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KEY WORDS:
Nanoparticles, Lysene, Sodium Alginates, 5-Fluorouracil, Ionic gelation method

LYSINE SODIUM ALGINATE CONJUGATES AS CONTROLLED DRUG DELIVERY VEHICLE FOR 5-FLUOROURACIL NANOPARTICULATE SYSTEM

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ABSTRACT

Nanomedicine can achieve tumor with spongy vasculature nearby the tumors by the enhanced permeability effect, so, the ligands inculcated at the plane of nanocarriers permit active targeting by requisite the receptors over-expressed by cancer cells or angiogenic endothilial cells. The objective of present work is development of carrier system of lysine with sodium alginate by chemical reaction. The developed carrier system utilize for formulate new colloidal drug carrier as nanoparticles (NPs) with 5-fluorouracil anticancer drug. The gelation or interlinkage of carrier system of sodium alginate with lysine display higher uptake in tumor tissue, as the scale of tumor increases, the uptake of this amino acid may also increases. The nanoparticles formulations evaluated diverse physicochemical parameters including particle size, drug loading capability, in vitro characteristics. Ionic gelation approach was used for the preparation of l-lysine conjugated sodium alginate nanoparticles due to the fact this system is non-poisonous, organic solvent free, handy and controllable. Ionic gelation approach is primarily based on the ionic interactions among the definitely charged primary amino group of the l-lysine conjugated sodium alginate and the negatively charged ions of the poly anion, including calcium chloride. These are the more extensively used ionic cross –linking agent due to its non-toxic and multivalent residences. This physical cross linking approach not only ignore the use of chemical cross-linking agents and emulsifying agents which are frequently toxic to organisms, but also avert the possibility of destruction to drugs, particularly biological agents.

INTRODUCTION

Cancer can result from ordinary proliferation of any of the exclusive forms of cells in the body, so there are more than a hundred awesome types of most cancers. These types of cancer could range substantially of their conduct and reaction to treatment. Cancer is a term used for illnesses in which odd cells divide without control and are able to invade different tissues. Cancer cells can spread to different parts of the frame through the blood and lymph structures [1]. A lot of demanding situations lie in advance for all those that are involved in cancer research and patient management. In most cancers, unusual cells divide in an uncontrolled manner and invade to other parts of the frame through the blood and lymph device. A single tumor cell surrounded by way of ordinary cells will mirror at a better charge than the other cells. Once a small cancerous mass has shaped, the ordinary cells will no longer be capable of compete with the tumor cells for the supply of nutrients from the blood move. The tumor cells will start displacing regular cells until the tumor reaches a spread-limited maximal size [2-3].One of the perfect methods overcome these problems may be exist with biodegradable polymeric providers consisting of nanoparticles. Nanoparticles may also emerge as one of the carrier systems for resolving the problems caused by infections. These infections can be refractory to conventional remedy and additionally widening the therapeutic margin of antibiotics presently utilized in clinical practice [4]. The effectiveness of most cancers therapy in strong tumors depends on good enough transport of the therapeutic agent to tumor cells. Inadequate delivery results in residual tumor cells and results in re-boom of tumors. Because of the specific attributes of the tumor microenvironment, it is viable to shape drug delivery systems that mainly target anti-cancer drugs to tumor [5]. Nanomedicine can arrive tumor probably through the leaky vasculature enclosing the tumors by utilizing the improved permeability impact (EPR).The ligands attached at the plane of nanocarriers enable active targeting by holding together the receptors over-expressed by cancer cells or angiogenic endothilial cells. An essential requirement of modern drug is the controlled delivery of drug or an energetic substance to the site of action in the body in an optimal concentration versus time profile. One attempt to achieve this goal is the improvement of colloidal drug provider referred to as nanoparticles (NPs) [6]. Alginates, natural hydrophilic polysaccharide derived from seaweed, consist of 1□4, connected D-mannuronic acid and L-glucuronic acid residues arranged as blocks of either sort of unit or as a random distribution of every type. Alginates do not gel considering they have poly (L-gluronic acids) which might be rigid. The gelation or crosslinkage is because of the stacking of the glucuronic acid blocks of alginate chains. Sodium alginate is the sodium salt of alginic acid. The sodium alginate is a flexible purposeful biomaterial for viscosity enhancement, stabilizer, matrixing agent, encapsulation polymer, bioadhesive and movie former in transdermal and transmucosal drug delivery. Alginate polymers are straight unbranched polysaccharides comprising of 1,4'-linked-b-Dmannuronic acid and a-L-gluronic acid. The design carried by those residues differs significantly and are organized in a block sample through the length of the chain [7]. Lysine is a vital amino acid and as a base, as are arginine and histidine. The □-amino institution frequently participates in hydrogen bonding and as a fashionable base in catalysis. (The □-amino group (NH3+) is hooked up to the fifth carbon beginning from the □-carbon, that is attached to the carboxyl (C=OOH) group. Lysine show higher uptake in tumor tissue, as the scale of tumor will increase, the uptake of this amino acid can even increases. Branched chain amino acid potentially utilize for proliferative and invasive activities in tumor. Tumor cells needed more glucose and amino acids than normal body

