https://doi.org/10.48047/AFJBS.6.14.2024.4912-4934

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

FORMULATION AND EVALUATION OF INSULIN GEL FOR THE TREATMENT OF DIABETES MELLITUS

Harshit Singhal [1*], Anupriya Adhikari[2], Shivanand Patil[3]

[1]Research Scholar, Department of Pharmaceutics, Shree Dev Bhoomi Institute of Education Science and Technology, Pounda, Dehradun, Uttarakhand, India

[2]Associate Professor, Department of Pharmaceutics, Shree Dev Bhoomi Institute of Education Science and Technology, Pounda, Dehradun, Uttarakhand, India

[3] Head, Department of Pharmaceutics, Shree Dev Bhoomi Institute of Education Science and Technology, Pounda, Dehradun, Uttarakhand, India

***Corresponding author:** Harshit Singhal, Department of Pharmaceutics, Shree Dev Bhoomi Institute of Education Science and Technology, Pounda, Dehradun, Uttarakhand, India

Volume 6, Issue 14, Aug 2024

Received: 05 June 2024

Accepted: 10 July 2024

Published: 15 Aug 2024

doi: 10.48047/AFJBS.6.14.2024.4912-4934

ABSTRACT: -

Diabetes mellitus is a chronic metabolic disorder characterized by high blood glucose levels due to inadequate insulin production or action. Conventional insulin therapy, primarily administered via subcutaneous injection, poses challenges such as patient discomfort, needle phobia, and poor compliance. This study aims to develop and evaluate a novel insulin gel formulation for transdermal delivery, offering a non-invasive alternative to injections. The insulin gel was formulated using biocompatible polymers that enhance skin permeability and ensure controlled release. The gel's physicochemical properties, including viscosity, pH, and drug content, were characterized to ensure stability and efficacy. In vitro skin permeation studies were conducted using Franz diffusion cells to assess the transdermal delivery efficiency of insulin. Pharmacodynamic evaluations in diabetic animal models demonstrated the gel's ability to lower blood glucose levels effectively, comparable to traditional injection methods. The study also examined the gel's safety profile, observing no significant skin irritation or adverse reactions. Overall, the insulin gel formulation presents a promising transdermal delivery system, potentially improving patient compliance and quality of life in diabetes management. Further clinical trials are necessary to confirm its efficacy and safety in human subjects. The viscosity, pH, and drug content of the insulin gel were assessed physiochemically after it was made with biocompatible polymers. Franz diffusion cells were used in in vitro research to measure the gel's skin penetration, while diabetic animal models were used in in vivo investigations to gauge the gel's effectiveness in decreasing blood glucose levels. The insulin gel had favourable physicochemical characteristics and a consistent drug release profile, according to the results. The transdermal delivery of insulin was found to be effective in the in vitro permeation investigations, and the in vivo studies showed a significant reduction in blood glucose levels that was similar to that of traditional insulin injections.

Keywords: - Diabetes mellitus, metabolic disease, insulin resistance, insulin gel.

INTRODUCTION: -

DIABETES MELLITUS: -

A class of illnesses known as diabetes mellitus impact how the body uses glucose, or blood sugar. An essential source of energy for the cells that comprise muscles and tissues is glucose [1-6]. Diabetes has different primary causes. However, diabetes can result in an excess of sugar in the blood regardless of the type you have. A blood sugar level that is too high can cause major health issues [7-11]. Diabetes, or diabetes mellitus, is a medical term used to describe a group of diseases related to how your body uses food as fuel [12-19]. Your body converts carbohydrates into glucose, a sugar that enters your circulation when you eat them. insulin is a hormone secreted by the pancreas that aids in transferring glucose from the bloodstream into your cells so they can use it as fuel. if you are untreated for diabetes, your body will not use insulin as it should. Excess blood glucose remains in the bloodstream, a condition commonly referred to as high blood sugar [20-26]. This may result in major health issues that could endanger life.

If you are untreated for diabetes, your body will not use insulin as it should. Excess blood glucose remains in the bloodstream, a condition commonly referred to as high blood sugar. This may result in major health issues that could endanger life [27-35].

Diabetes mellitus is a long-term metabolic disease marked by elevated blood glucose levels (hyperglycaemia) as a result of deficiencies in either the action or synthesis of insulin. Type 1 and Type 2 diabetes are the two main forms in which it typically presents itself, while there are additional varieties as well, including gestational diabetes and variations brought on by other factors [36-40].

Type 1 and type 2 diabetes are chronic diabetes diseases. Prediabetes and gestational diabetes are two diabetes disorders that may be treated. When blood sugar levels are greater than usual, prediabetes develops. However, the blood sugar isn't elevated enough to qualify as diabetes. Furthermore, if preventative measures are not implemented, prediabetes might progress to diabetes. Gestational diabetes develops in the course of pregnancy. However, when the baby is born, it can disappear [41,42].

Types 1 Diabetes: - When you have type 1 diabetes, your body is unable to produce the hormone insulin, which causes your blood glucose (sugar) level to rise too high. This occurs as a result of your body attacking the insulin-producing cells in your pancreas, which prevent you from producing any. To survive, we all require insulin. It performs a vital function [19]. It makes it possible for the blood glucose to go into our cells and power them. Your body still converts carbohydrates from food and drink into glucose even if you have type 1 diabetes. However, there isn't any insulin in your bloodstream when glucose enters your body, preventing it from entering your cells. The accumulation of glucose in your system subsequently causes elevated blood sugar levels.

Types 2 Diabetes: - When you have type 2 diabetes, your pancreas is unable to produce enough insulin or it is unable to do so effectively. This indicates that your blood sugar (glucose) levels Continue to rise. in the UK, types 2 diabetes affects about 90% of those with the disease. It's a dangerous condition that may last a lifetime [23].

Gestational Diabetes: - Pregnancy can cause diabetes to develop into gestational diabetes. Women who have never had diabetes before are affected. It indicates that you have hyperglycaemia and should take particular care of your developing baby. This entails maintaining an active lifestyle and eating healthy. Usually, it disappears once more after delivering delivery. A blood test is typically used to diagnose it between weeks 24 and 28 of pregnancy. During pregnancy, gestational diabetes develops. African Americans, Native Americans, Latinos, and those with a family history of diabetes are more likely to experience it. Although the illness is linked to a higher risk of getting diabetes later in life, it usually goes away after delivery [29].

The following are a few signs of both type 1 and type 2 diabetes:

Feeling thirstier than normal: - The body may lose more water due to the frequent urine required to eliminate excess sugar from the blood [31].
Frequent urination: - The kidneys attempt to filter out extra sugar from the blood when blood sugar levels are high. This may result in an increased frequency of urination, especially at night Trusted Source [32].
Regular hunger: -Individuals who have diabetes frequently don't obtain enough energy from their diet. Food is broken down by the digestive system into glucose, a simple sugar that the body needs as fuel. Not enough of this glucose enters the body's cells from the bloodstream in diabetics.
Fatigue: People with type 2 diabetes may experience changes in their energy levels and a sense of being weary. Diabetes tiredness is brought on by insufficient circulating glucose entering the body's cells.
Vision blurry: - Blood sugar excess can damage the small blood vessels in the eyes, resulting in blurry vision in one or both eyes. High blood sugar levels can also cause swelling of the eye lens, which can cause blurred vision but will go away when blood sugar levels drop. If a diabetic patient does not receive treatment, the damage to these blood vessels can worsen and eventually result in permanent vision loss.
Wounds and cuts heal slowly: -Elevated blood sugar levels have the potential to harm the body's blood vessels and neurons, so compromising blood flow. Because of this, even minor cuts and wounds might not heal for weeks or months. Infection risk is also increased by delayed wound healing.
Hand or foot tingling, numbness, or discomfort: -Elevated blood sugar levels can harm nerves and impair blood flow. Trusted Source. This might cause tingling, numbness, or pain in the hands and feet of those who have type 2 diabetes. mIt is referred to as neuropathy. If a person with diabetes is not treated, it may develop worse over time and result in more severe problems.
Darker skin patches: - Diabetes can also cause darker skin patches to appear in the groyne, armpit, or neck folds. These areas could have a silky, supple sensation. The name "acanthosis nigricans" refers to this skin disorder.
Novel drug delivery system: -
In comparison to conventional insulin administration techniques, the creation of a novel drug delivery system, like an insulin gel, intends to increase patient compliance, stability, and efficacy in the management of diabetes mellitus. A major area of attention in the management of diabetes mellitus has been the creation of innovative insulin medication delivery methods. Traditional insulin administration techniques, which mostly involve subcutaneous injections, have a number of disadvantages, such as the possibility of hypoglycemia, inconsistent absorption rates, and pain for the patient. Novel strategies seek to improve insulin therapy's overall effectiveness, patient compliance, and overall experience. Using insulin gels is one such potential strategy [43-50].
Nanogels are hydrogel particles that are smaller than nanometers and have a high surface area for encapsulating insulin and releasing it in a controlled manner. They can be engineered to target specific tissues and release insulin for a longer period of time. Hydrogels are three-dimensional, hydrophilic polymer networks that can hold large amounts of water [51-60].

- **Diffusion-Controlled Release:** As insulin diffuses out of the polymer network, it is progressively freed from the gel matrix.
- **Degradation-Controlled Release:** Over time, the encapsulated insulin is released as the polymer matrix breaks down.
- **Stimuli-Responsive Release:** In reaction to physiological changes, gels that react to particular stimuli (such as pH or glucose levels) can release insulin.

