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# Formulation and evaluation of mucoadhesive microspheres of repaglinide

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The aim of the current work to develop and evaluate mucoadhesive microspheres of Repaglinide using the emulsification solvent evaporation technique. Effects of formulation variables, i.e. polymer concentration and phase volume ratio on particle size, % mucoadhesion and drug release were investigated in this study. Scanning electron microscopy of microspheres with maximum drug content (Formulation CH1:8) demonstrated smooth surface spherical particles with a mean diameter of 64.78 ± 3.26 µm. The mean Particle size, % drug loading and mucoadhesion were found to vary by changing the formulation variables. Microspheres size was significantly increased as increasing the polymer concentration in the aqueous phase while the size of microspheres decreases as increase in volume of the continuous phase. Decrease in size of microspheres leads to decrease in mucoadhesion time, % drug loading and faster the drug release. It can be concluded that the present mucoadhesive microspheres can be an ideal system to deliver the Repaglinide in the sustained release manner for management of Type II Diabetes Mellitus.

***Keyword:*** Microspheres, Repaglinide, formulation variables, mucoadhesion, drug loading.

## 1. Introduction

Substantial efforts have recently been focused upon placing a drug or drug delivery system in a particular region of the body for an extended period of time [1]. From a technological point of view, an ideal Sustained Release Mucoadhesive (SRM) dosage form must have three properties. It must maintain its position in the mouth for a few hours, release the drug in a controlled fashion and provide the drug release in a unidirectional way towards the mucosa [1]. Microspheres form an important part of such novel drug delivery system. However, the success of these microspheres is limited owing to their short residence time at the site of absorption. It would, therefore, be advantageous to have means for providing an intimate contact of the drug delivery system with the absorbing membranes. This can be achieved by coupling bioadhesion characteristics to microspheres and developing bioadhesive microspheres [2, 7]. Bioadhesive microspheres have advantages such as efficient absorption and enhanced bioavailability of drugs owing to a high surface-to-volume ratio, a much more intimate contact with the mucus layer, and specific targeting of drugs to the absorption site [8, 9]. Mucoadhesive microspheres that are retained in the stomach would increase the drug absorption and decrease dosing frequency which provides better patient compliance as compared to conventional dosage forms.

Repaglinide is an oral hypoglycemic agent which acts by stimulating the release of insulin from pancreatic beta-cells by inhibition of potassium

efflux resulting in closure of ATP regulates K+ channels [10].

The bioavailability of the oral formulation was found to be 63% [11]. The effective control of diabetes type-II requires administration of Repaglinide 0.5 – 4 mg three times daily. Owing to its short biological half-life (1 hour) and low bioavailability (63%); it's necessary to develop a sustained release mucoadhesive dosage form of Repaglinide which adhere to the mucosa and release the drug in a sustained release manner.

These microspheres would prolong, relatively constant effective level of Repaglinide and improve patient compliance. Thus mucoadhesive microspheres of Repaglinide are suitable candidates for effective control of diabetes type-II.

The literature survey revealed that Sodium alginate and hydroxy propyl methyl cellulose (HPMC) are the polymer which shows good mucoadhesive properties, high drug entrapment efficiency and release the drug in a sustained release manner. Therefore, in the present study Repaglinide is selected as a model drug and sodium alginate and HPMC are chosen as a mucoadhesive polymer for design and evaluation mucoadhesive microspheres for treatment of diabetes type-II.

## 2. Material

Repaglinide was obtained as a gift sample from Glenmark Pharmaceuticals Ltd., Mumbai, INDIA. Sodium alginate was gifted from Cipla Pvt. Ltd, Goa India. HPMC was received as gift sample from Cadila Healthcare Ltd, Ahmadabad, INDIA. n-Hexane and span 20 were procured from the central drug house, New Delhi India. Liquid paraffin was procured from Loba Chemie Pvt. Ltd., Mumbai India. All the reagents were used of analytical grade.

