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Hydrogel Based on Poly (N-isopropylacrylamide) and Its Use as a Delivery System for Anti-Diabetic Medications: How It Swells and How It Works

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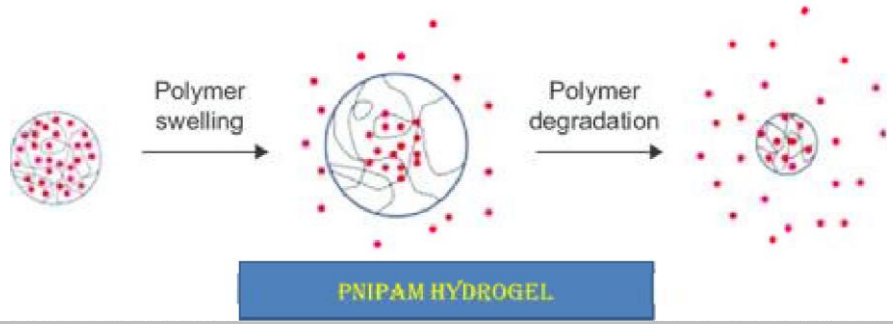
ABSTRACT

We directly weigh the hydrogel containing charantin from poly (N-isopropyl acrylamide) PNIPAM before soaking it in a fluid/drug solution in a temperature-controlled water bath to achieve the swelling kinetics. The research was conducted with the intention of using the findings as a delivery mechanism for drugs used to treat diabetes. The bitter melon fruit and leaves were subjected to a pH control process in order to extract charantin. An increase in the lower critical solution temperature (LCST) from 32-43 ℃ was seen in distilled water when the acrylamide concentration was varied between 3 and 12% during PNIPAM synthesis, suggesting a direct link. Hydrogel LCST was shown to decrease as the polarity of the solution increased, as demonstrated by the effects of switching between pure water, insulin, and charantin solutions. The hydrogels N1, N2, N3, and N4 had charantin loadings of 42.51, 44.57, 43.55, and 44.61 µg/mL, respectively, when immersed in a charantin solution with a concentration of 52.61 µg/mL. There is a physical connection between the hydrogel matrix and the charantin molecules, according to the hydrogels' FTIR spectroscopy characterization. Hydrogels exhibited diffusivity values between 1.48 x 10^4 and 5.08 x 10^4 M2/s, and release exponents of > 0.5 suggested non-Fickian diffusional release.

GRAPHICAL ABSTRACT



Keywords:
Charantin,
N-isopropyl acrylamide PNIPAM-based hydrogel Swelling kinetics

Introduction

Among the many benefits of controlled drug delivery systems are the following: better patient compliance, optimal medication use, reduced dosage required for administration, and maintenance of drug levels within the target range. Controlled drug delivery systems have previously been defined as drug formulations that are ready for any kind of administration and whose duration of action is entirely dictated by the delivery system. At the same time, these medications may have their release precisely regulated and directed to a particular organ or area of the body. When an active agent or medicine is meticulously mixed with a polymer—whether it be natural or synthetic—in a way that allows for the agent's controlled release from the material, this process is called controlled drug delivery. This active agent's release might be continuous for a long time, resulting in a long-term cyclicity, or it could be initiated by environmental factors or other external events. In a particular drug delivery system, the release of the drug may happen by any one of three mechanisms: diffusion, degradation, or swelling followed by diffusion. 1A hydrogel is a chemically or permanently stabilized gel that has a network of molecules bound together by covalent bonds. They are composed of biocompatible hydrophilic networks that may be built from both natural and synthetic materials, and they maintain the solid properties2. Poly (amidomines), hyaluronic acid, calcium alginate, photocross-linkable polymers, poly (L-lactide)-poly (ethylene oxide)-poly (l-lantide), gellan gum, and so on are only a few examples. 3 Because of their high water-holding capacity, hydrogels may expand and contract in response to

changes in temperature, ionic concentration, pH, or targeted antigens, among other physical and biological factors. 4, 5 Hydrogels' injectability, biodegradability, low cytotoxicity, mucus-adhesiveness, and turntable bio-adhesive characteristics make them very promising for a wide range of applications. Materials with improved properties for targeted medication delivery and tissue regeneration are therefore more appealing. 6-Charantin, a common cucurbitane-type triterpenoid in medicinal chemistry, may have anti-diabetic effects as a component. 7 Sitosterylglucoside and stigmasterylglucoside are the two main ingredients. Results showed that charantin has promise as a diabetic medication and a possible alternative to current options. 8 Using a high-performance thin-layer chromatographic technique, the amount of charantinha extracted from bitter melon fruits was measured. 9 Two of charantin's components cause noticeable shifts in blood sugar levels. This suggests that charantin could have additional active ingredients with hypoglycemic effects in diabetics. 10 Drug delivery systems based on hydrogels of poly (N-isopropyl acrylamide) and poly(N-isopropyl acrylamide) have been used in the treatment of diabetes. Microgels impregnated with insulin have also been created using this method. A dried polymer gel was rehydrated in a solution containing the appropriate ingredient to be loaded, and the hydrogel's network encased the insulin. The old encapsulation approach included pre-inflated gels that were then brought into contact with the substance to be loaded; however, this "breathing in" technique proved to be more effective at encapsulating the insulin. In addition, it is vital to highlight that the phases of insulin release are functions of heat changes if the PNIPAM gel is to be employed in future drug delivery systems. A different research generated core-shell microgels using PNIPAM (a biodegradable polymer) as the core and PNIPAM (a phenylboronic acid)–conjugated polymer) as the shell. 11 According to the paper, the precursor poly (N-isopropyl acrylamide-co-acrylic acid) gel shells allowed the degraded polymer segments to flow readily out of water when the temperature was room temperature.

