Molecular Pharmaceutics & Organic Process Research

Sami et al., J Mol Pharm Org Process Res 2018, 6:1
DOI: 10.4172/2329-9053.1000143

Research Article Open Access

Sustained Release of Ibuprofen by a Novel Formulated Hydrogel Containing Graphene Oxide

Amtul Jamil Sami*, Madeeha Khalid, Sitara Nasar, Hummad Ahmad Mangat and Yasha Nazir Butt

Institute of Biochemistry and Biotechnology, University of the Punjab, Lahore, Pakistan

***Corresponding author:** Amtul Jamil Sami, Institute of Biochemistry and Biotechnology, University of the Punjab, Lahore, Pakistan, Tel.: 923227845179; E-mail: 2amtuljamilsami@gmail.com

Received date: July 30, 2018; **Accepted date:** August 13, 2018; **Published date:** August 23, 2018

Abstract

Ibuprofen is a common hydrophobic drug with antipyretic and anti-inflammatory activity. The oral administration of ibuprofen is linked with gastric disturbances, which could be overcome by using a transdermal patch for site specific delivery. To bypass the gastro intestinal tack, it is required to use the transdermal route for drug delivery. The drug has poor solubility at acidic and physiological pH, which hinders the drug availability when used in dermal cream. For the sustained drug delivery, hydrogel was designed, containing Graphene oxide, chitosan and poly-acrylamide. The novel formulated hydrogel was characterized for physical properties using FTIR and SEM which confirms bonding pattern and dispersal of Graphene oxide layer on the top of hydrogel platform. The swelling properties were also studied at different pH values. The graphene oxide added hydrogels gels were able to facilitate the sustained release of Ibuprofen drug molecule, when avian skin was used as a model. Graphene Oxide (present in the hydrogel) interacts with the lipid bilayer membrane of avian skin model and enables the release of drug. The drug release was monitored by taking absorbance at 221 nm, after specific intervals. The results showed that the drug started releasing continuously, and kinetics studied. The FTIR analysis of the hydrogel before and after drug release is reveals that there was no physicochemical incompatibility between the drug and the hydrogel. It was confirmed from the results that chitosan, acrylamide, and graphene oxide based hydrogel could be successfully used for the sustained drug delivery of ibuprofen. This study could lead to the production of dermal patches for ibuprofen with minimum or no side effects. Tests showed that the released drug retained its biological activity. Such a method can be useful for the treatment of musculoskeletal inflammation which can be seen in diseases as gout and arthritis. To the best of our knowledge this is first report on the application of GO for sustained release of Ibuprofen through hydrogel when used as a dermal patch on an avian skin model.

Keywords: Ibuprofen; Graphene oxide; Transdermal patch; Hydrophobic drug

Introduction

Ibuprofen is commonly used for pain relief and prescribed as an antipyretic and anti-inflammatory agent [1]. It is a small molecule with a low molecular weight of 206 amu and hydrophobic in nature. The mechanism of drug involves inhibition of the Cyclo-oxygenase enzymes COX-1 & COX-2 that cause the production of prostaglandins (mediators of pain) [2]. It can be taken via oral, rectal or cutaneous routes but it is associated with side effects such as gastrointestinal problems, nausea, gastric ulceration etc. To avoid the side effects and improve the bioavailability of the drug, transdermal drug delivery is an ideal method [3]. Transdermal drug delivery is utilized to maximize the flux of drug to systemic circulation at the same time avoiding drug retention and first pass metabolism. Ibuprofen has a half-life of 1.5-2 hrs and a net charge of Zero. The solubility of ibuprofen is 0.020 μg/ml at neutral pH and is sparingly soluble at alkaline pH [4]. Due to the hydrophobicity the skin permeation of Ibuprofen is low and thus most of the topical formulations deliver very low effective concentration of the drug to the target [5,6]. Therefore it is required to engineer a material for formulating a hydrogel that could be used as a transdermal patch for the sustained release of this drug.

The network of hydrogels and high water content makes them biocompatible and desirable for applications in biomedical field [7]. For the synthesis of novel hydrogels selection of components is very crucial. The most important parameter for the selection of a polymer or a combination of polymers for a transdermal patch is based on the biocompatibility, swelling behavior, mechanical strength, ionic form of the drug and the type of physical properties that need to be engineered in a particular dermal patch. One attractive option among bio-based polysaccharides is chitosan, a linear polysaccharide co-polymer consisting of randomly dispersed β-1, 4 linked D-glucosamine and N-acetyl glucosamine [8,9]. It is a versatile polymer, as it is non-toxic, biodegradable and biocompatible. The hydrogels based on chitosan lack mechanical strength [9,10], to overcome this drawback a combination of Acrylamide or other synthetic polymers is desired. One of the most frequently used hydrophilic artificial polymers is polyacrylamide. This polymer is formed by the crosslinking of acrylamide with N, N, N', N'- methylene bisacrylamide. The polymer forms stable crosslinks between the chains, allowing hydration and maintain the porous structure at the same time. Polyacrylamide based hydrogels are usually pH sensitive and good candidates for drug delivery systems [11]. Dong and Hoffman synthesized acrylamide hydrogels for the targeted delivery of an enteric drug indomethacin in the intestine [12].

