DEVELOPMENT, EVALUATION, AND OPTIMIZATION OF GELLAN GUM BASED IN SITU GEL USING 3^2 FACTORIAL DESIGNS

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ABSTRACT

Among oral dosage form, liquid dosage forms are more prone to low bioavailability because of their quick transit from the gastrointestinal tract. Sustained release liquid formulation with efficacy can be produced using approach of In situ gel. The purpose of the present work was to develop oral in situ gelling system using Gellan gum for *in situ* gelation of ambroxol-HCl. The formulation variables like concentration of polymer and calcium chloride will be optimized using factorial design. Optimized formulations were prepared having desirability and evaluated for various parameter.

KEY WORDS: Gellan gum, Sustain release, Optimization

INTRODUCTION

Oral route is the most convenient and extensively used route for drug administration having high patient acceptability; primarily due to ease of administration.[1] High dosing frequency of drug with shorter half life can be avoided by sustained release formulation.[2] The drug profile data, such as absorption properties, half life determines the dose of drug in formulation.[3] Many attempts have been made to develop sustained-release preparations with extended clinical effects and reduced dosing frequency. The present investigation concerns the development of in situ gelling system using Gellan gum[4] which after oral administration are designed to prolong release of drug, Increase the drug bioavailability, and diminish the side effects of irritating drugs.[5]

MATERIAL AND METHODS

Ambroxol hydrochloride was obtained as a gift sample from Sehat pharma, Himatnagar. All other chemicals were used of analytical grade.

Preparation of Gellan gum based in situ gel

Gellan gum solutions of different concentration were prepared by adding gellan gum to ultrapure water containing sodium citrate and different concentration of calcium chloride while stirring on a magnetic stirrer. Ambroxol-HCl and Sodium propyl paraben was then dissolved in the resulting solution. Prepared sols finally stored in amber color bottles until further use. For 3^2 factorial designs, different levels of formulation variables are selected on the bases of preliminary trials.

Optimization by using 3^2 full factorial designs[3]

In the present study, a 3^2 full factorial design was employed for formulation containing in situ gelling polymers gellan gum. Optimization is carried out by studying effect of independent variables, i.e. Concentration of in situ gelling polymer (X_1) and the concentration of calcium chloride (X_2) on dependent variables. Three factorial levels coded for low, medium, and high settings (−1, 0 and +1, respectively) were considered for three independent variables as shown in Table 1. The selected dependent variables investigated were "n" value (Y1), percentage of drug released at 30 min (Y2), 4 hours (Y3), and 8 hours (Y4) and viscosity (Y5).

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1^2 + b_{22}X_2^2$$

Where, Y is the dependent variables, b_0 is the arithmetic mean response of the nine runs, and b_1 is the estimated coefficient for the factor X_1. The main effects (X_1 and X_2) represent the average result of changing one factor at a time from its low to high value. The interaction terms (X_1X_2) show how the response changes when two factors are simultaneously changed. The polynomial terms (X_1^2 and X_2^2) are included to investigate non-linearity. Formulation of desired characteristics can be obtained by factorial design application [6]

EVALUATIONS

Physical appearance and pH

All the prepared in situ solutions of Ambroxol-HCl were checked for their clarity and the type of the solutions. The pH of each of the solution of Gellan gum based in situ solutions of Ambroxol-HCl was measured using a calibrated digital pH meter at room temperature in triplicate.

Determination of viscosity

Viscosity of the samples was determined using a Brookfield digital viscometer at ambient condition. Increasing the concentration of a Gellan gum generally gives rise to increasing viscosity (*i.e.* thickening), and also as molecular weight of a solute increases viscosity.

***In-vitro* gelling capacity**

The formulations for their *in-vitro* gelling capacity were by visual method. The *in-vitro* gelling capacity of prepared formulations was measured by placing five ml of the gelation solution (0.1N HCl, pH 1.2) in a 15 ml borosilicate glass test tube and maintained at 37±1ºC temperature. One ml of formulation solution was added with the help of pipette in gelation solution. As the solution comes in contact with gelation solution, it was immediately converted into stiff gel like structure. The gelling capacity of solution was evaluated on the basis of time period for which formed gel remains as such. The *in-vitro* gelling capacity was graded in categories on the basis of gelation time and time period for which formed gel remains.[7]

Determination of drug content

The amount of ambroxol-HCl in each unit dosage form sample was determined by U.V. spectroscopy after sufficient dilution. The UV absorbance of the sample was determined at a wavelength of 245 nm. The drug content for batches was measured in triplicate and the average values are recorded.

