Comparative studies on Effect of Cations and Anions on controlled Release of Centchroman From Chitosan Microspheres

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الخلاصة:

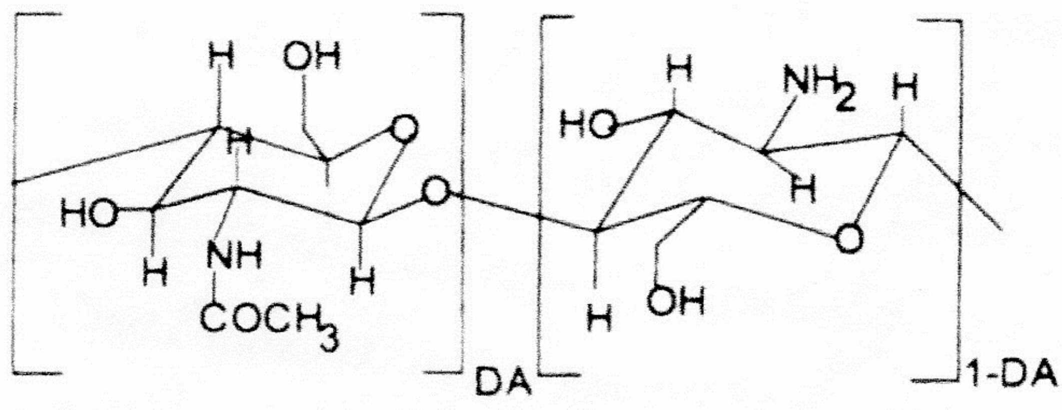
البوليمر الطبيعي الكايتوسان والمحور كيميائياً من الكايتين بعد إزالـة مجموعـة الاسـيتايل وتحويلها الى مجموعة امين اولي من البوليمرات الغير سامة، والملائمة بايولوجياً للاسـتخدامات البايولوجية داخل الجسم، كما ان قابليته على الالتصاق بالطبقة المخاطية للأمعاء مع قابليته على التحلل البايولوجي أهله للاستخدام في أنظمة إيصال الدواء والتحكم في زمن وكمية ضخ الـدواء بمعدل منتظم ضمن مديات حاجة الجسم الدقيقة. وقد تضمن البحـث دراسـة مقارنـة لحبيبـات الكايتوسان المايكروية المتشابكة بالالديهايد الثنائي والمضاف إليها انايونات وكاتايونـات مختلفـة الشحنة لمعرفة تاثيرها السلبي والايجابي على معدل انتفاخ الحبيبات المايكروية وعلى أعلى معدل تحميل للدواء مع حالة إطلاق منتظم للدواء لهذه الحبيبات المايكروية بالمقارنة مع تلك المحـضرة بدون إضافة الايونات الموجبة والسالبة. وقد لوحظ مـن نتـائج الدراسـة ان لتلـك الانايونـات والكاتايونات القابلية على التحكم في صفات الحبيبات المايكروية المصنعة وبالتالي تسهيل فرصـة تحضير حبيبات مايكروية بمعدل انتفاخ مطلوب وبمعدل تحميل عالي للدواء مع قابليـة اطـلاق منتظم للدواء وفي فترات زمنية منتظمة بالاعتماد على نوع الايون المـضاف لمحلـول تـصنيع الحبيبات المايكروية وعلى تركيز الايون المضاف. تم استخدام مانع الحمل سنتكرومان كنمـوذج دواء محمل على الحبيبات المايكروية المصنعة من البوليمر الطبيعي الكايتوسان.

Abstract

The Chitosan is a non-toxic, biocompatibale ploysacchride produced by deacetyltion of naturally occurring chitin. The mucoadhesitivity and biodegradability of chitosan have made chitosan as a choice material for developing various dosage forms for controlled delivery systems which are intended to deliver drugs at a controlled rate within a specified period of drug delivery. A comparative study on the degree of swelling and maximum loading and controlled drug released behavior were investigated for glutaraldehyde cross-linked chitosan microspheres in presence of different anions and cations and in the absence of these ions on burst and control release of centchroman. The addition of high charge density cations have reduced the amount of centchroman released in controlled manner, where as addition of anions has delayed the inception of controlled step of centchroman release and also decreased the amount centchroman released in this step.

Introduction :

Chitosan is a deacetylated derivative of the naturally occurring, biocompatible, biodegradable and non-toxic polysaccharide, which has shown potential application in various controlled release systems [1,2].
Chitosan found to be dependent on degree of deacetylation [3], molecular weight [4], and degree of cross- linking.



