RESEARCH ARTICLE

PREPARATION AND CHARACTERIZATION OF METHOCEL K4M, EUDRAGIT RLPO AND PEG 4000 LOADED CARBAMAZEPINE MICROSPHERES.

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Manuscript Info	*Abstract*
	Modulated carbamazepine prolonged release dosage forms, microspheres have been prepared by the emulsion solvent evaporation method using Methocel K4M, PEG 4000 and Eudragit RLPO. A 2^2 factorial design was made by central composite design where the amount of Methocel K4M and PEG 4000 were selected as independent variables. Thirteen formulations were prepared with MDT, $T_{50\%}$, $T_{80\%}$, and swelling index as dependent variables. Data were analyzed statistically using linear regression model by IBM SPSS version 22. Effect of varying amount of Methocel K4M and PEG 4000 on the drug content, entrapment efficiency, swelling index, surface morphology (SEM), and *in-vitro* drug release rate were evaluated. Formulations with high amount of Methocel K4M showed good swelling properties. In SEM studies microspheres appeared rough and spherical in shape. Increasing amount of PEG produced pores on the surface of the microspheres due to the diffusion of polymers towards the external phase thus showing better drug release (B1, B3 & B8). Further clarification on release mechanism from spherical particles were studied using the Kopcha kinetics, Weibull model and Baker-Lonsdale model. In Kopcha kinetics all the formulations had ratio of A/B greater than 1 where release was primarily controlled by a Fickian diffusion. Weibull Model has parameters that are more sensitive to release kinetic data, the value of β was <1 and T_d value was low showing enhance drug release. In Baker-Lonsdale model most spherical matrix had uniformly distributed drugs with consistent drug release by diffusion mechanism which was seen as linear graphs.

Introduction:-
In recent years, polymeric microspheres have been ubiquitously investigated as drug delivery system. Microspheres are small spherical particles, with diameters in the micrometer range (typically 1–1000 μm). Interest in polymeric microspheres as drug carriers is due to the fact that these systems are injectable, deliver drugs site specifically, release drugs in a controlled manner, minimize side effects due to dose fluctuation within the therapeutic range, moreover have other advantages, such as decrease dose frequency and improve patient compliance. The popular method for encapsulation of drugs within water soluble polymers is the emulsion solvent evaporation technique where stable emulsion is formed without compromising the activity of the drugs.

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Epilepsy is a chronic non communicable neurological disorder of the brain that affects people of all ages worldwide. Up to 75% of people with epilepsy can live a normal life, free from seizures, if they are appropriately treated with antiepileptic medicines. Carbamazepine (CBZ) a tricyclic iminostilbene derivative, is a first-line drug used in the treatment of epilepsy, trigeminal neuralgia, and bipolar disorders and is among the most important antiepileptic drugs. It is the drug of choice for simple and complex partial and secondarily generalized seizures as well as being a mood stabilizer in manic-depressive patients [1]. Carbamazepine, a BCS class II drug, is a water insoluble drug and its oral absorption is erratic. The rate of absorption and extent of bioavailability for such poor water soluble drug can be controlled by entrapping the drug in water soluble polymers as microspheres [2].

Hence, the present work was aimed to prepare sustained release carbamazepine microspheres using Methocel K4M, PEG 4000 and Eudragit RLPO which are biocompatible and permeable polymers. These release the drug slowly in the stomach for gradual absorption. The slow but complete drug release in the stomach is expected to increase bioavailability of the drug as well as its complete utilization which may result in, lower dosage and gastrointestinal side effects. A 2^2 factorial design was used to achieve the formulation where the independent variables were Methocel K4M, PEG 4000 and the dependent variables were $T_{50\%}$, $T_{80\%}$, MDT and swelling index. Linear regression analysis was performed to identify the best formulation and to validate the model by comparing the experimental results with the theoretical results of the responses using IBM SPSS version 22.

Materials and methods:-
Materials:-
Carbamazepine (Beximco Pharmaceuticals Ltd. Bangladesh), PEG 4000, Methocel K4M (Colorcon), Eudragit RLPO (Evonik Industries), Dichloromethane (MERK, Germany), Ethanol (Merk, Germany), Span 80 (Merk, Germany), n-hexane (Merk, Germany), liquid paraffin (Merk, Germany), and Sodium Lauryl Sulfate (Loba Chemie, India).

Methods:-
Preparation of carbamazepine microspheres:-
Weighed quantities of polymers and PEG were dissolved in 20 ml of mixed solvent system (MSS) consisting of dichloromethane and ethanol at 3:1 ratio. Then the required amount of carbamazepine was added and dissolved by sonication until a clear solution was formed. This solution was the internal phase. For the preparation of external phase, 50 ml liquid paraffin emulsified with 0.5 ml span 80 was taken in a 250 ml beaker and stirred using an over head stirrer. The internal phase was then slowly poured drop wise to the external phase, while stirring at 450 rpm held by the mechanical stirrer equipped with a three-blade propeller, at room temperature. The whole system was stirred for 3 hours and then the microspheres were separated by filtration, the excess of paraffin oil was eliminated by repeated washing (4 to 5 times) with 50 ml n-hexane and finally dried overnight in desiccators at room temperature to yield free flowing spherical products [3]. After drying, microspheres were kept in a 10 ml vial with proper identification and preserved in the desiccator.

Factorial design of experiments:-
In this present study the formulation of carbamazepine loaded polymeric microspheres was designed by 2^2 factorial central composite design (CCD) using Minitab 17. The independent variables were Methocel K4M and PEG 4000 whereas the dependent variables selected were MDT, $T_{50\%}$, $T_{80\%}$, and swelling index.

Table 1:-Formulation of carbamazepine microspheres by 2^2 factorial design using Methocel K4M, PEG 4000 and Eudragit RLPO.

