

Exploration of neem gum-chitosan and kheri gum-chitosan polyelectrolyte complex based film for transdermal delivery of protein/peptide

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ABSTRACT

Present investigation is continuation of author's previously published work. In the present investigation, the author has prepared neem gum-chitosan and kheri gum-chitosan polyelectrolyte complex transdermal film for the delivery of protein/peptide drug. Concentration of gum (neem gum and kheri gum) and chitosan was varied in each concentration while drug concentration kept constant. Albumin was used as a model protein drug. Transdermal films were fabricated using a solvent casting method without using any plasticizer and evaluated for various parameters viz. folding endurance, surface pH, weight variation, drug content, percentage moisture content, surface morphology, *in vitro* drug release and *ex vivo* drug permeation study. The study showed that films were successfully fabricated with good acceptable physical properties. *In vitro* drug release study and *ex vivo* drug permeation study showed that polyelectrolyte films were able to extend drug delivery up to 9 days. It can be easily concluded from the findings of the results that neem gum-chitosan and kheri gum-chitosan polyelectrolyte complex films can be easily prepared without using any plasticizer and able to deliver protein/peptide therapeutic agents for an extended period of time.

Keywords: neem gum; kheri gum; polyelectrolyte complex; transdermal film; protein/peptide delivery.

1. INTRODUCTION

Polysaccharide is played a significant role in the targeted therapeutic delivery of the drug. These are natural biomaterials which available at large scale and also less expensive in cost. They have properties of chemical modification and enables 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 [1]. They are biocompatible, biodegradable, non-toxic and inert in nature [1,2]. A new method is applied for the common transdermal and topical formulations area film-forming system. Polysaccharide polymer is used for the formulation of transdermal film. These have the ability of good mechanical strength, high adhesiveness, flexibility and proper drug release characteristics [3]. Film forming formulation is applied on the surface of the body or onto the skin because they are nonsolid dosage forms that produce a film *in situ* after they are applied.

These types of systems consist of drugs and film-forming excipients in a vehicle. The film forming method directly applied to the skin which can form a thin transparent film *in situ* by the method of solvent vaporization. The formed film is a polymeric material that can act as a matrix for the release of the sustained drug into the skin or it is a residual film that is fastly absorbed into the stratum corneum. After the loss of the volatile compound of vehicles from formulation and film formation on the skin surface, the drug concentration increases and reaches saturation level to attain the supersaturation level on the skin surface [4]. Thin films prepared by using natural polymers have been escalated due to disposal and environment problems that occur with synthetic material waste. Films can be developed with natural materials such as proteins, cellulose, polysaccharides or their composites which can help to solve the problem of disposal due to its non-toxic and naturally biodegradable properties [5]. In a study, it was evaluated that,

polyacrylamide grafted gum copolymer was used for the transdermal delivery of the drug by using electrical stimulus. This can act as drug reservoir gel and as rate controlling membranes. The thickness of membranes was increased by increasing the concentration of glutaraldehyde. Membranes were permeable to water vapors. The drug permeation from the formulation was enhanced in the presence of electric stimulus [6,7].

Chitosan is a cationic natural polymer obtained from alkaline deacetylation of chitin and chitin is the exoskeleton of crustaceans. It is the second most widely found naturally found polysaccharide. In acidic aqueous solution, chitosan becomes ionized due to the presence of positively charged amino groups. Naturally available gums contain -OH, -COOH, -CH₂OH etc. groups; they become negatively charged in the presence of aqueous medium. Oppositely charged gum polysaccharides and chitosan easily interact with each other and formed polyelectrolyte complex (PEC). Biocompatibility, non-toxicity, tolerability and biodegradability enriched the PECs to be used as drug delivery carriers for pharmaceutical and biomedical applications. In a study, Schneider *et al* discussed that polyelectrolyte films are also influenced cellular behavior such as adhesion, internalization, uptake and recolonization [8].

Gums have good aqueous solubility and easily degrade in physiological conditions of the body. Chitosan shows poor elasticity and physiological degradation and even remain intact in the body for more than 24 h. Strategy to develop polyelectrolyte complex between these two polymers adds the advantages of both polymers in the prepared hydrogel. It also improves structural, mechanical and tensile strength, bioadhesivity and affinity towards biological systems of hydrogel [9].

Chitosan based PEC has been used as drug delivery carriers e.g. tablet [10], periodontal film [11], Transdermal film

[12], ophthalmic film [13] and nanoparticles [14]. Polyelectrolyte complex films have been used for microencapsulation, biomedical engineering, filtration, nanocoating, capsule formulation and tissue science [15].

Protein/peptide can be administered by oral route due to the presence of proteolytic enzymes and acidic pH that further leads

to extensive degradation. Moreover, it requires a dosage form that prevents g.i. degradation and first pass metabolism and easily manufactured without any specific techniques and equipment.

To the best of our knowledge, it was the first attempt to study the protein/peptide delivery through chitosan-neem gum polysaccharide polyelectrolyte complex film.

