INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES
[ISSN: 0975-4725; CODEN(USA): IJPS00]
Journal Homepage: https://www.ijpsjournal.com

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Research Article

Development of Metronidazole Loaded Multiple Emulsion for Sustained Release Medication

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ARTICLE INFO

Published: 19 July 2025
Keywords:
Two-Step Emulsification, Prearranged Release, Sustained Way, Doses Prevalence
DOI:
10.5281/zenodo.16146966

ABSTRACT

Multiple emulsions are complex polydisperse structures in which each oil in water and water in oil emulsion exist simultaneously which may be equilibrated through lipophilic and hydrophilic surface-active agents respectively. These devices have additionally been hired as intermediate step inside the microencapsulation system and are the structures of increasing interest for the oral transport of hydrophilic capsules, which can be unstable in gastrointestinal tract like proteins and peptides. Method: on this have a study, metronidazole loaded with a couple of emulsions have been organized via the two-step emulsification method the use of span 60 and 20 for slow launch movement for amplification of bioavailability. Terrific evaluation parameters together with medicament and adjuvants interplay through FTIR, organoleptic developments, pH verification, in vitro medicament rescue observe, liquefaction, globule period, segment separation were studied for choice and upgradation of the product. Results: Freshly organized a couple of emulsions had been creamy white in color. There has been no trade in color in specific storage situations. Liquefaction is the signal of instability; it could be attributed to the passage of water from the internal segment to the outdoor section. FTIR study results indicate that there has been absence of any incompatibility inside the unfastened medicament along aggregate of excipients aggregate. The results proved that the lively medication, i.e. metronidazole without trouble is entrapped inside the system. Droplet sizes of emulsion structures had been ascertained by optical microscopy. In vitro launch it was revealed that medicament was released in a sustained way. Discussion: All three formulations maintained controlled for prearranged release of drugs in lively pharmaceutical factors like metronidazole. On this appearance we can finish that our technique is favorable for anaerobic dysfunctions for gradual launch of

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Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

medicament simultaneously it'll be benevolent for falling off doses prevalence.

INTRODUCTION

Multiple emulsions are heterogeneous systems in which dispersed phase consist of tiny droplets that have identical composition as the outdoor section. In double emulsion every dispersed droplet paperwork a vesicular shape with unmarried or more than one aqueous compartment separated from the watery phase through a layer of oil phase cubicles [1]. More than one emulsion also is termed as emulsions of emulsions, or double emulsions. Those forms of novel formulations are widely relevant for the development of bioavailability, or as an extended-release medication and predictable manner of medicament release [2]. multiple emulsion is framed using a combination of each hydrophilic and hydrophobic floor lively stores [3]. Multiple emulsion is polydisperse structures in which every oil in water and water in oil emulsion exists all collectively which can be stabilized with the resource of lipophilic and hydrophilic surface-lively marketers respectively [4]. Multiple emulsion is an emulsion in which water-in-oil (w/o) and oil-in-water (o/w) exist simultaneously. They amalgamate the houses of each w/o and o/w emulsions. The ones styles of emulsion are heterogeneous systems in which one immiscible liquid dispersed into every different form of notable droplets, which typically have diameters greater than 1μm [5]. Water-in-oil-in-water (W/O/W) multiple emulsions are the emulsification method wherein small water droplets are entrapped inside big oil droplets that in flip are dispersed in a continuous water section. Because of the presence of a reservoir segment internal droplets of every other section that can be used to extend release of lively factors [6,7].

Copolymers, polymers and surfactants and so forth are used to offer kinetic balance to keep their structure for particularly lengthy period due to the reality emulsions are thermodynamically wobbling approach [8]. The reason for a couple of emulsions is to offer a high yield of more than one droplet containing drug enticing in the innermost segment, and for this kind of gadget to have desirable stability in-vitro and the favored release trends in-vivo [9]. The droplet sizes of more than one emulsion (MEs), which may be isotropic, thermodynamically stable, apparent (or translucent) structures of oil, water, and surfactant-regularly in combination with a co-surfactant-generally variety from 10 to a hundred nm [10]. Splendid traits of a multiple emulsion as a drug shipping automobile consist of thermodynamic stability (extended shelf lifestyles), ease of manufacturing (0 interfacial anxiety), and nearly (spontaneous advent), optical isotropy, high floor location (immoderate solubilization potential), filtration-sterilization functionality, and rather small droplet length [11,12]. Additionally, the tiny droplets decorate membrane adhesion and supply medicine molecules in a regulated way [13]. Both w/o and o/w emulsions can coexist in an unmarried tool with many emulsions, which are probably unique issuer systems which are probably complicated and polydisperse in nature [14]. The objective of the existing observation is to increase the metronidazole containing multiple emulsions for sustained release motion for special anaerobic imperfection and useful for prevention of H. pylori infection in ulcerative colitis. Further, metronidazole containing multiple emulsions is likewise useful for flavor shielding for medicament like metronidazole, also useful for bioavailability improvement, enzyme immobilization.