Role of GO is also identified in the control of drug delivery. Recently it has been reported that GO interact with the lipid bilayer of the cell membrane so its addition can prove useful in delivering molecules like ibuprofen to the target tissues and it could be used as a docking system for the drug molecules. Graphene oxide based nano composite is a

promising tool to address these drawbacks, due to availability of several functional groups and 2 dimensional unique structures [13,14]. The physical properties of hydrogels can be tailored to facilitate the encapsulation or adsorption of drug molecule and its effective release. In this way drugs are efficiently transported to the network of the hydrogels and it also protects the drug from degradation until its release [15,16]. Dallavalle [17] reported that Graphene can Wreak Havoc with Cell Membranes. Further it was reported that molecular dynamics shows larger sheets of GO lie mainly flat on the top of the bilayer where they cause disorder with the membrane and create a patch of upturned phospholipids. The effect arises in order to maximize the interaction between hydrophobic moieties and is quantitatively explained in terms of flip-flops by the simulation analysis. Functionalization Pattern of Graphene Oxide Sheets that Control the Entry or Produces Lipid Turmoil in Phospholipid Membranes is reported by the same researchers [18]. Further Molecular dynamics, coarse-grained to the level of hydrophobic and hydrophilic interactions, shows that graphene oxide sheets, GOSs, can pierce through the phospholipid membrane and navigate the bilayer, under standard conditions. Herein, we, in the presented work successfully demonstrate the sustained release of hydrophobic drug Ibuprofen through a novel hydrogel, by the interaction of GO with the lipid bilayer, for the sustained release of drug.

Materials and Methods

Materials

Chitosan-200 (CS) with (90%-Degree of Deacetylation) was purchased from Biolog Biotechnologie und Logistik GMBH, Germany while ammonium persulfate (APS), Yeast, Tryptone, L.B. Agar, and Na-Alginate were purchased from Sigma Aldrich. Tetramethylethylenediamine (TEMED), N'N'-methylenebisacrylamide, Acrylamide (AM) and Glycerol (99%) were obtained from Uni-CHEM, Fluka-BioChemika, Acros-Organics and Omicron Sciences Limited respectively. Ibuprofen Tablets 400 mg were purchased from Aliud Pharma GmbH and Co.KG, Laichingen, Germany containing 400 mg Ibuprofen per tablet. Used zinc-carbon batteries were used as a source of graphite. Absolute ethanol, potassium hydroxide, acetic acid, and sodium hydroxide were purchased from Analar, BDH Company. All chemicals used in the experimental work were of analytical grade.

Formulation of CS/AM/GO hydrogel and drug loading

GO was purchased (Sigma) and dispersed in water by ultrasonication (13 kHz frequency). Chitosan was dissolved in 2% acetic acid. CS/AM/GO hydrogel was synthesized with the final composition of the components as chitosan 1%, GO 0.016%, acrylamide and bis-acrylamide 0.83%, glycerol 0.02% and TEMED 0.005%. The cross linking was initiated by adding freshly prepared solution of ammonium per sulfate. The hydrogel was casted in moulds (2.5 cm in diameter) and incubated at 55°C overnight. Fixing solution containing 96% ethanol, 4% glycerol and 0.1% KOH was added to the hydrogels and incubated for an extra hour. The hydrogels were then removed from the moulds, washed with distilled water, dried and stored in sterile bags at room temperature till further use. The method for the preparation of Ibuprofen loaded hydrogels was based on the method as described previously [19]. Each hydrogel was loaded with ten drug beads having 800 microgram of drug. Ibuprofen was estimated by UV method at 223 nm, as described previously [20].

Swelling properties

Swelling behavior of hydrogel was monitored using gravimetric analysis. Dried hydrogels were weighed. The hydrogels were allowed to swell in buffers of different pH (range: pH 2-10). After every 10 minutes interval, the gels were removed periodically, dried on filter paper, weighed and readings were continuously recorded. Swelling ratio was calculated using following formula:

$$Sw = \frac{M_t - M_0}{M_0}$$

Where Sw exhibits swelling (gg^{-1}) of hydrogel, M_0 represents weight of dry hydrogel; M_t is the weight of hydrogel after time interval t [21]. For swelling kinetics, recorded masses of CS/AM/GO hydrogel were used and were fitted into power-law expression equation 2, in order to determine the mechanism of water diffusion followed by hydrogels [22].

$$F(\%) = \frac{M_t}{M_{eq}} = kt^n$$

Where M_t and M_{eq} represents amount of solvent penetrated into gel at time (t) and at equilibrium state (eq) respectively, k is a constant related to the structure of polymeric network of hydrogel and n is diffusional exponent which describes mechanism of diffusion followed by hydrogel.

