RESEARCH ARTICLE

Preparation, Characterization of Bacterial Cellulose and Chlordiazepoxide Tablets for Oral Drug Delivery

Dada Kodalkar[1*], Ajit Kulkarni[2], Birendra Shrivastav[3], Harshal Tare[4]

[1]*Department of Pharmaceutical Chemistry, Jaipur National University, Jaipur, Rajasthan, India.*
[2]*Department of Pharmaceutics, Gourishankar Institute of Pharmaceutical Education and Research, Limb, Satara, Maharashtra, India.*
[3]*Department of Pharmaceutics, Jaipur National University, Jaipur, Rajasthan, India.*
[4]*Department of Pharmacognosy, Sharadchandra Pawar College of Pharmacy, Otur, Affiliated to Savitribai Phule Pune University, Pune, Maharashtra, India.*

Received: 08th October, 2022; Revised: 20th November, 2022; Accepted: 03rd December, 2022; Available Online: 25th December, 2022

ABSTRACT

Bacterial cellulose (BC) is a highly purified form of cellulose that offers several benefits, including an ultrafine fiber network, a high water retention capacity, and simple fabrication into desired forms. There has been a recent uptick in BC's focus on drug delivery for biomedical applications. Studies on prodrug design, controlled-release methods, and pill coating have all been conducted for BC. This research was done to determine if BC might be employed as a medication carrier in chemical and biological formulations. BC microparticles laden with the medication were produced *via* grinding. Clordiazepoxide hydrochloride (CDP) was used to test the microparticles' drug loading and release capabilities. Particle morphologies, drug loading efficiency, loaded drug physical state, and drug release behavior were all assessed for the manufactured microparticles. Amorphous microparticles were formed from BC microparticles. An scanning electron microscope (SEM) study found that the microparticles had a nearly round form with an average diameter of 400–500 µm. Drug loading was greater in the renewed BC microparticles (37.57 ± 0.22%) when the concentration of drugs was held constant at 10%. More than 85% of the medication was delivered for all formulations in the first 30 minutes.

Keywords: Bacterial cellulose, Chlordiazepoxide, Drug delivery, Immediate release, Microparticles.

International Journal of Pharmaceutical Quality Assurance (2022); DOI: 10.25258/ijpqa.13.4.11

How to cite this article: Kodalkar D, Kulkarni A, Shrivastav B, Tare H. Preparation, Characterization of Bacterial Cellulose and Chlordiazepoxide Tablets for Oral Drug Delivery. International Journal of Pharmaceutical Quality Assurance. 2022;13(4):408-412.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Dopamine, norepinephrine, and serotonin are neurotransmitters that are directly affected by antidepressants. It is possible to track the spread of these medicines throughout the globe by observing their rising prevalence in urban water systems. In addition, they have been found in non-urban streams, such as rivers and seas, as well. Various antidepressant medicines have been bioaccumulated in the tissues of certain indigenous aquatic creatures, such as some fish and mollusc. The spread of COVID-19 pandemic has boosted the incidence of sadness and anxiety throughout the globe, which led to increase in antidepressants consumption and their availability in the environment.[1-4]

A tricyclic antidepressant, chlordiazepoxide is a (7-chloro 2-methylamino)-5-phenyl-3-H-1,4 benzodiazepine 4-oxide tricyclic antidepressant. Gamma-aminobutyric acid (GABA) receptor complexes in the limbic system and temporal lobe include stereospecific binding sites for benzodiazepine binding sites. An increase in GABA-mediated chloride influx through the GABA receptor channel causes membrane hyperpolarization when the inhibitory neurotransmitter GABA binds more strongly to GABA receptor Benzodiazepines Benzodiazepines (BZDs).[5,6]

It's the first (benzodiazepine derivative) that has been approved for use in the treatment of psychiatric disorders. As a result of its pharmacological biotransformation into a variety of active metabolites, chlordiazepoxide has a convoluted metabolic route, including desmethylchlordiazepoxide, demoxepam, desmethyldiazepam, and oxazepam. This drug has an elimination half-life (t1/2β) of 5 to 30 hours in healthy adults, and a distribution volume of 0.25 to 0.50 liters/kg after a single dosage. The hepatic extraction rate is less than 5%. The first bioactive metabolite is formed when the parent molecule is eliminated.[7]

Elderly, cirrhotic, and disulfiram-using patients had slower clearance of chlordiazepoxide and a longer half-life