2. METHODOLOGY:

2.1. Preparation of multiple emulsions:

Training of multiple emulsions:

There are essential methods available for the education of multiple emulsions.

• One step emulsification
• Two-step emulsifications

The maximum common method of formulation is two-step emulsification methods (Double Emulsification method) from which excessive percentage of methods yield. Presently, a modified two-step emulsification technique is relevant for attaining huge quantity of stable more than one emulsion yield [15, 16].

More than one emulsion was organized by using step emulsification manner:

2.1.1. Training of primary emulsification:

10 ml distilled water generally consists of 25 mg of active pharmaceutical element (API), Metronidazole has been steadily added to 14 ml of oil medium containing primary emulsifying agent (Span 40) and 25 mg of metronidazole **(Table No 1)** with constant agitation at 5000 rpm for 5 min [17]. It offers primary emulsion **(Figure no 1)**.

Figure No 1: Primary emulsification

2.1.2. Instruction of secondary emulsification:

Secondary emulsification can be 20 ml of viscous primary emulsion is now emulsified even in addition with such outdoors aqueous answer containing secondary emulsifying agent (Tween 80) and 50 mg of Metronidazole **(Table no 1)** with constant stirring at a thousand rpm for 10 min [18]. All the formulations had evolved following the identical method.

Figure No 2: Multiple emulsions

Table 1: Design and formulation of multiple emulsions:

Sl No	Ingredients	F1	F2	F3
1.	Metronidazole	Primary emulsion (0.025g)	Primary emulsion (0.05g)	Primary emulsion (0.075g) Secondary emulsion (0.1g)

		Secondary emulsion (0.05g)	Secondary emulsion (0.1g)	
2.	Liquid paraffin	14ml	28ml	42ml
3.	Span 60	0.28 gm	0.56gm	0.84
4.	Tween 20	1ml	2ml	3ml
5.	Distilled water	30ml	30ml	30ml

3. Evaluation Parameters:

3.1. Physical look:

The API Metronidazole powder was tested for its organoleptic houses like coloration and odour.

3.2. Solubility assessment:

The sample became qualitatively tested for its solubility in diverse solvents. It changed into determined by taking 10 mg of API in 10 ml of solvent as water, methanol, and ethanol in small test tubes and nicely solubilized by the usage of shaking [19].

3.3. Fourier-redesign Infra-purple spectroscopy (FTIR):

The IR spectrum of drug substance modified into authenticated the use of IR spectroscopy. The presence of function peaks associated with structural developments of the drug molecule changed into stated [20,21].

3.4. Microscopic characteristics:

In this look at, microscopic characteristics of a couple of emulsion organized have been determined the use of light microscopes for the freshly prepared emulsions and for the emulsions stored at situations for 28 days [22, 23].

3.5. Organoleptic trends:

Freshly prepared number one and a couple of emulsions had been investigated organoleptically (color, liquefaction and phase separation). Organoleptic characteristics of more than one emulsion stored at considered one type of garage situation, i.e. coloration, liquefaction and section separation had been mentioned at diverse periods, i.e. zero h, 1 h, 1 day, three days, 7 days, 14 days, 21 days and 28 days [24,25].

3.6. pH observation:

The pH price of the freshly prepared emulsions and the emulsions stored at precise situations has been decided through a virtual pH-meter. pH measurements had been repeated for a couple of emulsions after 1, 3, 7, 14, 21 and 28 days of training [26,27].

