



“Formulation And Evaluation Of Combinational Drug Therapy Against Osteoarthritis”

PUSHPENDRA KUMAR¹, MUSKAN MISHRA¹, SWATI SAHU¹, ANISH KUMAR², DAMINEE³

¹APOLLO COLLEGE OF PHARMACY, DURG (491001), INDIA

²ATHRAVA COLLEGE OF PHARMACY, DURG

³SHRI RAWATPURA SARKAR IINSTITUTE OF PHARMACY, DURG

Abstract

Osteoarthritis is the most common form of joint disease and the knee is one of the most commonly affected joints.

Nanosphere is one of multiparticulate drug delivery system and prepared to obtained prolonged, controlled drug delivery to improved bioavaibility or stability and to target drug to specific site. Which enhance the therapeutic efficacy of drug.

Once such approach is using nanosphere as carrier for durg ,the target site drug deliver with specificity and controlled way in to the targeted site of body without any untoward effect. Drug can be targeted to specific site in body using nanosphere .The degree of targeting can be achived by localization of drug to a specific area in body(for example heart, lung etc.) to a particular group of cell(for example tumer cell.

The nanosphere are used in targeted drug delivery system we will improved the specificity of drug and reduce adverse effect, side effect ,toxic effect of drug and enhance the potency of drug such as chemotherapeutic agent and various cyto-toxic drug etc.

Key words:- Osteoarthritis , Diacerein, vitamin D3(cholecalciferol), Nanosphere,Evaluation of Nanospheres.

1.Osteoarthritis -

Osteoarthritis is the major chronic disease causing disability[1] Patients suffering from it feel pain and become unable to walk or work[2]. Mostly affected joints were hip, knee, hand and foot.[3]

Osteoarthritis is degenerative disease of joints involving cartilage damage and also damage to surrounding tissues.[4]Damage to articular cartilage, weakening of muscles and synovial inflammation may also be involved.[5]



Fig. Osteoarthritis

1.1 Classification of arthritis:[6]

- Hip Osteoarthritis:
- Knee Osteoarthritis:
- Hand Osteoarthritis:
- Foot Osteoarthritis

1.1.1 Hip Osteoarthritis:[7]

Hip arthritis is less common form of osteoarthritis.[8]It is 1.4% in Asia, 2.8% in Africa,[9] 10.1% in Europe and 7.2% in North America. In Korean population older than 65 hip osteoarthritis is found 2 % (15/686)[10]. In another recent study it showed that 19.6% in radiographic and 4.2% in symptomatic.[11]It also showed that men have higher prevalence in men in radiographic not symptomatic osteoarthritis.[12]Radiographically hip osteoarthritis is associated with the increased risk of mortality by all causes and also by cardiovascular diseases I white women.[13]However investigations by another study showed that there is no significance correlation.[14] Further studies are required in this regard.[15]

1.1.2Knee Osteoarthritis:[16]

Knee osteoarthritis according to Framingham study prevalence of knee osteoarthritis diagnosed by radiographic in participants above 45 years is 19.2%and above 80 years figure[17]reached to 43.7%.¹⁸ Dutch public health institute data shows that knee osteoarthritis in men is 15.6% and 30.5% in women above age of 55 years.[18]Another radiographic study in Korea showed knee osteoarthritis up to 38 % (265/696) in individuals

above 65 years of age.[19]Obesity was related to it. In Mlmo and Sweden among age of 56 to 84 years prevalence of radiographic knee osteoarthritis was 25.4% and symptomatic knee osteoarthritis was 15.4%.[20]

Knee osteoarthritis and frailty are associated with function limitation problems with the age [21]Frailty is found more associated with the knee osteoarthritis as compared to other types of osteoarthritis.[22]Data from china indicated that prevalence of knee osteoarthritis is two or times greater than Framingham osteoarthritis studies.[23] Five phenotypes are identified in knee osteoarthritis depending on clinical characteristics.[24]

These phenotypes are

- 1) Minimal joint disease phenotype,[25]
- 2) Strong muscle strength phenotype,[26]
- 3) Severe radiographic osteoarthritis phenotype,[27]
- 4) Obese phenotype,
- 5) Depressive mood phenotype.[28]

1.1.3 Hand Osteoarthritis:[29]

Symptomatic hand joint osteoarthritis is defined as if you have both symptoms and radiographic evidence for the presence of osteoarthritis.[30]In the survey conducted the presence of symptomatic osteoarthritis of hand was higher in women (26.2%) as compared to men (13.4%) in subjects aged between 72 to 100 years.[31]In another study it is reported that hand osteoarthritis is found more in women (67%) compared to men (54%) for at least one hand in subjects of age above 55 years.[32]

1.1.4 Foot Osteoarthritis[33]

By data it is evident that many of the obese people suffer from foot osteoarthritis or they are at risk of developing it.[34]A detailed review has also been published on foot osteoarthritis which stated that most studies are focused on radiographic study and its prevalence is between 0.1 to 61 % depending on age, sex and joints under focus.[35]In England a postal survey from adults of 50 or above resulted 72% response out of 26,705 mails and approximately 50% were suffering with disabling osteoarthritis of some kind out of four joints i.e., hip, knee, hand or foot.[36]

1.2 Risk Factors for osteoarthritis:

Person level risk factors

- 1) **Age-** Age is major risk factor for the arthritis.Increase in age is positively related to risk of osteoarthritis.[37]
- 2) **Sex-**Osteoarthritis is more prevalent in females as compared to males.[38]
- 3) **Socio-economic status-**There is increased risk of disease with lower socio-economic status.[39]

4) **Genetic Factor**-Hereditary character is considered very strong in development of osteoarthritis. Identification of genes associated with osteoarthritis can help to understand it further.[40] Studies showed that micro RNA miR-127-5p under expression was observed in knee osteoarthritis and there was also an increased expression of MMP-13.[41]

5) **Obesity**-Obesity is observed as a major factor in all the cases.[42] Obesity increases the osteoarthritis risk in both knee and hand.[43] Adipokines are known to disturb the homeostasis of joint tissues increasing the chances of osteoarthritis.[44] Adipokines are secreted by adipose tissues. With 5 kg weight loss, a 50% decrease in risk of symptomatic knee osteoarthritis was observed. Reduction of systemic levels of inflammation (C-reactive protein (CRP) and interleukin-6 (IL-6)) in obese people were achieved by loss of 5 kg body weight. According to study, body fat and waist to hip ratio is associated with the knee osteoarthritis, however weakly associated with hip osteoarthritis.[45]

6) **Bone mass and Bone mass**-High bone mass is directly linked with the osteoarthritis of hip and knee, and high bone mass is associated with bone sclerosis, so a hypertrophic phenotype will be suggested.[46]

7) **Smoking**-Smoking protects from the osteoarthritis according to some studies, but another study doubts and states that this may be false.[47]

1.3 JOINT ASSOCIATED RISK

1) Bone/Joint Shape:

A recent study shows that proximal shapes were different among osteoarthritis and normal persons among males. Reduced femoral offset,[48] more valgus neck shaft angle, increased hip height center and increased abductor angle are found associated with osteoarthritis. Mild and moderate osteoarthritis grades were associated with dorsal wedging of the third tarsal bone, while plantar wedging was associated with severe cases.[49] Subcondral bone early changes may predict the phenotype of osteoarthritis and help the clinician.[50]

2) Injury:

Injury is one of the most important risks for the osteoarthritis, especially for the knee, causing meniscal damage, rupture of the anterior cruciate ligament, or direct injury of articular cartilage.[51] In a study, the knee osteoarthritis was high among patients with reconstructed cruciate ligament injury.[52] Occupational knee osteoarthritis and previous history of knee injury are two most important factors.[53] Patients with reconstructed cruciate ligaments must manage weight, avoid excessive loading, to remain protected from osteoarthritis.[54]

3) Muscle Mass and Muscle strength:

Muscle weakness may cause osteoarthritis.[55] Specific role of muscle mass and muscle strength is still unclear. Women with more fat in intramuscular areas were found having pain.[56]

4) Joint load and alignment:

Knee alignment is the main prediction sign for the osteoarthritis.[57-60] Most of the literature suggests that knee alignment is very important with regard to osteoarthritis.[61-65] But still the findings are not

regular. Malalignment of metatarsophalangeal joint was linked with the osteoarthritis of metatarsal joint as well as knee joint and hip joint osteoarthritis.[66]

5) Occupation and Activities:

Previous studies showed that occupations involved in extreme pressure[67] and joint activity are linked with [68]osteoarthritis while moderate level activity is not linked with it. About hip osteoarthritis and occupation a comprehensive study is also present.[69-71]

6) Other joint level risks:

Leg length inequality was also reported to have some link but more study on it is needed.Crepitus in women was associated with the osteoarthritis.[72-75]Infrapatellar fat pad was also significantly and beneficially associated with the knee osteoarthritis.[76-79]

1.3 NANOSPHERE -

Nanospheres are matrix particles whose entire mass is solid.[80]These spherical particulate systems are characterised by a size between 10-200nm in diameter.

