**Research Article** **CODEN: IJRPJK** **ISSN: 2319 – 9563**

**International Journal of Research in Pharmaceutical and Nano Sciences**

**Journal homepage: www.ijrpns.com**

https://doi.org/10.36673/IJRPNS.2021.v10.i01.A08

# FORMULATION AND EVALUATION OF DOXORUBICIN HYDROCHLORIDE MICROSPHERES

**Barish*[1], E. Abraham Theodore[1], S. Princy[1], M. Mumtaj Begum[1], S. Aiswarya[1]**

[1*]Department of Pharmaceutics, RVS College of Pharmaceutical Sciences, Coimbatore, Tamil Nadu, India.

## ABSTRACT

The main objective of the present work was to formulate and evaluate Doxorubicin hydrochloride microspheres using polymers like HPMC and starch which will prolong the drug release leading to minimize the peak and valley effect in the plasma and provides patient convenience to perform the pre-formulation studies like Calibration curve of Doxorubicin hydro chloride and FIIR of Doxorubicin hydrochloride HPMC and starch to formulate a sustained release drug delivery of Doxorubicin microspheres containing 250mg of drug with polymers starch (Formula FS) and HPMC (Formula FH) respectively by Solvent evaporation method. Evaluation of microspheres to evaluate the post formulation parameters like appearance, solubility, SEM % yield, % entrapment efficiency, *in vitro* drug release, release kinetics and stability studies. The newly prepared microspheres were evaluated for morphology, vesicle size determination, percentage of drug encapsulation, drug leakage studies from vesicles, osmotic shock and *in vitro* release profile and came to conclusions to the point that microsphere enhances the therapeutic effectiveness of Doxorubicin producing prolonged activity and simultaneously minimizing the side effects.

## KEYWORDS

Microspheres, Solvent evaporation, *In vitro* release, Doxorubicin and Hydrochloride.

**Author for Correspondence:**

Barish,
Department of Pharmaceutics,
RVS College of Pharmaceutical Sciences,
Coimbatore, Tamil Nadu, India.

**Email:** princykananarjun@gmail.com

## INTRODUCTION

The concept of the advanced drug delivery systems, especially those offering sustained and controlled action of drug to desired area of effect attained great appeal for nearly half a century. However, prior to advent of improved alternative methods drug delivery system were considered only as a means of getting the drug into patient's body.

Actual practice of controlled release began with advent of timed release coating to the pills or solid drug particles in order to mask their unacceptable

taste or make them more palatable. The process of targeting and site specific delivery with absolute accuracy can be achieved by attaching bioactive molecule to liposomes, bio erodible polymer, implants, monoclonal antibodies and various particulate carriers (e.g.) Nano particles and microspheres. The micro particulate delivery systems are considered and accepted as a reliable means to deliver the drug to the target site with specificity micron size.

The term microspheres is defined as a spherical particles with diameters in the micrometer range typically 1mm to 1000µm (1mm) containing a core substance. Microspheres are in strict sense, spherical empty particles. The microspheres are characteristically free flowing powders consisting of proteins or synthetic polymers which are biodegradable in nature and ideally have a particle size less than 200nm. Solid biodegradable microspheres incorporating a drug dispensed as dissolved throughout particle matrix bare the potential for the controlled release of drugs. These carriers receive much attention not only for prolonged release but also for the targeting of the anticancer drugs to the tumors.

**Doxorubicin Hydrochloride**

In 1950 an Italian research company Farmitalia Research Laboratories began an organized effort to find anticancer compound from solid based microbes. A solid sample was isolated from the area surrounding the casel Del Monte a 13$^{th}$ Century Castle. A new strain of streptomyces peucetius which produced a red pigment was isolated and an antibiotic from this bacterium was effective against tumor in mice. The researchers named the compound Daunorubicin containing the name Dauni a pre-Roman tribe that occupied the area of Italy where the compound was isolated with French word for ruby *rubis* describing colour.

