Sustained delivery of Non-Steroidal Anti-Inflammatory drug for Wound dressing

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Abstract: Aim of this study is to fabricate and evaluate the composite wound dressing materials which contains drug and polymer. The successful incorporation and sustained release of Non-Steroidal anti-inflammatory drug (diclofenac sodium) from electrospun poly (vinyl Alcohol) nanofibrous scaffolds without the loss of structure was demonstrated. The morphology of the electrospun scaffold was found to be dependent on the drug and polymer concentration, applied voltage, Flow rate, Distance between the taylor cone and collector. Morphology of the scaffold was imaged using Scanning Electron Microscopy (SEM). The FTIR spectra of composite sheet shows characteristics peak of carboxylate functional group and Hydroxyl group. The electrospun scaffold is loaded with model drug bovine serum albumin (BSA) and drug releasing behaviour is studied using UV Visible double beam absorption spectra at one hour interval. The anti inflammatory drug release behavior from the electrospun composite also investigated, the maximum dosage of drug was released from the scaffolds after 1 h of incubation in water at 37 °C under mild magnetic stirring condition. The anti inflammatory drug released from these electrospun scaffolds was effective in their ability to (>90%). The non-woven nanofibrous biodegradable scaffolds and their capability for local delivery of drug increases their desired utility in biomedical applications.

Introduction

In the past 40 years, many long acting veterinary drug delivery technologies have been developed specifically for use in animals. Traditional dosage forms are typified by tablets, capsules, suspensions, etc . Following administration, such dosage forms immediately expose the drug to dissolution and subsequent absorption. The appearance of the drug in the blood is typically rapid and sustained for only a short duration depending upon the half-life of the drug. In contrast Drug delivery control systems is a specialised area dealing with formulating the drug in such a way that the formulation causes the entrapped drug to slowly release resulting in a long action for the administered drug. In majority cases, the physicochemical properties of the long acting formulation control the resultant blood profiles as opposed to the innate properties of the drug (1). Controlled release formulations have received increased attention in the pharmaceutical treatment of animals as they offer many advantages compared to previous forms of dosage, including low toxicity and improved therapeutic efficacy, when the drug is released at a controlled rate (2). 'Smart' delivery systems can have multifunctional characteristics to avoid biological barriers to successful targeting and they may also be 1.Time-controlled, 2.spatially targeted, 3.Self-regulated, 4. Remotely regulated and 5. Pre-programmed. It gives the possibility to maintain the concentration throughout the survival of causative agent in threshold level. Pharmacokinetic profile for traditional drug delivery and Sustained drug delivery is shown in the Figure. 1.
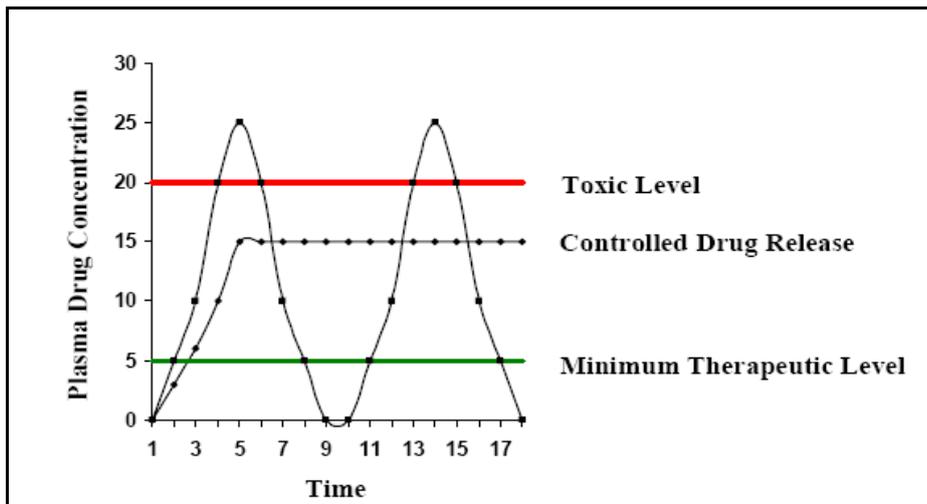
Fig.1.Pharmacokinetic Profile

The majority of veterinary drug delivery systems are produced from nondegradable polymers such as polyurethane, silicon, and acetate-vinylidene copolymers. These substances, approved by government regulators, are biocompatible, inert, and high-cost. Interest in biodegradable polymers as delivery control systems for veterinary applications has increased as they do not require removal following treatment. These biodegradable organizations suffer degradation and can be rapidly expelled by the body. They can provide remarkable benefits such as reductions in cost and stress on the animal. Currently, however, few biodegradable drug delivery systems are commercially viable for veterinary use as factors such as product price, regulatory burdens, and the uncertainty of some formulations have limited their development (3).

