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Review Article

APPLICATION AND CHARACTERIZATION OF *IN SITU* GEL

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ABSTRACT

Applications of in situ gel have been used for a variety of drug delivery routes, such as oral, ocular, rectal, vaginal and injection. Characterization of *in situ* gel was determined to ensure that the prepared preparation met the standard and it safe. This review describes every aspects of this novel application and characterization of *in situ* gel preparations, which present the readers an exhaustive detail and might contribute to research and development. In the chemical evaluation *in situ* gel determined the diffusion of the active substance of a compound by measuring its concentration. In physical evaluation of isotonic calculated by osmotic pressure, drug release was determined by melting point of the substance polymer, gel strength as measured by rheometer, homogenecity test determined by under the light, and stability test with environmental conditions setting. In microbiology evaluation determine if the preparations was contaminated or not, also be effective and safe. Ocular irritation studies-Draize Test us an animal mice or rabbit and determination of visual appearance, clarity, and pH is required. *In situ* gels offer the primary requirement of a successful controlled release product that is increasing patient compliance.

Keywords: *In situ* gel, Draize test, Drug delivery system

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INTRODUCTION

The drug development with new dosage forms has always been done to provide effective and easy to use by patients. In addition, the presence of new drug preparations may increase bioavailability and reduce side effects. One of the discovery is a breakthrough of gel preparations with unique characteristics, such as *in situ* gel. Over the last decades, an impressive number of novel temperature, pH, and ion induced *in situ* forming solutions have been described in the literature. Each system has its own advantages and drawbacks. The choice of particular hydrogels depends on its intrinsic properties and investigated therapeutic use.

In situ gel is a new drug delivery system. When applied, the system is carried out in contact with the body, *in situ* gel will undergo phase change to gel due to conditions of pH, electrolytes and temperature [1]. *In situ* gel produces a constant plasma drug profile in the body by sustaining the release of drug so it is attached and absorbed in gel form and is known to prolong the life of the drug in mucose [2]. Another advantage of *in situ* gel is easy to use, simple manufacturing at the factory, and improve both adherence and patient comfort by minimizing the frequency of its use [3]. Applications of *in situ* gel have been used for a variety of drug delivery routes, such as oral, ocular, rectal, vaginal and injection.

Oral

Theophylline, in the form of *in situ* gels, is administered orally to mice and rabbits with gellan gum as sustained release vehicle. The process of gel formation occurs in acidic conditions in the stomach. The *in situ* gel forms of theophylline increase bioavailability four to fivefold in mice and up to threefold in rabbits [4].

Ranitidine hydrochloride with a combination of gellan gum results in increased viscosity of the preparation as the gellan gum concentration increases. Oral in situ gel is sensitive to the environment. At the time of administration, *in situ* gel is present as a solution with a low viscosity, but in a sensitive environment, there is a change in the conformation of the polymer into gel form. use of *in situ* gel may extend the contact time between the drug and its absorption site in the stomach by slowly releasing the drug. Therefore, *in situ* gel is very useful for treating chronic diseases [5].

Ocular

Levofloxacin, as an antibacterial agent, is made in the form of *in situ* gel with additional encapsulation techniques by chitosan nanoparticles (CH-NPS). It has proven to be used as an effective carrier for treating eye infections [6]. The *in situ* gel formulation of brimonidine tartrate along with carbopol composition polymer 974P and hidroxy propyl methyl cellulose (HPMC) K4M gives sustained release profile so it greatly affects the duration of action of the drug and improves the activity of decreasing intraocular pressure better than drop preparations to treat glaucoma [7].

Rectal

In situ gel Ibuprofen of solid dispersion in combination with poloxamer 407 (thermosensitive), HPMC E5, and sodium alginate (mucoadhesive) is known to have a better effect than solid suppository when administered to rabbits. It is also produce higher plasma peak and bioavailability concentrations. In accordance with histopathologic results, it was shown that the use of 15 mg/kg dose *in situ* gel ibuprofen did not produce irritation [8].

Nimesulide (2%) was prepared in situ gel preparations with addition of HPMC (0.5%) as mucoadhesive polymer, sodium alginate (Alg-Na) and poloxamer 407 (18%) as a temperature sensing agent. Polyethylene glycol (PEG) is added for gelation temperature modification and drug release properties. The addition of mucoadhesive polymers aims to address the shortcomings of poloxamer 407 with low bioadhesive ability and high permeability in water. Combinations all result in acceptable drug release, appropriate gel forming temperature, and rectal retention at the administration site. The dosage of in situ gel 20 mg/kg does not indicate mucosal irritation. Serum concentrations, C (max) and area under curve (AUC) Nimesulide increased significantly compared with solid suppositories [9].

