International Journal of Scientific Engineering and Research (IJSER)
ISSN (Online): 2347-3878
Impact Factor (2020): 6.733

Design and Characterization of Diltiazem Hydrochloride Sustained Release Microspheres

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Abstract: ***The main aim of this study is to formulate and evaluate the control release Diltiazem micro capsules. The formulation is carried out using liquid paraffin as dispersion medium and poly-caprolactone as polymer by employing evaporation method. The specific speed, medium LP (ml), and the drug to polymer ratio is 1: 1, 1: 2, 1: 3 and 1: 4 are used for each batch were optimized. The droplet coalescence during stirring was prevented by adding magnesium stearate to the medium and the microspheres are solidified and hardened by using n hexane. Scanning electron microscopy and infrared spectroscopy were employed to understand the characteristics of prepared Diltiazem microspheres like drug loading, compatibility properties and micrometric enumeration. The Phosphate buffer of 7.4 pH was used to perform in-vitro drug release study. Various interpretations were done with the prepared Diltiazem microspheres.***

Keywords: Diltiazem microcapsules, microspheres, Liquid paraffin-hexane, scanning electron microscopy

1. Introduction

Sustained Release Delivery [1]
Sustained release is a type of drug delivery system which releases drug in a gradual way over an extended period of time, once after reaching the therapeutic concentration with a single dose of administration followed by the release of medicament continuously with the added incremental dose from the dosage form. However, the external environment plays a vital role in their therapeutic efficacy.

Advantages [2]
- Drugs with narrow therapeutic index can be administered safely.
- Improves patient's compliance and treatment efficiency.
- Drug can be utilized in a better way.
- The side effect of certain drugs can be minimized.

Disadvantages [3]
- Recovering a patient from hypersensitive reactions, poisoning or toxicity.
- Adjusting the dose by physicians.
- Chances of over dose.

Introduction to Microspheres
Microspheres are round, hallow, tiny and discrete solid particles made up of certain protective polymer materials which may be of natural polymers like gelatin and albumin; modified natural polymers like wax, fat, protein, gum and starch; synthetic polymer like polyglycolic acid and polylactic acid; or biodegradable synthetic polymers [**4, 5**].

Advantages of microspheres [6]
- Controls the release of drug in a pre-determined manner.
- Prolongs and provides steady state drug concentration.
- Can also be formulated as injectable dosage forms.
- Avoids the dose frequency.

Factors that influences the preparation of microspheres:
The choice of preparation technique and the selection of solvents and additives depends on the duration of treatment and quantity of drug polymer ratio. Other physio-chemical parameters that controls the microsphere preparation includes reproducibility, drug loading efficiency and the final product quality. The final preparation should be stable, non-toxic and possessing therapeutic efficiency with optimum particle size of the drug.

Techniques for microsphere preparation: [7, 8]

Single emulsion process
This manufacturing technique starts with the preparation of oil in water emulsion using suitable aqueous and organic phase, followed by solidification through extraction or evaporation process. The main disadvantage of this technique is water soluble drugs will have poor encapsulation capacity. Subsequently, the drug gets diffuses from oil to continuous phase. In order to overcome the disadvantage of poor encapsulation oil in oil emulsion method will be employed so as to the drug will get suspend in oil phase. Again evaporation process is continued till the formation of microemulsion.

Double emulsion process:
This manufacturing technique starts with the preparation of solid in oil in water emulsification for drugs that are not suitable for organic solvents or water in oil in water emulsification for hydrophilic drugs followed by evaporation process for the formation of micro particle.

Polymerization techniques
(a) Normal polymerization
This is a pure technique of polymerization that is initiated by heating a mixture of monomer of polymer and drug to be loaded which is finally moulded as microspheres of size less than 100µm. the production of polymer will be faster in this technique.

(b) Interfacial polymerization
In this technique the two immiscible liquid phase containing mixture of monomers which are allowed to react to form a film of polymer to envelop dispersing phase and a emulsified monomer in continuous phase. These two process leads to the formation of monolithic carrier.