Formulation code	Amount of carbamazepine (mg)	Eudragit RLPO (mg)	Methocel K4M (mg)	PEG 4000 (mg)
B 01	200	600	500	500
B 02	200	600	500	100
B 03	200	600	250	500
B 04	200	600	250	100
B 05	200	600	198.25	275
B 06	200	600	551.75	275
B 07	200	600	375	17.2
B 08	200	600	375	582.8

B 09	200	600	375	300
B 10	200	600	375	300
B 11	200	600	375	300
B 12	200	600	375	300
B 13	200	600	375	300

Characterization of microspheres:-
Swelling index:-
Swelling index of microspheres were measured by determining the extent of their swelling in 1 % SLS solution. Accurately weighed amount of microspheres (50 mg) were suspended in 5 ml solution and the study was done for 8 hours [4]. At specific time intervals of 30 min, 1st hour, 2nd hour, 3rd hour, 4th hour, 5th hour, 6th hour, 7th hour & 8th hour microspheres were taken out, dried on filter paper to remove excess fluid and then weighed. The swelling index was calculated according to the following equation:
Swelling Index (%) = $[(W_1-W_2)/W_1] \times 100$
Where, W_1 and W_2 is the weight of swelled microspheres and dried microspheres (mg).

Particle shape and surface analysis by scanning electron microscope (SEM) study:-
Shape and surface morphology of microspheres were observed under scanning electron microscopy (SEM). Small amount of microspheres was spread on a glass stub and the stub containing the sample was placed in the scanning electron microscope (JEOL JSM – 6490 LA, Japan). Then SEM photographs were taken at an acceleration voltage of 20 kV for 15 minutes. The drug-loaded microspheres were evaluated for their sphericity and surface smoothness under different magnification and photomicrographs of suitable magnifications [5].

***In-vitro* dissolution study of microspheres containing cabamazepine in 1 % SLS solution:-**
The dissolution studies of carbamazepine microspheres were carried out in a USP dissolution 8 station test apparatus type I (Basket type). 50 mg of microspheres were placed in 900 ml of dissolution medium (distilled water containing 1 % sodium lauryl sulfate) and were stirred at 100 rpm at 37 ± 0.5 °C. 10 ml aliquot was withdrawn from the dissolution medium at pre-determined intervals of 30 minute, 1st hour, 2nd hour, 3rd hour, 4th hour, 5th hour, 6th hour, 7th hour & 8th hour. At each interval, the withdrawn medium was replaced with an equivalent amount (10 ml) of fresh dissolution medium. Collected samples were filtered and then analyzed by measuring the absorbance through an UV spectrophotometer at 284 nm after suitable dilution to determine the amount of the carbamazepine released from the microspheres. The percentage of drug release was plotted *versus* time. Each experiment was repeated three times and their average were calculated to find percentage release for each batch.

Data analysis of release studies:-
Eight kinetic models including Zero order, First order, Higuchi plots, Korsmeyer-Peppas, Kopcha kinetics, Hixson Crowell plots, Weibull plots, and Baker and Lonsdale plots were applied to determine the rate and the mechanism of release of carbamazepine from the prepared microspheres.

Successive fractional dissolution time:-
To observe the release of drug from the formulations in different experimental conditions, MDT (mean dissolution time), $T_{25\%}$, $T_{50\%}$ and $T_{80\%}$ values were determined from dissolution data by the use of following equations:
$MDT = (n/n+1) \cdot K^{-1/n}$
$T_{25\%} = (0.25/k)^{1/n}$
$T_{50\%} = (0.5/k)^{1/n}$
$T_{80\%} = (0.8/k)^{1/n}$
Mean dissolution time (MDT) was measured to characterize the drug release rate from the formulations. Higher value of MDT refers to higher rate retardant efficiency of the polymer.

Statistical analysis:-
Analysis of variance (ANOVA) was determined using IBM SPSS software (Version 22, 2013 SPSS Inc., USA) for each batch of formulation to compare the dependent variables such as successive dissolution time, mean dissolution time and swelling index of all formulations [6].

Results and discussion:-
Physicochemical characteristics of carbamazepine loaded polymeric microspheres:-
Swelling index:-

A   B
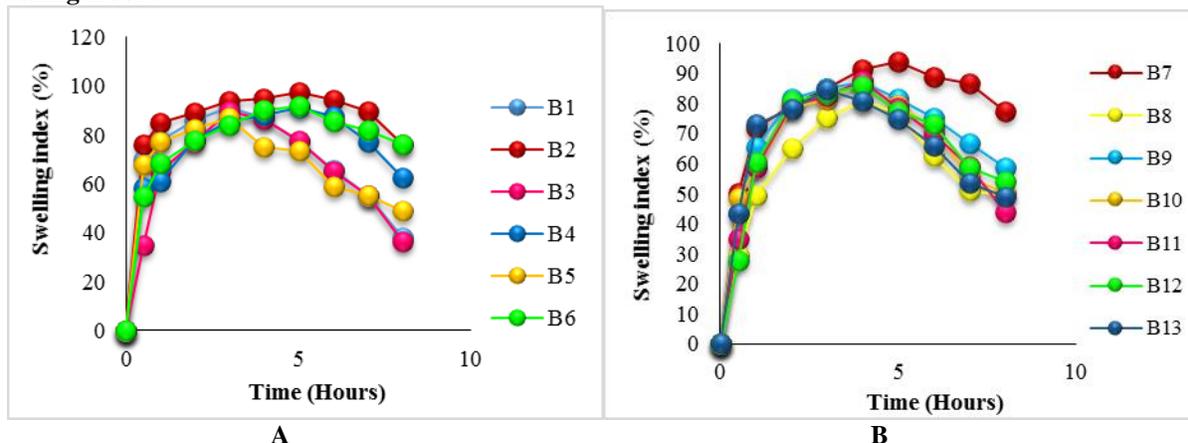
Fig. 1:-Comparison of swelling behaviors of carbamazepine microspheres.

