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Effect of different excipients on the release of vinpocetine from biodegradable polymeric implants of chitosan and sodium alginate

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Abstract
Vinpocetine is a drug which is reported to have cerebral blood-flow enhancing and neuroprotective effects. The purpose of this study was to prepare and evaluate a biodegradable implantable system of Vinpocetine to reduce its dosing frequency for lowering the chance of adverse effects and to improve patient compliance. Vinpocetine loaded Chitosan- Na Alginate implants were prepared of varying ratios of 60:40 and 70:30 with 50 mg drug load. Implants were placed into a crosslinking solution of Glutaraldehyde with exposure time of 30 minutes. Implants were evaluated for loading efficiency and in-vitro release studies. Implants formulated with 50 mg drug load in 60:40 ratio of Chitosan-Sodium Alginate produced the maximum sustained action. That is why 60:40 was considered as optimum ratio and six different excipients: Glyceryl mono-stearate, Cetyl Alcohol, Stearic Acid. Cetostearyl Alcohol, Methocel K 15 M and Kollidon 30 were used to prepare implants in 60:40 ratios with 30 minutes exposure time. All of the excipients were found to increase the loading efficiency of the implants. The results of in-vitro dissolution study were fitted to Higuchi, Korsmeyer-Peppas, Zero order and first order kinetics models to evaluate the release kinetics data. Implants were found to follow Zero order model implying controlled release. It was observed that drug release from the implants with Cetyl alcohol excipient showed maximum sustained action followed by stearic acid. Scanning Electron Microscope method was used to observe the surface morphology of implants to determine the surface integrity of the polymers before drug release and to observe the surface after drug release to correlate with the in-vitro results.

Keywords: Vinpocetine, chitosan, Na-alginate, biodegradable implants

1. Introduction
Implants are small sterile solid masses which consist of a highly purified drug and they can be with or without excipients which are intended for implantation in the body (usually subcutaneously) to provide continuous release of the drug over long periods of time. They are usually administered by a suitable injector or surgical incision [1]. In case of non-biodegradable implants, a second surgical procedure is needed to remove the devices. Although the invasive procedures are a major disadvantage of the administration, implants can be removed easily if early termination is required owing to adverse effects [2]. To overcome the drawbacks of conventional parenteral dosage forms, implantable delivery systems have been designed to reduce the frequency of dosing, to prolong the duration of action, to increase the patient compliance, and mainly to optimize pharmaceutically-related therapy [3].
Implants can be used as delivery systems for systemic or local therapeutic effects. For systemic therapeutic effects, implants are administered subcutaneously [1]. Incorporated drug is delivered from the implant and absorbed into the blood circulation [2]. Implants for local effects are placed into specific body sites. The term local injection is the drug administration to local compartments where the drug action occurs without being absorbed into the systemic circulation. Targeting implants aim to release a drug and have a therapeutic effect at the sites of implantation [4].
Implantable drug delivery system can be classified into three major categories: biodegradable or non-biodegradable implants, implantable pump systems, and the newest atypical class of implants. Biodegradable and non-biodegradable implants are available as monolithic systems or reservoir systems. The release kinetics of drugs from such systems depend on both the solubility and diffusion coefficient of the drug in the polymer, the drug load, as well as the in vivo degradation rate of the polymer, especially, in the case of the biodegradable systems [5].

Biodegradable polymers have been increasingly used in pharmaceutical applications. Ideally, biodegradable polymers would be: metabolized in the body and eliminated by normal physiological pathways; fabricated easily into the final forms; degraded into non-toxic substances that are non-mutagenic and non-cytotoxic; and cause no initiation of inflammatory processes after application, injection or insertion [6, 7, 8]. Another criterion to be considered for a polymer to be a suitable biodegradable polymer is the end product after degradation. The end products of aerobic degradation from biodegradable polymers should be carbon dioxide, water and/or minerals [9].

The advantage of biodegradable implants is that they degrade after they have fulfilled their function. Therefore, a second operation for removing implants is not necessary. Additionally, the healing process may be stimulated by the successive loss of the mechanical properties of the implant during degradation, corresponding with the increasing loading on the healing tissue [6, 7, 8].