Nanospheres can be biodegradable or non-biodegradable. Some of the biodegradable Nanosphere includes modified starch nanospheres, gelatin Nanosphere, polypropylene dextran Nanosphere, albumin Nanospheres and polylactic acid nanospheres. They can also be crystalline and amorphous. The administration of medication via these systems offers high advantageous since they can be ingested or injected. Also site specific delivery of drugs can be used for organ targeted release of drugs.[81]

The main objective for designing the nanospheres as a targeted drug delivery system is to:

- Control the particle size.
- Release of therapeutically active agents to achieve the site specific action at the therapeutically optimal rate and dose regimen.[82]

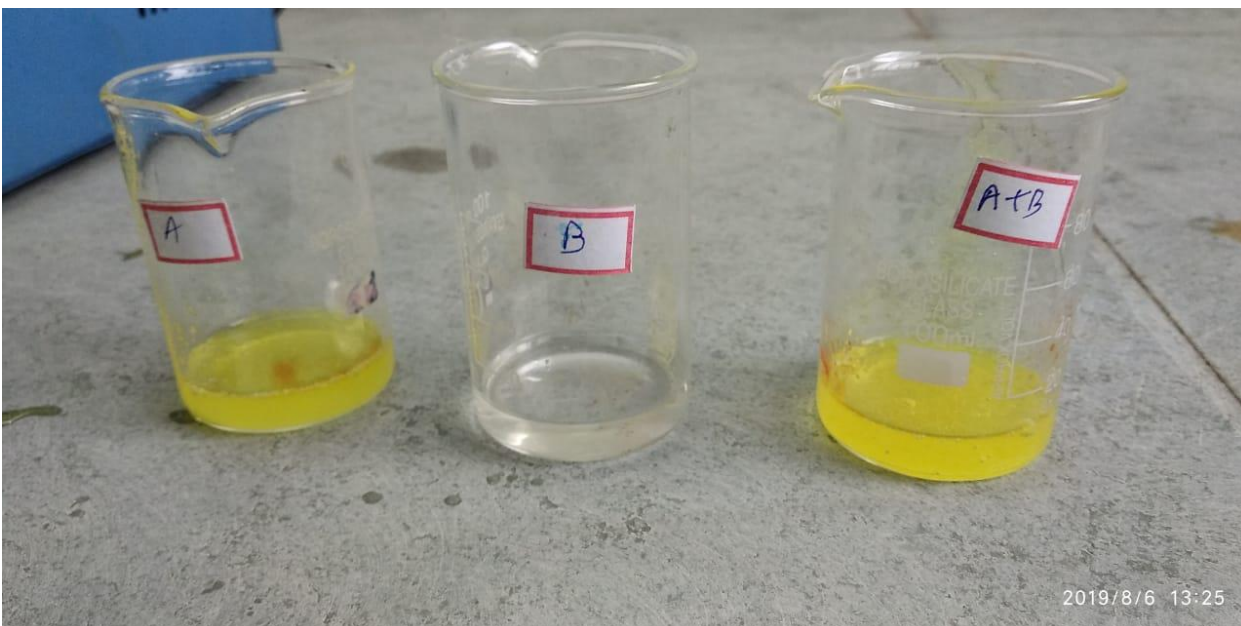


Fig.Nanosphere(sample A diacerein ,B Cholecalciferol,A+B diacerein+cholecalciferol)

1.3.1 ADVANTAGES OF NANOSPHERES

- ✓ Due to their ultra tiny volume the nanospheres can easily pass through the smallest capillary vessels.
- ✓ Nanospheres can be used to target the organs like liver, spleen, lungs, spinal cord as they easily penetrate the cells and tissue gap.[83]
- ✓ The duration in the bloodstream can be prolonged since they avoid rapid clearance by phagocytes.
- ✓ Nanospheres can be formulated for controlled release action.
- ✓ Reduction of toxicity.
- ✓ They can be administered via oral, nasal, parenteral route.
- ✓ Site specific targeting by attaching the ligands to the surface of the spheres.[84]

1.3.2 DISADVANTAGES OF NANOSPHERES

- It is difficult to handle nanospheres in liquid and dry form.
- They are prone to particle aggregation due to smaller size and larger surface area.

1.3.3 POLYMERS USED TO DESIGN NANOSPHERES

Various kinds of polymers are used to prepare the polymeric nanoparticle including nanospheres.[85] Both biodegradable polymers and their co-polymers such as Di-block, Tri-block, and multi-block or radial block copolymer structures have been used to prepare them and to encapsulate the active ingredients. Also the polymer must be compatible with our body i.e.; they should be non-toxic and non-allergent. The polymer of natural origin used for the preparation of nanospheres include albumin, gelatin, sodium alginate, chitosan.[86]Also synthetic polymers can be used such as poly(lactide co-glycolide) (PLGA), poly (methyl methacrylate), polyethylene glycol etc.

1.3.4 MECHANISM OF DRUG RELEASE

The delivery of the drug at the tissue site from these drug carriers can be achieved by:

- Rupture or degradation of polymer at the site of delivery due to enzymatic degradation which result in release of drug from the entrapped inner core.
- Swelling of polymer by hydration resulting in release of drug (figure 1)[87]
- Dissociation of drug from the polymer causing subsequent release of drug from the swelled polymeric nanoparticle.

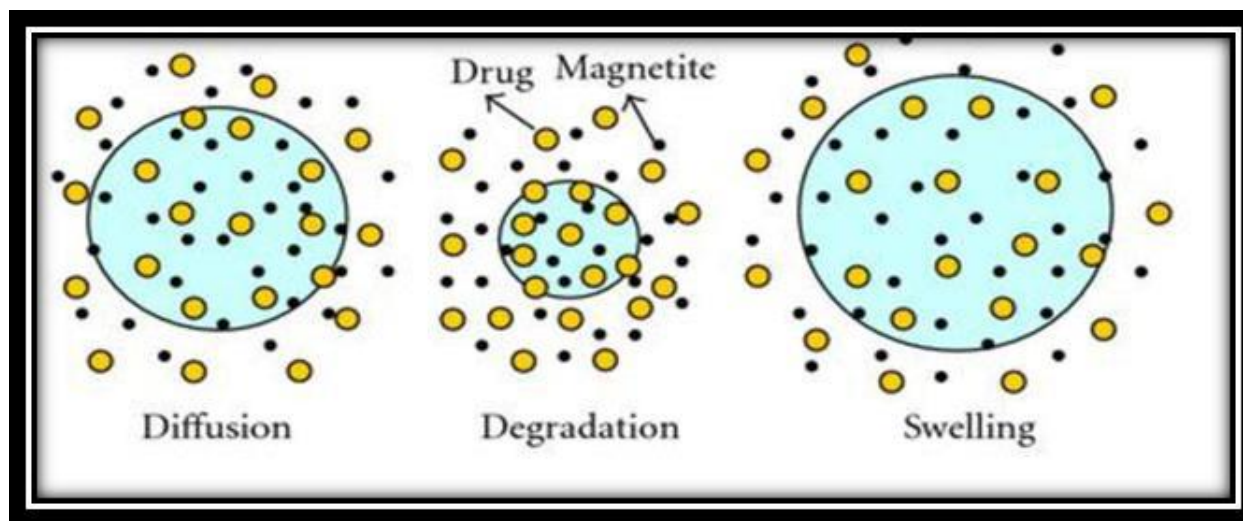


Figure 1: Mechanism of drug release.

1.3.5 PREPARATION METHODS FOR NANOSPHERES

Various preparation methods can be adopted for the preparation of nanospheres. The different methods include:

- Polymerisation(Emulsification polymerisation)
- Solvent displacement method (nanoprecipitation method)
- Solvent evaporation method
- Salting out
- Controlled gellification method
- Desolvation technique
- Ionic gelation method

1.3.5.1 Polymerisation method[88]

Generally it includes interfacial polymerisation and emulsification polymerisation. In this method polymer like polymethylmethacrylate and polycyanocrylate are emulsified i.e. emulsification polymerisation and interfacial polymerisation of polyalkylcyanoacrylate. Monomers are polymerized to form the nanospheres in an aqueous solution. After the completion of the polymerisation, the drug can be incorporated by dissolving in the polymerization medium or via adsorption onto the nanospheres (Figure 2). The obtained nanospheres are purified, centrifuged and finally freeze dried.[89]