**Author for Correspondence: dadakodalkar.94@gmail.com*

for (t1/2β). Internal injection is unpleasant and results in sluggish and unpredictable absorption of intramuscularly administered chlordiazepoxide. Two or more active metabolites of chlordiazepoxide are accumulated after many doses of the parent drug. Accumulation rates and levels are very variable across people.[8–10]

More time and resources are required in traditional formulations because of the several processing processes and high-priced equipment and energy inputs. Cost rises are a result of all of the variables listed above. The design of dosage forms requiring fewer excipients and processing stages and lower labor and equipment costs is thus critical. Bacterial cellulose (BC) could be used to create a streamlined polymer-based drug delivery system. BC, also known as bio-cellulose or microbiological cellulose, is cellulose in its purest form. It is produced in the form of enlarged membranes by some microorganisms belonging to the genera agrobacterium, rhizobium, sarcina, and gluconacetobacter. Because of the robust hydrogen connections both in between and within molecules, BC possesses unique macromolecular structural properties such as thin sub fibrils that produce microfibrils. The BC's hydrophilicity is accelerated by the addition of many hydroxyl groups on its surface. Because of their greater thickness and absence of a well-defined microfibrillar structure, PC fibers do not have the same amount of surface hydroxyl groups as nylon. As a result, Unlike PC, which is exclusively made up of the Ib form of cellulose, BC contains both Ia and Ib cellulose. In addition, BC has a greater degree of polymerization and a more crystalline structure than PC.[9-12]

Oral drug delivery matrices based on BC have yet to be investigated, even though it has been explored as a hydrogel for this purpose and in membrane form for the transport of medicines and proteins. Due to its exceptional properties, such as its high porosity and surface area, high transparency, and ability to retain water, BC is an excellent choice for the preparation of single excipient-based pharmaceutical formulations.[13–15]

An effort was made in the present work to solve the typical tablet dosage form issues by making single excipient-based BC matrixes filled with varied doses of medicines and conducting characterization and *in-vitro* release tests. Using BC as a matrix in oral medication administration, this study offers fresh insights into its prospective uses.

MATERIALS AND METHODS

Materials

Acetobacter Xylinum is used to make BC at the company's headquarters. Centaur Pharmaceuticals Pvt Ltd in India sent a complimentary sample of chlordiazepoxide HCL (CDP). hydroxypropyl methylcellulose (HPMC) was supplied by Dr. Reddy's Laboratory (hydroxypropyl methylcellulose). Midland and methylparaben are made by S.D. Fine Chemicals in Mumbai. Analytical-grade reagents were used for everything else.

Preparation of Granules

Table 1 shows three basic formulations, each containing varying amounts of cellulose polymorph and chlordiazepoxide HCl. 10, 50, and 90% (w/w) were the drug%. HPMC at a concentration of 2% was utilized as a dry binder in all formulations, with distilled water serving as the granulation liquid. At regular intervals, peristaltic pumping was utilized to provide precise doses of granulation liquid. The whole blend was mixed for five minutes at a rotor frequency of 430 rpm. Following the initial period of mixing, simultaneous measurements of liquid addition and power consumption began. The procedure ended when enough samples of granule diameters had been obtained.

Table 1: Formulations with different drug-to-excipient ratios

Ingredients	*Percentage per weight (% w/w)*		
Chlordiazepoxide HCL	10	50	90
Cellulose	88	48	08
HPMC	02	02	02

Preparation of BC and Chlordiazepoxide Tablets

An 8 mm concave-faced punch (R=5.5) with an edge was used in a 6-station rotating pill crusher to smash the tablets. It took three minutes of mixing in the turbula blender with 0.1% magnesium stearate (w/w) before compression. For safety reasons, no glidant was utilized, and just a handful of lubricants were utilized. A steel conical feeder was used to pour the material into the machine. Maintaining a constant turret rotation speed of 19 rpm during operation and making adjustments to filling depth and tablet thickness for each formulation ensured that tablets of all formulations had comparable physical properties, including porosity. Each of the tablets weighs 200 mg.

Characterization of Granules

Granules' micrometric characteristics a fine tablet's production relies heavily on powder flow. Determining bulk density, tap density, and the material flow parameters were evaluated based on the angle of repose of the grains and other factors.

