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Original Research Paper

DESIGN AND DEVELOPMENT OF KETOPROFEN PHARMACOSOMES FOR ORAL DELIVERY

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ABSTRACT

Pharmacosomes is a potential approach in vesicular drug delivery system which exhibits several advantages over conventional vesicular drug delivery systems. To improve the water solubility, bioavailability and minimize the gastrointestinal toxicity of ketoprofen, its pharmacosomes were prepared. Ketoprofen was complexed with phosphatidylcholine in various ratios using conventional solvent evaporation method. Pharmacosomes thus prepared were subjected to solubility and drug content evaluation, scanning electron microscopy, differential scanning calorimetry, X ray powder diffraction, *in-vitro* dissolution and *in-vitro* diffusion study. Pharmacosomes of ketoprofen were found to be disc shaped with rough surface in scanning electron microscopy. Solubility and *in-vitro* dissolution profile of pharmacosomes was found to be much better than ketoprofen. Drug content was found to be 99.8% and shown high % of drug loading. Differential scanning calorimetry thermograms and X-ray powder diffraction data's confirmed the formation of phospholipid complex. *In-vitro* diffusion rate of ketoprofen from pharmacosomes was significantly higher. After 24 h, maximum drug was released from formulation PC4 is 55.3%. The release experiments clearly indicated sustained release of ketoprofen from pharmacosomal formulations.

Keywords: Pharmacosomes, Vesicular systems, Control release, Solubility, Bioavailability.

INTRODUCTION

Ketoprofen is one of the most widely used therapeutic substances due to its analgesic, antipyretic and anti-inflammatory properties. Despite the proliferation in development of new non-steroidal anti-inflammatory drugs (NSAIDs), ketoprofen remains one of the most effective 'over-the-counter' drugs in the treatment of rheumatoid arthritis disease. Use of ketoprofen is associated with two major limitations; first, rare, but serious and sometimes fatal, gastrointestinal (GI) side-effects, including ulceration, and hemorrhage, especially in the elderly, and second, poor water solubility.[1] Different approaches have been applied to decrease NSAID-induced GI toxicity. For example, association of NSAIDs with phospholipids has been suggested to improve GI safety of these drugs. The presence of an adsorbed layer of surface-active phospholipids on the surface of the mucus that covers the surface epithelium is suggested to protect the GI tissues by providing a hydrophobic layer between the epithelium and the luminal contents. It has been reported that NSAIDs associated with zwitterionic phospholipids may reduce GI toxicity.[8]

Phospholipids (like phosphatidylcholine) play a major role in drug delivery due to its amphiphilic nature, which can modify the rate of drug release for the enhancement of drug absorption across

biological barriers. Developing of amphiphilic drug-lipid complexes or pharmacosomes may prove to be a potential approach for improving therapeutic efficacy of the drugs by improving the bioavailability (through improvement in solubility in GI fluid and permeation across the bio membranes).[3]

Pharmacosomes are defined as colloidal dispersions of drugs covalently bound to lipids, and may exists as ultrafine vesicular, micellar, or hexagonal aggregates, depending on the chemical structure of the drug lipid complex.[7] The salient features of pharmacosomes are, increased entrapment efficiency, easy removal of unentraped drug from the formulation, no loss of drug due to leakage, no problem of drug incorporation and no influence of uncaptured volume and drug-bilayer interaction on entrapment efficiency. These amphiphilic drug-lipid complexes are stable and more bioavailable with low interfacial tension between the system and the GI fluid, thereby facilitating membrane, tissue, or cell wall transfer, in the organism.[14] Besides GI toxicity of ketoprofen, it is also poorly water soluble, due to which its dissolution in GI fluid is very low, which in turn adversely affects the bioavailability (50–60% only). The association of ketoprofen with zwitterionic phospholipids, which may be both electrostatic and hydrophobic in nature, renders the phospholipids more water-soluble and the NSAID more lipid-soluble.[2]

Therefore, the present work aims to develop and characterize the pharmacosomes of ketoprofen, to improve the water solubility, bioavailability and minimize the gastrointestinal toxicity of ketoprofen for rheumatoid arthritis.

MATERIALS AND METHODS

Materials

Ketoprofen was obtained as a gift sample from Lutin Pharmaceuticals, New Delhi. All other chemicals were of analytical grade.