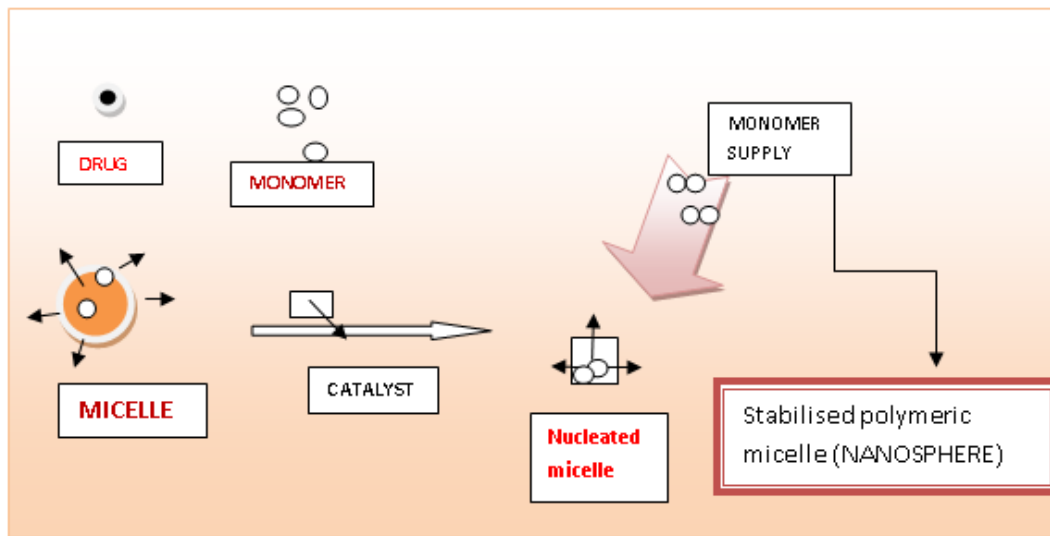


Figure 2: Polymerisation

1.3.5.2 Solvent displacement method

The solvent displacement method is also known as nanoprecipitation method. This method is based on displacement of the semi polar solvent followed by the interfacial deposition of a polymer. (Figure 3). The technique involves dissolving polymer in a water miscible solvent(organic). This solution is then added to an aqueous phase in the presence or absence of a surfactant which can induce precipitation of polymer and thus formation of nanospheres can occur.

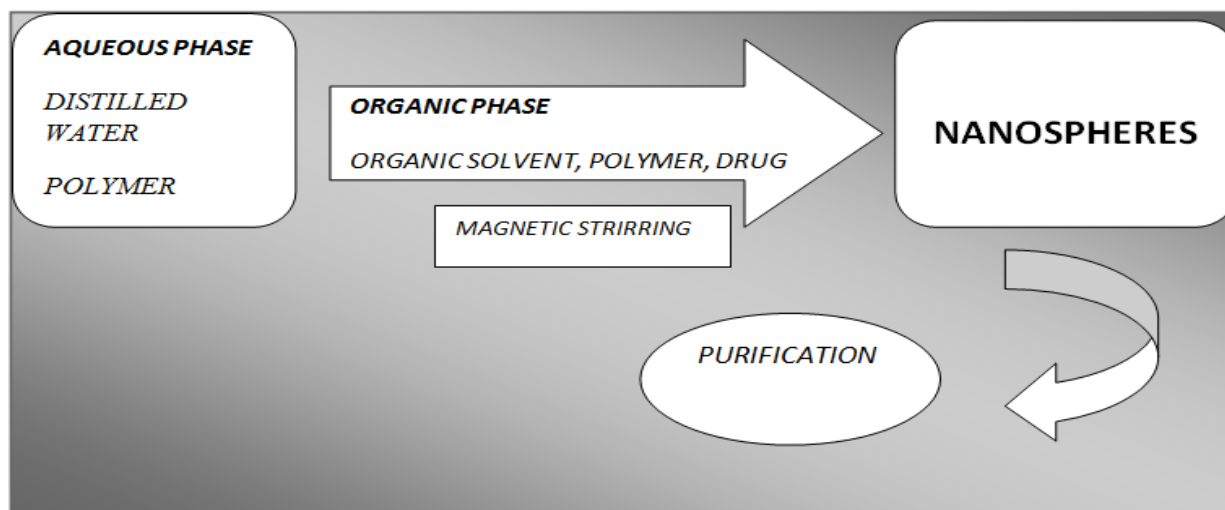


Figure 3: Solvent displacement.

1.3.5.3 Salting out

The polymer is dissolved in a suitable organic solvent. This is then added to an aqueous phase (that contains suitable emulsifier and high concentration of salts) under mechanical shear stress to induce emulsification. The salts used include magnesium chloride hexa hydrate (60% w/w) or magnesium acetate tetra hydrate (1:3 polymer

ratios). Pure water is then added to the formed o/w emulsion under mild stirring to enhance diffusion of organic solvent into aqueous phase to form nanospheres. Finally purified by centrifugation or cross flow filtration to remove salting out reagent.[90]

1.3.5.4 Controlled gellification method

This method is used to prepare sodium alginate nanospheres. Suitable amount of calcium chloride is added to sodium alginate solution to induce gellification. Then to this add poly-L-lysine in order to form a polyelectrolyte complex. The obtained nanospheres suspension was then stirred for 2hr. Finally the nanospheres are separated via centrifugation.[91]

1.3.5.5 Desolvation technique

The desolvation technique can be used to prepare nanospheres from natural polymer like albumin. Here the polymeric solution was prepared with polyethylene glycol solution (PEG). The drug after mixing dissolving with ethanol was added drop wise to prepared polymeric solution under magnetic stirring. After that suitable cross linking agent is added and cross linking process is continued for about 12hr. Finally the obtained suspension was centrifuged and lyophilized.[92]

1.3.5.6 Ionic gelation method

Ionic gelation or coacervation method can be used to prepare nanospheres from natural polymers like gelatin, sodium alginate, chitosan. In this method the aqueous solution of polymer and drug is taken. Due to the electrostatic interaction of the two aqueous phases, they form a coacervate having the particle size in nanometer range.[93]