- **Better Patient Compliance:** Fewer injections administered on a regular basis and the possibility of non-invasive delivery techniques (e.g., oral, nasal).
- **Controlled and Sustained Release:** Lowers the risk of hypoglycemia by providing a more consistent and extended insulin activity that resembles natural insulin release.
- **Diminished Adverse Reactions:** Reduced chance of adverse effects after injections, including lipodystrophy, discomfort, and infection.
- **Preliminary Research**: Examining insulin gel safety, pharmacokinetics, and pharmacodynamics in animal models.
- **Clinical trials:** Testing insulin gel formulations on humans to determine their safety, effectiveness, and acceptability by patients.
- **Formulation Optimisation:** Optimising gel formulations to enhance patient experience, release patterns, and insulin stability.

FUTURE PROSPECTIVE OF INSULIN GEL: -

Gels that are "smart"—that is, gels that can react to glucose levels—are still being researched. These gels would act as a closed-loop diabetes management system, releasing insulin in response to elevated blood glucose levels.

Nanotechnology: -

Better tissue penetration and dispersion may be possible with nanogels. It is being investigated whether using nanoparticles in gel formulations can improve the bioavailability and effectiveness of insulin.

Combination therapies: -

Including insulin and additional medicinal substances in a single gel formulation may offer a number of advantages, including reduced inflammation and better control of blood sugar.

- Insulin gel eliminates the need for injections by being used topically or orally. This non-invasive method can improve adherence to treatment plans and patient comfort.
- Injections can be frightening and painful for older people and youngsters, who may find gel formulations more user-friendly.
- The stability of insulin in gel form can be increased by advancements in formulation technology, guaranteeing that it stays effective for extended periods of time and under different storage settings.
- Insulin gels can be made to release insulin under regulated conditions, lowering the need for frequent injections and preserving stable blood glucose levels.
- A typical side effect of insulin therapy, hypoglycemia, may be less likely due to the gel's slow release of insulin.
- It is essential to make sure insulin gels adhere to strict regulatory requirements for both safety and effectiveness.

  It is necessary to control the cost of large-scale insulin gel production to guarantee patient affordability and accessibility.

DRAWBACK OF CONVENTIONAL SYSTEM OF INSULIN GEL FOR THE TREATMENT OF DIABETES MELLITUS: -

Numerous issues with the traditional insulin gel therapy approach for diabetes mellitus might affect both patient compliance and the system's efficacy. Below are a few major disadvantages:

1. **Variable Bioavailability and absorption:**
   - **Inconsistent Absorption:** Differences in skin characteristics, such as thickness, moisture level, and temperature, might cause irregular absorption of insulin from gels.

- **Low Bioavailability:** Compared to alternative techniques, such as subcutaneous injections, the bioavailability of insulin supplied using gels is frequently lower. This indicates that a reduced amount of the dose that is given enters the bloodstream and provides a therapeutic benefit.

2. **Problem with the application site:**
   - **Skin Irritation:** Extended usage of insulin gels may result in redness, itching, and irritation of the skin where the gel is applied.
   - **Variable Penetration:** Variations in skin characteristics (such as the existence of calluses or scars) might cause insulin to penetrate the skin differently, which can lead to uneven blood glucose management.
3. **Dosing Difficulties:**
   - **Difficulty with Dose Adjustment:** Accurate dose adjustments with insulin gels can be difficult, which makes it more difficult to customise the medication to meet the needs of each patient.
   - **Inaccurate Dosing:**Gels may not offer the exact dosage that injections may, which could result in an overdose or underdose.
   - **Application Mess:** Patients may not comply as much while applying insulin gel since it might be messy and inconvenient.
   - **Time-consuming:** While administering injections quickly, the application procedure could take longer.
4. **Sturdiness and Storage:**
   - **Storage Requirements:** In order to preserve stability, insulin gels may need to be kept refrigerated, which may be uncomfortable for patients.
   - **Limited Shelf Life:** Compared to insulin in injectable form, certain insulin gel formulations may have a lower shelf life.
   - **Effectiveness and Action's Start:**
   - **Delay in activity:** Compared to subcutaneous injections, the insulin gels' activity may start later and cause a delay in the drop in blood glucose levels.
   - **Reduced Efficacy:** As a result of the previously listed problems, insulin gels may not be as effective overall, which could result in less than ideal blood glucose control.
5. **Price and Availability:**
   - **Higher Costs:** Compared to conventional injectable insulin, insulin gels may be more costly to make and buy.
   - **Restricted Availability:** Patients' access to insulin gel therapy may be hampered by the product's possible limited availability.

ETIOLOGY AND RISK FACTOR OF DIABETES MELLITUS: -
MODIFIED RISK FACTOR: -

1. **Weight:** Having a high body mass index or being obese raises your risk of diabetes. Diabetes can be considerably lowered by losing 5% to 10% of your body weight in addition to engaging in regular physical activity. The more weight you reduce, the lower your risk is. A body mass index calculator can usually give you a target weight that is appropriate for your height. Acquire weight management skills [35].
2. **Physical activity:** Being sedentary is a major modifiable risk factor for diabetes and prediabetes. Frequent exercise reduces the risk of insulin resistance. This translates to improved insulin use by your body. Walking for at least 30 minutes a day, even at a quick pace, can significantly lower the risk of heart disease and diabetes. To improve your cardiovascular health overall, try to get at least 150 minutes a week of moderate-to-intense

aerobic exercise, or 75 minutes a week of vigorous-to-intense aerobic exercise (or both); and at least two days a week dedicated to muscular strengthening

3. **Blood pressure:** If left untreated, high blood pressure can harm the cardiovascular system and increase the risk of complications from diabetes [36]. Blood pressure of less than 130/80 mm Hg is recommended for those with diabetes and high blood pressure. Sub 120/80 mm Hg is considered normal blood pressure. Study up on high blood pressure and its management.
4. **Infections caused by viruses:** Researchers have discovered that certain viruses can cause the body's immune system to become hostile to the body, rather than supporting it in fending off illness and infection. This could lead to the development of type 1 diabetes [37].Type 1 virus is thought to be triggered by the German measles, coxsackie virus (which causes hand, foot, and mouth disease), and mumps.
5. **Geography:** It appears that residents of northern climates have a higher chance of contracting type 1 diabetes. According to many theories, people who reside in northern nations spend more time indoors, particularly during the winter, which puts them closer to one another and may increase the risk of contracting viruses [38].
6. **Smoking:** There are several methods, drugs, and internet resources available to assist you in quitting if you smoke. Discuss your best options with your medical team.
7. **Other autoimmune diseases:** Because it triggers the body's immune system to rebel against itself, type 1 diabetes is classified as an autoimmune disease. Type 1 may be more likely to develop if you have another autoimmune ailment since both conditions may have comparable HLA complexes. Multiple sclerosis, pernicious anaemia, and Graves' disease are a few more autoimmune diseases that can raise your risk of type 1.
8. **Certain Medical Conditions:** Dyslipidemia (abnormal cholesterol levels) and hypertension are two conditions that are frequently linked to an increased risk of diabetes. Diabetes risk can also be raised by a few endocrine conditions, such as acromegaly and Cushing's disease.
9. **Low birth weight and certain disorders**: -During foetal development can raise the risk of type 2 diabetes later in life. Birth weight and early life factors should also be considered. Commensurately, there is an increased risk associated with being born to a mother who had gestational diabetes [39].

NON- MODIFIABLE RISK FACTOR: -

1. **Family history: -** If any of your parents or siblings have diabetes, your risk of developing the condition is raised. Talk to your healthcare provider about your family's medical history to learn what it might mean for you.
2. **Race/Ethnic Background: -** Diabetes is more common in Black Americans, Asian Americans, Native Americans, Latino/Hispanic Americans, and Pacific Islanders.
3. **Age: -** Prediabetes and diabetes are more common in elderly adults. Most cases of diabetes in middle-aged adults develop after the age of 45. However, an increasing number of children and teenagers are receiving diabetes diagnoses from medical professionals [40].
4. **Gestational diabetes: -** If you were diagnosed with diabetes during your pregnancy, you have a higher chance of getting the disease again in the future. Women who suffer from PCOS (polycystic ovary syndrome) have an increased insulin resistance linked to type 2 diabetes increases the chance of getting the disease.
5. **Genetics:** certain genes may raise a person’s risk of developing diabetes. A person's physician can look up these genes [41].

RESULT:

STABILITY REAERACH

When hydrogels that are are used as medication carrier for topical application, the active ingredient molecule is shielded against enzymes and adverse pH changes while also enabling a gradual release of the active ingredient.

Stability investigations make it possible to evaluate a formulation's appropriateness during treatment. One of the most significant demands that the medication to be used safely and effectively is that the active ingredient not break down. The stability assessment findings for the hydrogels that are that were created are displayed in Table 1. There was a discernible shift in the hormone written material, whose might have been caused by condensation from the container pack (for low-stability drugs, the total volume of API ought to stay consistent by greater than 10%).