Structural analysis

FTIR spectrum of CS/AM/GO hydrogel before and after drug release was generated using IR Prestige-21-Shimadzu-Spectrometer. An average of 70-scans was set and spectral resolution was 4 cm^{-1} with effective range of 500 to 4000 cm^{-1} which determined the composition of formulated hydrogel. For Scanning electron microscopy S-3700N Hitachi with EDX attachment was used to examine the surface topology of the formulated CS/AM/GO hydrogels.

In-vitro cell culture analysis

In vitro cell culture studies were carried out using HeLa cell lines by direct contact method. Cells were grown in 48 well plates at 37°C in 95% air and 5% CO_2 concentration. The cells were seeded at 10,000 cells cm^{-2} density for 24 hours. After incubation fluorescein diacetate (FDA) staining was performed. Stock solution of FDA was prepared by dissolving 2mg/ml of FDA in dimethylsulfoxide (DMSO). 10 µl of FDA stock was diluted in PBS to make a working solution. 250 µl of working solution was added in wells and stained cells were observed under fluorescent microscope.

Drug release kinetics

To evaluate drug release kinetics from chicken skin model, Higuchi model was evaluated for drug release kinetics using following equation:

$$Q = A\sqrt{D(2Co - Cs)Cst}$$

Where, Q is the cumulative amount of drug released in time t per unit area, C_O is the initial drug concentration, C_S is the drug solubility in the matrix and D is the diffusion coefficient of the drug molecule in the matrix.

Biological activity of released drug

Inhibition of albumin denaturation technique was used to study anti-inflammatory activity of drug released through hydrogels by following formula [23].

Percentage inhibition=(Abs Control-Abs Sample)/(Abs Control) × 100

Anti-proteinase activity of drug released from hydrogels was monitored against trypsin [23]. Inhibition of trypsin activity was calculated using following formula.

Percentage inhibition=(Abs Control-Abs Sample)/(Abs Control) × 100

Results

The hydrogels were formulated studied on physicochemical, structural, and kinetic parameters to fully understand the potential of this novel formulation in sustained, transdermal delivery of ibuprofen (Figure 1).

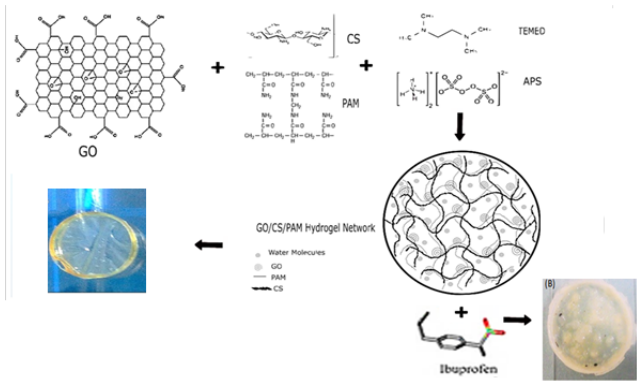

Figure 1: Schematic diagram of formulation of CS/AM/GO gels with and without drug beads.

Physical properties

The swelling properties of the synthesized hydrogels were analyzed. The hydrogels were soaked in buffer of pH ranging from acidic to basic.

Swelling of hydrogels at acidic pH was minimal and the gels degraded due to probable hydrolysis of biopolymers at acidic pH. At neutral pH the swelling ratio increased over time and the hydrogels maintained their architecture. At alkaline pH the swelling of hydrogel was fairly low as compared to neutral pH and reached a steady state in a much shorter duration after which the gels didn't uptake water (Figure 2).

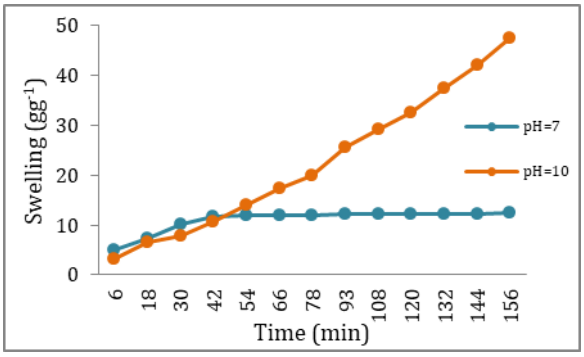

Figure 2: Swelling behavior of CS/AM/GO hydrogel as a function of pH.

Swelling kinetics analysis revealed a similar pattern of plot at pH 7 and pH 10 (Figure 3) the results are comparable to swelling behavior. The plot between the F(ln) and t(ln) indicated that there is a steady increasing trend in the swelling behavior of hydrogels at pH 7 whereas at basic pH (pH 10.0) the increasing trend is for a much shorter time period after which steady state is achieved.