## 3. Methods

### 3.1 Preparation of microspheres [7, 18]

Mucoadhesive microspheres of Repaglinide were prepared by an emulsification solvent evaporation method using various ratios of Sodium alginate and HPMC. For this, aqueous solution of drug and polymer is prepared. Then drug and polymer solution was added drop wise to the liquid paraffin containing 0.5% span 20 as an emulsifying agent with constant stirring. The constant stirring was carried out using a magnetic stirrer. The beaker and its content were heated at 80 $^0$C with constant stirring for 4 hrs until the aqueous phase was completely removed by evaporation. The liquid paraffin was decanted and collected microsphere were washed 5 times with n-hexane, filtered through whatman's filter paper and dried in hot air oven at 50 $^0$C for 2 hours. Table 1 shows the composition of various formulations of microspheres.

### 3.2 Surface morphology [19, 20]

The surface morphology and structure were visualized by scanning electron microscopy (SEM). The samples were prepared by lightly sprinkling the microspheres powder on a double side adhesive tape which already shucked to on aluminum stubs. The stubs were then placed into a fine coat ion sputter for gold coating. After gold coating samples were randomly scanned for particle size and surface morphology.

### 3.3 Particle Size [21, 22]:

Particle size analysis of drug-loaded microspheres was performed by optical microscopy using a compound microscope (Erma, Tokyo, Japan). A small amount of dry microspheres was suspended in n-hexane (10 mL). The suspension was ultra-sonicated for 5 seconds. A small drop of suspension, thus obtained was placed on a clean glass slide. The slide containing microspheres was mounted on the stage of the microscope and 300 particles were measured using a calibrated ocular micrometer. The average particle size was determined by using the Edmondson's equation D mean = $\Sigma nd/\Sigma n$, where n= number of microspheres observed and d= mean size range. The process was repeated 3 times for each batch prepared.

### 3.4 Drug entrapment efficacy [21]:

50 mg of microsphere were taken and the drug was extracted from microspheres by digesting for

24 hours with 10 ml of simulated gastric fluid (pH 1.2). During this period the suspension was agitated. After 24 hours, the solution was filtered and the filtrate was analyzed for the drug content. The drug entrapment efficiency was calculated using the following formula:

Entrapment efficiency = (Practical drug content/theoretical drug content) ×100

**3.5 *In-vitro* mucoadhesivity [7, 8, 23]:**

The mucoadhesive properties of the microspheres were evaluated by in vitro wash-off test as reported by Lehr et al. A 1-cm by 1-cm piece of rat stomach mucosa was tied onto a glass slide (3-inch by 1-inch) using thread. Microspheres were spread (∽50) onto the wet, rinsed, tissue specimen, and the prepared slide was hung onto one of the groves of a USP tablet disintegrating test apparatus. The disintegrating test apparatus was operated such that the tissue specimen was given regular up and down movements in a beaker containing the simulated gastric fluid (pH 1.2). At hourly intervals up to 10 hours, the number of microspheres still adhering onto the tissue was counted. Percent mucoadhesion was given by the following formula.

% mucoadhesion = (no. of microspheres remains / no. of applied microspheres) ×100

The observations are expressed in figure 2-4.

**3.6 *In-vitro* drug release [24, 25]:**

*In-vitro* drug release study was carried out in USP XXI paddle type dissolution test apparatus using simulated gastric fluid (pH 1.2) as dissolution medium, volume of dissolution medium was 900 ml and bath temperature was maintained at (37±1) °C throughout the study. Paddle speed was adjusted to 50 rpm. An interval of 1 hour, 10 ml of sample was withdrawn with replacement of 10 ml fresh medium and analyzed for drug content by UV-Visible spectrophotometer at 247 nm. All the experimental units were analyzed in triplicate (n=3). The cumulative percentage drug release was calculated using an equation obtained from a standard curve. The observations are expressed in figure 5 to 8 and table 3.

