International Journal of Medicine and Pharmaceutical Research

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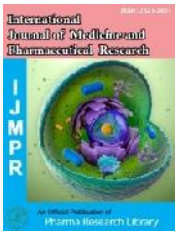

Research Article **Open Access**

Formulation and *In-Vitro* Evaluation of Doxorubicin Microspheres for the Treatment of Lymphoblastic Leukemia

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A B S T R A C T

In the present work, bioadhesive microspheres of Doxorubicin using Sodium alginate along with Carbopol 934, Carbopol 971, HPMC K4M as copolymers were formulated to deliver Doxorubicin via oral route. The results of this investigation indicate that ionic cross linking technique Ionotropic gelation method can be successfully employed to fabricate Doxorubicin microspheres. The technique provides characteristic advantage over conventional microsphere method, which involves an "all-aqueous" system, avoids residual solvents in microspheres. Other methods utilize larger volume of organic solvents, which are costly and hazardous because of the possible explosion, air pollution, toxicity and difficult to remove traces of organic solvent completely.. Micromeritic studies revealed that the mean particle size of the prepared microspheres was in the size range of 512-903µm and are suitable for bioadhesive microspheres for oral administration.Increase in the polymer concentration led to increase in % Yield, % Drug entrapment efficiency, Particle size, % swelling and % Mucoadhesion.The *in-vitro* mucoadhesive study demonstrated that microspheres of Doxorubicin using sodium alginate along with Carbopol934 as copolymer adhered to the mucus to a greater extent than the microspheres of Doxorubicin using sodium alginate along with Carbopol 971 and HPMC K4M as copolymers.The *invitro* drug release decreased with increase in the polymer and copolymer concentration..Based on the results of evaluation tests formulation coded T_4 was concluded as best formulation.

Keywords: Doxorubicin,Carbopol, Microspheres

A R T I C L E I N F O

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Article History: Received 28 May 2016, **Accepted** 30 June 2016, **Available Online** 10 August 2016

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PAPER-QR CODE

Citation: K. Lalitha. Formulation and *In-Vitro* Evaluation of Doxorubicin Microspheres for the Treatment of Lymphoblastic Leukemia. *Int. J. Med. Pharm. Res*., 2016, 4(4): 211-216.

1. Introduction

To achieve and then to maintain the concentration of drug administered within the therapeutically effective range needed for medication, it is often necessary to take this type of drug delivery systems several times in a day. This results in a fluctuated drug level and consequently undesirable toxicity and poor efficiency. The objective in designing a controlled release system is to deliver the drug at a rate necessary to achieve and maintain a constant drug blood level. This rate should be similar to that achieved by continuous intravenous infusion where a drug is provided to the patient at a rate just equal to its rate of elimination. This implies that the rate of delivery must be independent of the amount of drug remaining in the dosage form and constant over time, i.e release from the dosage form should follow zero-order kinetics.

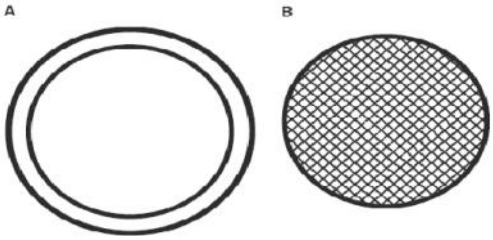

Figure 1

Microspheres: Microsphere carrier systems made from the naturally occurring biodegradable polymers have attracted considerable attention for several years in sustained drug delivery. Recently, dosage forms that can precisely control the release rates and target drugs to a specific body site have made an enormous impact in the formulation and development of novel drug delivery systems. Microspheres form an important part of such novel drug delivery systems. Microspheres have varied applications and are prepared using assorted polymers

Mucoadhesion:

The term "mucoadhesion" was coined for the adhesion of the polymers with the surface of the mucosal layer. Bio adhesions are a phenomenon in which two materials at least one of which is biological and are held together by means of interfacial forces

Advantages of mucoadhesive systems:

- Readily localized in the region applied to improve and enhance the bioavailability of drugs. E.g. Testosterone & its esters, vasopressin, dopamine, insulin and gentamycin etc.
- Facilitate intimate contact of the formulation with underlying absorption surface. This allows modification of tissue permeability for absorption of macromolecules. e.g. peptides and proteins.
- Prolong residence time of the dosage form at the site of application and absorption to permit once or twice a day dosing.