On the other hand, the collapsed structure of the PBA-modified PNIPAM-PBA nanoshells allowed them to retain a majority of the deteriorated core polymer chains under the same settings. A way to regulate its permeability by temperature and pH was found by raising the swelling degree of the PNIPAM-PBA shell when the temperature was lowered or the pH was raised. Charantin has been loaded into PNIPAM from bitter melon fruits and leaves in this research. Furthermore, for real-world use, we have ascertained the hydrogels' swelling and release characteristics when loaded with charantin.

Materials and Method

Sample collection

The leaves and fruits of bitter melon *(Momordicacharantin)* were collected fromSangere in Gerei Local Government Area, Adamawa State, Nigeria, and were identified by a plant taxonomist at the Department of Botany, ModibboAdama University of Technology, Yola, Adamawa State, Nigeria.

The leaves and fruits were brought to the laboratory and rinsed with water to remove the soil particles.

Sample preparation

The cleaning of the leaves and fruits of the bitter melon were done using distilled water before cutting them into small pieces and then oven dried at 50 °C for 24 hours. After which the dried sample waspulverized into a fine powder using a grinding machine, the sample was then stored at a temperature of 4 °C until it was ready for use. The leave fragments were further ground using mortar and pestle. The dry weight (W/W) was obtained bydrying the subsample at a temperature of 50 °C and then reweighed.

Extraction and purification of charantin

After being immersed in a water/ethanol solvent (50:50 v/v) for 72 hours, about 100 g of the fruit samples and leaves were removed. A buffer solution was used to change the mixes' pH levels. To remove the water/ethanol solvent, the extract was cooled in a coil submerged in water, collected, and then evaporated under vacuum. The charantin was isolated by passing the solutions over a silica gel column; further identification was carried out using a High-Performance Liquid Chromatography system using insulin standards. A UV-visible spectrophotometer operating at 734 nm was used to ascertain the charantin content.

The procedure outlined in the literature was used to purify the crude extract. The crude extract was supplemented with 5 mL of a 50:50 (v/v) mixture of methanol and water. After 15 minutes of sonication, the mixture was centrifuged at 8500 rpm to remove the precipitate and collect the liquid above. Next, 5 mL of a combination of 70:30 (v/v) methanol and water was added to the precipitate. The mixture was sonicated and centrifuged once more. Three milliliters of hexane was added to the precipitates from this stage, and the process was repeated. After this stage, the precipitate was redissolved in 200 mL of a chloroform-methanol combination that was 1:1 (v/v). The volume was then adjusted with methanol to the desired level, with 1 mL volume for extracts produced using a pressure liquid extractor (PLE) and 2 mL volume for those obtained using Soxhlet extraction. An American company called Millipore used a 0.45 m nylon membrane filter to filter the cleaned solutions. The analysis required the collection of all precipitates.

Preparation of PNIPAM-based hydrogels

Materials used in the synthesis of PNIPAM gelwere blended into powder.Free radical polymerization was used in the preparation of PNIPAM-based hydrogels were preparedby. The concentration of ammonium persulfate (APS) was 1.91 mol% while that of methylenebisacrylamide (MBA) 1.15 mol%.The equation below shows the amount of cross-linkedmethylenebisacrylamide (MBA) obtained.

$$\frac{\left(\frac{M_{MBA}}{M_{w(MBA)}}\right)}{\left(\frac{M_{NIPA}}{M_{w(NIPA)}}\right)} x\ 100\% = mol\ \% \quad [1]$$

From the equation, $\mathbf{M_{w\ (NIPA)}}$ = molecular weight of the monomer, NIPA $\left(113\ \frac{g}{mol}\right)$, $\mathbf{M_{NIPA}}$ = the initial amount of the monomer (0.87 g), $\mathbf{M_{w\ (MBA)}}$ = the molecular weight of the

cross-linker, **MBA** $\left(154\ \frac{g}{mol}\right)$ and M_{MBA} is the unknown amount of MBA (g) to be used in the gel polymerization.

The mole% of MBA was 1.15 %. The calculated amount of the MBA based on the 0.87 g of N-isopropylacrylamide (NIPA) monomer from equation (1) was found to be 0.0136 g. Furthermore, the amount of APS was obtainedby replacing MBA in equation (1) with APS. PNIPAM-based homopolymer, denoted by gel code A, was prepared by mixing 0.87 g of NIPA, 0.0136 g of MBA, and 0.0335 g of APS. The samples were then dissolved using 7.8 mL of distilled water, before been agitated vigorously until a homogenous mixture is obtained at 4-9 ˚C. The mixing process was exothermic hence the solution was then immersed in ice and sonicated, while nitrogen gas was bubbled through for 20 minutes at 10 bars pressure to remove all dissolved oxygen. The homopolymerwas initiated with 15 µL of N, N, N′, N′-Tetramethylethylenediamine (TEMED). The solution was then gentlyswirled for 5 seconds.

Free radical polymerization was then stopped by pouring the solution into cylindrical molds with a diameter of 5 mm and then opening the molds. To make the polymerized gels stronger, the samples in the cylindrical molds were submerged in a water bath maintained at a temperature of 24˚C for a duration of 12 hours. To ensure that no chemical residue remained, the samples were rinsed with deionized water ten times.