## 4. Results and Discussion

### 4.1 Particle size analysis:

Particle size analysis of different formulations was done by optical microscopy [20, 21]. The average particle size was found to be in the range of 28.43 to 64.78 µm. The mean particle size was significantly varied according to type of polymer used for the preparation of microspheres; this may be due to the fact that difference in the viscosity of the polymer solution13. Since high viscosity of the polymer solution requires high shearing energy for breaking of droplets of the emulsion. Microspheres containing HPMC are larger as compared to sodium alginate microspheres because HPMC solution has more viscosity at the same concentration. Particle size decreased with increase in volume of continuous phase due to the fact that increased in continuous phase, more efficiently utilized the energy produced by stirring, which leads to further decrease in droplet size of internal phase. Increase in concentration of polymer in the internal phase leads to increase in size of microspheres because at a higher concentration polymer solution have more viscosity which requires more energy to breaking the droplets of dispersed phase. Results of particle size analysis are shown in table 2.

### 4.2 Surface morphology:

The surface morphology of the mucoadhesive microspheres was examined by scanning electron microscopy (SEM). The SEM showed that the microspheres obtained from all the formulations are spherical with smooth surface. The SEM showed that sodium alginate produced spherical with smooth surface microspheres due to their high solubility in water [13, 14]. The SEM of microsphere of formulation C1:8 are shown in figures 1

### 4.3 Drug entrapment efficiency:

Drug content in different formulations was estimated by the U V Spectrophotometric method. Percent drug loading efficiency of

microspheres was found in the range of 62.13 to 76.5 % (table- 2). Formulation CH1:8 containing blend of sodium alginate and HPMC showed maximum % drug loading about 76.5 % because these microspheres have larger size as compared to other formulations. Whereas formulation H1:16 containing HPMC showed the minimum % drug loading about 63% because these microspheres are small in size, which results more loss of drug from surface during washing of microspheres. Increase in polymer concentration of internal phase also increases in drug entrapment of microspheres. Rank order of percentage drug loading of various formulations was found to be as follows:

CH1:8>C1:8>C1:12>CH1:12>C1:16>CH1:16>H 1:8>H1:12>H1:16

**4.4 *In-vitro* mucoadhesivity test:**

To assess the mucoadhesive property of microspheres, In-vitro wash-off test was performed for all the formulations. In the mucoadhesion process, it is necessary for swelling and expansion of the polymer chain since interpenetration and entanglement of the polymers and the mucous networks are considered to be responsible for adhesion13. Therefore, bioadhesives should swell and expand rapidly when they come in contact with water. Adhesion of polymer with the mucus membrane is mediated by hydration in the case of hydrophilic polymer. Upon hydration these polymers becomes sticky and adhere to mucus membrane. A high percentage of adhesion indicates that microspheres have excellent mucoadhesion to mucosal tissue. Sodium alginates are interacts with the mucin, resulting in adhesion of the polymer to the mucin. Formulation H1:8 containing HPMC showed the highest mucoadhesivity. Formulation C1:16 containing sodium alginate showed the shortest mucoadhesion time due to the small size of microsphere which takes short time for solubilization. The results of the percentage mucoadhesivity test of all the formulations are expressed in figure 2, 3 and 4.

**4.5 Drug release study:**

Drug release form these microspheres were slow, extended and dependent on the type of polymer and concentration of polymer used. The rate of release of drug from the bioadhesive microspheres was slow and found to further decrease with an increase in drug to polymer ratio. Formulation H1:16 containing HPMC showed the fast drug release due to rapid swelling property in a dissolution environment (0.1 N HCl). Dissolution medium permeation into the microspheres is facilitated due to high swelling action of the HPMC which leads to more medium for the transport of the drug is available. While HPMC microspheres showed the least drug release. A drug release form microsphere is significantly affected by the size of microspheres. Increase in polymer concentration leads to increase in size of microspheres thus drug release from microspheres having low drug to polymer ratio found to significantly decrease. FormulationC1:16 shown fastest drug release among all the formulation due to fact that these microspheres are small in size. Results of the drug release study are expressed in figure 5 to 8.

