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Application of Gellan gum in targeted delivery of sulfasalazine to the colon based on sensitivity to PH

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Abstract

Targeted drug delivery is one of the most important branches of pharmaceutical science, and its importance for researchers lies in increasing drug efficacy and reducing drug toxicity through drug delivery carriers. Drug delivery system formulations can increase drug safety by reducing systemic side effects, preventing drug release into the stomach and damaging it, and preventing drug distribution to healthy tissues. They also prevent drug degradation, mask the bitter taste of the drug, and reduce costs. The aim of this study is to use the drug sulfasalazine to the colon in a drug delivery system based on azo hydrogels, which we cover with gellan polysaccharide, and to show its effect on the digestive system of the body and the treatment of inflammatory bowel disease. To do this, first, distilled water was added to the sample beaker where we poured the gellan and mixed using a magnetic stirrer. Heating was performed for one hour to completely dissolve the gellan. Then, the ground drug was added while dissolving the gellan. Stirring continued for three hours and then the samples were emptied into a plastic container and placed in the refrigerator. 30 ml of deionized water was added to 10 ml of each sample, then they were titrated separately using a 10 M sodium hydroxide solution and a 10 M hydrochloric acid solution. The dialysis bag was cut into several pieces and then the bottom of the bag was closed with a piece of suitable thread that was completely cleaned and sterilized with PBS buffer and 20 ml of the samples were added to each bag. For each sample, 25 ml of isotonic PBS buffer with a pH = 0.7 was added to a 50 cc Falcon tube, and the dialysis bag and its contents were immersed in the Falcon tube. Then, the optical absorption or ultraviolet light was read using a spectrophotometer in both environments inside the dialysis bag and outside the bag during different hours. The cytotoxicity test is performed by several methods: NRU, CFU, MTT, XTT. In this study, we used the MTT test to investigate the toxicity of the substances on cell viability. We used the FTIR analysis test to identify unknown substances, determine the concentration and quality of the sample. This study, while confirming slow release, showed that the maximum drug release was within 48 hours in an environment similar to physiological conditions and with isotonic conditions. The successful development of nanoparticles for oral administration could change the treatment paradigm of many diseases and have a significant impact on future treatment outcomes.

Keywords: Drug release, inflammatory bowel disease, sulfasalazine, drug delivery, colon, gum gall

Introduction

Inflammatory bowel disease (IBD) is a common digestive disease that causes inflammation and ulceration of the lining of the large intestine and small intestine. It can be painful, debilitating, and in some cases life-threatening. The inflammation can be limited to the intestinal wall or spread throughout the entire intestine, eventually leading to other serious illnesses in different parts of the digestive tract and anus. Sulfasalazine is one of the main and most effective drugs for the treatment of inflammatory bowel disease. It is an anti-inflammatory drug that contains two components: 5-aminosalicylic acid and sulfapyridine, which are linked by an AZO bond and are taken orally. Drug delivery is a method in which, by using medical methods and combining them with an engineering perspective, drugs or therapeutic molecules

in general can be delivered to the desired target, i.e. the area under treatment, in a more effective way. Drug delivery to the colon is a new strategy that has received much attention. Selective release of drugs to the colon can not only control the required dose, but also reduce the systemic side effects caused by high doses. Peptide, protein, oligonucleotide drugs, and vaccines can be suitable candidates for this route. Drug release from the colonic route and through the absorption of cells in this route is another method of delivering drugs that are absorbed in small amounts from the previous parts of the digestive tract. However, there are some obstacles to choosing this route for drug delivery.

In this study, to deliver sulfasalazine to the colon, we coated it with gellan polysaccharide in a drug delivery system based on azo hydrogels.

Materials and method

The gellan sample was poured into the beaker and distilled water was added to it (Table1). Then, the beaker was covered and mixing was performed using a magnetic stirrer. Heating was continued at 90°C for one hour until the gellan was completely dissolved. It is also worth noting that 90°C is the temperature required to dissolve gellan gum.

After one hour, the ground drug was added to the gellan while dissolving it. Then, it was stirred for 3 hours using a magnetic stirrer. The prepared samples were emptied into plastic containers and stored in the refrigerator.

To 10 ml of each sample, 30 ml of deionized water were added, then titrated separately using 0.1 M hydrochloric acid and 0.1 M sodium hydroxide solutions. The numbers in Table 2 are in ml of acid or base used to reach the target PH. A pH meter was used to measure the final pH of the samples.

The dialysis bag was cut into 17 cm pieces. Then the bottom of the bag was closed with a piece of suitable thread thoroughly cleaned and sterilized with PBS buffer. 20 ml of the samples were added to each dialysis bag.

For each sample, 25 cc of isotonic PBS buffer with a pH of 0.7 was added to a 50 cc Falcon tube, and the dialysis bag and its contents were immersed in the Falcon tube.

Then, the absorbance of O.D. (Absorbance) or UV light at a wavelength of 359 nm (λ 359 nm) was read using a spectrophotometer in both environments inside the dialysis bag and outside the bag at 1, 2, 4, 8, 24 and 48 hours.

Cytotoxicity test

Cell experiments were performed at the Institut Pasteur Center in the cell culture laboratory. Fibroblast cells were obtained from the Institut Pasteur and Iran cell bank and cultured in DMEM culture medium containing 10% FBS and 1% penicillin and streptomycin antibiotics in a 1:1 ratio. Then, the grown cells were detached from the culture medium with trypsin and centrifuged at 1500 rpm for 10 minutes at 4°C. Then, 200 microliters of cell suspension were cultured in 96-well BIOFIL microplates for 24 hours, with 5000 cells. Next, each sample was added to each well in the order shown in the table with mixed culture medium.

The microplates were placed in an incubator for 24, 48, and 72 hours at 37 degrees Celsius, 95% humidity, and 5% CO_2 concentration. After 24, 48, and 72 hours, the effect of each sample on the aforementioned cell line was examined using the MTT colorimetric assay.

Cytotoxicity testing is performed according to ISO10993-5 standard and is performed using three methods: NRU test, CFU test, MTT test and XTT test. The most common method in evaluating cytotoxicity is measuring cell viability using the MTT or -3 (4,5 - dimethylthiazol-2-yl -) 2,5-diphenyltetrazolium bromide method. After the incubation period, 37 microliters of MTT solution were added to each well and the microplates were kept in the incubator for another 3 hours. Finally, the resulting precipitate was dissolved using dimethyl sulfoxide, and the optical absorbance of the solution was read at a wavelength of 570 nm using an Elisa Reader.

Result and Discussion

FTIR Result:

The images of FTIR spectra of six samples including gellan gum, sulfasalazine drug, hydrogel P1 to P4 are given. As you can see in the diagram related to sample GG, peaks at wavelengths of 3753/89, 3413/42, 2924/70, 2144/09, 1617/83, 1418/66, 1301/03, 1237/85, 1154/90, 893/08, 810/43 and 612/66 can be seen. According to the standard peak bank and the interpretation performed by the device software (Diagram 4 1 (B); compounds such as two types of sodium salt of alginic acid 4 and two types of cellophane have been detected.