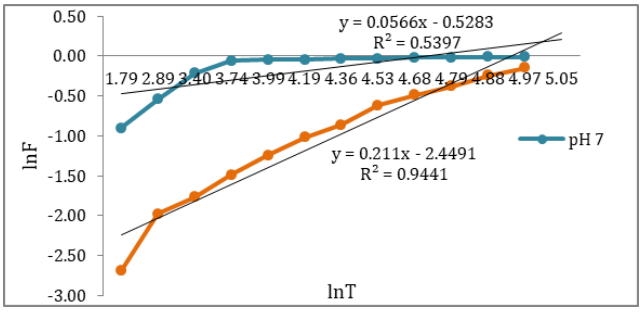

Figure 3: Swelling kinetics of CS/AM/GO at different Ph.

Swelling kinetic parameters including 'F' the fractional uptake, kinetic constant 'K', and 'n' diffusion/release exponent was calculated using Korsmeyer Peppas equation. The mechanism of diffusion was determined by the value of diffusion/release exponent. The hydrogels at both basic and neutral pH followed non-fickian mechanism of diffusion (Table 1).

`Hydrogel	pH	Mt(g)	F (fractional uptake of swelling)	ln (F)	N (Slope)	K (Intercept)	R2 (Regression)	Mechanism Type
CS/AM/GO	7	5.023	0.4		0.0566	-0.5283	0.5397	Less Fickian
		7.2	0.581	-0.9				
		10..012	0.81	-0.54				
		11.647	0.94	-0.213				

		11.83	0.954	-0.06				
		11.99	0.96	-0.047				
		12.034	0.97	-0.046				
		12.09	0.976	-0.03				
		12.156	0.98	-0.029				
		12.19	0.984	-0.019				
		12.243	0.988	-0.016				
		12.3	0.99	-0.012				
		12.392	1	-0.01				
		3.24	0.07		0.211	-2.4491	0.9441	
		6.534	0.137	-2.69				
		7.9	0.17	-1.98				
		10.62	0.22	-1.77				
		13.9	0.29	-1.49				
		17.22	0.36	-1.24				
	10	19.9	0.42	-1.02				Less Fickian
		25.6	0.54	-0.86				
		29.1	0.613	-0.618				
		32.53	0.68	-0.49				
		37.3	0.785	-0.38				
		42.043	0.9	-0.24				
		47.5	1	-0.15				

Table 1: Swelling Kinetics.

The value of diffusion constant was calculated to be 0.53 for the gels at pH 7.0 whereas for gels swelled at pH 10.0 the value of diffusion coefficient was 0.944 which follows that the hydrogels followed non-fickian swelling mechanism at all pH values.

Structural analysis

FTIR analysis revealed the bonding pattern of gels and confirmed change in bonding patterns before and after drug release from CS/AM/GO hydrogel (Figure 4). FTIR spectra of GO/CS/AM hydrogels before and after drug release are shown in Figure 4. Peaks at 2883.58 cm^{-1}, 2889.58 cm^{-1} and 2951.09 cm^{-1} exhibit C-H stretching. C-H bending peaks are demonstrated at 1321.24 cm^{-1}. Broad stretching peaks for N-H and O-H are represented by the peaks at 3290.56 cm^{-1} and 3356.14 cm^{-1}. Peaks at 1458.18 and 1560.41 highlight primary N-H bending. C-NH_2 stretching is shown by peaks at 1099.43, 1041.56 and 1037.7 cm^{-1}. Peak at 1421.54 cm^{-1} represents the presence of C-O-C functional groups. Peak at 655.80 demonstrates HC=CH presence.

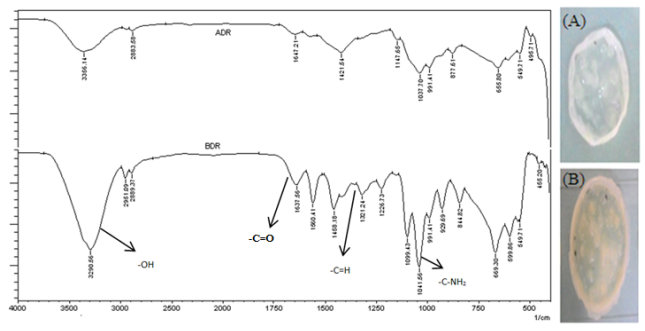

Figure 4: FTIR spectra of CS/AM/GO hydrogels, A indicates hydrogel after drug release, B indicates hydrogel before drug release

Presence of C-ring was confirmed by the peak at 991.41 whereas; peak at 844.82 suggests – NH_2. Figure 4 shows intense peaks at 3290 cm^{-1}, 1637.56 cm^{-1} and 1321.24 cm^{-1} which suggest abundance of O-H, C=O and C-H respectively due to the presence of ibuprofen in

GO/CS/AM hydrogel. Lesser number of peaks with lower intensity has been monitored in Figure 4 due to the release of drug. Reduction in the peak intensity at 1037.70 cm^{-1} describes the removal of C-NH_2 from the hydrogel during ibuprofen release.