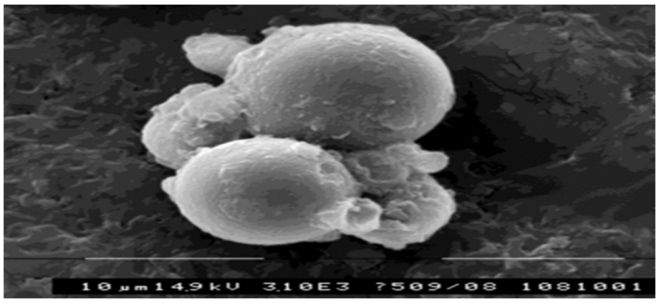


**Fig 1:** SEM of formulation C1:8 showing population of microspheres

**Table 2:** % yield, %drug entrapment & particle size of microsphere.

| Formulation code | % yield | Particle size(µm) | % Drug entrapment |
|---|---|---|---|
| F1 | 72.40±1.43 | 42.65 | 73.43 |
| F2 | 76.76±2.43 | 51.23 | 77.54 |
| F3 | 73.20±2.56 | 45.65 | 76.54 |
| F4 | 73.55±2.31 | 37.87 | 75.54 |
| F5 | 66.41±2.12 | 41.12 | 65.43 |
| F6 | 70.12±1.54 | 52.75 | 71.65 |
| F7 | 81.43±2.43 | 62.43 | 85.43 |
| F8 | 77.65±1.87 | 54.23 | 81.73 |
| F9 | 78.43±2.41 | 56.43 | 79.23 |

**Table 3:** % mucoadhesion, $t_{50}$ & $t_{50}$ of repaglinide release from microsphere.

| Formulation | % mucoadhesion after 1hr | T50 of drug release | T50 of drug release(min) |
|---|---|---|---|
| F1 | 77.32±2.12 | 230 | 522 |
| F2 | 81.98±1.54 | 273 | 521 |
| F3 | 79.98±2.13 | 254 | 483 |
| F4 | 83.87±2.87 | 238 | 487 |
| F5 | 79.65±1.43 | 212 | 514 |
| F6 | 88.98±1.65 | 283 | 492 |
| F7 | 89.65±2.76 | 243 | 498 |
| F8 | 81.87±1.87 | 287 | 439 |
| F9 | 85.24±2.65 | 292 | 470 |