Vaginal

Clindamycin HCl is prepared in *in situ* gel form with the addition of gellan gum as activated gelling polymer and HPMC (0.1%) ions as bioadhesive proven to produce non-irritating, bioadhesive preparations with good retention properties. The result of the formula had good transparency (refractive index 1.335-1.337),

display, clarity, and drug levels of 98.1-101% with accumulated drug release reached 98.9% after 12 h of use [10].

Treatment of vaginal candidiasis using clotrimazole *in situ* gel formed with mixture of poloxamer 407 and 188, also HPMC K100M or E50 is known to produce gel with good retention properties, which is the remaining of gel formulations remains in the vaginal mucose even after 24 h of application [11].

Injection

Simvastatin as a treatment of osteoporosis was made as biodegradable *in situ* gel with sub-cutaneous administration using chitosan polymer. Chitosan was used as biodegradable polymer and beta-glycerol phosphate disodium salt hydrate as a buffering agent to reach gelation process at pH and body temperature. The results showed the development of simvastatin *in situ* gel, which was administered subcutaneously, was effective for the treatment of osteoporosis [12].

Doxorubicin (DOX) with the addition of *zein* was made into *in situ* gel preparation administered through intratumoral injection. Doxorubicin is a drug commonly used for the treatment of colorectal cancer but has great side effects. The results of the test showed there was an effective accumulation of *in situ* gel DOX as an inhibitory agent in tumors with low concentrations of the drug in the blood and normal organs which potentially reduce the side effects of the drug [13].

Chemical evaluation

A chemical evaluation is needed to determine which drug or drug compound is efficacious to meet the required standards and to ensure safety in terms of contaminants [14].

Diffusion

Evaluation of this defuse system using the principle, testing the diffusion of the active ingredient of the gel preparation using a diffusion cell by measuring the concentration of the active ingredient in the receiving fluid at certain intervals [14].

Physical evaluation

Isotonic evaluation

Tonicity is related to the osmose pressure provided by a solution of a dissolved substance or solid [15]. Body fluids or eye fluids provide the same osmose pressure with normal osmose saline or 0.9% NaCl. A solution with a solute amount/more solute of body fluid/eye fluid has a greater osmose pressure and this solution is called a hypertonic solution [16]. Conversely, when the number of solutes is less so that the lower osmose pressure is called isotonic. Body fluids including eye fluids contain a number of solutes which can lower the freezing point of a solution of 0.52 °C. Similarly, 0.9% NaCl solution can reduce freezing point to 0.52 °C. Therefore 0.9% NaCl solution and body fluids are called isotonic. Several ways can be used to calculate the isotonic value (tonicity) of a solution, among others: a. Decrease of freezing point b. Equivalent NaCl Example of isotonic calculation with frost drops given an eyewash solution containing 1% boric acid. For 1% boric acid causes a decrease in freezing point of 0.29 °C [17]. Calculate the NaCl to be added to obtain an isotonic solution [18].

Drug-release

Evaluation of drug preparation is one drug drug release in the body. By knowing the time devastated and the polymer components used we can design the drug as per the needs of pharmacotherapy [19].

Gel strength

Based on research, the power of the gel depending on gelling agent to the mechanism used in the formula of a material. Gel strength can be measured with a rheometer. Placed in an aqueous gel or container and then given the pressure slowly so that the tool is immersed in the gel. Change the load on the tool can be measured as the strength of the gel [20, 21].

Homogeneity

Based on the research, examination of its homogeneity of a material can be done by putting the preparation between two glass objects then observed particle roughness under light. It aims to find out if the entire substance used in the formula is already spread or homogeneous [22].

Stability studies

Based on research, testing of stability aimed to know the time of storage and the use of a material. The sample is placed in a climatic chamber with a temperature of 40 °C and 75% RH for approximately one month. After a few months, the sample analyzed associated pH, viscocity, clarity, drug content, rheological, and *in vitro* dissolution [23, 24].