Methods

Ketoprofen-PC complex was prepared by associating ketoprofen with an equimolar concentration of PC (80 % purity grade of soya-phospholipids). The equimolar concentration of PC (in 1:1 molar ratio) and ketoprofen were placed in a 100 ml round bottom flask and dissolved in dichloromethane. The solvent was evaporated off under vacuum at 40^0C in a rotary vacuum evaporator (Tecnai-6100, JASCO). The dried residues were collected and placed in vacuum desiccators overnight and then subjected to characterization.

Drug Content Uniformity

To determine the drug content in ketoprofen-PC complex, complex equivalent to 100 mg was weighed and added to a volumetric flask with 100 ml of pH 7.4 phosphate buffer saline. The volumetric flask was stirred continuously for 24 hr on a magnetic stirrer. Dilutions were made suitably and measured for the drug content at 260nm UV spectrophotometrically by Lambda 25 Perkin Elmer UV/Visible Spectrophotometer.[15]

Solubility

To determine the change in solubility due to complexation, solubility of ketoprofen and ketoprofen-PC complex was determined in pH 7.4 phosphate buffer saline and n-octanol by shake flask method. 50 mg of ketoprofen (and 50 mg equivalent in case of complex) was taken in a 100 ml conical flask. 50 ml of pH 7.4 phosphate buffer saline was added and then stirred for 15 minutes. The suspension was then transferred to 250 ml separating funnel with 50 ml of n-octanol and was shaken well. Separating funnel was allowed to stand for about 30 minutes. Concentration of the drug was determined from the aqueous layer spectrophotometrically at 260 nm.

Vesicle Size and Size Distribution Analysis

The effect of phospholipids concentration on the size and distribution of pharmacosomes vesicles was investigated by using Malvern Zetasizer (Centre for Pharmaceutical Nanotechnology, Pharmaceutics Dept. NIPER).

Vesicle Shape Determination

The surface morphology (roundness, smoothness, and formation of aggregates) of pharmacosomes were studied by Scanning Electron Microscopy (SEM).

Drug Exipient Interaction Studies (Compatibility Studies)

To check any kind of interaction of drug with phospholipid is determined by IR. IR spectra for ketoprofen, soya-lecithin and mixture were obtained on an IR spectrometer in the transmission mode with the wave number region 3500- 1000 cm^{-1}. IR spectra of ketoprofen and phospholipid (soya-lecithin) and mixture was carried out with IR spectrophotometer (Perkin-Elmer, USA).[16]

Differential Scanning Calorimeter (DSC)

Thermograms of ketoprofen, phosphatidylcholine (80%), and ketoprofen-PC complex were recorded using a differential scanning calorimeter. The thermal behavior was studied by heating 2.0 ± 0.2 mg of each individual sample in a covered sample pan under nitrogen gas flow. The investigations were carried out over the temperature range 40-280º with a heating rate of 10ºC/min.

X-Ray Powder Diffraction Analysis (XRPD)

The crystalline state of ketoprofen in the different samples was evaluated with X-ray powder diffraction. Diffraction patterns were obtained on a Powder X-ray diffractometer. The X-ray generator was operated at 40 KV tube voltages and 40 mA of tube current, using the Ka lines of copper as the radiation source. The scanning angle ranged from 1 to 60^0 of 2θ in step scan mode. Ketoprofen- phosphatidylcholine complex were analyzed with X-ray diffractions.

In-Vitro Dissolution Studies

In-vitro dissolution studies of ketoprofen complex as well as plain ketoprofen were performed in triplicate in a USP (XXIII) six station dissolution test apparatus, type II at 100 rpm and at 37°C. An accurately weighed amount of the complex equivalent to 100 mg of pharmacosomes was put into 900 ml of pH 7.4 phosphate buffer. Samples (3 ml each) of dissolution fluid were withdrawn at different intervals and replaced with an equal volume of fresh medium to maintain the sink conditions. Withdrawn samples were filtered and diluted suitably and then analyzed spectrophotometrically at 260nm.

Drug Release Kinetic Data Analysis

The release data obtained from various formulations were studied further for their fitness of data in different kinetic models like Zero order, Higuchi's. In order to understand the kinetic and mechanism of drug release, the result of *in-vitro* drug release study of pharmacosomes were fitted with various kinetic equation like zero order as cumulative percent release vs. time, higuchi's model as cumulative percent drug release vs. square root of time and in first order equation, a graph is plotted between time on x-axis and log cumulative percentage of drug remaining to be released on y-axis and in Korsmeyer plot, a graph is plotted between log T and log percent cumulative release.