## MATERIAL AND METHODS

Doxorubicin hydrochloride from Medicare Remedies Pvt. Ltd., Mumbai starch from Merck specialties. Pvt. Ltd., Mumbai HPMC from sd fine chemicals. Chloroform from sd fine Chem. Ltd., light liquid paraffin from Fischer chemicals. Tween 80 from SD fine chem. Ltd., Petroleum ether 40°-60°C from SD fine Chem. Ltd., Disodium hydrogen Phosphate from Reachem laboratory Chemicals Pvt. Ltd., Potassium dihydrogen phosphate spectrum reagent and chemical Pvt. Ltd., Sodium chloride SD fine Chem. Ltd., Mumbai Potassium bromide merck specialties Pvt. Ltd., Germany.

**Methods**

**Pre-formulation Studies**

**Preparation of Standard Graph**

Standard graph of Doxorubicin hydrochloride in O.IN HCL buffer - pH 1.2 100mg of the Doxorubicin hydrochloride was accurately weighed and transferred to 100 ml standard flask. To this small quantity of 0.1 N HCL buffer was added and the drug was made to dissolve. Then the solution was made up 100ml (1000mg/ml). This was considered as stock solution. From the stock solution 10ml was pipetted out to 100ml standard flask and it was diluted up to 100ml (100mg/ml) From 10 to 50mg/ml solution was prepared and the absorbance for the solution was determined at 233nm by Elico make UV spectrophotometer.

**Standard graph of Doxorubicin hydrochloride in phosphate buffer pH 7.4**

100mg of Doxorubicin hydrochloride was accurately weighed and transferred to a 100ml standard flask. To this small quantity of phosphate buffer was added and the drug was made to dissolve. Then the solution was made up to 100ml (1000µg/ml). This was considered as stock solution. From the stock solution 10ml was pipette out to 100 ml standard flask and it was diluted up to 100ml (100mg/ml). From this 10 to 50mg/ml solution was prepared and the absorbance for the solutions was determined at 235nm by Elico make UV spectrophotometer.

**Compatibility studies (FTIR spectroscopy)**

FTIR spectra of Doxorubicin hydrochloride, polymers (starch, HPMC) and the microspheres were obtained in potassium bromide pellets a moderate scanning speed between 2000-4000cm$^{-1}$ in Schimadzu FTIR spectrophotometer.

Stability studies were performed using IR spectrophotometer. The IR spectrum of pure drug and physical mixture of drug and polymer were studied. The characteristic absorption peak of Doxorubicin hydrochloride were obtained as wave numbers 1758cm-1, 1600cm-1, 1475cm-1, 669cm-1 (KBr disk). The peak obtained in the spectra of each formulation correlation with the peak of drug spectrum. This indicates that the drug in compatible with the formulation components.

## FORMULATION OF MICROSPHERES

### Solvent evaporation method

Non aqueous solvent evaporation method was employed for the preparation of microspheres. The polymer was dissolved in 20ml of chloroform and stirred until a homogenous solution was formed. Core material Doxorubicin hydrochloride was added to the polymeric solution and mixed thoroughly with magnetic stirrer. The resulting mixture was then added in a thin stream to 100ml of light liquid paraffin contained in a 450ml beaker while stirring at 2000rpm. The solution was stirred for 1.5 hours to allow the solvent to evaporate completely and the microspheres were collected by filtration through whatman's filter paper. The microspheres were washed repeatedly with petroleum ether (40°-60°C) until free from oil. The collected microspheres were dried at room temperature overnight and subsequently stored in desiccators over fused silica gel. The core (Doxorubicin hydrochloride) and the Coat material (starch HPMC) were mixed in the ratio of 1:2 respectively.

## EVALUTION OF MICROSPHERES

### Physical appearance of the drug

Doxorubicin hydrochloride microspheres were physically examined for their colour, odour, shape and size of the particle.

### Determination of Solubility

The solubility of Doxorubicin hydrochloride were determined by shaking an excess amount of drug in measured volume of water, methanol, acetone benzene chloroform, petroleum ether etc., at 25°C for 2 hours.

