RESEARCH ARTICLE

Effect of carbopol gel in stable liposomes and their enhanced antipyretic effect

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The modified liposomes formulated with carbopol (CP) polymer might be promising for their enhanced stability and antipyretic action. The formulation of liposomes was performed to obtain the minimum concentration of the gel to enhance the stability of the vesicles and to prove the efficacy of the liposome in promoting the antipyretic activity of the drug. The liposome formulation was found to be more effective than the marketed antipyretic drug. A 1.5% CP could be considered as the minimum concentration for the gel to maintain the liposome stable than a 0.5% CP polymer. The surfactant, Polysorbate 80, helped to form spherical vesicles and CP polymer-1.5% w/w was found to show enhanced stability of the formulation. The concentration of 1.5% CP seemed to give the necessary mechanical strength to the liposomal formulation. A drug delivery system by the modified liposomes may be considered for further investigation for validating and standardizing this formulation for the controlled drug release system toward enhancing maximum therapeutic efficacy.

Key words: *Antipyretic activity and carbopol polymer, paracetamol liposomes*

INTRODUCTION

A very potent drug, Paracetamol is known for its analgesic and antipyretic activity. It is being marketed under various trade names like Paracep, Calpol, etc. It is widely formulated as a tablet dosage form. A newer approach of designing the liposomal gel using carbopol (CP) polymer for its stability and enhanced antipyretic effect is a facile one. Liposomes act as carrier vehicles for dermal, transdermal, oral and intravenous controlled drug delivery devices.[1] Liposomes are closed vehicles of phospholipid bilayers or lamellae enclosing an aqueous layer and cores are able to encapsulate hydrophobic and hydrophilic ingredients into their structure.[2] The hydrophilic surface of the liposomes provides prolonged circulation of the drug carrier.

Phosphatidylcholine, also known as lecithin, is the main structural component derived from both natural and synthetic sources. It is a mixture of glycolipids, triglycerides and phospholipids.[3] Cholesterol, sterol lipid, another structural component found in the cell membranes of all body tissues, is considered for formulating liposomes, which results in simulating the cell biomembranes.

Polymer used is CP, which is a polymer of acrylic acid cross-linked with polyalkenyl ethers or divinylglycol.[4] Carbomers readily absorb water, get hydrated and swell. The hydrophilic nature and its cross-linked structure and its insolubility in water make CP a potential candidate for use in a controlled release drug delivery system. It has a very good water sorption property. It swells in water up to 1000 times its original volume and 10 times its original diameter to form a gel when exposed to a pH environment above 4.0-6.0. Its pKa is 6.0-0.5.[5] Polysorbate 80 (commercially known as tween 80, a trade mark of Uniqema/ICI), is a nonionic detergent and emulsifier derived from polyoxylated sorbitol and oleic acid. It is stable in its weak acids or bases.[6] Liposomal vehicles fuse on to the skin surface and the intimacy of contact between the drug-loaded bilayers and the skin is probably mediated via calcium bridges. Topically applied liposomal formulations, particularly those prepared from lipid mixtures of composition similar to the stratum corneum, may act as a drug delivery system for the treatment of skin disorders.[7] In a study, the deposition of topically applied gels of free and liposomally entrapped triamcinolone on rabbit skin were compared and found that application of the liposomal gel resulted in a concentration of triamcinolone acetonide approximately five times larger in the epidermis and three times higher in the dermis than application of

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DOI: 10.4103/0973-8398.56308

the free drug gel.[8] A study was conducted to develop and characterize hydrophilic gels containing chlorophyllin (CHL)-loaded liposomes as well as to evaluate their stability. The chlorophilin-loaded liposomes were found to be highest in the gel containing nonhydrogenated lecithin liposomes followed by the gel containing hydrogenated lecithin liposomes and liposome-free gel, indicating that the encapsulation of CHL in liposomes led to a greater stability of CHL.[12]