In-Vitro Diffusion Study

In-vitro diffusion study was carried out by using diffusion cell model using egg membrane. Membrane was equilibrated for an hour in phosphate buffer pH 7.4. A small magnetic bead was placed in receptor cell containing buffer saline solution maintained at 25 rpm. High vacuum grease was applied to cell to prevent leakage. The permeation cell was placed in thermostat water bath at 37^0C. The diffusion cell was then placed on a magnetic stirrer with a small magnetic needle for uniform distribution of the phosphate buffer. Then isolated membrane was mounted between the compartments of the diffusion cell, with the epidermis facing upward into the donor compartment. Formulation was placed on upper portion of cell. Sample volume of definite volume (5 ml) was removed from the receptor compartment at regular intervals of 0, 1, 2, 4, 6, 8, 12 & 24 hours and an equal volume was replaced by fresh dissolution medium. Samples are to be filtered through filtering medium and analyzed by UV-spectrophotometer. Membrane was removed at the end of the diffusion study. Plots of cumulative amount of drug in receptor compartment versus time were plotted.

Stability Studies

The optimized pharmacosomal formulation was selected and stored in tightly closed glass vials at room temperature and in refrigerator (4 ± 2ºC).[6]

Specimen (0.5 gm) from each sample was withdrawn at an interval of one week and analyzed to determine the leakage rate. The samples were analyzed at predetermined time intervals visually and under optical microscope for appearance of drug agglomerates.

RESULTS AND DISCUSSION

In the present experiment ketoprofen-phospholipid complex (pharmacosomes) were prepared by a simple and reproducible method. Accepted uniformity of drug content among the batches was observed with all formulations and ranged from 98 to 99%. The results indicate that the process employed to prepare pharmacosomes in this study was capable of producing formulations with uniform drug content and minimal drug variability. Good percent loading of the drug makes the delivery of drug clinically feasible. Solubility of formulation was much higher than drug. By the mechanism of wetting and dispersion, increase solubility. Table1 provides solubility data. The amphiphilic nature of complex had shown increased bioavailability. The increase in solubility of ketoprofen in the complex can be explained by the solubilization resulted from the formation of micelle in the medium and by the amorphous characteristics of the complex.

Results indicate that vesicle size was dependent on the composition of lipid bilayer. As the concentration of soya lecithin in the pharmacosomes was increased from ratio 1 to 2, there was an increase in the vesicle size. This was due to the formation of micellar structure instead of vesicles, which have smaller size than vesicles.[12]

The result of the vesicle size measurement was well correlated with the report of Lobenberg *et al.* (1996), that vesicle size increased as soya-lecithin concentration was increased.

Pharmacosomes were found to be of disc shaped with rough surface morphology. Complexes were found to be as free flowing particles. As the phospholipids are natural component their different purity grades may have different effects in shape and surface morphology. The surface was found to be sticky in the complexes prepared with low purity grades (40 %) of phospholipids (Figure 1).

On the other hand the surface of the complexes prepared with the high purity grades of phospholipids (80 %) show rough, non-sticky and free flowing nature as in the present study.[13]

The size and shape of the DSC curves are useful in determining the crystallinity of the drug and the carrier. The results of the DSC test confirmed the association of ketoprofen and phosphatidylcholine (PC) in the complex as both peaks representing ketoprofen and phosphatidylcholine changed position.

The presence of unique peak in the complex is entirely different from the positions of peak of ketoprofen and phosphatidylcholine.

It is evident that the original peaks of phytoconstituent and phospholipids disappear from the thermogram of complex and the phase transition temperature is lower than that of phospholipids. The acetamin and indomethacin also showed the significant changes in the DSC thermogram when these aryl acetic acid derivatives were complexed with the 1,2-dipalmitoylphosphatidylcholine.[9] Figure 2 showed thermograph of Ketoprofen. In Figure 3 thermographs showed changed position of peaks that confirmed the formation of complex in the pharmacosomes.[11] DSC data of the prepared complex showed a noticeable reduction in the enthalpy as well as the melting point. These results are well supported by various previous studies done on the phospholipid complexes of some phytoconstituents like silybin, puerarin curcumin, naringenin and gallic acid.[10] The XRPD of ketoprofen complex revealed a broad peak similar to PC indicating that the ketoprofen was in amorphous form in phospholipid complex (Figure 4). The disappearance of ketoprofen crystalline diffraction peaks confirmed the formation of phospholipid complex.

