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Non-invasive drug delivery system for the delivery of protein/peptide using neem gum and its derivatives

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ABSTRACT
The present investigation is continuation of author's previous work. In previous work, the author was prepared acrylamide grafted copolymer of neem gum and carboxymethylatedneem gum derivatives. Neem gum polysaccharide (NGP) and its derivatives viz. acrylamide grafted neem gum (NGP-g-Am) and carboxymethylated neem gum (CMNGP) were explored as film forming agent for transdermal delivery of protein/peptide drug (albumin). It was observed that films were not prepared at all the concentrations of NGP with a given concentration range. Studies show that film cannot be prepared using CMNGP even at ahigher concentration of polymer (2 % w/v solution). So only acrylamide grafted neem gum based film were prepared and evaluated. Transdermal films were prepared by using solvent casting method. The developed films were evaluated for various parameters such as drug content, folding endurance, thickness, weight variation, surface pH, moisture uptake, *in vitro* drug release study and *ex-vivo* permeation study. The films showed more than 300 folding endurance which demonstrated the good mechanical strength of film. It was also observed that after permeation studies small cracks were also formed in the films. Fabricated films were able to deliver drug upto 7 h. Drug release study and drug permeation studies showed that formulations followed zero order and KorsmeyerPepass model of kinetics. It can be concluded from the findings of the results that acrylamide graft copolymers of neem gum were able to deliver protein/peptide drugs through transdermal route.

Keywords: *transdermal film; neem gum; acrylamide grafting; protein delivery; polysaccharide.*

1. INTRODUCTION

The material which widely occurred in nature or extracted from the plant, animal and microbes source are known as natural polymers [1]. Natural polymers are used for vital pharmaceutical applications such as excipients, prosthetics, drug delivery, imaging and bone tissue engineering [2,3]. Polysaccharide plays significant role in targeted therapeutic delivery of drug. These are natural biomaterials which available at large scale and also less expensive in cost. They have ability for chemical modification and enable easy constructions of particles and hydrogel for delivery purposes. These can also increase the aqueous solubility of the drug and enhances the stability of drugs and other unstable therapeutics such as proteins [4]. Gums have a property of film-forming and used in the preparation of breath films, cough strips, transdermal film, buccal film and sore throat strips. Natural polymers are biodegradable, biocompatible, less toxic in nature and easily available [5]. There are some limitations with natural polymers are high degree of variability in natural materials, complex structure, expensive extraction process and chances of microbial contamination [6,7].

Polysaccharides have been exploited as drug delivery carrier to modulate release behavior and intensify therapeutic effect of API. Polysaccharides have inherent potential to deliver drug topically to the skin, which adheres to the body, forming a thin transparent film and provide delivery of the active ingredients to the body tissues. Gums are the backbone of the film forming system. To achieve the desired film properties, these polymers can be used alone or in combination with other film forming polymers. These polymers are generally forming a clear flexible film at skin temperature [8]. These have the ability of good mechanical strength, high adhesiveness, flexibility and proper drug release characteristics [9].

Thin films prepared by using natural polymers have been escalated due to disposal and environment problems that occur with synthetic material waste. The release mechanism of the drug from these polymers is occurred by degradation, diffusion and swelling which control the drug release from the device. Natural polymers can be used as the means of achieving predetermined rates of drug delivery. They are biocompatible, biodegradable, non-toxic and inert in nature [7]. Due to hydrophilicity and viscous solution of gums, they may be easily prepared in water and cast to develop films or patches of different dimensions. Grafting is a novel tool to prepare tailored materials with prerequisite properties [10].

Blooming development in the utilization of polysaccharides and their modified derivatives for drug delivery carrier, open a promising area for researchers. Acrylamide grafted xanthan gum has been utilized to prepare transdermal films for topical drug delivery. Prepared filmswere found to be slightly opaque, smooth, flexible, and permeable to water vapor. Drug atenolol was also uniformly distributed in the matrix. Atenolol release was extended upto 24 h through the grafted transdermal films [11].