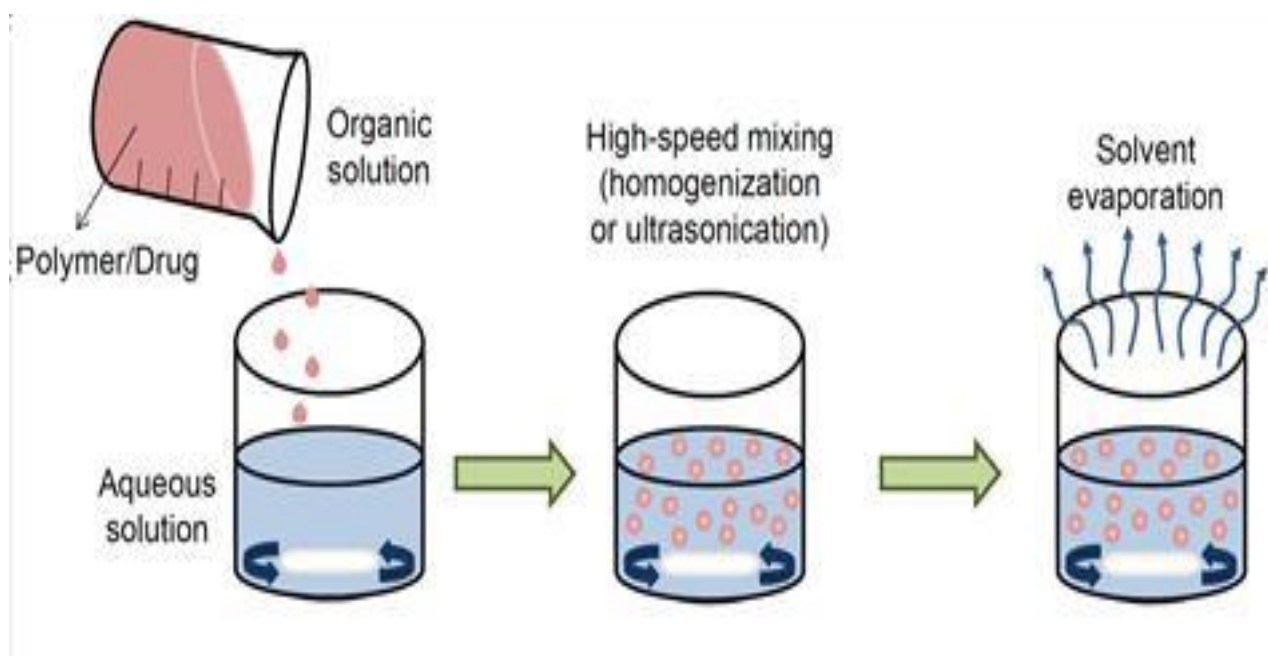


Figure : solvent evaporation

2. LITERATURE REVIEW

- 1) Ibrahim Niba et.al(2018) reported in their study that The combination of nanotechnology and polymers are extremely useful in various applications which led to development of polymeric nanoparticle like Nanospheres.
- 2) Nirupama M.P.et.al(2018) experimented in their study that Nanosphere Lithography is economical and simple process for preparation of Nickel (Ni) Nanoparticle Arrays on plain glass, TCOcoated glass and silicon substrates.
- 3) Murata Yuki et.al(2018) experimented in their study that statistical data were expressed as the mean \pm standard deviations.
- 4) HamidReza Omraniet.al(2018) studied in their study Cholecalciferol in high doses can be administered to control secondary hyper parathyroidism and vitaminD(25OH) deficiency in hemodialysis patients .
- 5) Kesharwani Adarsh et.al(2017) studied in their study Solubility is one of the important parameters to achieve desired concentration of drug in systemic circulation for pharmacological response.
- 6) Girish C.Mohan et al(2017) reported in their study diacerein significantly opposes pro-atherogenic and pro-inflammatory effects of IL-1 on multiple genes in ECs and KCs.
- 7) Claudia R.L. Cardoso.et al(2017)studied in their study Diacerein reduced mean HbA1c levels, with peak of effect at the 24th week of treatment.
- 8) Qiong Wang et. al (2016) experimented in their study that The as-prepared hollow ZnSnO₃ nanospheres have an average diameter of 120 -150nm and the shell thickness is about 20nm. It was found that the sensor, based on hollowZnSnO₃ nanospheres, showed high response, good selectivity, and stability as well as fast response and recovery time toward ethanol gas at a low temperature, suggesting the potential applications as advanced gas-sensing materials.
- 9) Miriam Méndez-del Villar.et.al(2016)) experimented in their study that All patients who were eligible after enrollment completed the 90 days of the pharmacological intervention.
- 10) Gupta Khemchand et.al(2016) experimented in their study that β -cyclodextrin is a suitable carrier for the preparation of Diacerein solid dispersions. crystalline to an amorphous form which is responsible for the enhanced solubility
- 11) Karel Pavelka.et.al (2016) reported in their study The overall analysis of randomised controlled clinical studies and meta-analyses confirmed the efficacy of diacerein in the symptomatic treatment of knee and hip OA.
- 12) Yadollahi Roya.et. al(2015) experimented in their study that Therapeutic nanosuspensions produced by various techniques such as high pressure homogenisation, media milling, and emulsification.
- 13) Walla'a A Osman et.al(2015) studied in their study compare the anti-inflammatory effect of diacerein, a selective inhibitor for production and activity of IL-1 β with diclofenac sodium, a member of NSAIDs

commonly used in treatment of OA, separately and in combination on an experimental model of osteoarthritis in rats induced by monoiodoacetate (MIA).

- 14) Tania S.A. et.al(2015) found that in their study found that the symptomatic benefit of diacerein in participants with OA of the knee or hip was minimal or none when compared with placebo. Minimal benefit was noted in terms of joint space narrowing for hip OA, and was uncertain for knee OA. Adverse effects related to the gastrointestinal tract (diarrhoea) were frequent, and safety concerns could make use of this drug non-beneficial
- 15) Shah Jay et.al(2014) studied in their study Both the drug Diacerein and Oxaceprol, statistically found to reduce VAS and Lequesne scale individually, so both the drug individually effective in Osteoarthritis of knee joint. The mean reduction in score was found to statistically insignificant between the drugs so, both the drugs are equally effective in Osteoarthritis of knee joint.

3. Aim & Objective:

The objective of our work was to develop novel drug delivery system (nanosphere) of Diacerein in combination with vitamin D3 using appropriate polymers.

1. To study the compatibility profile of combinational drug and polymers.
2. To prepare, optimize and evaluate nanosphere of Diacerein, vitamin D3 and their combination.
3. To the Pharmacokinetics profiles of prepared nanospheres.

4. Plan of work:

- ❖ Literature survey
- ❖ Selection and procurement of suitable drug and excipients
- ❖ Pre-formulation study
 - Detection of melting point
 - Solubility study
 - Detection of absorbent maxima U.V
 - FTIR spectra
 - Preparation of Calibration curve
- ❖ Preparation of Nanospheres formulation
- ❖ Evaluation of final formulation
 - Drug entrapment efficiency
 - Particle size analysis
 - Determination of drug content

- In vitro drug release by Dialysis method.
- ❖ Result and discussion
- ❖ Conclusion
- ❖ References

5. Drug Profile

5.1 Diacerein-

Diacerein[94] also known as diacetylrhein is a drug used in the treatment of osteoarthritis. Chemically it is 4, 5-diacetyloxy-9,10-dioxo-anthracene-2-carboxylic acid. It is slow acting symptomatic treatment of osteoarthritis by inhibiting interleukin-1, which has demonstrated efficacy on functional manifestation of osteoarthritis and on the structural component.[95]

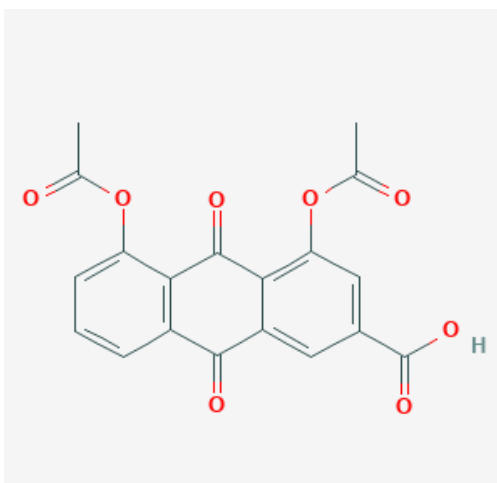


Figure: chemical structure of Diacerein

Formula	:C ₁₉ H ₁₂ O ₈
Mol. Mass	:368.294g/Mol
Routes	:Oral
Excretion	:Renal(30%)
Solubility	:insoluble in water
Appearance	: A crystalline solid
Melting point	:217.50 ⁰ C to 235.68 ⁰ C
pH	:4.5 to 6.0

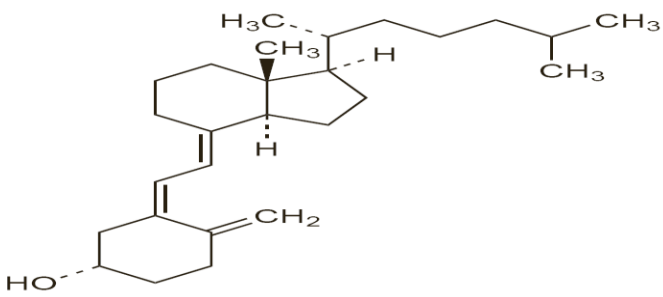
Table :-List of Marketed Formulated Product list of Diacerein

DCR 50MG CAP	PACIFIC INDIA	TABLETS
JAIZZY 50MG CAP	ST MORISON	TABLETS
JENCER TAB	JENBURKT PHARMA	TABLETS
JOCART D TAB	PEGASUS FARMACO	TABLETS
JOINCERIN CAP	TORRENT PHARMACEUTICALS LTD	TABLETS
KNEESAFE TAB	MAVERICK PHARMA	TABLETS
KNEECERIN 50MG TAB	EUGENE PHARMA	TABLETS
LEECRIN TAB	LEEFORD HEALTHCARE	TABLETS
LEFAX 50MG CAP	ADLEY LAB	TABLETS
KAVCEREIN 50MG TAB	KAVYA HEALTHCARE	TABLETS
KICEREIN 50MG TAB	KINDCARE PHARAMACEUTICALS	TABLETS
MORECERIN 50MG CAP	MOREPEN LABORATORIES LTD	TABLETS
MEDOCERIN 50MG CAP	MEDOPHARM	TABLETS
MAXRENE 50MG TAB	MAXLIVE PHARMACEUTICALS PVT LTD	TABLETS
EZYCERIN 50MG CAP	RAJ DISTRIBUTORS	TABLETS
GEMCERIN 50MG TAB	ALKEM LABORATORIES LTD	TABLETS
GENUCERIN 50MG TAB	DRUTO	TABLETS
GLACART D TAB	GLADCARE FORMULATIONS PVT LTD	TABLETS
GLOCERIN 50MG TAB	GLOBUS REMEDIES LTD	TABLETS
HILIN CAP	DR REDDYS LABORATORIES LTD	TABLETS
DJC 50MG TAB	SYSTOPIC LABORATORIES PVT LTD	TABLETS
DORCERIN TAB	CANDOR BIOTECH LIMITED	TABLETS
FEMORIN 50MG CAP	FEMGRACE FORMULATIONS	TABLETS
ELTRODAR CAP	ELDER PHARMACEUTICALS LTD	TABLETS
FITJOINT CAP	EMCURE PHARMACEUTICALS LTD	TABLETS
DIACER 50MG CAP	CADILA PHARMACEUTICALS LTD	TABLETS

5.2 VITAMIN D₃ (cholecalciferol)

Vitamin D is a secosteroid produced in the skin from 7-dehydrocholesterol under the influence of ultraviolet irradiation. Vitamin D is also found in certain foods and is used to supplement dairy products. Both the natural form (vitamin D₃, cholecalciferol) and the plant-derived form (vitamin D₂, ergocalciferol) are present in the diet.