Visual and tactile observations of all the formulations in Fig. 1 confirmed that swelling was dominant in all the formulations due to the presence of Methocel, a hydrophilic polymer as it undergoes swelling in presence of liquid solvent forming a polymeric chains [7]. This mechanical feature of surface hydrated polymer forms a viscous barricade that plays a crucial role in the whole drug release rate.

In-vitro release kinetics of carbamazepine loaded microspheres:-
To determine the release profile of the microspheres, the dissolutions of all the batches were carried out in 1% SLS for 8 hours at 100 rpm and the release rate was calculated. Eudragit RLPO was used as release retardant because it offers pH independent drug release and has highly permeability which could allow the gastrointestinal fluid penetration inside the microspheres. Methocel K4M is a hydrophilic matrix forming polymer which enhances drug release in a controlled rate, moreover it inhibits recrystallization of carbamazepine to carbamazepine dihydrate (CBD) in the gastrointestinal tract, which is one of the major rate-limiting steps in bioavailability of oral dosage forms. Polyethylene glycol (PEG 4000) was used as a channeling agent to improve the drug release properties of the microspheres [8]. From the release profile it was observed that there was a burst release initially from 10-20%. Further analysis of the release profiles also revealed that changes in the amount of Mehocel K4M and PEG 4000 had a great effect on the release rate of carbamazepine from the microspheres. So, the release rate of carbamazepine from the microsphere can be modulated by adjusting the amount of Methocel and PEG (Table 2).

Table 2:-Cumulative percent release of formulations at different time intervals.

Time (Hours)	Cumulative % of drug released													
	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	B13	Teg CR
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.5	16.8 8	13.0 8	14.4 8	10.7 4	11.8 8	15.2 1	11.1 3	16.3 9	18.2 0	12.2 2	13.7 8	20.3 4	14.7 8	26.9 2
1	27.6 7	16.5 6	26.9 6	19.9 2	19.7 5	21.3 3	18.3 6	30.0 2	24.7 0	22.4 6	28.2 6	31.4 2	21.8 8	38.4 5
2	35.7 6	32.4 4	37.3 9	27.6 6	28.3 9	28.4 8	23.5 2	41.8 2	35.1 2	30.9 7	40.0 9	44.1 8	31.8 3	53.7 9
3	42.5 7	33.6 3	47.1 5	32.6 5	37.7 8	33.4 9	26.8 1	47.7 9	43.3 9	45.4 6	45.6 6	48.5 4	38.2 9	58.3 9
4	49.2 9	41.4 9	58.0 6	36.9 1	43.1 6	38.2 8	33.6 8	53.5 3	49.0 0	58.6 0	49.1 7	52.8 3	42.9 6	60.8 8
5	56.5 5	45.6 2	66.5 9	39.6 5	48.3 8	41.7 3	36.3 7	60.3 6	53.6 7	60.7 4	53.7 0	55.1 5	52.1 6	63.9 6
6	63.4	47.6	68.4	41.5	53.6	43.8	41.9	68.3	57.1	64.1	57.2	56.6	56.6	66.3

	1	6	9	4	5	4	2	1	2	5	8	1	5	9
7	68.52	56.37	71.85	42.76	60.49	46.79	43.99	73.06	61.74	64.96	61.15	60.84	60.09	69.06
8	72.15	58.99	74.58	43.60	68.58	47.98	45.16	76.37	67.90	65.77	62.54	65.87	60.95	71.97

