



## Formulation and in-vitro evaluation of Propranolol HCl mucoadhesive vaginal beads for contraceptive purposes

Eman Gomaa<sup>1\*</sup>, Mennatullah M. Faisal<sup>1</sup>

<sup>1</sup> Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Zagazig University, Zagazig 44519, Egypt

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### ABSTRACT

**Purpose:** Based on the clinical findings on the efficacy of propranolol Hcl as a potent spermicide, the present study was designed to develop new sustained-release intravaginal propranolol-HCl-loaded alginate beads for contraceptive purposes. **Method:** Mucoadhesive vaginal beads were developed using an ionotropic gelation method with different ratios of the mucoadhesive polymer (Na alginate) to the cross-linking agent (calcium chloride). The prepared beads were examined and evaluated for their particle size, pH, entrapment efficiency % and yield percentage, in-vitro bioadhesion, and percentage of swelling and tested for stability.

**Results:** The prepared formulations revealed micro-sized beads with a yield percentage exceeding 90%, entrapment efficiency was much influenced by the drug: polymer ratio as well as CaCl<sub>2</sub> concentration, pH ranged from 4.8±0.1 to 5.6±0.1 which was acidic enough to cause inhibition of sperm motility and cause spermicidal action, and satisfactory swelling percentage with optimum bioadhesive strength for good intravaginal application. The in vitro release of the drug proceeded over 8 hours with different release percentages according to the incorporated polymer and crosslinking agent. The critical impacts of increasing polymer concentration on increasing the EE% and mucoadhesion were emphasized. The developed beads showed acceptable stability results under the studied storage conditions.

**Conclusion:** Propranolol HCl-loaded alginate beads were suitable for vaginal application and suggested a promising formulation for spermicidal contraceptive purposes.

## 1. Introduction

At the present time, population growth restraint is one of the most important issues that occupy the world health arena. This placed on the shoulders of

scientists in the field of the pharmaceutical industry the necessity of manufacturing and developing pharmaceutical dosage forms to confront this issue.

From that point forward, it became imperative to design and formulate a new safe, economical and safe vaginal contraceptive [1].

Spermicides are reversible, temporary, non-prescription methods of contraception. The word *spermicide* means "sperm killing", and its mechanism is based on establishing a physical barrier hindering sperm motility besides its negative impact on the sperm structure. These physical barrier contraceptives have an advantage over the traditional hormonal ones by not affecting or disturbing a woman's menstrual cycle, and the chance for pregnancy returns by just their stop. Barrier methods are also cheap compared with the hormonal ones. The manufacturing of spermicides has evolved over decades, from the first use of gum, animal dung, and different acidic compounds, passing by using a mixture of quinine sulfate, cocoa butter, and lactic acid, till the development of a classic spermicide dosage formulation which composed of an active ingredient and inactive base. [2]

The serious side effects of various spermicidal contraceptives make them unacceptable for use, despite being safe contraceptives. Nonoxynol-9 is a surfactant considered the main active ingredient and the most potent spermicide causing disruption in the sperm membrane structure. Unfortunately, as a spermicide it causes irritation of vaginal mucosa and genital ulcerations and this consequently may give a risk for sexually transmitted infections as HIV. [3]

Therefore, current recommendations have appeared to direct the pharmaceutical industry towards the formulation of vaginal spermicides with no detergent-type membrane toxicity which could present a superior clinical benefit over the detergent-type spermicides available right now. [4]

The key advantage of vaginal drug delivery is the avoidance of harsh gastric environment and the ability to bypass hepatic first-pass metabolism caused by oral route for drug delivery. All commercial spermicidal products are short-acting products; either foam, creams, gel, vaginal suppositories, and vaginal film [5], so the main target of the present study is to formulate long lasting vaginal contraceptive acting as a physical barrier.