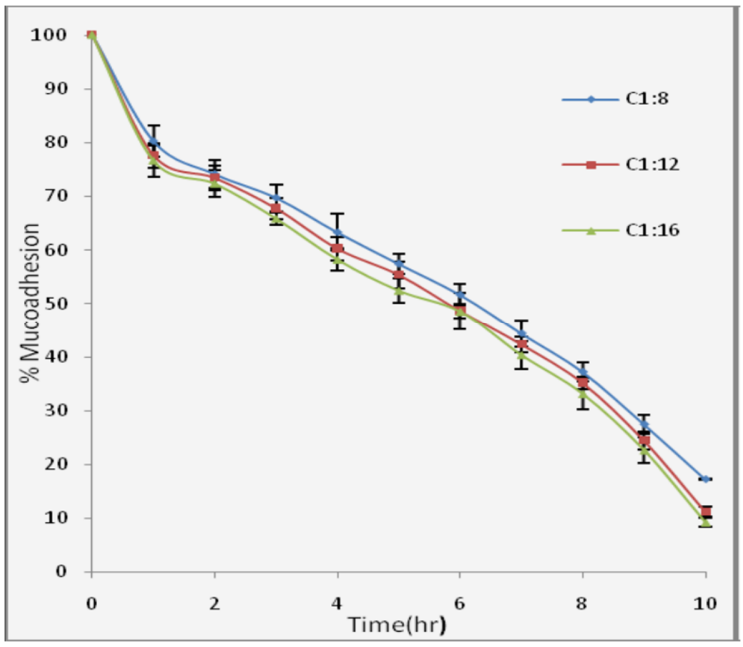


**Fig 2:** Comparative % mucoadhesion of formulations C1:8, C1:12 & C1:16

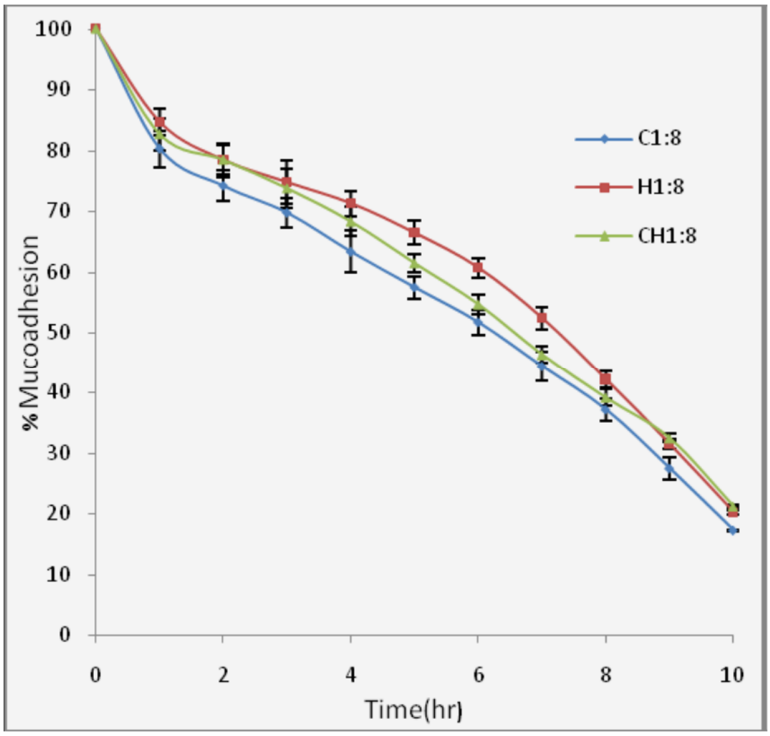


**Fig 3:** Comparative % mucoadhesion of formulations C1:8, H1:8 & CH1:8

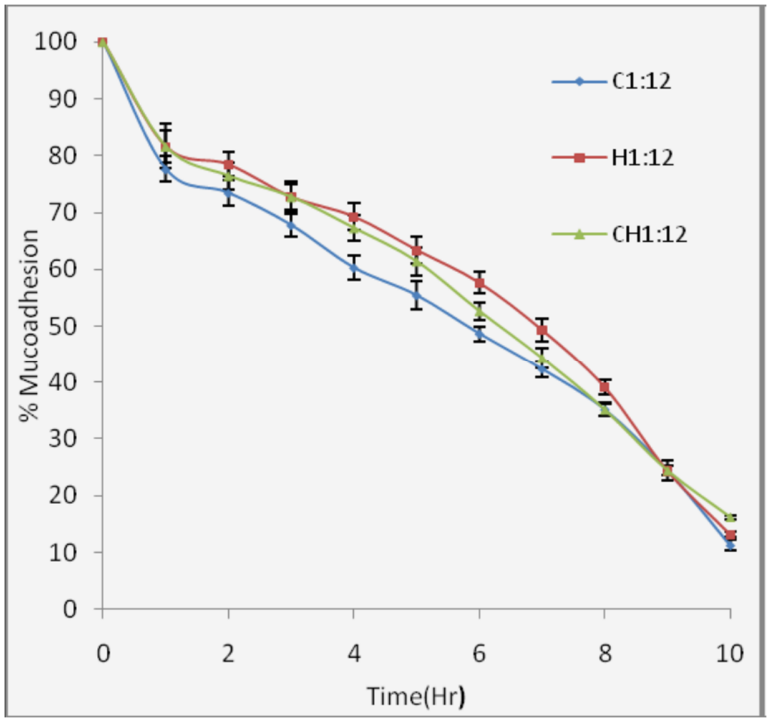