Particle Size Determination

Wet granulates were dried for around 10 hours at 40°C in a dish dryer before being sieved in ISO-norm sieve oscillator-type equipment with eight selected sieves ranging in mesh size from 1000 m to 90 m. (Heraeus Instruments). To calculate the particle size distribution, we divided the sample's entire mass by the sieves' total weight. Further compression with a desired granular particle size between 125 and 710 m (i.e., cumulative samples from sieves 125 to 500 μm).

Scanning Electron Microscopy (SEM)

Clinical Development Plan (CDP) and cellulose were photographed using a JSM 6390 scanning electron microscopy with a 10-kV accelerating voltage (JEOl, Japan).

Moisture Content

In order to determine the quantity of moisture that was lost during drying, thermogravimetric analysis was utilised (Mettler-Toledo LP16M, Mettler Instruments). During granulation and immediately before compression, 1 to 2 g samples were warmed at 105°C for 20 minutes. As a proportion of the overall weight, the water loss was calculated.

$$\text{Moisture sorption capacity (\%)} = \frac{W_2 - W_1}{W_1} \times 100$$

Bulk Density

Weighing and measuring the mass and size of an uncompacted pre-sieved powder mix were used to determine its bulk density. The bulk density unit is g/mL, which is defined by

Db = M V0

To calculate the bulk volume of powder, we need to know its mass, M, and its volume, V_0, or its total volume, a.k.a. its density.

Tapped Density

Mechanical tapping was used to determine the density of the powder mix by putting a known quantity of powder blend into an open graduated cylinder and tapping it. After tapping, the powder's compact volume was measured. G/mL (g/mL) is another way to express tapped density.

Dt = M Vt

Where M is the amount of powder and Vt is the amount of powder that was tapped.

Angle of Repose

The angle of repose was measured using the funnel method. The powder made a cone at the top when it fell out of the funnel. The angle of repose was calculated using the diameter and height of the pile and the following formula.

tan θ = h/r

where h is the height of the pile of powder
r = the powder pile's diameter
θ = the angle of repose

Compressibility Index (Carr's Index)

It is possible to calculate Carr's index (CI) using the bulk and tap densities. Material flowability increases linearly with compressibility.

CI = (TD-BD) x 100/TD

where, TD is the tapped density and BD is the bulk density.

Hausner's Ratio

As the tapped density increases, so does its bulk density. For this reason, Hausner observed that this ratio might be used to predict powder flow qualities. Favorable flow qualities are indicated by an index value lower than 1.25 (20% of Carr's index).[16-18]

Hausener's Ratio = Tapped density/Bulk Density

Evaluation of BC and ChlordiazepoxideTablets

General Appearance

Each formulation batch's tablets were examined to see how they looked in general. Shape and color were shown to be the most important visual appearance factors.

Uniformity of Weight

Randomly picked twenty tablets were weighed one at a time. The mean mass was also taken into consideration. The proportion of tablets that de*via*ted from standard specs was computed and compared.

Weight Variation Test

A 20 pills from each composition were weighed using an electronic scale to examine weight variance. Weight (WA) measurements were taken and averaged. The formula for calculating percentage weight change was as follows. The weights of the pills were weighed and their averages and standard de*via*tions were determined.

% Weight variation = (WA–WI) x 100/ WA

Thickness

From the representative sample, twenty random pills were measured for thickness with a digital vernier caliper. The data were looked at to find out what the average thickness was and what the standard de*via*tion was.

Hardness

The tablet's hardness was measured with Monsanto's hardness tester. From each batch 6 tablets were tested to see how hard they were, and the average and standard de*via*tions were written down.

Friability Test

The device used for the friability test was used to weigh and place 10 tablets from each batch (Roche Friabilator). While the machine was running, tablets were turned at 25 rpm for four minutes. After 100 turns, the tablets were taken out, wiped clean, and weighed again. The amount of weight loss was used to figure out how fragile it was.

Note that no tablet should stick to the walls of the device. If this is the case, dust the walls with talcum powder. Because of this, there should also be no caps.
% Friability was calculated as follows

% Friability = (W1 – W2) x 100/W1

Where W1 is the weight of the 20 tablets at the beginning.
W2 = The 20 tablets' final weight after the test.
Most of the time, values of friability below 0.8% are fine and acceptable.