Propranolol HCl, a  $\beta$ -adrenergic blocker, has already been approved by FDA for human use, and is indicated in the treatment of cardiovascular symptoms. Recently, this drug was proven to have an impressive in-vitro inhibition of human sperm motility and has the same in-vivo effect in rats, regarding to acting as a local anesthetic or its membrane stabilizing activity rather than its  $\beta$ -blocking potential. A concentration of 0.3 mM of propranolol HCl could inhibit sperm movement by 50% (IC<sub>50</sub>) with no risk of eliciting side effects as those recorded from other contraceptives. [6]

For its clinical efficacy and tolerability, several studies have already been established. Zipper and co-workers, (1983) [7], declared the potency and efficacy of 80 mg propranolol HCl vaginal contraceptive tablets on 198 fertile women for 11 months, with no reported adverse effects [7]. Based on a previous study conducted to observe any possible side effects of propranolol HCl tablets after insertion into the vagina on six healthy women, None of them complained of vaginal irritation, sensation or any other related symptoms. No postural symptoms, breathlessness or wheezing were noticed. (1)

Many other studies to justify the absence of any serious side effects were conducted and no complain was reported or noticed. [8]

Based on the previous clinical trials and the prevalence of propranolol HCl efficacy and safety as a novel spermicidal contraceptive at a dose of 80 mg [7], the purpose of the present study is to prepare, and in-vitro evaluate long-lasting mucoadhesive pharmaceutical formulations of propranolol HCl for vaginal administration to overcome the poor retention of the conventional marketed formulations. [9]

## 2. Materials and Methods

Propranolol HCl was supplied as a gift from (Sigma Aldrich Co.Ltd. (UK), [AstraZeneca-Egypt), Sodium alginate was obtained from was kindly supplied by (E.P.I.C.O), Egypt. Calcium chloride was purchased from (El-Nasr Co.,Egypt)

### 2.1 Preparation of Na alginate mucoadhesive vaginal beads

Propranolol HCl-loaded beads were prepared using the ionotropic gelation method. Exactly 80 mg of

Propranolol HCl was dispersed in Na alginate solution at different concentrations as illustrated in Table (1), and magnetically stirred at 100 rpm till uniform dispersion was obtained. The prepared dispersion was sonicated to be free from any air bubbles produced during the stirring step. A syringe of a needle size of 22G was filled with the dispersion through which it is extruded dropwise at a constant rate into a solution containing various concentrations of Calcium chloride as a cross-linking agent stirred at 200 rpm. Microdroplets or beads were set under stirring for 15 minutes, then filtered, washed with buffer, and finally kept at room temperature for 24 hrs to be dried. [10; 11]

**Table 1:** Composition of different Propranolol HCl-alginate beads formulations

Formula	Drug: Na alginate	CaCl <sub>2</sub> %
F1	1:1	1
F2	1:2	1
F3	1:3	1
F4	1:1	2
F5	1:2	2
F6	1:3	2
F7	1:1	3
F8	1:2	3
F9	1:3	3

## 2.2 Evaluation of alginate beads Formulations

### 2.2.1 Particle size determination

The particle size of the formulated alginate beads was determined using the laser light diffraction technique (Particle size analyzer, Malvern Instrument, Mastersizer 2000). The average size was calculated by measuring the diameter of 20 beads from each formulation. The mean weights and diameters of the beads were measured, [11]

### 2.2.2 Yield percentage %

The yield percentage of each formula was calculated on a weight basis with respect to the weight (theoretical and experimental weight). The yield was calculated as per equation:

$$\text{yield percentage} = \frac{\text{the weight of recovered beads}}{\text{weight of (drug + polymer)}} \times 100\%$$

### 2.2.3 Entrapment efficiency %

The method is based on measuring the filtrate after filtration of alginate beads equivalent to 80 mg of Propranolol Hcl from each batch. Samples were measured spectrophotometrically at 289 nm (against Blank beads treated in the same manner).

The entrapment efficiency (EE%) was evaluated by determining the amount of free drug according to the following equation:

$$\text{EE (\%)} = [(\text{Total drug used} - \text{amount of free drug})/\text{total drug used}] \times 100 \% \quad [12]$$

### 2.2.4 Swelling index

Certain weight of the prepared beads was withdrawn, and immersed in phosphate buffer pH 4.5. Every 1 hour, beads were withdrawn and weighed again. The swelling index was expressed as a percentage of weight gained by the dry beads. The percentage of weight gain of beads was determined according to the equation:

$$\text{Swelling Index (SI)} = [(\text{Wt} - \text{W0})/\text{W0}] \times 100 \% \quad [13]$$

where, Wt= weight of swollen beads at time t

W0= weight of dry beads at t=0

### 2.2.5 Surface pH Measurement

The pH of the formulated gels was measured directly after preparation by a digital pH meter.