In a study, electroresponsive transdermal hydrogel films were prepared using polyacrylamide-g-xanthan gum and poly (vinyl alcohol) was formulated for the release of ketoprofen. It was observed in the study that drug permeation across the skin was get increased in the presence of electric stimulus as compared

to passive diffusion and was found to be dependent upon the applied electric current strength and crosslinked density of transdermal film [12].

Delivery of peptides through transdermal route allows the avoidance of both gastrointestinal degradation and hepatic first-pass metabolism of short half-life drugs and allowing administration of drug by an easily accessible and non-invasive route. This can decrease the amount of potential drug-drug interactions with combined therapies and also lead to better patient compliance as compared to injection due to the ease of use, self-administration and less frequent dosing. Transdermal delivery of drug characterized by the prolonged, continuous and rate-controlled drug release unique to these systems [13].

In the present investigation, neem gum polysaccharide, acrylamide grafted neem gum polysaccharide and carboxymethylatedneem gum polysaccharide were explored to prepare Transdermal film for the delivery of protein/peptide (albumin). The present investigation was supposed to describe the effect of NGP, NGP-g-Am and CMNGP on release behavior of albumin from transdermal film.

NGP-g-Am has been synthesized abd characterized by author previously[14].

2. MATERIALS AND METHODS

Material: Neem gum was purified and its derivatives *viz.* acrylamide grafted neem gum and carboxymethylated neem gums were prepared as discussed in our previous publication [15]. Albumin and glycerine were purchased from Merck Specialties Private Limited, Mumbai India.

Method: Transdermal films were prepared using a solvent casting method. NGP and NGP-g-Am were used as the film-forming agent. Different concentrations of polymers were used to prepare the Transdermal film (Table 1). As shown in table 1, gum solution (20 ml) was prepared using double distilled water. After keeping still for 24 h, 1 ml of glycerine was added into the polymer solution (20 ml). The solution was kept for stirring for 45 min at 100 rpm and 40 ˚C. After stirring, 10 mg of protein-based drug (bovine serum albumin) was added into the solution and stirred for 15 min followed by sonication for 5 min, poured into a mold and dried.

Table 1.Formula to prepare transdermal film.

Formulation*	Polymer (mg)
N1/NA1	100
N2/NA2	125
N3/NA3	150
N4/NA4	175
N5/NA5	200
N6//NA6	225
N7/NA7	250
N8/NA8	275
N9/NA9	300

*where N and NA denotes the formulation prepared using NGP and NGP-g-Am, respectively.

Evaluation of polymer-based films: The films were developed and evaluated for different parameters like drug content, folding endurance, thickness, weight variation, surface pH, moisture uptake, i*n vitro* drug release study and *ex-vivo* permeation study [16,17,18,19,20].

Physical Appearance: All the films were visually observed for any defect.

Folding endurance: A specific area of films was cut and folded at the same place until it was broken. The number of times the films could be folded without breaking gave the value of folding endurance.

Thickness of films: The thickness of the prepared films was measured by screw gauge at five different points and the average was calculated with standard deviation.

Surface pH: The films were allowed to moist by keeping them in contact with distilled water for a few minutes. The surface pH was then measured with the help of a pH meter.

Weight variation: The films were subjected to weight variation by individually weighing 1*1 cm^2 films, selected randomly and the average was calculated with standard deviation.

Drug content: All formulations 1*1 cm^2 films were dissolved in phosphate buffer (pH 6.8) and shaken properly for the 24 h using a magnetic stirrer. After filtration and dilution with phosphate buffer, drug content (%) was measured spectrophotometrically at a wavelength of 202 nm. Drug content is defined as the amount of drug present in the formulation. The drug content was determined by the below Eqn. 1.

$$\text{Drug content} = \text{Concentration} * \text{Dilution factor} \qquad \text{Eqn. 1}$$

Percent moisture uptake: The prepared films were weighed individually and kept in desiccators containing potassium chloride at 35 ˚C for 24 h. After 24 h the films were reweighted and determine the percent moisture uptake by using the following Eqn. 2.

$$\text{Moisture intake (\%)} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \qquad \text{Eqn. 2}$$

Surface morphology: Surface morphology of prepared formulation was studied using scanning electron microscope. A scanning electron microscope (SEM) is a type of electron microscope that produces images of a sample by scanning the surface with a focused beam of electrons. The SEM uses a focused beam of high-energy electrons to generate a variety of signals at the surface of solid specimens.