It is important to know if the drug delivery system is intended for the treatment of domestic animals or livestock, the foremost applications of veterinary medicine. In Drug delivery for veterinary, biodegradable systems use as microspheres and implants, including *in situ,* have been evaluated for temperature and ectoparasitic standard, growth, vaccine and antibiotics delivery, and use as antiparasitic factors and steroids for fertility control (4). Beside these, Nanoparticles and nanoformulations provide massive advantages regarding drug targeting, delivery and release. The nanotechnological applications in human medicine, food and nutrition are only applicable after the prototype is certified nontoxic by invitro and invivo laboratory testing. So, after confirming the nontoxic behavior of prototype, nanotechnology possesses these applications for domestic animals also. So, nanotechnology may offer a breakthrough in clinical veterinary medicine and animal health.

Electrospinning has been extensively explored as a simple and versatile technique to produce micro and nano-fibers of polymers because it provides a potential way to fabricate continuous nanofibers with different structural designs. A typical electrospinning setup is comprised of a reservoir for polymer solution, pump, capillary spinneret, and high voltage power supply. The electrospinning process consists of applying a strong electrostatic field supplied by the high voltage source to polymer solution as it exits the spinneret connected to its reservoir. Under the impact of the electrostatic field, a pendant shape bead of the polymer solution at the capillary tip is deformed into a Taylor cone shape. When the surface tension is overcome by the electrostatic forces generated by the electric potential a fine charged jet is ejected and moves toward the grounded collector, polymer fibers accumulate on the collector (5).

The Electrospun nanofibers have been explored as a system for drug delivery control and sustained release applications, the active ingredients are dissolved in the polymer solution and incorporated into nanofibers by using a one-step method like electrospinning (6). The nanofibers structure possesses the advantages of having a three-dimensional open porous medium with high surface area, what enhance therapeutic efficacy and minimize toxicity (7). Due to the high surface area and the porous structure of these nanofibers applications in drugs delivery systems such as the liberation of anti-neoplastic (8), antibiotics (9), and pheromone agents have been suggested. Other appealing advantages reported to nanofibers obtained by electrospinning are high encapsulation efficiency, high loading capacity, and cost-potency.

Poly(vinyl alcohol) is a well-known, eco-friendly, biodegradable polymer owing to its desirable properties such as biocompatibility, nontoxicity, and appropriate mechanical properties. Because of these properties, PVA is used in some biomedical applications such as implants of artificial organs, soft contact

lenses, cardiovascular devices, and cartilage skin. PVA polymer is suitable for blending various polymers and additives in electrospinning process to achieve uniform nanofibers (10).

Anti-inflammatory painkillers like diclofenac are sometimes called non-steroidal anti-inflammatory drugs (NSAIDs), or just 'anti-inflammatories'. Anti-inflammatories are drugs that have the capacity to control inflammation, act as analgesics, and prevent fever. Diclofenac is used to treat painful conditions such as arthritis, gout, dental pain, migraine, sprains and strains, and pain after surgical operations. It eases pain and minimizes inflammation. Diclofenac sodium is recommended for human and animal use in cases of pain, fever, and inflammation (11).

The release of active agents from electrospun fibers is reported to follow nearly zero-order kinetics due to the degradation mode of the fibers (12). Moreover some researchers registered a burst release for active agents in electro spun nana fibers via diffusion of the drug on or nearby the fiber's surfaces. In this sense, the present study aims to investigate the morphology characteristics of diclofenac sodium encapsulated in poly(vinyl alcohol) fibers obtained by electrospinning, and investigates the release characteristics of diclofenac sodium encapsulated in PVA nanostructured membranes.

Materials and Methods

Materials

Poly(vinyl alcohol) (PVA) and Diclofenac sodium were obtained from Sigma-Aldrich. Deionized water was used to prepare the polymeric solutions. The chemical assembly of polymer and drug are illustrated in Figure 2.