SEM analysis was conducted to analyze the architecture of the hydrogel and also for the comparison in the morphology of hydrogel with and without ibuprofen beads at molecular level (Figure 5).

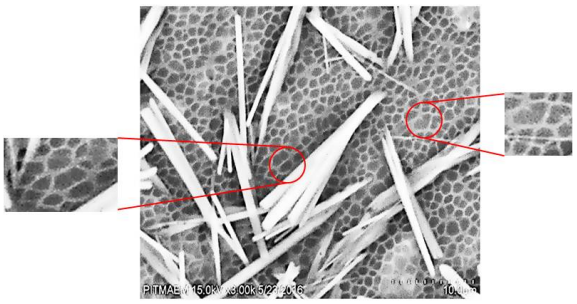

Figure 5: SEM micrographs of CS/AM/GO hydrogels, A Indicates drug beads loaded hydrogel at 1000X magnification, Hole defect of graphene oxide is also indicated.

SEM results shows high concentration of chitosan embedded in fine polymerized surface of polyacrylamide in hydrogel without drug (Figure 5). Needle-like structures of ibuprofen were observed in micrographs of drug loaded hydrogel at 1000X magnification (Figure 5). Homogenous structure of polyacrylamide can be seen on which amorphous aromatic rings of GO nanoparticles are clearly visible in micrographs at 3000X magnification (Figure 5).

In-vitro cell culture analysis

Biocompatibility of hydrogel was analyzed through HeLa cell culture. Analysis was carried out by direct method in which HeLa cells were grown on hydrogel coated wells. Fluorescent microscopy was used to analyze cell adhesion and morphology. Cells were successfully attached to the hydrogel and remained viable. Moreover, morphology of cells was also comparable to that of control under microscope, which confirms the biocompatible nature of CS/AM/GO hydrogel (Figure 6).

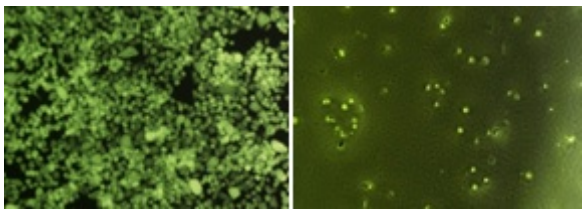

Figure 6: *In-vitro* cell compatibility using HeLa cell lines, A indicates control group, B indicates cells growing on hydrogel.

Drug release kinetics

Chicken skin model was used to assess the applicability of CS/AM/GO hydrogels as transdermal drug delivery vehicle. 740 µg/ml of drug was released from hydrogel via skin model in 150 mins as shown in Figure 7.

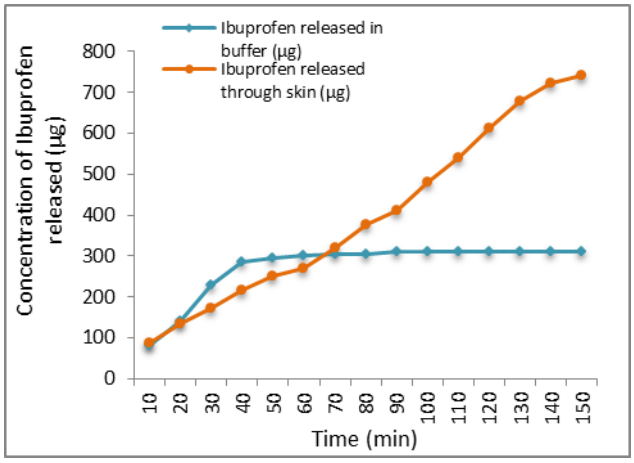

Figure 7: Drug release from CS/AM/GO hydrogel with and without chicken skin model

The drug release data from avian skin shows sustained release of ibuprofen from the hydrogel through skin (Figure 7) whereas the drug release in simple buffer system indicates that a low amount of drug was released and a steady state was achieved very early (40 mins) [24-26].

Kinetics studies showed that drug release from hydrogel through avian skin followed higuchi model Figure 7.

The drug release followed the Higuchi model of release kinetics as indicated by the drug release data (Figure 8). Results are in agreement with two hydrophobic drugs Carbidova and lapidova, as per mathematical model.

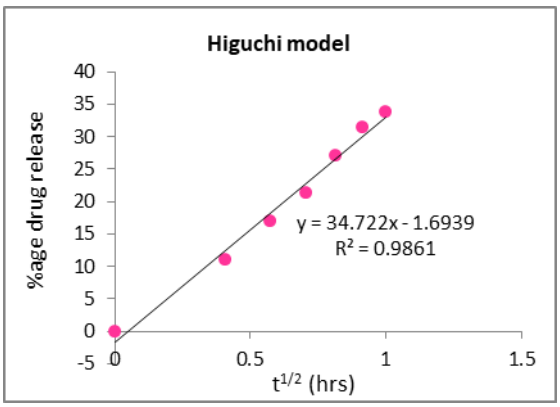

Figure 8: Drug release kinetics of CS/AM/GO hydrogel, indicates Higuchi model.