After that, the wet gels were sliced into cylindrical and disc-shaped samples with a 5 mm height and diameter. The next step was to immerse the samples in deionized water, which was changed regularly for a duration of two weeks. After being extracted from the deionized water, the samples were dried in a controlled laboratory setting at 29˚C to eliminate any remaining moisture.

PNIPAM-based co-polymer hydrogels were also made using the same process as the PNIPAM homo-polymer. Nevertheless, copolymer gels were formed by copolymerizing the co-monomer species Acrylamide (AM) and Butyl Methylacrylate (BMA) with PNIPAM. When starting samples of PNIPAM co-polymer hydrogels with 5 or 10 mol% of Butyl Methylacrylate (BMA), respectively, 5 and 10 µL of Tetramethylethylenediamine (TEMED) were used. On the other hand, when starting samples with 5, 10, or 15 mol% of Acrylamide (AM), 20, 30, or 40 µL of TEMED were used. The purpose of the controlled addition of TEMED was to make the hydrogels more transparent by lowering their turbidity.

Scanning electron microscopy was used to study the gel microstructures, as the transport properties of traditional PNIPAM-based gels are largely influenced by the morphologies of the gels' interior matrices.

Table 1: Materials Used for the Gel Preparation and Their corresponding functions in Gel Polymerization.

Gel materials and the grade of reagent	Active functions in Polymerization of PNIPAM-based Gel
N-Isopropylacrylamide (NIPA), 97 %	Monomer
N,N,N′,N′-Tetramethylethylenediamine (TEMED), 99 %	Catalytic agent alters polymerization rate of used with APS
N,N′-Methylene bisacrylamide (MBA), 99 %	Cross-linker
Ammonium persulfate (APS), 98 %	initiating radical
Acrylamide (AM), 99.8 %	Hydrophilic Compound
Butyl Methylacrylate, 98 %	Hydrophobic Compound

Table 2. The Composition of the Gel Formed is as Presented in table 3.3.1 and it's Configuration.

Gel Code	P(NIPA) (mol%)	BMA (mol%)	AM (mol%)	APS (g)	MBA (mg)	TEMED (µL)
N1	100	-	-	0.034	0.014	5
N2	97	-	3	0.034	0.014	10
N3	94	10	6	0.034	0.014	15
N4	91	10	9	0.034	0.014	5
N5	88	5	12	0.034	0.014	10
N6	97	-	3	0.034	0.014	15
N7	94	5	6	0.034	0.014	5
N8	91	10	9	0.034	0.014	10

P(NIPA)- Isopropylacrylamide, Am- Acrylamide, BMA- Butylacrylamide, APS Ammonium persulfate, TEMED- N,N, N′,N′-tetra Methylehylenediame.

Lower critical solution temperature (LCST) determination

Modulated differential scanning calorimeter (DSC)-TA Instruments 2920 was used in obtaining the lower critical solution temperature (LCST) values of the gels.To attain equilibrium, Gel samples were first immersed in their respective media such as charantin solution and de-ionized water for a minimum of 24 hours. To simulate release processes and charantin loading, charantin-loaded gels been released and taken after 48 hours of in vitro studies, while the control gels were first immersed in de-ionized water before been transferred to phosphate buffer solution (PBS). Using deionized water heating at 38 °C/min and temperature ranging from 15 to 55 °C under a dry nitrogen atmosphere (flow rate: 40 mL/min) thermal analyses of these gels were carried.All statistical analyses were conducted using Sigma Plot for Windows Version 2.03 (SPSS). Each experiment was carried out at least two more times to attain a higher degree of precision and accuracy.

Charantin loading

The vacuum-dried gels were immersed in a charantin solution for 4 days at a temperature of4 ºC and at a fixed concentration. The studies show the maximum loading of charantin within 4 days. The total charantin loaded was determined by the mass change of charantin in the solution before and after loading. Each loading experiment was carried out in triplicates. The charantin content in the loading solution was analyzed using high-performance liquid chromatography (HPLC, Waters 2690D). A Zorbax GF250 column (4.6 mm 25 cm, Agilent) was used as the separation column for

Drug/fluid concentrations

Samplesof BB and Charantin were each accurately weighed to 0.1g. This 0.1 g ofcharantinwas at first dissolved with 2 mL methanol (100%) giving a stock solution of 5 mg/mL. The solutionof 5 mg/mL was thenattuned with PBS to give a final concentration of 2.5 mg/mL. BB and Charantin were prepared using the same procedures. Nevertheless, the initial dissolution of BB and Charantin were done in 2 mL each of ethanol and DMSO, while 38 mL PBS was used to adjust each solution to obtain final concentrations of 2.5 mg/mL. The ratios of DMSO:PBS, methanol:PBS, and ethanol, DW, was each be 5:95 v/v% in the final drug/sample solutions.

Filtration of sample and drug solutions wascarried out twice using filter paper to remove fragments. A final concentration of 2.5 mg/mL (Charantin and BB) and DWwas used in the characterization of the swelling properties of the hydrogels.