Figure 2: Chemical structures: poly(vinyl Alcohol) (PVA) (a) and diclofenac sodium (b).
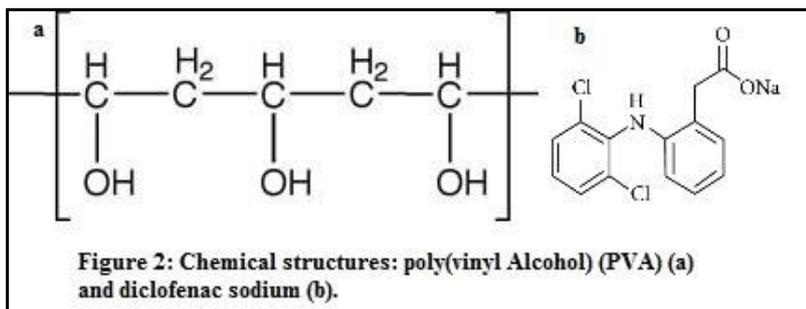
Preparation of Solutions

To prepare solutions, amounts of PVA were weighed and dissolved in Deionized water (8% wt) under vigorous stirring for several hours until complete dissolution was attained. Diclofenac sodium, an anti-inflammatory drug was also weighed and added to PVA solution in the concentration of (0.5% w/v) to study its controlled release.

Electrospinning

The electrospinning setup used in this study consisted of a 5 mL plastic syringe, 21G needle (i.d.: 0.84 mm), a high-voltage supply (Flow controller :KD Scientific 781220,Power supply :Zeonics). which can generate positive direct current voltages up to 99 kV. The needle was connected to the high-voltage source, positioned at a fixed distance from the grounded collector (tip-to-collector distance–TCD), and the polymer solution was injected at a constant rate. The applied voltage was adjusted at 15 kV. The solution was delivered to the blunt needle tip via syringe pump to control the solution flow rate. Fibers were collected on an electrically grounded aluminum foil placed at 15 cm vertical distance to the needle tip. The above spinning conditions were found being the best condition to make PVA based nanofibers in our previous report (13, 14). A schematic representation of Electrospinning set up is given in Figure. 3. Electrospun nanofibers have been deposited on aluminum foils. The fiber mats were than collected by peeling off and stored in a desiccator.

Figure 3. Electrospinning Setup
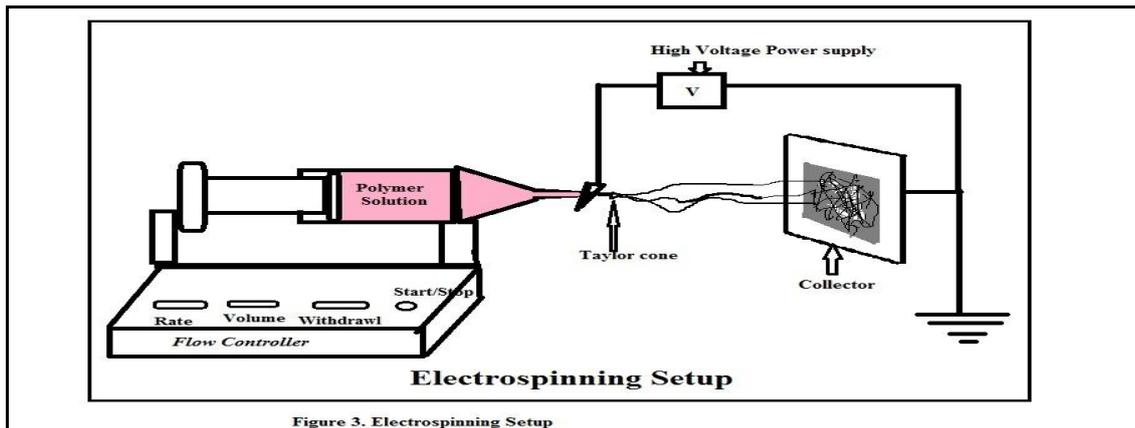
Scanning Electron Microscopy (SEM) Characterization

Samples for morphology characterization were prepared by cutting samples of the mats with a scissor and fixed on aluminum stubs using double-sided adhesive tape and sputter coated. Samples were analyzed for surface characterization using a Scanning Electron Micro-scope (VEGA3 TESCAN) at a voltage of 20 kV

FTIR study

The structure of nanofibers was analyzed by FT-IR spectra. Powder Samples were mixed with KBr to make pellets. IR spectra in the absorbance mode were filed using FT-IR spectrometer, and fiber sample was studied by sensor, connected to a PC, and the data was analyzed by IR Solution software.