cells [8]. Hence, the objective of the work was to formulate lysine conjugated sodium alginate nanospheres of 5-fluorouracil with the aid of ionic gelation technique. The prepared NPs formulations investigated with physicochemical characteristics such as particle size, drug loading ability, in vitro release characteristics. This physical cross linking method not only avoids using chemical cross-linking agents and emulsifying agents that are often toxic to organisms, but also prevents the possibility of harm to drugs, specifically organic retailers.

MATERIAL AND METHOD

Synthesis of Lysine conjugated sodium alginate

Sodium alginate (1%) solution was prepared in 100 ml of refined water with the aid of extended magnetic stirring in a beaker. Various quantities of sodium metaperiodate dissolved in a 100 mL of distilled water were dissolved to the solution and had been agitated magnetically in the dark at 25°C for 6 h. The solution changed into then dialyzed in opposition to distilled water (2.5 L) with various change of water until it was free from periodate (48 h). The solution became stirred for 24 h at dark. The response becomes stopped by means of including 1ml Ethylene glycol. 2.5gm sodium chloride brought Ppt happens after addition of extra amount of ethyl alcohol. Ppt happens after addition of extra amount of ethyl alcohol. Ppt turned into collected, redissolved in distilled water and freeze dried the product oxidized sodium alginate [9]. The prepared alginate dialdehyde (ADA) reacted with Lysine with methanol and 2% acetic acid with the aid of a Schiff's Base response. The l-lysine conjugated alginate answer became then frozen at -5°C, lyophilized and saved in a desiccator in the freezer at 4°C. The dried conjugated sodium alginate become powdered the use of glass mortar-pastel and surpassed via sieve wide variety 60 and stored in desiccator until similarly use. The presumptive substructure and synthetic pathway of sodium alginate Lysine is proven in figure 1.

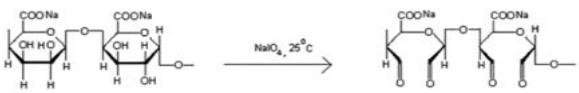


Figure 1: Reaction of lysine sodium alginate conjugate

Characterization lysine sodium alginate conjugate: The identification and characterization of the prepared compound was carried out in terms of various parameters i.e. physical appearance, solubility determination, thermogram (Differential scanning calorimeter), infra red spectroscopy etc.

Physical appearance: The synthesized compound was subjected to physicochemical characterization like nature and color.

Solubility: Solubility can be defined as spontaneous interaction of two or greater materials to from a homogeneous molecular dispersion. The solubility of the lysine conjugated alginate changed into tested in various aqueous and non-aqueous solvents. A specific amount of compound (10 mg) was tried to dissolve in 10 ml of each investigated solvent at room temperature. The solubility became determined handiest through visual inspection.

DSC Analysis: The DSC appears at changed into performed by the usage of JADE Perkin Elmer differential scanning calorimeter with thermal analyzer. Accurately weighed samples (□□ three mg of samples) had been located in sealed aluminium pan, earlier than heating underneath nitrogen float (20 ml/min) at a scanning charge of 10 °C consistent with min from 25 to 300°C. An empty aluminium pan used as a reference. Thermogram of the synthesized conjugate, sodium alginate, lysine alginate dialdehyde, and physical mixture (alginate dialdehyde + lysine) were received.