**Fig 4:** Comparative % mucoadhesion of formulations C1:12, H1:12 & CH1:12

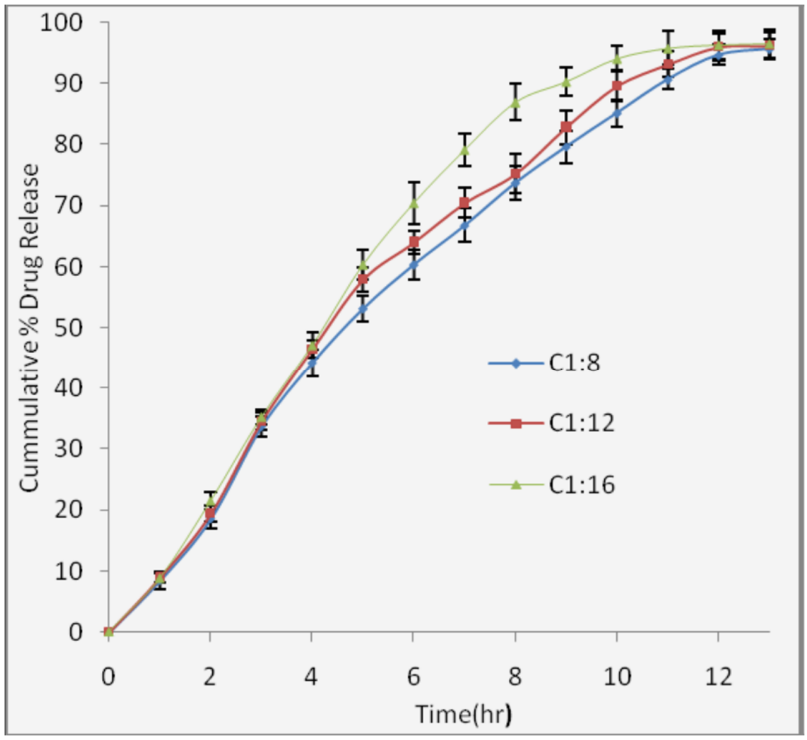


**Fig 5:** Cumulative % drug release from formulation C1:8, C1:12 & C1:16

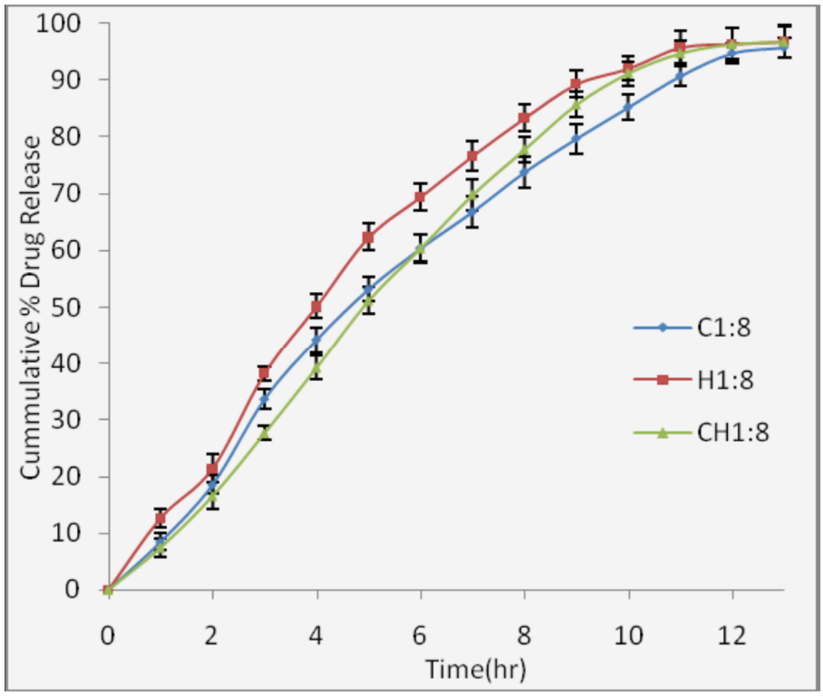


**Fig 6:** Cumulative % drug release from formulation C1:8, H1:8 & CH1:8

