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# Physico-chemical characterization of *Rasagarbha Pottali* - An Approach to Standardization

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## ABSTRACT

In *Rasashastra* among the four *Kalpa*'s, *Pottali Rasakalpas* have unique place in *Ayurvedic* therapeutics because of their qualities, very compact in structure and quick acting due to its high potency. Here a research work is carried out on *Rasagarbha Pottali* by molten sulphur method for about 6hrs *Paka* and analysed with classical and modern parameters. Similarly, an attempt was also made to differentiate the structure of the drug before *Paka* (RGPK) and after *Paka* (RGP). Where particle size found to be in RGP is 475.3nm. In SEM-EDAX there is presence of C, Au, Hg, S and Si. XRD showed HgS and Au<sub>2</sub>S compounds with cubic in structure. FTIR proved that it has Organic Compounds with functional groups like Alkyl halides, Aromatics, Alkenes, Amines. Along with these other physico-chemical tests, NPST were carried out to show the standard values of final product. Overall, a remarkable difference was observed in the samples RGPK and RGP.

**Key words:** *Rasagarbha Pottali*, Physico-chemical parameters, XRD, SEM-EDAX, FTIR, NPST.

## INTRODUCTION

In *Rasashastra*, *Pottali Rasayana* is superior, innovative in the method of preparation, administration and has showed its remarkable excellence in unique dosage than other *Rasakalpas*.

As per *Rasayoga Sagara*, there are mainly four types of *Rasagarbha Pottali*<sup>[1]</sup> explained. Among them one is selected for preparation, which is a *Sagandha*, *Sagni*, *Bahirdhooma*, *Gandhaka Jarita*, *Pottali Kalpana*. It consist *Hingulotha Parada* of 1 *Karsha*, *Gandhaka* of 1 *Tanka*, *Swarnatantu Khanda* of 6 *Rattika* and *Kajjali* of 4 *Pala* separately. Here

*Gandhaka Drava Paka* method has been carried out and analysed for *Pottali Siddhi Lakshana* on ancient and modern Parameters. 6hrs *Paka Kala* was needed for completion of process, where pilot study followed by 3 main study carried out which is cited at *Sapna et.al*. After obtaining all the *Siddhi Lakshana*, it was analysed for Physico-chemical analysis and all through instrumental techniques to check genuinity and aiding towards the standardization of the final product RGP.

## OBJECTIVES OF THE STUDY

To carry out analytical study of *Rasagarbha Pottali Kajjali* (RGPK) and *Rasagarbha Pottali* (RGP).

## MATERIALS AND METHODS

### Materials

Classical organoleptic parameters were done in TGAMC Ballari and other Qualitative and Quantitative chemical tests, Estimation of Mercury, Sulphur and Gold of RGPK and RGP were done at Quality Control Lab, A.L.N. Rao Ayurvedic Medical College, Koppa.

X-ray diffraction was done at MIT Manipal and SEM-EDX, Particle size, FTIR at IISc, Bangalore.

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### Preparation of Rasagarbha Pottali

Firstly *Parada* is triturated with *Shuddha Swarna Tanutantu Khanda* till *Dhatu Pisti* is formed, then *Shuddha Gandhaka* is added to the above compound, then again trituration is carried out till *Kajjali Lakshana* appears. The *Samaguna Kajjali* of required quantity is prepared separately and added to the above *Dhatugata Kajjali*, trituration is continued till *Kajjali Lakshana* appears, then it is subjected to *Bhavana* with *Kumari Swarasa* for 7 times and made into *Shikarakara* after drying this *Pottali* will be kept in centre of four folded silk cloth. Then *Pottali* is subjected to *Gandhaka Drava Paka* where the entire procedure was carried out for 6hrs with *Mridu Agni* is maintained i.e. 150°- 250°C, till *Pottali Siddhi Lakshanas* appears. The pilot and main study was carried out the details of this can be cited at sapna.et.al.<sup>[2]</sup>

The *Pottali* before *Paka* RGPK and after *Paka* RGP has been analysed on classical and modern parameters, Physico-chemical parameters, Quantitative and Qualitative modern parameters like XRD, SEM-EDAX, FTIR and NPST.