Compatibility study

The FT-IR spectrum of the obtained sample of the drug was compared with the standard FT-IR spectra of the pure drug. FT-IR spectroscopy was carried out to check the compatibility between drug and polymer.

***In-vitro* drug release study**

The drug release study was carried out using modified USP XXVI paddle apparatus at 37 ± 0.5 º and at 50 rpm using 900 ml of pH 1.2 buffer as a dissolution medium (n=3) as per modified paddle dissolution test. In situ gels equivalent to 60 mg of ambroxol-HCl were used for the test. 10 ml of sample solution was withdrawn at predetermined time intervals, filtered through a 0.45 µ membrane filter, dilute suitably and analyzed spectrophotometrically at 245 nm. In vitro drug release study of Ambroxol-HCl was shown in Figure 3.The values of drug release at 4 hrs & at 8 hrs for in situ gels from batches F1 to F9 are calculated and release exponent n in Korsmeyer and peppas model was calculated.[8, 9, 10]

Formulation and evaluation of optimized batches of in situ gel of both polymers

The computation for optimized formulation was carried using software, DESIGN EXPERT 7.1.6 (STAT-EASE). Optimised batches of in situ gel with desirability 1 was prepared using concentration of both independent polymers obtained by setting goals for each response to generate optimal condition of response. Optimized batch was evaluated for various parameters.

Gel integrity test of optimized batch of in situ gels

In vitro gel integrity of optimized batch was checked in simulated gastric condition. In situ gelling solution containing single dose was added in previously weighed china dish containing 0.1 N HCl. Weight of gel formed is recorded after decantation of liquid from china dish. Gel formed in china dish was added in 500 mL beaker containing 0.1 N HCl (1.2 pH). Around 100 plastic beads of 3-mm diameter were incorporated into gastric juice to mimic food particulates in human stomach. Fluid in beaker was rotated by road attached to stirrer at 30-40 rotation per minute. Integrity of gel was checked after 2 hour. Experiment was also performed in absence of polymer beads. At the end of 2 hours difference in weight of gel in both conditions was observed and gel integrity was checked.

Effect of variations in gastric pH on gelation of ambroxol-HCl in situ gelling formulations[11]

The in situ gel formed in 50 ml of gastric acid buffer (pH ranging from 1.0- 3) was used for this study. From each formulation the gel portion from the buffer was separated and the excess buffer was blotted out with a tissue paper. The weight of the gel was recorded and weight was calculated and reported for each gel formed in gastric acid buffer.

RESULT AND DISCUSSION

Appearance and pH

Clarity of all the formulations was found to be satisfactory. The pH of the formulations was found to be satisfactory as depicted in table 2 and was in the range of 6.5 -7.5. The formulations were liquid at room temperature and at the pH formulated.

In-vitro Gelation Studies

Table 2 shows the gelling capacity of all formulations and is depicted as + (gels after few minutes and dissolves rapidly), ++ (gelation immediate, remains for few hours only) and +++ (gelation immediate, remains for extended period).

Drug content

Figure 1 and Table 2 **s**hows the percent drug content for formulations. The drug content was found to be in acceptable range for all the formulations indicating uniform distribution of drug.

Viscosity

The formulation should have an optimum viscosity that will allow ease of administration as a liquid (drops), which would undergo a rapid sol-to-gel transition. Table 1 also shows the viscosity (cp) of formulations from G1 to G9. The viscosity increased in proportion with gelling agent. This may be attributed to the higher viscosity of Gellan gum.

Compatibility study

The IR spectrum of the pure Ambroxol Hydrochloride sample was recorded by FTIR spectrometer. Preformulation studies were carried out to study the compatibility of pure drug Ambroxol-HCl with the polymer Gellan gum and other excipients. The individual IR spectra of the pure drug and combination with polymers are shown in the Figure 2-3.