## 2.3 In-vitro Disintegration Capsules

*In-vitro* disintegration time was estimated after filling the prepared vaginal beads into hard gelatin capsules for ease of application, by the watch glass method. Each capsule was fitted to the center of a watch glass having a diameter of 11cm and immersed in a water bath at  $37 \pm 1^\circ\text{C}$  then 4 ml phosphate buffer was poured. The disintegration time was defined as the time of the first release of the beads from the capsules. [14]

## 2.4 In-vitro drug release study

For determination of the % release of drug from alginate beads, an open diffusion cell was used. A semi-permeable cellophane membrane was soaked overnight in Sørensen's phosphate buffer of pH 4.5 and stretched over the open-ended diffusion cell. An

accurate amount of formulation was used to be equivalent to 80 mg of Propranolol HCl was fitted to the membrane between the empty diffusion cell and receptor compartment containing 100 ml of phosphate buffer (pH 4.5) which was preheated and maintained at  $37 \pm 1^\circ\text{C}$  in a thermostatic shaker bath. The tube's height was adjusted to set the level of the membrane just below the surface of the release medium. The whole assembly was shaken at 100 rpm during the entire time of diffusion, where samples of 3 ml were withdrawn from the receptor compartment and replaced with equal volumes of fresh medium solution after 30, 60, 120, 180, 240, 300, 360 and 480 min [15]. The amount of Propranolol HCl was determined at 289 nm [16].

### 2.5 Kinetic analysis of the release data

The in-vitro release data were analyzed according to zero, first and Higuchi diffusion models in order to describe their release model based on their correlation coefficients. The model that recorded the highest correlation was used for the assessment of the drug release rates [17].

### 2.6 Stability Studies

Propranolol HCl-loaded beads were subjected to various temperatures as per ICH guidelines and tested for their stability. Samples were weighed, wrapped in butter paper, and classified into two sets for storage at either ambient room temperature ( $27 \pm 2^\circ\text{C}$ ) or elevated temperature ( $45 \pm 2^\circ\text{C}$ ) for a period of 4

weeks. Beads were examined physically for any change in color, texture or drug content. The drug solutions were further scanned to observe any possible spectral changes. [18; 19]

### 2.7 Statistical analysis

All values were expressed as a mean  $\pm$  S.D. Statistical analysis was performed using one-way ANOVA using GraphPad InStat software (GraphPad Software, CA, USA). The level of significance was set at  $p < 0.05$ .

## 3. Results and discussion

As previously reported, introducing a polymeric solution of sodium alginate onto a solution of calcium chloride would be accompanied by the formation of a gel, that could be explained on the basis of the exchange of sodium ions by calcium ions and the polymer cross-linked together. When the alginate is longer in contact with the calcium chloride solution, a more rigid gel will be formed and more calcium ions cross-links [20; 21].

### 3.1 Evaluation of the prepared vaginal alginate beads Formulations

#### 3.1.1 Particle size determination:

The formulated beads were almost spherical in shape having a uniform particle size distribution and a mean particle size within a range of  $1300 \pm 10.2$  to  $1350 \pm 11.9 \mu\text{m}$ . The particle size distribution varied according to different compositions (Table 2).