In Vitro Drug Release

The amount of diclofenac sodium released was monitored using the following process: 50 mg of spun film was placed in a 50 mL Bovine Serum Albumin of pH 6.8 and shaken in a water bath at room temperature (37°C). Each hour, samples collected from the system (each 3.5 mL) for analysis and returned to the release medium (15). The releasing medium was quantified for the amount of the released diclopenac sodium. Each measurement was carried out in triplicate and the cumulative amount of diclofenac sodium released over time was calculated. The cumulative release profiles of diclopenac sodium were expressed as unit percentage of drug release during different times (1, 2, 3, 4, 5, 6, 7, and 8h).

Results and Discussion

Scanning Electronic Microscopy

Figure 4 shows the scanning electron micrographs of the PVA nanofibre without diclofenac sodium after electrospinning. The electrospinning process constructs relatively smooth nanofibers. Dewdrop or agglomerated nanofibers cannot be observed in the obtained mats. As can be seen in this figure, the solution has result in a smooth morphology for nanofibers.

Figure 4. SEM micrograph of the PVA nanofibre without diclofenac sodium
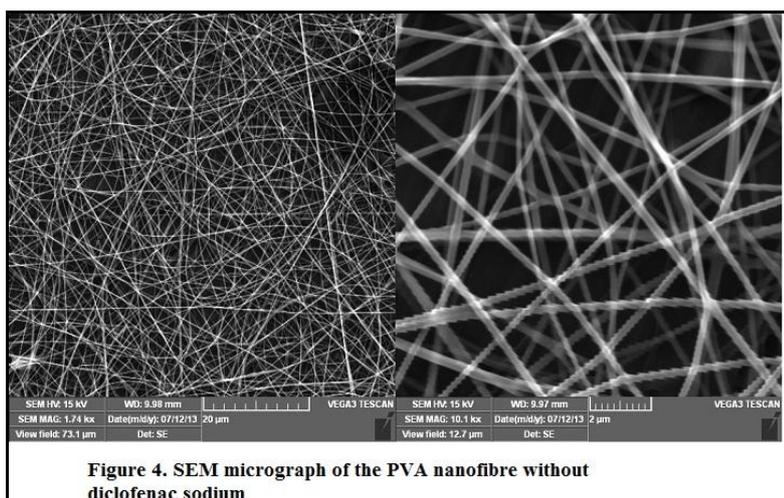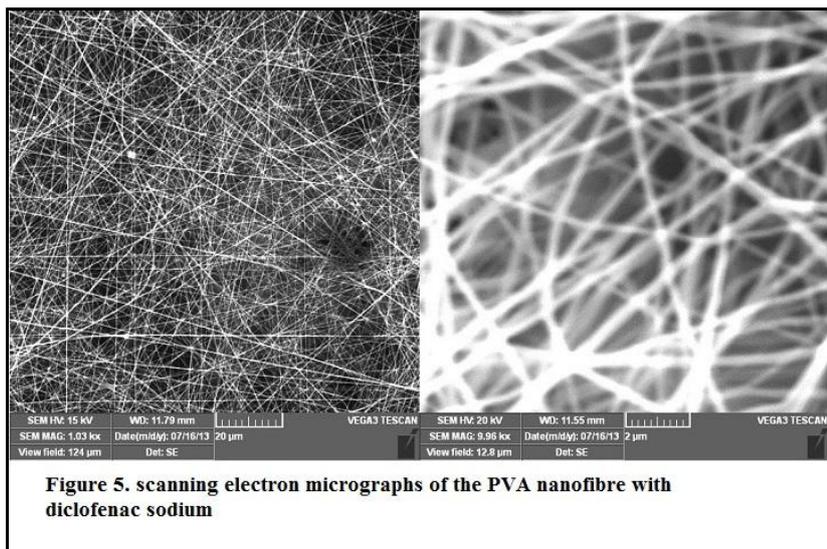
Figure 5. scanning electron micrographs of the PVA nanofibre with diclofenac sodium