Drug Release: Eggshell membrane was used to determine the drug release of various formulations i.e. polyelectrolyte complex based films.

Preparation of egg membrane: Egg of chicken was taken to prepare the egg membrane. An orifice was made at one end of the egg and through opening,yolk was completely removed. The shell of the egg was kept in a beaker containing water with concentrated HCl. The heat was provided to the beaker and waited until bubbling ends and foam vanishes. Only the membrane was left in the beaker as the eggshell contains calcium carbonate that releases as foam when it comes in contact with HCl.

***In vitro* drug release:** In the isolated egg membrane 1*1 cm^2 film was kept and tied properly. The drug release was determined in IP phosphate buffer pH 6.8 for 5 days. At a fixed time of interval 5 ml of the sample were withdrawn from the main solution and 5 ml of the buffer was added at the same time interval. Analysis of the

sample was done using UV spectrophotometer at wavelength 202 nm.

Kinetic studies of drug release: As discussed in our previous study, kinetics of drug release were determined [15,21].

***Ex vivo* skin permeation studies:** Modified Franz diffusion cell was used to evaluate *ex vivo* drug diffusion from films. Diffusion cell had 2.52 cm^2 diffusion area and 20 ml receptor volume. IP phosphate buffer 6.8 was kept in the receptor compartment. The goat intestine membrane was used as a biological membrane. At different time interval aliquots were withdrawn and replaced with fresh buffer. Drug permeation was determined by using UV spectroscopy.

Kinetic study for *ex vivo* permeation studies: Kinetic studies of drug permeation were also determined for *ex vivo* skin permeation studies carried out using goat intestine membrane.

3. RESULTS

Transdermal drug delivery system is mainly used to eliminate the first pass metabolism that occurs due to oral dosage form. In the present study, Neem gum and its derivative were used to prepare transdermal film for the protein/peptide drug release.It was observed that films were not prepared at all the concentrations of NGP with a given concentration range. Films were found to be ruptured, non-uniform and broken. Preliminary studies show that film cannot be prepared using CMNGP even at ahigher concentration of polymer (2 % w/v solution). NGP-g-Am based films were fabricated and used for the characterization. Table 2 shows the results of various characterization parameters of films fabricated using NGP-g-Am such as folding endurance, thickness of film, tensile strength, weight variation, pH, moisture uptake, drug content.

The film of acrylamide graft copolymers of NGP was formulated and evaluated for the various parameters. The folding endurance determines the ability of film to endure stress based rupture during application and use. The folding endurance of films was found to be more than 300 that demonstrates that they are of good mechanical strength. The thickness of the film was increased in the formulation as the concentration of polymer increased in the formulation. The thickness of the films was found 0.81±0.02 (NA1) to 1.43±8.03 mm (NA9). The pH of the films was found to be 7.0±00 (NA1) to 7.1±0.01 (NA5 and NA7). Since the normal pH of the skin was found to be 6.8, so it is cleared that the formulated film would not cause any irritation. The weight variation of films was found to be 0.98 (NA4 and NA5) to 1.24 % (NA9). Values show that the process of film fabrication is efficient. The drug content of the produced film was found in the range of 98.38±0.97 (NA8) to 99.13±0.96 % (NA4) for NGP-g-Am based films. Significantly less variation in drug content shows that the process of fabrication is efficient. The moisture uptake of the NGP-g-Am based films was found 9.13±0.87 (NA2) to 10.28±0.98 % (NA5).