**Table 2:** Characterization of different Propranolol HCl-alginate beads formulations

Formulation	Particle size ( $\mu\text{m}$ )	Yield percentage	EE%	Swelling index %	Mucoadhesive strength ( $\times 10^3 \text{ dyne/cm}^2$ )	pH
F1	$1300 \pm 10.2$	$92.2 \pm 1.8$	$86.2 \pm 1.2$	$55.3 \pm 5.5$	$15.2 \pm 0.5$	$5.3 \pm 0.1$
F2	$1315 \pm 9.1$	$90.5 \pm 1.5$	$88.3 \pm 1.3$	$62.6 \pm 6.8$	$16.1 \pm 0.3$	$5.5 \pm 0.1$
F3	$1320 \pm 15.2$	$95.5 \pm 1.5$	$90.5 \pm 0.8$	$70.5 \pm 3.1$	$17.3 \pm 0.5$	$5.0 \pm 0.2$
F4	$1310 \pm 10.5$	$93.3 \pm 1.3$	$92.8 \pm 1.3$	$73.3 \pm 2.4$	$22.2 \pm 0.4$	$4.8 \pm 0.1$
F5	$1320 \pm 12.2$	$95.1 \pm 1.1$	$95.5 \pm 1.5$	$76.4 \pm 1.8$	$23.4 \pm 0.6$	$5.6 \pm 0.1$
F6	$1325 \pm 15.1$	$96.5 \pm 1.5$	$96.3 \pm 0.9$	$80.2 \pm 2.2$	$24.2 \pm 0.4$	$5.5 \pm 0.02$
F7	$1320 \pm 10.5$	$94.2 \pm 0.8$	$80.1 \pm 1.3$	$96.3 \pm 3.3$	$33.2 \pm 0.3$	$5.1 \pm 0.1$
F8	$1335 \pm 12.2$	$95.5 \pm 0.5$	$82.2 \pm 1.5$	$106.2 \pm 6.5$	$35.5 \pm 0.2$	$4.9 \pm 0.1$
F9	$1350 \pm 12.9$	$98.2 \pm 2.2$	$83.1 \pm 0.5$	$125.5 \pm 4.5$	$37.2 \pm 0.6$	$5.3 \pm 0.2$

At each concentration examined for CaCl<sub>2</sub>, the mean bead size increased by increasing the drug: Na alginate ratio from 1:1 to 1:3, and it was noticed that their shape became almost spherical with an increase in their mean diameter based on the increase in micro-viscosity of the polymeric dispersion [22]. These results were in accordance with Kashid *et al.* [2016] [23]

On the other hand, increasing the concentration of CaCl<sub>2</sub> from 1% to 3% was accompanied by a marked decrease in beads' size and these results were in accordance with the results declared by Manjanna *et al.* [2009] [24]. These results can be explained on the basis of the gelation process initiated when a drop of alginate solution comes in contact with Calcium ions, where the latter penetrates into the interior of droplets. At the same time, water is squeezed out of the interior of droplets, as a result, contraction and shrinking of the beads and subsequent decrease in their particle size is achieved [25].

### 3.1.2 Yield percentage

The total yield percentage of propranolol HCl-loaded beads fall within the range of 90.5±1.5 w/w to 98.2±2.2 % w/w as shown in Table (2). Results revealed that upon increasing ratio of Na alginate from 1:1 to 1:3, the yield percentage significantly increased.

### 3.1.3 Entrapment Efficiency %

The percentage of drug entrapped in all formulations was found to be fairly satisfactory. It was obvious that the % EE increased by increasing the drug: polymer ratio at the same concentration of cross-linking agent. This can be explained on the basis of increasing the amount of Na alginate is accompanied by an increase in the number of available active binding sites in the polymeric chains and consequently, increased cross-linking degree [24].

It was observed in Table 2 that increasing Calcium chloride concentration from 1% to 2% w/v was accompanied by an increase in the percentage of the entrapped drug from 86.2±1.2 % (F1) to 96.3±0.9% (F4), and this could be explained as the higher the Ca<sup>2+</sup> ions concentration would increase the degree of cross-linking of the polymer and consequently the compactness of the formed matrices. Possible saturation of Calcium binding sites in the guluronic acid chain was noticed after any further increase in Calcium chloride concentration to 3%w/v, so

preventing further Ca<sup>2+</sup> ions entrapment and hence cross-linking was not altered with a higher concentration of Calcium chloride solution [25]. Another explanation was reported by Sankalia *et al.* [2005] [26] who found that water-soluble Calcium chloride would form a significant number of pores on the surface of beads through which the drug molecules would leach into the medium.

### 3.1.4 Swelling index

Swellability is an indicative parameter for the rapid availability and greater flux of drug solution for diffusion [27]. Results demonstrated that with the increase of drug: polymer ratio from 1:1 to 1:3; the swelling percentage also increased from 55.3±5.5 (F1) to 125.5±4.5 (F5) [13]. On the other hand, an increase in the concentration of CaCl<sub>2</sub> was accompanied by a significant decrease in the swelling percentage of beads.

