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PREPARATION AND CHARACTERIZATION OF CELECOXIB LOADED GELATIN MICROSPHERES FOR CONTROLLED AND TARGETED DRUG DELIVERY IN VIVO STUDIES

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ABSTRACT

In the present study, the gelatin microspheres were formulated in such a way that it could control the drug release based on disease conditions or by using external stimuli. The Celecoxib loaded gelatin microspheres were formulated for intra- articular injection, thereby localizing the drug in the arthritic knee. All formulated batches were free from residual glutaraldehyde and the method by which the microspheres were formulated was completely eliminated the glutaraldehyde residue. Sodium metabisulphite was used to terminate glutaraldehyde cross-linking and to remove unreacted glutaraldehyde. In conclusion, the gelatin microspheres loaded with Celecoxib showed promising results in reducing joint swelling in arthritic knee without drug induced toxicity. The results obtained from this study indicated the scope of gelatin microspheres in the effective treatment of arthritis.

Key Words: Celecoxib, gelatin microspheres, arthritis.

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INTRODUCTION

The goal of a sustained release dosage form is to maintain therapeutic blood levels of the drug for an extended period of time. The onset of its pharmacological action is often delayed and duration of its therapeutic effect is sustained. It prolongs the drug release over an extended period of time, but not necessarily at a predetermined rate (e.g., coated granules). Control release drug delivery system has a meaning that goes beyond the scope of sustained action. It delivers the drug at a predetermined rate, locally or systemically for a specified period of time. It provides localization of the drug at an active site. It implies a predictability and reproducibility in the drug release kinetics. The rate profile is predictable as well as reproducible from one unit to another. Targeted Drug Delivery drugs after administration in a conventional immediate or controlled release dosage form will be distributed throughout the body, typically leading to uptake by cells, tissues and organs (non-target region) other than where their pharmacological receptors (target region) are located. This leads to unwanted side effects in the non-target region and more quantity of drug to be administered to maintain desired concentration in the target region. For example, after oral administration, the

quantity of drug available for absorption may be reduced due to various factors such as dissolution, solubility, pH, enzymes and microbial flora. After absorption from GIT, the drug enters into systemic circulation where it undergoes metabolism, distribution and excretion. To produce therapeutic effect, the drug must interact with its pharmacological receptors where the drug concentration may be less because of metabolism and distribution to non-target regions.

Administration of drug by parenteral routes may overcome the problem associated with GIT, but deactivation of the drug and dose related toxicity are frequently observed. Furthermore, it cannot be assured that after administration the drug will be reaching in adequate concentration to its desired destination. There are many diseases that are poorly accessible to drugs. These include rheumatoid arthritis, disease of central nervous system, some cancers and intractable bacterial, fungal and parasitic infections. The treatment of these diseases often require frequent dosing and high doses which can lead to toxic effects, inappropriate pharmaco disposition, untoward metabolism and other deleterious effects. Targeted drug delivery system is the one which delivers the drug only to its site of action and not to the non-target organs or tissues. This will enhance the therapeutic effectiveness and reduce the toxicity to other healthy tissues.

NSAIDs are the most frequently prescribed class of therapeutic agents for the treatment of arthritis and their side effects associated with gastrointestinal (GI) tract present a relatively major problem for the public. To overcome this problem, various approaches have been taken, including development of enteric-coated (EC) and sustained release (SR) NSAIDs. After over 20 years of clinical use, it is evident that novel drug delivery systems have not solved the problem of NSAID induced GI side effects. There is no convincing clinical evidence in the literature suggesting safer profiles for NSAID modified release formulations**.** Celecoxib is one of the drugs of choice to treat arthritis because of its potential anti-inflammatory and analgesic activity and this is the only approved NSAID available for parenteral delivery. Because of shorter biological half-life, Celecoxib should be given frequently to maintain its therapeutic activity. It also has high percentage of protein binding and it undergoes pre-systemic metabolism. To overcome these problems many authors were developed sustained release formulations with an intention to maintain effective Celecoxib concentration for prolonged period. To overcome the toxicity produced by the Celecoxib and to get prolonged therapeutic effect, in the present study, gelatin microspheres are formulated to target the drug at its site of action. To do so the drug is incorporated in gelatin microspheres, which can control/prolong the drug release depending upon disease condition or by external stimuli. Gelatin is well known biocompatible and biodegradable polymer, widely used in various pharmaceutical applications and hence selected for the present study (1-3).