2. MATERIALS AND METHODS

Chitosan (molecular weight: 190,000-310,000 Da) was procured from Merck Specialties Private Limited, Mumbai India. Acetic acid was supplied by S.D. Fine Chemicals Mumbai India. All the chemicals were used as supplied, without any purification. In experiments, HPLC grade water was used. Drug Albumin was purchased from Merck Specialties Private Limited, Mumbai India. As described in our previous publication, crude neem gum polysaccharide (NGP) and kheri gum polysaccharide (KGP) were collected and purified using water-based extraction process [16,17].

2.1. Preparation of Film.

Different concentrations of gum (NGP/KGP) and chitosan were used to prepare Transdermal film. As shown in table 1, gum (NGP/KGP) solution (20 ml) was prepared using double distilled water. Chitosan solution (20 ml) was prepared using 10 % acetic acid solution as a solvent. After keeping still for 24 h, polysaccharide solution (20 ml) was added into cationic polymer solution (20 ml). The solution was kept for stirring for 15 min at a temperature of 40°C. After stirring, protein-based drug (bovine serum albumin) was added into polyelectrolyte solutions and stirred for the next 15 min followed by sonication for 5-10 min, poured into mold and dried.

Table 1. Formula to prepare Transdermal film

Formulation	Polysaccharide (NGP/KGP) (mg)	Anionic polymer (mg)
N1/K1	100	150
N2/K2	100	100
N3/K3	100	50
N4/K4	200	150
N5/K5	200	100
N6/K6	200	50
N7/K7	150	150
N8/K8	150	100
N9/K9	150	50

2.2. Evaluation.

2.2.1. 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, *in vitro* drug release study and *ex-vivo* permeation study [18,19,20].

2.2.2. Physical Appearance.

All the films were visually observed for any defect.

2.2.3. 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.

2.2.4. 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.

2.2.5. 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.

2.2.6. Weight variation.

The films were subjected to weight variation by individually weighing 1*1 cm² films, selected randomly and the average was calculated with standard deviation.

2.2.7. Drug content.

All formulations 1*1 cm² 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

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

2.2.8. 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 reweighed and determine the percent moisture uptake by using the following Eqn. 2.

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

2.2.9. Surface morphology.

The 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.

2.2.10. Drug Release.

Egg shell membrane was used to determine the drug release of various formulations i.e. polyelectrolyte complex based films.

2.2.11. Preparation of egg membrane.

Egg of chicken was taken to prepare 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 egg shell contains calcium carbonate that releases as foam when it comes in contact with HCl.

2.2.12. In vitro drug release.

In the isolated egg membrane 1*1 cm² film was kept and tied properly. The drug release was determined in IP phosphate buffer pH 6.8 for 5 days. At 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.

2.2.13. Kinetic studies of drug release.

As discussed in our previous study, the kinetics of drug release was determined [21].

2.2.14. Ex vivo skin permeation studies.

Modified Franz diffusion cell was used to evaluate *ex vivo* drug diffusion from films. The diffusion cell had 2.52 cm² 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.

3. RESULTS

Besides electrostatic interaction, hydrophobic and hydrophilic interactions also take place between chitosan and neem gum polysaccharide. As discussed by Recillas *et al*, complex stability depends upon temperature, concentration of ionic polymers, ionic strength, density of charge, pH and temperature [22]. In the present investigation all the factors were kept constant except the concentration of chitosan and gum. Self-assembly of oppositely charged polymer in dilute solution results in complexation during film formation.

Table 2 and Table 3 shows characteristics of NGP-Chitosan and KGP-Chitosan polyelectrolyte complex films, respectively. Folding endurance of all the prepared films was found >300. It showed the good strength of prepared films. pH of the film was found in the range 6.5±0.01 to 6.6±0.02 and 6.7±0.00 to 6.8±0.01, respectively for NGP-Chitosan and KGP-Chitosan polyelectrolyte films, respectively. The normal pH of skin is 6.8. It shows that prepared film shall not show any irritation when applied over the skin. The thickness of films was found to be 0.81±0.00 to 0.83±0.02 mm and 1.00±0.01 to 1.03±0.02 mm, respectively for NGP-Chitosan and KGP-Chitosan polyelectrolyte complex films. Weight variation of films was found to 0.82 to 1.36 % for NGP-Chitosan polyelectrolyte complex films and 0.31 to 0.32 % for KGP-Chitosan polyelectrolyte complex films. Significantly less weight variation shows that the process of film fabrication was quite efficient to produce uniform films. Drug content is defined as amount of drug present in the formulation. Drug content of prepared

2.2.15. Kinetic study for *ex vivo* permeation studies.

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

films was found in the range of 98.57±0.26 to 99.72±1.02 % and 97.7±1.21 to 98.8±1.49 % for NGP and KGP based films; respectively. Good value of drug content showed that the process of preparation was efficient.