This could be explained on the basis of the existing cross-linked polymers, where the lower cross-linked polymers have longer chain lengths and are easy to expand, while the higher cross-linked polymer, chain length is smaller and difficult to swell. [28]

### 3.1.5 Mucoadhesive strength measurements

Results of mucoadhesion revealed that as the drug: polymer ratio increases, there is a corresponding increase in mucoadhesion results. On the contrary, there was a non-significant (p<0.5) increase mucoadhesion, by increasing the concentration of crosslinking agent CaCl<sub>2</sub> at the same drug/ polymer ratio. This may be attributed to surface charge density and its pivotal role in mucoadhesion [29]. It

was also reported that increasing cross linking degree is accompanied by a decrease in the surface negative charge on the alginate beads and a decrease in its mucoadhesion efficiency. [30]

### 3.1.6 pH measurement

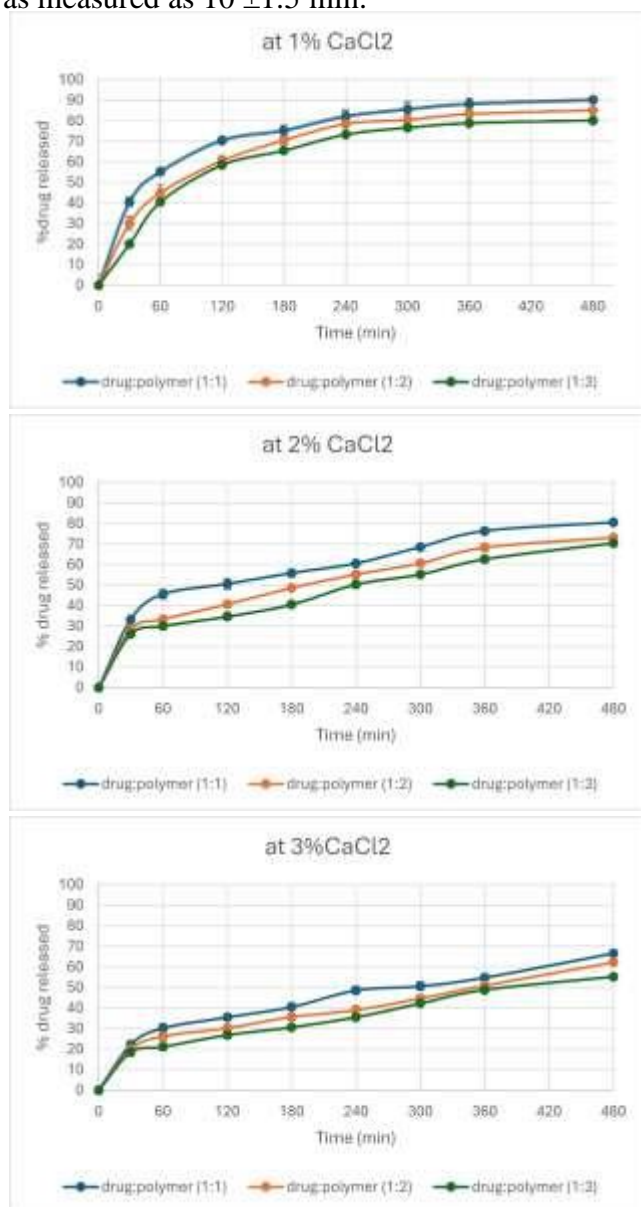
Based on clinical reports, the optimal pH for sperm viability ranges from 7.0 to 8.5, and a marked reduction in sperm motility is reported at pH of less than 6.0. [23]

All beads formulations in the present study showed a pH range from 4.9±0.1 to 5.6±0.1 which is acceptable for administration in the vaginal cavity and

potentiates the toxic acidic environment to sperm in the vagina.

### 3.1.7 Disintegration test of the capsule

The disintegration time is defined as the time point at which the beads released from the capsules, and it was measured as  $10 \pm 1.5$  min.



**Figure 1a:** Effect of different concentration of Na alginate on release of drug from 1% CaCl<sub>2</sub> alginate beads.

**Figure 1b:** Effect of different concentration of Na alginate on release of drug from 2% CaCl<sub>2</sub> alginate beads.

**Figure 1c:** Effect of different concentration of Na alginate on release of drug from 3% CaCl<sub>2</sub> alginate beads.