Transport measurementand swelling ratios

Swelling ratios of PNIPAM-based hydrogels Charantin and BB were determined using dried hydrogels while the dissolution of solvents BB and Charantin were carried out using aqueous solvents. The swelling ratios of PNIPAM- based hydrogels were determined by immersing the hydrogels into different solutionsof 5 mL volume, i. e. 2.5 mg/mL of charantin and BB or 5 mL DW, to absorb drugs/fluids. The swelling and release activities of the loaded gels were then be observed. The average swelling ratios (SR_A) were obtained by soaking the PNIPAM -based hydrogels in DW, insulin,and BB at temperatures between 28 -48 ˚C.[13]
carrying out theBSA analysis, with the column temperature
 [3]
set at 25 ºC. The mobile phase flow rate (PBS, pH 7.4) was at
1.0 mL/min while the UV detection was set at 210 nm. For charantin analysis, a Symmetry 300 C_4 column (3.9 mm 15 cm, Waters) was employed. The column temperature was set at 25 ºC. 0.1% trifluroacetic acid (TFA) in water with 0.1% TFA in acetonitrile. Was used in preparing the mobile phases. The separation was performed in a gradient mode, in which the volume ratio of 0.1% TFA in water to 0.1%

TFA in acetonitrile was changed from 75:25 to 68:32 within 15 min. The flow rate of the mobile phase was 1.0 mL/min. The UV detection was set at 220 nm as describedabove.
The experimental protein loading was obtained from the equation below:

Experimental Drug% = $\frac{\text{Mass of Drug Loaded}}{\text{Mass of loaded drug + Mass of dry gel}}$ (2)

From Equation $\frac{m_t}{m_i} = 4\left(\frac{D}{\pi\delta^2}\right)t^n = kt^n$ is defined as the volume (mL) of charantin solution absorbed per mg of the dry gel during charantin loading, and C is the concentration of the charantin loading solution (mg/m).
Equation (2) was based on the following assumptions:

1. All the charantinwere dissolved in the water in the gel.
2. The charantin concentration in the gel was equal to that of the external charantin loading solution.
3. There was no charantin interaction with the polymer matrix.

From equation (3), $\mathbf{M_t}$ is the mass of the gel at time ***t*** while
$\mathbf{M_o}$ is the mass of the dried gel at time, $\mathbf{t = 0}$.
The fluid/drug uptake in the gels was obtained from the relative gel mass[14]

$$\text{[illegible]} = \text{[illegible]}/\text{[illegible]} \quad [4]$$

From equation (4), $\mathbf{M_s}$ is the swollen mass at a given temperature and $\mathbf{M_t}$ is the mass of the hydrogel at the time, ***t****.The* The equilibrium volume ratio was obtained from:

$$D_s = \frac{k\pi\delta^2}{4} \quad [5]$$

From equation (5), $\mathbf{D_o}$ and **D** represents diameters of the hydrogels before and after equilibrium swelling respectively.

Strain-induced in hydrogels due to swelling

Brannon-peppas and Peppashave given much explanation to the swelling dynamics of hydrogels. With resConcerningensional transport, the gel swelling is associated with the twinges in length. ε denotes strain, as it is induced in the gel as a result of swelling, is given by equation $D = D_o\exp\left(\frac{-E_a}{RT}\right)$ $\ln D = \ln D_o - \frac{E_a}{RT}$ (6) to be:

ε [6]

Where *l* denotes the length at any given time, *t* while l_o
denotes the initial length.

Drug/fluid release from pnipa-based hydrogels

Table 3(b): the percentage of charantin extracted from 100 g of leave and fruit sample of bitter melon.

Sample	% of Charantin Extracted
Fruit	4.05
Leaves	9.89

Peppas and his co-workers were able to establish an equation for drug releasemodellinmodelingich assumes dependent power law function of the form:the solvent. It was reported 9.65% in leaves and 3.21% from the fruits of Momordicacharantia which is lower than the ones obtained. The higher values of the charantin can be attributed to better separations by the use of a better centrifuge at 8500 revolutions per minutes for 15 minutes which was carried out under a controlled pH of 4.5 as the results of the extraction is shown in Table 3(a).

[7]

From equation (7), $\frac{m_t}{m_i}$ is the fraction fluid/drug release, the geometric constant of the release systemis thus represented as ***k*****,** fluid/drug release exponent as ***n***, depicting the release mechanism, is the initial mass of the swollen hydrogel prior to drug release, [illegible] is the mass of the hydrogel at time, **t** during drug elution δ is the thickness of the gel and *D* is the diffusion coefficient. Equation (7) is applicable to systems in which diffusion occurred within the polymeric networks. Constants *k* and *n* were gotten from the linear form to give;

$$\text{[illegible]}\ (\text{[illegible]}/\text{[illegible]}) = \text{[illegible]} + \text{[illegible]}\ln\text{[illegible]} \quad [8]$$

From the above of equation (8), the intercepts and slopes of the plots of ln (m_t/m_i) versus ln **t, k** and **n** were obtained respectively. The intercepts on theln(m_t/m_i) axis were equal to ln (k). The diffusion coefficients, D_s, were obtained from:

$$V_{eq} = \frac{\left(\frac{\pi D^2}{4}\right)}{\left(\frac{\pi D_s^2}{4}\right)} = \frac{D^2}{D_s^2} \quad [9]$$

Where***k*** denotes the geometric constant of the release system,***π***represents the mathematical constant reflecting the ratio of a circle circumference to its diameter while ***δ*** is the thickness of the gel. The diffusion coefficients were determined at temperatures ranging from 28 to 48 ˚C. Arrheniusequationwas used in the determination of the activation energies for the different gels. This gives;

[10a]

and

[10b]

In the two equations above, the diffusion coefficientis represented as ***D***, the diffusion constant at room temperature is denoted as $\frac{l-l_o}{l_o}$ ***D_o***, the universal gas constant as ***R***, temperature ***T*** while ***E_a*** is the gel activation energy. The activation energy for each sample was obtained from the slope of a linear plot *of* ln Dversus $T^{-1}(K^{-1})$.