TABLE 1 :Physicochemical and pharmaceutical characteristics of cholecalciferol

Chemical name:	(5Z,7E)-9,10-Secocholesta-5,7,10(19)-trien-3 β -ol
Molecular formula:	C ₂₇ H ₄₄ O
Structural formula: Structural	
Molecular mass:	384.6
Melting point:	84.5°C
Properties	White or almost white crystals, which are sensitive to air, heat, and light. Practically insoluble in water; freely soluble in alcohol; soluble in trimethylpentane and in fatty oils. Solutions in solvents without an antioxidant are unstable and should be used immediately. A reversible isomerization to pre-cholecalciferol takes place in solution, depending on temperature and time. The activity is due to both compounds. Store under nitrogen in airtight containers at a temperature of 2 degrees to 8 degrees. The contents of an opened container should be used immediately. Protect from light.
Polarity	Log P = 7.5

Pharmacology

Vitamin D is the product of a cholesterol-like precursor (7-dehydrocholesterol) after it has been irradiated by ultraviolet light (UV). The most well-known effect of vitamin D is maintenance of normal blood levels of calcium and phosphate, which are in turn needed for the normal mineralisation of bone, muscle contraction, nerve conduction, and general cellular function in all cells of the body. In addition vitamin D has widespread effects on cellular differentiation and proliferation, can modulate immune responsiveness, and central nervous system function and may act as a chemo preventive agent against several malignancies including cancers of the prostate and colon.

Pharmacokinetics

Vitamin D is either obtained by UV radiation of the skin, or provided via food. Absorption of ingested vitamin D occurs with the aid of bile salts primarily in the lower third of the small intestine. Vitamin D enters the lymphatic system and then the blood stream. Further transport of vitamin D occurs through the lymph where the ingested vitamin is carried in the chylomicron fraction to the liver. As time passes, vitamin D increasingly associates with α_2 -globulins, to which it is tightly bound. Vitamin D₃ originating from the photolysis reaction occurring in the skin is directly transported into the liver by the vitamin D binding protein. Vitamin D is typically located in 4 organs: the liver, as free vitamin D, the mucosa of the small intestine, the bone, and the first third of the kidney proximal tubule, but it has been shown that vitamin D is also present in nuclei of neurons in adult rat and mouse brains. Calcidiol is stored in the liver. Lesser amounts are distributed to adipose tissue and stored as vitamin D₃ at these sites for later release into the circulation. The half-life of vitamin D in adipose tissues is about 2 months. Calcidiol shows a half-life of 15 days whereas calcitriol shows a half-life of approximately 15 hours. The vitamin

D receptor (VDR) has been found in over 36 cell types where it is thought to initiate physiological responses.

Vitamin D needs to be metabolised first in the liver to calcidiol and subsequently in the kidneys to become calcitriol, the biologically active form. In the liver, this hydroxylation is mainly carried out by two enzymes in the rat, one found in the microsomes and the other in the mitochondria of hepatic cells. Not only is this hormone produced in the kidneys as previously thought. It has been demonstrated that it is produced in over 10 extra renal organs. Further hydroxylation occurs prior to elimination. Many metabolites of vitamin D₃ have been isolated from plasma. The elimination of vitamin D metabolites occurs in the urine and faeces.[96]

Pharmacodynamics

The MAH (Marketing Authorisation Holder) has provided an overview of general pharmacodynamic properties of vitamin D. The pharmacodynamic section is considered sufficiently described. Section 4.5 of the SmPC reflects most of the interactions of vitamin D with other medicinal products. The interactions with orlistat, actinomycin, imidazole have also been added.[97]

6.Pre-Formulation study-

6.1 SOLUBILITY PROFILE:

The spontaneous interaction of two or more substances to form a homogenous molecular dispersion is called as solubility. Solubility is a chemical property referring to the ability for a given substance ,the solute, to dissolve in a solvent.It a measured in terms of the maximum amount of solute dissolved in a solvent at equilibrium. The resulting solutopn is called saturated solution.1mg of drug taken and dissolved in the different type of solvent.The solubility of drug are shown on below table.

a) Solubility Profile of cholecalciferol

S.N	Solvent	Obervation
1	Water	Insoluble
2	Ethanol	Soluble
3	Acetone	Soluble
4	Benzene	Soluble
5	Ether	Soluble
6	Ethlacetate	Soluble

b) Solubility Profile of Diacerein,

S.N	Solvent	Obervation
1	Ethanol	Soluble
2	Acetone	Soluble
3	Benzene	Soluble
4	Ether	Soluble
5	Ethlacetate	Soluble
6	Aquos buffer.	Slightly soluble
7	Water	Slightly soluble

6.2 Partition coefficient determination

The ratio of the concentration of a solute in two immiscible or slightly miscible liquid,or in two solid,when it is in equilibrium across the interface between them.

Procedure

- ❖ About 500mg of Diacrine are transferred into three labeled stoppered glass bottle or separating funnel.
- ❖ Around 50 ml of benzene and around 50 ml of water are added to a bottle.
- ❖ After tightly stopper the bottle they are vigorously shaken every 3 to 5 min. for about 1 hr. The stopper is removed to release the pressure.
- ❖ The bottles are then kept undisturbed to get the layers separated. The lower layer is aqueous, the upper layer is organic phase (benzene).
- ❖ 10 ml of benzene layer is pipette out into a conical flask containing around 30 ml water.
- ❖ The solution is titrated against N/10 NaOH using phenolphthalein as indicator.
- ❖ Similarly 10 ml of aqueous layer is pipette out into a conical flask and titrated against N/50 NaOH using phenolphthalein as indicator.
- ❖ Similarly done with vit. D3

6.3.U.V Spectroscopy-

Apparatus and Instrumentation-

A Shimadzu UV 1800 series Spectrophotometer was used with 1 cm matched quartz cells. Single pan electronic balance (Shimadzu, ATY 224) was used for weighing purpose. Sonification of the solutions was carried out using an Ultrasonicator (Spectra lab UCB 40, India). Calibrated volumetric glasswares (Borosil) were used in this study.

6.3.1. For Diacrine

Preparation of Standard Solution

The standard stock solution of diacrine was prepared by transferring, accurately weighed, 100 mg of API to 100 ml with Hydroethanolic solution (water 50 ml + ethanol 50 ml) of volumetric flask.

Take a 10 ml of solution and dissolve the further 90 ml of Hydroethanolic solution. The standard stock solution was further diluted with the solution to get the concentration of 10 µg/mL.

Spectral Scanning and Wavelength Selection

The standard solution of diacerein (10 µg/mL) was scanned in the range of 400-200 nm. against ethanol solvent as blank after baseline correction. The absorbance maximum was observed at 265 nm. Different working standards were prepared between 1-5 µg/mL. Various wavelength ranges were tried and the final range between 246-266 nm was selected on the basis of linear relationship between area and corresponding concentration and the horizontal axis. The horizontal axis was selected by entering the wavelength range over which the area has to be

calculated. The wavelength range from 262.0- 250.0 nm was selected which showed good linearity between area under curve and concentration.

6.3.2 For cholecalciferol

Preparation of Standard Solution

The standard stock solution of cholecalciferol was prepared by transferring, accurately weighed, 100 mg of API to 100 ml with Hydroethanolic solution (water 50ml+ethanol 50 ml) of volumetric flask.

Take a 10 ml of solution and dissolve the further 90 ml of Hydroethanolic solution. The standard stock solution was further diluted with the solution to get the concentration of 10 μ g/mL.

Spectral Scanning and Wavelength Selection

The standard solution of cholecalciferol (10 μ g/mL) was scanned in the range of 400-200nm. against ethanol solvent as blank after baseline correction. the absorbance maximum was observed at 256. nm . Different working standards were prepared between 1-5 μ g/mL. Various wavelength range were tried and final range between 246-266nm was selected on the basis of linear relationship between area and corresponding concentration and the horizontal axis. The horizontal axis was selected by entering the wavelength range over which the area has to be calculated. The wavelength range from 262.0- 250.0 nm was selected which showed good linearity between area under curve and concentration.

6.4 Fourier Transform Infrared Spectroscopy (FTIR) Studies :

The infrared (IR) spectra were recorded using an FTIR spectrophotometer by the KBr pellet method in the wavelength region between 3500 and 1000 cm^{-1} . The spectra obtained for Diacerein and mixtures of cholecalciferol were compared to check compatibility of drug with each other.

6.5 Detection of Melting Point: Melting point of pure drug and excipient was determined by capillary method. Melting point of diacerein and cholecalciferol was found to be 217.50 $^{\circ}$ C and 84.5 $^{\circ}$ C.

Solubility studies: The solubility study of diacerein and cholecalciferol was determined.

a) Solubility Profile of cholecalciferol

S.N	Solvent	Obervation
1	Water	Insoluble
2	Ethanol	Soluble
3	Acetone	Soluble
4	Benzene	Soluble
5	Ether	Soluble
6	Ethlacetate	Soluble

b)Solubility Profile of Diacerein,

- Soluble in organic solvent such as ethanol,ether.
- Springly soluble in aqueous buffer.

Preparation of calibration curve of Pure drug:

The calibration curve was plotted by taking different concentration of drug on x-axis and absorbance on y-axis and is shown in figure of Diacerein and Cholecalciferol. The drug (Diacerein & Cholecalciferol) obeys Beer's law in the concentration range of 1-5 mcg/ml with coefficient of correlation (R^2) = 0.999 and (R^2) = 0.999. The calibration curve was determined at λ_{Max} at 262 nm and 256 nm.