Chemical structure of chitosan

The effect of these characteristics has been studied on loading and releasing of various drugs [5], which need controlled and prolonged delivery. Chitosan beads using glutaraldehyde as cross- linker have been investigated for various delivery systems [1,2], but the effect of addition of anions and cations in such system has not been investigated yet [6-7]. The addition of cations and anions may provide further opportunity in controlling the loading and release characteristics of the beads by controlling the electrostatic interactions between the residual amino groups of the chitosan and added ions. The drug centchroman is a nonsteriodal contraceptive used orally could be loaded on chitosan cross- linked microspheres an studied as controlled drug release [8].

Experimental :

The glutaraldehyde cross- linked chitosan micropheres have been prepared by spray drying procedure using 1% (w/v) solution of chitosan (MW=1135000 Dalton) in 2%(w/v) acetic acid. The spray- dried microspheres were cross- linked by keeping them in 6%(w/v) solution of glutaraldehyde in water. After three hours these microspheres were separated and vacuum dried at 50°C [9].

To compare the effect of cations and anions, the microspheres were prepared by adding a calculated amount of these ions in solution of chitosan before spraying it to form microspheres.

The degree of swelling of microspheres was studied by keeping calculated amount of these microspheres in a phosphate buffered solution (PH = 7.4) at constant temperature (25±0.1°c) and determining percent increase in the weight of microspheres in comparison to their initial weight (Wo) at predetermined time intervals. Loading of centchroman in chitosan cross- linked microspheres and chitosan cross- linked microspheres with ions was carried out by keeping these microspheres for about 48h in a buffered solution (PH 5) containing calculated amount of the centchroman.

The amount of centchroman loaded on these microspheres was estimated by determining the remaining concentration of centchroman in the filtrate atλmax=275nm [2]. The release of centchroman from the loaded microspheres has been studied spectrophotometrically by using (ashimadzu uv-210A double beam spectrophotometer).

Results and discussion :

The chitosan used in preparation of microspheres was of (1135000 Dalton) molccular weight and degree of deacetylation (62%). The microspheres were prepared at fixed concentration of glutaraldchyde (6%w/v). The prepared microspheres have also shown significant variations on loading and release profile on varying the solution pH due to the variation in degree of swelling hence solution pH was also maintained constant while studying the effect of addition of cations and anions on loading and release profile of the centchroman on the prepared microspheres. To compare the effect of addition of cations (Na^{+}, Ca^{+2} and Fe^{+3}) and anions (Cl^{-}, $SO4^{-2}$ and $HPO4^{-2}$) on characteristics of the microspheres, the concentration of these ions in the microspheres was taken 2%(w/w) of weight of chitosan [10]. The loading and release profile of the microspheres have shown dependence on the degree of swelling of microspheres in presence of cations (Na^{+}, Ca^{+2} and Fe^{+3}) and anions of different charge density (Cl^{-}, $SO4^{-2}$ and $CO3^{-2}$) have been compared with microspheres of cross- linked chitosan without these additives Table (1).

Table -1: Effect of addition of cations and anions on degree of swelling behavior of microspheres

Time (hr)	swelling in presence of different type of cations %				swelling in presence of different type of anions %		
	Blank	**NaCl**	**CaCl2**	**FeCl3**	**Na2HPO4**	**Na2SO4**	**Na2CO3**
0	—	—	—	—	—	—	—
20	145	217	270	315	115	90	170
40	250	288	300	290	235	180	275
60	250	263	252	228	273	227	280
80	250	212	201	168	264	233	262
100	250	167	153	107	255	240	235
120	250	120	104	48	244	246	212

Weight of microspheres =100mg in 20ml of solution¸ PH = 7.4 ·
Amount of cations / anions = 0.2% (w/w) of chitosan¸ Temp. = 25°C