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Phase separation coacervation techniques:
This method produces a reservoir type system which separates two immiscible liquid phase from micromolecular solution. Here, one incompatible polymer will be added to the polymer solution containing drug so that the phase separation occurs and drug particle gets separated and forms microspheres.

Spray drying:
This preparation method is accompanied by spray drying and atomization where the drug will be added to a polymer solution and mixed with volatile organic solvent and homogenized. The homogenized final liquid is then atomized through a pre-determined automiser on a good surface, where the volatile matter is evaporated under room temperature and leading to the formation of microspheres which was harvested by scrapping.

Solvent extraction:
In this technique the microsphere formation is done by removal of organic solvent by fixing a suitable temperature so that the water or aqueous phase gets removed from the mixture of phases.

Pharmaceutical application of microsphere [9, 10, 11, 12, 13, 14]
a) Topical porous microspheres
b) Targetting drug delivery system
c) Oral drug delivery system
d) Nasal drug delivery system
e) Monoclonal antibodies mediated microspheres targeting improves the antibody's compatibility and efficiency with other salts.
f) Protects vaccine against toxins or any other microorganisms.
g) Implantable devices
h) Imaging of lung tumours
i) Gastro-retentive drug delivery system

Plan of Work
The present study covers:
1) Preparation of microspheres by solvent evaporation technique.
2) Optimization of process parameters.
   a) Speed of the propeller
   b) Volume of the medium
   c) Concentration of polymers solution
   d) Amount of magnesium stearate used.
   e) Evaluation of Diltiazem microspheres by following study.
   f) Percentage yield
   g) Particle size enumeration
   h) Entrapment efficiency
   i) In-vitro drug release study
   j) Release kinetics-mechanism of drug release from microspheres.
   k) Scanning electron microscopy (SEM)
   l) Fourier transform infrared spectroscopy (FTIR)

2. Materials and Equipments

All chemicals used in the study were procured from standard source. The equipment and instruments used here is calibrated and established protocols were followed to conduct the experiments.

Equipments

Equipment	Source/ Model:
1. Spectrophotometer	Shimadzu UV-1800
2. Dissolution apparatus	Electro lab-dissolution apparatus, Mumbai. TDT-06P
3. Sonicator	Pci, 1.5 Lit 50, Mumbai
4. Propeller	Remi equipment, Mumbai
5. Cyclo Mixer	Remi equipment, Mumbai
6. Shaker	Remi equipment, Mumbai
7. Digital Balance	Shimadzu AUY 220

Materials

Chemical	*Source/ Supplier*
Sodium hydroxide	Finar chemicals, Ahmedabad.
Potassium dihydrogen phosphate	Finar chemicals, Ahmedabad.
Magnesium stearate	Qualikem Fine chemicals, New Delhi.
Dichloro methane	Finar chemicals, Ahmedabad.
Poly-(Ɛ-caprolactone)	Sigma Aldrich, Germany.
Diltiazem hydrochloride	Ranbaxy laboratories Pvt. Ltd., Hyderabad.

Standard graph of Diltiazem HCl in pH 7.4 phosphate buffer: [17]
The stock solution was prepared by accurately weighing 10 mg of Diltiazem which is dissolved in 10ml of 7.4 pH phosphate buffer. From the above stock solution 1ml was taken and made up to 10ml with the same phosphate buffer and treated as working standard. From here the serial dilution were made by taking 1, 2, 3……. .9 and 10ml and transferred to separate test tubes having the concentrations of 2µg, 4µg, 6µg……. .18µg and 20µg/ml by diluting upto the mark and these solutions were screened by spectrophotometer at 237 nm for its absorbance. Finally the absorbance versus concentration was plotted in a graph.

Preparation of Diltiazem Hydrochloride Microsphere: [18]
Emulsion evaporation method was employed to prepare microspheres. A polymer solution was prepared by dissolving polycaprolactone of weighed amount in 15 ml of Dichloro-methane and then, 50 mg of magnesium stearate and diltiazem hydrochloride of weighed amount is added to it. The prepared polymer phase is added to 270 ml of light liquid paraffin and this is rotated at a speed of 1000rpm for 4 hours continuously for formation of discrete rigid microspheres. Finally, the microspheres are filtered and air dried and repeatedly washed with n-hexane. The microspheres were collected and kept in desicator for further use.