Chitosan is a poly cationic polysaccharide, synthesized by alkaline de acetylation of natural chitin. Due to its nontoxicity, biocompatibility and biodegradability, it is also an attractive polymer for parenteral delivery of drug and biologically active compounds [10]. Decrease in the rate of drug release from chitosan-based systems, and the gel formation of chitosan can be achieved by a crosslinking process for example, glutaraldehyde and divalent anions as crosslinking agents [11, 12]. Complex of chitosan and alginate has been created via ionic interaction between the amine groups of chitosan and the carboxyl groups of alginate [13]. A more effective controlled-release was observed when using the complex of chitosan and alginate to produce the drug release system when compared to chitosan or alginate alone. A longer duration of drug release was found when the complex was used [14, 15. 16].

Vinpocetine is reported to have cerebral blood-flow enhancing and neuroprotective effects, and is used as a drug in Eastern Europe for the treatment of cerebrovascular disorders and age-related memory impairment [17]. Vinpocetine is widely marketed as a supplement for vasodilation and as a nootropic for the improvement of memory and cerebral metabolism. Vinpocetine has been identified as a potent anti-inflammatory agent that might have a potential role in the treatment of Parkinson's disease and Alzheimer's disease [18].

The purpose of this study was to prepare an implantable form of Vinpocetine to overcome the drawbacks of the conventional dosage form because conventional preparation will lead to large fluctuation in drug plasma concentration and side effects on human body. If Vinpocetine is given to patients under 25 years of age with high dosing frequency it might hamper the natural development of the brain and also cause drug dependency.

2. Materials and Methods

2.1 Materials

Vinpocetine was provided by Eskayef Bangladesh limited. All the chemicals and reagents used in this study were of analytical grade. Their experimental mixtures were prepared in standard volumetric flasks about 30 minutes prior to recording the data. Suitable storage conditions were maintained to store the working chemicals and reagents.

2.2 Methods

2.2.1 Implant preparation

Biodegradable implants of Vinpocetine were prepared by the use of two biodegradable polymers Chitosan and Sodium Alginate. Implants were prepared using 50 mg drug with 2 different polymer ratios (60:40 and 70:30) as well 50 mg drug load with different excipients.

Implants were prepared by using 100ml of 1% acetic acid solution to dissolve 4.167g of chitosan. The solution was stirred until no large chunks remained and then blended until it was homogenous. 100ml of distilled water was used to dissolve 4.167g of Na Alginate. The solution was stirred until no large chunks remained and added to the blended chitosan solution. Drug Vinpocetine was then dispersed to the Chitosan and Sodium Alginate solution. After being mixed with ultra-sonication, the mixture was poured into petridish. Then they were allowed to set by placing inside a refrigerator at -32 ºC for 24 hours. After that, implants were cut into 1 cm width and 1 cm length square shape by NT cutter.

Then implants were placed into a crosslinking solution of Glutaraldehyde for hardening. Then they were washed with methanol and distilled water respectively. After hardening they were allowed to place it in aseptic cabinet for air drying for few minutes.

Table 1: Formulation of implant preparation

Ingredients	Polymer ratio 60:40	Polymer ratio 70:30
Drug (Vinpocetine)	50 mg	50 mg
Polymers (Chitosan and Na Alginate solution)	4.167g	4.167g
1% Acetic Acid	100 ml	100 ml
Distilled Water	100 ml	100 ml

Table 2: Formulation Chart of Implants with Different Excipients

Name of Formulation	Drug loading	Excipient Loading	Polymer ratio	Excipients
F1	50mg	None	60:40	None
F2	50mg	None	70:30	None
F3	50 mg	50 mg	60:40	Glyceryl mono-stearate
F4	50 mg	50 mg	60:40	Cetyl Alcohol
F5	50 mg	50 mg	60:40	Stearic Acid
F6	50 mg	50 mg	60:40	Cetostearyl Alcohol
F7	50 mg	50 mg	60:40	MethocelK15M CR
F8	50 mg	50 mg	60:40	Kollidon 30