Observable beads can be noticed in the Figure 5.This could be attributed to low viscosity and/or high conductivity of the solution. Many experiments have shown that a minimum viscosity for each polymer solution is required to yield fibers without beads. At a low viscosity, it is common to find beads along the fibers deposited on the collection plate. When the viscosity increases, there is a gradual change in the shape of the beads from spherical to spindle-like until a smooth fiber is obtained as shown in Fig. 5. At a lower viscosity, surface tension has a dominant influence along the electrospinning jet causing beads to form along the fiber. When the viscosity is increased which means that there is a higher amount of polymer chains entanglement in the solution, the charges on the electrospinning jet will be able to fully stretch the solution with the solvent molecules distributed among the polymer chains (16). The surface tension has a part to play in the formation of beads along the fiber length. The viscosity of the solution and its physical properties will determine the range of elongation. In turn, it will have an effect on the diameter of the resultant electrospun fibers. The PVA polymer containing diclofenac sodium at demonstrated morphology predominantly composed of nanofibers with diameters of approximately 400 nm (17).

FTIR Analysis

The representative FTIR absorption spectrum of the PVA/ diclopenac sodium scaffold in the 4000 cm^{-1} to 500 cm^{-1} range is shown in fig. 6. As other major components, OH- ions are identified by observation of the wide and intense band from about 3700cm^{-1} to 1700 cm^{-1}. As shown in Figure, the characteristic absorption bands of PVA occur at 3291.49 cm^{-1} (stretching of OH), 2928.50cm^{-1} (asymmetric stretching of CH_2), and 1142.80cm^{-1} (stretching of CO from crystalline sequence of PVA). The principle IR peaks of pure diclofenac sodium appeared at wave-numbers of 1283.14 cm^{-1} and 1304.22 cm^{-1} and they resulted from C-N stretching. The peaks at 1507.81 cm^{-1} and 1574.81 cm^{-1} resulted from stretching of the carboxylate functional group (C=C and C=O), respectively (18). These characteristic peaks were identified in the drug-loaded nanofibre (Fig. 6) with no appreciable changes in frequencies. Thus it can be inferred that there was no chemical interaction between the drug and polymer in electrospun nanofibres.