Fig. 2:-Comparative zero order plots of all formulations, B1- B13 withTegretol CR.
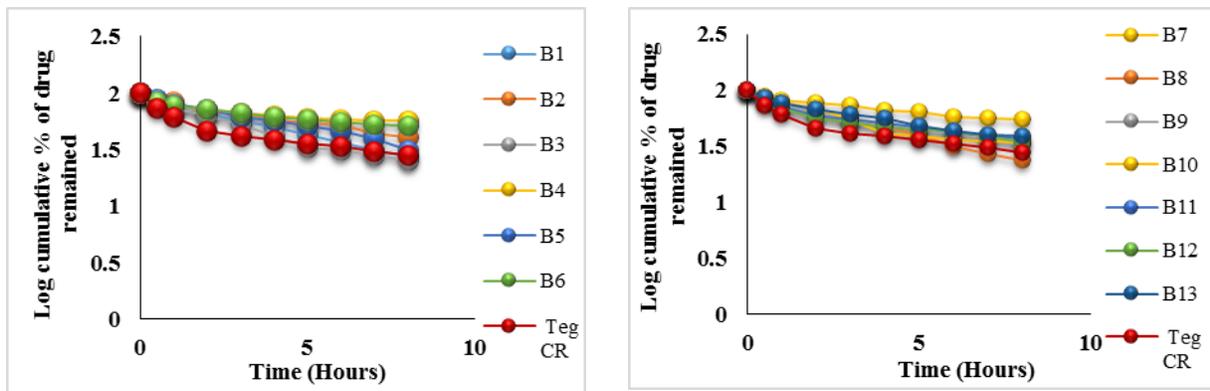
Fig. 3:-Comparative first order plots of all formulations, B1- B13 with Tegretol CR.
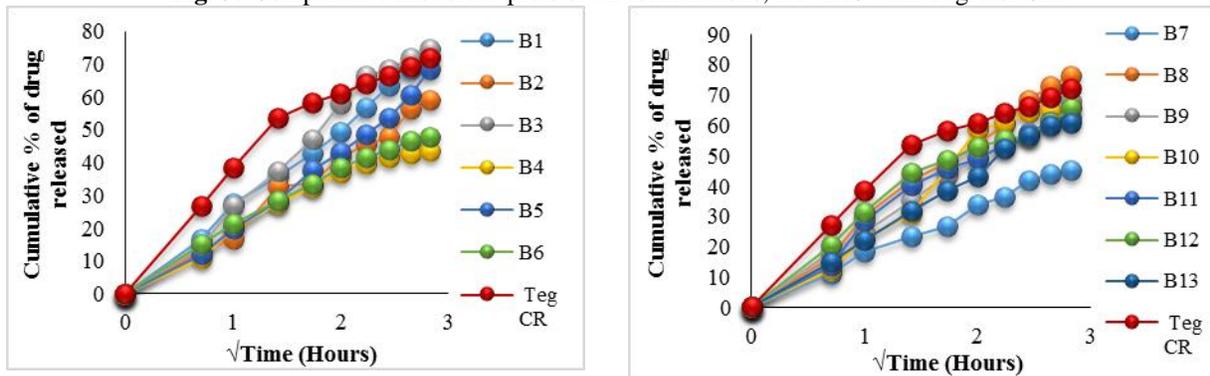
Fig.4:-Comparative Higuchi plots of all formulations, B1- B13 with Tegretol CR.
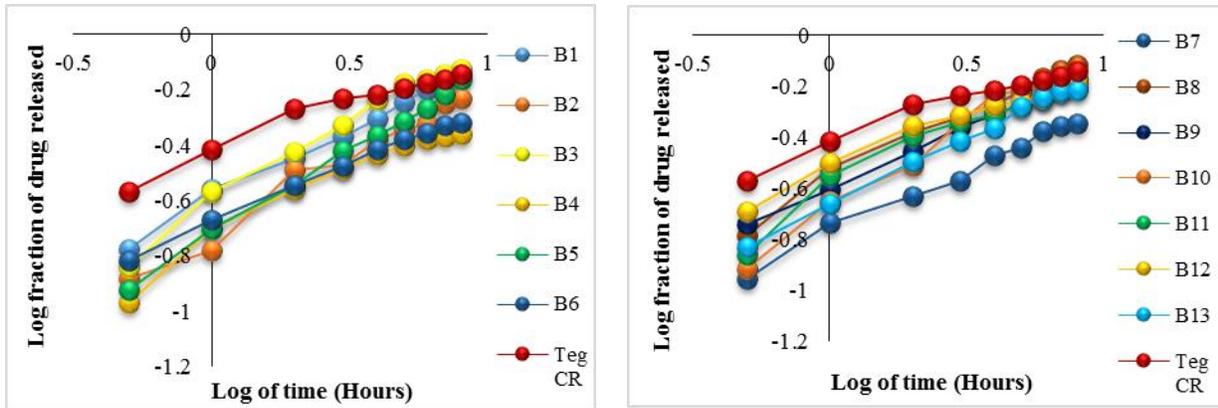
Fig. 5:-Comparative Korsmeyer-Peppas plots of all formulations, B1- B13 with Tegretol CR.
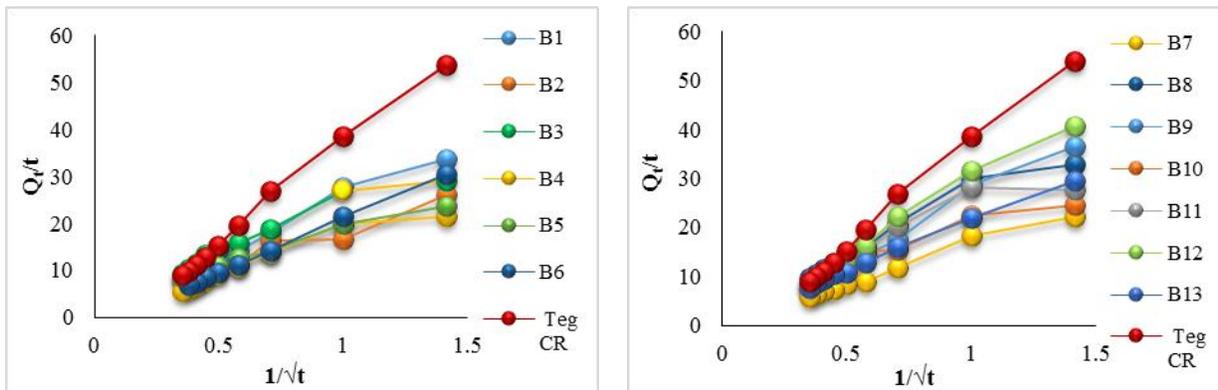
Fig. 6:-Comparative Kopcha plots of all formulations, B1- B13 with Tegretol CR.
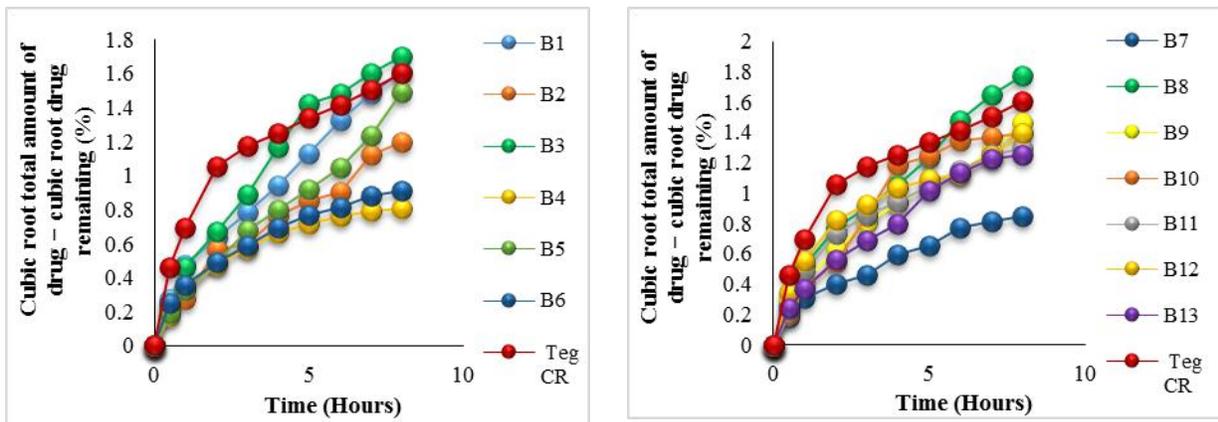
Fig. 7:-Comparative Hixson Crowell plots of all formulations, B1- B13 with Tegretol CR.
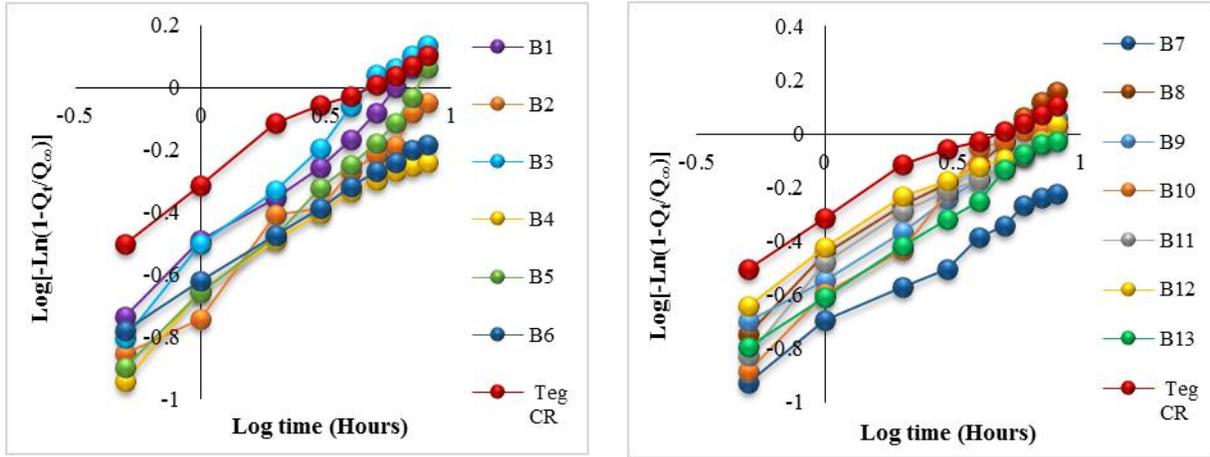
Fig. 8:-Comparative Weibull plots of all formulations, B1-B13 with Tegretol CR.
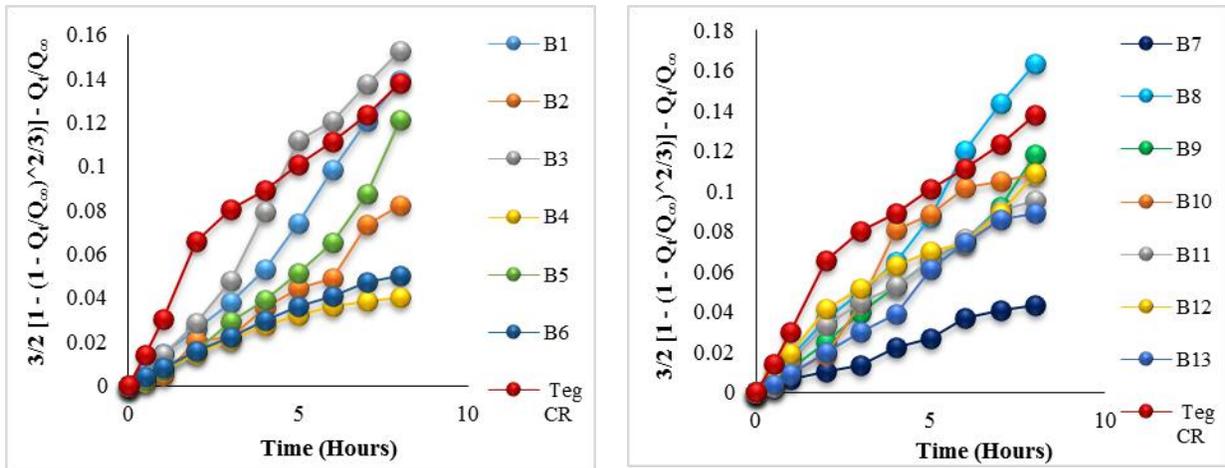
Fig. 9:-Comparative Baker and Lonsdale plots of all formulations, B1- B13 with Tegretol CR.