S.No.	Concentration(μ g/mL)	Absorbance(nm)
1	0	0
2	1	0.1015
3	2	0.2044
4	3	0.2956
5	4	0.3958
6	5	0.4752

Table: Standard curve of Cholecalciferol

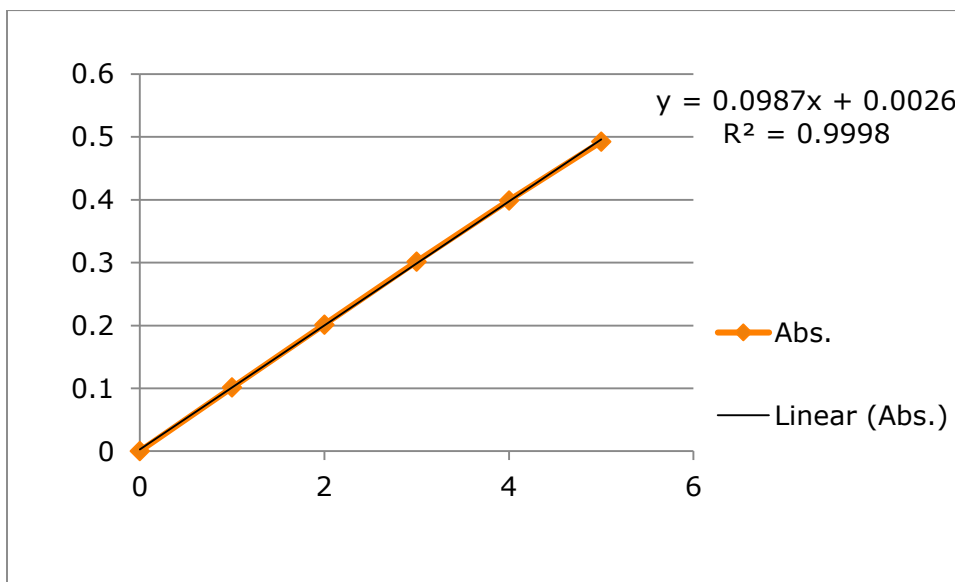


Figure:Calibration Curve of Cholecalciferol

S.no	Concentration(µg/mL)	Absorbance(nm)
1	0	0
2	1	0.1017
3	2	0.2637
4	3	0.3697
5	4	0.4963
6	5	0.5552

Table:Standard curve of Diacerine

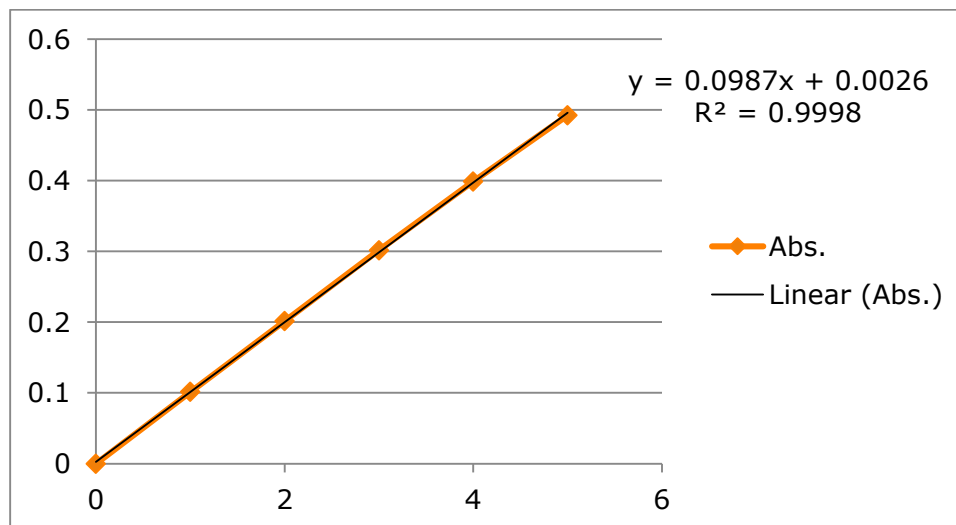
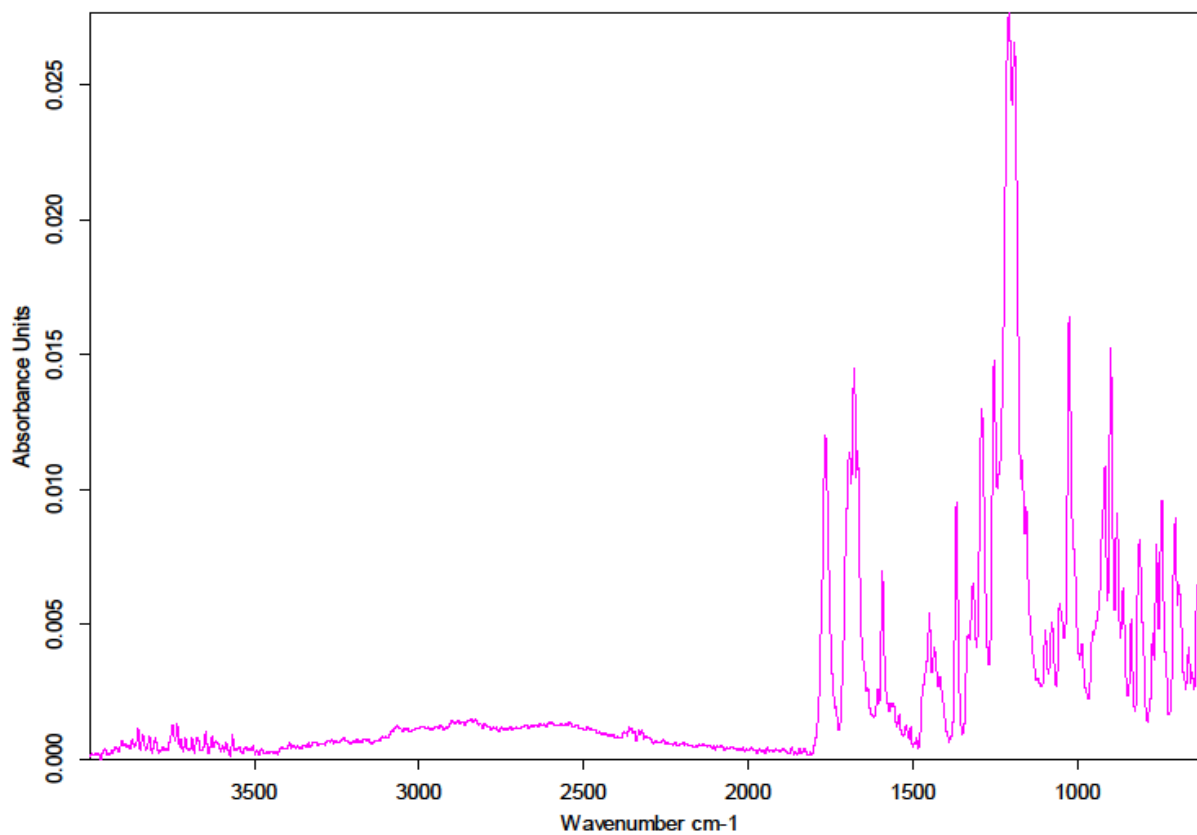


Figure:Calibration Curve of Diacerine

FTIR Study:

The samples were crushed with KBr and form pellets. For estimation of functional groups of a drug, IR (Infrared spectroscopy) is the most suitable analytical technique. Ratio of drug and KBr 1:10:10. IR spectrum has two distinct regions such as functional group region $3500-1000\text{cm}^{-1}$

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fig. FTIR of Diacerein, with vitamin D3

7. Materials and methods

Diacerein used was a gift sample from Cipla Pvt. Ltd. Mumbai and cholecalciferol from sigma Raipur. HPMC and sodium alginate were obtained from and All other chemicals used were of analytical grade.

7.1. Preparation of nanospheres

Diacerein and cholecalciferol were prepared by solvent dissolution techniques. Sodium alginate was dissolved in aqueous solution at various concentrations such as 1.0, 2.0, 3.0, 4.0, 5.0 mg/ml. Under magnetic stirring at room temperature, then added HPMC slowly in prepared sodium alginate solution. Drug Diacerein are added in solution. pH was adjusted to 6.0 by adding 0.1 M NaOH. The stirring was continued for about 30 min. The resultant nanoparticles suspensions were centrifuged at $12000 \times g$ for 30 min using C24 centrifuge.

7.2. Drug entrapment efficiency

Drug content was determined by centrifugation method. The redispersed nanoparticles suspension was centrifuged at 15000 rpm for 40 min at 25°C to separate the free drug in the supernatant. Concentration of Diacerein and cholecaliferol in the supernatant was determined by using UV-visible spectrophotometer at 266 nm after suitable dilution. The drug entrapment efficiency (% EE) was determined using the relationship in equation.

7.3. Particle size analysis -The size of nanoparticles was analyzed using a Zetasizer, Ver. 6.01 (Malvern Instrument Ltd) at Pune, Bharti vidyapeeth (Deemed to be University) Poona College of Pharmacy. The Nanosphere was placed in the sample measured as previously explained.