### Scanning Electron Microscopy

Particle size and shape characteristics can be determined by microscopic method using scanning Electron Microscope. The SEM provides magnifications ranging from 20 X to 30,000 X with a resolution approximately 25 A° to determine detailed particle surface morphology as well as individual particle surface characteristics.

### Percentage of Practical Yield

$$\text{Practical yield} = \frac{\text{Mass of microspheres obtained}}{\text{Total weight of drug and polymer used}} \times 100$$

### Percentage entrapment efficiency of Doxorubicin Hydrochloride Microspheres by UV - spectrophotometer

100 mg of the sample was soaked in 100ml of phosphate buffer solution (pH 7.4). Overnight it is passed through membrane filter. Entrapment efficiency was estimated by measuring the absorbance in UV - Visible Spectrophotometer at 235nm. The entrapment efficiency of the microspheres was calculated from the absorbance obtained by the sample.

The quantity of Doxorubicin hydrochloride present in 100mg of the sample taken was calculated by the formula

$$\% \text{ Entrapment efficiency} = \frac{\text{Calculated drug content}}{\text{Theoretical drug content}} \times 100$$

### *In-Vitro* Dissolution Studies

*In-vitro* dissolution studies are carried out using USP apparatus type II (Lab India, Mumbai, India). The dissolution medium consists of 900 ml of acidic buffer of pH 1.2 and phosphate buffer of pH 7.4 respectively. 100mg equivalent of Doxorubicin hydrochloride containing microspheres were filled in hard gelatin capsules and dissolution test was being carried out at 100rpm at 37°C ± 05°C. Aliquots of 5ml were withdrawn after every 1 hour for the entire period of time. The samples with draws were replaced with fresh dissolution medium equilibrated at the same temperature. The drug released at different time intervals from the dosage form is measured by the UV spectrophotometer by

measuring the absorbance for the sample solution at 235nm for Doxorubicin hydrochloride. The dissolution characteristics of each formula were studied after accounting for the loss in the initial concentration of Doxorubicin hydrochloride while changing the buffer. The release studies for each formulation were conducted in pH 1.2 acidic buffer and pH 7.4 phosphate buffers indicating the reproducibility of the results.

**Release Kinetics**

Various kinetic models were used to describe the *in vitro* release kinetics. The zero order rate describes the system where the drug release rate is independent of its concentration. The First order describes the release from system where release rate is concentration dependent Higuchi (1963) described the release of drugs from insoluble matrix as a square root of time dependent process based on Fickian diffusion.

**Zero Order Kinetics**

**Zero order release would be predicted by the following equation**

At = Ao - Kot when the data is plotted as cumulative percent drug release versus time, if the plot is linear then the data obeys zero- order kinetics and its slop is equal to zero order release constant Ko.

**First Order Kinetics**

**First order release could be predicted by the following equation**

Log C = log Co - Kt/2.303. When the data plotted as log cumulative percent drug remaining versus time, yields a straight line, indicating that the release follow first order kinetics. The constant 'K', can be obtained by multiplying 2.303 with the slope value.

**Higuchi's Model**

Drug release from the matrix devices by diffusion has been described by following Higuchi's classical diffusion equation

Q = [D€/τ (2A-€Cs) Cst] ½ When the data is split according to equation i.e., cumulative drug release versus square root of time yields a straight line indicating that the drug was released by diffusion mechanism. The slope is equal to 'K' O (Higuchi's 1963).

**Stability Studies**

Stability is defined as the ability of a particular drug as dosage form in a specific container to remain with its physical chemical therapeutic and toxicological specifications. The optimized formulation was selected for the stability studies. The purpose of stability testing is to provide evidence on how quality of a drug substance are drug producing varies with time under the influence of variety of environmental factors such as temperature, humidity and light and enables recommended storage conditions retest periods and shelf lives are determined. The formulations were filled with capsules and packed in screw capped bottles and study was carried out by keeping at

25° ± 2°C and 60 ± 5% RH

40° ± 2°C and 60 ± 5% RH

The duration of study was for period of 6 months samples were withdrawn in 0, 3 months and 6 months were analyzed for surface morphology and drug content.