Thickness and tensile strength of the films were found directly proportional to the concentration of polymer. Drug content was found to be in the range of 98.47±0.95 (NA1) to 99.13±0.96 % show that process of film fabrication is efficient. Moisture uptake was determined to indicate how the film behaves during the initial stage of drug release. The moisture uptake was found to be 9.13±0.87 (NA2) to 10.28±0.98 % (NA5). The incorporation of higher amount of polymer also significantly not changed the pH of the formulation; it range from 7.0±0.00 to 7.1±0.01.

Figure 1 shows the SEM images of films. It was also observed that after permeation studies small cracks were also formed in the films. Figure 1 (b) represents rupturing of the film after *in vitro* drug release study. Swelling and unequal thickness of the film can be observed after *ex vivo* permeation study Figure 1 (c).

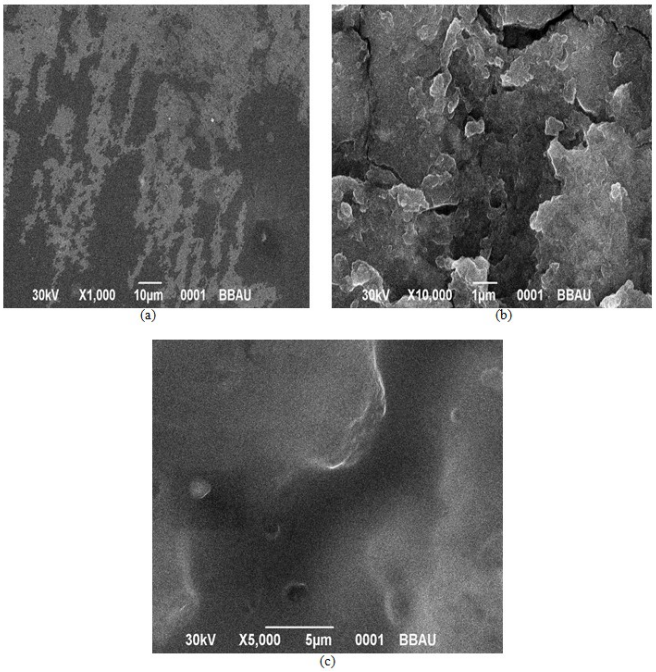
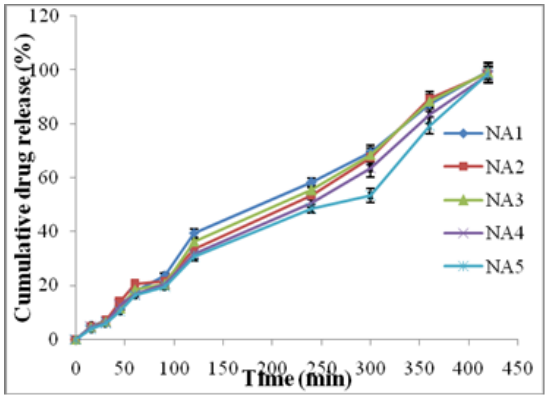


Figure 1. SEM images of the film (a): film before drug release study (NA1), (c) film after drug release study (NA1) and (d) film after drug permeation study (NA1).

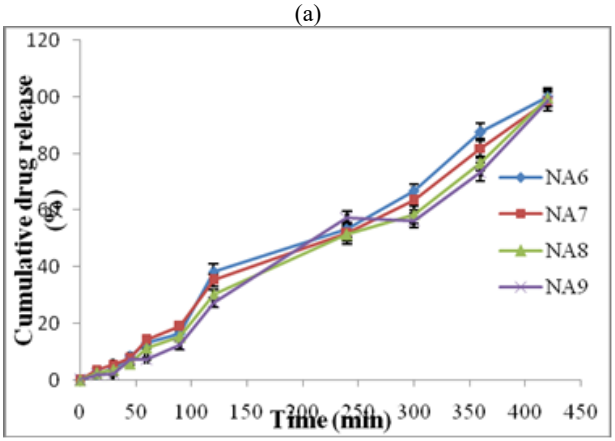
Figure 2 represents the *in vitro* drug release pattern of films prepared using acrylamide graft copolymers of NGP. Drug release study was performed using an egg membrane as a biological barrier. It was found that drug (bovine serum albumin) was released in a sustained manner within 7 h. *Ex vivo* permeation study was performed by using goat intestinal membrane as a biological barrier. Results predict that used drug can be easily permeated through the biological membrane (Figure 3).