Unlike liposomes, chemical bonding between the drug and phospholipids in the development of pharmacosomes might have resulted into a significant change of its X-ray diffraction. These results are well supported by previous studies done with the phospholipid complexes of

diclofenac and naringenin. The phospholipid complexes of puerarin, insulin and salmon calcitonin also supported the results obtained.[17] Formulations were subjected to *in vitro* drug release studies using pH-7.4 Saline Buffer. For the optimization of drug concentration that could be incorporated into the vesicles, the pharmacosomal formulations were subjected to *in vitro* drug release. Values of percentage cumulative amount of ketoprofen diffusion are summarized in the Figure 5. Ketoprofen complex showed 87.9% of drug release at the end of 24 h in dissolution study in pH-7.4 saline buffer. It was concluded that the phospholipid complex of ketoprofen may be of potential use for improving the solubility of ketoprofen and hence its bioavailability. The complexes may also reduce GI toxicity of the drug. Phospholipids being an amphiphilic surfactant increased the solubility of the drug by the action of wetting and dispersion. And that's why the dissolution profile of the complex was found to be improved. The release experiments clearly indicated sustained release of ketoprofen from pharmacosomal formulations.[4]

In order to define a model, various models are designed, which will represent a better fit for formulation. The first order rate describes the release from system, where release rate is concentration dependent. Zero order rates describes release rate independent of drug concentration.

Highest drug release in 1:4 ratios. All the formulations followed mixed order kinetics. In vitro drug release profile could be best expressed by Higuchi equation ($r^2 = 0.996$).

To confirm the diffusion mechanism, the data were fitted into korsmeyer–peppas equation. All the formulations showed good linearity (R^2: 0.925 to 0.998) with slope (n) values ranging from 0.522 to 0.595, indicating that non-Fickian diffusion (anomalous) was the predominant mechanism of drug release from all the formulations.[5] For the optimization of drug concentration that could be incorporated into the vesicles, the pharmacosomal formulations were subjected to *in-vitro* drug release through egg membrane and the % amount of drug permeated was calculated. Values of percentage cumulative amount of ketoprofen diffusion across egg membrane are summarized in the Figure 6. The release rate of ketoprofen from pharmacosomes was significantly higher. After 24 h, maximum drug was released from formulation PC4 is 55.3%. The release experiments clearly indicated sustained release of ketoprofen from pharmacosomal formulations. From the results, it was found that pharmacosomes incorporated into residues does not change for 60 days.

No drug agglomerates appear even after 60 days at refrigerated temperature (4 ± 2^oC) as shown in Table 2.

CONCLUSION

In the present study ketoprofen–phospholipid complex (pharmacosomes) were prepared by a simple and reproducible method. The physicochemical investigations showed that ketoprofen formed a complex with phospholipids with better solubility and dissolution profile. The phospholipid complex of ketoprofen may be of potential use for improving bioavailability. As the phospholipid complexes have also been reported to reduce the GI toxicity of the drugs, the phospholipid complex of ketoprofen may also be useful or minimizing the GI toxicity of ketoprofen.

The pharmacosomes may be developed for other NSAIDs with poor bioavailability and GI side effects. Thus, the formulated pharmacosomes seem to be potential candidate as an oral controlled drug delivery system in this era of novel and controlled drug delivery systems. The developed formulations are expected to improve the patient compliance, form better dosage regimen and provide optimum maintenance therapy to rheumatoid arthritis patients.

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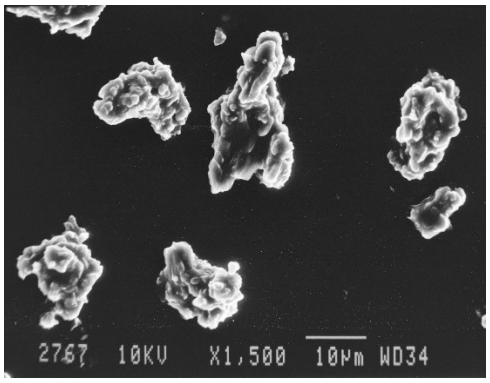
Table 1: Evaluation of pharmacosomes