Conditions	**Weeks**	**Color**	**Phase Separation**	**pH**	**Drug Content**	**Viscosity η (30 s−1) [mPa × s]**
5 ± 3 °C	2	No color change	No	7.11 ± 0.05	99.5% ± 0.5	3940 ± 204
	4	No color change	No	7.12 ± 0.03	99.6% ± 0.5	3960 ± 114
25 ± 1 °C	2	No color change	No	7.16 ± 0.05	99.7% ± 0.4	4080 ± 199
	4	No color change	No	7.15 ± 0.02	100.5% ± 0.2	4091 ± 200
5 ± 3 °C	2	No color change	No	7.16 ± 0.04	99.2% ± 0.4	4320 ± 180
	4	No color change	No	7.11 ± 0.03	99.8% ± 0.3	No color change
25 ± 1 °C	2	No color change	No	7.09 ± 0.04	99.8% ± 0.2	4680 ± 202
	4	No color change	No	7.11 ± 0.01	101.2% ± 0.5	4820 ± 200

Table No. 1 Represent Stability investigations of a formulation's appropriateness during treatment

Images of hydrogel at different temperature with different week:-

Fig No.1 (Temp. 5 ± 3 °C) 2 weeks

Fig No.2 (Temp. 5 ± 3 °C) 4 weeks

Fig No.3 (Temp. 25 ± 1 °C) 2 weeks

Fig No.4 (Temp. 25 ± 1 °C) 4weeks

Fig No.5 (Temp. 5 ± 3 °C) 2 weeks

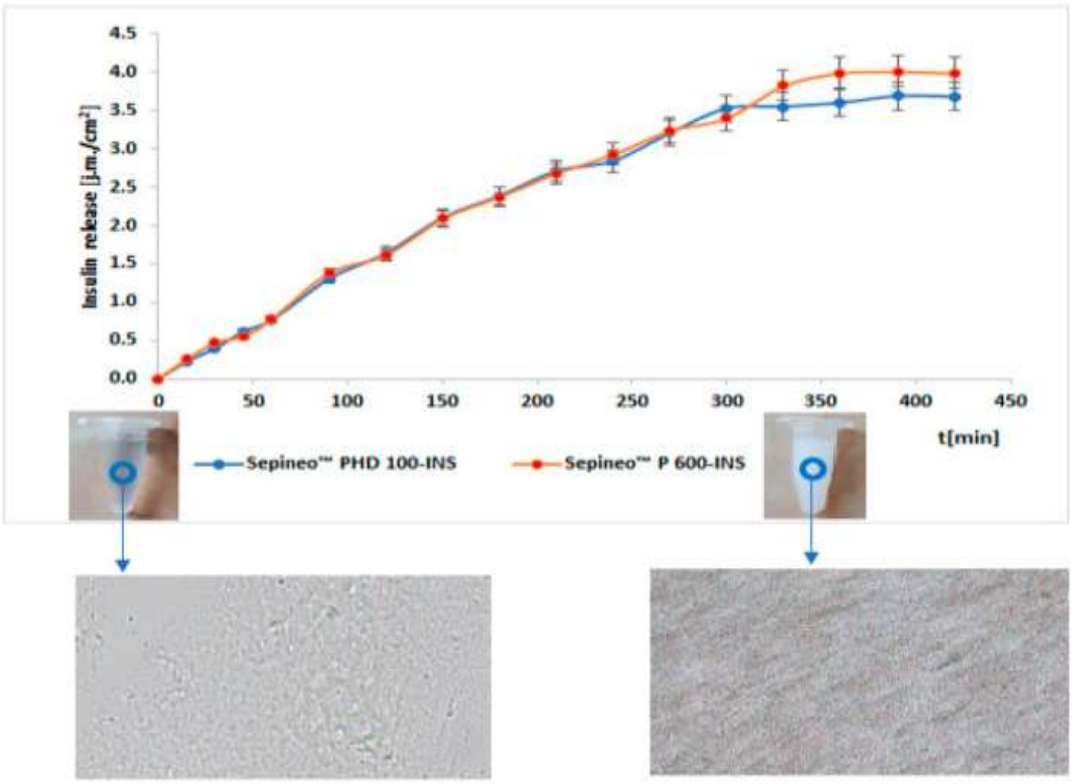
Fig No.6 (Temp. 5 ± 3 °C) 4weeks

Fig No.7 (Temp.25 ± 1 °C) 2 weeks

Fig No.8 (Temp.25 ± 1 °C) 4 weeks

INSULIN SECRETION IN VITRO:

Investigations on in vitro pharmaceutical availability show the fact that the cross-linked polymeric architecture causes glucose to be delivered over an extended period of time as shown in Figure 1. Following 1 hours, the hydrogels based on SepineoTM P -600 and SepineoTM PHD -100 produced 4.01 IU/cm2 (53.36%) and 3.69 IU/cm2 (47.4%) of insulin, respectively. Following this period, the amount of the degraded polymers probably rose, increasing the structure's viscosity and obstructing the release of more hormone via the membrane.



Graph No. 1 The results obtained from the mathematical modeling of rheogram.

According to guidelines from the European Medicines Agency (EMA) and the Food and Drug Administration (FDA) in the USA, the comparable nature of the published profiles (Figure 1) has been assessed using the distinction (f1) and resemblance (f2) component technique. It turned out that the previously examined release of insulin characteristics for the SepineoTM P 600 and SepineoTM PHD 100 matrices were comparable. The parameter f2 was 99.78 (takes a value larger than 50 and near to 100), whereas f1 is 4.39 percent (ranges from 0 to 15).

Using computational models, the produced hydrogels that are' insulin release patterns were examined by comparing three parameters: the model selection criterion (MSC), the AIC (Akaike information criterion), and the coefficient of determination (R2) (Table 3). It is assumed that API release follows a kinetic model, where the parameters with the greatest values are R2 and MSC, while the parameters with the lowest values are AIC [41]. The Peppas-Sahlin model [42] is followed by the hormone released from the produced hydrogel matrix, according to an analysis of the data in Table 1. The kinetics of API release from hydrogels are characterized by this model. Diffusion of the active ingredient and polymer chain relaxation underpin the release mechanism. The negligible impact of kPS1 (kPS1 = -0.220) is determined by its negative value. the release of insulin as a function of Fick's diffusion mechanism as opposed to polymer chain relaxation (kPS2 = 0.121). Super case II transport caused by erosion and polymerization relaxation is the main mechanism. It is most likely caused by the electrostatic interactions between molecules during swelling and diffusion.

Kinetics Models	**Equation**	**Hydrogel**	**Parameters**	**$R^2_{adjusted}$**	**AIC**	**MSC**

Zero Order model	$f = k_0 \times t$	G1-INS	$k_0 =$ 0.012	0.9790	−9.0372	3.719
		G2-INS	$k_0 = 0.011$	0.9369	7.1041	2.6294
First Order model	$f = 100 \times [1 - e^{-k1 \times t}]$	G1-INS	$k_1 =$ 0.001	0.9813	−10.6466	3.8347
		G2-INS	$k_1 = 0.001$	0.9412	6.0386	2.7004
Higuchi model	$f = k_H \times t^{0.5}$	G1-INS	$k_H =$ 0.184	0.8933	13.7068	2.0952
		G2-INS	$k_H = 0.181$	0.9123	12.0342	2.3007
		G1-INS	$k_{KP} = 0.030$	0.9946	−27.2065	5.0176
			$k_{PS2} = 0.121$			
Korsmeyer-Peppas model	$f = k_{KP} \times t^n$	G2-INS	$k_{KP} = 0.046$			
			n = 0.749	0.9796	−8.9155	3.6973
		G1-INS	$k_{PS1} = -0.220$	0.9961	−30.7982	5.2741
			$k_{PS2} = 0.121$			
			m = 0.324			
Peppas-Sahlin model	$f = k_{PS1} \times t^m + k_{PS2} \times t^{(2 \times m)}$	G2-INS	$k_{PS1} = -0.973$	0.9882	−16.3418	4.1924
			$k_{PS2} = 0.561$			
			m = 0.217			
Hixson-Crowell model	$f = 100 \times [1 - (1 - k_{HC} \times t)^3]$	G1-INS	$k_{HC} = 0.0$	0.9805	−10.1033	3.7959
		G2-INS	$k_{HC} = 0.0$	0.9398	6.3962	2.6766
		G1-INS	$k_{HB} = 0.0$	0.9797	−8.6180	3.6869
			n = 58.345			
Hopfenberg model	$f = 100 \times [1 - (1 - k_{HB} \times t)^n]$	G2-INS	$k_{HB} = 0.0$	0.9366	8.0568	2.5659
			n = 58.939			
Baker–Lonsdale model	$3/2 \times [1-(1 - F/100)^{2/3}] - F/100 = k_{BL} \times t$	G1-INS	$k_{BL} = 0.0$	0.8915	13.9423	2.0784
		G2-INS	$k_{BL} = 0.0$	0.9108	12.2931	2.2834

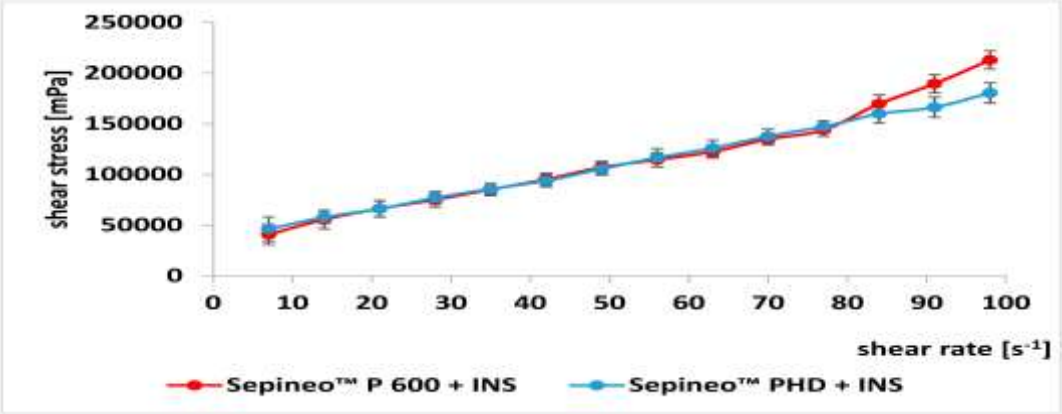
Table 2 presents mathematical models that explain the release kinetics of insulin from hydrogels.