Mechanism of mucoadhesion:

A complete understanding of how and why certain macromolecules attach to a mucus surface is not yet available, but a few steps involved in the process are generally accepted, at least for solid systems. Several theories have been proposed to explain the fundamental mechanism of adhesion. A general mechanism of mucoadhesion drug Delivery system is show.

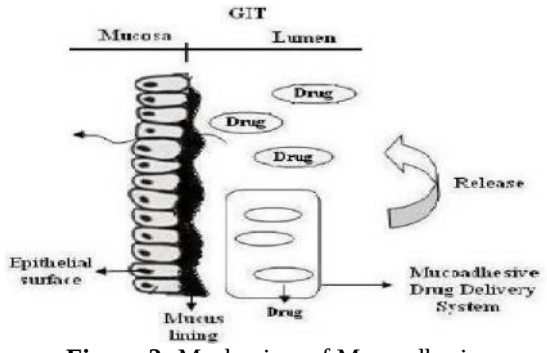

Figure 2: Mechanism of Mucoadhesion

Theory of mucoadhesion:

A) Electronic theory
B) Wetting Theory
C) Adsorption Theory
D) Mechanical Theory
E) Cohesive Theory
F) Diffusion theory

Polymers used for mucoadhesive system

Hydrophilic polymers:

The polyelectrolyte's have greater mucoadhesive property when compared with neutral polymers.

Anionic polyelectrolyte's:

Ability to exhibit strong hydrogen bonding with the mucin present in the mucosal layer.

Cationic Polyelectrolyte's:

Used for developing mucoadhesive polymer due to its good biocompatibility and biodegradable properties

Non-Ionic Polymers: Used for mucoadhesive properties

Hydrogels: have the ability to hold water within its porous structure

Thiolated polymers: Which can substantially improve the mucoadhesive properties of the polymers

Lectin-based polymers:

Lectins are proteins which have ability to reversibly bind with specific sugar carbohydrate residues and are found in both animal and plant kingdom

Methods of preparation of mucoadhesive microspheres:

1) Air suspension
2) Spray drying
3) Polymerization
4) Coacervation
5) Solvent evaporation
6) Wet inversion technique
7) Hot melt microencapsulation
8) Iontropic gelation technique
9) Orifice ionic gelation method

Application of mucoadhesive microspheres:

Microsphere in vaccine delivery have a specific advantage like improved antigenicity by adjuvant action, modulation of antigen release, stabilization of antigen. Vaccine delivery for treatment of diseases like hepatitis, influenza, pertusis, ricin toxoid, diphtheria, Passive targeting of leaky tumour vessels, active targeting of tumour cells,antigens, by intra arterial / intravenous application.

Mechanism of Action:

Doxorubicin has antimitotic and cytotoxic activity through a number of proposed mechanisms of action: Doxorubicin forms complexes with DNA by intercalation between base pairs, and it inhibits topoisomerase II activity by stabilizing the DNA-topoisomerase II complex, preventing the religation portion of the ligation-religation reaction that topoisomerase II catalyzes.

2. Materials and Methods

Doxorubicin from the Pellets india private Ltd. Hydrophilic polymers: Anionic polyelectrolyte's Non-Ionic Polymers Non-Ionic Polymers Hydrogels Thiolated polymers Lectin-based polymers are from Srinivasa scientifics Hyderabad.

Method of Preparation

Ionotropic gelation method:

Batches of microspheres were prepared by ionotropic gelation method which involved reaction between sodium alginate and polycationic ions like calcium to produce a hydrogel network of calcium alginate. Sodium alginate and the mucoadhesive polymer were dispersed in purified water (10 ml) to form a homogeneous polymer mixture. The API, Doxorubicin (100 mg) were added to the polymer premix and mixed thoroughly with a stirrer to form a viscous dispersion. The resulting dispersion was then added through a 22G needle into calcium chloride (4% w/v) solution. The addition was done with continuous stirring at 200rpm. The added droplets were retained in the calcium chloride solution for 30 minutes to complete the curing reaction and to produce rigid spherical microspheres. The microspheres were collected by decantation, and the product thus separated was washed repeatedly with purified water to remove excess calcium impurity deposited on the surface of microspheres and then air-dried.