Formulation Code	Solubility (mg/ml) (n=3)	Drug content (%) (n=3)	Particle size (nm)
PC1	7.6±0.15	98.8±0.43	233
PC2	8.58±0.26	97.3±0.25	390
PC3	9.19±0.20	98.3±0.15	433
PC4	9.59±0.29	98.5±0.17	578
PC5	3.04±0.23	97.2±0.23	177
PC6	3.67±0.32	99.4±0.28	290
PC7	4.4±0.32	99.8±0.49	345

Table 2: Stability studies of pharmacosomes

Parameters	Time (in days) and storage temperature	Observations
Agglomerates of drug	20(1)	Not appeared
	20(2)	Not appeared
	40(1)	Not appeared
	40(2)	Appeared
	60(1)	Not appeared
	60(2)	Appeared



(a)

(b)

Figure 1: Scanning Electron Microscopy of PC4 (a) and PC7 (b)

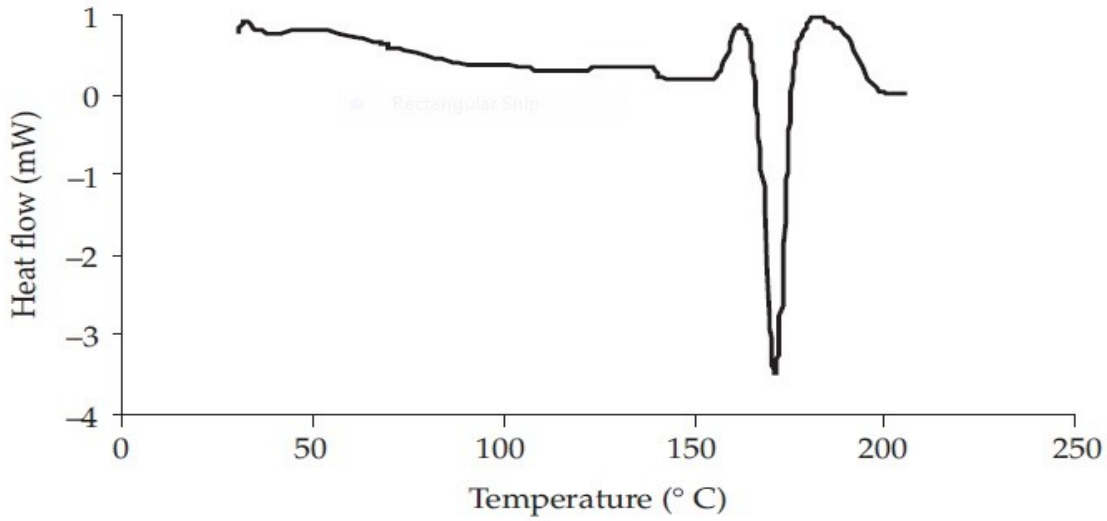
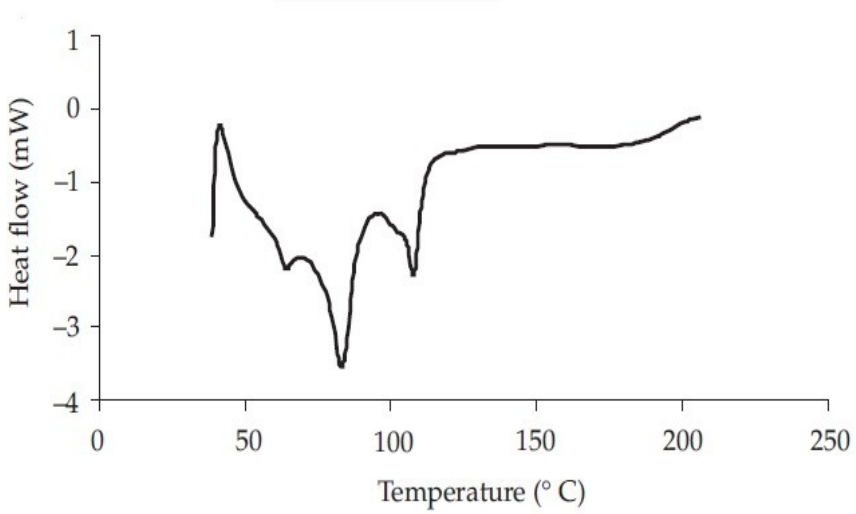
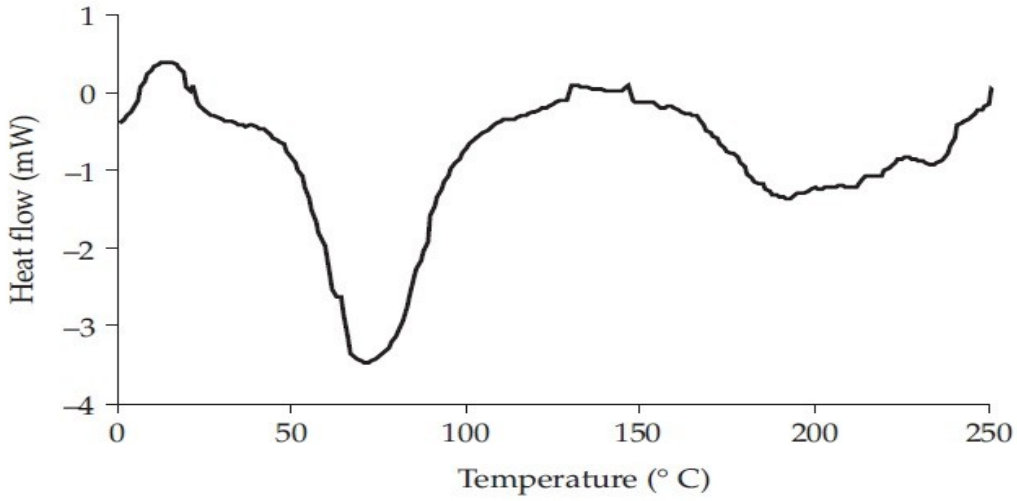