## RESULTS AND DISCUSSION

Doxorubicin is an anti-neoplastic agent which is used in the treatment of various cancerous disease. The half-life of doxorubicin was 1 to 3 hours. The present study was to develop pharmaceutically elegant stable cost effective and quality improved formulation and evaluation of doxorubicin hydrochloride microspheres.

The sustained release microspheres prepared by using HPMC and starch using solvent evaporation method. It was concluded that polymers HPMC and starch are best suited with sustained release microspheres. The UV scanning spectroscopy study of doxorubicin hydrochloride microspheres was 7.4 phosphate buffer and pH 1.2 acidic buffers. It was found that the maximum absorption at 235nm. From the SE analysis the microspheres formulated were spherical with rough surfaces.

**Scanning Electronic Microscopic studies (SEM Analysis)**

Encapsulation efficacy was used as a primary criterion in evaluation of quality of microspheres. The formulation FH showed more efficiency to entrap the drug when compared to the formulation FS. It was summarized from the data and graph obtained that the formulation FH showed release of doxorubicin hydrochloride at 24 hours. The formulations FH and FS showed first order kinetics.

**Table No.1: *In vitro* drug release profile of doxorubicin hydrochloride microspheres**

| S.No | Time (Hours) | % Cumulative Drug Release | |
|---|---|---|---|
| | | Formulation FH | Formulation FS |
| 1 | 2 | 5.06 | 6.28 |
| 2 | 4 | 9.16 | 11.11 |
| 3 | 6 | 13.11 | 16.03 |
| 4 | 8 | 18.24 | 22.68 |
| 5 | 10 | 23.05 | 26.14 |
| 6 | 12 | 33.85 | 39.22 |
| 7 | 14 | 40.01 | 46.61 |
| 8 | 16 | 48.73 | 53.23 |
| 9 | 18 | 53.66 | 57.64 |
| 10 | 20 | 60.53 | 64.79 |
| 11 | 22 | 64.16 | 67.17 |
| 12 | 24 | 66.70 | 72.13 |