3.7. In vitro release profile:

The in vitro drug launch examines changed into achieved in an easy 500 ml beaker using dialysis membrane (good day Media, thickness-200 mm) In vitro drug launch of each components become finished at 37±2°C in PBS, pH 6.8, as dissolution media for a length of 30 h. In a 100 ml beaker, 50 ml dissolution media come to be taken. The weighed number of components (5 mg) changed into reconstituted in 1 ml dissolution media and changed into saved dialysis luggage. ends of the dialysis bag were tightly bound with cotton threads and the bag changed into hanged in the beaker with the help of a pitcher rod just so part of dialysis bag with the components should dip into the dissolution media. The beaker became stored on a magnetic stirrer [28]. Stirring modified into maintained at three hundred rpm with the assistance of a magnetic bead at 37±2°C. Sampling has become conducted by retreating 1 ml with the assistance of micropipette from the beaker

containing drug release medium and 1 ml clean medium modified into modified. Samplings were performed on the different predetermined time intervals (0.5, 1, 2, 3, four, five h). The samples have been analyzed in a UV-VIS spectrophotometer at 278 nm. The awareness of drug at every time factor became calculated from the calibration curve [29,30].

4. RESULTS AND DISCUSSION:

4.1. Physical appearance:

The medicament (Metronidazole) powder becomes tested for organoleptic homes like coloration and odor. And it became located that Metronidazole was whitish crystalline powder.

5. Solubility observation:

The sample changed into qualitatively tested for its solubility in numerous solvents. It was decided with the aid of taking 10 mg of free powder API sample in 10 ml of solvent as water, ethanol, ether & acetone in small test tubes **(Table no 2)** and properly solubilized by way of shaking **(Figure 3)**.

Table 2: Evaluation of solubility:

Sl. No.	Solvents	Solubility
1.	Water	Soluble
2.	Ethanol (Fig No.5)	Soluble
3.	Acetone (Fig No.6)	Sparingly soluble
4.	Ether (Fig No.7)	Insoluble

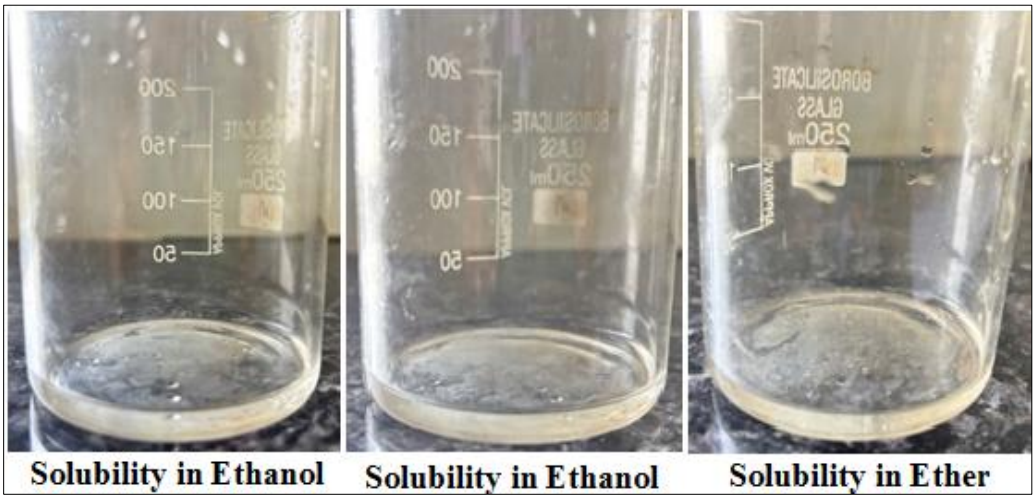

Figure No 3: Solubility in different organic solvents

5.1. Colour:

Freshly prepared more than one emulsion was creamy white in shade. There has been no exchange in color in exceptional garage situations. This suggests that the formula became strong at specific storage situations up to 28 days. There has been little trade in color of samples stored at 400C (in oven) and the coloration has become yellowish white. The exchange in color seemed to be on the 21st day and persisted up to twenty-eight days. The change in shade on the stop of the commentary length can be because of the oily section separation that is promoted at extended temperatures (Table no 3).