Experimental Designing

The factorial design was carried out using the software DESIGN EXPERT® version 7.0.2.8 (Stat-Ease Inc., Minneapolis, USA). A quadratic model was obtained after analyzing data. Values of $p<0.05$ indicate model terms are significant. The statistical model comprising incorporated interactive and polynomial terms was utilized to evaluate the response. Once the uncoded values of factor levels were applied and response quadratic model was performed using DESIGN EXPERT® version 7.0.2, equations were obtained. Values of coefficients are mentioned in table 5. The resulted equations for all five dependent variables—Y1 (Viscosity), Y2 (n), Y3 (X30 min), Y4 (X4 hrs), and Y5 (X8 hrs)—in terms of coded factors are presented below.

Formulation of optimized batches of in situ gel of both polymers

In optimization overlay graph highlights area of operability as shown in figure 5. Desirability 1.0 indicated optimum formulation of Gellan gum based in situ gel was prepared using 0.5% Gellan gum and 0.02% Calcium chloride. Formulation was evaluated for various parameters as shown in table 4.

Effect of variations in gastric pH on gelation of Ambroxol HCl in situ gelling formulations[11]

Gelation of In situ gelling of optimized formulation of both polymers was checked in gastric buffer with different pH in range of 1-2.5. Gelation of in situ gelling system was checked in pH 2.5 gastric buffer containing Calcium simulating In vivo condition. Weight of gel formed in different pH was recorded in table 5. Results show weak gelation with increase in pH of gastric buffer, but gelation at pH 2.5 in presence of calcium simulated to in vivo condition shows good gelation.

Gel integrity study of gellan gum based in situ gelling system

In situ gel formed in stomach is subjected to both hydrodynamic mixing and

mechanical force. Beads impact to in situ gel producing mechanical force. Hydrodyanamic pressure was created by rotation of gastric fluid. It was observed as shown in Table 6 that Presence of polymer beads does not show significant attrition effect by hydrodyanamic or mechanical force acting on gel that shows integrity of gel in Gastrointestinal tract.

CONCLUSION

Gellan gum based in situ gelling system undergoes sol-gel transition under influence of acidic pH in presence of calcium ion. Sodium citrate helps maintain fluidity before administration by complexation of calcium chloride which becomes free at acidic pH. Very little concentration of divalent cation was required for formulation.

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Table 1: Factorial design of Gellan gum

Batch No.	Variables levels in coded form		Value of n	Viscosity (cp)	% Drug release At 30 minutes	% Drug release At 4 hrs	% Drug release At 8 hrs
	X_1	X_2					
G1	-1	-1	0.289	124	52.4	97.02	97.02
G2	-1	0	0.279	142	44.8	83.29	95.8
G3	-1	+1	0.327	176	38.8	75.8	97.3
G4	0	-1	0.369	258	32.5	68.72	96.09
G5	0	0	0.495	272	20.2	56.8	92.4
G6	0	+1	0.473	292	18.9	50.86	83.2
G7	+1	-1	0.292	308	32.2	60.88	96.4
G8	+1	0	0.477	339	18.3	47.84	81.11
G9	+1	+1	0.465	380	18.2	44.79	76.23

Translation of coded levels in actual units

Variables level	Low (-1)	Medium (0)	High (+1)
Concentration of Gellgum (X_1)	0.25 %	0.5 %	1.0 %
Concentration of Calcium chloride (X_2)	0.01 %	0.02 %	0.03 %

Note: All the batches contained the constant amount of drug as 60 mg/10 ml, viscosity measured at 120 rpm and having the same pH 7.0 $\pm$ 0.3.

Table 2: Evaluation parameters of Gellan gum in situ gel formulation

Formulation	pH	Drug content (%)	In vitro gelation
G1	7.1	97.02	+
G2	7.3	94.85	+
G3	6.8	96.78	++
G4	6.9	95.92	+
G5	7.1	97.78	+++
G6	7.0	97.64	+++
G7	7.2	96.22	+
G8	6.9	97.95	+++
G9	6.9	95.75	+++

(+: poor, ++: good, +++: excellent)