Rate control membrane is key deciding factor to control drug release composition and psychochemical nature of Transdermal film. Drug release also depends on porosity of film. Grafting decreases all the molecular space and hinders the movement of albumin.

Banga *et al* have been utilized polyacrylamide based Transdermal drug delivery system from the delivery of peptide drug [22]. In a study, Mundargi *et al* was discussed that chain length of polymer of side chain length of copolymer (after grafting) has a direct impact on the release of drug. Grafting of polymer decreases drug release due to the increase in chain length and decreases in intermolecular effect. As found in the present investigation, Mundergi *et al* were also observed initial rapid release of drug due to direct exposure of surface drug into the diffusion media [23].

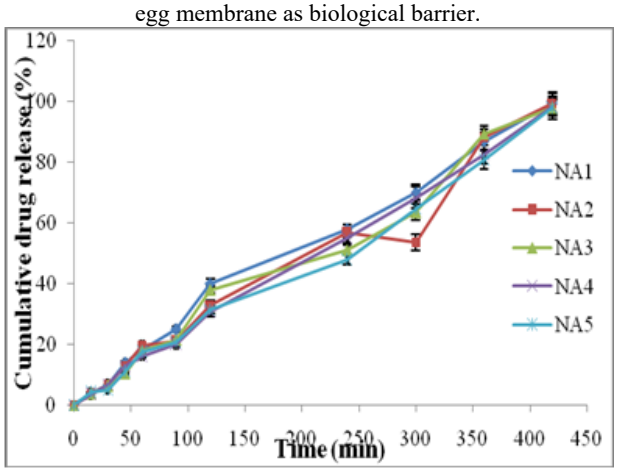


(a)

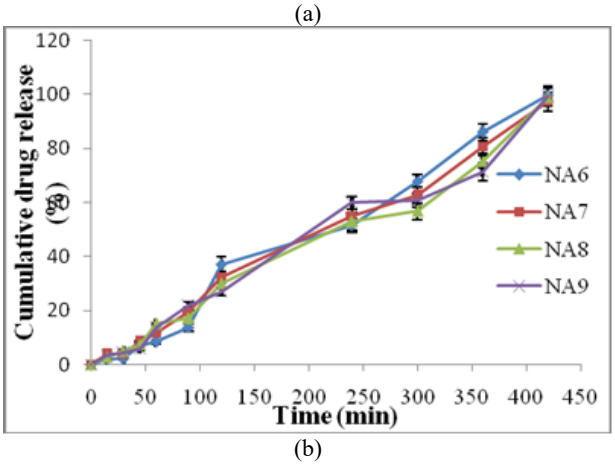


(b)

Figure 2. *In vitro* drug release of NGP-g-Am based formulations using egg membrane as biological barrier.



(a)



(b)

Figure 3. *Ex vivo* skin permeation studies of NGP-g-Am based films using goat intestinal membrane.

Native polymer is more hydrophilic in nature than acrylamide grafted polymer and it was proved in our previous study by contact angle determination. Polymeric films are gaining more attention for the targeted delivery of active pharmaceutical ingredients.

Due to hydrophilic nature of polymer, formation of loose channels takes place within the matrix. Furthermore, formation of loose channels leads to reduction in diffusion path length for the API, thereby higher rates of drug release through the polymeric matrix. Initial drug release is due to migration of surface albumin into dissolution media. Hydrophilic polymers easily solubilize with less swelling into dissolution media and forms large pores to promote drug release. Swelling and erosion of polymeric matrix also attributed to drug release.