Objective of the study is to develop gelatin microspheres loaded with celecoxib, which can control/prolong the drug release depending on disease condition or by external stimuli.

MATERIALS AND METHOD

Determination of Celecoxib: Preparation of calibration curve

A standard solution of Celecoxib (1 mg/ml) in phosphate buffer (pH 7.4) was prepared with (100 µg/ml) and without collagenase. A suitable volume of solution was diluted to various concentrations ranging from 2.5 to 35 µg. These standard samples were analyzed at 251 nm using UV visible spectrophotometer (Shimadzu 1601).

Preparation of saturated solution of glutaraldehyde in toluene

Equal quantity of aqueous glutaraldehyde solution and toluene was taken in a separating funnel and shaken for 1 hour to allow the saturation of glutaraldehyde in toluene. Then the aqueous phase and toluene phase was separated. Thus, obtained toluene saturated with glutaraldehyde was used to cross-link gelatin microspheres.

Preparation of gelatin microspheres loaded with Celecoxib (4-6)

As given in the Table-1, required quantity of gelatin was dissolved in 3 ml of phosphate buffer (pH 7.4) by heating to 60ºC. Specified quantity of Celecoxib was dissolved separately in 3 ml of phosphate buffer (pH 7.4) by heating and added to gelatin solution. Then the mixture was added drop wise to 100 ml of sesame oil with 1% w/v span 80 preheated to 60ºC and emulsified by stirring with the help of hand blender (5,000 rpm/3 min). Then the stabilized emulsion was stirred with the help of a stirrer attached to a motor (Remi, India, approx.1000 rpm). Required quantity of glutaraldehyde-saturated toluene solution (1, 5 and 10 ml) was added drop wise and the stirring was continued at room temperature for required time period (2, 4 and 6 h). The cross-linked microspheres were collected by filtration by using Whatman filter paper (No: 41). After filtration, the microspheres were washed with anhydrous ether to remove sesame oil. Then it was washed with 3x10 ml of 5% w/v sodium metabisulphite, 2x10 ml water and 2x10 ml of isopropyl alcohol. After washing, the *microspheres were dried at 45ºC, transferred to glass vials and stored in desiccator.*

Table-1 Formula and yield of gelatin microspheres

Batch No.	Amount of gelatin used (mg)	Amount of Celecoxib used (mg)	Yield[a] (mg)	Theoretical loading of Celecoxib (%)
GM_0	1500	-	1466 □ 82	0
GM_1	1500	180	1630 □ 43	10.7
GM_2	1500	380	1770 □ 52	20.2
GM_3	1500	650	1980 □ 65	30.2

Effect of proteolytic enzyme on formulated microspheres

Gelatin microspheres prepared with various drug loading, extent and duration of cross- linking were separately added to 25 ml of phosphate buffer containing collagenase, in 100 ml conical flask. The flasks were kept in an incubator at 37ºC for 6 h and shaken occasionally. At different time intervals, one ml of sample was taken and analyzed for drug content by spectrophotometer at 251 nm. After each sampling, same quantity of fresh phosphate buffer was replaced.

RESULTS AND DISCUSSION

The microencapsulation process adopted in the study produced good yield of microspheres with free flowing nature. The usage of sesame oil with span 80 was found to be effective in dispersing aqueous globules containing drug and gelatin and produced good yield of microspheres. The stirring conditions were optimized to get the required size (below 50 □m) by observing the globule size under the microscope. The cross- linking of gelatin microspheres were done by using glutaraldehyde-saturated toluene solution. At the end of preparation, by adding sodium metabisulphite, which was used to neutralize unreacted glutaraldehyde, the cross-linking was terminated

Determination of Celecoxib

The drug released by proteolytic degradation was estimated spectrophotometrically at 251 nm by using calibration curve (Table-2).