Table 3: polynomal equation of dependant variable

Dependant variable	Polynomal equation
n	$0.46 + 0.043X_1+0.049X2+ 0.029X_1X_2 -0.084X_1^2 -0.041X_2^2$
Viscosity	$270.44+ 97.50X1+ 26.33\ X2 + 5.0X_1X_2-29.17X1^2 +5.33X_2^2$
Drug release in ½ hour	$20.93 -11.22X_1-6.87X_2 -0.10X_1X_2+ 10.25\ X_1^2 +4.40\ X_2^2$
Drug release in 4 hour	$56.33 -17.10X_1-9.19X_2 -1.28\ X_1X_2 +9.48\ X_1^2 +3.71X_2^2$
Drug release in 8 hour	$89.72 -6.06X_1 -5.46X_2 -5.11X_1X_2 +0.080X_1^2+1.27X_2^2$

Table 4: Evaluation parameters for optimized batch

parameters	**Result**
Drug content (%)	97.2
Release exponent (n)	0.502 (R^2 = 0.994)
Viscosity (CP)	272
Drug release in 4 hours (%)	56.8
Drug release in 8 hours (%)	92.4

Table 5: Effect of gastric pH on gelation of optimized batch of gellan gum

pH	**Weight of gel formed in different gastric pH buffer (gm)**
1.0	12.3
1.5	12.1
2.0	10.5
2.5	4.6
3.0	2.5
2.5* (pH 2.5 with presence of calcium)	10.8

Table 6: Gel integrity test of optimized formulation of gellan gum

Batch	**Trial**	**Initial weight of gel (Gm)**	**Weight of gel after 2 hour (Gm)**	**% weight loss**
Gellan gum based in situ gel	With poymer beads	6.5	5.5	15.38
	Without Polymer beads	6.2	5.5	11.29