Abbreviations: f, quantity of drug release; G1, SepineoTM P 600 with insulin; G2, SepineoTM PHD with insulin; t, the time The following are the experimental parameters: n, release exponent; kPS1, Peppas–Sahlin release constant (constant for Fickian diffusion); kPS2,

constant for Case II relaxational mechanism; m, diffusion exponent; kHC, Hixson–Crowell release constant; kHB, Hopfenberg release constant; kBL, Baker Lonsdale release constant; R2adjusted, adjusted correlation coefficient; AIC, Akaike Information Criterion; MSC, Model Selection Criteria.

ANALYZING RHEOLOGY:

Understanding the rheological properties of hydrogels is crucial for their development, management, control of technical procedures, and acceptance by patients of the formulation. The viscosity and thinness, two rheological factors, can have a big impact on how a medication releases as well as acts. All formulas' flow charts are quite similar (Figure 2). The criteria for using mathematical structures to describe the flow curves are displayed in the following table. The higher R2 values indicate that the Herschel–Bulkley model best fits the results of experiments flow profiles. Comparing the hydrogel as determined by SepineoTM P 600 points with the hormone insulin to SepineoTM PHD 100 with a hormone called insulin the hydrogel exhibits a three percent reduced yield stress (τ0, the force needed to overcome Van der Waals-type cohesive forces and commence flow). The K-factor, a fluidity indicator Compared to SepineoTM PHD /100 with glucose, there was a 33% reduction in yield stress (τ0, the force needed to overcome Van der Waals-type cohesive forces and start flow). G1-INS has a 14% lower K-factor (a measure of fluid viscosity) than G2-INS. This is most likely because SepineoTM PHD 100 has bigger particles. A pseudoplastic flow type (n = 1, Newtonian flow; n > 1, dilatant flow; n < 1, pseudoplastic flow) is indicated by a parameter value of n < 1



Graph No. 2 Prepared formulation flow rheograms are shown.

Formula Code	Herschel–Bulkley				Ostwald-de Waele			Bingham		Casson		
		τ0	N	K	R2	n	K	R2	τ0	R2	τ0	R2
G1-INS	32200	0.993	2.84	0.912	0.930	3.76	0.760	74282	0.639	4701	0.688	
G2-INS	48000	0.945	3.30	0.983	0.538	15.5	0.901	33006	0.880	15712	0.905	

Table No. 3 Represent the formulation flow of rheograms

Symbols: τ0, the Yield stress [mPa]; K, the consistency index [Pa*sn]; n, the flow behavior index; R2, regression coefficient.

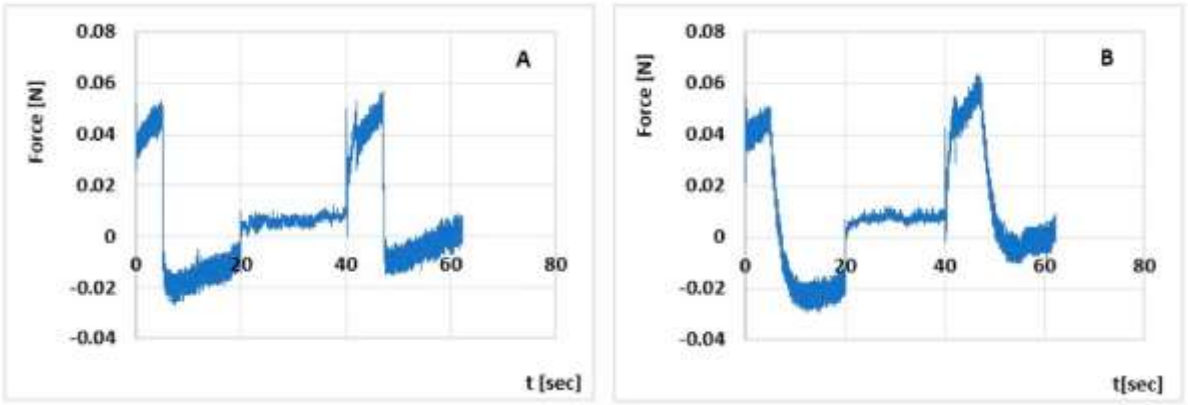
The produced hydrogels with insulin were found to have non-Newtonian flow, be shear-thin, and have a propensity toward yield stress determined by their flow curves (Figure 2, Table 5). The breakdown of the polymer crosslink leads to shear thinning. Lower viscosity readings are the result of the specimen's intermolecular communication bond breaking rate breaking faster than the the sample's re-formation rate during shearing. According to Karavana et al, formulations made of relaxed, entangled long-chain polymer molecules under shear stress detangle and liberate fluid that is trapped in molecule coils. Thus, there is a decrease in perceived viscosity. Thixotropy is a sign of viscosity. Thixotropic characteristics of hydrogels were revealed via rheological investigation. The surface area enclosed by curves (an ascending curve with increasing shear rates and a descending curve with decreasing shear rates) is an indicator of thickness. Its hydrogel architecture has optimally recovered, as indicated by the tiny surface areas of the hysteresis loops. The region between the two lines has a pair of values: G2-INS: 6888.7 Pa*s−1, G1-INS: 4764.8 Pa*s−1.

Hydrogel	**η (30 s^{-1}) [mPa × s]**	**η (50 s^{-1}) [mPa × s]**	**η (100 s^{-1}) [mPa × s]**
G1-INS	3241 ± 50	1949 ± 91	1387 ± 71
G2-INS	3772 ± 87	2070 ± 101	1462 ± 113

Table No. 4 Represent the hydrogel with insulin show non-Newtonian flow

INVESTIGATION OF SURFACE PROFILES:

Topical medications that are semi-solid should be able to be applied to the skin with ease, squeeze out of the unit package, and stay in the bed of the wound without leaking. Therefore, the correct mechanical properties, such as hardness, elasticity, adhesiveness, and cohesiveness, should be present in the created hydrogels. Controlling the mechanical resistance to stress and, as a result, evaluating how they perform under use conditions (squeezing out of the packing, spreading on the skin surface) are made feasible by their examination. Table 6, Figures 3 and 4, and the analysis' findings are displayed.



Graph No. 3 SepineoTM P 600 with insulin (A) and SepineoTM PHD 100 with insulin (B) texture profile analyses (TPAs).

The hydrogel's deformation force can be ascertained by the test of hardness. Regarding ease of application, both formulations have a lower desired hardness. When combined with insulin, SepineoTM PHD 100 and P 600 exhibit harder surfaces by 4% (Hardness 1; NS) and 12% (Hardness 2; $p < 0.05$), respectively. This suggests that the G2-INS composition has a more compact matrix [53,54,55]. Ozcan further confirmed that the

hydrogel's toughness improves as its viscosity does. Additionally, the sample straining strength of SepineoTM PHD 100 is 9% greater (cohesiveness G2-INS vs. G1-INS is 1.671 and 1.579, respectively; $p < 0.05$). Following the application of hydrogel, or hydro higher "cohesiveness" results indicate structural healing.

The amount of pore water between the chains of polymers varies with deformation and load testing. According to Saurez et al., a gel-like substance with greater cohesiveness has a stronger structural bond with water. The more effective action of the formulation at the application site is influenced by cohesiveness. Additionally, the G2-INS hydrogel demonstrated a 1.5-fold ($p < 0.05$) increase in cohesiveness—the capacity to stick to the packaging and engage with the surrounding tissues. Conversely, the manner in which a hydrogel remodels after being compressed and deformed during a predetermined period of time is known as its elasticity. The G2-INS formulation's decreased elasticity is indicated by its higher "elasticity" characteristic of 0.716 compared to the G1-INS formulation's 0.693 ($p < 0.05$). Different temporal relaxant characteristics are observed in the preparations under investigation.

The reason is most likely because of the variations in porosity that define the hydrogel structure. When a spatially cross-linked polymer is completely saturated, the polymeric linkages form an interconnected porous structure that fills with water. The hydrogel maturation period, cross-linking density, and formulations component type and concentration all affect the porosity structure. The functional qualities of both formulations remain unaffected by the observed variations in the magnitude of the evaluated mechanical parameters of the tested formulations. The sensory qualities of both formulations are excellent. Nanoparticles may be taken out of the packaging packing and readily dispersed throughout the skin.