The diagram is for the SS sample, or the drug sulfasalazine with the closed formula (S5O4N14H18C). This drug, which is used in tablet form, in addition to the active ingredient; usually additives are added to it during the drug production process. Therefore, the desired tablet was made into powder form and after preparation, it was given to the device in crystalline form. According to diagram ,this drug has created numerous peaks due to its multiple functional groups, including: 88 / 3061, 62 / 3027, 1 / 2918, 66 / 2822, 84 / 2761, 55 / 1676, 25 / 1635, 80 / 1615, 34 / 1585, 59 / 1536, 74 / 1484, 15 / 1463, 68 / 1393, 48 / 1358, 26 / 1279, 94 / 1262, 78 / 1199, 85 / 1172, 47 / 1127, 00 / 1080, 27 / 1042, 55 / 963, 98 / 935, 19 / 793, 30 / 767, 40 / 728, 92 / 707, 24 / 664, 24 / 649, 81 / 613, 92 / 572 and 47 / 519.

According to the standard peak bank and the interpretation performed by the device software (Figure 4 2 (B);)) compounds such as sulfasalazine 1 with the molecular formula C18H14N4O5S1 and catalog number S883; molecule -4,9one; Khellin-5-g] chromen-furo[3,2-5H-methl-7-Dimethoxy with the molecular

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formula 5O12H14C and Cas Number: 2049-67-4 Figure(4,2); the next substance is the molecule 2,5-Dihydroxybenzaldehyde with the molecular formula 3O6H7C and Cas Number: 1-26-Cas Number: 5469 and finally -2-Chloromethyl-1hylnaphthalenemet with the molecular formula 1Cl11H12C and Cas Number: C5,320.

Table 1: Estimated parameters of the length-weight relationships for four species of fish from Gollapalli and Jeedipalli Reservoirs, Andhra pradesh.

Family	Species & IUCN status	No. of fish examined	Total length±SD; Range	Total weight±SD; Range	aa	b	R2	Growth pattern
Cyprinidae	*Labeo catla* (LC)	25	28.41±4.37 (20-37.5 cm)	823.96±326.78 (418-1560gm)	0.54	2.176	0.787	Negative allometry
	L. rohita (LC)	39	28.70±4.20 (20-36.8 cm)	764.89±247.81 (354-1345gm)	0.577	2.132	0.981	Negative allometry
	L. calbasu (LC)	19	28.15±3.52 (23-36.7 cm)	739±208.20 (456-1135 gm)	0.463	2.202	0.907	Negative allometry
Cichlidae	*O. niloticus* (LC)	173	27.82±3.33 (22-40.8 cm)	666.85±197.25 (350-1232 gm)	0.395	2.226	0.763	Negative allometry
	Total	256						

*LC- Least concern.

Table 2: Estimated parameters of the length-weight relationships for four species of fish from Gollapalli and Jeedipalli Reservoirs, Andhra Pradesh.

Family	Species & IUCN status	No. of fish examined	Total length±SD	Total weight±SD	In a	b	R2	Growth pattern
Jeedipalli Reservoir, Andhra Pradesh								
Cyprinidae	*Labeo catla* (LC)	17	29.1±4.38	853.7±326.57	-0.334	2.21	0.739	Negative allometry
	L. rohita (LC)	25	28.26±4.27	740.8±246.20	-0.2027	2.108	0.883	Negative allometry
	L. calbasu (LC)	6	28.05±3.77	772.8±253.12	-0.6315	2.42	0.75	Negative allometry
Cichlidae	*O. niloticus* (LC)	112	28.01±3.61	685.27±214.89	-0.558	2.34	0.841	Negative allometry
Gollapalli Reservoir, Andhra Pradesh								
Cyprinidae	*Labeo catla* (LC)	8	26.95±4.24	760.6±340.0	-0.294	2.206	0.847	Negative allometry
	L. rohita (LC)	14	29.48±4.11	807.78±253.98	-0.326	2.192	0.737	Negative allometry
	L. calbasu (LC)	13	28.2±3.5	723.38±193.62	-0.190	2.097	0.786	Negative allometry
Cichlidae	*O. niloticus* (LC)	61	27.48±2.76	633.03±155.92	0.135	1.84	0.474	Negative allometry

Table 3: Condition factor of fish species in two reservoirs.

Sl. No.	Reservoir	Family	Fish species	K	Kn
1	Jeedipalli Reservoir	Cyprinidae	*Labeo catla*	3.41±0.74	1.01±0.17
			Labeo rohita	3.26±0.56	1.00±0.10
			Labeo calbasu	3.43±0.35	1.00±0.058
		Cichlidae	*Oreochromis niloticus*	3.07±0.41	1.17±0.157
2	Gollapalli Reservoir	Cyprinidae	*Labeo catla*	3.82±0.94	1.01±0.19
			Labeo rohita	3.11±0.41	1.00±0.087
			Labeo calbasu	3.21±0.43	1.00±0.086
		Cichlidae	*Oreochromis niloticus*	3.04±0.38	1.01±0.141

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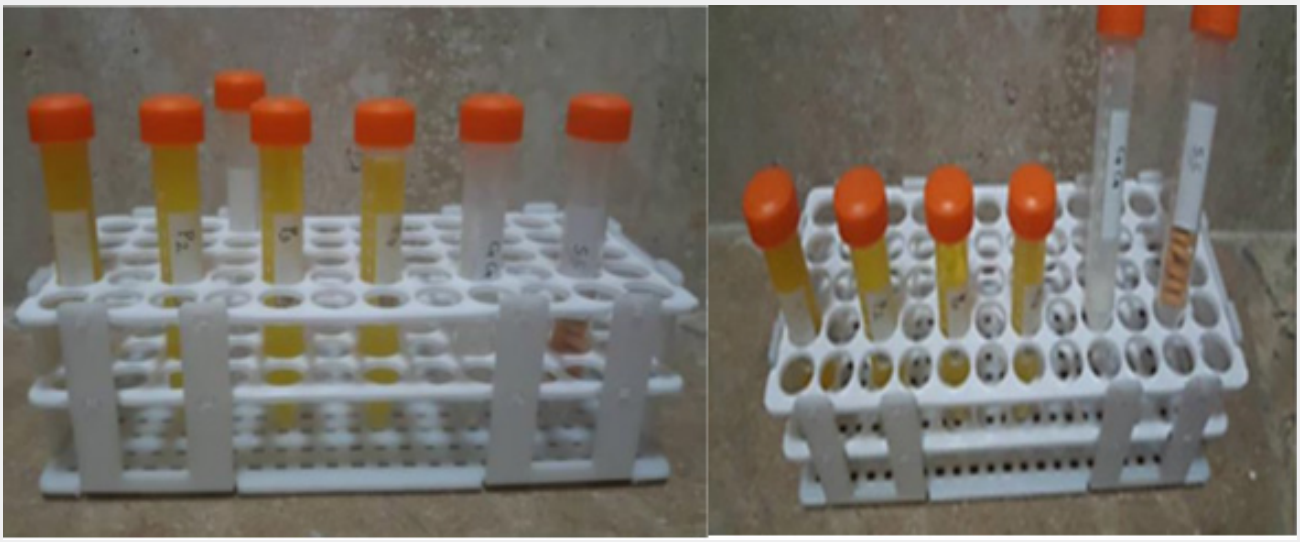