In vitro release study

UV-Vis absorption spectra, spectrum drug release of diclopenac sodium are observed. The rate of drug release from the nanofibre in Bovine Serum Albumin is directly related to the rate of inflation. Light with an appropriate wavelength can cause oscillation in the conduction electrons of diclopenac sodium. The interaction of light with the electrons of diclopenac sodium leads to a phenomenon known as Surface Plasmon Response, which results in optical absorption peaks. Bovine Serum Albumin solutions containing diclopenac anti inflammatory drug show characteristic optical absorption spectra in the UV-Vis region.
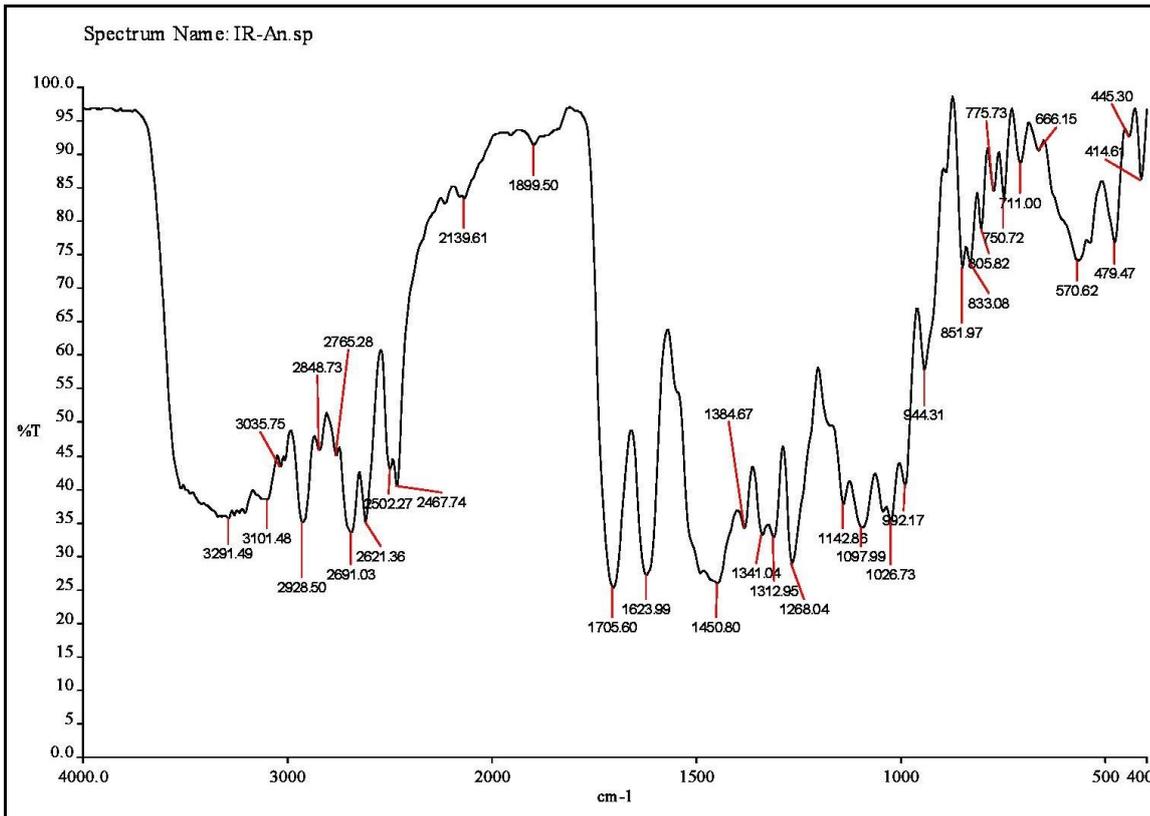
Figure 6. FTIR absorption spectrum of the PVA/ diclopenac sodium scaffold
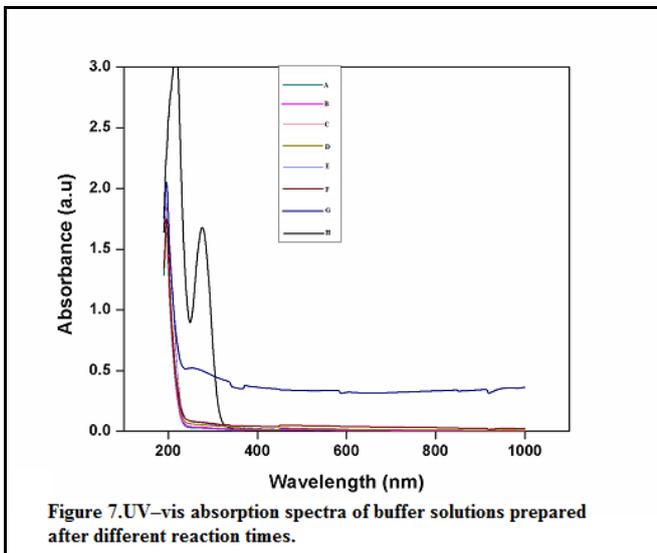

Figure 7 shows the UV–vis absorption spectra of Bovine Serum Albumin solutions prepared after different reaction times. It can be observed that (i) at the early stages of the reaction (after 1 h) the plasmon band is broaden, which indicates that there was no substantial reduction occurred. (ii) extending the reaction time to up to 7 h leads to enhancement of the plasmon intensity, bell shaped plasmon band at appeared, which is taken as indication of the released drug from nanofibre mat. (iii) further increase in the reaction time, up to 8 h, was accompanied by insignificant increase in the absorption intensity. It can be seen that there was an absorption band with a peak around 200–320 nm, which corresponded to the SPR absorption band of the drug. In Bovine Serum Albumin, an increase in the diclopenac sodium concentration resulted in an observed increase in the intensity of the bands. No absorption of any kind was observed for the base Bovine Serum Albumin solution.

Cumulative profile study

Table.1.Standard calibration curve for diclofenac sodium in Bovine Serum Albumin:

| Concentration of diclopenac sodium (mg/ml)	Absorbance
0	0
2	0.055
4	0.12
6	0.186
8	0.246
10	0.31

Dissolution media for Diclofenac sodium was chosen to be Bovine Serum Albumin of 6.8 PH. A UV spectrophotometer method was developed and calibration curve was constructed in the Bovine Serum Albumin of PH 6.8 as the solvent system. Table 1 shows the standard calibration value of diclofenac sodium in BSA. Linear plot with R^2 value (0.999) and slope of 0.031 was obtained when absorbance value of the working standard solutions were plotted against concentration (19). Calibration curve is represented in Fig. 8