Results and Discussion

Extraction of charantin

The result of the extraction of charantinis presented in

Table 3(a), which shows that charantin is higher in leaves sample which is 9.89% and lower in fruit of *Momordicacharantia* and has the extractable value of 4.05%. The extractability of charantin is very vital and in the preparation of diabetic drugs of different types. This is becauseintermolecular reactions occur higher temperature causes (dipole-dipole and hydrogen bonding) within the solvent to reduce, resulting in higher molecular motion and making the solute to dissolve easily in

The left sample is shown to have the highest amount of extractability of charantin using ethanol and these values of charantin are higher than previously presented and could be attributed to the centrifuge used that is responsible for total separation at 8500 revolutions per minute for 15 minutes. This extraction was done under a controlled pH of 4.5.

Purification of charantin

The high-performance liquid chromatography of charantin was carried out for both the fruit and leavea sample of charantin with the peak that grows and retains symmetry at
4.318 at a peak area of 1987. 62317 for the standard insulin where the sample is given in Table 4, the standard was used to plot a standard calibration curve to determine the concentration of charantin for both samples as given in Table 4.

Table 4: Retention time and a peak area of insulin leave and fruit sample of character from HPLC.

Sample	Retention time [Mins]	Area [MAμ*S]
Insulin	4.318	1987.6317
Leave	4.023	1475.0937
Fruit	4.291	1070. 7437

The percentage (%) concentration of charantin in the fruit and leave sample of Momordica*charantia* was found to be 56.90% and 76.73% respectively using the standard calibration curve in Figure 2.

The peak of insulin 4.205 was reported by Ansri*et al.* which agrees to the result obtained.These where the concentration of charantin in the left sample and fruit sample
were found to be 74.69 μg/mL and 52.61 μg/mL respectively.

Poly (N-isopropylacrylamide) hydrogel preparation

Preparation of poly (n-isopropylacrylamide) hydrogel was synthesized from the materials and their roles in gel polymerization as given in Table 1. This was achieved by varying the concentration of acrylamide from 3% to 12%.

Characterization of poly (N-isopropylacrylamide)

Lower critical solutions temperature of PNIPAM-based hydrogel (LCSTs)

The result for the determination lower critical solution temperature and the activation energy of PNIPAM is as presented in Table 5. The result of the LCST of PNIPAM-based hydrogels susuggestshata heat trigger mechanism can

be used in managing the release of drugs from the thermo- sensitive hydrogels such that the control of the LCST makes theapplication in drugs delivery systemsof PNIPAM–based hydrogels attractive.Table 4.0 summarizes the LCSTs of the P (NIPA)-based hydrogels. The LCSTs for a homopolymer
soaked in distilled water was 32.8 ℃ white 32 ℃, and 34.6 ℃ was reported.[17] Withinthecrease, in the concentration of acrylamide from 3% to 12% there is an increase in lower
critical solution temperature from 32–43 ℃.
It was found that when N1 was soaked in distilled water, insulin and Charantin, the LCSTs of the hydrogel was found to be 32.8 ℃, 34.6 ℃ and 36.0 ℃ in distilled water, insulin
solution and charantin solution respectively.

Table 5: Activation energy and the lower critical solution temperature of P (NIPA)

Gel	Activation Energy (KJ/mole)	Lower Critical Solution Temperature (℃)
N1	120±8	32±1.9
N2	164±7	36±1.7
N3	166±7	37±1.5
N4	289±16	43±1.1
N5	118±4	30±1.5

N1 = (100 mol% P (NIPA), N2 = (93% P NIPA-3% AM), N3 = (94% P NIPA – 6%AM), N4 = (88% P NIPA – 12%AM), N5 = (91% P NIPA – 9%AM).

The LCST of PNIPAM increases as the gel changes from one solution to another. It was found to be 32.8 ℃ in distilled water whereas the gel loaded with the charantin solution was
found to be 36.0 ℃ this shows that there is an interaction between the charantin and the hydrogel matrix. The value of the LCST of PNIPAM in insulin solution was found to be

34.6 ℃ which is lower than that of charantin this could be attributed their difference in molecular size and the polarity of the solution The effect of the three different solutions on LCSTs of PNIPA (N1 which is a homopolymer 100 mol % of PNIPA) is presentedin Table 6. However, charantin was found to increase the LCST of the hydrogel from 32.0 ℃ in distilled water, to 34.6 ℃ in insulin and 36.0 ℃ in charantin solution. Hence the incorporation of more hydrophobic or hydrophilic co-monomer will increase or decrease the LCSTs of the hydrogel as reported.[18] The LCST of the blank (PNIPAM) in distilled water shows agreement with those of Heskin and Guilletas well as Dhara and Chaheri.[19, 20] This can be related to the complexation or salting out of the gel with the loaded charantin increasing the hydrophilicity of the gel matrix. The result of the LCSTs of PNIPAM-based hydrogel suggests that the release of drugs from the thermo-sensitive gel is useful for application as for a drug delivery vehicle for an anti-diabetic agent. The hydrogel and the charantin interaction is responsible for the increase LCST as it is represented in Figure 3.

Table 6: Lower Critical Solution Temperature of P-NIPA Loaded in Some Solutions.

Gel	Distilled water(℃)	Insulin solution (℃)	Charantin solution (℃)
A	32.8	34.6	36.1
B	32.9	34.5	36.0
C	32.8	34.7	36.0

The fourier transformed infrared (FTIR) spectroscopy

The results of the Fourier transformed infrared (FTIR) Spectroscopy of the materials utilized in this study are stretching vibration, while 704.87 and 920.66 cm^{-1} are due to methylene sp^3 C-H bending vibration (i.e., α carbons).