Table 3:-Interpretation of release rate constants and R-squared (regression coefficient) values for different kinetics of the carbamazepine loaded microspheres.

Formulation code	Zero order		First order		Higuchi		Korsmeyer-Peppas		
	K_0	R^2	K_1	R^2	K_h	R^2	K_{kp}	n	R^2
B1	8.07	0.93	-0.07	0.99	22.49	0.979	0.252	0.507	0.99
B2	6.66	0.93	-0.05	0.97	21.08	0.987	0.188	0.554	0.98
B3	8.83	0.91	-0.07	0.98	28.26	0.99	0.244	0.582	0.98
B4	4.84	0.83	-0.03	0.88	16.04	0.971	0.179	0.482	0.95
B5	7.69	0.96	-0.06	0.99	23.91	0.99	0.19	0.61	0.99
B6	5.14	0.86	-0.03	0.92	16.88	0.987	0.210	0.416	0.99
B7	5.11	0.92	-0.04	0.93	16.33	0.974	0.167	0.494	0.98
B8	8.54	0.91	-0.07	0.99	27.34	0.994	0.267	0.522	0.98
B9	7.28	0.91	-0.05	0.97	18.33	0.988	0.258	0.462	0.99
B10	8.14	0.88	-0.06	0.93	26.21	0.966	0.210	0.622	0.97
B11	6.86	0.84	-0.05	0.93	22.61	0.973	0.243	0.500	0.94
B12	6.54	0.80	-0.05	0.90	21.95	0.959	0.299	0.389	0.96
B13	7.08	0.92	-0.05	0.97	22.57	0.987	0.301	0.511	0.99
Teg CR	6.965	0.74	-0.06	0.87	23.96	0.929	0.376	0.335	0.95

Table 4:-Interpretation of release rate constants and R-squared values for different kinetics of the microsphere containing carbamazepine.