The microspheres prepared with FeCl3 have shown highest degree of swelling (315%) in comparison to pure chitosan (217%) at a contact time of 20 hours of microspheres with buffer solution but the degradation by erosion of these microspheres have started after 40 hours where as microspheres prepared with NaCl have shown degradation after 60 hours and in case of microspheres prepared without these additives, the degradation was after 120 hours Table (1). The variation in swelling and degradation of microspheres in presence of added cations has been due to the existence of repulsive force between chitosan segments, which have acquired positive charge from the added cations. The Fe^{+3} ions have developed high positive charge on chitosan surface, hence microspheres have shown maximum swelling (315%) and early degradation in the solution Table (1). The swelling behavior of microspheres with added anions has also been affected as clear from the swelling data given in Table (1). Although selected anions are bivalent but due to the difference in their charge density, they have shown significant variations in degree of swelling [11]. The microspheres prepared with $SO4^{-2}$ ions have been found to be compact and shown low degree of swelling Table (1) without any degradation upto a contact time of 120 hours. The microspheres prepared with $HPO4^{-2}$ $CO3^{-2}$ anions were less compact and shown initially high degree of swelling but shown degradation at a contact time of 60 hours in comparison to chitosan cross – linked microspheres and microspheres with $SO4^{-2}$ Table(1). The effect of degree of swelling and degradation of microspheres has shown clear relation- ship with the added anions. Thus it is clear that the presence of added anions in the microspheres has controlled the swelling behavior, hence may be useful in controlling the loading and release profile of encapsulated centchroman in the microspheres. To investigate the effect of added cations and anions on the extent of loading and release of centchroman were recorded as shown in Table – 2& 3.

Table – 2 : Effect of addition of cations and anions on loading of centchroman (CT) on microspheres.

[C T] mg	Amount of centchroman loaded on 100mg of microspheres in 20ml						
	Blank	NaCl	$CaCl_2$	$FeCl_3$	Na_2HPO_4	Na_2SO_4	Na_2CO_3
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
20	15.0	14.0	17.0	18.8	13.0	12.2	13.5
40	30.0	26.6	25.0	22.0	25.5	25.0	27.0
60	37.5	27.5	25.0	22.0	32.0	37.5	30.0
80	37.5	27.5	25.0	22.0	32.0	41.0	30.0
100	37.5	27.5	25.0	22.0	32.0	41.0	30.0

Time for loading 48hr , PH = 5 , Temp. = 25°C.

Table – 3 : Effect of addition of cations and anions on release profile of centchroman from microspheres.

Time (hr)	Amount of centchroman released from 100mg of microspheres						
	Blank	NaCl	$CaCl_2$	$FeCl_3$	Na_2HPO_4	Na_2SO_4	Na_2CO_3
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10	1.2	2.3	3.6	4.4	1.1	0.9	1.7
20	3.5	6.8	10.8	13.2	3.2	2.5	5.0
30	6.7	13.7	17.9	22.0	6.4	5.0	10.0
40	11.1	20.6	25.0	-	10.7	8.4	16.7
50	15.5	27.5	-	-	16.0	12.5	32.3
60	19.9	-	-	-	21.3	17.6	30.0
80	28.7	-	-	-	31.9	29.3	-
100	37.5	-	-	-	-	41.0	-

Release medium = 20ml phosphate buffer solution of PH = 7.4

The extent of loading of centchroman has shown variation with the type of cations and the type of anions as similar to the variation observed with degree of swelling of microspheres in presence of these ions. The loading of centchroman has been studied taking 100mg microspheres in 20ml buffered solution (PH = 5) containing different amount of centchroman ranging from 20mg to 100mg at constant temperature 25°C. The microspheres with Fe^{+3} ions have shown initially high loading (18.75mg) at an initial concentration of (20mg) in the solution but overall loading of centchroman Table (2) was low with these microspheres at a high concentration of the centchroman (100mg) in the solution. Similarly microspheres with carbonate anions have shown high loading in the beginning Table (2) but overall loading at high concentration of centohroman was high only with microspheres prepared with SO_4^{-2} ions. The sulfate ions microspheres have shown a loading of (41mg) of centchroman in comparison to microspheres prepared with cross – linked chitosan with out additives (37.5mg).

The variation in loading behavior of microspheres has shown direct dependence on the variation in degree of swelling of microspheres and observed conpactness in the microspheres was due to the enhanced electrostatic interactions in the presence of added anions and cations. The releasing behavior of the microspheres has be studied keeping (100mg) of microspheres in phosphate buffered solution (PH = 7.4) for different time intervals. The microspheres prepared with $FeCl_3$ salt have shown initially a burst release of centchorman Table (3) and total loaded centchroman was released without showing any significant region of sustained and controlled release in its release profile (Fig – 1)but microspheres prepared with NaCl or without adding any anions or cations have shown slow and linear release of centchroman in beginning Table (2) and upto a time interval of (30 hours) with NaCl and (40 hours) without additive Table (3) after that they shown sustained release of centchroman for a period of (20 and 60 hours) respectively (Fig–1). The microspheres with anion have also shown significant variation in initial release of centchroman Table (3) and in the controlled region of the release profile (Fig–2). The microspheres with sulfate anions (Na_2SO_4) have shown a slow release of centchroman for relatively a longer period of 70 hours and there after shown a significantly high region of controlled release of 30 hours (Fig – 2).