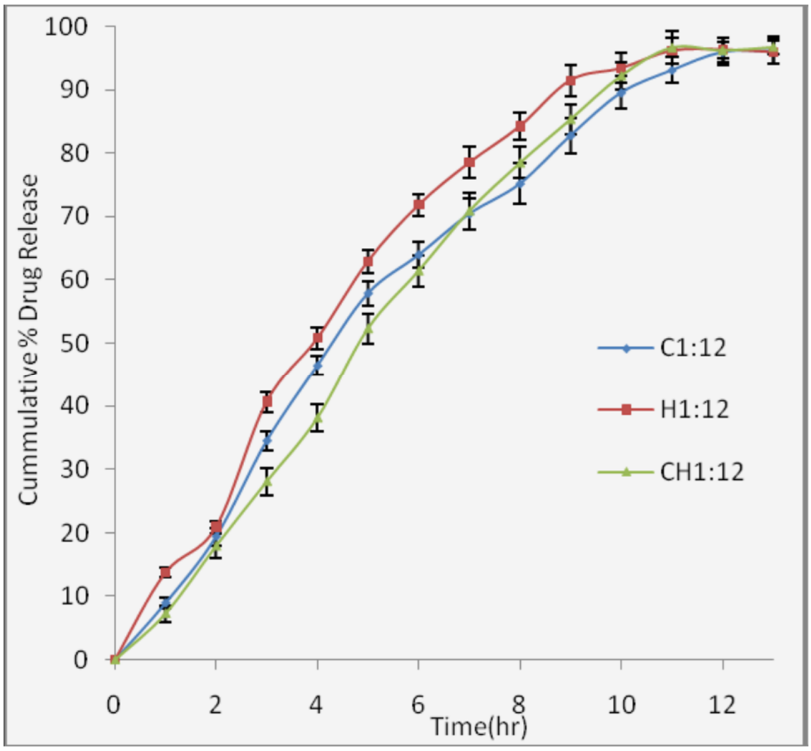


**Fig 7:** Cumulative % drug release from formulation C1:12, H1:12 & CH1:12

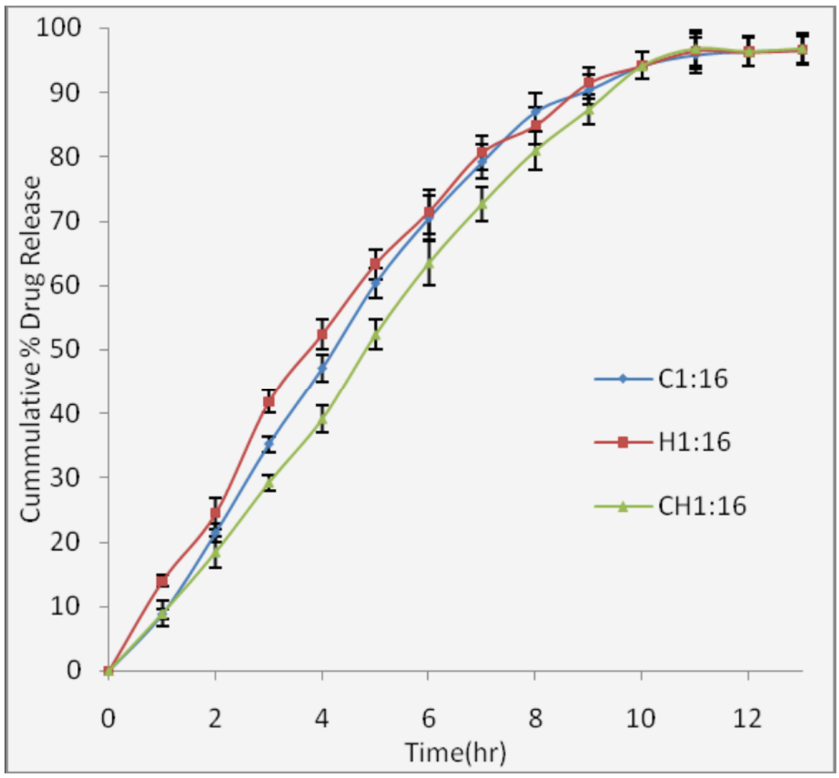


**Fig 8:** Cumulative % drug release from formulation C1:16, H1:16 & CH1:16

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