The drug polymer ratio used for the study is shown in the below table.

S. No.	Drug polymer ratio	Mgs (mg)	Medium LP (ml)	Speed (rpm)
1	1: 1 (F1)	100	270	1000
2	1: 2 (F2)	100	270	1000
3	1: 3 (F3)	100	270	1000
4	1: 4 (F4)	100	270	1000

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Characterization of Diltiazem microsphere

Particle size:
The optical microscopy method is used to determine the mean particle size of prepared microspheres. Before starting the evaluation process, the eye piece of microscope was calibrated with the stage micrometer for its perfection in report. The microspheres were observed by mounting them on a slide. The diameter of 200 microspheres was calculated from each batch prepared and the photos were taken for visualizing the shape of them.

Encapsulation efficiency / Drug content determination:
Sonication was done for microcapsules with drug for 20 minutes that are suspended in water after making as a powder. For extracting the drug from micro capsules, the resultant suspension was shaken for 20 minutes using Rotatory shaker. The final extract solution is passed through a Millipore of 0.45 µm. The UV-spectrophotometer at 237nm was used to determine the drug content in the extract. The encapsulation efficiency of the microcapsule was calculated using the following formula,

$$Encapsulation\ efficiency = \frac{Actual\ drug\ content}{Theoritical\ drug\ content}\ X\ 100$$

Scanning electron microscopy (SEM): [16]
Scanning electron microscope was used to determine the morphological character of prepared microspheres. This study is done only before microspheres dissolution. Prior to microscopic examination, the samples were coated using gold palladium to a thickness of 200 Å.

In-vitro drug release studies:
A USP dissolution study apparatus containing 900ml of 7.4pH phosphate buffer is taken and it is thermostatically maintained at 37± 0.5 ˚C, operated at 100 rpm, 100mg of microcapsule was cautiously placed into the dissolution medium, periodically 5ml of the sample was withdrawn from the dissolution medium and it was replaced with same 5ml of buffer at specific time interval. Then the drug release is studied using slope value of standard calibration curve.

Kinetics of drug release:
The drug release mechanism of prepared micro capsules was calculated by using various kinetic equations with the help of data obtained from in-vitro drug release study. The kinetic models used were;
Zero order equation

$$Q_t = K_o\, t$$

First order equation

$$= \ln Q_0 - K_1 . t$$

Higuchi equation based on Fickian diffusion

$$Q_t = K . S . \sqrt{t} = K_h \sqrt{t}$$

Peppas and Korsenmeyer equation

$$Mt/M_\infty = k\ .\ t^n$$

Where,
t = time
Q_t = amount of drug release in time t
K_0= Rate constants of zero order equation
K_1= Rate constants of first order equation
K_h= Rate constants of higuchi rate equation
S = Surface area of microsphere.
Mt = Amount of drug release at time t
M_∞= Amount of drug release at time ∞

FTIR studies: [19]
The FTIR spectroscopy is used for studying the interaction between drug and the polymer for the compatibility of drugs with excipients. The method employed in this study is pellet method. Here, the potassium bromide is used to make pellet with drug a disc using dies. Finally, in the spectrophotometer, the discs were placed for recording the spectrum of drug loaded microspheres placebo microspheres, polymer and drug.