7.4 Determination of drug content

Twenty five mg of the prepared nanoparticles were weighed and dissolved in 5 ml of lactic acid and made up to 25 ml with phosphate buffer (pH 7.4). From the aliquots, 1 ml was taken and diluted to 10 ml with the buffer and the absorbance was measured in UV-Vis spectroscopy at 220 nm. From the absorbance's total drug content in the batches were calculated.

7.5 Zeta potential measurement

Zeta Potential of optimized liposomes obtained from ethanolevaporation and rotary evaporator method was determined using Zeta sizer 300HSA (Malvern instrument, Malvern, UK).

7.6 *In vitro* release studies

The *in vitro* drug release of drug Diacerein and vit D3 from Nanospheres formulations was carried out by using dialysis membrane in 7.4 pH phosphate buffer for 12 hrs. The *in vitro* release profile of obtained for formulation, are shown in Table 8.4 and in Fig8.4 respectively.

The comparisons of *in vitro* release profile of diacerein and vit D3 formulation. The cumulative percentage release of combinational drug from the prepared nanospheres was varied from depends upon the drug ratio for 12 hrs are shown 8. 4 and in fig. no.8.4.

Kinetic Treatment of Dissolution Data

In order to describe the kinetics of the release process of drug in the different formulations, models were fitted to the dissolution data of optimized formulations using linear regression analysis. In order to study the exact mechanism of drug release, drug release data was analyzed according to Zero Order Kinetics; first order kinetics, Higuchi square root equation, Hixon -Crowell equation. The criterion for selecting the most appropriate model was chosen on the basis of goodness of fit test.

Zero Order Kinetics

Drug dissolution from pharmaceutical dosage forms that do not disaggregate and release the drug slowly assuming that area does not change and no equilibrium conditions can be represented by the following equation.

$$Q_t = Q_0 + K_0t$$

Q_t is the amount of drug dissolved in time t

Q_0 is the initial amount of drug in the solution K is the zero order release constant

First Order Kinetics

The application of this model to drug dissolution studies used to describe absorption and/or elimination of drugs. To study the first order release rate kinetics the release rate data were fitted to the following equation.

$$\text{Log } Q_t = \text{log } Q_0 + K_1t / 2.303$$

Q_t is the amount of drug released in time t

Q_0 is the initial amount of drug in the solution K_1 is the first order release constant.

Higuchi Model

Higuchi developed several theoretical models to study the release of watersoluble and low soluble drugs incorporated in semi-solid and/or solid matrixes. Mathematical expressions were obtained for drug particles dispersed in a uniform matrix behaving as the diffusion media, the equation is

$$Q_t = K_H \cdot t^{1/2}$$

Q_t is the amount of drug released in time t K_H is higuchi dissolution constant.

Higuchi describes drug release as a diffusion process based in the Fick's law, square root time dependent.

Korsmeyer and Peppas Model

This model is generally used to analyze the release of pharmaceutical polymeric dosage forms, when the release mechanism is not well known or when more than one type of release phenomena could be involved.

$$M_t / M = K \cdot t^n$$

M_t / M is the fraction of drug release K is the release constant

t is the release time

n is the diffusion exponent for the drug release that is dependent on the shape of the matrix dosage form.

Release Exponent (n)	Drug Transport Mechanism
$n = 0.5$	Fickian diffusion or square root of time kinetics
$0.5 < n < 1$	Anomalous (non- Fickian) diffusion,
$n=1$	Case -II transport
$n>1$	Super Case II transport

8. Results and discussion

8.1 Drug entrapment efficiency

S.No.	Concentration ($\mu\text{g/ml}$)	Absorbance(nm)
1.	5	1837.65
2.	10	3786.3
3.	15	5412.85
4.	20	7138.4
5.	25	9087.15

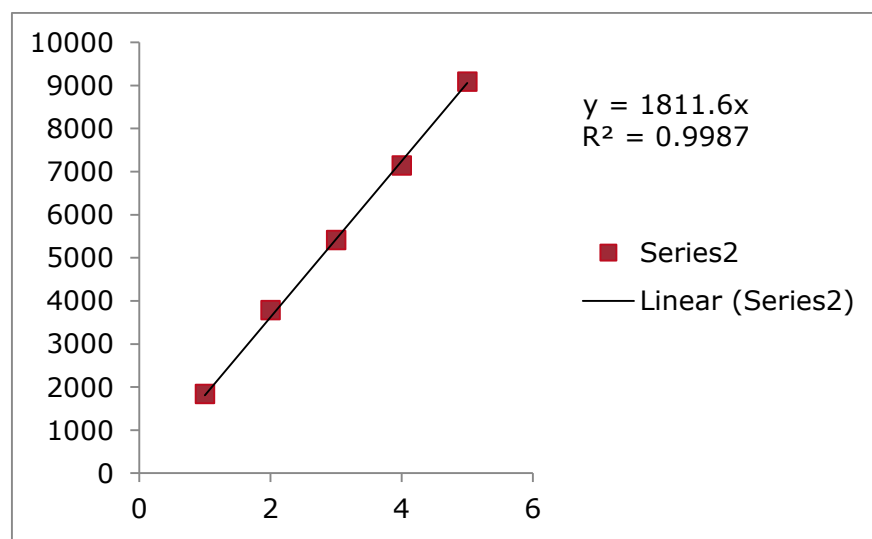
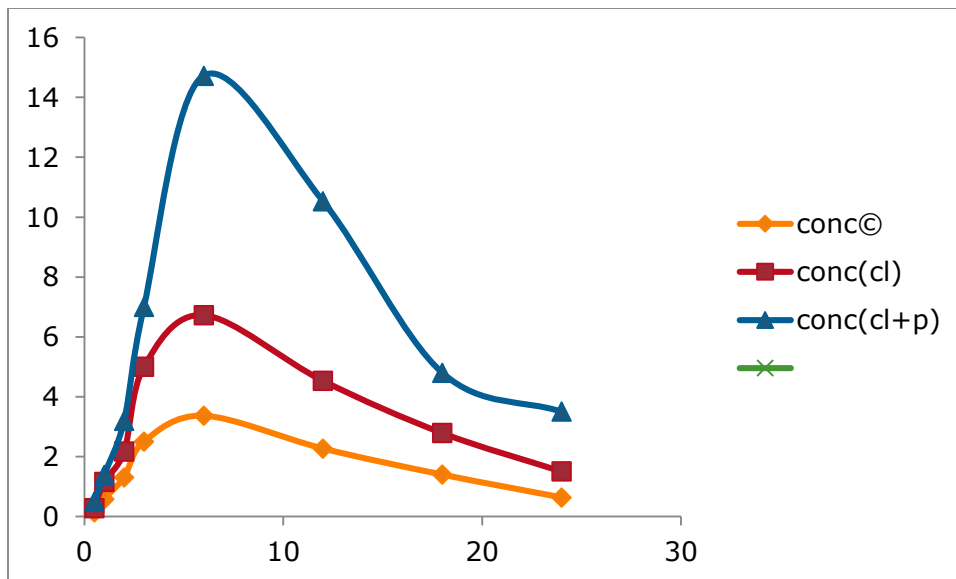


Fig standard graph of Diacerein and Vit D3 by HPLC

S.no	Concentration($\mu\text{g/mL}$)	Absorbance(nm)
1	0	0
2	1	0.1017
3	2	0.2637
4	3	0.3697
5	4	0.4963



PDC vs.Time

Plasma drug concentration**8.2 Particle size data-** Average particle size of combinational drug nanoparticles

Formulation code	Z- Average (d.nm)
F1	126.7
F2	149.9
F3	165.9
F4	126.5
F5	227.5
F6	189.9
F7	116.8
F8	146.7
F9	146.9