Fourier transformed infrared spectra: FTIR Spectra (systronic) of drug, sodium alginate lysine conjugates and drug loaded NPs were recorded in potassium bromide pellets. All the above discussed powder become blended with dried and floor potassium bromide (Merck – FTIR - grade), pelletized at 10.2x 10^4 pa and the spectrum changed into recorded among 2000 – 500cm^{-1} and 4000 – 450 cm^{-1} using a excessive electricity ceramic supply and DLATGS detector similarly, the NPs and the sodium alginate powder have been analyzed by means of FTIR spectral analysis.

Preparation of nanoparticles by controlled Ionic gelation method

The calcium chloride solution in different concentrations was added continuously to 10ml of lysine conjugated sodium alginate alternatives to produce gellification. The nanoparticles suspension obtained was continuously stirred with mechanical homogenizer at 500 rpm at 3h to shape a polyelectrolyte complicated and saved in a single day for stabilization. The nanoparticles were separated via ultra centrifugation at 20000 rpm for 45 minutes and beneath vacuum to structure a flaky mass, which on redispersion in sterile water for injection composed distinct particles.

Optimization of l-lysine conjugated sodium alginate nanoparticles: L-lysine conjugated sodium alginate concentration have been varied at the constant time as maintaining different parameters with calcium chloride concentration, temperature of l-lysine conjugated sodium alginate solution. Lysine conjugated sodium alginate solution was varied from 0.75% w/v to 2.0 % w/v while calcium chloride answer concentration changed into various from 0.05% w/v to zero.5% w/v and effects recorded as suggest particle size, mean PDI and zeta potential.

Characterization of nanoparticles

Determination of particle size: The nanospheres were spread over a glass slide and dried under vacuum at room temperature (25°C). The sample became shaded in a cathodic evaporator with a gold layer 20nm thick. The diameters of all the spheres in every area have been calculated by the help of a JSM-6400 scanning electron microscope (Tokyo, Japan) [10]

Zeta potential: Zeta potential become measured for each method using big bore capillary cells within the Zetasizer Nano-Zs (Malvern Instruments) 1 ml of nanoparticle suspension from the preparation medium became sampled out and diluted to 8ml with 0.9% (M/V) sodium chloride solution prepared in distilled water for optimal signal intensity. Three formulations have been noted to get the common zeta capacity for distinctive formulations.

Determinations of drug content: The amount of drug present in the clean supernatant after centrifugation was determined in phosphate buffer (pH 7.4) by ultracentrifugation method [11]. The centrifugation was separated for free drug from nanoparticles from nanoparticles and used to estimate the drug loading of the nanosparticles. The final colloidal suspensions were extremely centrifuged at ten thousand rpm at 25 ± 2°C for half of an hour. The supernatant turned into analyzed for drug content by measuring the absorbance at 266 nm the use of UV spectrophotometer.

***In-vitro* release studies:** The drug release estimation turned into carried out with diffusion cell. A cellophone dialysis membrane became fixed to one stop of permeation cellular as ex-vivo study. The nanoparticles suspension became positioned in the cell donor compartment and the cell turned into immersed in a beaker containing 75ml of phosphate buffer (pH 7.4) act as a receptor compartment. The cell became immersed to a intensity of 1cm under the surface of the receptor compartment containing solvent. The medium inside the receptor compartment turned into continuously agitated using a magnetic stirrer at 50 rpm with temperature

of 37 ± 5°C. The aliquots of 1ml of the sample from the receptor compartment became taken at each predetermine time durations upto duration of 24 h and each time replaced with fresh buffer. The samples withdrawn were estimated spectrophotometrically at 266 nm.