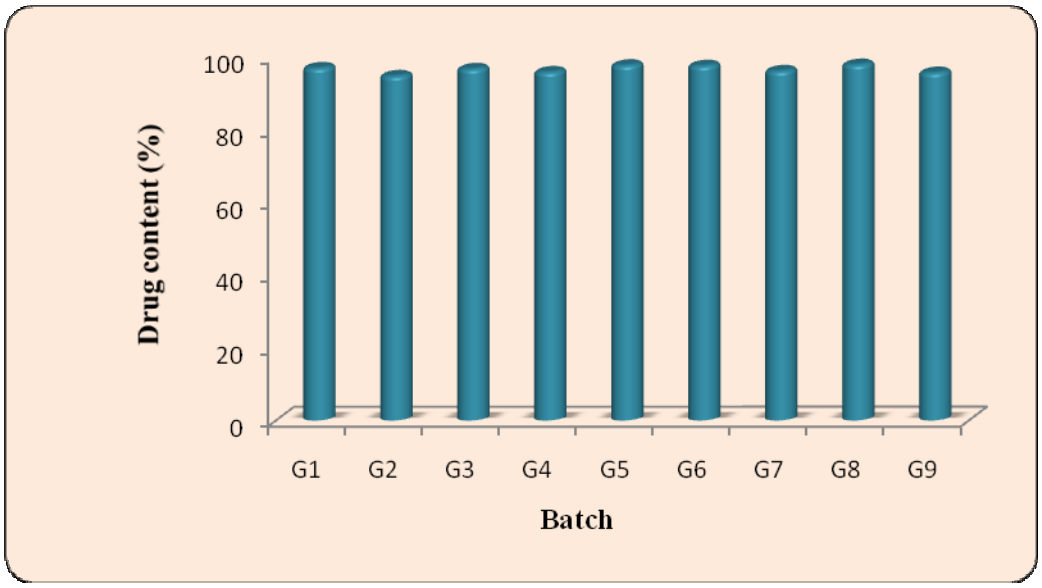

Figure 1: Drug content of Gellan gum based in situ gels batch F1-F9

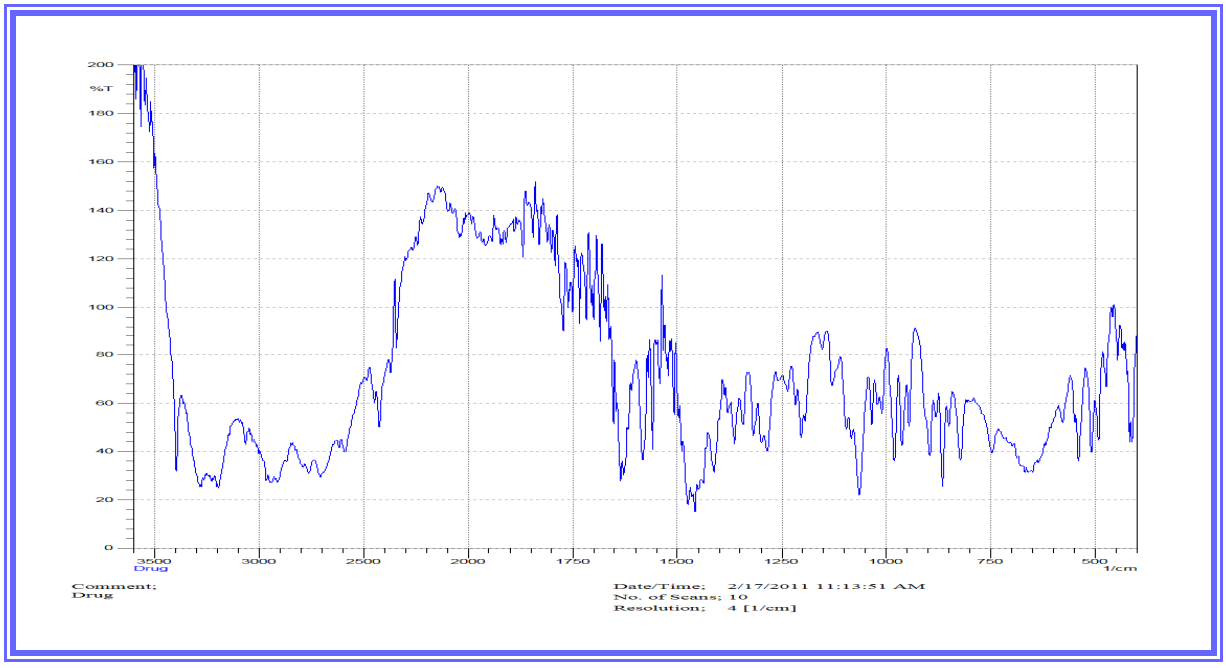

Figure 2: FTIR of drug

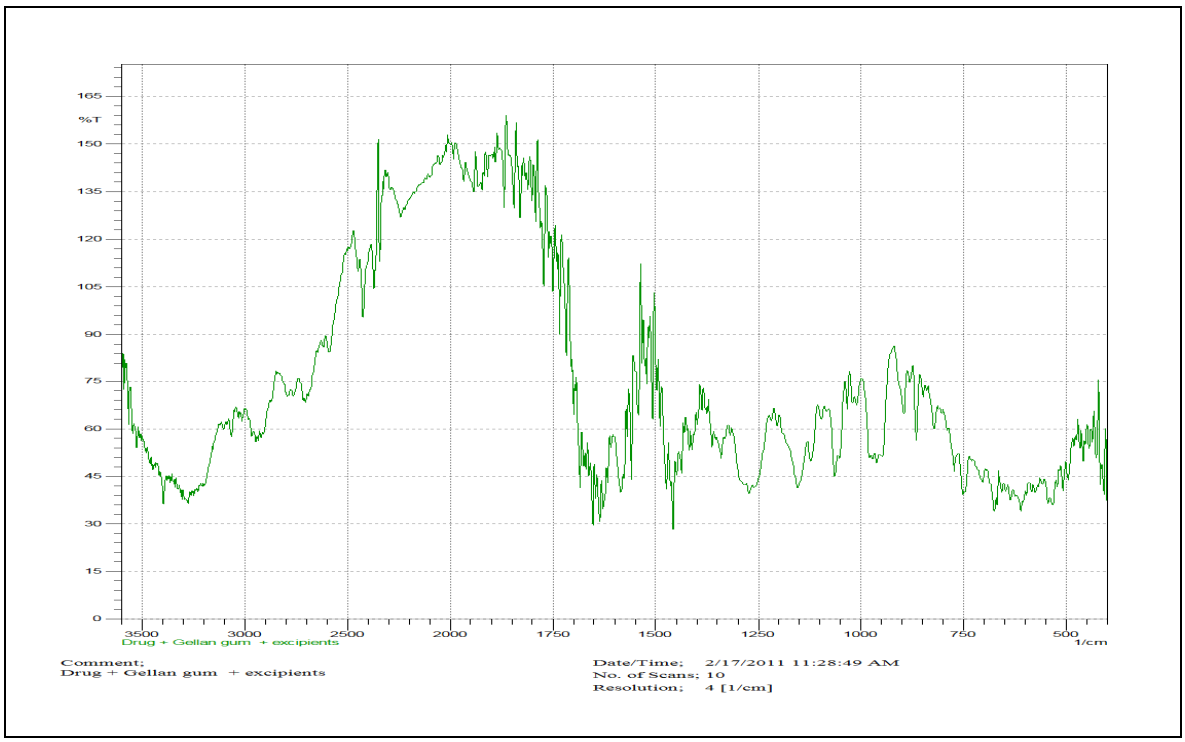