Stability Study

High-density polyethylene closed covers were used to keep an optimized batch of clorazepoxide and BC tablets for short-term stability studies per the International Committee for Harmonization (ICH) guidelines. *In-vitro* dissolution, appearance, and drug content were assessed at 6 and 12 weeks of storage.[19-22]

RESULT AND DISCUSSION

Particle Size Determination

Figure 2 and 3 shows a comparison of the three formulas' total particle size distributions. The range or dispersion of similar dimensions was lower for the composition with the greatest cellulose concentration than in the other two drugs. For 50% weight drug granules, the PSD was more closely aligned with the distribution of 90% weight

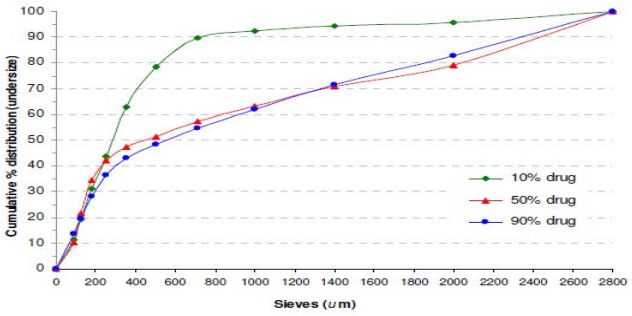

Figure 1: Cumulative percental frequency *undersize* distribution curves

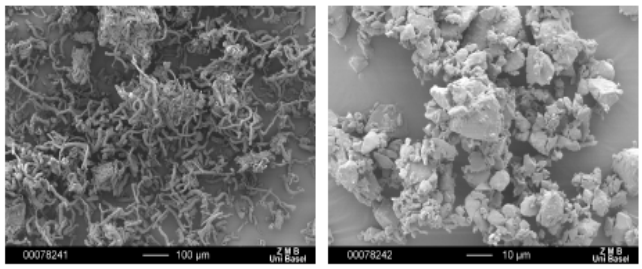

Figure 2: SEM Pictures of BC and Chlordiazepoxide Granules

Table 2: Physical Properties of Granules

Formulations	*Angle of repose (°)*	*Bulk Density (g/mL)*	*Tapped Density (g/mL)*	*Carr's Index (%)*	*Hausner's ratio*	*Particle Size D10 (µm)*	*Particle Size D90 (µm)*	*Loss on Drying*
F1	26.43	0.375	0.442	15.15	1.17	47.76	280.76	5.12
F2	28.77	0.533	0.617	13.61	1.15	35.87	365.87	5.09
F3	19.29	0.434	0.497	12.67	1.14	38.09	396.54	5.87
F4	21.25	0.520	0.582	10.65	1.11	43.76	368.98	5.43
F5	26.43	0.412	0.483	14.69	1.17	23.76	354.98	5.30

drug granules than with the 10% weight drug granules. Chlordiazepoxide HCL had a larger effect on agglomeration than cellulose in this formulation. As a result, 55.48% of the medication volume exceeded the cellulose content (42.46%).

Scanning Electron Microscopy (SEM)

UICEL particles have a broad contact area because of long fibres (aggregated and non-aggregated), as seen in Figure 2. Because it is made up of much smaller particles than cellulose, chlordiazepoxide HCl powder has an aggregated structure that may be used to make an array of different materials. A high surface-to-to-mass ratio supports the cohesion of chlordiazepoxide HCl, which is why it was discovered to be a microfine powder (90% of the particles were smaller than 45 m).

The granules of BC and Chlordiazepoxide were tested for their angle of repose, bulk density, tapped density, Carr's index, and drug content. All batches of granules had an angle of repose of less than 35° and a Carr's index value of less than 21, which means that they could flow and be compressed well to a medium degree. All the batches had a Hausner's ratio of less than 1.25, meaning they flowed well. All of the different types of granules had at least 90% of the drug in them.

Evaluation of BC and Chlordiazepoxide Tablets

Granules with higher concentrations of chlordiazepoxide HCl were projected to be softer, which resulted in tablets with smoother surfaces and fewer fractures and fissures. There are few bumps or protrusions can be seen. Fissures seemed to expand when the amount of cellulose in the composition was raised, and the major granules could be clearly seen on the surface, separated by dips. According to Narayan and Hancock, the MCC particles' very irregular form and surface morphology prevented tight packing during compression, resulting in a compact surface's high roughness. The brittle