Moisture uptake indicated that the increase in the concentration of hydrophilic polymer was directly proportional to the increase in moisture uptake of the films. The moisture uptake of the films varies from 8.80±0.39 to 12.92±0.42 % for NGP-Chitosan films while 7.01±1.01 to 7.12±0.87 % for KGP-Chitosan films. Data of moisture uptake studies easily predict that NGP is more hydrophilic in nature as compared to KGP. Formulation N4 showed maximum drug content (99.72±1.02) and selected as optimized formulations.

In the current scenario, safety is a major concern for regulatory bodies. Fabricated PEC based film was made up of biodegradable polymers and leads to eliminate safety concerns in terms of biodegradability.

Morphological characterization also predicts homogeneous nature of film without any defects, voids and bubble formation. As depicted in SEM images (figure 1), films were flat. The same results were also observed by Maciel *et al* [23]. SEM image (Figure 1) showed a uniform surface of film prepared using NGP/KGP-Chitosan polyelectrolyte complex. Figure 1 showed SEM images of film (a): film before drug release study (N4), (b) film before drug release study (K7), (c) film after drug release study (N4) and (d) film after drug permeation study (N4).

Table 2. Characteristics parameters of the transdermal film prepared by using NGP-Chitosan polyelectrolyte complex

Formulation	Folding endurance	Thickness of film (mm)	Tensile strength (Kg/cm ²)	Weight variation (%)	pH	Moisture uptake (%)	Drug content (%)
N1	>300	0.82 ±0.01	0.82 ±0.00	1.03	6.5 ±0.01	12.25 ±0.82	99.54 ±1.43
N2	>300	0.81 ±0.00	0.79 ±0.01	1.11	6.5 ±0.01	11.05 ±0.56	98.89 ±0.98
N3	>300	0.83 ±0.02	0.81 ±0.01	0.98	6.6 ±0.02	10.38 ±0.83	98.57 ±0.26
N4	>300	0.81 ±0.01	0.78 ±0.00	0.82	6.5 ±0.01	11.89 ±0.35	99.72 ±1.02
N5	>300	0.82 ±0.02	0.79 ±0.01	1.02	6.5 ±0.01	12.92 ±0.42	98.57 ±0.92
N6	>300	0.83 ±0.01	0.81 ±0.01	1.00	6.6 ±0.02	10.33 ±0.43	98.77 ±0.33
N7	>300	0.81 ±0.01	0.80 ±0.01	0.92	6.5 ±0.01	11.07 ±0.89	99.44 ±1.32
N8	>300	0.82 ±0.01	0.81 ±0.00	1.36	6.6 ±0.00	9.47 ±0.90	99.23 ±0.39
N9	>300	0.81 ±0.00	0.81 ±0.01	1.08	6.6 ±0.01	8.80 ±0.39	98.85 ±0.83