RESULTS AND DISCUSSION

The present investigation was involved for preparation of nanoparticles by controlled gellification for model drug 5-fluorouracil as anticancer drug. The formulations were prepared with biocompatible (l-lysine conjugated sodium alginate) conjugate. This complex was safe and prepared a mild and simple reaction procedure. The conjugate presented good dispersibility in water and the L-lysine anchored sodium alginate nanoparticles depict a controlled release pattern with the model drug. Sodium alginate is a disachharide consisting of □-D mannuropyranosyluronic acid and □-L-gulopyranosyluronic acid units. Sodium alginate is oxidized by sodium periodate and yields alginate dialdehyde. The amino groups of lysine were reacted with aldehyde groups derived from the oxidation of alginate to formed Schiff's base. In order to analyze the conjugate, successive tests such as FT-IR and DSC were performed. These identification parameters are very important to confirm the formation of conjugate after reaction. The obtained conjugate was white amorphous in nature and it has the melting point in the extent of 71°C to 85°C. Solubility of the l-lysine conjugated sodium alginate was checked qualitatively in methanol, ethanol, chloroform, petroleum ether, di-ethyl ether, ethyl acetate, 0.1N HCL, 0.1 N NaOH, distilled water, phosphate buffer (pH 7.4), acetone, dimethyl sulphoxide (DMSO), tetrahydrofuran (THF). The conjugate was found to be soluble in 0.1 N HCl, distilled water and 1% acetic acid solution. The solubility of the conjugate was analysed qualitatively in different polar and non-polar solvent showed that the conjugate was soluble in water, whereas insoluble in other solvents. The DSC is very important tool in the analysis of thermal properties of compounds. The DSC thermogram of alginate dialdehyde showed a broad endothermic peak at 70 °C which shows the melting of alginate dialdehyde. The endothermic peak at 75°C indicated the melting point of sodium alginate while new endothermic peak at 65°C indicated the melting point of l-lysine conjugated sodium alginate (Figure 1-5).

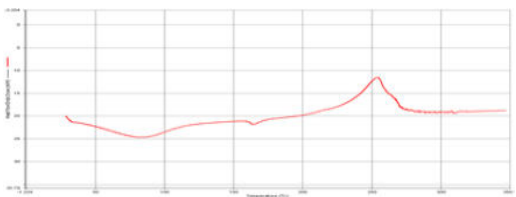


Figure 2: DSC Thermogram of sodium alginate

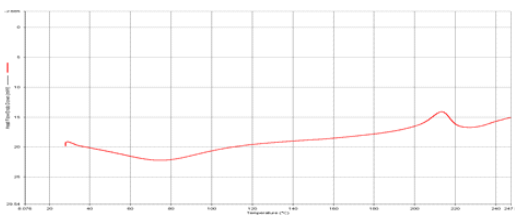


Figure 3: DSC Thermogram of Alginate Dialdehyde

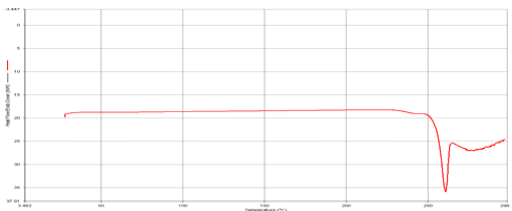


Figure 4: DSC Thermogram of l-lysine

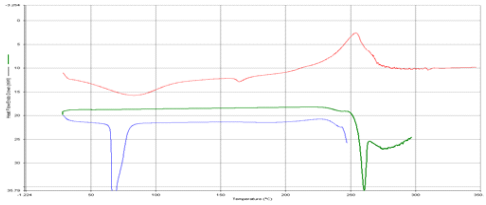


Figure 5: Overlay of DSC Thermogram of l-lysine conjugated sodium alginate

Identification of the L-lysine conjugated sodium alginate was done by IR Spectra. To confirm this linkage the IR spectroscopic analysis was performed and the characteristic absorbtion peak at 1590 cm-1 of resultant imine bond. The peak at 3365.76 cm-1 represented stretching vibration at the secondary amine (N-H bonding) and stretching respectively which showed that successful coupling reaction was taken place between aldehyde group of alginate dialdehyde and amino group of l-lysine. The peaks at 1622.13 cm-1 in the spectra of l-lysine represented the primary amine N-H bending which was greatly reduced in the spectra of l-lysine conjugated sodium alginate again confirmed the coupling reaction between l-lysine and oxidized alginate or alginate dialdehyde (Figure 6-9 and Table 1-3). The above formed conjugate is utilized for the nanoparticle formation; the method used in the development of nanoparticles is the ionotropic gelation method.