Albumin is a hydrophilic drug and interaction with hydrophilic polymer leads to higher value of drug release. Movement of drug from the surface causes formation of numerous pores and channels. These pores improved the drug release from matrix. In case of acrylamide grafted copolymer these pores are easily filled due to polymer swelling and subsequent retardation in drug release takes place. In case of ungrafted polymer (NGP) solution causes pore formation and dimension of pores simultaneously increases because of relaxation of polymer. Polymer relaxation inhibited in case of grafted polymer.

The *in vitro* release of drug from film preparations was examined and observation was shown in Table 3. The kinetics of drug permeation study of the formulation was shown in Table 4. As observed in Table 3, all the NGP-g-Am based formulations followed zero order release kinetics except NA4 and NA5 (Kosermeyer-Peppas kinetics). Drug permeation study of NGP-g-Am also showed that most of the formulations (NA1, NA4, NA5, NA6, NA7 and NA9) followed zero order drug permeation kinetics while NA2, NA3 and NA8 followed Kosermeyer-Peppas kinetic of drug permeation.

The kinetic study of the drug release showed that all the formulation followed zero order release kinetics except formulation NA4 and NA5 (KorsmeyerPeppas Kinetics). In the sure way formulation NA2, NA3 and NA8 showed Korsmeyer-Peppas Kinetics of drug permeation while other formulation followed zero order kinetics of drug permeation. It can be concluded from the findings that drug release as well as drug permeation from most of the formulation does not depend upon the concentration of drug present in the formulation at any time. It can be concluded from the findings of results that acrylamide graft copolymer of Neem gum (NGP-g-Am) can be utilized for the preparation of Transdermal film for the delivery of protein and peptide drug (bovine serum albumin as model protein). Prepared formulations were able to deliver drug for 7 h in a sustained manner. NGP-g-Am based formulations followed zero order and KorsmeyerPeppas kinetic of drug release.

NGP-g-Am based films showed Zero order and Korsmeyer-Peppas kinetics of drug permeation.

Raghavendra *et al* also prepared transdermal film using acrylamide grafted xanthan gum to deliver drug [24]. Polyacrylamide graft xanthan gum was also used for the controlled release of ketoprofen [25]. The acrylamide graft copolymer of sodium alginate has been synthesized by researchers and further utilized to prepare membrane controlled Transdermal drug delivery system [26]. Research and simultaneous literature survey easily elicit the fact that acrylamide graft copolymer of the polysaccharide can be synthesized and utilized for the preparation of transdermal drug delivery system.

Table 2. Characterization parameters of films fabricated using NGP-g-Am

Formulation		Thickness of film (mm)	Tensile strength (Kg/cm^2)	Weight variation (%)	pH	Moisture uptake (%)	Drug content (%)
NA1	>300	0.81±0.02	1.10±0.00	1.14	7.0±0.00	9.93±0.91	98.47±0.95
NA2	>300	0.85±0.01	1.12±0.01	1.20	7.0±0.00	9.13±0.87	98.96±1.35
NA3	>300	0.91±0.02	1.15±0.01	1.02	7.0±0.01	9.37±0.96	98.86±0.89
NA4	>300	1.08±0.03	1.15±0.00	0.98	7.0±0.01	10.27±1.11	99.13±0.96
NA5	>300	1.18±0.02	1.16±0.01	0.98	7.1±0.01	10.28±0.98	99.04±1.48
NA6	>300	1.25±0.02	1.19±0.01	1.12	7.0±0.01	10.01±1.04	99.01±1.28
NA7	>300	1.31±0.01	1.20±0.01	1.08	7.1±0.00	10.04±0.97	98.83±1.27
NA8	>300	1.39±0.02	1.21±0.00	1.18	7.1±0.01	9.13±1.18	98.38±0.97
NA9	>300	1.43±0.03	1.34±0.01	1.24	7.1±0.00	10.17±1.02	99.02±1.20

Table 3. Kinetics of drug release of the formulation (NA1-NA9)