UV ABSORPTION SPECTRUM OF A HYDROGEL LOADED WITH INSULIN; -

Measuring the UV absorption spectrum of a hydrogel treated with insulin is usually the first step in creating a hydrogel insulin UV absorption graph. In addition to ensuring appropriate encapsulation and release qualities, this is done to investigate the interactions between insulin and the hydrogel matrix.

Prepare Hydrogel Samples: Create hydrogels both insulin- and non-insulin-containing.

Calculate UV Absorption Utilize a UV-Vis spectrophotometer to gauge the samples' absorption over a variety of wavelengths.

Capture Information: Gather information for the absorption spectra.

Make a graph: Plot the absorbance (AU) on the y-axis and the wavelength (nm) on the x-axis.

0.1, 0.15, 0.12, 0.11, 0.14, 0.13, 0.12, 0.1, 0.09, 0.08, 0.07

S.NO	WAVELENGTH (nm)	ABSORPTION FOR HYDROGEL WITHOUT INSULIN (AU)
1	200	0.1
2	220	0.15
3	240	0.12
4	260	0.11
5	280	0.14
6	300	0.13
7	320	0.12
8	340	0.1
9	360	0.09
10	380	0.08
11	400	0.07

Table No.5 Absorption for hydrogen without insulin

S.NO	WAVELENGTH (nm)	ABSORPTION FOR HYDROGEL WITH INSULIN (AU)
1	200	0.1
2	220	0.2
3	240	0.18
4	260	0.25
5	280	0.3
6	300	0.28
7	320	0.26
8	340	0.2
9	360	0.18
10	380	0.15
11	400	0.1

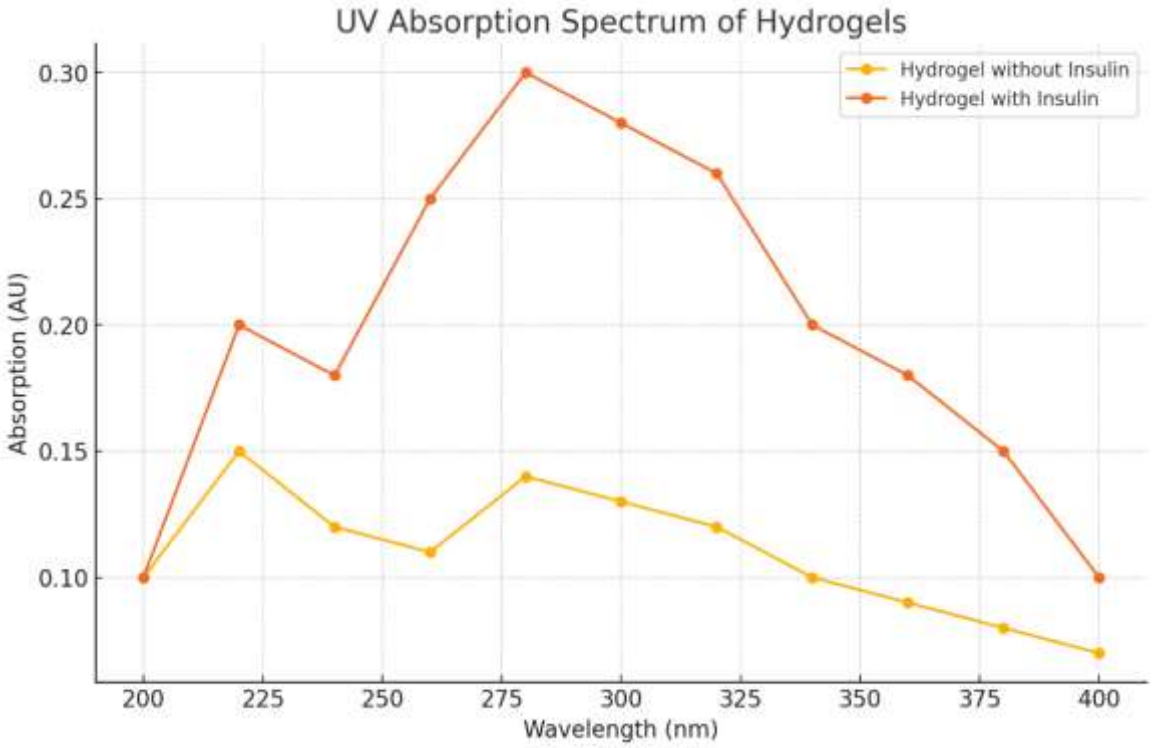
Table No.6 Absorption for hydrogel with insulin

FTIR ANALYSIS OF INSULIN HYDROGELS:

Finding the Functional Groups: Insulin and the hydrogel matrix's functional groups can be found using FTIR. Insulin has side chains with particular functional groups (such phenylalanine, tyrosine, and cysteine residues) and amide bonds (derived from its peptide structure). Polymers with different infrared absorption spectra, such as polyethylene glycol, polysaccharides, or PEG, may also make up the hydrogel matrix.

Measuring Variations in the Hydrogel Structure During Gelation: FTIR is a useful tool for monitoring gel formation. Certain IR absorption bands may shift or vary in intensity as insulin and the hydrogel components interact (for example, through cross-linking or physical entrapment), signaling the creation of new connections or interactions.

Characterizing Cross-Linking: In the event that the insulin hydrogel undergoes cross-linking (for instance, by means of chemical or physical agents),



Graph No. 4 UV absorption Spectrum of hydrogels

FTIR SPECTRUM OF INSULIN:-

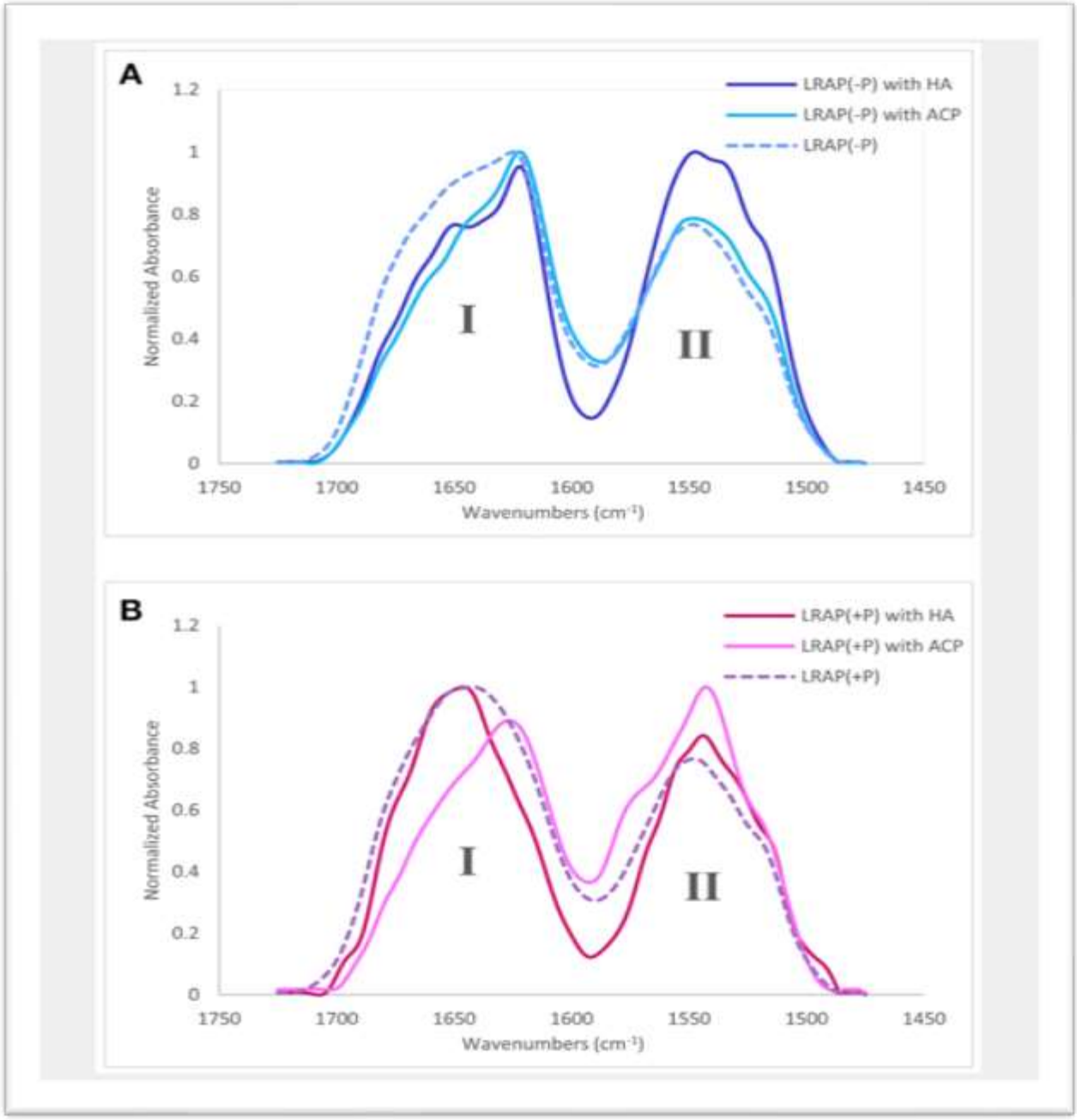
1. **Amide I Region (1700-1600 cm^{-1})**:
    - **Peak Assignment**: Amide I band around 1650 cm^{-1} (C=O stretching vibration).
2. **Amide II Region (1600-1500 cm^{-1})**:
    - **Peak Assignment**: Amide II band around 1550 cm^{-1} (N-H bending and C-N stretching).
3. **Amide III Region (1300-1200 cm^{-1})**:
    - **Peak Assignment**: Amide III band around 1250 cm^{-1} (C-N stretching and N-H bending).
4. **Water Absorption Region (3700-3000 cm^{-1})**:
    - **Peak Assignment**: Broad O-H stretching band around 3200-3500 cm^{-1}.