## OBSERVATIONS AND RESULTS

### Analysis using classical parameters

*Pottali Siddhi Lakshana* like *Vyoma Varna* of *Gandhaka*,<sup>[3]</sup> metallic sound when banged against *bhanda* with burning of silk cloth. Different views are mentioned in *Rasa Shastra* texts regarding the duration of *Paka* of *Pottali*. They are *Yamardha*, *Yamaka*, *Chiram*, *Dwighatika* or up to the attainment of *Vyomavarna* of *Gandhaka*, considering all these and from pilot study it was concluded that 6hr is needed to attain all these *Siddhi Lakshana*.<sup>[2]</sup> Other classical parameters like *Rekha poornata*, *Varitara*, *nischandra* for RGPK and RGP is tabulated in Table 1.

**Table 1: Classical Parameters for Analysis of RGP**

Test	RGPK	RGP
<i>Rekha Purnatva</i>	When fine powder of RGP <i>Kajjali</i> was rubbed between the thumb and	When fine powder of R.G.P was rubbed between the thumb and index finger it entered the furrows

	index finger it entered the furrows of the fingers.	of the fingers.
<i>Varitaratva</i>	When finely powdered RGP <i>Kajjali</i> was carefully Sprinkled into a test tube containing water, <i>Kajjali</i> was floating on the surface of water.	When finely powdered R.G.P. was carefully Sprinkled into test tube containing water, it was floating on the surface of water.
<i>Nischandratva</i>	Lustre less i.e., No shining particles was observed.	There was no shining particle in the finely powdered R.G.P. even when it was rubbed between thumb and index finger and made wet, observed in the bright Sunlight.

### Namboori Phased Spot Test (NPST)<sup>[4]</sup>

In Namburi Phased Spot test sensitivity of reactions at different time intervals is measured unlike the chromatography of chemistry.

The continuous chemical reactions taking place gradually between 2 chemical substances on a static media at a fraction of second are easily detected by their distinct colour changes and the pattern of spot which is specific to each rasa formulation.

**Table 2: Observations during NPST of RGPK and RGP**

Sample	I Phase (0-5min)	II Phase (5-20)	III Phase (24hrs)
RGPK	Immediate central brick red spot and red periphery developed. Central ring started fading with brown ring	Periphery is on large area.	Fading of Central spot with

	in periphery appeared.		light brown margin.
RGP	Immediate central brown spot and red periphery developed. Central brown ring started fading with small brown ring.	Central red spot with dark brown periphery.	Fading of Central spot with light brown margin.



Fig. 3: RGPK- Phase III

Namboori Phased Spot Test



Fig. 1: RGPK- Phase I



Fig. 4: RGP- Phase I



Fig. 2: RGPK- Phase II



Fig. 5: RGP- Phase II



Fig. 6: RGP- Phase III

**ANALYTICAL STUDY<sup>[5]</sup>**

**Organoleptic characteristics**

The specific characters that are mentioned in our classics for evaluating the qualities of preparation by color, touch, fineness, taste, odor, etc. were noted through *Gnyanendriya* in all the samples.

**Table 3: Organoleptic of RGPK and RGP.**

Parameters	RGPK	RGP
Color	Black	Black
Taste	Tasteless	Tasteless
Odor	Characteristic	Characteristic
Touch	Amorphous	Amorphous

**Physico-chemical Parameters<sup>[6]</sup>**

The physical tests are carried out as per pharmacopeial standard techniques which helps measuring the physical property that is characteristically related to the component. Then the convectional chemical analysis was carried out by Volumetric and Gravimetric method analysis.

**Table 4: Physico-Chemical characters of RGPK and RGP.**

Parameters	RGPK	RGP
Total Ash	15.00%	8.55%
Acid insoluble ash	1.00%	0.50%
Water soluble ash	1.00%	0.50%

Loss on drying	0.00%	0.00%
pH	5.04 ±0.10	5.84 ± 0.10
Mercury	15.00%	21.25%
Sulphur	9.35%	15.05%
Gold	0.15%	0.12%