Figure 2: DSC Thermogram of Ketoprofen



(a)



(b)

Fig. 3: (a) Phospholipid, (b) and Ketoprofen-Phospholipid Complex (PC4)

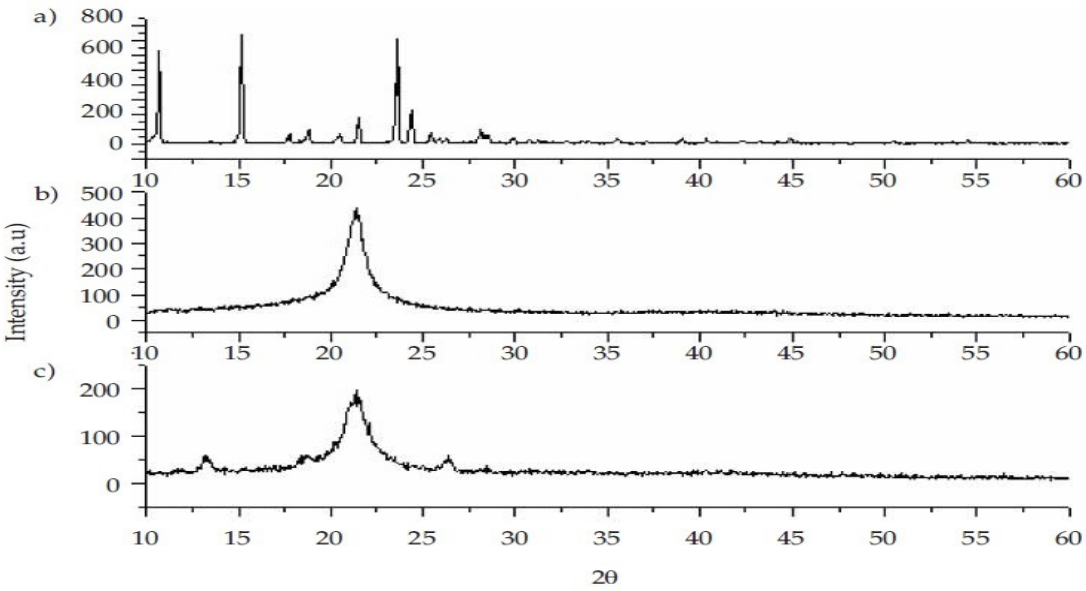


Figure 4: XRPD study of Ketoprofen (a), phospholipid (b), ketoprofen-complex (c) (PC4)