Figure 1: Hydrogels P1, P2, P3, P4 (GG: gellan gum, S: sulfasalazine)

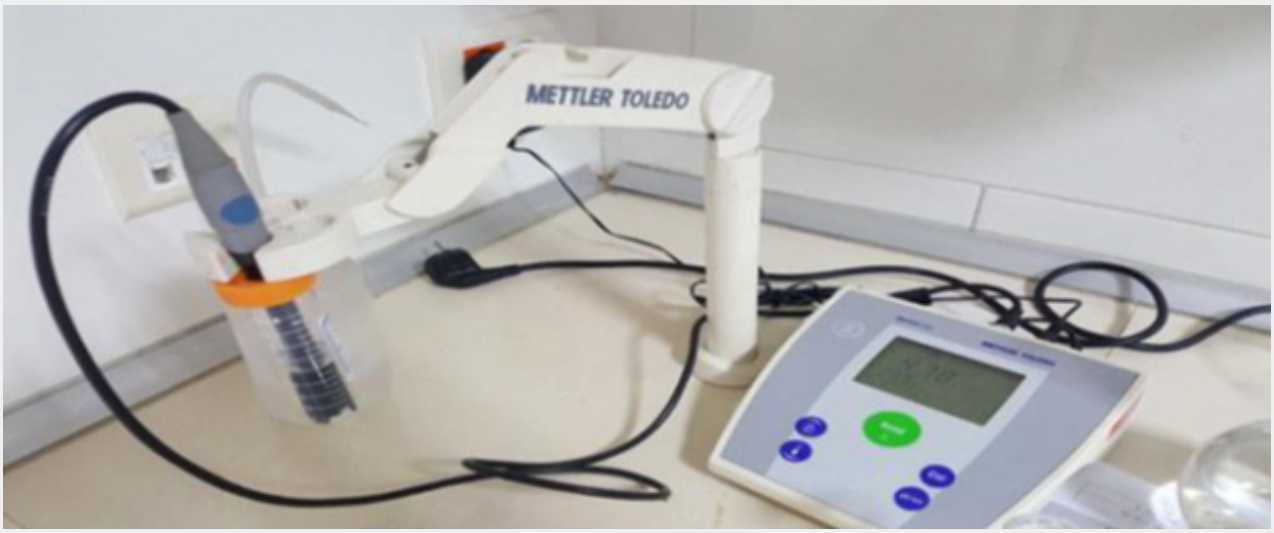

Figure 2: Measuring the pH of samples with a pH meter

Figure 13 is the FTIR spectrum of the hydrogel product P2. The characteristic peaks observed in this material were at wavelengths of 3432/98, 43/1998, 1593/09, 1040/82, 698/96, 666/67, 636.85 and 497.49. In Figure (4 4) B), the compounds identified are as follows: silicon tetrabromide or tetrabromosilane with the formula 1Si4Br and 4-66-Cas Number: 7789; titanium IV chloride with the formula 1Ti4Cl and 0-45-Cas Number: 7550; pullulan P2000 and pullulan P800 were sodium salts of alginic acid. Figure (4 5) is also the FTIR spectrum of the hydrogel. P3. In Figure (A), the wavelengths 83 / 3727, 16 / 3626, 15 / 3379, 42 / 1628 and 38 / 1038 can be mentioned as indicators. Figure 4 5 (B) (During the interpretation made using the standard spectrum bank, similar compounds as before, namely alginic acid (sodium salt), Chipboard, L-Glucose and pullulan P2000, have been mentioned. Obviously, other compounds present in this hydrogel have been eliminated due to the restrictions imposed.

The graph also shows the infrared spectrum of hydrogel P4, which used the lowest amount of gellan gum in this series of experiments. The wavelengths with the most noticeable

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peaks were 16/3231, 11/2148, 78/1986, 77/1043, 74/604, and 60/462. In diagram (4 6) B) also compounds such as vanadium oxytrichloride with the formula 1V1O3Cl and 6-18-Cas Number: 7727; glycerol with the formula C3H9O3; propanol-2-Difluro-1,3 with the formula 1O2F6H3C and Cas Number: 7-18-7417; (Methoxymethoxy)-2 Ethanol with the formula 3O10H4C and 2-72-Cas Number: 121 and diethyl chlorophosphate with the formula 1P3O1Cl10H4C and 8-73-Cas Number: 590 are mentioned.

Figure 3: Dialysis bag containing sample

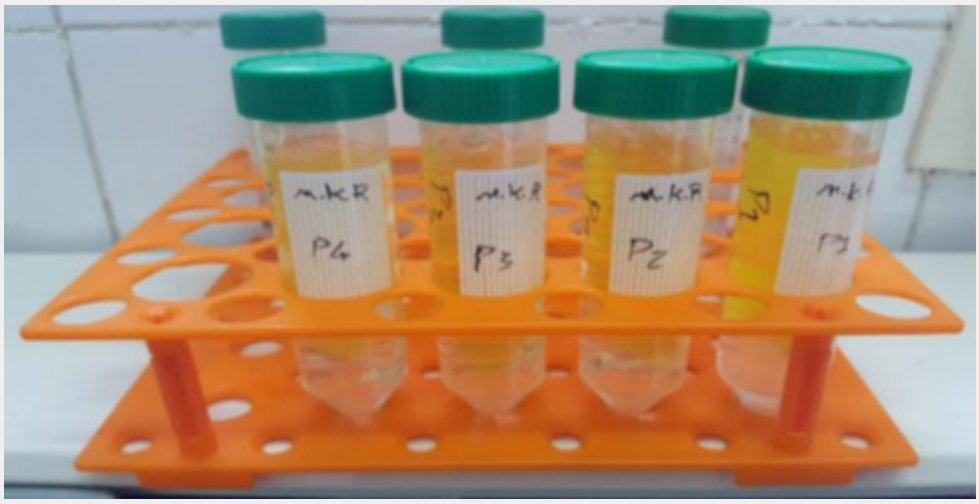

Figure 4: Dialysis bag immersed in Falcon tube containing isotonic PBS buffer

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Figure 5: Measuring the light absorption of samples at a wavelength of 359 nm using a spectrophotometer.