3. Results and Discussion

Standard graph of Diltiazem HCl in pH 7.4 phosphate buffer:
As per the procedure, a standard graph was plotted using absorbance and concentration in (µg/ml). A straight line was obtained with R^2 value of 0.999 and the line equation was

$$y = 0.052x + 0.008$$

Concentration (µg/ml)	Absorbance
2	0.116
4	0.229
6	0.321
8	0.439
10	0.525
12	0.628
14	0.730
16	0.840
18	0.952
20	1.062

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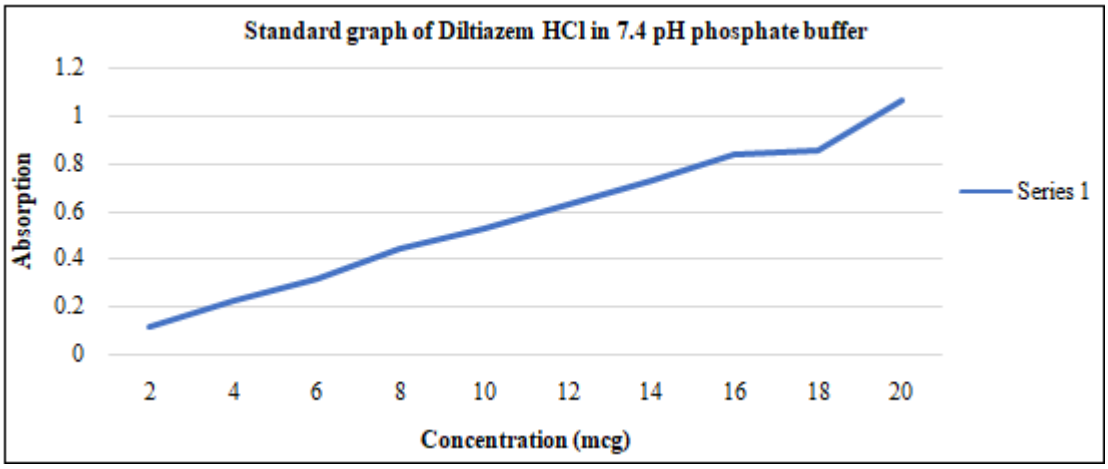

Results of optimization of formulation variables: [15]

In our study, the entrapment efficiency of Diltiazem was increased by formulating as solvent evaporation technique, where to the oil phase another oil phase with drug is dispersed. Following to it, evaporation and separation is done using *n hexane*. Microspheres are produced using light liquid paraffin (starting with 100 mL and increased to 270 mL) as dispersion medium. The volume of dispersion medium is increased to produce proper spherical shape to the microspheres. As there will be no formation of microspheres under 500 rpm and produces irregular shapes for more than 1000 rpm, the stirring speed was optimized to 800-1000 rpm. The droplet coalescence during stirring was prevented by adding 100 mg of magnesium stearate to the medium. The microspheres are solidified and hardened by using *n hexane*.

Encapsulation efficiency (EE):

By increasing the concentration of polymer in the preparation the entrapment efficiency of the drug was increased. Based on experimentation with different drug polymer ratio, the entrapment efficiency of Diltiazem was found to be 57-73% and concludes that more the polymer concentration more the entrapment efficiency is and entrapment efficiency is also depending on other phenomenon like batch size and stirring rpm.

S. No.	Drug polymer ratio	Mgs (mg)	Shape	Medium LP (ml)	Speed (rpm)	% yield Mean ± SD	% EE Mean ± SD
1	1: 1 (F2)	100	Spherical	270	1000	67.09 ± 1.36	57.42 ± 1.95
2	1: 2 (F2)	100	Spherical	270	1000	72.32 ± 2.25	62.50 ± 0.95
3	1: 3 (F3)	100	Spherical	270	1000	79.12 ± 0.14	69.06 ± 1.02
4	1: 4 (F4)	100	Spherical	270	1000	82.4.72 ± 3.65	67.09 ± 1.36

Particle size:

The optical microscopy method is used to determine the mean particle size of prepared microspheres. Based on the observations obtained after the study, it is concluded that the size of emulsion droplets and polymer concentration increases with increase in viscosity to produce a higher microsphere size.

S. No.	Formulation (code)	Mean ± SD (µm)
1	F1	± 1.36
2	F2	67.09 ± 1.36
3	F3	67.09 ± 1.36
4	F4	67.09 ± 1.36

Morphological study of Diltiazem microsphere: [16]

Scanning electron microscope was used to determine the morphological character of prepared microspheres. Based on observation under SEM the microspheres are found to have rough porous surface and exhibit to produce spherical shape. Hence, concluded that the mechanism of the prepared Diltiazem microspheres is found to be diffusion controlled. The observation of various formulations prepared were shown in the figure1, 2, 3, 4 and 5.