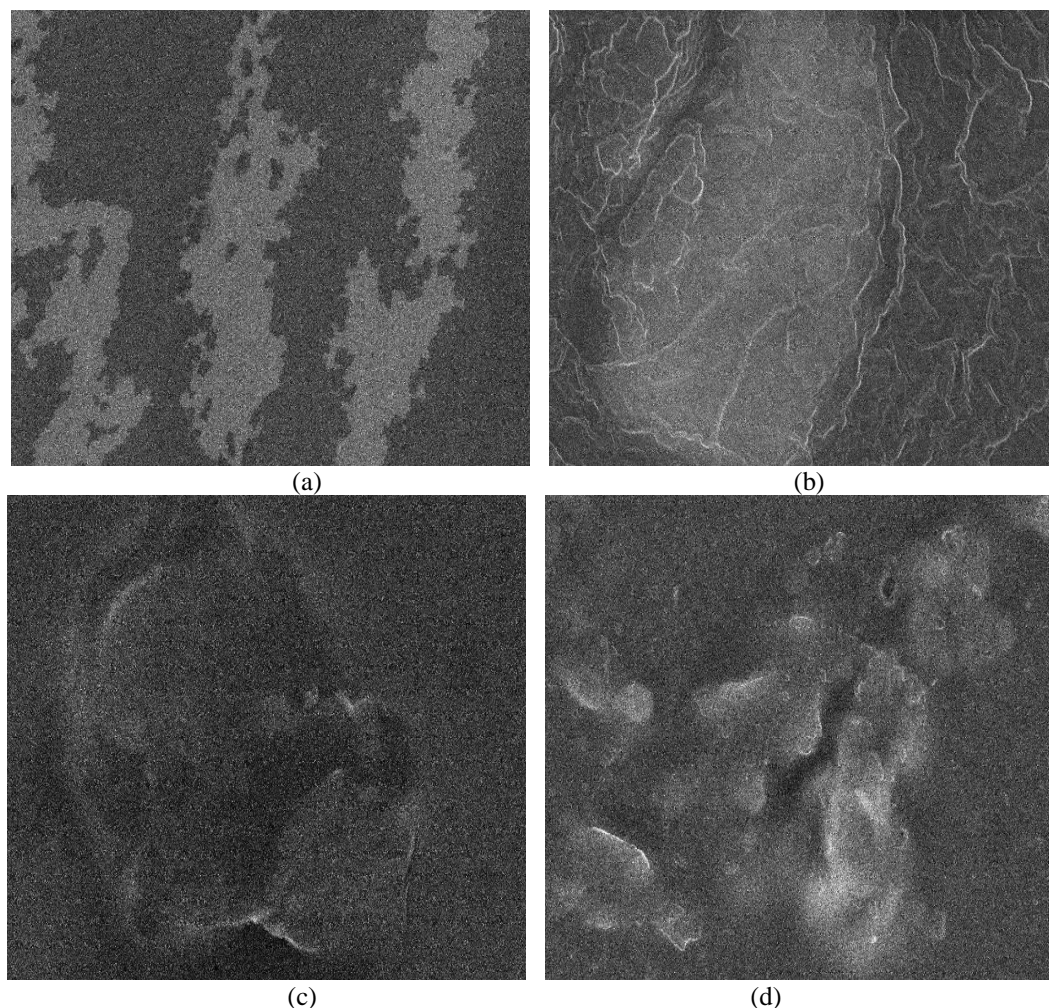


Figure 1. SEM images of the film (a): film before drug release study (N4), (b) film before drug release study (K7), (c) film after drug release study (N4) and (d) film after drug permeation study (N4)

Table 3. Characteristics parameters of the transdermal film prepared using KGP-Chitosan polyelectrolyte complex

Formulation	Folding endurance	Thickness of film (mm)	Tensile strength (Kg/cm ²)	Weight variation (%)	pH	Moisture uptake (%)	Drug content (%)
K1	>300	1.03 ±0.02	0.83 ±0.03	0.31	6.7 ±0.00	7.12 ±0.87	98.7 ±1.03
K2	>300	1.01 ±0.01	0.82 ±0.03	0.32	6.7 ±0.00	7.09 ±0.97	97.7 ±1.21
K3	>300	1.03 ±0.01	0.82 ±0.02	0.31	6.8 ±0.01	7.08 ±0.89	98.4 ±1.42
K4	>300	1.00 ±0.01	0.84 ±0.03	0.33	6.8 ±0.01	7.07 ±0.83	98.5 ±1.82
K5	>300	1.02 ±0.01	0.83 ±0.01	0.31	6.7 ±0.02	7.03 ±0.93	98.8 ±1.49
K6	>300	1.03 ±0.01	0.83 ±0.02	0.32	6.8 ±0.01	7.01 ±1.01	98.8 ±1.36
K7	>300	1.02 ±0.02	0.84 ±0.01	0.32	6.8 ±0.00	7.08 ±0.93	98.8 ±1.84
K8	>300	1.02 ±0.01	0.81 ±0.01	0.32	6.8 ±0.00	7.10 ±0.97	97.9 ±1.38
K9	>300	1.01 ±0.01	0.81 ±0.02	0.31	6.8 ±0.00	7.07 ±0.77	98.1 ±1.53

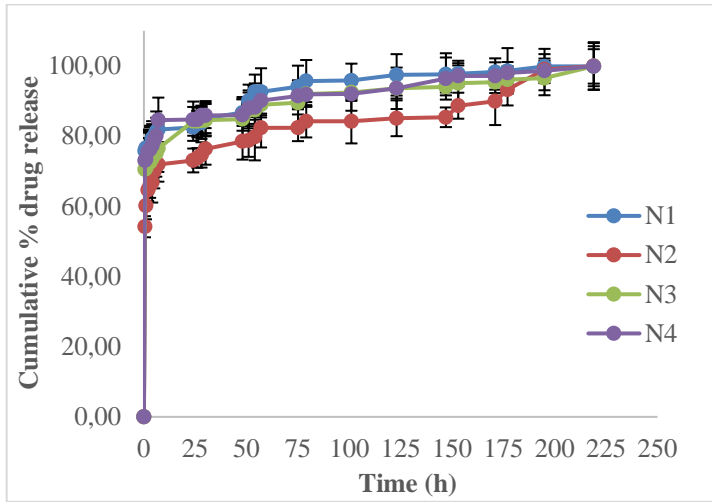
Data of *in vitro* drug release from NGP-Chitosan polyelectrolyte complex fabricated film is shown in Figure 2. It was found that N2, N7 and N9 release 50-60 % drug within 30 min time while other formulations release 70-75 % drug in the same time period. After 30 min, all the fabricated films were releases drug in a sustained manner up to 9 days.