Biological activity of released drug was estimated by the method [23]. The Released Drug retained full biological activity, as it displayed protease inhibition Activity and Anti-inflammatory effect when measured against BSA solution. The results confirmed that the fabrication process of hydrogel did not harm the intrinsic properties of

drug. Ibuprofen released from formulated CS/AM/GO hydrogel had considerable anti-inflammatory and anti-protease activity.

Discussion

Ibuprofen (isobutylphenylpropanoic acid) is a weak acidic non-steroidal anti-inflammatory drug (NSAID), commonly used as anti-pyretic and analgesic. Ibuprofen has a biological half-life of 1.3-3hrs [18]. This research focuses on the synthesis and functionality of graphene oxide for providing better solubility and targeted drug delivery, of a common analgesic hydrophobic Drug Ibuprofen ($C_{13}H_{18}O_2$) with a molecular weight of 206 amu, and a net charge of zero. Ibuprofen is a weak acid and does not dissolve at acidic or neutral pH, however at alkaline pH its pendant groups get protonated and it gets solubilized. The drug has poor solubility in water that is 21 mg/L (at 25°C). Poor solubility of drug is a main hindrance in the formulation of transdermal patch for ibuprofen. It is required to apply a strategic approach for the release of drug through the dermal patch to the skin. Due to hydrophobicity it poses a limitation of insolubility, administration problems, dose dependent side effects and systemic toxicity. Graphene oxide based nanocomposite is a promising tool to address these drawbacks, due to availability of several functional groups and 2 dimensional unique structures. We synthesized 'smart' graphene oxide nanostructures hybrid with Chitosan and polyacrylamide, with a stimulus-response mediating sustained drug release, when it comes in contact with skin.

Graphene is a flat monolayer of carbon atoms with several unique properties including unparalleled thermal conductivity, remarkable electronic properties, and most attractively higher planar surface and superlative mechanical strength that are required in biotechnological applications. Drug delivery using graphene and its derivatives has aroused major interest in this emerging field. Chitosan is an animal derived biopolymer (biocompatible and biodegradable) with noted haemostatic properties with the body. Chitosan has shown good potential for use in drug-eluding polymeric devices, having been used before as a diluent in polymer-coated oral tablets, which allow sustained delivery of various drugs. It is the linear (1-4)-2-amino-2-deoxy-β-D-glucan, having a degree of acetylation close to 0.20, is currently isolated from marine chitin.

As GO has ample phenol hydroxyl, epoxide and carboxylic functional groups to allow for better bonding to other molecules (a large surface area, excellent dispersion within water and other aqueous mediums, low nanotoxicology) and is easy to manufacture, it is considered highly interesting in the biomedical field including drug delivery. When conjugated to GO, hydrophobic drugs have been shown to be dispersible in water solutions while maintaining their original potency. Hydrogels were synthesized (Section 2.2) and swelling properties of the synthesized hydrogels were analyzed. Swelling behavior depends on the type of polymers used in formulation whereas, for ionic polymers swelling depends on ionic interactions among the ionic species of polymer. Chitosan is a hydrophilic polymer and presence of amino groups at its terminals increases its swelling abilities. pKa of amino group of chitosan is ~6.5, so it is protonated in alkaline medium and exhibits increased swelling ratio at high pH [24]. Polyacrylamide, being a cationic polymer is also protonated at basic pH where it shows maximum swelling. When chitosan is grafted into polyacrylamide along with GO (a two-dimensional mosaic of hydroxyls, epoxides, carbonyls, and aromatic rings), an ampholytic hydrogel containing both cations and anions is prepared, which shows higher degree of swelling at alkaline pH due to abundance of ionizable groups in gel matrix (Figure 2) [11]. Swelling kinetics reflects the mechanism of diffusion followed by the hydrogel for uptake of solvent. Hydrogel exhibits lesser Fickian diffusion property at pH 7 which tells that diffusion rate is much lower than rate of polymer relaxation in neutral medium. However, gel shows non-Fickian diffusion behavior at alkaline pH which is suggestive of relatively higher rate of diffusion than polymer relaxation at pH 10 (Figure 2). Similar results of swelling are reported by Kumbar et al., [25] and results are in agreement for the hydrogels based on similar materials [11].

FTIR spectra of GO/CS/AM hydrogels before and after drug release are shown in Figure 4. Peaks at 2883.58 cm^{-1}, 2889.58 cm^{-1} and 2951.09 cm^{-1} exhibit C-H stretching. C-H bending peaks are demonstrated by peaks at 1321.24 cm^{-1}. Broad stretching peaks for N-H and O-H are represented by the peaks at 3290.56 cm^{-1} and 3356.14 cm^{-1}. Peaks at 1458.18 and 1560.41 highlight primary N-H bending. C-NH_2 stretching is shown by peaks at 1099.43, 1041.56 and 1037.7 cm^{-1}. Peak at 1421.54 cm^{-1} presents the presence of C-O-C functional groups. Peak at 655.80 demonstrates HC=CH presence. Presence of C-ring was confirmed by the peak at 991.41 whereas; peak at 844.82 suggests –NH_2.