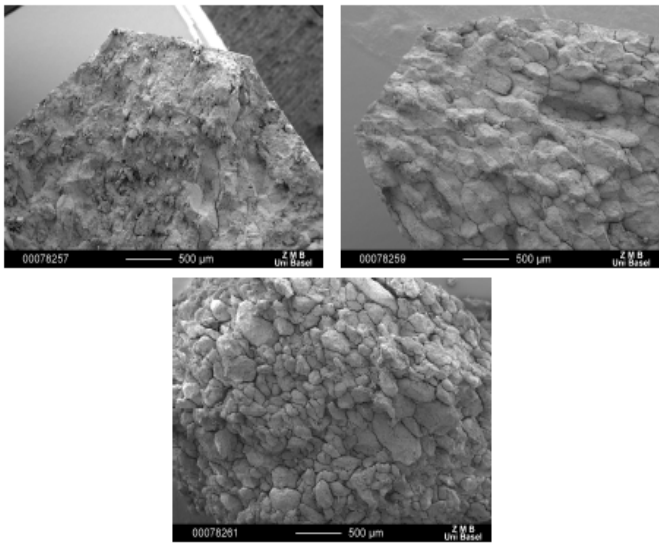

Figure 3: SEM Pictures of BC and Chlordiazepoxide internal Tablets

powders generated smooth-surfaced compacts, whereas ductile materials formed rough-surfaced ones.

An analysis of tablet weight, hardness, thickness and friability as well as medication content is presented in Table 3 for your perusal and review. Because they ranged in weight from 198.76 to 210.32 mg, all of the pills in the various batches met the regulatory standards for weight consistency. Hardness varied from 5.01 to 6.76 kg/cm^2 and friability values were slightly less than 0.8%, indicating that the matrix tablets were compacted and hard. From 2.88 to 3.40 mm, the tablets' thickness varied. Thus, the physical properties of the prepared tablets were found to be almost completely under control.

Table 3: Physical Evaluation of BC and Chlordiazepoxide Tablets

Formulations	*Hardness (kg/cm2)*	*Thickness (mm)*	*Weight (mg)*	*Friability (%)*	*Drug content (%)*
F1	5.66 ± 0.55	3.20 ± 0.20	118.8 ± 1.64	0.12	101.22 ± 0.88
F2	5.50 ± 0.31	3.37 ± 0.25	120.4 ± 0.54	0.39	95.28 ± 0.80
F3	4.41 ± 0.60	3.32 ± 0.89	119.0 ± 0.43	0.37	95.35 ± 1.14
F4	4.08 ± 0.30	3.33 ± 0.25	119.2 ± 0.83	0.58	99.53 ± 1.87
F5	5.00 ± 0.44	3.38 ± 0.73	120.5 ± 0.80	0.77	96.34 ± 2.18

Stability Study

Over the course of 12 weeks, the hardness, swelling index, and release study of the new formulations were all looked at. During the 12-week stability assessment, there were no big differences between the three batches in terms of hardness, swelling index, or *in-vitro* release. There are three stable formulations (F-5).

CONCLUSION

All BC and chlordiazepoxide Pill formulations demonstrated quick drug release in our testing, with more than 85% of medication released in the first 30 minutes. Reduces blood level fluctuations, dose-related side effects, and costs, which in turn improves patient compliance and therapeutic efficacy. According to ICH recommendations, a stability study was carried out, and no physical changes or considerable drug loss were found. In other words, the formulation has shown to be stable throughout testing. As a result, the polymer might be used as a novel effective medication-release retardant with improved patient compliance.