2.2.2 Implant analysis
The amount of drug that was actually loaded inside implants during fabrication process was determined by spectrophotometric analysis. For determining the drug content of Vinpocetine loaded implants, first the implant was weighted and then crushed in a mortar and pestle. Then it was dissolved in 2ml Acetic Acid by vigorous ultra-sonication. Then 2 ml of Acetonitrile, 4 ml hot buffer and 2 ml Acetic Acid added for precipitating the polymer and extracting the drug in solvent. That means the total volume of Acetic Acid, Acetonitrile and phosphate buffer (pH 7.4) ratio is 40:20:40. Then it was centrifuged at 4000 RPM for 15 minute to separate the solid material. Clear supernatant was withdrawn and it was analyzed at 274 nm (λmax of Vinpocetine) in UV spectrophotometer. Vinpocetine concentration was calculated from the standard curve. Excipient incorporated Vinpocetine loaded in Chitosan-Na Alginate polymeric implants were also analyzed in the same manner

2.2.3 Loading efficiency calculation
The loading efficiency of implant depends on a number of factors related to the drug, polymer and solvent properties: the lipophilicity or hydrophilicity of the drug and the polymer, the solubility of the drug, polymer in the solvent, the physicochemical properties of the solvent like log partition co-efficient, miscibility with the aqueous outer media and viscosity are the determining factor for the implant forming process and corresponding drug loading [19].
Chitosan-Sodium Alginate biodegradable polymeric implants were analyzed for actual drug content against the theoretical drug content. The percentage of loading efficiency (%LE) of implants was determined with the formula:
%LE= (LD/AD) x 100
Here,
LD is the amount of loafed drug in the implant and
AD is the amount of added drug in the formulation.

2.2.4 Effect of Excipients on Loading Efficiency of Chitosan-Sodium Alginate polymeric Implants
The effect of incorporation of different excipient on drug loading efficiency of Vinpocetine was studied for 50 mg drug load. The excipient load was same as the drug load. The changes in the loading efficiency were probably caused by the respective excipients.

2.2.5 *In-Vitro* Dissolution of Biodegradable Implants
After formulation of implants, in-vitro dissolution studies of the implants were carried out in static condition in order to observe the drug release profile for Vinpocetine implants.
There 3 implants for each formulation were taken, and their weight recorded. They were then transferred to rubber capped glass vessels containing 100 ml phosphate Buffer, pH 7.4 at predetermined time interval, 3 ml of sample is withdrawn from the dissolution vessels using 5ml conventional disposable syringe, after mild stirring of the dissolution vessels for few second to ensure uniform distribution of drug throughout the dissolution medium. 3 ml of fresh medium (phosphate buffer, pH 7.4) was then added to the dissolution vessels to replace the withdrawn sample to maintain the sink condition. The withdrawn samples were then analyzed for determining the percentage of release of drug by UV spectrophotometer at 274 nm (λmax of Vinpocetine in phosphate buffer, pH 7.4), after subsequent dilution of the samples. All data were used in statistical analysis for the determination of mean, standard deviation and release kinetics.

Fig 1: In-vitro dissolution study in glass vessels

2.2.6 Drug Release Kinetics of Implants Based on Chitosan-Na Alginate Polymeric ratio
The kinetics of Vinpocetine from varying Chitosan-Na Alginate Polymeric ratios was determined by finding the best fit of the release data to Higuchi, Korsmeyer-Peppas, Zero order and First order plots. The release rate constant of each model was calculated by linear regression analysis. Co-efficient of determination and R^2 values were used to calculate the accuracy of the fit.

2.2.7 Scanning Electron Microscopy Analysis
SEM was used to observe the surface morphology of Vinpocetine biodegradable polymeric implants with Chitosan and Na alginate with 60:40 polymeric ratios to determine the surface integrity of the polymers before drug release and to observe the surface after drug release to correlate with the in-vitro results.