### 3.2 In-vitro drug release study

Figure 1 shows the % released of Propranolol HCl from the prepared bead formulations, and it is clear that there is a significant reduction ( $p < 0.5$ ) in the % of

drug released upon increasing the drug: polymer ratio from 1:1 to 1:3, meanwhile, increasing the crosslinking % is accompanied by lowering in the % of drug release. This may be explained by the tight junction between guluronic acid residues after gelation or cross-linking of Na alginate with CaCl<sub>2</sub>, which increases the length of the diffusion layer path the drug takes to release [31]. This can be explained on the basis that greater cross-linking resulted in a more rigid gel network with smaller pore size, which retard the penetration of dissolution medium into the beads, which in turn decreased the drug release rate. [32]

### 3.3 In-vitro release kinetics

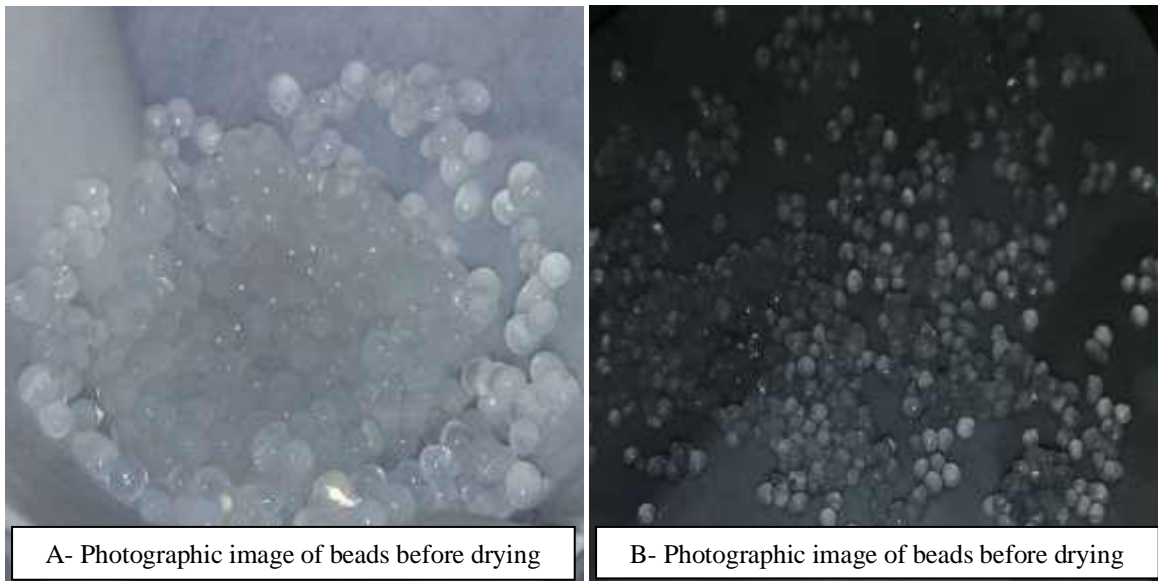
The in-vitro release data of the drug generated a linear relationship between the amount released and the square root of time with a good correlation of coefficient ( $r^2$ ) for all formulations. Results are shown in Table 3 demonstrated that release percentage data obeyed the diffusion mechanism [33]. Results suggested that diffusion plays a pivotal role in the release of the drug from formulations.

### 3.4 Stability results:

A stability study was conducted by storing the selected formulations at 40°C and 27°C for 1 month. There was a negligible change in the tested parameters which ensured the good stability of the prepared beads.

**Table 3:** Kinetic data of the release of Propranolol HCl from Ca+2 alginate bead formulations in phosphate buffer (pH4.5) and at 37°C.

Formula	Zero	First	Diffusion	Observed order
F1	0.8682	0.9507	0.9756	Diffusion
F2	0.8783	0.9585	0.9753	Diffusion
F3	0.8389	0.9319	0.9435	Diffusion
F4	0.9619	0.9628	0.9678	Diffusion
F5	0.9865	0.9883	0.9965	Diffusion
F6	0.9533	0.9801	0.9994	Diffusion
F7	0.9629	0.9810	0.9885	Diffusion
F8	0.9865	0.9774	0.9873	Diffusion
F9	0.9956	0.9800	0.9934	Diffusion