Spreading coefficient

Spreading coefficient was determined by a device or an apparatus. The device consist of a ground glass slide that was fixed on the wooden block. Each formulation of emulgel weighting about 2 g was placed and study on this ground slide. Gel preparation was then sandwiched between this slide and second glass slide having same dimension as that of the fixed ground slide. The second glass slide is provided with the hook. Weight of 1 g was placed on the top of the two slides for 5 min to expel air and to provide a uniform film of gel between the two slides. Measured quantity of weight was placed in the pan attached to the pulley with the help of hook. The time (in seconds) required by the top slide to separate from ground slide was noted. A shorter interval indicates better spreading coefficient [25].

The result is calculated by using the following formula:

$$S = \frac{(M \times L)}{T}$$ With: S= Spreading coefficient

M = Weight tied to upper slide

L = The length of the glass slides

T = Time taken to separate the slides.

Here are the composition of simulated tear fluid

1. Sodium chloride: 0.670 g

2. Sodium bicarbonate: 0.200 g

3. Calcium chloride dihydrate: 0.08 g

4. Deionized water: 100 g

Viscosity

The purpose of this study was to formulate in situ gel that previously we know that gels show thixotropic behavior, so that rheological research should be done [26].

Opthalmic solutions or eye drops that use polymers to increase viscosity can improve the bioavalability of the stock. Raising the viscosity works to slow the settling of particles and at the same time, the viscosity to maintain their suspense [27].

The formulation must be done before and after the gelation process using a Brookfield viscometer (RVT model) in a small adapter volume or a Cone and Platform geometry viscometer (Brookfield RVCP DV-III) [28]. Formulations that have viscosities ranging from 5 to 1000 mPas in solution form and also after being converted to gel, should be 50 to 50000 mPas. This test needs to be done at room temperature and temperature 37 °C. *In situ* gel preparations should show pseudoplastic flow and Newtonian before and after gelation [29]. The gel formulation *in situ* should be well formulated, so administration to the patient is good, especially in ocular administration [30].

However, viscosity agents have the disadvantage of making blurred vision and leaving residue on the eyelids. Overly high viscosity can also cause difficulties in screening used for [27].

Dissolution

In vitro release data will provide information about the system under test conditions. *In vitro* test conditions were made as closely as possible with *in vivo* conditions. This study will provide data on residence time, and related parameters of pharmacokinetics. These values will help in predicting the vivo performance of the dissolution system [31].

Dissolution profile using zero order. First-order Higuchi and Korsmeyer Models to ensure pharmacokinetic modeling. The Korsmeyer-Peppas equation describes a relationship that describes drug release from polumeric to know the mechanism of drug release. N-values are used to characterize the release mechanism. The value of 0.45 ≤ n corresponds to a Fickian diffusion, whereas a value of 0.45<n<0.89 non Fickian transport. If the value of n = 0.89 is relational and n>0.89 of case II transport [31].

Gel *in situ* dissolution test was performed using a Franz diffusion device and as a phosphate buffer solution (pH 7.4). pH phosphate buffer 7.4 will simulate lachrymalfluid. Temperature can be adjusted and maintained, 37±0.5 °C with rotation speed at 100 rpm. Take samples at intervals of time, then analysis using spectrophotometry for drug content [32].

Texture analysis

The purpose of texture analyses is to provide information about mechanical properties of samples, namely hardness, compressibility and adhesiveness. These properties can be directly correlated with Sensory parameters *in vivo* and therefore, are valuable in the development of product with desirable attributes that contribute to patient acceptability and compliance [33]. The consistency, firmness and cohesiveness of *in situ* gelling system is assessed by using a device called texture profile analyzer. Higher values of adhesiveness of gels are needed to maintain intimate contact with mucus surface. Texture analysis provides information on mechanical properties of gel [34].

Determination of visual appearance, clarity, and pH

Visual appearance were determined by various ways, the first method is to visually check the product under fluorescent light with black and white as the background in a cabinet that have sufficient light. Second method involve checking the pH with a device called pH meter. Each formulation was check by dispersing 2.5 g of the formulation in 25 ml of purified water. The pH meter must be calibrated before use with buffered solution at pH 4 and 7 [35]. And lastly, gelling capacity of formulations was evaluated in order to identify the formulations suitable for use as *in situ* gelling systems. Gelling capacity was determined by mixing the formulation with simulated tear fluid in the proportion 25:7 and examined visually [25].

