosity	400	400	400	500
Syringability	+	+	+	+
pH	7.2	7.3	7.1	7.2

***In -vitro* diffusion study**

The drug release profile of all formulations was studied in buffer media (pH 7.4 and Alcohol 20%) by using K-Cell. The cellulose membrane used as a barrier. The data were shown in Figure 2. The drug release profile was studied for 24 hrs. The drug release data shows that the drug release of all formulations in the following order, Fe > Fd > Fc > Fb > Fa > Marketed formulation. The amount of the drug diffused out was relatively small, indicating that the water layer of the multiple emulsion shows a stable diffusion barrier, thus the drug was released mainly by permeation through this membrane. Slightly faster drug diffusion was observed with a low concentration of the surfactants. Since the multiple emulsions containing higher concentration of surfactants include the smaller droplet size and high viscosity. The comparative drug diffusion studies were carried out, comparison with the marketed formulation

and shown the percentage drug release of marketed formulation more than the multiple emulsions.

Thus, drug incorporated in to the internal oil phase of the O/W/O multiple emulsion was relatively stable and induces the prolong release of the Hydroxyprogesterone caproate from the multiple emulsion. The different kinetic equations were applied to interpret the release rate of Hydroxyprogesterone caproate studies of multiple emulsion formulation 'Fa' follows first order kinetics and Korsmeyer's peppas (r^2) values shows 0.9616 and 0.9925 respectively.

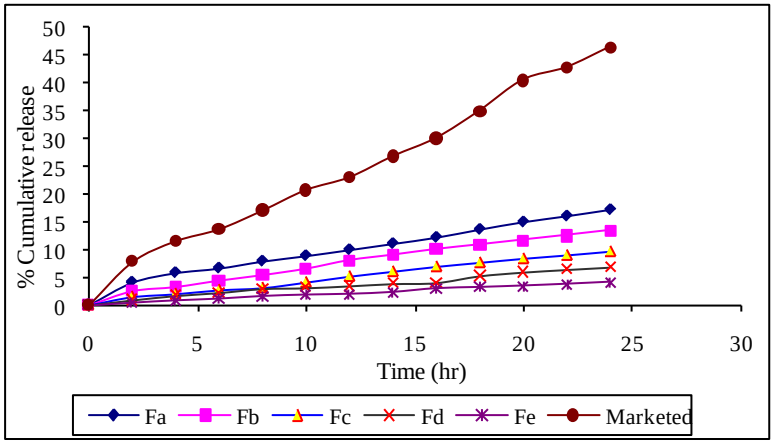

Fig. 2: In -Vitro drug release through cellulose membrane

CONCLUSION

The present work was undertaken with the aim to design multiple emulsions for intramuscular drug delivery of hydroxy progesterone. The O/W/O multiple emulsions were prepared. The most promising use of multiple emulsion system is in the area of sustained release drug formulations because of the extra partioning step for the release of drug. The vegetable oils were taken as oil phase because the drug is lipophilic and it has solubility in vegetable oil. The selection of the surfactants was made on the basis of the HLB required for the preparation of O/W/O multiple emulsions by using HLB scale. The soybean oil and the non-ionic surfactants spans (hydrophobic) and Tweens (hydrophilic) were selected for the preparation of multiple emulsions. From the evaluation parameters it concluds that incorporating 5% hydrophilic surfactant and 5% hydrophobic surfactant could developed the stable formulation. The 'Fa' preparation shows the significant results, and it was selected as an optimized formulation on the basis of low viscosity and excellent syringability. *In-vitro* diffusion study reveled that multiple emulsion sustained the release of drug more than the marketed preparation. It can be predict that the formulation sustained the release up to seven days.

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