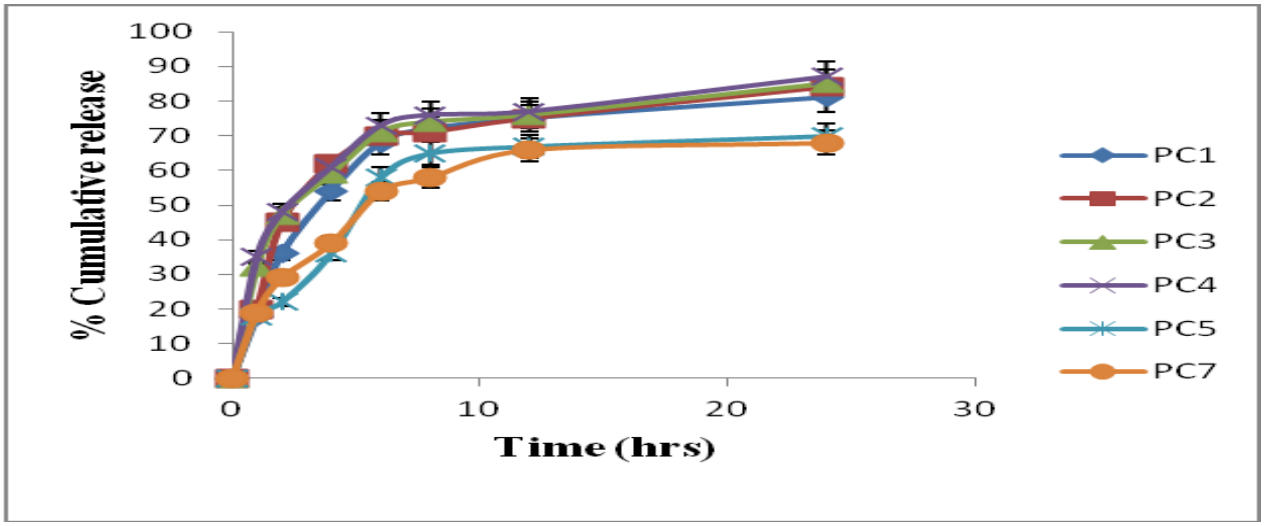


Figure 5: *In-vitro* release of pharmacosomes

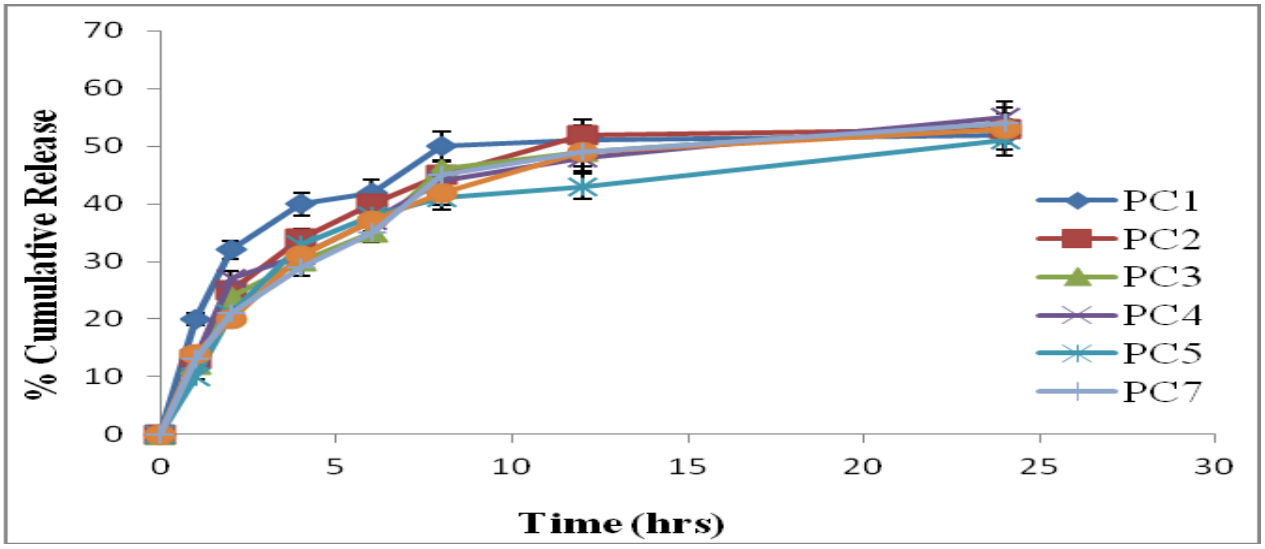


Figure 6: *In-vitro* diffusion of pharmacosomes

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