Figure 4(B) shows intense peaks at 3290 cm^{-1}, 1637.56 cm^{-1} and 1321.24 cm^{-1} which suggest abundance of O-H, C=O and C-H respectively due to the presence of ibuprofen in GO/CS/AM hydrogel. Lesser number of peaks with lower intensity has been monitored in Figure 4(A) due to the release of drug. Reduction in the peak intensity at 1037.70 cm^{-1} describes the removal of C-NH_2 from the hydrogel during ibuprofen release. Similar results have been reported by [25].

To confirm the bonding pattern Scanning electron micrography (SEM) was conducted. SEM results shows high concentration of chitosan embedded in fine polymerized surface of polyacrylamide. Needle-like structures of ibuprofen were observed in micrographs of drug loaded hydrogel at 1000X magnification (Figure 5) [27]. Homogenous structure of polyacrylamide can be seen on which amorphous aromatic rings of GO nanoparticles are clearly visible in micrographs at 3000X magnification (Figure 5). Hexagonal porous GO is visible in the micrographs indicating uniform nanosheets. Hole defect in reduced Graphene oxide has been reported by [27].

The results from SEM microscopy indicate that the hydrophobic aromatic plane and the presence of defect holes in reduced sheets of GO. It is thought that during the contact with the skin lipid bilayer probably spread over the rGO surface with their tails associating closely with the hydrophobic aromatic plane, prefer to form a layer of continuous membrane covering the whole rGO sheet including defect holes. There is a possibility that the strong association between rGO sheets and lipid tails further influences the melting behavior of lipids.

In vitro cell culture studies of the gels indicate that these are biocompatible. HeLa cells were seeded and grown on the hydrogels and visualized by FDA staining. The microscopic analysis revealed the cells growing in the hydrogels. The rounded morphology of the cells was similar to the cells growing in the media without the hydrogels. So the gels could be effectively used in animal models and human clinical trials.

This work reveals a dramatic effect of the local structure and surface property of rGO sheets on the substrate-directed assembly and subsequent phase behavior of the supported lipid membranes. The drug release profile of the hydrogel containing encapsulated ibuprofen indicated 310 μg of drug release over a period of 150 minutes at

physiological pH. It is important to note that only 30% of the drug released from the gel in buffer and the plateau for released was achieved within 60 minutes of the drug release experiment. The drug release followed Higuchi model of release kinetics as indicated by the drug release data (Figure 7). The drug release data from avian skin shows steady release of ibuprofen from the hydrogel through skin. A steady increase in the amount of ibuprofen in the donor compartment containing buffer, 300 µg of drug released in 60 minutes was observed This suggests that the drug released from the hydrogel, permeated the skin and released into the buffer. Several factors could contribute towards the skin permeation and steady release of drug one important factor is the amount of drug loaded in the hydrogel. Considerable amount of drug loading in the hydrogel results in sustained release [26]. The release of drug and skin permeation also depends on the thermodynamic activity and solubility of ibuprofen. At neutral pH the partition coefficient of the drug containing hydrogel and the buffer is high which aids the permeation of drug through skin. One other important factor is the addition of reduced graphene oxide in the hydrogel. As described earlier the structure of GO interacts with the hydrophobic tails of the phospholipid bilayer of the cells this induces the formation of a temporary pore in the membrane by a flip flop mechanism this can be one the major factors contributing towards the sustained drug release profile through skin (Figure 7) [26-28]. The drug release through skin followed zero order kinetics that further validates the constant and sustained drug release over time (Figure 8).

Conclusion

CS/AM/GO hydrogels can be used as transdermal patches for controlled and localized ibuprofen delivery due to their biocompatible, non-toxic nature and anti-microbial properties by the virtue of GO. The presence of GO facilitates the release of ibuprofen from the hydrogel therefore the novel formulated gel could be used a dermal patch for the sustained release of the drug.