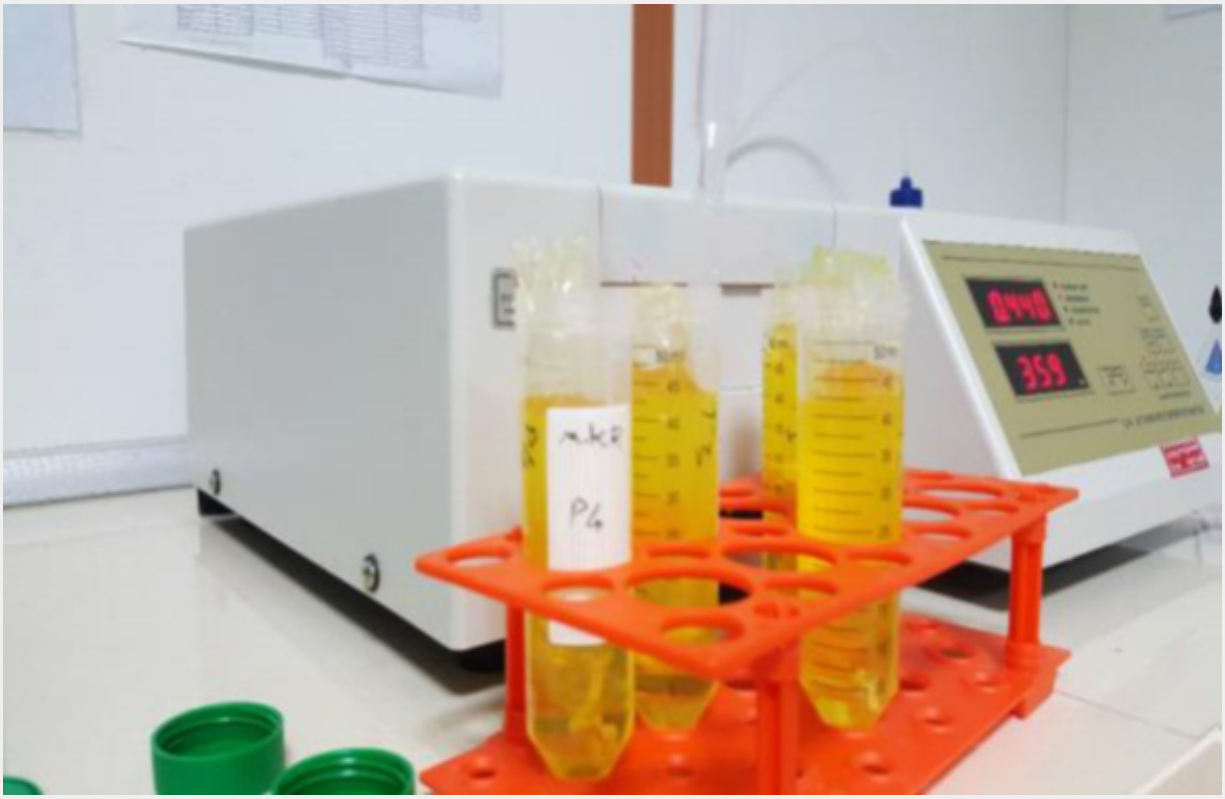

Figure 6: Measuring the light absorption of samples at a wavelength of 359 nm using a spectrophotometer

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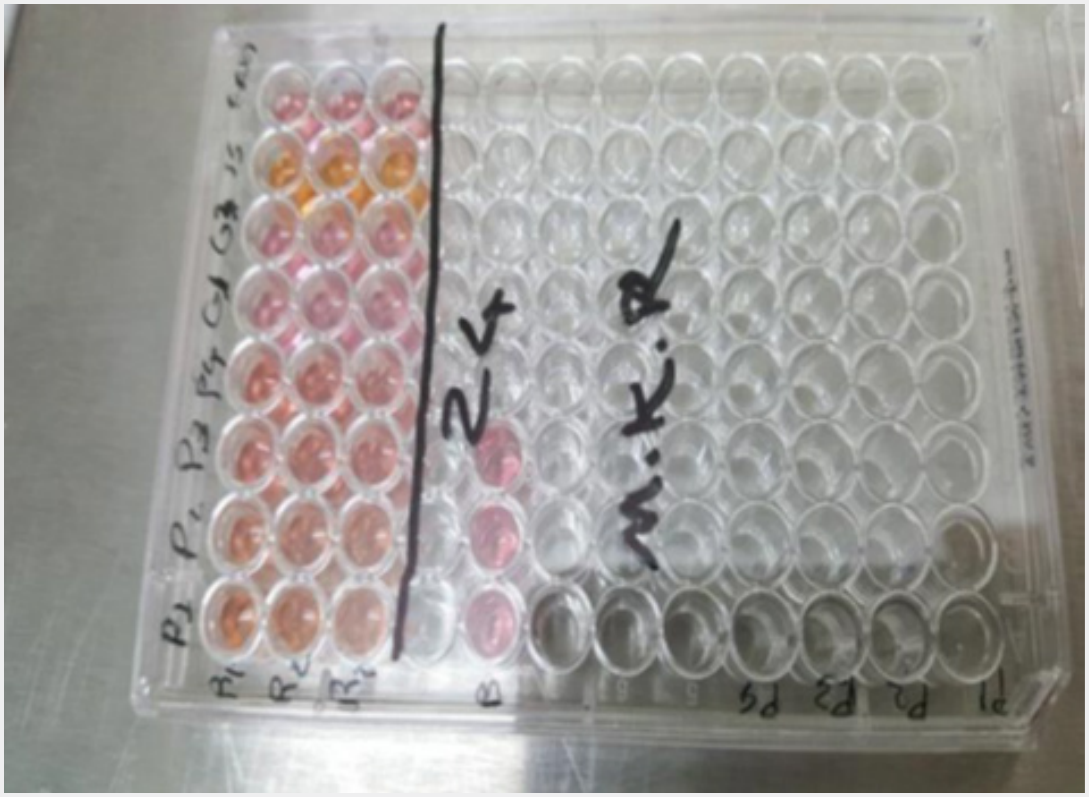