3. Results and Discussion
3.1 Results
Loading efficiency of the formulations are displayed in Table 3 and 4

Table 3: Drug loading efficiency of Chitosan-Sodium Alginate Polymeric Implants

Implants of varying polymer ratios with no excipients	Loading Efficiency (%)
F1 (60:40)	75.96
F2 (70:30)	74.91

Table 4: Effect of Excipients on Vinpocetine Loading Efficiency of Chitosan-Sodium Alginate Polymeric Implants

Excipients	Loading Efficiency (%)
Glyceryl Monostearate (F3)	80.32
Cetyl Alcohol (F4)	94.83
Stearic Acid (F5)	99.27
Cetostearyl Alcohol (F6)	75.97
Methocel K15 M (F7)	86.79
Kollidon 30 (F8)	84.06

Drug Release profile of Implants Based on Varying Chitosan-Na Alginate Polymer Ratio

The implants were formulated with two polymer ratios, namely 60:40 and 70:30 of Chitosan-Na Alginate composition and were subjected to 30 minute exposure to Gluteraldehyde for hardening.

Table 5: Time taken for drug to be released from Chitosan-Na Alginate polymeric implants at different polymer ratios

Polymer ratio of implants	Time (Days) taken for drug release to be completed from implants
60:40 (F1)	55
70:30 (F2)	40

Time taken for drug to be released from Chitosan-Na Alginate Polymeric implants of varying ratios are shown in Figure 2

The formulation containing Chitosan-Na Alginate in the ratio 60:40 showed better sustained effect.

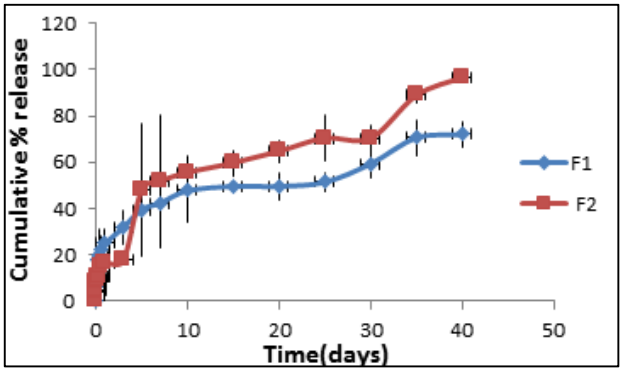

Fig 2: Comparison of cumulative release profile of Vinpocetine from different polymeric ratios of Chitosan-Sodium alginate (60:40 and 70:30)

From these profiles it can be seen that release occurred in the same pattern among the two ratios of the implants. These formulations showed sustained release action. But the 60:40 polymeric ratio of 50 mg Vinpocetine drug loaded implants showed more sustained action because release of drug occurred slowly.

Effect of excipients on drug release profiles of implants with polymeric ratio 60:40

In vitro release of implants formulated with different excipients was observed for 60 days.

The results are displayed in Table-6 and Figure-3.

Table-6: Amount of drug release from different formulations after 60 days.

Formulations with different excipients	Amount of drug released (%)
Glyceryl Monostearate (F3)	100
Cetyl Alcohol (F4)	68.42
Stearic Acid (F5)	84.40
Cetostearyl Alcohol (F6)	100
Methocel K15 M (F7)	92.10
Kollidon 30 (F8)	99.25

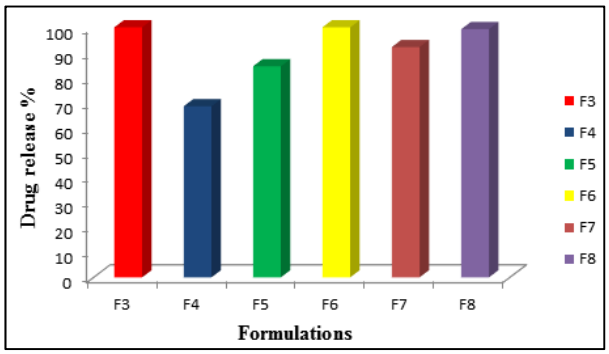

Fig 3: Amount of drug release from different formulations after 60 days.