Relaxation of incorporated polymer also leads to the dissolution of matrix. Skin moisture may promote ionization of

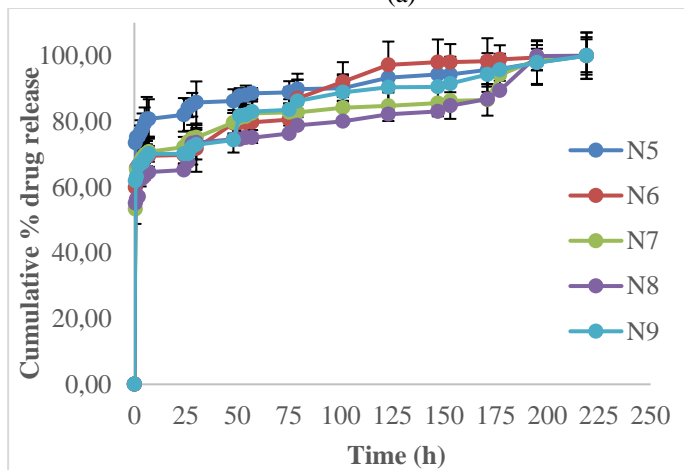
polymeric ionizable groups and leads to improved dissolution. In this study, the high solubility of albumin supports their statement. Gan and Wang had described that initial immediate release of proteinaceous drug from formulation may depend upon desorption of albumin from surface followed by diffusion and disintegration of PEC. As in the case of Transdermal film, relatively less moisture is available, so disintegration was not taken placed that further leads to extended drug release from the matrix [24].

Studies showed that both gum polysaccharide and chitosan have good film forming properties.

pH of the skin is 6.5-6.8 and pKa of chitosan is about 6.3. It reduces the chances of the protonation of chitosan and leads to significant swelling. As a result release of albumin becomes inhibited due to large diffusion path.



(a)



(b)

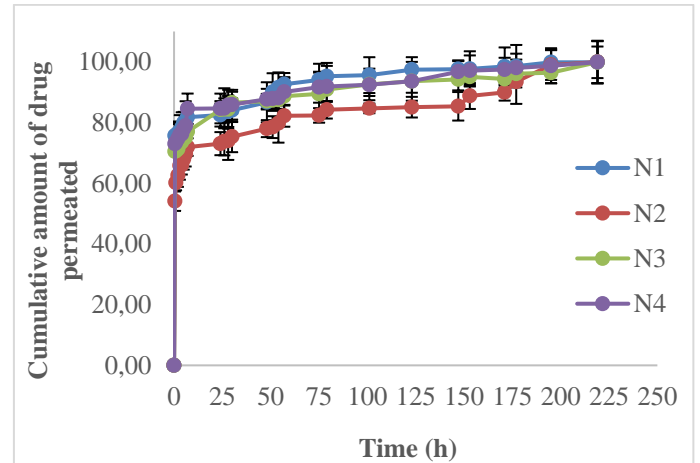
Figure 2. *In vitro* drug release study of NGP-Chitosan polyelectrolyte complex fabricated films using egg membrane as a biological barrier

Results of *ex vivo* drug permeation studies of NGP-chitosan polyelectrolyte complex fabricated film is depicted as Figure 3. The result showed that formulation N2, N6, N8 and N9 permeate 50-60 % bovine serum albumin within 30 min followed by sustained permeation of drug up to 9 days. Rest of the formulations was permeating 65-75 % drug within 30 min, followed by sustained permeation of drug up to 9 days. So, all the fabricated

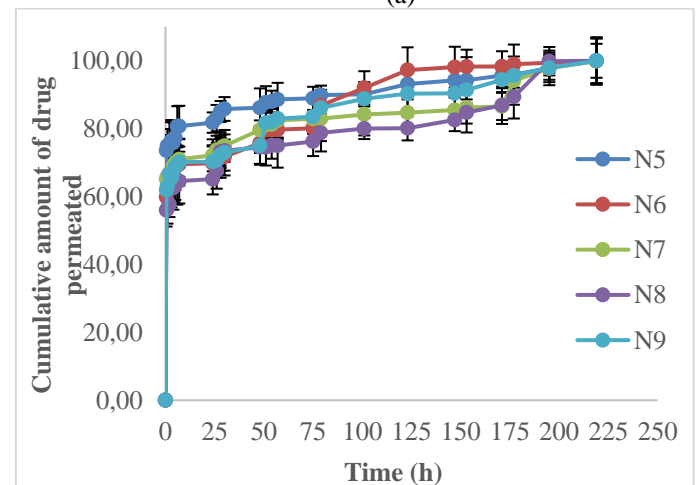
films were found efficient to deliver drugs for an extended period of time.

Table 4 showed kinetics of *in vitro* drug release of film fabricated using NGP-Chitosan polyelectrolyte complex. It was concluded from the regression coefficient that N2, N6, N7, N8 and N9 followed Baker Lonsdale kinetics of drug release while N1, N3, N4 and N5 followed Kosermeier Peppas kinetics of drug release.

Table 5 showed kinetics of *ex vivo* drug permeation study of film fabricated using NGP-Chitosan polyelectrolyte complex. Formulations N2, N7 and N8 followed Baker Lonsdale kinetics of drug permeation. N1, N4, N5, N6 and N9 followed Hixson-Crowell kinetics of drug permeation while only N3 followed Kosermeier Peppas kinetics of drug permeation.



(a)



(b)

Figure 3. *Ex vivo* skin permeation studies of NGP-chitosan polyelectrolyte complex fabricated films using chicken intestinal membrane

Table 4. Kinetics of *in vitro* drug release of the formulation (N1-N9).