Gelling capacity

Gelling capacity of formulation is determined by placing a drop of the formulation in a vial containing 2.0 ml of freshly prepared simulated tear fluid (STF) with the composition of the artificial tear fluid is Sodium chloride 0.670 g, Sodium bicarbonate 0.200 g, Calcium chloride dihydrate 0.008 g, and purified water q. s. 100 g) [36], and time taken for its gelling is noted [1]. It can also be determined by placing a drop of formulation in the vial containing 2 ml of phosphate buffer 7.4. Note the time required to form a gel and also note the time taken by the formed gel to dissolve [37]. It can also be determined by using visual method the *in vitro* gelling capacities were determined. The colored solutions such as (Amaranth dye, Congo red dye and indigo blue dye) can be used by dissolving 1g of dye in distilled water and mixed with the formulation of *in situ* gel. The *in vitro* gelling capacity of the formulations were measured by placing 5 ml of gelation solution (STF fluid 7.4) in the glass test tube and maintained the temperature at 37 °±2 °C. 1 ml of dye solution is taken in the pipette and added into the gelation solution (STF fluid 7.4); it immediately converted into stiff gel like appearance. The *in vitro* gelling capacities were evaluated by the appearance of stiffness of the gel. And the time period for which the gel converted into stiff gel remains as such. Further, the color was added to give the visual appearance to the gel. Based on the 3 categories the *in vitro* gelling capacity time period was calculated [38].

1. (+) Gel forms after few min, disperses rapidly.

2. (++) Immediately gelation occurs, remains for few h.

3. (+++) immediately gelation occurs, remains for extended period of time

Microbiology evaluation

Sterility testing

Based on research done by the sterility test, observe the turbidity of growth medium and compared with positive and negative control. According to the Indian Pharmacopoeia and British Pharmacopoeia, microbial growth media used is thioglycolate medium and soy-bean digest medium. This testing is done with the technique of aseptic work to avoid contamination of the environment. The method used is the direct inoculation by way of inoculating the sample into liquid media by comparison (1:10) and the media is incubated. Incubation time can be done 7–14 d with different temperature; thioglycolate medium (30 °–35 °C) and soy-bean digest medium (20 °–25 °C) to identify anaerobic and aerobic microbial growth [14, 39-41].

Antifungal studies

Based on research associated efficiency testing, antifungal performed according to the purpose of pharmacological active substances in preparations. On testing, antifungal used saboured dextore dissolved in hot water and media was autoclaved for 15 min. Insert organism namely *Candida albicans* and *Aspergillus fumigatus* in media in order and put a sample test with mikropipet and let sit for 30 min and then incubated at a temperature of 25 °C for 24 h. The diameter of the inhibition zones jamus is measured and compared to the positive and negative control [42].

Antibacterial activity

Based on the research by Meshram and Thorat, testing was conducted to find out the effectiveness of antibacterial or antibiotic active substances used in the preparation of the gel *in situ* in concentrations that can be referred to as antibacterial. Testing is done by comparing the results of the growth of bacteria from samples with standard antibiotics [43].

Ocular irritation studies-draize test

For the ocular irritation test, Albino rabbit (Newzeland white rabbit) are used as test species. One eye is designated the test eye; the contralateral eye serves as a matched control and is usually left untreated. Single drop approximately 0.04 ml is instilled into the lower conjunctival *cul-de-sac*; normal blinking is allowed, although the eyelids can be held together for several seconds after instillation. Observations was done at 1, 24, 48, 72 h one week after exposure. Ocular changes were graded by a scoring system that includes rating any alterations to the eyelids, conjunctiva, cornea, and iris [44].

Statistic

The result form experiment mucoadhesive strength and release studies were analysed by statistical multivariate test. To get significant difference, using various SPSS software and considered significant at $p<0.05$ [1].

To test long-term storage conditions can use one-way anova analysis of viscosity data using prism with significance value ($p>0.05$). But there is no general rules regarding this statistical test. This test is based on the data obtained [45].

CONCLUSION

Evaluation of *in situ* gel is determined to ensure that the prepared preparation meets the standard and is safe. In the chemical evaluation *in situ* gel determined the diffusion of the active substance of a compound by measuring its concentration. In physical evaluation of isotonic calculated by osmotic pressure, drug release is determined by melting point of the substance polymer, gel strength as measured by rheometer, homogenecity test determined by test under light, and stability test with environmental conditions setting. In microbiology evaluation determine if the preparations is contaminated or not, also be effective and safe. Ocular irritation studies-Draize Test us an animal (mice or *et al.*) and determination of visual appearance, clarity, and pH is required. For analytic test about statistic determination with SPSS software with significant $p<0.05$ that means data can be accepted.

AUTHORS CONTRIBUTIONS

All the author have contributed equally

CONFLICT OF INTERESTS

Declared none

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