Results of Release Kinetic Study

All of the formulations followed zero-order release kinetic. The zero-order plots of the formulations are displayed in Figure-4 and the release rate constants and R^2 values are displayed in Table-7.

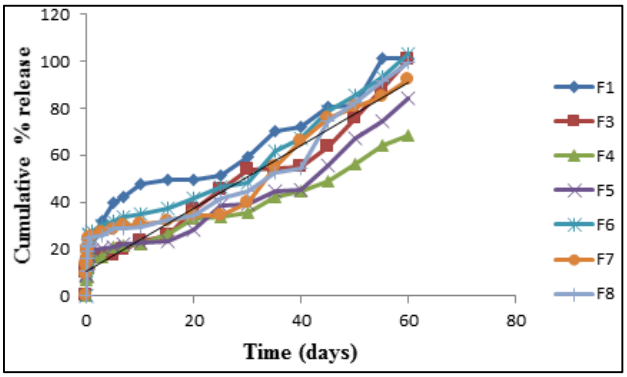

Fig 4: Zero order plot of the formulations.

Table 7: Comparison of Release Rate constants of the formulations by Zero order kinetics model with R^2 values

Formulations	Release rate constants	R^2 Values
F1	1.418	.903
F3	1.33	.971
F4	1.01	.959
F5	1.05	.946
F6	1.309	.945
F7	1.209	.931
F8	1.227	.929

Scanning Electron Microscope Analysis Results

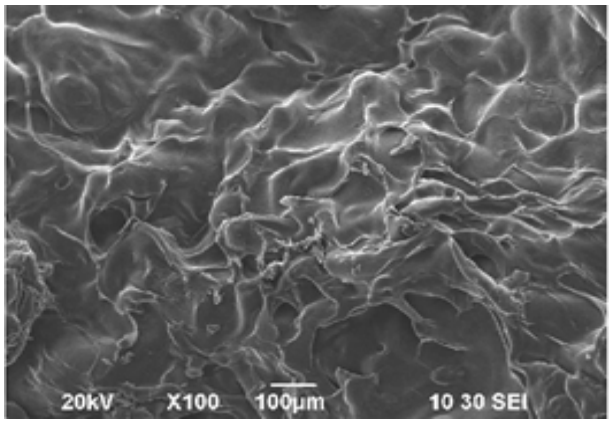

Fig 5: Surface morphology before drug release (100 times magnification)

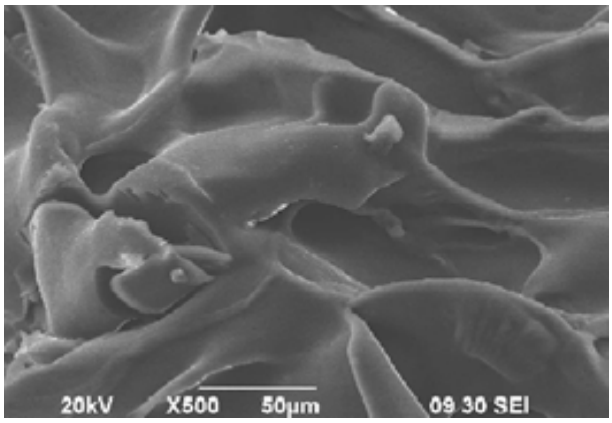

Fig 6: Surface morphology before drug release (500 times magnification)

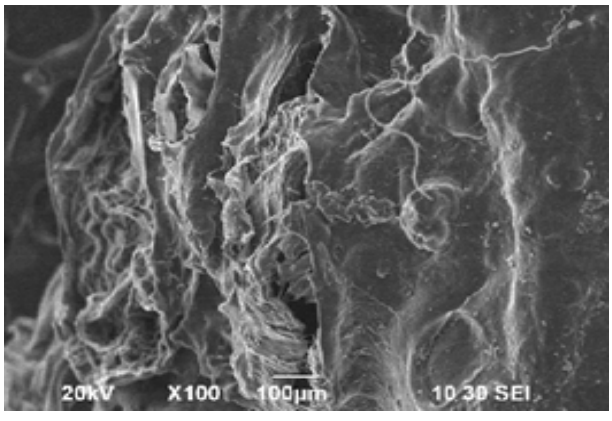

Fig 7: Surface morphology after drug release (100 times magnification)