Figure 7: Cell culture of cell suspension for 24 hours

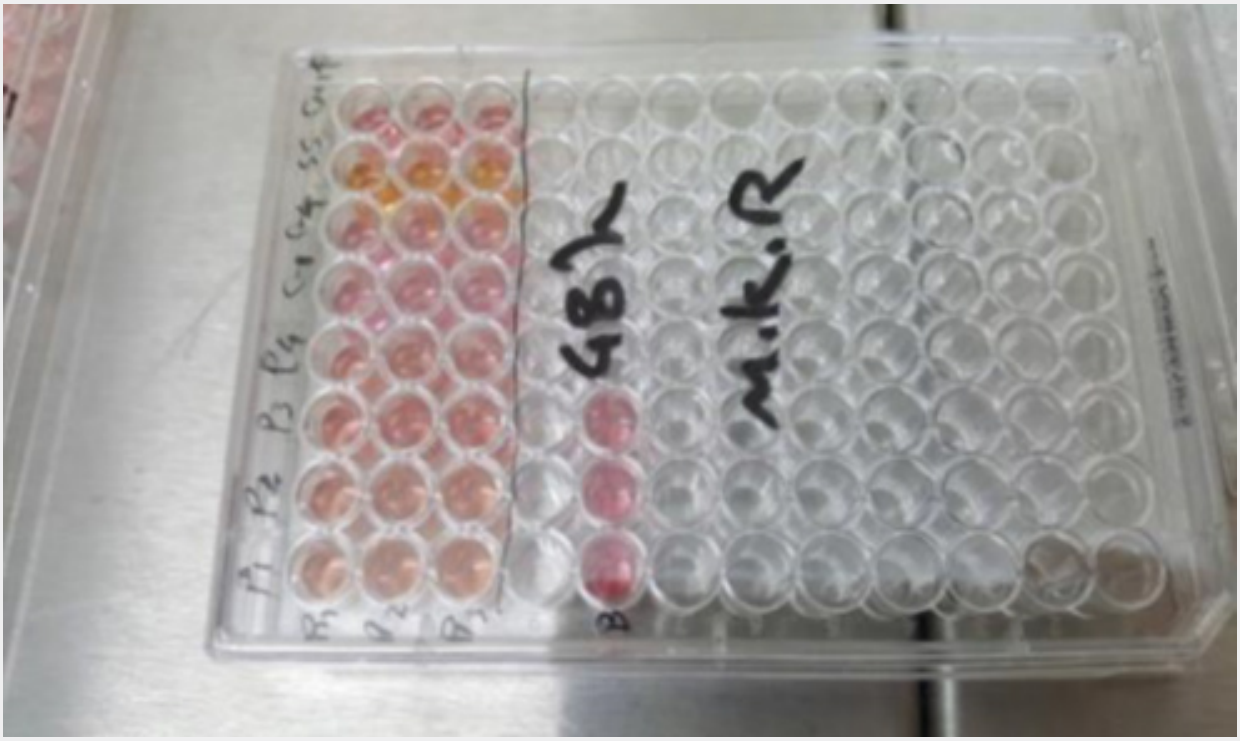

Figure 8: Cell suspension after 48 hours

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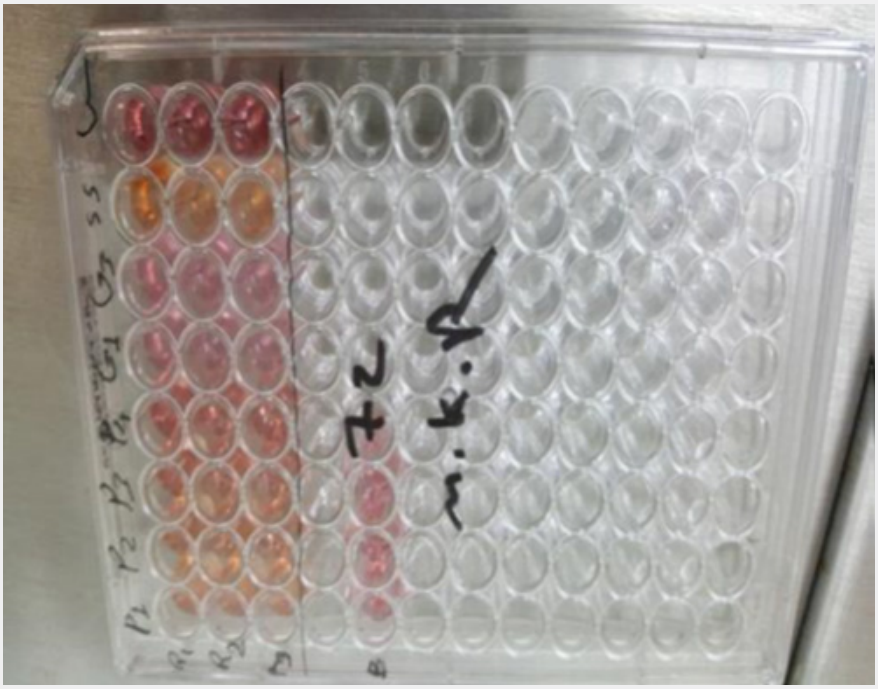