In another study, release of calcein and griseofulvin (GRF) from control (gels in which solutes are dissolved) and liposomal gels was studied using agarose-assisted immobilization as a technique to separate gels from drug-receptor compartments. They concluded that calcein and GRF release from control CP gels is faster compared with hydroxylethyl–celluloseand mixture gels. The same is true for calcein in liposomal gels. CP gel rheological properties were found to be significantly different (compared with the other gels), implying that these characteristics are important for drug diffusion from the gels.[13] In a study conducted by Pavelić *et al.,* they have designed and evaluated a new vaginal delivery system for the local treatment of vaginitis. Liposomes containing two commonly applied drugs in the treatment of vaginal infections, namely clotrimazole and metronidazole, were prepared by the proliposome and the polyol dilution methods. Storage stability studies have proved the ability of the CP 974P NF gel to preserve original size distributions of the incorporated liposomes. All the experiments performed confirm the applicability of bioadhesive liposome gels as a novel delivery system for the local therapy of vaginal infections.[14]

Mucoadhesive liposomes were prepared by coating multilamellar liposomes with CP in a similar manner to that used in the preparation of chitosan-coated liposomes (CS-Lip) The overall pharmacological effect of CP and CS-Lip was evaluated by means of the area under the plasma calcium concentration curve and was 2.4 and 2.8 times higher than that of negatively and positively charged non-Lip, respectively.[15] A novel topical benzoyl peroxide (BP) gel formulation containing liposomal BP was shown to significantly reduce local irritation relative to its nonliposomal BP gel (plain BP gel) preparation and also to improve clinical efficacy (almost twofold) in the treatment of acne. BP liposomes were prepared, optimized and formulated into a CP 934 gel base. The liposomal gel formulation of both the drugs significantly reduced the local adverse effects, thereby improving patient compliance.[16]

In our study, the formulation of liposomes was performed to obtain the minimum concentration of CP gel to enhance the stability of the vesicles and to prove the efficacy of the liposome in promoting the antipyretic activity of the drug.

MATERIALS AND METHODS

Using lecithin and cholesterol in a molar ratio of 1:2, which was dissolved in 5 ml of organic (ether) and 3 ml of polysorbate 80, phospholipid vesicles were prepared having drug as the dispersed phase in the aqueous solution.[9] Various concentrations of CP: 0.5, 1, 1.5, 2 and 2.5% were prepared by the cold method.[9]

Preparation of the liposomal gel

Liposomes were prepared based on the solvent evaporation method.[9] About 100 mg of Paracetamol was dissolved in 100 ml of water. A series of dilutions were made to get a strength of 10 mcg/ml. The absorbance of the resulting solution was measured at 257 nm using a visible spectrometer (Elico-SL159). Lecithin and cholesterol, at a molar ratio of 1:2, were dissolved in an organic solvent – ether and 3 ml of polysorbate 80 was added. The contents were homogenized at 5000 rpm until the organic solvents got evaporated. The prepared standard solution was added to the homogenizer jar and homogenized lightly for the drug entrapment in the phospholipid vesicles. Five similar liposomes were prepared as such. Various concentrations (0.5, 1, 1.5 and 2%) of the CP gels were prepared in water and gently stirred for the gel to swell. Five milliliters of triethanolamine and water were alternatively added with continuous stirring to form a transparent gel until the gel was alkaline. The prepared liposomes were transferred to the CP gel of varying concentrations.

Liposome particle size analysis

The formulation was mounted on a glass slide and viewed under a calibrated microscope (Medilux – 2117 R II; Kyowa–Getner, Ambala, India).The liposomes were analyzed for their size distribution using a compound microscope at 100X to observe for their spherical nature after their suitable dilution.[10] The mean diameter of the liposomal particles was found to be 275.10 + 10 μm in the range of 178.5-455 μm.

Determination of the *in vitro* drug release kinetics

The *in vitro* release of the drug in a free form was determined from the different formulations using a dialysis bag. A known quantity of the gel (1 g drug equivalent of the prepared test and standard gels) formulation was weighed and filled in a dialysis sac (Sigma Chemical Company, St. Louis, MO, USA) and sealed in a glass vial under a constant magnetic stirring. This dialysis tube was suspended in the release medium of 0.1N hydrochloride solution. The medium was maintained at 37°C with stirring at 100 rpm, maintaining a proper sink condition. The samples were withdrawn and were serially diluted with 0.1N hydrochloric acid (to simulate gastric media) to get a final concentration of 1 mcg/ml. The amount of Paracetamol in the media was determined using a visible spectrometer (ELICO-SL159) at 247 nm.[11] The release study was conducted at various intervals of 1, 2 and 3, 4 weeks, first and second month, respectively, after storing at 25°C at 60% relative humidity.