Formulation	Zero order kinetics		First order kinetics		Higuchi Kinetics		Kinetics Baker Lonsdale Kinetics		Hixson-Crowell Kinetics		KorsmeierPeppas Kinetics		n
	R ²	K ₀	R ²	K ₀	R ²	K ₀	R ²	K ₀	R ²	K ₀	R ²	K ₀	
N1	0.321	0.155	0.082	0.002	0.460	0.027	0.908	0.945	0.829	0.012	0.922	0.136	0.056
N2	0.475	0.185	0.113	0.002	0.626	0.032	0.937	0.704	0.753	0.010	0.917	0.222	0.078
N3	0.355	0.161	0.089	0.002	0.495	0.028	0.909	0.878	0.753	0.010	0.980	0.166	0.057
N4	0.317	0.151	0.081	0.002	0.458	0.027	0.912	0.912	0.784	0.010	0.955	0.140	0.055
N5	0.303	0.144	0.079	0.002	0.434	0.026	0.912	0.921	0.726	0.009	0.946	0.138	0.051
N6	0.570	0.219	0.131	0.002	0.678	0.036	0.954	0.768	0.923	0.014	0.818	0.228	0.088
N7	0.449	0.178	0.108	0.002	0.555	0.029	0.943	0.711	0.722	0.010	0.881	0.209	0.070
N8	0.557	0.200	0.130	0.002	0.664	0.032	0.918	0.692	0.735	0.011	0.890	0.268	0.091

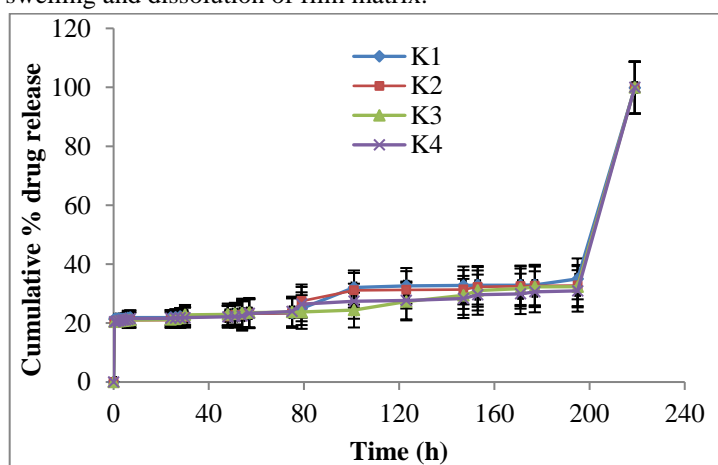
Exploration of neem gum-chitosan and kheri gum-chitosan polyelectrolyte complex based film for transdermal delivery of protein/peptide

Formulation	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
N9	0.519	0.198	0.119	0.002	0.642	0.033	0.916	0.778	0.835	0.011	0.866	0.225	0.082

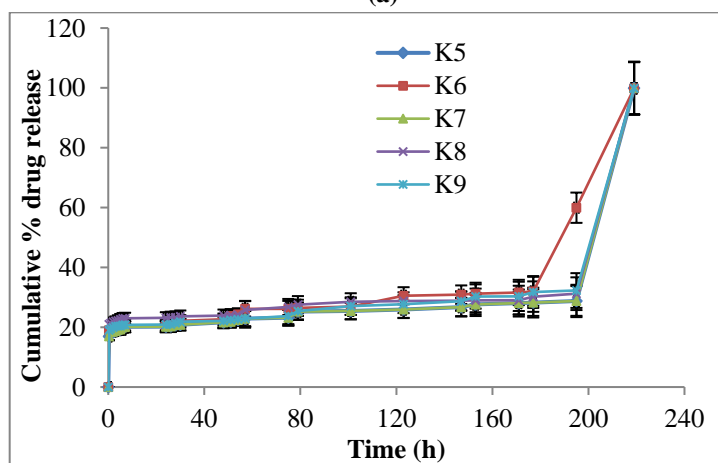
Table 5. Kinetics of *ex vivo* drug permeation of formulation (N1-N9)

Formulation	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
N1	0.329	0.157	0.083	0.002	0.451	0.027	0.908	0.942	0.939	0.011	0.927	0.139	0.057
N2	0.492	0.190	0.117	0.002	0.607	0.031	0.935	0.701	0.857	0.008	0.927	0.229	0.082
N3	0.348	0.159	0.088	0.002	0.499	0.028	0.908	0.877	0.903	0.007	0.985	0.166	0.065
N4	0.327	0.154	0.083	0.002	0.446	0.026	0.910	0.911	0.965	0.008	0.961	0.144	0.057
N5	0.311	0.147	0.080	0.002	0.424	0.025	0.910	0.920	0.953	0.007	0.944	0.142	0.052
N6	0.572	0.220	0.131	0.002	0.679	0.036	0.925	0.768	0.971	0.013	0.803	0.227	0.087
N7	0.437	0.171	0.102	0.002	0.578	0.030	0.908	0.811	0.853	0.006	0.884	0.210	0.070
N8	0.553	0.198	0.129	0.002	0.671	0.033	0.915	0.700	0.737	0.008	0.884	0.267	0.090
N9	0.521	0.199	0.120	0.002	0.638	0.033	0.915	0.777	0.965	0.009	0.877	0.227	0.083

Based on the highest regression coefficient, it can be concluded that all the PEC films followed Higuchi kinetics of albumin release. It shows that drug release depends upon the swelling and dissolution of film matrix.