INTERPRETATION:-

- **Amide Bands**: Peaks in the amide I, II, and III regions confirm the presence of insulin and provide information about its secondary structure.
- **Hydrogen Bonding**: The intensity and shape of these bands provide insights into hydrogen bonding and protein conformation.
- **Water Content**: The broad O-H stretching band indicates the presence of water, essential for maintaining the hydration state of insulin.

GRAPHICAL PRESENTATION:-

- **X-axis**: Wavenumber (cm^{-1}) representing the range from 4000 cm^{-1} to 400 cm^{-1}.
- **Y-axis**: Absorbance or Transmittance (%).
- **Legend**: Different spectra can be overlaid for comparison (e.g., insulin alone, insulin in hydrogel, insulin with graphene).



Graph No. 5 Represent the FTIR Spectrum

Presenting the FTIR spectrum graphically allows for a visual representation of insulin's molecular structure and its interactions within different matrices such as hydrogels or composite materials like those incorporating graphene. This format helps researchers and practitioners quickly grasp the spectral characteristics and changes due to formulation or environmental conditions, aiding in the development and characterization of insulin-based biomaterials for various biomedical applications.

DISCUSSION:

The particular kind of substrates utilized has a major impact on how well insulin therapy works for wounds. The right polymer selection affects both the preparation's ideal skin contact and the hormone's release over an adequate amount of time. It is imperative that the polymer matrix is non-toxic, biodegradable, patient-tolerated, and insulin-incompatible. Conversely, the patient should have easy access to the formulation at a reasonable cost. We are researching the development of an insulin hydrogel carrier based on these limitations. The preformulation study

of hydrogels based on Carbopol® UltrezTM 10, Carbopol® UltrezTM 30, methylcellulose, and glycerol ointment with insulin.
The hue of the SepineoTM P 600-INS system was matt and milky, but the chemical composition of the SepineoTM PHD 100-INS was transparent. The products that were kept for four weeks at 25 ± 1 °C and 5 ± 3 °C both exhibited the necessary quality.
There were no visible physical alterations that would suggest decreased stability (Table 2). As we previously showed using circular dichroism, the structure of insulin was stable at the point of absorption into the polymeric matrix. Quitério's group, who created adrenaline-loaded poly-DL-lactide/glycolide (PLGA) nanoparticles, likewise verified that homogenization had no influence on alterations in the secondary structure of insulin. The addition of Polysorbate 80 to the SepineoTM P 600-based hydrogel considerably improves its stability.
The hydrogels under investigation are non-Newtonian, shear-thinning fluids having a yield anxiety, or an upper limit on the amount of force needed to start the flow process, according to rheological study. The main benefit for cutaneous application is that the formulations exhibit typical hydrogel features with poor polymer–polymer interactions. The yield stress value enables the selection of ideal settings for technological processes (mixing, filling of unit packs), and it connects to the preparation's distribution qualities (spreadability on the skin). The flow curves that were produced best fit the Herschel–Bulkley theoretical model. There is no shear striping and the curves are monotonic. The hydrogel qualities obtained from rheological measurements were validated by texturing profile analysis. The SepineoTM PHD displayed the greater cohesiveness and hardness parameters.

They investigated the rheological properties of insulin-containing hydrogels that are using Carbopol® UltrezTM 10, Carbopol® UltrezTM 30, methylcellulose, and glycerol ointment in our earlier study. The rheograms' typical course for shear-thinning non-Newtonian fluids with a flow limit was shown via analysis. Lower shear rate analysis of the hydrogels verified their extremely dynamic viscosity, which is a feature of pseudoplastic liquids. Furthermore, a hysteresis loop-characterized thixotropy effect was seen in the hydrogels under study. Similar rheological characteristics were seen in an insulin-based intranasal hydrogel produced by Von Zuben et al. The hydrogen gel was categorized as a liquid that was shear-thinning, or pseudoplastic ($n < 1$). The viscosity of the finished.
The formulation’s initial decreased resistance to flow following shear impacts dosing convenience. An intranasal insulin hydrogel (HuminsulinTM) based on chitosan and polyvinyl alcohol (PVA) was created by Agarwal et al. Like other polymer-based the hydrogels, the composition also exhibited the properties of a pseudoplastic shear-thinning fluid. The characteristics of semi-solid drug formulations that have been mentioned enable the development of a thick aqueous barrier at the therapeutic site that has long-lasting, efficient adherence.
The study's TPA characteristics are characteristic of hydrogels that are used in therapeutic uses. Similar outcomes were achieved in our previous study for insulin hydrogels based on Carbopol® UltrezTM 30 (referred to as U30) for three of the textural parameters evaluated. The adhesiveness values for G1-INS, G2-INS, and U30 are 0.25 mJ, 0.3 mJ, and 0.33 mJ, in that order. G1-INS: 1.579, G2-INS: 1.671, U30: 1.638 were the figures for cohesion, whilst G1-INS: 0.693, G2-INS: 0.716, U30: 0.696 are the values for elasticity. Hardness 1 (G1-INS: 0.053, G2-INS: 0.055, U30: 0.061) and toughness 2 (G1-INS: 0.057, G2-INS: 0.064, U30: 0.074) measurements showed differences. Based on carbohydrates® UltrezTM 30, the results indicate that hydrogels containing epinephrine have a higher hardness by 15% and 11% (Hardness 1) and 30% and 16% (Hardness 2), respectively.
A decreased quantity of hormones produced (47.4% vs. 53.36%) was influenced by the stronger matrix makeup and increased viscosity of the SepineoTM PHD 100-INS formulation (by 16%

at η = 30 s−1). The strongly linked together polymer structure affects the postponed discharge of API, as reported by Huang et al., Additionally, the chemical that is active may occasionally not emerge entirely. The Peppas-Sahlin pharmacokinetics model predicted the extended release of insulin from the proposed formulation in an experimental pharmacological accessibility research. Numerous writers have verified that the speed at which the medication releases from a hydrogel matrix is correlated with the carrier's inflammation, so it's contingent upon the physical composition of the polymer employed.

The studied hydrogels that are have a very elastic structure. Additionally, by creating crosslinks between the polymer molecules, the hydrophobic softener glycerol reduces the swelling ratio. Water's ability to operate as a drug diffusion channel is thus restricted. Using cellulose dialysis membrane Spectra/Por® 2/MWCO of 12–14 kDa, we demonstrated in our previous study that insulin release compared to hydrogel made from Carbopol® UltrezTM 10 (H1-INS) and Carbopol® UltrezTM 30 (H2-INS) happened corresponding to the Higuchi model, whereas from hydrogel based on methylcellulose (H3-INS) and glycerol ointment (H4-INS) was according to the Korsmeyer–Peppas model.

Even though Actrapid, a quick-acting glucose, was used, the hormone release profile was different. The H1-INS formulation took 3 hours to release 70% of the INS, the H2-INS formulation took 3 hours to release 65% of the INS, and the H3-INS and H4-INS formulations took 9 hours to release 75% of the INS with 6 hours to release 60% of the INS, correspondingly. Von Zuben et al suggested Hydroxyethyl cellulose. as a foundation for creating an insulin hydrogel formulation. Novolin R® is a short-acting insulin solution that's utilized. A synthetic cellulose acetate membrane served as the model membranes. Kinetics based on the Weibull model showed that 90.74% of INS were evacuated after 4 hours. The release of insulin (HuminsulinTM) from chitosan (CS) was investigated by Agrawal et al. [66].1% CS, 4% PVA; 2% CS, 3% PVA; 3% CS, 2% PVA; 4% CS, 1% PVA; and 5% CS, 0% PVA polymer based on polyvinyl alcohol, also known as (PVA). Within six hours, 60–90% of the hormone was released through the egg's membranes in vitro. The findings of four distinct investigations indicate that the characteristics of the hydrogel matrices utilized have a major influence on the quantity of insulin released and the rate at which this process happens. However, further study is necessary to show that the insulin release profile is independent of the type of business insulin preparation employed.

Since the growth hormone that would be discharged through the hydrogel has already low amounts, it may be hypothesized that the IRS (insulin receptor substrate) receptors' affinity for insulin enables the production of cellular responses. The normal growth and operation of the skin are influenced by the insulin action mediated by IRS-1 and IRS-2 under physiological settings. The brief half-life (2.5–5 minutes) of topical insulin administration is a drawback. While avoiding the necessity for repeated administration of the medication, this issue is resolved by the hydrogel's sustained absorption of insulin.

The Strat-M® membrane is the membrane of choice for the in vitro investigation of insulin pharmaceutical availability from hydrogels. Its application in studies of API release from a semi-solid drug formulation has significantly increased recently, according to a review of the literature. Furthermore, the use of synthetic membranes in drug release experiments is advised by the European Medicines Agency. Without lot-to-lot fluctuation, the Strat-M® membrane was found to be a successful substitute to both animal and human skin. It eliminates the moral dilemmas raised by using animals in preclinical research while closely resembling the multilayered structure of natural skin. It is possible to permeabilize hydrophilic and lipophilic molecules with the Strat-M® membrane.