**Figure2:** A- Photographic image of beads before drying, B- Photographic image of beads after drying

#### 4. Conclusions

Propranolol Hcl, beta-blocker antihypertensive drug works by relaxing blood vessels and slowing the heart rate, has been recently proven as an effective spermicidal agent. It can be effectively incorporated in vaginal preparation to inhibit the sperm motility. The formula is based on the development of alginate-based beads of sufficient mucoadhesion to release the drug over a prolonged period of time. Many factors as polymer and cross-linking agent concentrations were optimized. The prepared beads showed acceptable physical properties in terms of particle size, yield index, entrapment efficiency, swelling index and mucoadhesion as well as in-vitro release. Na alginate-based beads may be a promising vaginal dosage form for administration of propranolol HcL as a safer and more effective spermicidal agent.

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#### Conflict of Interest:

The authors declare that they have no conflict of interest.

#### Author contribution

All authors contributed equally in Methodology, Writing – review & editing, original draft, Visualization.

#### References

1. Tasdighia E., Azara Z.J. and Mortazavi S.A.: Development and In-vitro Evaluation of a Contraceptive Vagino-Adhesive Propranolol Hydrochloride Gel. *Iranian Journal of Pharmaceutical Research* (2012), 11 (1): 13-26
2. Richard E. , Kristin H. : *Contraception, in Human Reproductive Biology* (Fourth Edition), 2014
3. Courtney A. S., Barnhart K.: *Contraception, in Yen and Jaffe's Reproductive Endocrinology* (Eighth Edition), 2019
4. Katzung B, Masters S and Trevor A. (eds.) *Basic and Clinical Pharmacology*. 11th ed. McGraw-Hill, New York (2009) 155-159.
5. Duffy D.M., Archer D.F.: *Female Reproduction, in Encyclopedia of Reproduction* (Second Edition), 2018
6. Srivastava S and Coutinho E. Adrenergic antagonist propranolol as a novel, effective spermicide: An NMR study. *Int. J. Pharm. Pharm. Sci.* (2010) 4: 196-200.
7. Zipper J, Wheeler RG, Potis DM and Rivera M. Propranolol as a novel effective spermicide: preliminary findings. *Br. Med. J.* (1983) 287: 1245-1246.
8. Patel LG, Warrington SJ and Pearson RM. Propranolol concentration in plasma after insertion into the vagina. *Br. Med. J.* (1983) 287: 1247-1248.
9. Farhan J, Mohd M, Roop K and Mushir A. Development and in-vitro evaluation of an acid buffering bioadhesive vaginal gel for mixed vaginal infections. *Acta Pharm.* (2008) 58: 407-419.
10. Khan AD, Bajpai M. Formulation and evaluation of floating beads of verapamil hydrochloride. *Int J PharmTech Res* 2011;3:1537-46. 15.
11. Rasel MA, Hasan M. Formulation and evaluation of floating alginate beads of diclofenac sodium. *Dhaka Univ J Pharm Sci* 2012;11:29-35
12. Patel J., Patel D. and Raval J.: Formulation and Evaluation of Propranolol Hydrochloride-Loaded Carbopol-934P/Ethyl Cellulose Mucoadhesive Microspheres. *Iran J Pharm Res.* 2010 Summer; 9(3): 221–232.
13. Iswariya VT, Soujanya GL, Santhosh R, Kumar AP, Lingaswamy J, Satyanarayana K. Design and development of in vitro characterization of floating microspheres of ranolazine. *Eur J Pharm Med Res* 2016;3:220-32
14. Abass HA, Kamel R. Formulation and evaluation of in situ forming polymeric drug delivery systems for mixed vaginal infection. *Br J Pharm Res* 2014;4:2281-95.
15. Parhi R, Suresh P. Alginate-ploxamer beads for controlled release of metoprolol succinate. *Turk J Pharm Sci* 2012;12:59-66.
16. Kalita B, Saikia K, Kalita B. Formulation and evaluation of metronidazole microspheres-loaded bioadhesive vaginal gel. *Asian J Pharm Clin Res* 2017;10:418-24
17. Patel YL, Sher P, Pawar AP. The effect of drug concentration and curing time on processing and properties of calcium alginate beads containing metronidazole by response surface methodology. *AAPS PharmSciTech* 2006;7:86
18. Gulzar M., Udupa. N.: Development and