8.3 DRUG CONTENT

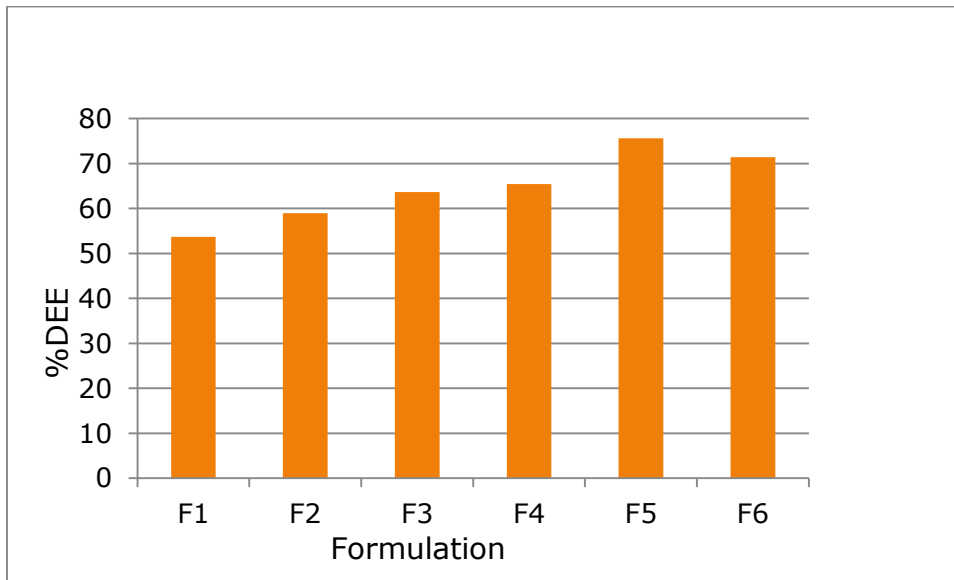


Fig: Graph of drug content

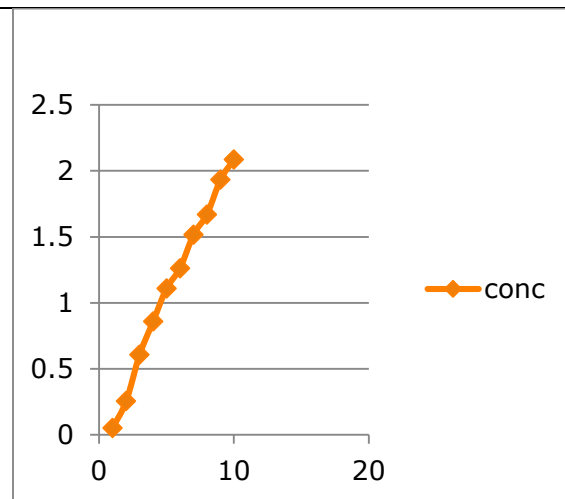
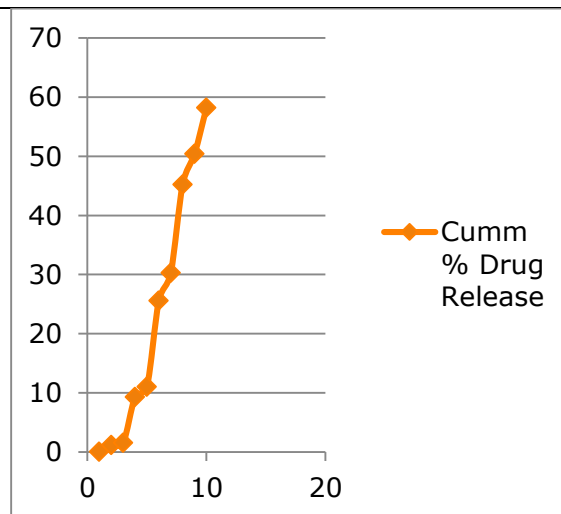
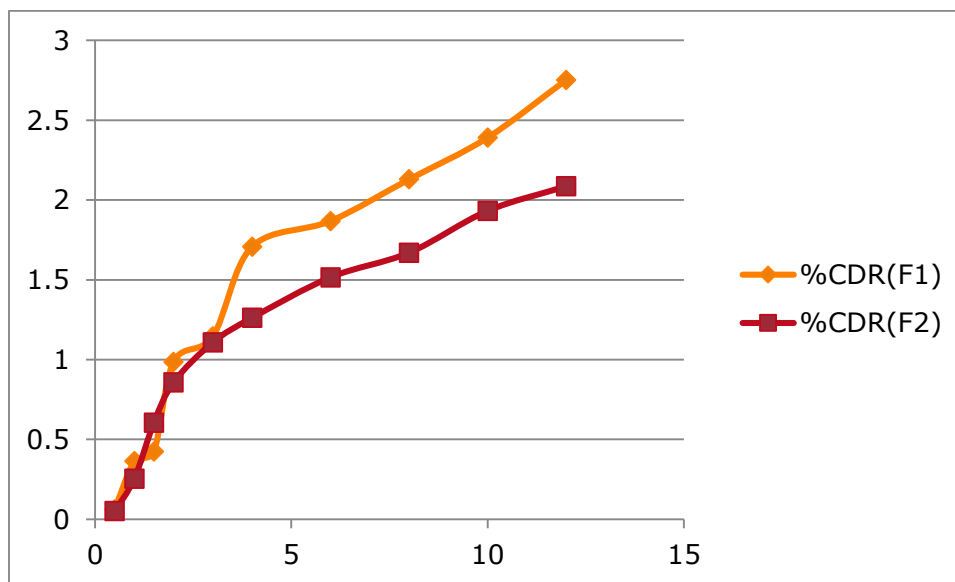
8.3 Zeta potential of formulation of cholecalciferol

Table- Zeta potential of formulation

S.No	Formulation	Zeta potential (mV)
1.	Drug loaded formulation	26.30 ±0.243

8.4 Table *In vitro* release profile of Nanospheres formulation F1 and F2

S.No.	F1		F2
	Time (hr)	%CDR	%CDR
1.	0.5	0.061	0.052
2.	1	0.364	0.254
3.	1.5	0.425	0.606
4.	2	0.986	0.858
5.	3	1.147	1.108
6.	4	1.708	1.262
7.	6	1.869	1.516
8.	8	2.130	1.670
9.	10	2.391	1.932
10.	12	2.752	2.086

Cumulative % drug release of diacrine loaded and diacrine+vit.D3 nanospheres formulation (F1&F2)**Fig In vitro release profile of diacrine****Fig In vitro release profile of cholecalciferol****Invitro drug release profile of the formulations (F1 & F2)****Time vs. cumulative drug release****8.5 Release kinetics**

Drug release data obtained with all four formulations was analysed according to following four kinetic models:

- %CDR Vs Time (Zero order rate kinetics)
- Log cumulative percent drug retained Vs Square root of Time (First order rate kinetics)
- %CDR Vs Square Root of Time (Higuchi model)
- Log % CDR Vs Log Time (Kosmeyer-Peppas model)

Calculated regression co-efficient for different formulations are shown in Table these values were compared with each other for model fitting equation. The model giving a regression coefficient close to unity was taken as order of release. The best fit model was observed to (zero order release) for formulation.

Table Best Fit Model for all formulations

Formulations	Zero order	First order	Higuchi matrix	Peppas plot	Best fit Model
	R^2	R^2	R^2	R^2	
F1	0.9465	0.8636	0.884	0.7044	Zero order
F2	0.9859	0.9749	0.9353	0.8322	Zero order

8.6 Drug encapsulation efficiency

The encapsulation efficiencies of the formulations are shown in Table given below. The results of encapsulation efficiency of the formulations was found to be almost similar without any significant differences, but it can be observed that , as a particle size was increased encapsulation efficiency was also increased.

Table - Drug encapsulation Efficiency of nanospheres formulations

S.No	Formulation	Encapsulation efficiency (%)
1.	F1	59%
2.	F2	61%

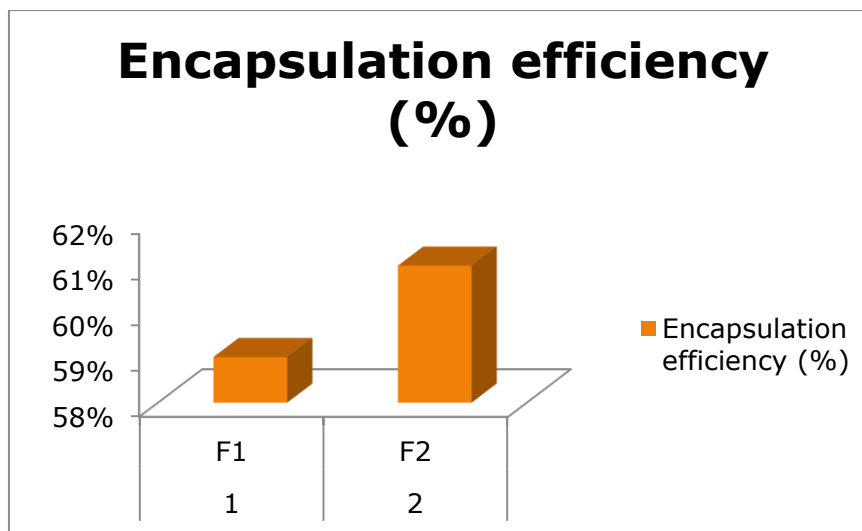


Fig no. 8.6

9. Conclusion-Based on drug content, drug entrapment efficiency, and *in vitro* release, formulation was selected as an optimum formulation. Stability studies were carried out for the selected formulation. The stability studies showed that maximum drug content and closest *in vitro* release to previous data was found for formulation stored at 4°C and room temperature. Thus nanosphere of combinational drug (Diacerein and cholecalciferol) with 2:1 ratio was found to be spherical, discrete and free flowing and able to sustain the drug release effectively.

Careful selections of various procedures are critical, firstly to achieve stabilization during formulation.

These findings indicate the suitability of formulation procedure for preparation of formulation of poorly water soluble drug. FT-IR studies confirm the compatibility of curcumin with the various excipients used in our study.

Dissolution profile studies demonstrated the significant improvement of Diacerein and vit D3 performance from Nanospheres compared to pure drug of Diacerein and vit D3.

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