However, the controlled region shown by microspheres prepared with additives was low in comparison to cross- linked microspheres without any additives (Figs-1 & 2).

Conclusion :

The presence of anions and cations in the microspheres has controlled the characteristics of the chitosan microspheres and provided a large opportunities to prepare microspheres with desired swelling , loading and release behavior by controlling the type and concentration of anions and cations in the casting solution of the microspheres.

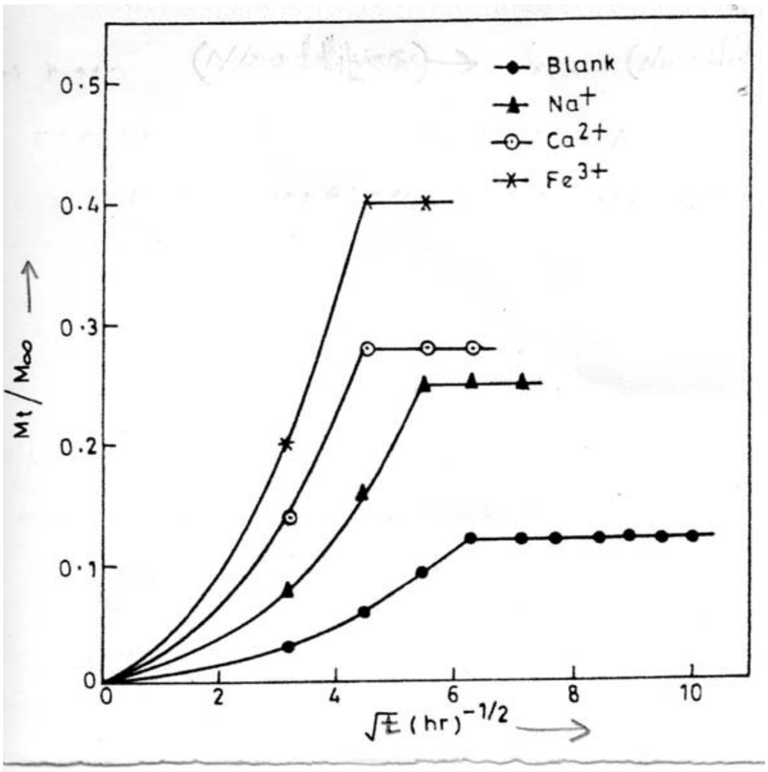


Fig -1 Release kinetic of centchroman in presence of cations

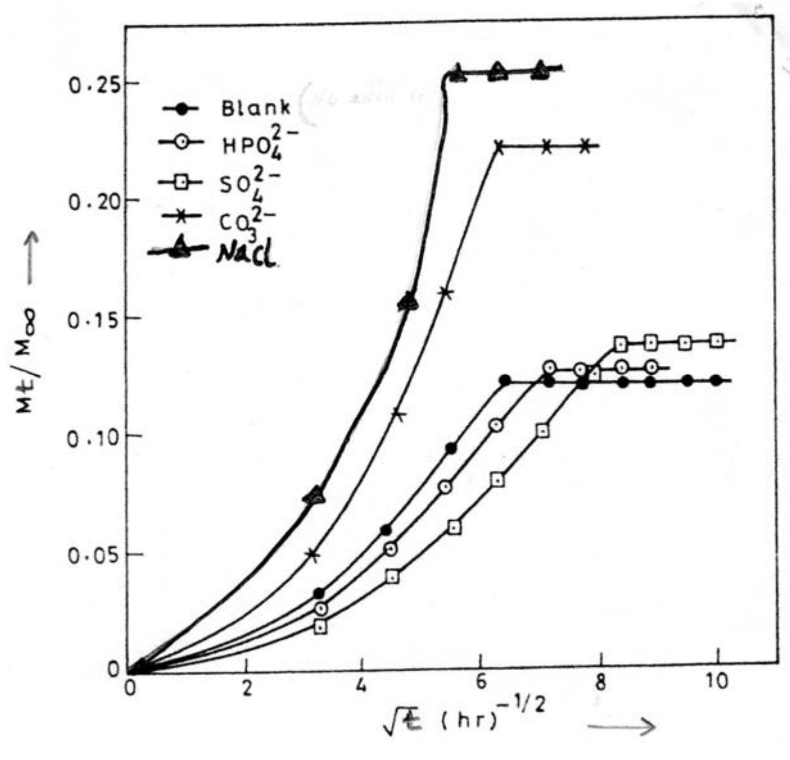


Fig -2 Release kinetic of centchroman in presence of anions

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