Formulation code	Kopcha				Hixson-Crowell cube root law		Weibull model				Baker and Lonsdale model	
	A	B	A/B	R^2	K_{HC}	R^2	α	β	R^2	T_d (Hrs)	K_{BL}	R^2
B1	24.40	0.57	42.51	0.98	0.19	0.98	3.43	0.67	0.99	6.21	0.02	0.99
B2	17.02	1.61	10.57	0.96	0.14	0.96	4.47	0.68	0.98	9.15	0.01	0.98
B3	19.40	4.11	4.72	0.94	0.21	0.96	3.51	0.78	0.99	5.00	0.02	0.99
B4	16.19	0.90	18.09	0.93	0.09	0.86	5.03	0.56	0.97	17.64	0.01	0.96
B5	15.19	3.23	4.71	0.99	0.17	0.98	4.78	0.76	0.99	7.93	0.01	0.95
B6	23.08	2.05	11.24	0.99	0.10	0.90	4.22	0.50	0.99	17.74	0.01	0.99
B7	16.35	0.14	115.14	0.98	0.10	0.94	5.47	0.58	0.99	18.91	0.01	0.99
B8	23.87	2.04	11.71	0.95	0.21	0.97	3.18	0.71	0.99	5.09	0.02	0.99
B9	27.67	1.46	18.90	0.99	0.16	0.96	3.42	0.16	0.96	8.96	0.01	0.99
B10	15.08	5.03	3.00	0.91	0.18	0.91	4.16	0.79	0.98	6.01	0.02	0.95
B11	21.00	2.08	10.10	0.88	0.18	0.91	3.53	0.63	0.96	7.30	0.01	0.99
B12	32.06	2.73	11.76	0.99	0.14	0.87	2.78	0.52	0.98	7.26	0.01	0.97
B13	20.56	0.84	24.48	0.99	0.15	0.96	4.07	0.66	0.99	8.49	0.01	0.99
Teg CR	43.29	5.99	7.23	0.99	0.17	0.83	2.08	0.48	0.98	4.59	0.02	0.95

Drug release from matrices may involve processes of diffusion, erosion, and leaching or dissolution. From Table 3 & 4 it can be seen that none of the batches followed zero order, batch B1-B3, B5, B8, B9 and B13 followed first order, Batch B1-B9, B11 and B13 followed Higuchi model, Batch B1, B5 and B8 followed Hixon Crowell cube root law, and Batch B1-B3, B5-B10, and B13 followed Korsmeyer – Peppas model [9, 10, 11].The values of release exponents (n) for batch B1-B3, B5, B7-B10, B13 was >0.45 which indicate that the drug was released from the formulations by following non-Fickian release mechanism more specifically both diffusion and erosion controlled release mechanism wheras B6 had n values <0.45 which mean the drug release followed Fickian release mechanism (diffusion controlled). As a result the formulation followed both diffusion and erosion mechanism. For further clarification the data were fitted to Kopcha mathematical model [12]. This finding was supported by evaluation of the ratios of the exponents A/B (i.e., diffusional factor A and erosional factor B) derived from the Kopcha model which were greater than 1 in all cases [13]. The data in Table 4 clearly shows that the value of A is far greater than that for B, suggesting that drug release from the microspheres was primarily controlled by a diffusion process. The plots for Kopcha kinetics can be seen in Fig. 6.

Weibull model [14] was used to determine and compare the dissolution release profiles of the formulations as shown in Table 4 and Fig. 8. Graphical representation of Log [-Ln (1 - Q_t / Q_∞)] *verses* Log time gives a linear relation where the R^2 values were 0.97-0.99 in all the formulations except B11 (R^2 = 0.96). The calculated Weibull parameter β was <1 for all the formulations, indicated a parabolic curve with steeper initial slope that was consistent with the exponential. If the β value was >1 then an S-shaped graph would be seen. The time parameter, T_d, can be calculated from α and β parameters (α = $(T_d)^β$) and represents the time interval necessary to dissolve 63.2% of the drug [14]. From the data in Table 4 the T_d value is lowest for Teg CR. Thus drug release was faster in case of the Tegretol compared to the formulation. A higher T_d value indicates slower release [15]. Highest T_d value was obtained in B7 which shows that the drug release is slowest in this formulation. This could be possible because of less amount of channeling agent (PEG) compared to the other formulations. Formulation B8, B3 and B1 shows quiet similarity with the market product i.e. fast release the possible reason could be the high concentration of PEG that enhanced drug release.

Baker Lonsdale model was used to linearize the controlled release of drug from a spherical matrix [16]. Where formulation B1, B2, B3, B6-B9, and B11-B13 had R^2 values of 0.97- 0.99 i.e. the graphical plots were linear, showed in Fig. 9, thus describing release profiles of drugs from matrices with uniform drug distribution.

Table 5:-Successive fractional dissolution time of thirteen formulations of carbamazepine microsphere and commercial product Tegretol CR tablet.