Table-2 Absorbance (251 nm) of Celecoxib in phosphate buffer (pH 7.4) with and without collagenase

S No	Concentration µm/ml	Absorbance	
		With collagenase	Without collagenase
1.	0	0	0
2.	5	0.17	0.16
3.	10	0.33	0.31
4.	15	0.50	0.47
5.	20	0.66	0.62
6.	25	0.83	0.77
7.	30	1.02	0.93

The effect of drug loading on proteolytic degradation of gelatin microspheres

The effect of drug loading on proteolytic degradation of gelatin microspheres was shown in Fig-1. As the drug loading increased, the degradation increased. By the end of 6 h, at 100 □g/ml collagenase, the gelatin microspheres loaded with 30% drug released approximately 20% of the entrapped drug. This may be due to more surface area of gelatin microsphere, which resulted in faster dissolution of the poorly entrapped drug due to high loading, collagenase activity

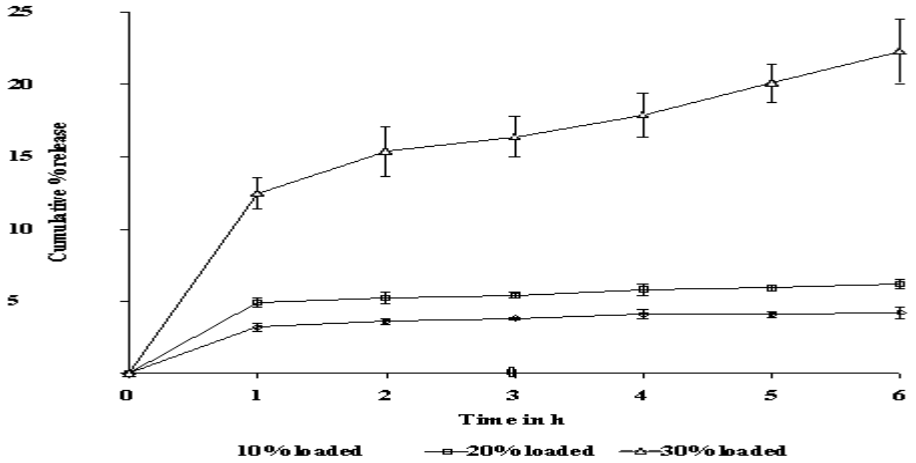

Fig-1 The effect of drug loading on proteolytic degradation of gelatin microspheres

From the Fig-1, it can be concluded that microspheres prepared by crosslinking with 10 ml of glutaraldehyde-saturated toluene for a duration of 6 h, seem to be optimum to withstand proteolytic enzymes and capable of releasing the drug for a prolonged period. The microspheres prepared with higher quantity of cross-linking agent and duration may be irrational, which might produce very slow release of the drug and result in ineffective therapy. Hence, gelatin microspheres prepared as per the formula by using 10 ml of glutaraldehyde-saturated toluene solution and cross-linking duration of 6 h were selected for further studies

Drug loading, entrapment and encapsulation efficiency

The drug present in the microspheres must be completely extracted by suitable method during the determination of drug content. Sodium hydroxide solution was used to digest the gelatin in order to extract the encapsulated

Celecoxib. Since the drug is soluble in sodium hydroxide, it is possible to get complete extraction of drug from the microspheres. Gelatin microspheres/magnetic microspheres showed good loading, entrapment and encapsulation efficiency as given in Table-3 and 4. Higher drug loading lowered the percentage of entrapment and encapsulation, which indicates the wastage of drug during microencapsulation process. This may be due to surface drug present in the microspheres with higher drug loading, which might be removed during washing and recovery and thus reducing the % of entrapment and encapsulation.