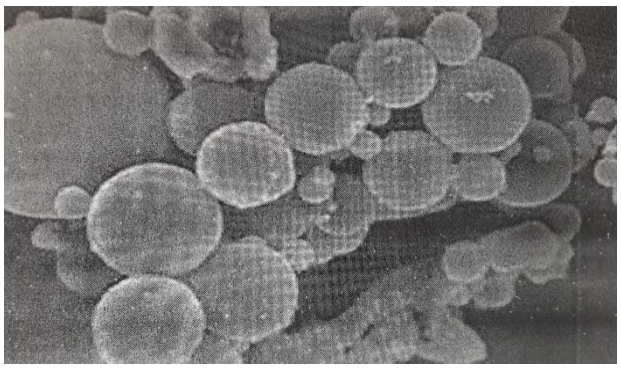

Figure 1: SEM photography showing the surface morphology of the microspheres.

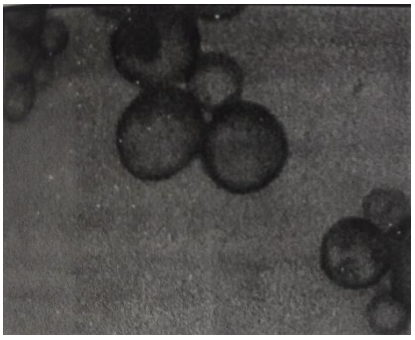

Figure 2: F1 Formulation of Microspheres

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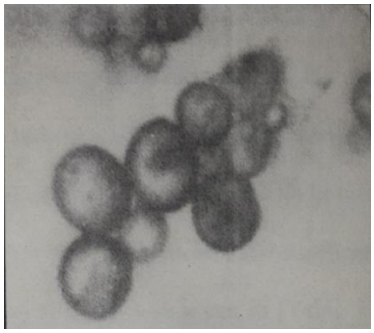

Figure 3: F2 Formulation of Microspheres

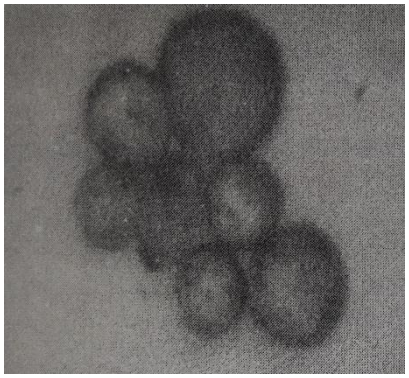

Figure 4: F3 Formulation of Microspheres

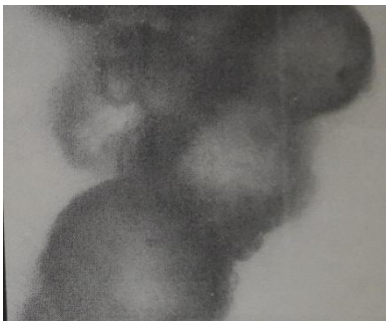

Figure 5: F4 Formulation of Microspheres

In-vitro Drug release studies:

As the surface of microspheres consist of drug particles the Diltiazem microspheres exhibits bust effect I in-vitro drug release study. However, the first plasma concentration of Diltiazem is achieved with the initial bust effect and the release of drug is governed by polymer drug ratio. From the results obtained, it is concluded that the increase in polymer concentration decreases the rate of drug release. Out of various kinetic models calculated, the highest correlation obtained were mentioned in table.