Animal studies

All the animal experiments were carried out after the prior approval from the Institutional Animal Ethical committee. Wurster rat (200-250 g) of four groups each containing two

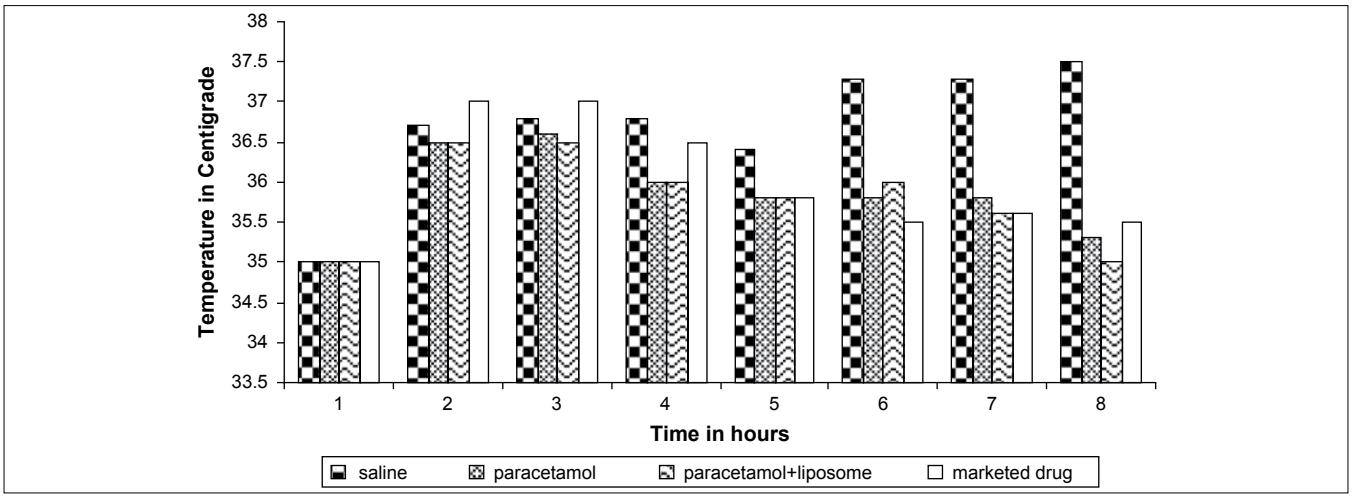

Figure 1: Plot showing *in vivo* animal study–antipyretic action

rats were taken. To all the four groups of animals, 0.5 ml (6% aqueous dispersion) of yeast was administrated via the subcutaneous route. After 15 min, various drug solutions were administrated orally through an oral feeding needle. The first group was administrated orally with saline 0.9% w/v (control). The second group was administrated with Paracetamol drug solution (Paracetamol – 1 mg/ml) orally. The third group was administrated with liposomal drug solution orally containing Paracetamol – 1 mg/ml. The fourth group was administrated with marketed Paracetamol drug solution (1 mg/ml) orally. The control of rise in body temperatures was recorded at various time intervals (0, 1, 2, 3, 4, 5, 6, 7 h). Similarly, three such cycles were performed after providing 1 week of wash out period and the average results were tabulated and the plot of concentration verses temperature was made.

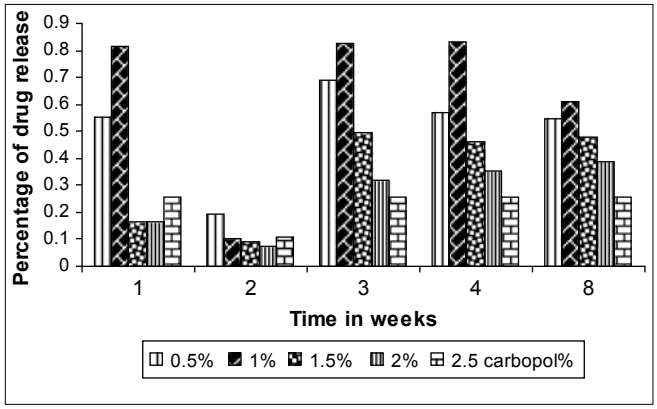

Figure 2: Plot showing *in vitro* release of varying concentrations of prepared carbopol gels at varying time intervals

RESULTS AND DISCUSSION

The modified liposomal gel seems to show enhanced permeability, faster onset of action and enhanced antipyretic action compared with the other formulation used in antipyretic study. The liposomal gel entrapping the drug Paracetamol were able to control the rise in body temperature at a faster rate than the other Paracetamol/ control formulation viz saline solution, Paracetamol drug solution and marketed formulation, and this was evident from Figure 1. This ensures that liposome-entrapped Paracetamol matrixed in 1.5% of CP gel was more efficient in regaining or controlling the elevating body temperature of the animal than the other positive (marketed formulation) and negative control (saline solution).