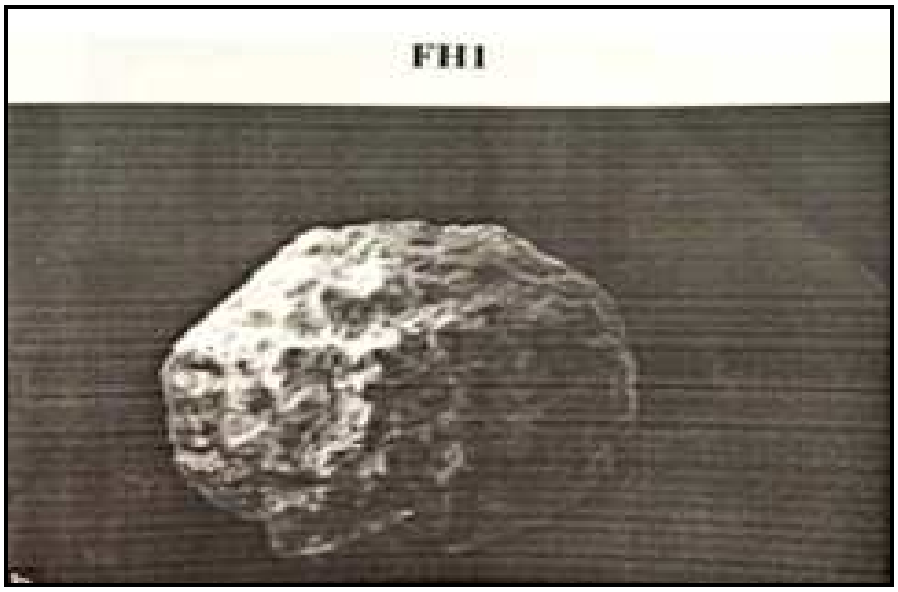


**Figure No.1: SEM analysis of *Doxorubicin Hydrochloride loaded HPMC microspheres***

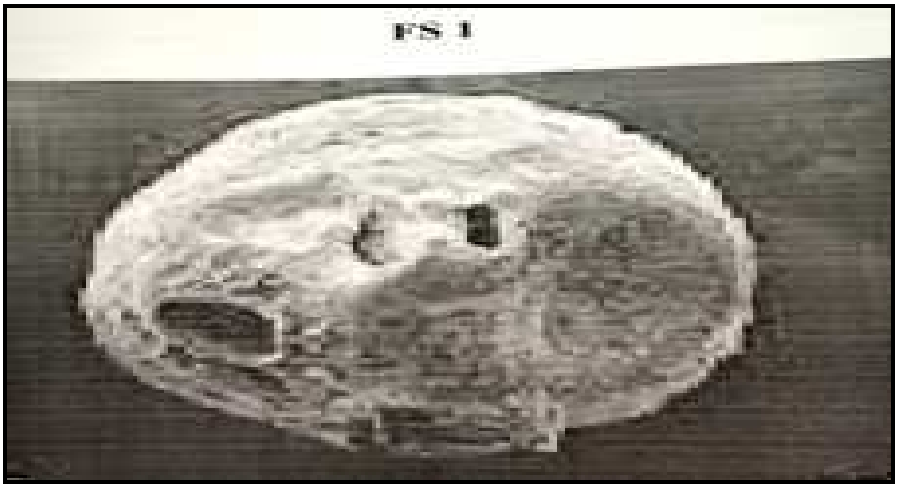


**Figure No.2: SEM analysis of *Doxorubicin Hydrochloride loaded Starch microspheres***

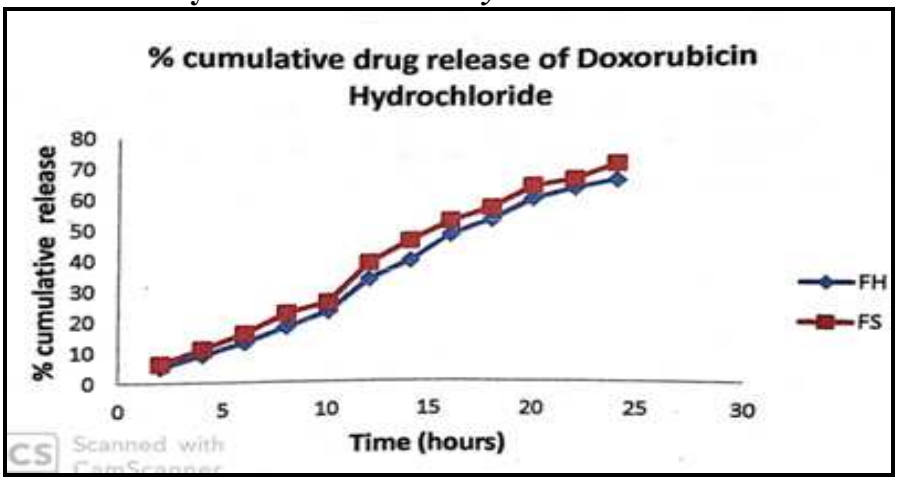


**Figure No.3: Comparative dissolution studies of FH and FS**

## CONCLUSION

The drug doxorubicin hydrochloride was selected for the study because of its availability. Doxorubicin hydrochloride is one of the most widely used anti-cancer agents used in the treatment of various cancers including Breast cancer.

The formulated microspheres were analyzed for various parameters including *in vitro* release and stability studies.

The SEM analysis revealed that the microspheres formulated were spherical in shape with rough surfaces. The percentage practical yield of formulation FH (formulated with HPMC) was found to be 66.70% and formulation FS was found to be 72.13%. The microspheres formulated using HPMC and starch is analyzed for their bulkiness. The bulkiness of microspheres prepared using HPMC was found to be more when compared to that starch.