Batch code	$T_{25\%}$ (Hours)	$T_{50\%}$ (Hours)	$T_{80\%}$ (Hours)	MDT (Hours)
B1	0.99	3.87	9.78	5.11
B2	1.67	5.85	13.66	7.29
B3	1.04	3.43	7.69	4.15
B4	2.01	8.46	22.43	11.59
B5	1.57	4.89	10.56	5.77
B6	1.52	8.06	24.94	12.53
B7	2.27	9.24	23.92	12.42
B8	0.88	3.32	8.17	4.29
B9	0.93	4.18	11.56	5.92
B10	1.32	4.04	8.59	4.72
B11	1.06	4.25	10.87	5.66
B12	0.63	3.74	12.53	6.23
B13	0.69	2.69	6.76	3.54
Teg CR	0.30	2.34	9.52	4.65

From the Table 5 it is seen that the MDT is highest for formulation B4, B6 and B7 due to comparatively less amount of PEG and more amount of Methocel. The MDT is low in formulations that have high amount of PEG limiting the retarding effect.

Surface morphology study:-
The SEM images in Fig. 10 and 11 revealed the surface morphology and shape of carbamazepine microspheres prepared from Methocel K4M, PEG 4000 and Eudragit RLPO. In the two batches, B8 and B11 the amount of Methocel K4M and Eudragit RLPO were same but the amount of PEG 4000 was 582.80 mg in B8 and 300mg in B11. So changes in the morphology and shape were possibly due to the varying amount of PEG 4000.
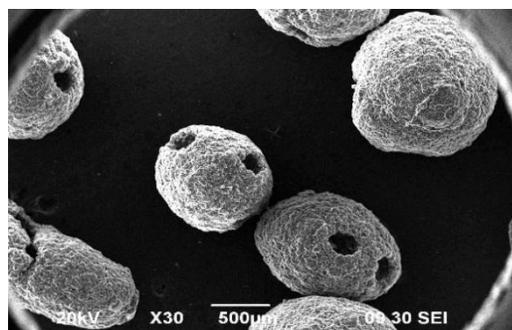
SEM image of B8 at X30 SE
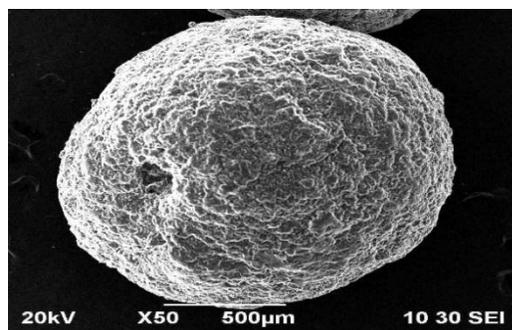
Magnification of B8 image at X50 SE
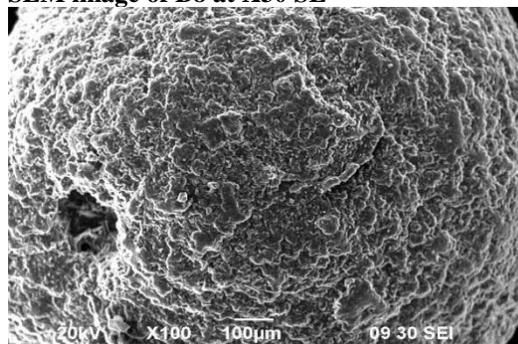
Magnification of B8 image at X100 SE
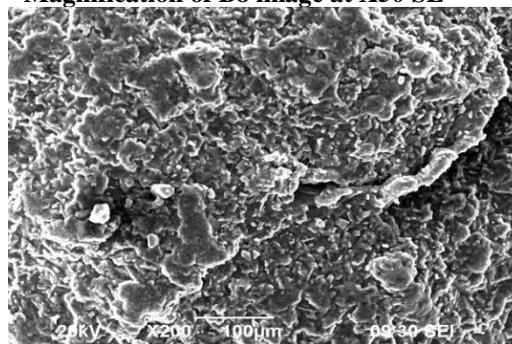
Magnification of B8 image at X200 SE

Fig. 10:-Effect of drug and polymers on shape and surface morphology of formulation B8.

Magnification of B11 image at X30 SE

Magnification of B11 image at X75 SE

Magnification of B11 image at X100 SE

Magnification of B11 image at X200 SE
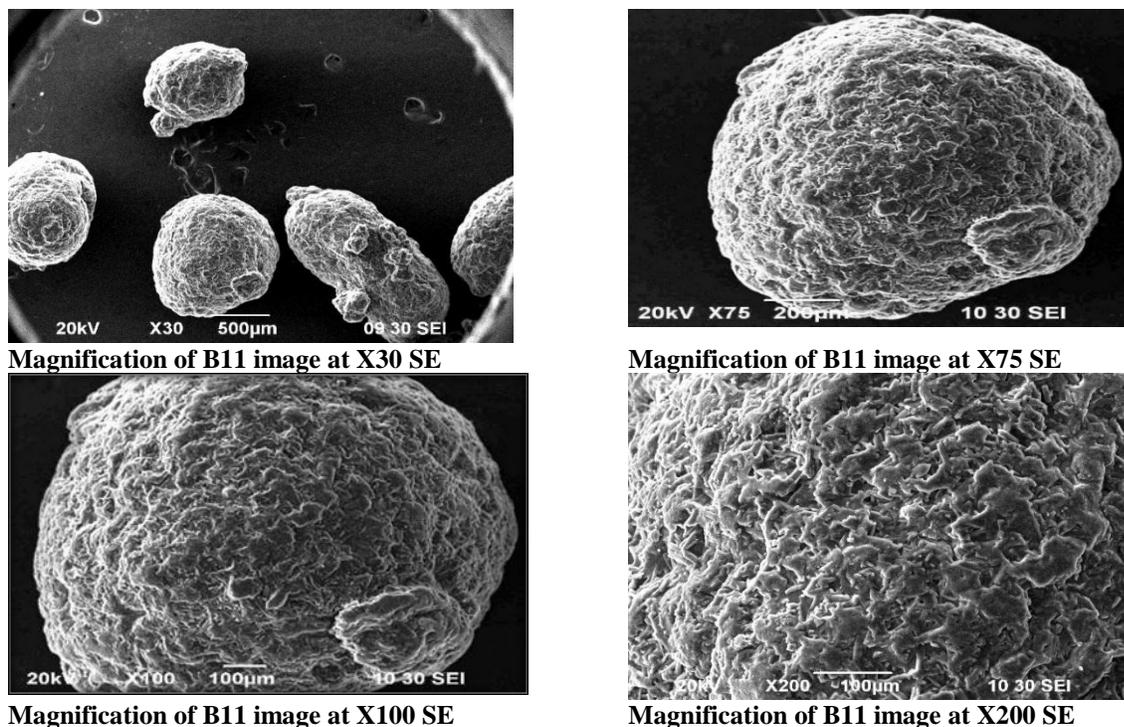
Fig. 11:-Effect of drug and polymers on shape and surface morphology of B11 microspheres.