For the fruit sample of *Momordicacharantia*, the sharp peaks between 2928.86 – 2866.31 cm^{-1}can be associated with the sp^3 C-H at the methylene linkage. presented in Figures 4 to 8.-1
The small and doublet peaks at 2367.31 cm^{-1} are due to For insulin, the broad absorption peak at 3385 cm are due to O-H of acids with overlapping C-H stretch, and C≡C stretching vibrations, 2123.93 cm^{-1} are due to symmetry the stretching absorption by C≡N, and the smallpeak at 1741.93 cm^{-1} is due to stretching vibrations of C=O.The peak at 1596.53 and 1655.50 cm^{-1} are due to C=C stretching vibration while the peaks between 1373.72 – 1451.93 $cm^{-1}$and 1158.62–250.95 cm^{-1} are due to sp^3 C-H bending vibration and C-O stretching vibration respectively.

For leave sample of *Momordicacharantia*, the peakat 3370.57 cm^{-1} is due to O-H stretching vibration with C-H overlapping. The peaksbetween 2868.14-2934.33 cm^{-1} are due to sp3 C-H stretching vibration. The peak at 1745.64 cm^{-1} is due to C=O stretching vibration while the peaks at 1651.11, 1455.35 and 1161.54 cm^{-1} are due to C=N stretching vibration, sp^3 C-H bending vibration and C-O stretching vibration, respectively.

The broadband at 3434.10 cm^{-1} is due to O-H (alcohol) stretching vibration, while that at 3199.96 is due to sp^2 C-H stretching vibration. The peak observed at 2942.44 cm^{-1} is due to sp^3 C-H stretching vibration,and the smallest peak at 2372.07 cm^{-1} is due to the stretching vibration of C≡N. The peak observed at 1611.83 cm^{-1} is due to C=O stretching vibration, the peaks between 1330.60-1447.11 cm^{-1} are due to sp^3 C-H bending vibration and peaks between 1047.26–1097.69 cm^{-1} are due to C-O stretching vibration. The peak observed at 683.96 is due to sp^2 C-H bending vibration.

The broadband stretching at 3435.86 cm^{-1} is due to O-H stretching, and that at 3200.76 is due to sp C-H stretching. The peak at 2939.16 cm^{-1} is due to sp^3 C-H stretching vibration, while the peaks between 2105.08–2370.57 $cm^{-1}$are due to C≡C stretching vibrations. The peak at 1607.93 cm^{-1} is due to C=O stretching vibration while the peaks between 1335.34-1443.91 cm^{-1} and 1048.86-1118.28 cm^{-1} are due to sp^3 C-H bending vibration and C-O stretching vibration, respectively.The peakat 600.00 cm^{-1} is due to sp^2 C-H bending vibration when the hydrogel was soaked for 48 hours in charantin solution.

Charantin loading

What you see in Table 7 is the end outcome of loading PNIPAM-based hydrogel with charantin. The hydrogels with the following charantin concentrations were identified. The overall concentration of charantin in the hydrogel loaded with N1, N2, N3, and N4 was 52.61 µg/mL.
Gel N4 had the greatest loading charantin. Possible causes include a low charantin loading ability (N1), a high level of interaction between the hydrogel matrix and the charantin molecules (high adsorption), and a high concentration of acrylamide (reduced pore space in the gel). Low charantin adsorption by the hydrogel may be due to the gel's wide pore space, which in turn is caused by inadequate interaction. The results of this investigation demonstrate that the hydrogel matrix and the charantin molecules do interact, contradicting the prior assumption made by Coulghet al. that there is no contact. 21

Table 7 shows that N4 has the highest loading ability whereas N1 has the lowest drug loading ability and it can be attributed to larger pore spaces of N1. The higher loading ability is as a result of higher cross-linking of the hydrogel. Hence,the higher the acrylamide concentration,the higher the charantin loading of a hydrogel.

Table 7: Summary of Charantin Loading of Hydrogel

Gel code	The concentration of charantin solution left (µg/mL.)	Concentration loading to the gel (µg/mL.)
N1	10.10	42.51
N2	8.04	44.57
N3	9.06	43.55
N4	8.00	44.61

Release of charantin

The release kinetics at 28 ℃, 32 ℃ and 34 ℃is presented in Figure 9 (a-c) and the kinetic release of charantin from PNIPAM shows that at 28 ℃, the hydrogel for N1, N2,
N3,and N4 are -0.70, -0.70, -0.49 and 0.46, respectively with
N1 and N2 having the lowest whereas N4 has the highest value and the negative sign signifies that as the time increase the concentration of charantin in the hydrogel decreases. At
32 ℃, N3 has the highest value,and N1 has the value of -0.49 and -0.69, respectively, whereas with a temperature increase up to 34 ℃, N3 has the highest valueof -0.68 and lowest value of -0.84.

From the result obtained from the kinetic release, it shows
that the 28 ℃, N3 and N4 < are less than –0.5 and at 32 ℃ only N3<0.5 but all other values are more significant than
0.5 which can be easily be approximated to unity.

At 28 ℃, for the hydrogel N1 between 16-24 hours, there is the high release of charantin and between 40-48 hours, the lowest release of the charantin loaded on hydrogel with the rate of change of concentration per unit time as - 0.67 µgmL^{-1}S^{-1}. The hydrogel N2 shows the rate of release at 0.742 µgmL/s and the highest release is between 16-24 hours with change in concentration From 32.23 µg/mL to 17.93 µg/mL and least releasewas observed between 40-48 hours. The gel N3 show the highest release rate between 24-32 hours unlike N1 and N2 which change in concentration was between 31.27-17.27 µg/mL and the lowest release was observed between 8-16 hours, Which corresponds to 40.43-37.67 µg/mL. N4 shows the highest release between 24-32 hours similar to that of N3 which shows changes in concentration
between 22.14-11.30 µg/mL. This was reflected that at 28℃, the higher the concentration of acrylamide, the longer the time it takes for charantin to be released.