5.2.Liquefaction:

For more than one emulsion, while no liquefaction became observed in them samples kept at 8 °C (in refrizerator) and 25°C (in oven) throughout 21 days, moderate liquefaction becomes discovered inside the samples saved at 40°C (in oven) on 21st day **(Table no 4)**. Liquefaction is the signal of instability; it can be attributed to the passage of water from the internal phase to external phase.

Table 4: Evaluation of Liquefaction:

- ✓ **'+'**= Sign of Liquefaction.
- ✓ **'-'** = No sign of Liquefaction.

4.5. Phase separation:

For the multiple emulsions, no segment separation was seen in the samples kept in any storage conditions, besides mild section separation beginning at the 21st day **(Table no 5)**.

Table 5: Evaluation of Phase Separation parameter:

Time	F1 (W/O/W)	F2 (W/O/W)	F3 (W/O/W)
0 hr	-	-	-
1 hr	-	-	-
24 hrs	-	-	-
72 hrs	-	-	-
7 days	-	+	+
14 days	-	+	+
21 days	+	+	+

- ✓ **'+'= Sign of Liquefaction.**
- ✓ **'-' = No sign of Liquefaction.**

4.6.Globule size:

Globule sizes of emulsion structures can be decided with the aid of optical microscope, laser diffraction strategies, electron microscope or through coulter counter. Optical microscopy turned into used on this study. The a couple of droplets may additionally coalesce with the opposite oil drops, inner aqueous droplets may be expelled for my part, more than one drop can be expelled, inner globules may coalesce earlier than being expelled out ensuing in the shrinkage of inner droplets or water might also pass from the outside section to the internal aqueous segment resulting in the swelling of internal droplets accompanied by way of complete rupture of droplets (Figure No. 4).

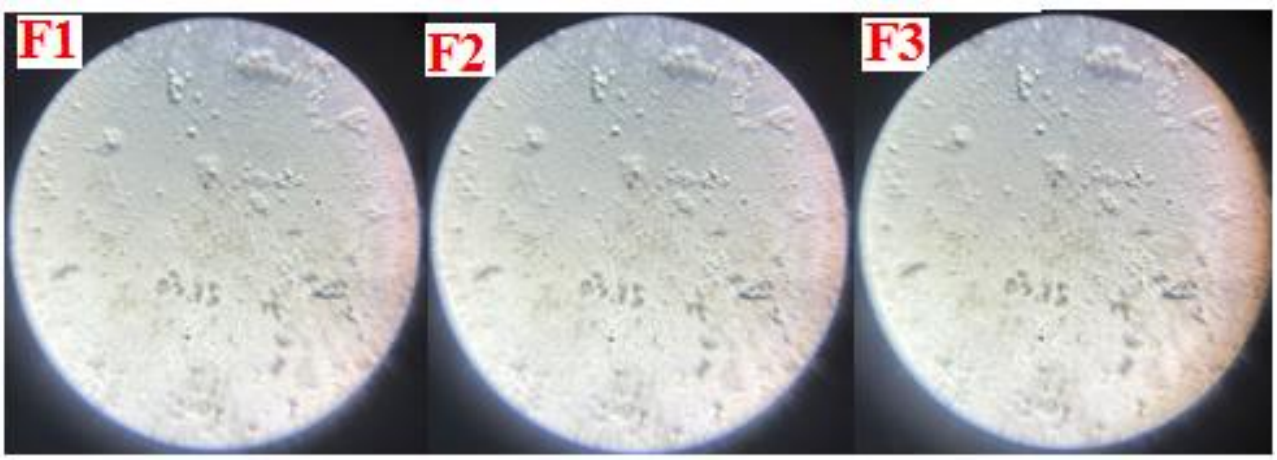

Figure 4: Globule size of three formulations

4.7.Drug-Excipient Interaction Study by FTIR

FTIR spectroscopy for a component is executed to research interactions (if any) between the drug and the excipients at the extent of practical agencies. in the present study (**Figure No 5)** suggests the FTIR spectra of every man or woman chemical such as drug, their physical mixture and the formulations