The surfactant – polysorbate 80 – helped to form spherical vesicles and CP polymer-1.5% was found to show enhanced stability of the formulation. The concentration of 1.5% CP seemed to give mechanical strength to liposomal formulation thus preventing any leakage of drug from vesicles till 8 weeks than the other gel concentration viz 0.5,1, 2 and 2.5 % w/w, and this could be clearly represented in Figure 2.

The liposomal formulation of Paracetamol matrixed in 1.5% CP gel was much superior in keeping the drug stable and intact within the liposomes thus preventing the degradation of the lipid carrier during the storage period, and this was evident from the *in vitro* studies [Figure 3]. The liposomal gel of Paracetamol (1.5% CP) showed satisfactory *in vitro* release after storage till 8 weeks. The liposome formulation was found to be more effective than the marketed antipyretic drug and 1.5% of CP could be considered as the minimum concentration for the gel to maintain the liposomes in a stable state than other CP concentration as antipyretic drug delivery for analgesic.

Thus, this optimized liposomal formulation matrixed in 1.5% CP gel would be considered most suitable toward faster onset of action and stability for the drug than the conventional drug dosage forms.

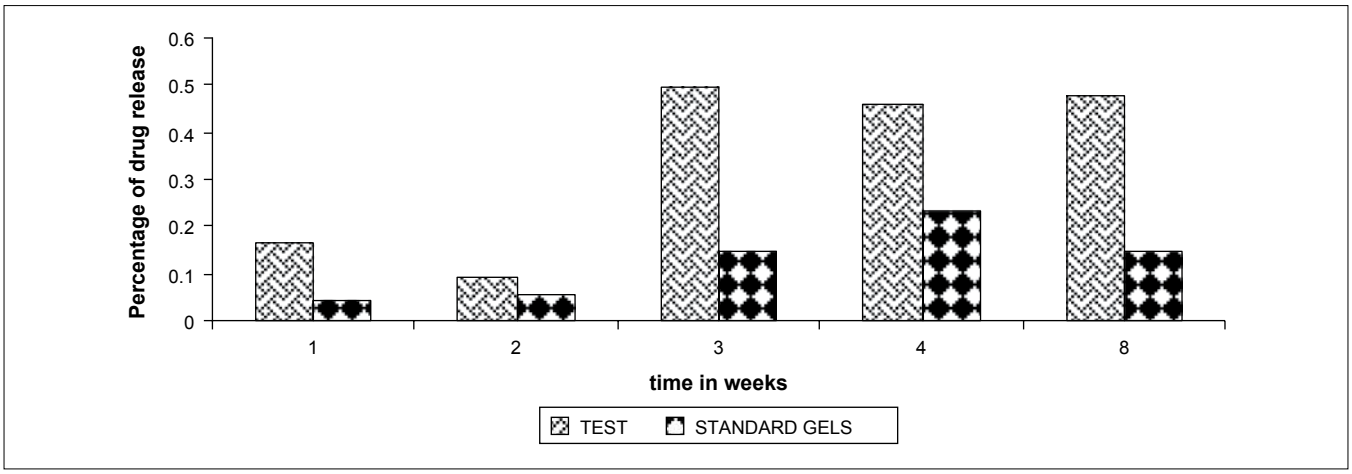

Figure 3: Plot showing *in vitro* release of 1.5% carbopol gel during a period (8 weeks)

CONCLUSION

The formulated liopsomal gel having CP of 1.5% concentration would be stable and keeps the medicament for a longer time period from the leaching process of the lipid bilayer. Thus, highly degradable, potent drug can be formulated in this CP gel liposomal drug delivery thus enhancing the potency of the drug and protecting the drug's therapeutic efficacy till the desired period.

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Source of Support: Nil, **Conflict of Interest:** None declared