The kinetic release at 32 ℃ are shown in Figure 9 (b). The hydrogel N1 shows the highest charantinrelease between 24- 32 hours with change in concentration between 25.89-7.39 µg/mL whereas the leastrelease of charantin from N1 was shown between 40-48 hours corresponding to 5.29-3.25 µg/mL change in concentration. For the gel N2, the highest release rate was between 16-24 hours and the lowest release at this particular temperature is observed between 0-8 hours which is between 43.55-42.59 µg/mL. The hydrogel N4 shows the highest release between 16-24 hours, whereas the lowest release of charantinwas observed between 40-48 hours which correspond to 4.45-4.28 µg/mL.

The kinetic release of charantin from PNIPAM at 34 ℃ is shown in Figure 9 (c). The hydrogel N1 shows the highestrelease between 24-32 hours and lowest release between 0-8 hours corresponding to 25.89-7.39 and 42.51-
40.37 µg/mL, respectively. The hydrogel N2 shows its highestrelease between 40-36-22-03 µg/mL whereas the slowest release can be observed between 0-8 hours which has a change in concentration between 44.57-43.83 µg/mL. The highest release of charantin from PNIPAM N3 is observed between 24-32 hours,andthe lowest is observed between 40-
48 hours corresponding to 34.38-19.98 and 11.98-11.06 µg/mL, respectively which is unlike the one observedfor N4 which is at 16-24 hours corresponding to 35.58-24.98 µg/mL and the lowest is as the same as observed in N3 which is between 40-48 hours and its correspond to 4.45-4.28 µg/mL. The release profiles of charantin from PNIPAM gels synthesized with 3% cross linker at temperatures above and below the LCST. During the first 8 h, the release rate of
charantin was the highest at 34 ℃, followed by 32 ℃. When the gel came into contact with PBS above the LCST, a temperature gradient was generated across the polymeric matrix. This resulted in the collapse of the gel surface faster than the interior of the gel matrix, forming a dense polymeric skinlayer. The skinlayer hindered the outward diffusion of the protein solution, causing a build-up of hydrostatic pressure inside the gel. This pressure would eventuallysqueezeout the water containing the protein as time passes. For charantin release at 34 ^{0}C, there was an initial burst release of surface encapsulated charantin accompanying the formation of a dense skin layer.

Diffusion coefficient of PNIPA in different fluids at 34 °C.

Table 8 displays the results of the PNIPA diffusion coefficient in various fluids. Hydrogels N1, N2, N3, and N4 were tested in solutions of distilled water, bromophenol blue, and charantin for their diffusion coefficients. The hydrogel N1's diffusion coefficient, 100 PNIPA The measurement of homopolymer in bromophenol blue was 1.56x10-10 m2/s, whereas in phenol blue it was 2.50 x 10-10 m2/s. It was discovered that bromophenol had the greatest diffusion coefficient, whereas pure water had the

lowest. This finding agrees with the one made by Caiet al.22. In distilled water, bromophenol blue, and charantin solutions, the diffusion coefficient values of the N2 hydrogel are 4.70 x 10-9 m2/s, 1.10 x 10-8 m2/s, and 5.08 x 10-8 m2/s, respectively. This, in turn, has shown to charantin at both the greatest and lowest concentrations in distilled water.

Distilled water was determined to have the hydrogel N2 value. The diffusion coefficient for hydrogel N3 is 1.60 x 10^5 m2/s in distilled water and 5.08 x 10^4 m2/s in charantin, according to the results. An extreme value of 4.42 x 10-8 m2/s was observed in pure water for the hydrogel N3 concentration. Diffusion rates of N4 were 1.44 x 10-8 and 1.48 x 10-9 m2/s, respectively, in distilled water and charantin, with the latter exhibiting the lowest rates.

The diffusion is greatest in hydrogel N2 (1.44 x 10-8 m2/s) and lowest in hydrogel N1 (1.56 x 10-10), when tested in distilled water. N3 has the greatest bromophenol blue diffusion coefficient at 8.09 x 10-10 m2/s, whereas N1 has the lowest at 4.42 x 10-8 m2/s. The charantin diffusion coefficient ranges from 2.5 x 10-10 m2/s in N1 to 5.08 x 10-8 m2/s in N2. These values represent the extremes of the distribution. When inflated, the majority of hydrogels utilized in biomedical applications have a mesh size ranging from 5 nm to 100 nm. Since these scales are much bigger than the majority of pharmaceutically-used tiny molecular weight medicines, their diffusion in the enlarged matrices remained unimpeded. The expansion of the gel regulated the solute transport. The medications are able to disperse into the gel network due to their tiny hydrodynamic radii. In order to comprehend the swelling gel's network structure and its ability to serve as a medication delivery carrier, it is essential to be familiar with its features.