Figure 9: Cell suspension after 72 hours

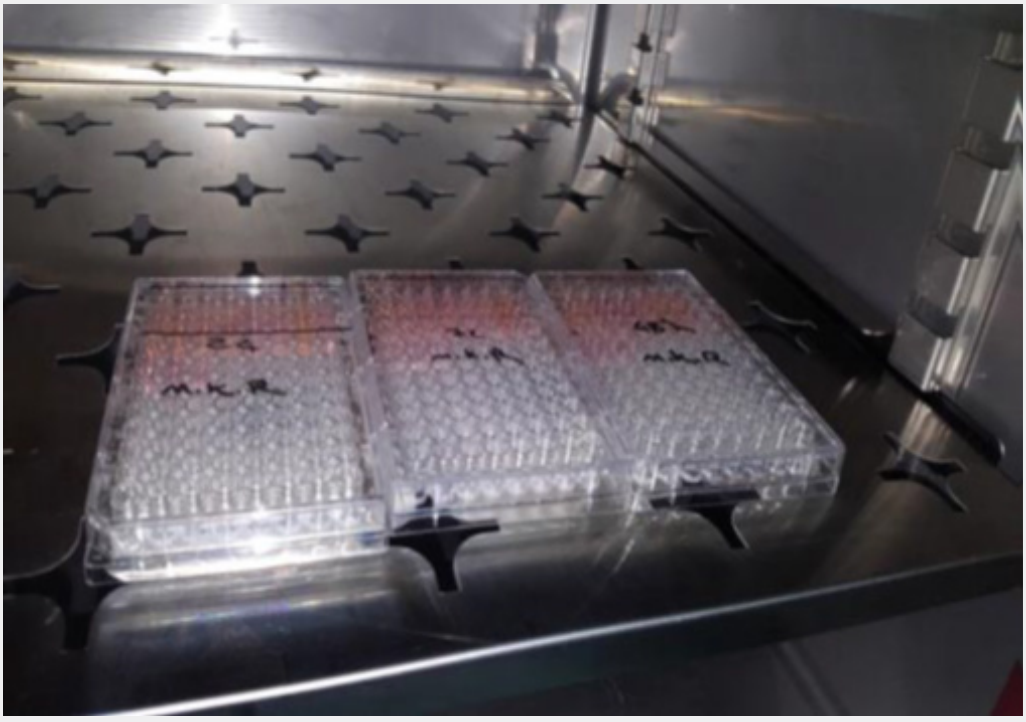

Figure 10: Incubate suspensions for 24, 48 and 72 hours

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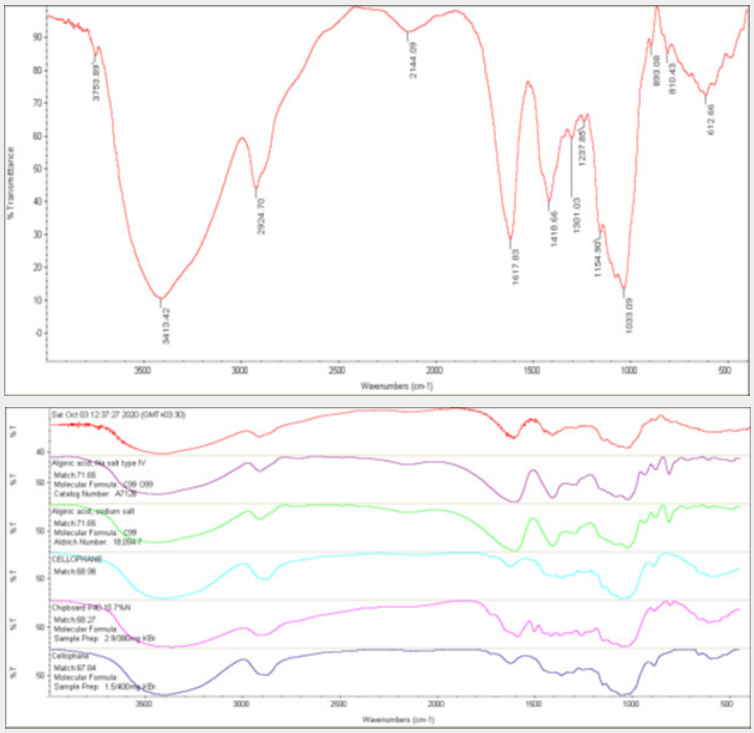

Figure 11: FTIR spectrum images in the wavenumber range of 400-4000-1-cm for pure gellan gum powder (GG) sample (A) Peaks obtained from the percentage of transmittance (IR spectrum) and (B) Interpretation of peaks based on the spectral bank obtained from standards

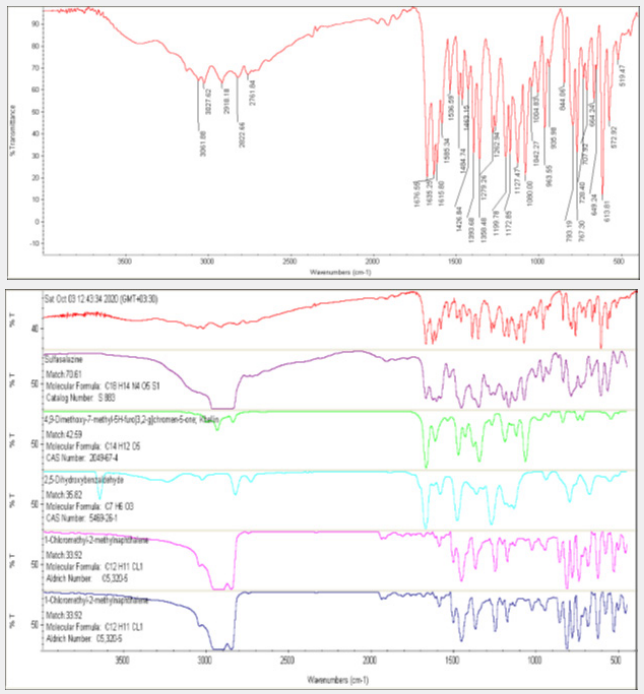

Figure 12: FTIR spectrum images in the wavenumber range of 400-4000-1-cm for the drug sample Sulfasalazine (SS) (A) Peaks obtained from the percentage of transmittance (IR spectrum) and (B) Interpretation of peaks based on the spectral bank obtained from standards

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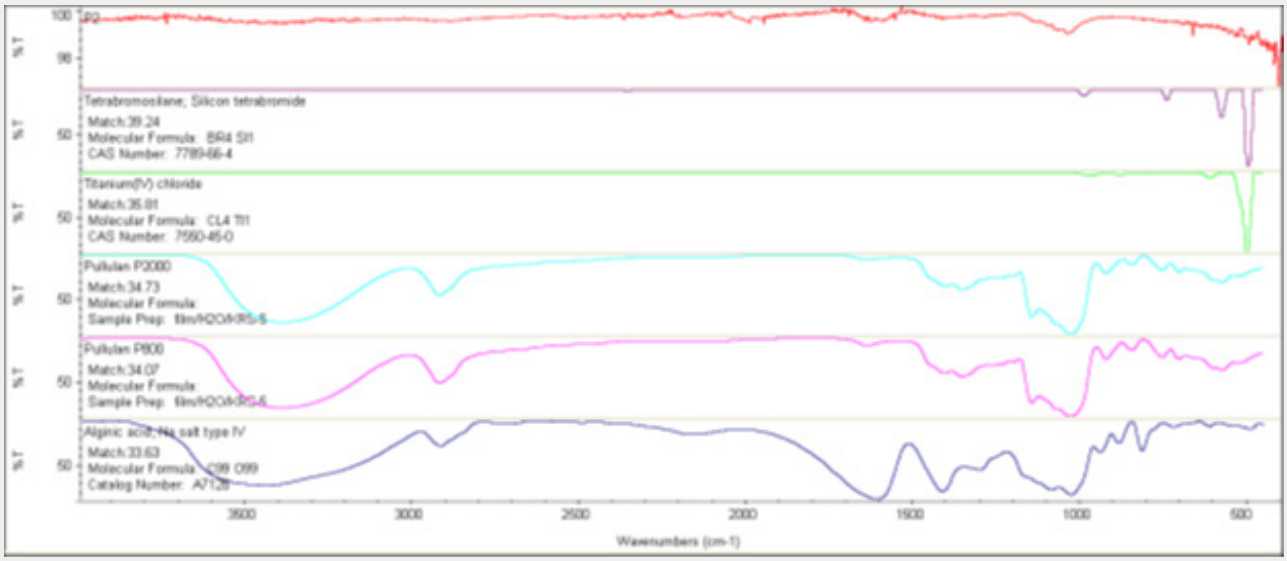

Figure 13: FTIR spectrum images in the wavenumber range of 400-4000-1-cm for hydrogel containing GG mg/ml 5/7 (P2) (A) Peaks obtained from the percentage of IR transmittance and (B) Interpretation of peaks based on the spectral bank obtained from standards