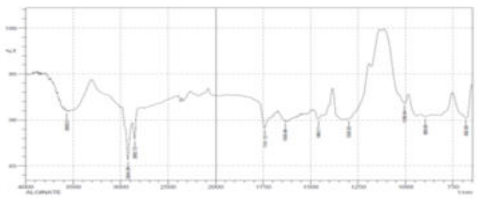


Figure 6: IR spectrum of sodium alginate

Table 1: IR spectrum of sodium alginate

S. No.	Wave Number (cm^{-1})	Characteristic Absorption
1	1006.84	=C-H bending (alkene)
3	1741	-C=O Stretching (carboxyl)
4	1460	C-H bending
5	3500	N-H Streching
6	2852	C-H symmetric stretching

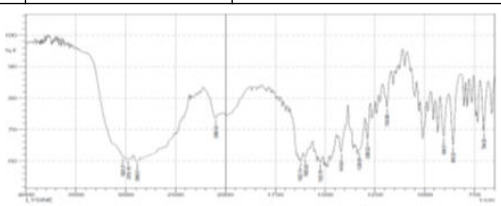


Figure 7: IR spectrum of l-lysine

Table 2: IR spectrum of l-lysine

S. No.	Wave Number (cm-1)	Characteristic Absorption
1	1190.08	C-N stretching
2	1622.13	N-H stretching
3	2976.16	C-H stretching
4	3026.31	O-H stretching

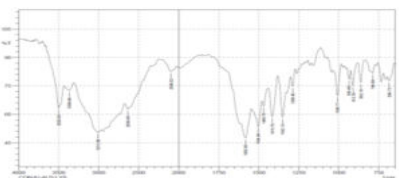


Figure 8: IR spectrum of Lysine conjugated sodium alginate

Table 3: IR spectrum of Lysine conjugated sodium alginate

S. No.	Wave Number (cm-1)	Characteristic Absorption
1	682	-C-H bending
2	3369.64	-N-H stretching
3	1583	-N-H bending
4	1635	-C=O Stretching
5	2620-2700	-H-C=O Stretching

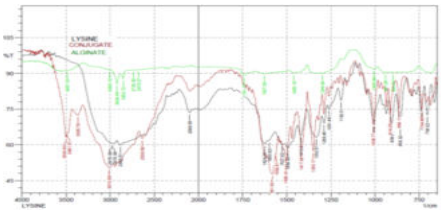


Figure 9: Over lay spectra of l-lysine, sodium alginate and alginate dialdehyde

Table 4: Preparation of nanoparticles (Nps)

F. Code	Conc. of modified Sodium alginate (%w/v)	Conc. of Calcium Chloride (%w/v)
F1	0.75	0.05
F2	1	0.1
F3	1.25	0.15
F4	1.5	0.2
F5	1.75	0.25
F6	2	0.5

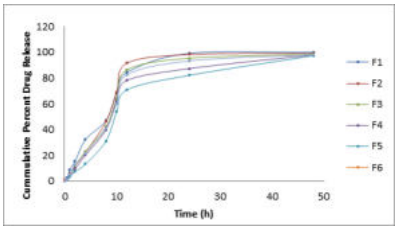
The particle size, zeta potential and polydispersity index was determined by the Malvern Zetasizer (DTS version 4.10, Malvern, UK), the size was 221 nm to 257 nm is the size of optimized formulations, the zeta potential was recorded to be -0.5 mV to -1.48 mV which clearly reveals the stability of system. The parameters like entrapment efficiency, loading efficiency and in-vitro release profile was also calculated using 5-Fluorouracil as a noble drug, the in-vitro release was determined in phosphate buffer (pH 7.4) using dialysis membrane (molecular weight cut off 12 kDa). Release data shows that 64% drug released in 48 h, initially burst release of 28% was observed in first 4 h was obtained and the coefficient of regression for higuchi model was obtained to be 0.917 which is very near to unity it means that the drug release follows the Higuchi model, in this model the drug released by the diffusion process from the polymeric matrix of nanoparticles. The movement of drug from nanoparticles followed first order kinetics over a 24 h period. Drug loading is another factor that influenced the drug dispersion rate from the nanospheres. Generally, increasing the drug content in the nanospheres increased. The values of 'k', 'n' and 'r' for five different batches are reported in Table 2, and the 'n' value was within 0.5132 to 0.5583 9 (Figure 10 a-d). The results of kinetic analysis reveal that the release of drug from nanostructured formulation followed Fickian diffusion.