Table-3 Physical and chemical parameters of gelatin microspheres loaded with Celecoxib

Batch No.	Percentage of drugloading		Percentage of drug entrapment[a]	Percentage of drug encapsulation[a]	Average particle size[a] µm (n=200)
	Theoretical	Actual[a]			
GM_0	-	-	-	-	21.7
GM_1	10.7	9.8 □ 0.9	91.6 □ 8.4	88.7 □ 8.2	29.2
GM_2	20.2	18.3 □ 1.3	90.6 □ 6.4	85.2 □ 6.1	31.6
GM_3	30.2	26.7 □ 1.8	88.4 □ 5.9	81.3 □ 5.5	34.4

Table-4 Physical and chemical parameters of gelatin magnetic microspheres loaded with Celecoxib

Batch No.	Percentage of drug loading		Percentage of drug entrapment[a]	Percentage of drug encapsulated[a]	Average particle size µm (n=200)
	Theoretical	Actual[a]			
MG	-	-	-	-	15.3
MG_0	-	-	-	-	22.5
MG_1	10	9.1 □ 0.85	91 □ 8.5	89.4 □ 8.3	25
MG_2	21	18.7 □ 1.6	89 □ 7.6	84.8 □ 7.3	27.9
MG_3	30.8	24.9 □ 2.1	80.8 □ 6.8	77.8 □ 6.5	30.6
MG_4	10	8.9 □ 0.74	89 □ 7.4	74.9 □ 6.2	2.4

***In Vivo* Studies**

The primary objective of this study was to target Celecoxib at its site of action for the effective treatment of arthritis. Three different routes were selected to target the drug in knee joint. As expected, since the microspheres directly injected to the synoviam, the intra- articular administration of microspheres showed the maximum targeting efficiency. About 81.2% of intra-articularly administered drug was recovered from the injected joint after one day of post injection. The remaining might be released/distributed/excreted during the time period between the administration and estimation of drug. As shown in the Table 8.1, the intra-articular injection showed maximum targeting than the other methods. About 70.7% of injected dose was recovered from the target site after intra-arterial injection and only about 4.4% of injected dose was recovered from the target site after intravenous administration. The gelatin microspheres formulated for intra-arterial and intra-articular administration showed good targeting efficiency and selected for i*n vivo* studies. The targeting efficiency of microspheres by intravenous administration was markedly low, which might be due to phagocytosis of administered microspheres by the macrophages and reticulo endothelial system.

The cross-section of femoral artery of rabbit that received intra-arterial injection of gelatin magnetic microspheres is shown in Figure-2, 3. The diameter of femoral artery isolated near the knee region was between 590 and 1204 µm. The size of magnetic microspheres formulated for intra-arterial administration was well below this size and hence successfully injected without any blockage of blood circulation. The presence of magnetic microspheres was seen in the photograph as black spherical aggregated particles which also confirmed the targeting efficiency of the formulated magnetic microspheres by this route of administration.

Table-5 showing targeting efficiency of prepared microspheres

Preparation	Dose injected (mg)	Dose recovered (mg) mean □ SE (n=5)	% of drug targeted
Intra-articular	10	8.12 □ 0.74	81.2
Intra-arterial	10	7.07 □ 1.7	70.7
Intravenous	10	0.44 □ 0.23	4.4