This study contributes to the ongoing effort to identify hormone topical delivery methods that are both secure and efficient. From a pharmacy prescription room standpoint, the suggested

insulin formulation can be made for a particular patient who has a dry wound (occlusive action) or a wet and leaking wound (absorption of excess fluid, cooling). Materials that are more sophisticated and preparations, such as sponge dressing, electrospun polymeric fibers, transdermal drug delivery systems, and nano-drug delivery structures, are also being researched for the long-term delivery of bioactive insulin. Li et al., for instance, studied the use of insulin in silk fibroin dressings. In just 28 days, 90.7% of the hormone was extracted from the formulation.

Insulin-delivering chitosan nanoparticles were coated over electrospun poly (ε-caprolactone)/Collagen in a work by Ehterami et al. Within 14 days, 64.36% of the insulin in the formulation had been released overall. It is crucial to remember that maintaining the sterility of the prepared product is one of the main obstacles in the development of insulin formulation technology. Diabetes is susceptible to changes in pressure, temperature, and radiation. The hormone's inactivation is impacted by the sterilizing techniques currently in use, which could cause the hydrogel to lose its viscosity.

CONCLUSION

The development of a secure carrier that can successfully carry the hormone to the wound bed is a limiting factor for topical treatment with insulin. Preformulation investigations show that the new formulations offer extended release of insulin and are stable. The ideal application features of the insulin hydrogels based on SepineoTM P 600 and SepineoTM PHD 100 are demonstrated.

The development of a secure carrier that can successfully carry the hormone to the wound bed is a limiting factor for topical treatment with insulin. Preformulation investigations show that the new formulations offer extended release of insulin & are sustainable. The ideal usage characteristics of the insulin hydrogels based on SepineoTM P 600 and SepineoTM PHD 100 are demonstrated. Hydrogels have seen some promising advancement in recent years. To satisfy the various needs of daily living, however, further knowledge on the logical control of the hydrogels' structure and properties is needed.

The synthesis and application of hydrogels are growing increasingly sophisticated over time. Some drawbacks were revealed by hydrogels, including exhaustion and cracking under prolonged load and prolonged exposure to harsh climatic conditions. These restrictions are ascribed to the hydrogel network's architecture and chemical makeup. Double connected connections can be created to get around these restrictions.

Low conductance might occasionally present an unforeseen difficulty when using hydrogels in real-time applications. Because conducting elements like nanofillers have a higher surface area and work in concert with other components to produce hybrid hydrogel networks, they can reinforce the conductivity, mechanical strength, and optoelectronic properties of hydrogels. Conducting polymer compounds, metal oxide, sulfide, phosphate, carbon nanotubes, carbon dots, and nanotechnology are examples of nanofillers that are utilized to increase conductivity. Percolation, also theory states that hydrogels can be combined with specific electroactive elements, like nanofillers, to create flexible electrochemical devices. To create flexible devices, nevertheless, the ratio of hydrogels that are to electroactive materials needs to be taken into account. Furthermore, more precisely, nanofillers in terms of channel interaction, ionic diffusion, and stability enhance the porous networks of hydrogel electrolytes during cycling.

FUNDING

Nil

CONFLICT OF INTEREST

None

REFERENCES:

1. Ostróżka-Cieślik A, Przybyła M, Wójcik W, Birówka K, Majczyna M, Dolińska B. Review of Research in Developing Hydrogels with Insulin to Promote Wound Healing. InMedical Sciences Forum 2023 Mar 29 (Vol. 21, No. 1, p. 17). MDPI.
2. Yang P, Wang X, Wang D, Shi Y, Zhang M, Yu T, Liu D, Gao M, Zhang X, Liu Y. Topical insulin application accelerates diabetic wound healing by promoting anti-inflammatory macrophage polarization. Journal of cell science. 2020 Oct 1;133(19):jcs235838.
3. Wang J, Xu J. Effects of topical insulin on wound healing: a review of animal and human evidences. Diabetes, Metabolic Syndrome and Obesity. 2020 Mar 13:719-27.
4. Sun S, Zhang L, Liu J, Li H. [Retracted] Insulin Topical Application for Wound Healing in Nondiabetic Patients. Computational and mathematical methods in medicine. 2021;2021(1):9785466.
5. Chen X, Liu Y, Zhang X. Topical insulin application improves healing by regulating the wound inflammatory response. Wound Repair and Regeneration. 2012 May;20(3):425-34.
6. Nathan C. Neutrophils and immunity: challenges and opportunities. Nature reviews immunology. 2006 Mar 1;6(3):173-82.
7. Li C, Yu T, Liu Y, Chen X, Zhang X. Topical application of insulin accelerates vessmaturation of wounds by regulating angiopoietin-1 in diabetic mice. The international journal of lower extremity wounds. 2015 Dec;14(4):353-64.
8. Dhall S, Silva JP, Liu Y, Hrynyk M, Garcia M, Chan A, Lyubovitsky J, Neufeld RJ, Martins-Green M. Release of insulin from PLGA–alginate dressing stimulates regenerative healing of burn wounds in rats. Clinical Science. 2015 Dec 1;129(12):1115-29.
9. Ostróżka-Cieślik A. The potential of pharmaceutical hydrogels in the formulation of topical administration hormone drugs. Polymers. 2022 Aug 14;14(16):3307.
10. Atiyeh BS, Ioannovich J, Al-Amm CA, El-Musa KA. Management of acute and chronic open wounds: the importance of moist environment in optimal wound healing. Current pharmaceutical biotechnology. 2002 Sep 1;3(3):179-95.
11. Li S, Dong S, Xu W, Tu S, Yan L, Zhao C, Ding J, Chen X. Antibacterial hydrogels. Advanced science. 2018 May;5(5):1700527.
12. Zhao Z, Xie H, An S, Jiang Y. The relationship between oxygen permeability and phase separation morphology of the multicomponent silicone hydrogels. The Journal of Physical Chemistry B. 2014 Dec 18;118(50):14640-7.
13. MADDEN MR, NOLAN E, FINKELSTEIN JL, YURT RW, SMELAND J, GOODWIN CW, HEFTON J, STAIANO-COICO LI. Comparison of an occlusive and a semi-occlusive dressing and the effect of the wound exudate upon keratinocyte proliferation. Journal of Trauma and Acute Care Surgery. 1989 Jul 1;29(7):924-31.
14. Svensjö T, Pomahac B, Yao F, Slama J, Eriksson E. Accelerated healing of full-thickness skin wounds in a wet environment. Plastic and reconstructive surgery. 2000 Sep 1;106(3):602-12.
15. Burek M, Waśkiewicz S, Awietjan S, Wandzik I. Thermoresponsive hydrogels with covalently incorporated trehalose as protein carriers. Reactive and Functional Polymers. 2017 Oct 1;119:105-15.
16. Maggioni D, Cimicata A, Pratico A, Villa R, Bianchi FM, Busoli Badiale S, Angelinetta C. A preliminary clinical evaluation of a topical product for reducing slight rosacea imperfections. Clinical, Cosmetic and Investigational Dermatology. 2020 Apr 21:299-308.
17. Bonacucina G, Cespi M, Palmieri GF. Characterization and stability of emulsion gels based on acrylamide/sodium acryloyldimethyl taurate copolymer. Aaps Pharmscitech. 2009 Jun;10:368-75.