Figure 8. Standard calibration curve for diclofenac sodium in phophate buffer
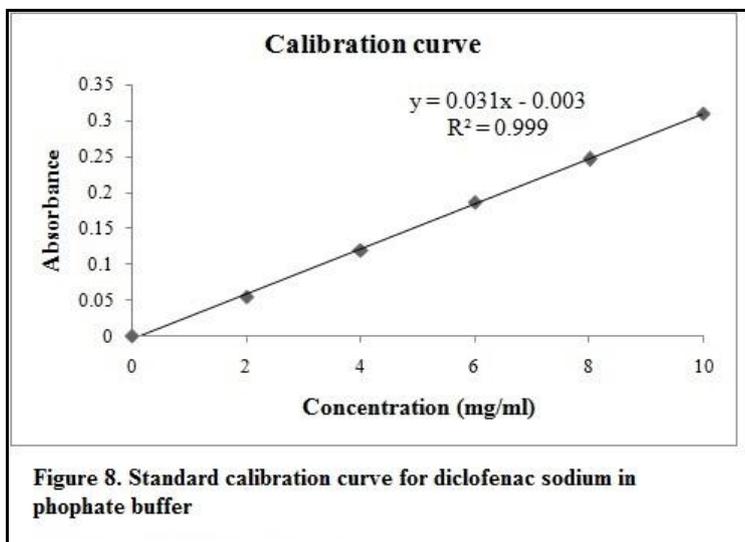
For determining the percentage of drug release, the samples were withdrawn at 1 h intervals. The samples were filtered and suitably diluted to determine the absorbance at 290 nm using UV/ Visible single-beam spectrophotometer (20). Concentration of drug released was calculated from the standard curve and tabulated (Table 2). Cumulative % of drug released vs. time (zero order release plot) was shown in fig. 9

Table 2. Cumulative percentage of drug release during different reaction time:

Time (h)	Absorbance at 290 nm	Concentration of diclofenac sodium (mg/ml)	Cumulative % of drug release
0	0.000	0.00	0.0
1	0.009	0.387	7.7
2	0.02	0.74	14.82
3	0.026	0.968	21.92
4	0.044	1.5161	30.3
5	0.077	2.48	49.6
6	0.095	3.161	63.22
7	0.118	3.9	78
8	0.146	4.84	96.12

Figure 9. Cumulative % of drug released vs. time
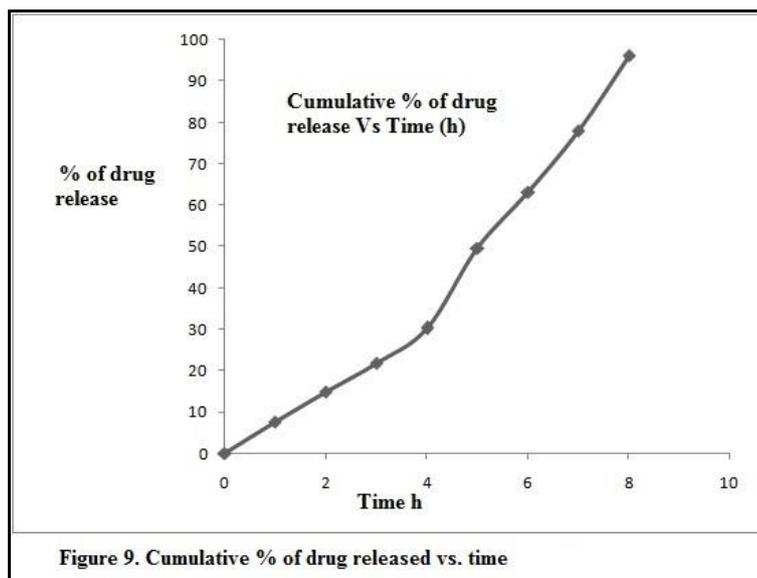
This phenomenon is anticipated as the release rate from the PVA nanofibre mat is diffusion dependent. Moreover, anti-inflammatory drug delivery depends on drug concentration in the nanofibers in which increasing amounts of drugs gave rise to higher release rates. The proportion of diclopenac sodium in the nanofibre increased, the overall time for release of the drug from the fibre was also increased. Drug releases from nanofibre membrane depend on drug dissolution, drug diffusion or both.

Conclusion

The PVA nanofibers containing Diclofenac sodium were produced by electrospinning. The formation of electrospun nano fibers was confirmed by obtaining SEM micrographs. The in vitro release of diclofenac sodium depends concentration in the nanofibers. The development of PVA membranes encapsulating diclofenac sodium could be useful in commercial markets with potential applications in controlled-release devices in human and veterinary medicine.

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