Batch	Kinetics												
	Zero order kinetics		First order kinetics		Higuchi Kinetics		Baker Lonsdale Kinetics		Hixson-Crowell Kinetics		KorsmeyerPeppas Kinetics		
	R^2	K_0	R^2	K_0	R^2	K_0	R^2	K_0	R^2	K_0	R^2	K_0	n
NA1	**0.990**	0.231	0.840	0.0046	0.98	0.057	0.269	0.002	0.968	0.006	0.988	2.441	0.934
NA2	**0.991**	0.229	0.838	0.0046	0.967	0.057	0.171	0.002	0.931	0.006	0.985	2.452	0.935
NA3	**0.993**	0.233	0.852	0.0046	0.972	0.057	0.189	0.002	0.951	0.006	0.987	2.490	0.949
NA4	0.818	0.139	0.868	0.0046	0.962	0.055	0.237	0.002	0.954	0.005	**0.988**	2.503	0.946
NA5	0.975	0.215	0.855	0.0046	0.937	0.053	0.094	0.001	0.931	0.004	**0.986**	0.962	0.962
NA6	**0.983**	0.233	0.805	0.0046	0.969	0.061	0.465	0.005	0.932	0.006	0.982	2.975	1.154
NA7	**0.990**	0.227	0.777	0.0046	0.926	0.050	0.155	0.004	0.966	0.005	0.983	2.690	1.021
NA8	**0.986**	0.226	0.852	0.003	0.951	0.056	0.423	0.004	0.977	0.004	0.983	3.026	1.146
NA9	**0.980**	0.226	0.834	0.003	0.947	0.057	0.580	0.006	0.974	0.004	0.971	3.314	1.262

Table 4. Kinetics of drug permeation study of the formulation (NA1-NA9).

Batch	Kinetics												
	Zero order kinetics		First order kinetics		Higuchi Kinetics		Baker Lonsdale Kinetics		Hixson-Crowell Kinetics		KorsmeyerPeppas Kinetics		
	R^2	K_0	R^2	K_0	R^2	K_0	R^2	K_0	R^2	K_0	R^2	K_0	n
NA1	**0.989**	0.230	0.803	0.0046	0.983	0.057	0.009	0.00	0.872	0.010	0.985	2.514	0.968
NA2	0.967	0.223	0.814	0.0046	0.941	0.055	0.015	0.000	0.824	0.010	**0.979**	2.531	0.961
NA3	0.983	0.229	0.830	0.0069	0.960	0.057	0.005	0.000	0.906	0.006	**0.985**	2.566	0.981
NA4	**0.998**	0.229	0.848	0.0069	0.973	0.057	0.035	0.000	0.981	0.005	0.994	2.566	0.997
NA5	**0.990**	0.221	0.844	0.0069	0.960	0.055	0.051	0.001	0.963	0.005	0.979	2.577	0.975
NA6	**0.985**	0.245	0.819	0.0069	0.959	0.060	0.514	0.007	0.956	0.006	0.964	3.328	1.282
NA7	**0.993**	0.227	0.789	0.0069	0.928	0.050	0.155	0.004	0.976	0.005	0.976	2.783	1.059
NA8	0.985	0.220	0.852	0.003	0.951	0.054	0.423	0.004	0.977	0.004	**0.987**	2.826	1.068
NA9	**0.981**	0.222	0.843	0.003	0.957	0.055	0.580	0.006	0.985	0.004	0.970	2.822	1.067

4. CONCLUSIONS

The acrylamide graft copolymer of neem gum (NGP-g-Am) can be utilized for the preparation of transdermal film for the delivery of non-invasive delivery of protein/peptide. Experimentation work easily predicts that NGP and CMNGP cannot be used for the preparation of Transdermal film at a given concentration range. It can be concluded that NGP-g-Am) have potential to form transdermal film for controlled delivery of protein/peptide molecules (albumin). Prepared films had good mechanical strength, non-irritant in nature and able to deliver albumin for an extended period of time i.e. 7 h. In future, optimized formulations would be evaluated for preclinical studies.

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6. ACKNOWLEDGEMENTS

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