18. Khalil EA, Majid SA, Suaifan GA, Al-Akayleh FT, Sallam AS. Physicochemical characterization of emulgel formulated with SepineoP 600, SepineoSE 68 and cosolvent mixtures. Pharmaceutical development and technology. 2016 Jul 3;21(5):519-27.
19. Khan BA, Ahmad S, Khan MK, Hosny KM, Bukhary DM, Iqbal H, Murshid SS, Halwani AA, Alissa M, Menaa F. Fabrication and characterizations of pharmaceutical emulgel co-loaded with naproxen-eugenol for improved analgesic and anti-inflammatory effects. Gels. 2022 Sep 22;8(10):608.
20. Badruddoza AZ, Yeoh T, Shah JC. API-polymer interactions in Sepineo P600 based topical gel formulation-impact on rheology. International Journal of Pharmaceutics. 2022 Jun 10;621:121824.
21. Eudralex GM. The Rules Governing Medicinal Products in the European Union–Volume 4-EU Guidelines to Good Manufacturing Practice–Medicinal Products for Human and Veterinary Use–Annex 1–Manufacture of Sterile Medicinal Products. European Commission: Brussels, Belgium. 2008 Nov 25.
22. Yoshida R, Kaneko Y, Sakai K, Okano T, Sakurai Y, Bae YH, Kim SW. Positive thermosensitive pulsatile drug release using negative thermosensitive hydrogels. Journal of Controlled release. 1994 Nov 1;32(1):97-102.
23. BROWN F, GRIFFITHS E, HORAUD F, PETRICCIANI JC. Quality of biotechnological products: Stability testing of biotechnological. Developments in biological standardization. 1998(93):211-9.
24. Ostróżka-Cieślik A, Maciążek-Jurczyk M, Pożycka J, Dolińska B. Pre-formulation studies: Physicochemical characteristics and in vitro release kinetics of insulin from selected hydrogels. Pharmaceutics. 2021 Aug 6;13(8):1215.
25. Cardia MC, Carta AR, Caboni P, Maccioni AM, Erbì S, Boi L, Meloni MC, Lai F, Sinico C. Trimethyl chitosan hydrogel nanoparticles for progesterone delivery in neurodegenerative disorders. Pharmaceutics. 2019 Dec 6;11(12):657.
26. A. Mumuni M, E. Calister U, Aminu N, C. Franklin K, Musiliu Oluseun A, Usman M, Abdulmumuni B, Y. James O, C. Ofokansi K, A. Anthony A, C. Ibezim E. Mucin-grafted polyethylene glycol microparticles enable oral insulin delivery for improving diabetic treatment. Applied sciences. 2020 Apr 11;10(8):2649.
27. Zhang Y, Huo M, Zhou J, Zou A, Li W, Yao C, Xie S. DDSolver: an add-in program for modeling and comparison of drug dissolution profiles. The AAPS journal. 2010 Sep;12:263-71.
28. Ostróżka-Cieślik A, Maciążek-Jurczyk M, Pożycka J, Dolińska B. Pre-formulation studies: Physicochemical characteristics and in vitro release kinetics of insulin from selected hydrogels. Pharmaceutics. 2021 Aug 6;13(8):1215.
29. Neupane R, Boddu SH, Renukuntla J, Babu RJ, Tiwari AK. Alternatives to biological skin in permeation studies: Current trends and possibilities. Pharmaceutics. 2020 Feb;12(2):152.
30. Ponmozhi J, Dhinakaran S, Varga-Medveczky Z, Fónagy K, Bors LA, Iván K, Erdő F. Development of skin-on-a-chip platforms for different utilizations: Factors to be considered. Micromachines. 2021 Mar 10;12(3):294.
31. Joshi V, Brewster D, Colonero P. In vitro diffusion studies in transdermal research: A synthetic membrane model in place of human skin. Drug Dev. Deliv. 2012 Mar;12:40-2.
32. Kaur L, Singh K, Paul S, Singh S, Singh S, Jain SK. A mechanistic study to determine the structural similarities between artificial membrane Strat-M™ and biological membranes and its application to carry out skin permeation study of amphotericin B nanoformulations. AAPS pharmscitech. 2018 May;19:1606-24.

33. Haq A, Goodyear B, Ameen D, Joshi V, Michniak-Kohn B. Strat-M® synthetic membrane: Permeability comparison to human cadaver skin. International journal of pharmaceutics. 2018 Aug 25;547(1-2):432-7.
34. Uchida T, Kadhum WR, Kanai S, Todo H, Oshizaka T, Sugibayashi K. Prediction of skin permeation by chemical compounds using the artificial membrane, Strat-M™. European Journal of Pharmaceutical Sciences. 2015 Jan 25;67:113-8.
35. Milanowski B, Wosicka-Frąckowiak H, Główka E, Sosnowska M, Woźny S, Stachowiak F, Suchenek A, Wilkowski D. Optimization and evaluation of the in vitro permeation parameters of topical products with non-steroidal anti-inflammatory drugs through Strat-M® membrane. Pharmaceutics. 2021 Aug 20;13(8):1305.
36. Arce FJ, Asano N, See GL, Itakura S, Todo H, Sugibayashi K. Usefulness of artificial membrane, Strat-M®, in the assessment of drug permeation from complex vehicles in finite dose conditions. Pharmaceutics. 2020 Feb 19;12(2):173.
37. R. Varges P, M. Costa C, S. Fonseca B, F. Naccache M, de Souza Mendes PR. Rheological characterization of carbopol® dispersions in water and in water/glycerol solutions. Fluids. 2019 Jan 4;4(1):3.
38. Zakaria AS, Afifi SA, Elkhodairy KA. Newly developed topical cefotaxime sodium hydrogels: antibacterial activity and in vivo evaluation. BioMed research international. 2016;2016(1):6525163.
39. European Medicines Agency. Guideline on the investigation of bioequivalence. Committee for Medicinal Products for Human Use (CHMP). 2010 Oct 3.
40. Food FD. Drug Administration. Guidance for Industry: Bioavailability and Bioequivalence Studies Submitted in NDAs or INDs—General Considerations. Draft Guidance. US Department of Health and Human Services. Center for Drug Evaluation and Research (CDER). 2014.
41. Alatzoglou FE, Vassaki M, Nirgianaki K, Tripodianos E, Turhanen P, Demadis KD, Papathanasiou KE. Surface-modified silica hydrogels for the programmable release of bisphosphonate anti-osteoporosis drugs: The case of etidronate. Materials. 2023 Apr 26;16(9):3379.
42. Bruschi ML. Strategies to modify the drug release from pharmaceutical systems. Woodhead Publishing; 2015 Jun 16.
43. Bharti. AD., Keservani R. K., Sharma. AK., Kesharwani Rajesh, K., & Mohammed GH., 2012. Formulation and in vitro characterization of metoprolol tartrate loaded chitosan microspheres. Ars Pharmaceutica, (53-3), 13-18.
44. Keservani, R. K., & Sharma, A. K., 2018. Nanoemulsions: Formulation Insights, Applications, and Recent Advances. Nanodispersions for Drug Delivery, 71-96.
45. Keservani, R. K., Sharma, A. K., & Ramteke, S., 2010. Novel vesicular approach for topical delivery of baclofen via niosomes. Lat Am J Pharm, 29, 1364-1370.
46. Keservani, Raj K. and Gautam, Surya Prakash., 2020. Formulation and evaluation of baclofen liposome vesicles using lecithin, ARS Pharmaceutica, 61 (3), 175-180.
47. Keservani, Raj K. and Gautam, Surya Prakash., 2022. Skeletal muscle relaxant activity of different formulation of span 60 niosome, ARS Pharmaceutica, 63 91, 32-44.
48. Khairnar, S. J., Ahire, E. D., Jagtap, M. R., Surana, K. R., Kshirsagar, S. J., & Keservani, R. K., 2024. Management and Prevention of Diseases by Flavonoids. In Advances in Flavonoids for Human Health and Prevention of Diseases (pp. 47-71). Apple Academic Press.
49. Khulbe, P., Singh, D. M., Aman, A., Ahire, E. D., & Keservani, R. K., 2023. The emergence of nanocarriers in the management of diseases and disorders. Community Acquired Infection, 10.

50. Sharma, A. K., Keservani, R. K., & Kesharwani, R. K. (Eds.). 2018. Nanobiomaterials: applications in drug delivery. CRC Press.
51. Keservani, R. K., Sharma, A. K., &Kesharwani, R. K. (Eds.). 2019. Nanocarriers for brain targeting: principles and applications. CRC Press.
52. Sharma, V. K., Koka, A., Yadav, J., Sharma, A. K., & Keservani, R. K., 2016. Self-micro emulsifying drug delivery systems: A strategy to improve oral bioavailability.
53. Behera, J., Keservani, R. K., Yadav, A., Tripathi, M., & Chadoker, A., 2010. Methoxsalen loaded chitosan coated microemulsion for effective treatment of psoriasis. International Journal of Drug Delivery, 2(2).
54. Keservani R K, Praveena R, Pawar S V, Devi R, Tiwari S W, Patel V K, Guru P V., Uddin N., Insilico Efficacy of Quercetin As A Therapeutic Potential For Colorectal Cancer, Afr.J.Bio.Sc. 6(Si2) (2024), 3322-3329.
55. Komu, L.T., Dharmalingam, S., Kumar, S., Keservani, R. K., Joshi, N., Rajesh, K.S., Sakarkar, S.N., Vippamakula, S., Mehta, F.F., 2023. Formulation Development and Evaluation of Thermo-Responsive BIOGELS: Nose to Brain Approach, JCHR, 13(3), 839-844.
56. Nataraja, B. T., Dharmalingam, S., Keservani, R. K, Gupta, A. K, Pulipati, S, Bhor R. J., Singh, A.K., Singh, S.K., Boddeda, B., 2023. Silver Nanoparticle Formulation Development and Evaluation: In-Vitro Anti-Microbial Evidences, JCHR, 13(3), 1389-1396.
57. Jaiswal, M., Gupta, A.A., Singh, AK., Keservani, R.K., Kachave R.N., Amrutkar S.V., 2023. Simultaneous Estimation of Olmesartan Medoxomil and Hydrochlorothiazide in Bulk Drug and its Tablet Formulation by RP-HPLC, Eur. Chem. Bull., 12 (11), 74 – 86.
58. Gautam, S. P., Keservani, R. K., Gautam, T., Gupta, A. K., & Kumar Sharma, A., 2015. An alternative approach for acetylation of amine terminated polyamidoamine (PAMAM) dendrimer. Ars Pharm. 56(3): 155-159.
59. Khambete, H., Keservani, R. K., Kesharwani, R. K., Jain, N. P., & Jain, C. P., 2016. Emerging trends of nanobiomaterials in hard tissue engineering. Nanobiomaterials in Hard Tissue Engineering, 63-101.
60. Keservani, R. K., Bandopadhyay, S., Bandyopadhyay, N., & Sharma, A. K., 2020. Design and fabrication of transdermal/skin drug-delivery system. In Drug Delivery Systems (pp. 131-178). Academic Press.