with or without the drug. feature peaks of the individual components had been observed within the bodily combo of the pure drug and the drug with excipients (**Figure No 5)**. As for example, the drug metronidazole has proven its feature peak at wave no 3099.03 cm^{-1}, indicating the presence of secondary amine for variable intensity bending vibration of NH2 and N-H wagging. in the drug loaded more than one emulsion formulation confirmed feature peaks at the wave no 3145.9 cm^{-1}. Inside the components containing drug, no characteristic standard band of metronidazole become determined as seen in the physical mixture. The absence of the peak or band of the drug in the system shows that the drug turned into encapsulated and unfastened drug became not to be had at the floor of the formula. even though the feature peaks or bands of the drugs and the person excipient were gift inside the drug excipients aggregate, there had been minor versions of several of the peaks inside the following degrees 1218 to 1308 cm^{-1}. there has been no moving found in excipient combination with aggregate of pure loose drug at the side of mixture of excipients combinations wavelength degrees consisting of 3308 cm-1, 2916 cm-1, 2849 cm-1, 1735.1 cm-1, 887.1cm-1and 7212 cm-1. Minor transferring of peaks in wave numbers 1472 cm-1 to 1097 cm-1, has been discovered. Therefore, minor bodily interactions due to vulnerable hydrogen bond, Vander Waals' force, and many others. appear to exist among the excipients, and between the free API like metronidazole. For designing the system development of metronidazole loaded a couple of emulsions, FTIR study results revealed that there has been absence of any incompatibility inside the unfastened dug in conjunction with combination of excipients combination. The outcomes proved that the lively medicament i.e. metronidazole without difficulty entrapped in the method.

Fourier-Transform Infra-Red spectroscopy (FTIR):

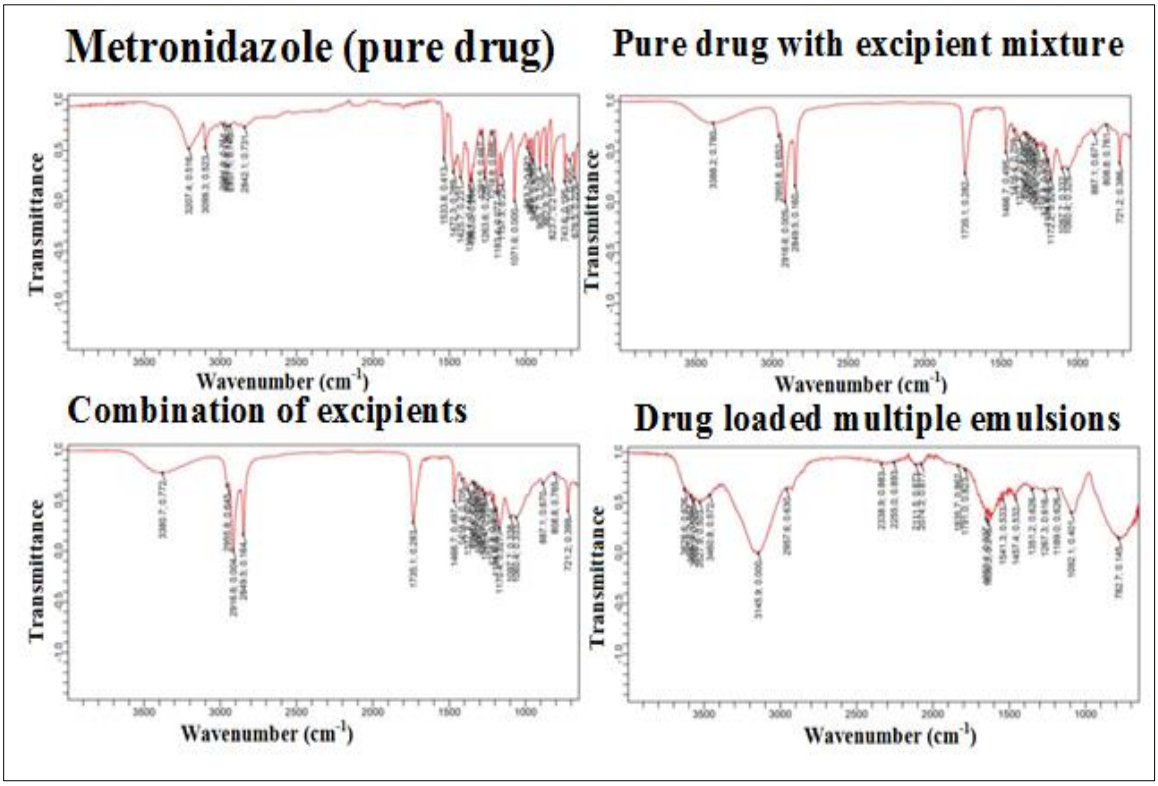

Figure no 5: Drug-excipient interaction by FTIR

4.8 pH values:

pH values of skin range between 7.4 and 5.5 are the common pH of the pores and skin. Therefore, the formulations supposed for dermal software should have a pH cost round this range.