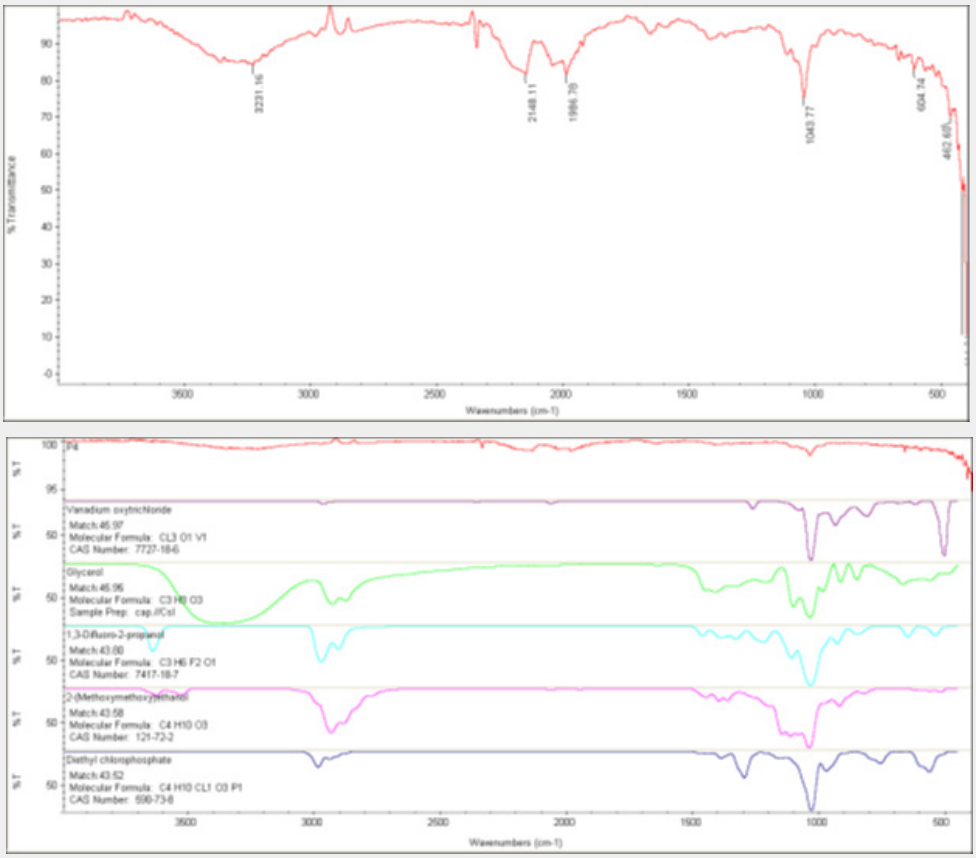

Figure 14: FTIR spectrum images in the wavenumber range of 400-4000-1-cm for hydrogel containing 2.5 mg/ml GG (P4) (A) Peaks obtained from the percentage of transmittance (IR spectrum) and (B) interpretation of peaks based on the spectral bank obtained from standards

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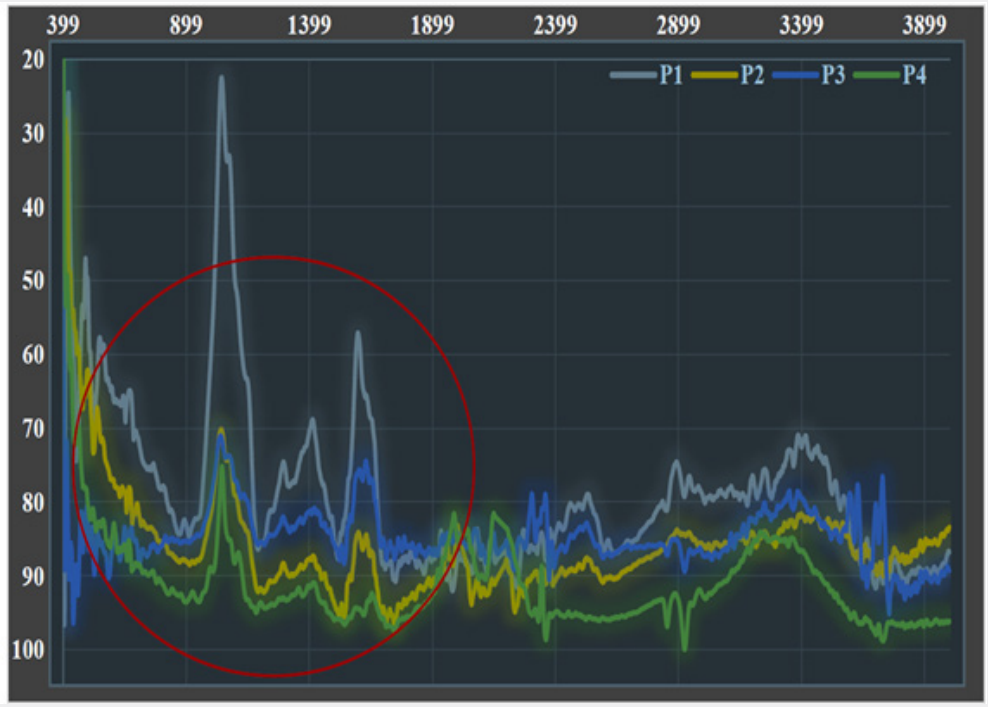

Figure 15: Comparative diagram of four hydrogels P1, P2, P3 and P4. The area enclosed in the red circle has clearly affected the absorption of the spectrum due to the gellan gum.

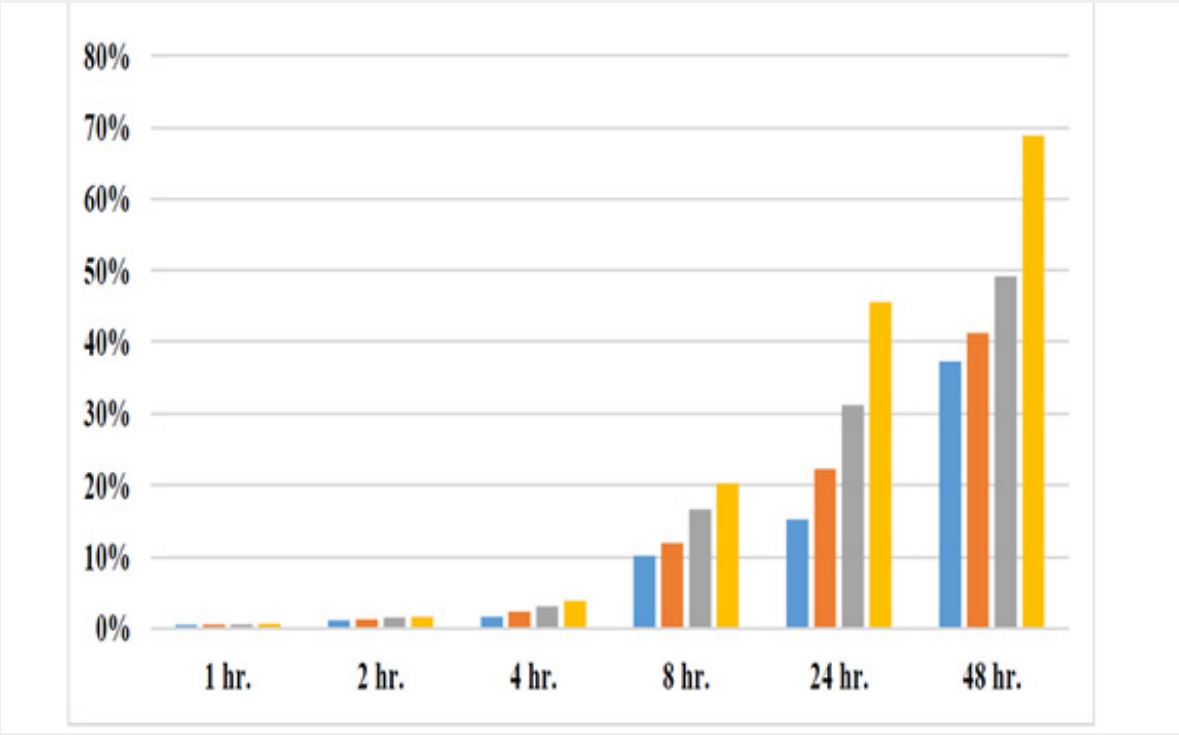

Figure 16: Graph of the dependence of the percentage release of sulfasalazine on time