In Formulation B8, the microspheres were almost spherical but the surface was rough with two pores on it (Fig. 10), the possible reason for this could be the high concentration of PEG 4000 (compared to B11). PEG is partially soluble in the oil phase and thus during the matrix formation the migration of PEG 4000 from the internal phase had led to the formation of pores in the microspheres [17]. Moreover the surface roughness could be due to the slow evaporation of solvent or high permeability of Eudragit RLPO [18]. In Formulation B11 as showed in Fig. 11, the microspheres were almost spherical, the surface was rough but had no holes on it and the size of the microspheres was found to be non-uniform. The possible reasons for surface roughness could be due to the slow evaporation of solvent or high permeability of Eudragit RLPO and Methocel K4M [19]. In case of rough surface there is more chance of wetting and contact of water compared to smoother ones. The uneven texture might cause holding of moisture at crests, cracks, or ridges. This persistence of moisture for more time might cause weakening of the matrix system. Presence of holes enhance drug release since these type of areas facilitate the entrance of dissolution medium to the microsphere. The presence of such holes might cause leaching of the matrix system of the particles. Hence, from the SEM images it can be concluded that the surface morphology of the microspheres vary with changes in polymer type and concentration.

Statistical analysis and model development:-
The regression parameters of the developed model and graphical interpretation for each response with statistical significance were calculated by using *IBM SPSS version 22 2013.*

Table 6:-Analysis of variance.

Dependent variables	Source	DF	SS	MS	*F*-value	*p*-value	Comment
MDT (Hours)	Model	5	98.590	19.718	5.253	0.025	Significant
	Error	7	26.274	3.743	-	-	-
	Total	12	14.864	-	-	-	-
	Lack of fit	3	21.498	7.166	6.002	0.058	Insignificant
	Pure error	4	4.776	1.194	-	-	-
	R^2= 79 %, Adjusted R^2= 69.3 %						
T$_{50\%}$ (Hours)	Model	5	50.548	10.11	9.11	0.006	Significant
	Error	7	7.71	1.10	-	-	-

	Total	12	58.26	-	-	-	-
	Lack of fit	3	6.07	2.02	4.94	0.078	Insignificant
	Pure error	4	1.64	0.41	-	-	-
	R^2= 86.8 %, Adjusted R^2= 77.3 %						
$T_{80\%}$ (Hours)	Model	5	375.74	75.15	4.58	0.036	Significant
	Error	7	114.89	16.41	-	-	-
	Total	12	490.62	-	-	-	-
	Lack of fit	3	92.83	30.94	5.61	0.065	Insignificant
	Pure error	4	22.06	5.51	-	-	-
	R^2= 76.6 %, Adjusted R^2= 59.9 %						
Swelling Index at the 8th (Hours)	Model	5	1930.32	386.06	6.57	.014	Significant
	Error	7	411.18	58.74	-	-	-
	Total	12	2341.50	-	-	-	-
	Lack of fit	3	285.84	95.28	3.04	.155	Insignificant
	Pure error	4	125.34	31.33	-	-	-
	R^2= 82.4 %, Adjusted R^2= 69.9 %						

The *F*-values were 5.253, 9.11, 4.58 and 6.57 respectively. The *p*-values were found to be 0.025, 0.006, 0.036 and 0.014 respectively and all these *p*-values are <0.05 (significance level) indicating that the overall model has significant capacity to explain variation in response variables of MDT (Hours), $T_{50\%}$ (Hours), $T_{80\%}$ (Hours) and swelling index at 8th (Hours). Insignificant lack of fit (*p*-value >0.05) also reflects the model adequacy as it can be seen from the Table 6. The R^2 values were 79%, 86.8 %, 76.6 % and 82.4 % which means the model can explain 79%, 86.8 %, 76.6 % and 82.4 % variations for MDT (Hours), $T_{50\%}$ (Hours), $T_{80\%}$ (Hours) and swelling index at 8th (Hours) respectively.

Conclusion:-
The present study was aimed to prepare sustained release microspheres for delivery of carbamazepine that will provide controlled drug release and prevent the re-crystallization of drug in GIT. The W/O emulsion solvent diffusion method was utilized in the preparation of carbamazepine microspheres. The microspheres prepared by this method were found to be of non-uniform in size and provided better controlled release profile of carbamazepine that could prevent the re-crystallization and carbamazepine dihydrate formation. The drug release from microspheres was found to be dependent on the concentration of PEG 4000 and Methocel K4M. The swelling behavior of the formulations were studied where an increase in swelling behavior was observed due to Methocel K4M which could be a good retardant. Methocel K4M and Eudragit RLPO increased the surface roughness due to their permeability characters, and PEG increased the porosity on the microsphere that offered high surface area for dissolution, which could have been the reason for enhanced drug release. Carbamazepine is a high dose drug which requires large quantity of excipient that may be the hurdle for preparation of sizeable dosage form. Further investigations are required to reduce the amount of polymer in the microspheres that can provide maximum drug loading and acceptable dosage form. Moreover, *in-vivo* behavior of the formulation should be evaluated to prove its clinical efficacy.

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