Time (Hrs)	F1	F2	F3	F4
0	0	0	0	0
1	6.82	9.03	8.73	6.53
2	11.23	1.61	12.54	9.76
3	19.61	21.43	19.76	13.53
4	23.41	29.78	25.69	17.82
5	27.72	38.34	32.72	23.8
6	31.43	47.84	39.83	27.67
8	39.64	55.34	43.72	35.87
10	47.38	67.28	52.37	40.3
12	55.42	73.65	63.41	54.6
16	69.43	81.34	71.73	65.6
20	80.423	95.27	83.46	78.29
24	96.34	---	90.47	86.24

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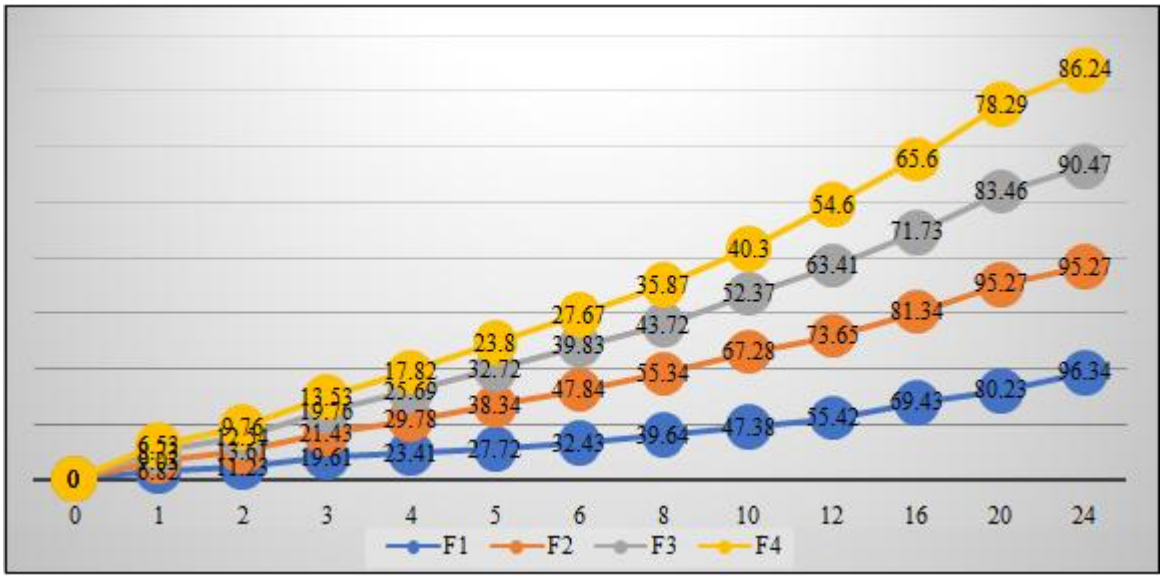

Fourier transforms infrared radiation measurement (FTIR)

The stability of Diltiazem microspheres was best fitted with absorbance peak of 1366 cm^{-1} for ether, 1725 cm^{-1} for carbonyl group of placebo microspheres and absorbance peak of carbonyl group at 1710 cm^{-1}. The spectrum peak points of Diltiazem microspheres during FTIR is listed in the below table

Formulation	Group	Wavelength (cm^{-1})
Diltiazem HCl	-c=o -c-o -c-N -c-S	1725 cm^{-} 1366 cm^{-1} 1046-1187 cm^{-1} 732 cm^{-1}
Diltiazem microspheres	-c=o -c-o -c-N -c=H -c-S	1720 cm^{-1} 1375 cm^{-1} 1172 cm^{-1} 2944 cm^{-1} 739 cm^{-1}

4. Conclusion

Results of the present study demonstrate that the prepared microcapsules prolong the drug release up to 24 hours. This can be expressed to reduce the frequency of administration and decrease the dose-dependent side effects associated with repeated administration of conventional Diltiazem tablets. The in-vitro dissolution study also showed that the drug release was mainly depends on the polymer proportion in the formulation. The FTIR of the present study clearly indicated that there are no drug and excipient interactions. Microscopic study of the prepared microcapsules (optimized formulation) showed the spherical shaped microspheres can be encapsulated as a capsules form for oral administration. Further in-vitro and in-vivo correlation studies are said to be essential to have better dosage form in future.

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