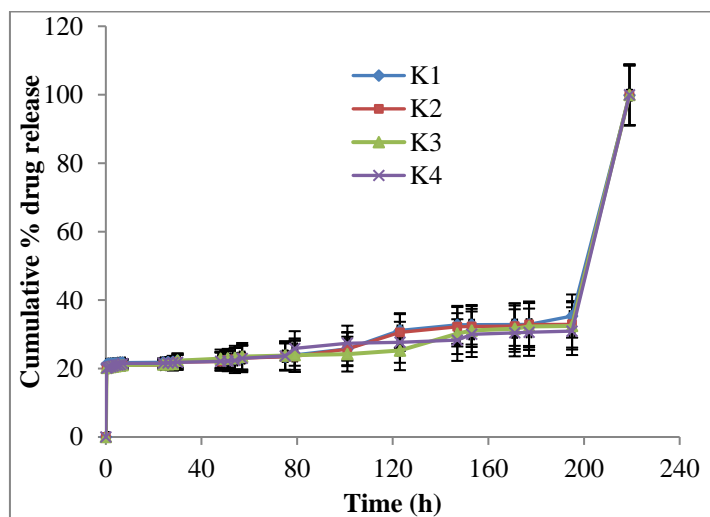


(a)

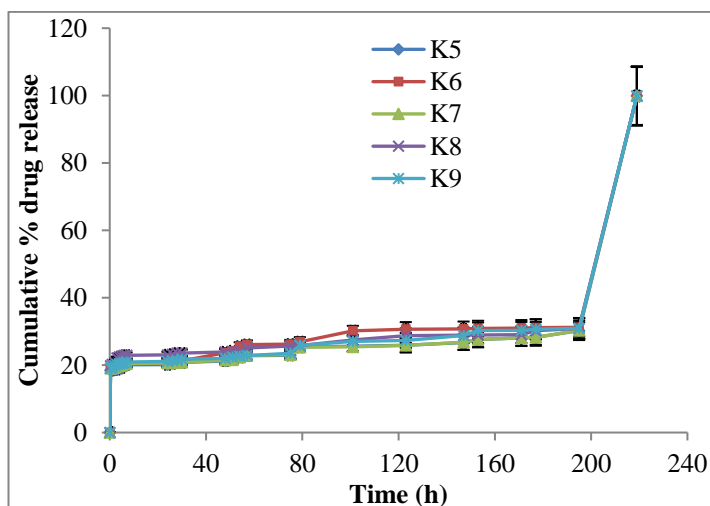


(b)

Figure 4. *In vitro* drug release study of KGP-Chitosan polyelectrolyte complex fabricated films using egg membrane as a biological barrier



(a)



(b)

Figure 5. *Ex vivo* skin permeation studies of KGP-chitosan polyelectrolyte complex fabricated films using chicken intestinal membrane

In the present study, albumin was used as model protein drug and it has good aqueous solubility. The presence of water soluble drug in the PEC matrix may enhance the solubility and dissolution of PEC matrix. As discussed by Dubey *et al* Higuchi model assumes extensive drug dissolution, pseudo steady state and

constant diffusivity. Higuchi model also states the diffusion through water filled pores [25].

In a study, Meng *et al* prepared chitosan-alginate films using sulfadiazine for wound dressing purpose. Prepared films were able to control the drug release up to 4 days. It showed that PEC films can control drug release for extended time period [26]. Chitosan-carboxymethyl starch based microparticles were used to deliver bovine serum albumin. Prepared microparticles followed zero order kinetic of drug release [27].

Figure 4 showed *in vitro* drug release pattern of KGP-Chitosan polyelectrolyte complex fabricated films. It can be concluded from the data that all the fabricated films were releases 16-21 % albumin up to 30 min followed by sustained release of the drug up to 29- 35 % albumin in 8 days. A burst release of the drug (98-99 %) was observed on 9th days.

Figure 5 showed *ex vivo* skin permeation studies of KGP-Chitosan polyelectrolyte complex fabricated films. *Ex vivo* skin permeation studies showed almost the same drug permeation pattern as observed in *in vitro* drug release.