Table 8: Diffusion Coefficients for the Hydrogels in Different Fluids at 34 ˚C

Gel Codes	Diffusion coefficients in Distilled Water (m^2/s)	Diffusion coefficients (m^2/s) in Bromophenol blue	Diffusion coefficients (m^2/s) in Charantin
N1	1.56×10^{-10}	8.09×10^{-10}	2.50×10^{-10}
N2	4.70×10^{-9}	1.10×10^{-8}	5.08×10^{-8}
N3	1.60×10^{-9}	4.42×10^{-10}	7.90×10^{-9}
N4	1.44×10^{-8}	1.16×10^{-9}	1.48×10^{-9}

PNIPAM N-Isopropylacrylamide, Acrylamide, Gel codes, ^{N1}P (NIPA) Homopolymer (100 mol% of P (NIPA)), ^{N2}P (NIPA-co-AM) (93-3 mol%), ^{N3}P (NIPA-co-AM) (94-6 mol%), ^{N4}P (NIPA-co-AM) (88-9 mol%).

Diffusivity D of PNIPAM-loading with charantin drugs release exponent (n) and the geometric constant of PNIPAM-based hydrogels (k) at 34 ℃

The result for *n, k* and *D* were obtained as summarized in Table 9. The fraction release of charantinwas exponentially related to the time of drug release for hydrogels that are cylindrical n=0.5 and above to unify corresponding to Fickian diffusion.

While the release rate was then defended on t^{-05}. This represents diffusion- controlled release when *n* lies between 0.5 and 0.7 (i.e. 0.49<n<0.7), non-Fickiandiffusional release (anomalous transport) as reported that occurs, while n=0.7 corresponds to case II transport (for a cylindrical sample).

The value of ***n*** obtained isgreater than 0.49 but less than 0.71. The release exponent (*n*) provided some insights into the mechanism of the release of charantin form PNIPAM-based hydrogels withthe *n*values showing an anomalous transport mechanism at 34 ℃. This implies that as the exponential release increases, the geometric constant which contains the diffusion term decreases. The coefficient of charantin diffusion from P (NIPA) could be used to control the release of the anti-diabetic agent.

The diffusion kinetics of PNIPAM-hydrogels studies and the drug release rates were rate governed by the earlier time approximation, while the diffusion rate of drug molecules was strongly influenced by temperature. Fickianbehavior is observed in N1, N2, N3 and N4.

The small hydrodynamic radii of drug molecules allow the drug (charantin) to diffuse through the gel network. Hence, the diffusion coefficient *D* is independent of the drug concentration. The release of the drug from the polymer is fractionally and exponentially related to the release time. The release exponent*n* corresponds to the mechanism of drugs release. When n=0.5, correspond to fickian diffusion and the release rate is time depending on $t^{-0.5}$ This relation can be seen in Table 9.

Table 9: Diffusivity, D, of P (NIPA)-loaded Charantin, Drug Release Exponent (n), and Geometric Constant of P (NIPA)-Based Hydrogels (K) at 34˚C

Gel Codes	Geometric constants (K)	Release exponents (n)	Diffusion Coefficients D (m^2/s)
N1	0.01 ± 0.003	0.5 ± 0.03	2.50×10^{-10} ± 0.180
N2	0.01 ± 0.010	0.6 ± 0.035	5.08×10^{-8} ± 0.490
N3	0.01 ± 0.005	0.6 ± 0.035	7.90×10^{-9} ± 0.479
N4	0.01 ± 0.003	0.7 ± 0.04	1.48×10^{-10} ± 0.573

Swelling ratio

Figure 10 displays the swelling ratio result. Hydrogels made of PNIPAM were subjected to a charantin solution, bromophenol blue, and distilled water as solvents. Hydrogels were allowed to expand in this solvent for 30 hours until the swelling reached a saturation point or the solvent equilibrated.
The ability of hydrogels based on PNIPAM to swell was shown to be temperature dependent. The effects of temperature on swelling are shown by the findings from this. However, when the cross-linked structure's retroactive force equalizes the thermodynamically driven swelling force, the result is equilibrium.
The expansion of the hydrogels makes it easier for different solutes to be transferred through them by enlarging the spaces between the cross-links and the size of the mesh. Hydrogels' cross-linking and network structure, as well as their ability to serve as drug delivery vehicles, may be better understood with knowledge of their swelling feature.

Figure 10 shows that compared to inbromophenol blue and charantin, PNIPAM hydrogels expand much better in distilled water. Equally beneficial to the swelling ratio was the acrylamide. Nevertheless, the swelling ratio is highest in N4 owing to the high concentration of acrylamide in this hydrogel, and it is also greater in copolymers. Distilled water showed the greatest swelling ratio for all of the hydrogels, followed by charantin and finally bromophenol. The compound's swelling ratio in a given solvent is directly proportional to its polarity, hence this is the result of solvent polarity.

Conclusion

Charantin extraction results showed that the left sample had about twice as much charantin as the fruit sample, suggesting that ethanol, when employed under control at 8500 revolutions per minute of centrifuge speed, improves extractability and guarantees complete separation. The retention times for insulin leave, meal, and purified charantin samples are 4.318, 4.023, and 4.291 seconds, respectively.
There are 74.69 /mL of ground per/mm and 52.61 µg/mL of concentrations on the leaf sample.
Hydrogels based on PNIPAM exhibit enhanced cross-linking and water absorption at increasing concentrations, according to the results of their manufacture. A gel containing the active substance will be formed as a result. The lower critical solution temperature varies with the molecular weight or size of the solvent and increases as the hydrophobicity does.

Charantin loading demonstrates a marginally improved efficacy. Swelling ratios indicate that hydrogels swell better in distilled water compared to charantin or bromophenol blue, and FTIR data show that a bond stretch subsequently fades after charantin loading. Additionally, we noted what may be a robust interaction between the charantin molecules and the hydrogel matrix.

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