References

1. Davies NM (1998) Clinical pharmacokinetics of ibuprofen. Clin Pharmacokinet 34: 101–154.
2. Kakuta H, Zheng X, Oda H, Harada S, Sugimoto Y et al. (2008) Cyclooxygenase-1-selective inhibitors are attractive candidates for analgesics that do not cause gastric damage. design and in vitro/in vivo evaluation of a benzamide-type cyclooxygenase-1 selective inhibitor. J Med Chem 51: 2400–2411.
3. Hoare TR, Kohane DS (2008) Hydrogels in drug delivery: Progress and challenges. Polymer 49: 1993–2007.
4. Ashley GW, Henise J, Reid R, Santi DV (2013) Hydrogel drug delivery system with predictable and tunable drug release and degradation rates. Proc Natl Acad Sci 110: 2318–2323.
5. Li J, Mooney DJ (2016) Designing hydrogels for controlled drug delivery. Nat Rev Mater 1: 16071.
6. Garzón LC, Martínez F (2004) Temperature dependence of solubility for ibuprofen in some organic and aqueous solvents. J Solut Chem 33: 1379–1395.
7. Berth G, Dautzenberg H, Peter MG (1998) Physico-chemical characterization of chitosans varying in degree of acetylation. Carbohydr Polym 36: 205–216.
8. Liechty WB, Kryscio DR , Slaughter BV, Peppas NA (2010) Polymers for drug delivery systems. Annu Rev Chem Biomol Eng 1: 149–173.
9. Bhattarai N, Gunn J, Zhang M (2010) Chitosan-based hydrogels for controlled, localized drug delivery. Adv Drug Deliv Rev 62: 83–99.
10. Gupta P, Vermani K, Garg S (2002) Hydrogels: from controlled release to pH-responsive drug delivery. Drug Discov Today 7: 569–579.
11. Denkbas EB, Ottenbrite RM (2006) Perspectives on: chitosan drug delivery systems based on their geometries. J Bioact Compat Polym 21: 351–368.
12. Zhang Y, , , , et al. (2017) Bio-inspired layered chitosan/graphene oxide nanocomposite hydrogels with high strength and pH-driven shape memory effect. Carbohydr Polym 177: 116–125.
13. Hung H, Holbrook RJ, Rotz MW,Glasscock CJ, Manshukhani ND et al. (2014) Graphene oxide enhances cellular delivery of hydrophilic small molecules by co-incubation. ACS Nano 8: 10168–10177.
14. Zhang L, Xu B, Wang X (2016) Cholesterol extraction from cell membrane by graphene nanosheets: A computational study. J Phys Chem B 120: 957–964.
15. Muthoosamy K, Bai RG, Manickam S (2014) Graphene and graphene oxide as a docking station for modern drug delivery system. Curr Drug Deliv 11: 701–718.
16. Devi N, Narzary A (2012) Release dynamics of brufen from a drug-loaded polymer hydrogel containing polyvinyl alcohol, 2-acrylamide-2-methylpropane sulfonic acid and acrylamide. Int J Polym Mater 61: 821–833.
17. Dallavalle M, Bottoni A, Calvaresi M, Zerbetto F (2018) Functionalization Pattern of Graphene Oxide Sheets Controls Entry or Produces Lipid Turmoil in Phospholipid Membranes. ACS Applied Materials & Interfaces 10: 15487-15493.
18. Dallavalle M, Calvaresi M, Bottoni A, Franco M, Zerbetto F (2015) Graphene Can Wreak Havoc with Cell Membranes. ACS Applied Materials & Interfaces 7: 4406-4414.
19. Sami AJ, Khalid M, Jamil T, Aftab S, Mangat SA et al. (2018) Formulation of novel chitosan guargum based hydrogels for sustained drug release of paracetamol. International journal of biological macromolecules 108: 324-332.
20. Sadeghi M (2011) Pectin-based biodegradable hydrogels with potential biomedical applications as drug delivery systems. J Biomater Nanobiotechnology 2: 36-40.
21. Drużyńska M, Czubenko (2012) Mechanism of water diffusion into noncrosslinked and ionically crosslinked chitosan membranes. Prog Chem Appl Chitin Deriv 17: 59–66.
22. Peppas NA, Franson NM (1983) The swelling interface number as a criterion for prediction of diffusional solute release mechanisms in swellable polymers. J Polym Sci Part B Polym Phys 21: 983–997.
23. Leelaprakash G, Dass SM (2011) Invitro anti-inflammatory activity of methanol extract of Enicostemma axillare. Int J Drug Dev Res 3: 189-196.
24. Ruvalcaba M, Sánchez J, Becerra F, Cruz L, González (2009) Swelling characterization and drug delivery kinetics of polyacrylamide-co-itaconic acid/chitosan hydrogels. Express Polym Lett 3: 25–32.
25. Kumbar SG, Soppimath KS, Aminabhavi TM (2003) Synthesis and characterization of polyacrylamide-grafted chitosan hydrogel microspheres for the controlled release of indomethacin. J Appl Polym Sci 87: 1525–1536.
26. Pathan B, Setty CM (2009) Chemical penetration enhancers for transdermal drug delivery systems. Trop J Pharm Res 8: 173-179.
27. Shariare MH, Blagden N, de Matas M, Leusen FJ, York P (2012) Influence of solvent on the morphology and subsequent comminution of ibuprofen crystals by air jet milling. J Pharm Sci 101: 1108–1119.
28. Rui L, Liu J, Li J, Weng Y, Dou Y et al. (2015) Reduced graphene oxide directed self-assembly of phospholipid monolayers in liquid and gel phases. Biochim Biophys Acta BBA-Biomembr 1848: 1203–1211.