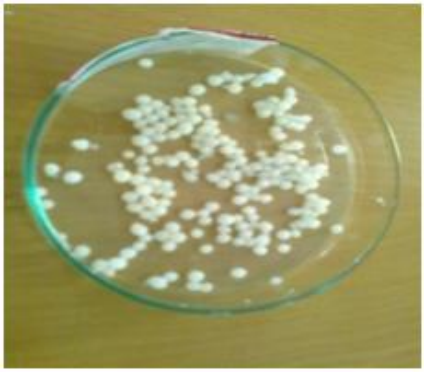

Figure 3: Mechanism of Action

3. Results and Discussion

Determination of λmax

A solution of 10µg/ml of doxorubicin was scanned in the range of 200 to 400nm. The drug exhibited a λmax at 269nm in simulated gastric fluid pH 1.2 and had good reproducibility

Drug Entrapment Efficiency

Percentage Drug entrapment efficiency of Doxorubicin ranged from 82.66 to 88.66% for microspheres containing sodium alginate along with Carbopol 934 as copolymer, 53.2 to 76.66% for microspheres containing sodium alginate along with Carbopol 971 as copolymer and 66.73 to 79.2% for microspheres containing sodium alginate along with HPMC K 4 M as copolymer

Particle Size Analysis:

Microspheres containing sodium alginate along with carbopol 934 as copolymer had a size range of 512µm to 826µm, microspheres containing sodium alginate along with carbopol 971 as copolymer exhibited a size range between 517µm to 834µm and microspheres containing sodium alginate along with HPMC K 4 M as copolymer had a size range of 664µm to 903µm

Swelling ratio:

The swelling ratio is expressed as the percentage of water in the hydrogel at any instant during swelling. Swellability is an important characteristic as it affects mucoadhesion as well as drug release profiles of polymeric drug delivery systems.

In-Vitro Mucoadhesion Test: The rank of order of mucoadhesion is carbopol 934 > HPMC K 4 M >carbopol 971. The results of in-vitro mucoadhesion test are compiled

***In-Vitro* Drug Release Studies**:

Dissolution studies of all the formulations were carried out using dissolution apparatus USP type I. The dissolution studies were conducted by using dissolution media, pH 1.2. The formulations T_1,T_2,T_3 and T_4 containing Sodium alginate along with Carbopol 934 as copolymer showed a maximum release of 92.66% after 9 hours, 90.66% after 10 hours, 90.6% after 11 hours and 97.66% after 12 hours.

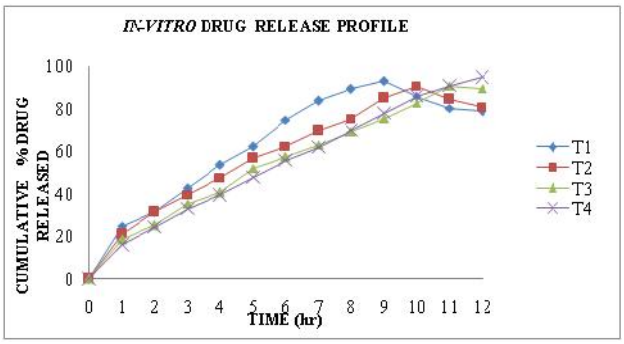

Figure 3: Comparison of In-Vitro drug release profile of Doxorubicin microspheres containing sodium alginate along with carbopol 934 as copolymer.

4. Conclusion

In the present work, bioadhesive microspheres of Doxorubicin using Sodium alginate along with Carbopol 934, Carbopol 971, HPMC K4M as copolymers were formulated to deliver Doxorubicin via oral route. The results of this investigation indicate that ionic cross linking technique Ionotropic gelation method can be successfully employed to fabricate Doxorubicin microspheres. FT-IR spectra of the physical mixture revealed that the drug is compatible with the polymers and copolymers used. Micromeritic studies revealed that the mean particle size of the prepared microspheres was in the size range of 512-903µm and are suitable for bioadhesive microspheres for

oral administration. Increase in the polymer concentration led to increase in % Yield, % Drug entrapment efficiency, Particle size, % swelling and % Mucoadhesion The *in-vitro* mucoadhesive study demonstrated that microspheres of Doxorubicin using sodium alginate along with Carbopol934 as Copolymer adhered to the mucus to a greater extent than the microspheres of Doxorubicin using sodium alginate along with Carbopol 971 and HPMC K4M as copolymers. Based on the results of evaluation tests formulation coded T_4 was concluded as best formulation.