Table 6. Kinetics of *in vitro* drug release of the formulation (K1-K9)

Formulation	KINETICS												
	Zero order kinetics		First order kinetics		Higuchi kinetics		Baker Lonsdale kinetics		Hixson-Crowell kinetics		Korsmeyer Peppas 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
K1	0.478	0.153	0.540	0.029	0.409	1.321	0.904	0.265	0.914	0.001	0.604	5.420	0.090
K2	0.473	0.153	0.564	0.032	0.409	1.303	0.905	0.253	0.933	0.001	0.663	5.649	0.096
K3	0.428	0.145	0.5	0.027	0.366	1.315	0.909	0.253	0.962	0.001	0.672	5.482	0.081
K4	0.402	0.140	0.478	0.027	0.347	1.324	0.905	0.254	0.967	0.001	0.694	5.395	0.075
K5	0.393	0.141	0.521	0.029	0.346	1.279	0.906	0.254	0.961	0.001	0.833	5.492	0.085
K6	0.572	0.183	0.665	0.048	0.528	1.161	0.922	0.216	0.930	0.007	0.491	6.714	0.169
K7	0.393	0.141	0.521	0.029	0.346	1.279	0.904	0.237	0.961	0.001	0.833	5.942	0.085
K8	0.371	0.131	0.451	0.025	0.331	1.374	0.922	0.216	0.944	0.000	0.798	4.977	0.066
K9	0.436	0.147	0.539	0.029	0.377	1.297	0.912	0.262	0.983	0.001	0.728	5.688	0.081

Table 7. Kinetics of *ex vivo* drug permeation of formulation (K1-K9)

Formulation	KINETICS												
	Zero order kinetics		First order kinetics		Higuchi kinetics		Baker Lonsdale kinetics		Hixson-Crowell kinetics		Korsmeyer Peppas 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
K1	0.467	0.152	0.513	0.029	0.394	1.318	0.905	0.264	0.929	0.001	0.572	5.395	0.084
K2	0.465	0.152	0.533	0.029	0.395	1.300	0.906	0.252	0.946	0.001	0.624	5.623	0.09
K3	0.430	0.146	0.5	0.027	0.366	1.315	0.905	0.254	0.962	0.001	0.672	5.482	0.081
K4	0.408	0.141	0.478	0.027	0.347	1.324	0.906	0.254	0.967	0.001	0.694	5.395	0.075
K5	0.398	0.141	0.515	0.066	0.347	1.279	0.906	0.229	0.973	0.001	0.811	5.970	0.086
K6	0.462	0.152	0.665	0.048	0.528	1.161	0.904	0.237	0.767	0.001	0.491	6.714	0.169
K7	0.386	0.138	0.479	0.027	0.334	1.297	0.908	0.236	0.977	0.001	0.763	5.714	0.077
K8	0.374	0.132	0.462	0.025	0.333	1.361	0.918	0.248	0.947	0.000	0.828	5.069	0.068
K9	0.423	0.145	0.527	0.029	0.368	1.300	0.911	0.238	0.978	0.001	0.751	5.675	0.086

Ex vivo skin permeation studies showed that all the prepared formulations followed Hixson-Crowell model (Table 6 and Table 7). The Hixson-Crowell model assumed that surface area and diameter of the drug matrix change with time. The Hixson-Crowell model applies to all pharmaceutical dosage, where the dissolution occurs in planes that are parallel to the drug surface if the formulation dimensions diminish proportionally, in such a manner that the initial geometrical form keeps constant at all the time. In a study Lefnaoui *et al* has utilized chitosan sodium alginate polyelectrolyte film as a carrier to deliver drug through Transdermal route. During characterization they observed that prepared films were able to deliver drug in a controlled manner.

Lefnaoui *et al* prepared a transdermal film of chitosan-alginate polyelectrolyte complex using ketotifen fumarate as drug. They observed that drug release followed Korsmeyer-Peppas model of kinetics. Results are against the present investigation, where Higuchi kinetics was followed [28].

Chitosan-carbopol polyelectrolyte film has been prepared and showed promising characteristics in terms of flexibility and mechanical strength [29].

PEC formation also not required any catalyst or initiator and eliminate the chance of subsequent toxicity. In other words, a smart PEC based film was synthesized and successfully utilized to deliver protein/peptide (albumin) drug.

4. CONCLUSIONS

The present investigation, explored the utilization of neem gum-chitosan and kheri gum-chitosan polyelectrolyte complex

based Transdermal film for protein/peptide drug delivery. Albumin was used as model protein molecules in the study. Transdermal

films were fabricated without using any penetration enhancer. It can be concluded from the data that gum-chitosan polyelectrolyte complex film can be easily fabricated for the noninvasive delivery of the proteinaceous drug. Prepared Transdermal films were able to

extend drug release up to several days. In the future, fabricated films would be utilized for *in vivo* studies to develop *in vitro- in vivo* correlation.

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6. ACKNOWLEDGEMENTS

Authors are thankful to USIC, Babasaheb Bhimrao Ambedkar University, Lucknow for SEM analysis. Present work is continuation of previously published work “Rishabha Malviya. Non-invasive drug delivery system for the delivery of protein/peptide using neem gum and its derivatives. *Biointerface Research in Applied Chemistry*. Volume 10, Issue 3, 2020, 5460 – 5465”.



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