Figure 3: FTIR of drug + polymer + excipient

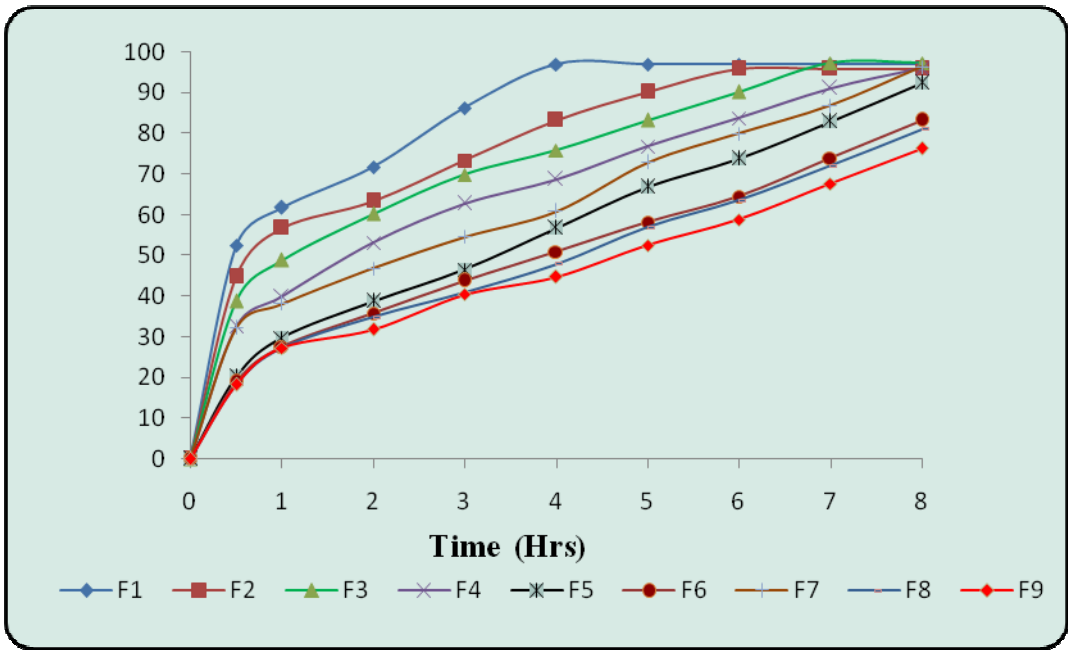

Figure 4: In vitro drug release study of gellan gum based in situ gelling system

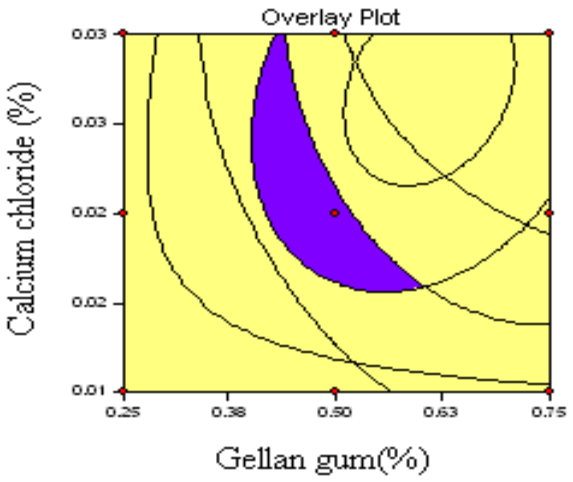

Figure 5: Overlay graph showing operability