REFERENCES

1. Amin MC, Abadi AG, Katas H. Purification, characterization and comparative studies of spray-dried bacterial cellulose microparticles. Carbohydrate polymers. 2014 Jan 2; 99:180-9.
2. Apfelbach R. Instinctive predatory behavior of the ferret (Putorius furo L.) modified by chlordiazepoxide hydrochloride (Librium®). Psychopharmacology. 1978 Jan;59(2):179-82.
3. Badshah M, Ullah H, Khan AR, Khan S, Park JK, Khan T. Surface modification and evaluation of bacterial cellulose for drug delivery. International journal of biological macromolecules. 2018 Jul 1; 113:526-33.
4. Badshah M, Ullah H, Khan SA, Park JK, Khan T. Preparation, characterization and *in-vitro* evaluation of bacterial cellulose matrices for oral drug delivery. Cellulose. 2017 Nov;24(11):5041-52.
5. Bencan Z, Sledge D, Levin ED. Buspirone, chlordiazepoxide and diazepam effects in a zebrafish model of anxiety. Pharmacology Biochemistry and Behavior. 2009 Nov 1;94(1):75-80.
6. Brauer R, Alfageh B, Blais JE, Chan EW, Chui CS, Hayes JF, Man KK, Lau WC, Yan VK, Beykloo MY, Wang Z. Psychotropic medicine consumption in 65 countries and regions, 2008–19: a longitudinal study. The Lancet Psychiatry. 2021 Dec 1;8(12):1071-82.
7. Ford JL. Design and evaluation of hydroxypropyl methylcellulose matrix tablets for oral controlled release: A historical perspective. In Hydrophilic matrix tablets for oral controlled release 2014 (pp. 17-51). Springer, New York, NY.
8. Greenblatt DJ, Shader RI, MacLeod SM, Sellers EM. Clinical pharmacokinetics of chlordiazepoxide. Clinical pharmacokinetics. 1978 Oct;3(5):381-94.
9. Greenblatt DJ, Shader RI, MacLeod SM, Sellers EM. Clinical pharmacokinetics of chlordiazepoxide. Clinical pharmacokinetics. 1978 Oct;3(5):381-94.
10. Köhler-Forsberg O, Gasse C, Hieronymus F, Petersen L, Christensen RH, Nierenberg AA, Østergaard SD. Pre-diagnostic and post-diagnostic psychopharmacological treatment of 16 288 patients with bipolar disorder. Bipolar Disorders. 2021 Jun;23(4):357-67.
11. Li FQ, Hu JH, Deng JX, Su H, Xu S, Liu JY. *In-vitro* controlled release of sodium ferulate from Compritol 888 ATO-based matrix tablets. International journal of pharmaceutics. 2006 Nov 6;324(2):152-7.
12. Maddiboyina B, Jhawat V, Sivaraman G, Sunnapu O, Nakkala RK, Naik MH, Gulia M. Formulation Development and Characterization of Controlled Release Core-in-cup Matrix Tablets of Venlafaxine HCl. Current Drug Therapy. 2020 Oct 1;15(5):503-11.
13. Rabeea SA, Merchant HA, Khan MU, Kow CS, Hasan SS. Surging trends in prescriptions and costs of antidepressants in England amid COVID-19. DARU Journal of Pharmaceutical Sciences. 2021 Jun;29(1):217-21.
14. Salari M, Khiabani MS, Mokarram RR, Ghanbarzadeh B, Kafil HS. Development and evaluation of chitosan based active nanocomposite films containing bacterial cellulose nanocrystals and silver nanoparticles. Food Hydrocolloids. 2018 Nov 1; 84:414-23.
15. Tiwari SB, Murthy TK, Pai MR, Mehta PR, Chowdary PB. Controlled release formulation of tramadol hydrochloride using hydrophilic and hydrophobic matrix system. aaps Pharmscitech. 2003 Sep;4(3):18-23.
16. Ullah H, Badshah M, Correia A, Wahid F, Santos HA, Khan T. Functionalized bacterial cellulose microparticles for drug delivery in biomedical applications. Current pharmaceutical design. 2019 Sep 1;25(34):3692-701.
17. Ullah H, Badshah M, Mäkilä E, Salonen J, Shahbazi MA, Santos HA, Khan T. Fabrication, characterization and evaluation of bacterial cellulose-based capsule shells for oral drug delivery. Cellulose. 2017 Mar 1;24(3):1445-54.
18. Ullah H, Santos HA, Khan T. Applications of bacterial cellulose in food, cosmetics and drug delivery. Cellulose. 2016 Aug;23(4):2291-314.
19. Vidyadhara S, Rao PR, Prasad JA. Formulation and evaluation of propranolol hydrochloride oral controlled release matrix tablets. Indian journal of pharmaceutical sciences. 2004;66(2):188.
20. Walker AJ, Croker R, Curtis HJ, MacKenna B, Goldacre B. Trends in antidepressant prescribing in England. The Lancet Psychiatry. 2021 Apr 1;8(4):278-9.
21. Wen H, Li X, Li Y, Wang H, Wang Y, Wang T, Pan W, Yang X. *In-vitro* and *in-vivo* evaluation of controlled-release matrix tablets of highly water-soluble drug applying different mw polyethylene oxides (PEO) as retardants. Drug development and industrial pharmacy. 2018 Apr 3;44(4):544-52.
22. Zeng WM. Oral controlled release formulation for highly water soluble drugs: drug–sodium alginate–xanthan gum–zinc acetate matrix. Drug development and industrial pharmacy. 2004 Jan 1;30(5):491-5.