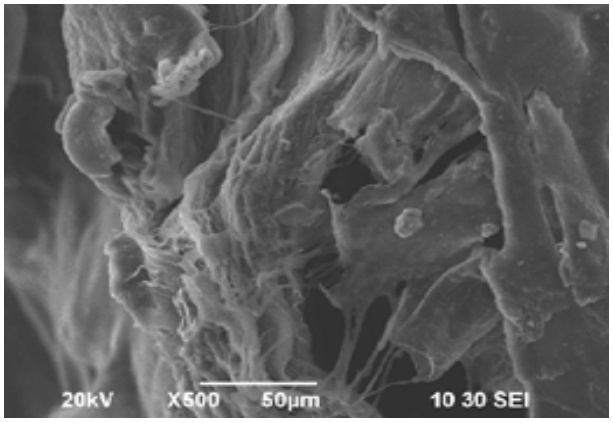

Fig 8: Surface morphology after drug release (500 times magnification)

Figure 5 and 6 displays a 100 times and 500 times magnified polymeric implant micrograph before drug release respectively. Figure 7 and 8 displays 100 times and 500 times magnified polymeric implant micrograph after drug release.

3.2 Discussion

The aim of the research was to explore the scope of sustaining the release of drug by Chitosan-Sodium Alginate combination biodegradable implants. The active ingredient selected was Vinpocetine. Implants were formulated in varied ratios of Chitosan and Sodium Alginate.

At first, Drug release profile of implants prepared by using Chitosan and Sodium alginate in two different ratios of 60:40 and 70:30 was observed by placing the implants in phosphate buffer (pH 7.4 at 37 ºC). It was observed that the implants with Chitosan-Sodium Alginate in the ratio 60:40 produced the maximum sustained action for 55 days. Due to this reason 60:40 polymeric ratio of Chitosan-Sodium alginate was chosen to prepare the formulations which had different excipients.

The effects of different excipients were studied on loading efficiency and drug release profile. Loading efficiency was found in the range between 75.97%-99.27% in different formulations. The highest loading efficiency was found with stearic acid (99.27%) and the lowest with Cetostearyl alcohol (75.97%). Loading efficiency was increased by incorporating excipients.

The effects of different excipients with 60:40 ratios on drug release profile were studied for 60 days. It was observed that drug release from the implants with Cetyl alcohol excipient produced the maximum sustained action followed by stearic acid.

The results of in-vitro dissolution study were fitted to Higuchi, Korsmeyer-Peppas, Zero order and first order kinetics models to evaluate the kinetics data. Implants were found to follow Zero order model implying controlled release.

In Figure 5 and 6 the SEM micrograph of Vinpocetine loaded polymeric implant surface before drug release was smooth and non-porous. Good polymeric integrity was observed in those figures. Figure 7 and 8 being more porous and rough we can say that very low amount of drug was remaining after drug release which also comply with the figures.

4. Conclusion

Polymeric drug delivery systems are an attractive alternative to control the release of drug to obtain defined blood levels over a specified time. The patients suffering from some

disease conditions often benefit from such long-term drug delivery systems.

In this research we observed that Vinpocetine loaded Chitosan-Sodium Alginate combined biodegradable implants exhibited long term drug release under *in vitro* conditions. The drug of choice has displayed potential to be of convenience to patients and this system shows sufficient promise as a candidate for further development so further work should be carried out in this area.

5. Acknowledgement

The authors are thankful to Eskayef Bangladesh Ltd. for providing Vinpocetine and the University of Asia Pacific for providing the space and lab equipment for this research.

6. Conflict of Interest

The authors declare there is no conflict of interest among them.

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