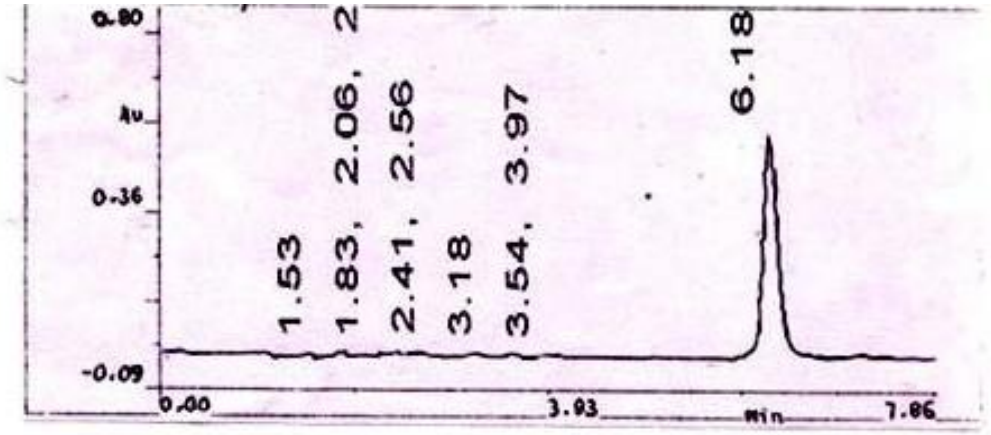

Fig-2 HPLC chromatogram of Celecoxib Standard drug dose injected

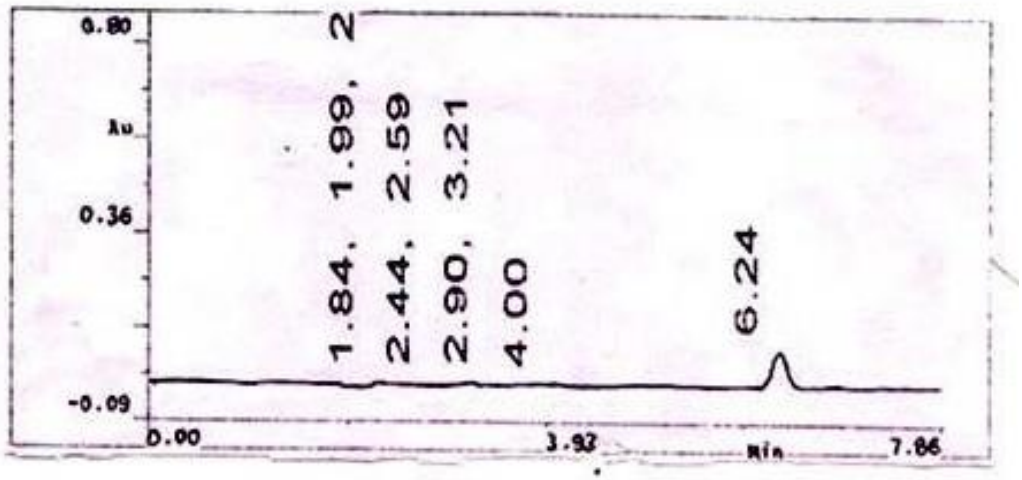

Fig-3 HPLC chromatogram of Celecoxib gelatin microspheres

CONCLUSION

In the present study, the gelatin microspheres were formulated in such a way that it could control the drug release based on disease conditions or by using external stimuli. The Celecoxib loaded gelatin microspheres were formulated for intra- articular injection, thereby localizing the drug in the arthritic knee. The Celecoxib loaded gelatin magnetic microspheres were formulated for intra-arterial and intravenous administration and were localized at the site of action by keeping a magnet near target site. The microspheres were formulated by using emulsification/cross- linking technique. Glutaraldehyde in toluene was used as cross-linking agent and the unreacted glutaraldehyde was removed by the treatment with sodium metabisulphite. The formulated microspheres were characterized by drug loading, percentage of entrapment/encapsulation and by various analytical techniques such as optical microscopy, scanning electron microscopy, particle size analysis, FT-IR spectroscopy, differential scanning calorimetry, X-ray diffraction and atomic absorption spectroscopy. Gelatin microspheres meant for intra-articular injection and gelatin magnetic microspheres meant for intra-arterial injection were formulated with theoretical loading of 10, 20 and 30% w/w. Gelatin magnetic microspheres meant for intravenous injection were formulated with a theoretical loading of 10% w/w. Formulated microspheres with different percentage of loading and magnetite content showed good entrapment (above 81%) and encapsulation efficiency (above 75%). The magnetite content of magnetic microspheres was 27-29% w/w. The average particle sizes of formulated gelatin microspheres for intra-articular and intra-arterial administration were about 29-34 and 22-30 µm, respectively. The average particle size of magnetic microspheres meant for intravenous administration was 2.4 µm. The size distribution of gelatin microspheres meant for intra-articular injection and intra-arterial injection were narrow and within a range of 1-60µm. The size range of gelatin magnetic microspheres for intravenous administration was between 0.1 and 5 µm. The particle sizes of microspheres were well within the injectable range through desired routes with 20-27 gauge needle. All formulated batches were free from residual glutaraldehyde and the method by which the microspheres were formulated was completely eliminated the glutaraldehyde residue. Sodium metabisulphite was used to terminate glutaraldehyde cross-linking and to remove unreacted glutaraldehyde. In conclusion, the gelatin microspheres loaded with Celecoxib showed promising results in reducing joint swelling in arthritic knee without drug induced toxicity. The results obtained from this study indicated the scope of gelatin microspheres in the effective treatment of arthritis.

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