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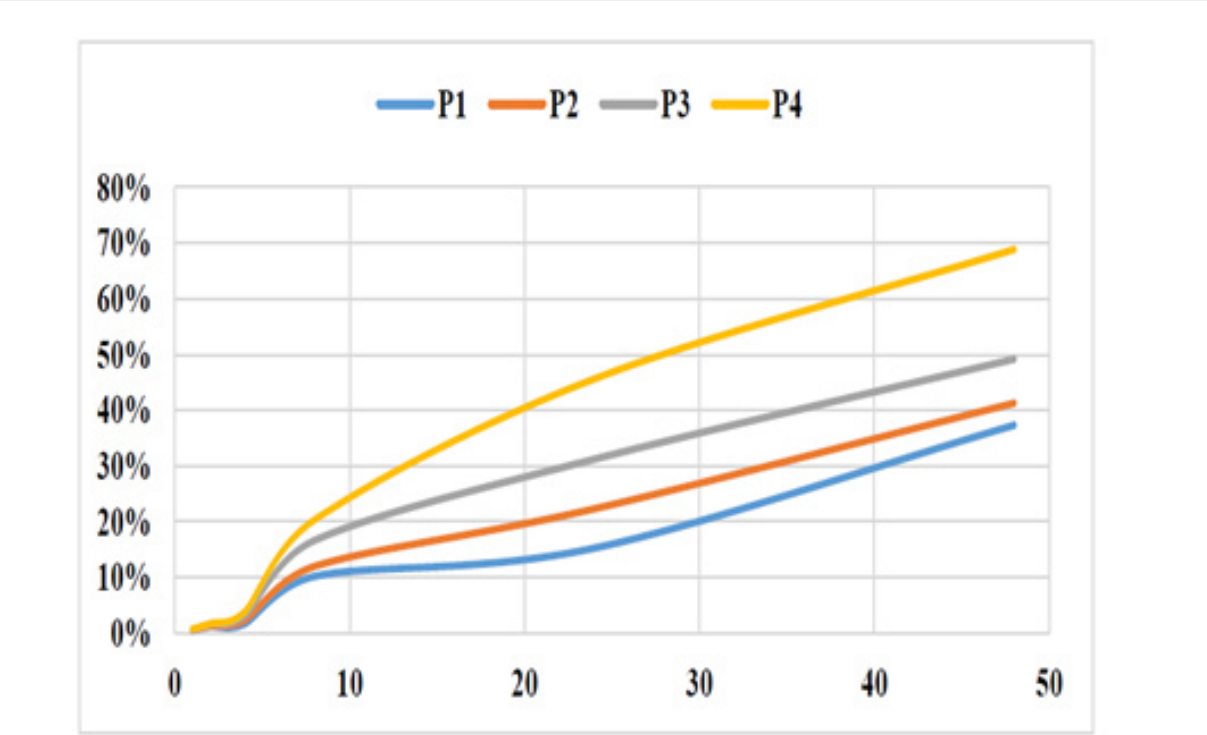

Figure 17: Linear diagram of the release of sulfasalazine in four products P1, P2, P3 and P4, separately for each product.

Figure 15 compares the four infrared Fourier transform spectroscopy spectra of the four produced hydrogels in reverse order of the Y axis. In the area enclosed by the red circle, it can be seen that the spectra are in complete agreement and only the amount of the transmitted spectrum in the wavenumber range of 500-2000 cm changes clearly. This difference in the transmitted spectrum is of course visible in all parts of the spectrum, but it is more pronounced in this range.

Resistance test PH

Solutions resist changes in pH according to the functional groups, the interaction of molecules together, and the ionization strength. Table (1) shows the amounts of 0.1 molar solution of each of the titrant solutions (hydrochloric acid and sodium hydroxide) used in milliliters. As you can see, the amount of solution required to change the pH is much greater with distance from the initial pH of each solution.

The four hydrogel products prepared differ only in the percentage of gellan gum. Since gellan is a molecule that gives the prepared material a gelling property; it is expected to play a role in drug release in physiological and chemical environments. The percentage of sulfasalazine released from the product to the outside of the semipermeable membrane was calculated. In order to simulate the conditions of the living environment, the dialysis bag was immersed in physiological serum solution and the absorption of electromagnetic waves at a wavelength of 359 nm was measured using a spectrophotometer at different times.

The graph shows the drug release rate in standard conditions and in the presence of a semipermeable barrier of a dialysis bag with a cut-off of 14 kDa over time in a linear manner. As you can see, with increasing time, the drug release ratio in product P1, which has the highest GG content, becomes further away from product P4, which has the lowest GG content, indicating the effectiveness of GG in releasing the drug more slowly. Also, in the first 4 hours of the study, all four products showed almost the same behavior, but in the eighth hour and after that, the difference between the different products became significant.

Conclusion:

Many new drugs are only available by injection. Alternative methods of injection, especially oral route, are highly desirable compared to the injectable route due to their convenience and patient compliance. Although oral route presents many challenges due to the presence of gastrointestinal barriers, polymeric nanoparticles can be used to overcome pH and enzyme barriers, but intestinal permeability barriers remain a significant challenge. So far, many methods have been used to overcome the intestinal epithelial barrier to effectively deliver biologics and nanodrugs. In vitro experiments to investigate salt release from hydrocolloids appear to be reliable, acceptable, and reproducible and can be compared with different gel structures and various

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other materials in the future. Mucoadhesives, using the intestinal mucus layer, prolong the residence time in the intestine and increase the concentration of drugs near the surface of epithelial cells. In addition, many mucus adhesives are permeabilizers, which open the tight junctions between epithelial cells so that drugs and pharmacological agents can cross this barrier. Other approaches have focused on targeting natural transcytic pathways, including M cells, the vitamin B12 pathway, and the FcRn pathway. Recent studies have shown that targeting transcytic pathways can efficiently deliver drugs and nanodrugs orally, but further investigation is needed before these technologies can be used clinically. Oral drug delivery systems are being developed for many different applications, such as oral drug delivery of chemotherapeutic agents for cancer treatment, local drug delivery to the intestine for the treatment of inflammatory bowel disease, and oral mucosal vaccination. Many proteins, especially insulin for the treatment of diabetes, have been loaded into nanoparticles for oral administration. The successful development of nanoparticles for oral administration could change the treatment paradigm of many diseases and have a significant impact on future therapeutic outcomes. This study, while confirming slow release, showed that the maximum drug release occurred within 48 hours in an environment similar to physiological conditions and with isotonic conditions.

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