From the *In vitro* dissolution studies the % release from the formulation FH was found to be 66.70% at 24 hours and that of formulation FS was found to be 72.13% at 24 hours. It was concluded that formula FH was found to be better sustained release when compared to formula FS.

## ACKNOWLEDGEMENT

The authors wish to express their sincere gratitude to Department of Pharmaceutics, RVS College of Pharmaceutical Sciences, Coimbatore, Tamil Nadu, India for providing necessary facilities to carry out this research work.

## CONFLICT OF INTEREST

We declare that we have no conflict of interest.

## REFERENCES


1. Martin A, Swarbrick J, Cammarata A. Physical pharmacy: Physical chemical principles in the pharmaceutical sciences, *Philadelphia (Pa.): Lea and Febiger,* 3rd Edition, 1983, 492-520, 592-638.
2. Patel Samirkumar, Para Chand and Talsania Maulik. Formulation, development and evaluation of microspheres containing duloxetine hydrochloride, *Inter Jour of Res in Pharma and Bio Sci,* 4(2), 2013, 2229-3701.
3. Gabinwang, Shihjungliu, Stevewen Neng, Err-Chengchan. The Release of Cefazolin from biodegradable PLA/PGA beads, *Intern Jour of Pharm,* 273(1-2), 2004, 203-212.
4. Parke E, Maniar M, Shah J C. Biodegradable polyanhydride devices of cefazolin sodium bupiracaine and taxol for local drug delivery preparation - Kinetics and mechanism of *in vitro* release, *Jour of Contro Rele,* 52(1-2), 1998, 179-189.
5. Fallon M, Shafer W, Jacob E. Cefazolin microspheres to treat localized Methicilin resistant staphyloccus aureus infection in rats, *Jour of Surgi Res,* 86(1), 1999, 97-102.
6. Noveen Konda, Arvind G, Sumit Sha, Prashanth P. Formulation and evaluation of non pegylated Doxorubicin liposomal drug delivery system, *Inter Jour of Pharm and Pharma Sci,* 5(2), 2013, 541-547.
7. Jacob Elliot, Cierny George Fallon Michael M C Neill Jaes Sidneys, George S. Evaluation of biodegradable Cefazolin Sodium microspheres for the prevention of infection in rabbits with experimental open tibital fractures stabilized with internal fixation, *Journal of Orthopaedic Research,* 11(3), 1993, 404-411.
8. Kate A Cholewka, Lisa L, Loannides-Demos, Lisa Liolios, Philip Paul, W. John Spicer Allan J Mc Lean. Cephalosporin clinical concentrations -time profile modeling and invitro bactericidal effect as Escherichia coli, *Journal of Antimicrobial Chemotherapy,* 44(4), 1999, 471-476.
9. Chourasia M K, Jain S K. Potential of gaur gum microspheres for target specific drug release of colon, *Journal of Drug Targeting,* 12(7), 2004, 435-442.
10. Shanthi Chalasani, Vanitha Prakash K, Ravi Pratap Pulla, Radhika Tekula, Manasa E and Umashankar B. Development and validation of Doxorubicin Helin bulk and its pharmaceutical dosage form by visible spectrophotometry, *International Journal of Pharma Sciences,* 3(3), 2013, 216-218.
11. Mohini Chaurasia, Manish Chaurasia, Nithin K Jain, Aviral Jain, Vandana Soni, Yaswant Gupta, Sanjay Jain. Crossed linked guar gum microspheres a viable approach for improved delivery of anti-cancer drugs for the treatment of colorectal cancer, *AAPS Pharma Science Tec,* 7(3), 2006, 359-374.


**Please cite this article in press as:** Barish *et al.* Formulation and evaluation of doxorubicin hydrochloride microspheres, *International Journal of Research in Pharmaceutical and Nano Sciences,* 10(1), 2021, 64-70.