Table 5: Optimization result prepared nanoparticles (Nps)

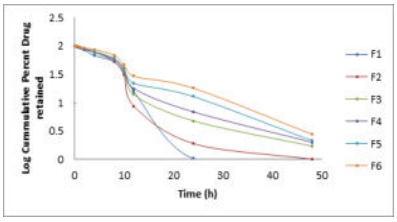
F. Code	Mean Particle size (nm)	Mean PDI	Zeta (Mev)	Drug content
F1	221	0.009	-0.51	99.02
F2	232	0.015	-0.86	99.13
F3	238	0.072	-1.12	98.92
F4	242	0.129	-1.31	99.01
F5	244	0.069	-1.39	100.09
F6	257	0.095	-1.48	99.01

Table 6: in-vitro study of nanoparticulate system

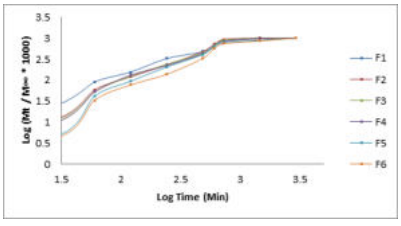
Time (h)	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0
0.5	2.6	1.206	1.106	1.006	0.482	0.421
1	8.89	5.72	5.12	5.02	4.06	3.15
2	15.34	11.67	11.97	12.68	9.21	7.34
4	32.12	22.23	22.93	21.43	19.98	13.11
8	47.23	46.31	42.31	41.31	39.89	31.23
10	68.23	68.98	64.18	59.98	61.89	53.67
12	84.21	91.34	86.21	82.21	77.99	70.76
24	98.98	98.11	95.23	93.12	87.21	81.98
48	99.89	99.05	98.3	98.01	97.87	97.24



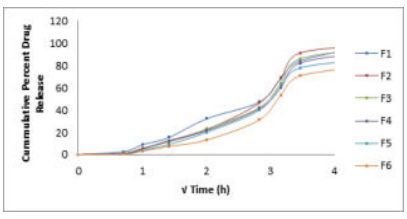
10.a) Zero-order kinetics



10.b) First-order kinetics



10.c) Korsmeyer's Peppas kinetic plot



10.d) Higuchi plot

Figure 10: in-vitro study of nanoparticulate system

CONCLUSION

The developed sodium alginate nanoparticles of 5-fluorouracil have been found to be an powerful and natural carrier in terms of distinct particle size, optimum drug loading efficiency, satisfactory in vitro release characteristics. L-Lysine is an essential amino acid. The amino groups of L-lysine are exceedingly reactive and participate in reactions on the active centers of enzymes. Lysine indicates better uptake in tumor tissue, as the scale of tumor will increase, the uptake of this amino acid will increase. The L-lysine conjugated sodium alginate changed into synthesized by using the response between alginate dialdehyde and L-lysine. The solubility of the conjugate turned into analyzed qualitatively in special polar and non-polar solvent showed that the conjugate become soluble in water, while insoluble in different solvents. The solubility of the conjugate turned into

analyzed qualitatively in one of a kind polar and non-polar solvent confirmed that the conjugate became soluble in water, whereas insoluble in different solvents. The in-vitro release became determined in phosphate buffer (pH 7.4) using dialysis membrane released controlled way and the coefficient of regression for higuchi modelwas found by means of the diffusion process from the polymeric matrix.

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