4.9 *In vitro* release study:

All three formulations (F1, F2 and F3) revealed that from those formulations drug metronidazole release in sustained manner inside one-of-a-kind time periods. outcomes indicated within Fig No. 9. F3 releases the medication of 88 % of drug release inside five h, simultaneously F1 and F2 release the medicament of metronidazole 74% and 66 % of drug release within 5 h (Figure 6). It proved that our formula, this is metronidazole loaded more than one emulsion maintained preprogrammed or predetermined action for anaerobic dysfunctions in the human body.

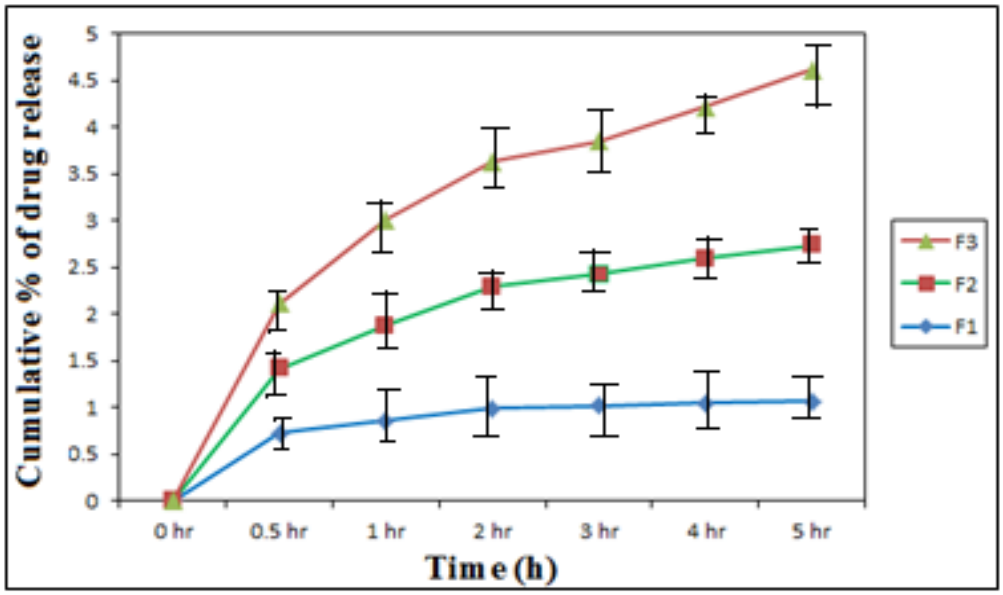

Fig No.6: *In vitro* release study of multiple emulsions

5.CONCLUSION:

One of the more sophisticated remedy delivery strategies for boosting the one-of-a-kind elements of the medicaments, along with taste, appreciation, launch rate, and bioavailability, is more than one emulsion. The dispositions encompass some modern formulations that decorate medication control and palatability through integrating them into immediate formulations. A complex polydisperse component called a multiple emulsion is made up of one emulsion blanketed into another. It can be used for a selection of functions, consisting of taste masking, sustained launch, delivering risky pills, and stopping capsules from getting into the surroundings. All three formulations maintain constant shipping of medicine for predetermined launch of lively pharmaceutical factors like metronidazole. On this look we can finish that our formulation is favorable for anaerobic dysfunctions for chronic launch medicament simultaneously it's going to supportive for subsidiary doses incidence. It is going to be serviceable for lessening toxicity and audition for centered action all through therapy of ill health.

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HOW TO CITE: Dr. Niladri Shekhar Dey*, Shreya Pal, Puja Saha, Amit Samanta, Pankaj Kumar Ram, Md Shahjaha, Development of Metronidazole Loaded Multiple Emulsion for Sustained Release Medication, Int. J. of Pharm. Sci., 2025, Vol 3, Issue 7, 2661-2670. https://doi.org/10.5281/zenodo.16146966