- Characterization of suppositories containing nimesulide. *Indian J Pharm Sci* 62(3); 2000:196-199
19. Velmurugan S, Ali MA. Formulation and evaluation of maraviroc mucoadhesive microspheres by ionotropic gelation method. *Int J Pharm Pharm Sci* 2013;5:294-2
  20. Zhao Y, Carvajal MT, Won Y, Harris MT (2007) Preparation of calcium alginate microgel beads in an electrodispersion reactor using an internal source of calcium carbonate nanoparticles. *Langmuir* 23: 12489-12496.
  21. Fathy M, Safwat SM, el-Shanawany SM, ShawkyTous S, Otagiri M (1998) Preparation and evaluation of beads made of different calcium alginate compositions for oral sustained release of tiaramide. *Pharm Dev Technol* 3: 355-364.
  22. Das, M. K. and Senapati, P. C. (2008), "Furosemide-loaded Alginate Microspheres Prepared by Ionic Cross-linking Technique: Morphology and Release Characteristics", *Indian J. Pharm Sci.* 70(1): 77–84.
  23. Kashid, P., Doijad, R., Shete, A., Sajan, S. and Bhagat, A. (2016), "Studies on Rebamipide Loaded Gastroretentive Alginate Based Mucoadhesive Beads: Formulation & In-vitro, In-vivo Evaluation", *Pharm Methods.*; 7(2): 132-138.
  24. Manjanna, K. M., Shivakumar, B. and Pramod, K. T.M. (2009), "Formulation of oral sustained release aceclofenac sodium microbeads", *Int.J. of Pharm Tech Res.*; 1(3): 940-952.
  25. Akifuddin, S. K., Abbas, Z., Marihal, S., Ranadev, A. K., Kumar, S. I. H. and Kulkarni, R. (2013), "Preparation, Characterization and In-vitro Evaluation of Microcapsules for Controlled Release of Diltiazem Hydrochloride by Ionotropic Gelation Technique", *J. of Applied Pharma. Sci.*; 3 (4):035-042.
  26. Sankalia, M. G., Mashru, R. C., Sankalia, J. M. and Vijay, B. S. (2005), "Papain entrapment in alginate beads for stability improvement and site-specific delivery: physicochemical characterization and factorial optimization using neural network modeling", *AAPS Pharm. Sci. Tech.*; 6 (2):209-222.
  27. Roy, V., Rathore, D. and Dahima, R. (2014), "Formulation and evaluation of controlled release glibenclamide beads by ionotropic gelation technique", *Scholars Res Lib Der Pharmacia Lettre.*; 6(5):70-77.
  28. Mane, S., Ponrathnam, S. and Chavan, N. (2015), "Effect of Chemical Cross-linking on Properties of Polymer Microbeads: A Review", *Canadian Chem Transactions.*; 3(4):473-485.
  29. Chickering, D.E., Mathiowitz, E.(1995), "Bioadhesive microspheres: I. A novel electrobalance-based method to study adhesive interactions between individual microspheres and intestinal mucosa", *J.Control. Release.*; 34: 251–261.
  30. Jahan, S. T., Sadat, S. M., Islam, M. R., Azam, A. T. Z. and Chowdhury, J. A. (2012), "Effect of Various Electrolytes on Theophylline Loaded Sodium Alginate Beads Prepared by Ionic Cross Linking Technique", *Dhaka Univ. J.Pharm. Sci.* 11(2): 181-189.
  31. Jelvehgari, M., Mobaraki, V. and Montazam, S. H. (2014), "Preparation and Evaluation of Mucoadhesive Beads/Discs of Alginate and Algino-Pectinate of Piroxicam for Colon-Specific Drug Delivery via Oral Route", *Jundishapur J. Nat Pharm Prod.*; 9(4): 1-10.
  32. El-Kamel, A. H., Al-Gohary, O. M. N., Hosny, E. A. (2003), "Alginate diltiazem hydrochloride beads: optimization of formulation factor, in vitro and in vivo availability", *J. Microencapsul.*; 20:211–225
  33. Abou El Ela Ael S, Hassan MA, El-Maraghy DA (2014) Ketorolac tromethamine floating beads for oral application: Characterization and in vitro/in vivo evaluation. *Saudi Pharm J*22: 349-59.