Table 1: Prepared Formulation of Bioadhesive Microspheres

S.No.	Formulation code	Drug: Polymer Ratio	Polymer ratio
1	T_1	1:2.5	Na alginate:Carbopol 934 (1.5:0.5)
2	T_2	1:3	Na alginate:Carbopol 934 (2:1)
3	T_3	1:3.5	Na alginate:Carbopol 934 (2.5:1)
4	T_4	1:4	Na alginate:Carbopol 934 (3:1)
5	T_5	1:2.5	Na alginate:Carbopol 971 (1.5:0.5)

Table 2: Calibration curve data for Doxorubicin in stimulated gastric fluid pH 1.2

Conc. (µg /ml)	Absorbance
2	0.051
4	0.110
6	0.163
8	0.221
10	0.290

Table 3: Percentage yield and percentage drug entrapment efficiency of the prepared microsphere

S.No.	Formulation code	% yield	Drug Content (mg)	% Drug entrapment efficiency
1	T_1	80	12.40	82.66
2	T_2	83.33	12.66	84.4
3	T_3	85	12.70	84.66
4	T_4	88	13.29	88.66
5	T_5	62.22	8.07	53.2

Table 4: Particle size data of T4

Particle Size Range (µm)	Midpoint Size Range (d)	Frequency (n)	Average Particle Size (µm)
500-600	550	6	826 µm
600-700	650	12	
700-800	750	16	
800-900	850	32	
900-1000	950	34	
		∑n=100	

Table 4: Percentage swelling of the prepared microspheres

S.No.	Formulation Code	Initial (Wt)	Final (Wt)	Percentage Swelling
1	T_1	10	12.8	28
2	T_2	10	14.2	42
3	T_3	10	16.2	62
4	T_4	10	18.5	85
5	T_5	10	12.4	24

Table 5: Percentage mucoadhesion of the prepared microspheres

S.No.	Formulation Code	No. of microspheres		Percentage mucoadhesion
		Initial	Final	
1	T_1	20	13	65
2	T_2	20	14	70
3	T_3	20	15	75
4	T_4	20	17	85
5	T_5	20	12	60

Table 5: *In-Vitro* drug release data of Doxorubicin microspheres containing sodium alginate along with carbopol 934 as copolymer

Time (h)	Cumulative Percent of Drug Released				
	T_1	T_2	T_3	T_4	T_5
0	0	0	0	0	0
1	24.88	21.11	18.66	15.88	27.77
2	31.55	31.55	25.11	24.22	36.44
3	42.44	39.77	35.44	32.66	43.77
4	53.55	47.77	40.66	39.33	54.66
5	62	56.66	52	47.55	64.01
6	74.66	62.44	57.33	55.77	75.77
7	83.55	69.55	63.11	61.77	84.65
8	89.33	75.33	69.11	69.55	90
9	92.66	84.66	75.33	77.55	92.22
10	85.55	90.66	82.66	85.55	84.88
11	80.22	84.22	90.66	90.66	79.55
12	78.88	80.88	89.55	97.66	77.55

Table 6: Release kinetics studies of the prepared formulations

Formulation code	**Release model**								
	Zero order		**First order**		**Higuchi matrix**		**Koresmeyer-peppas**		
	K	**R^2**	**K**	**R^2**	**K**	**R^2**	**N**	**K**	**R^2**
T_1	21.6	0.797	1.923	0.720	-0.313	0.912	0.556	1.388	0.925
T_2	16.39	0.908	1.991	0.890	-3.945	0.970	0.595	1.326	0.983
T_3	10.45	0.976	2.062	0.945	-8.966	0.975	0.673	1.233	0.991
T_4	7.434	0.990	2.118	0.914	-12.25	0.962	0.743	1.171	0.996
T_5	24.34	0.768	1.897	0.689	2.624	0.903	0.498	1.442	0.914

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