**Instrumental methods of analysis**

**X-Ray diffraction analysis<sup>[7]</sup>**

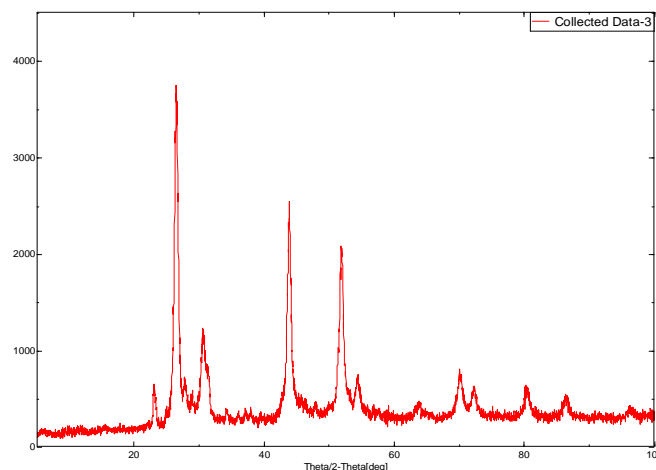
XRD method determines the arrangement of atoms within a crystal with the help of x-rays. On analysis XRD peaks in this study were identified to be as HgS and Au<sub>2</sub>S compounds with both cubic in structure.

**RGPK:** Totally 14 peaks were identified in RGPK at different angles (2θ) from 23.072 to 96.09. In that 6 strong peaks in HgS and 5 peaks Au<sub>2</sub>S were matching with (2θ) angles when compared to standard X – ray powder diffraction file.

**Table 5: XRD Results of RGPK and RGP**

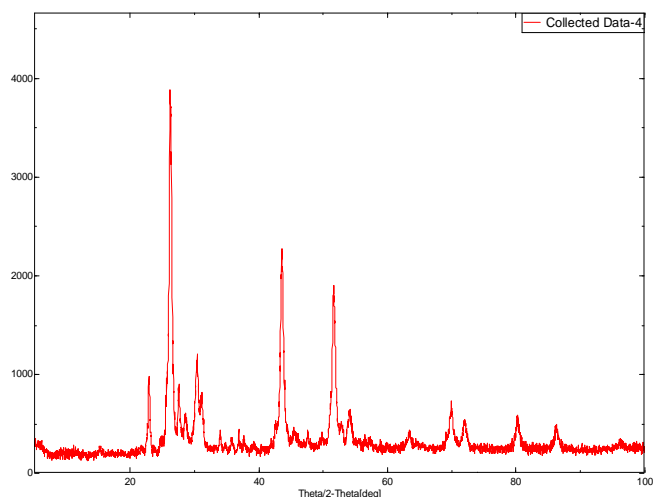
Sample	Compound Name	Chemical Formula	Crystal Structure
RGPK	Metacinnabar	HgS	Cubic
	Gold Sulphide	Au <sub>2</sub> S	Cubic
RGP	Metacinnabar	HgS	Cubic
	Gold Sulphide	Au <sub>2</sub> S	Cubic

**Fig. 7: XRD Graph- RGPK**



**RGP:** Totally 20 peaks were identified in RGP at different angles ( $2\theta$ ) from 22.86 to 96.34. In that strong 9 peaks of HgS and 5 peaks Au<sub>2</sub>S were matching with ( $2\theta$ ) angles when compared to standard X – ray powder diffraction file.

**Fig. 8: XRD Graph-RGP**



**SEM-EDAX:**<sup>[8],[9]</sup> SEM-EDAX of both samples were carried out and elements present in those is tabulated in Table 6.

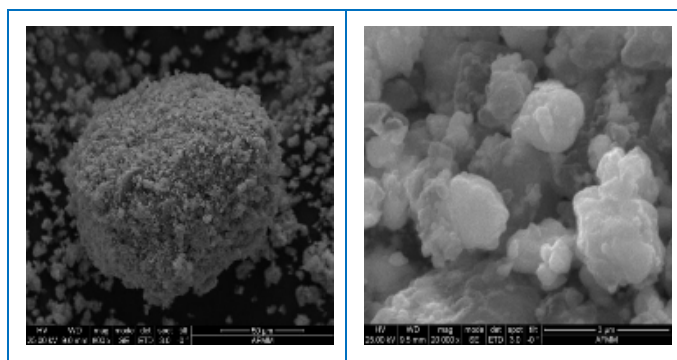
**Table 6: SEM-EDX of RGPK**

Element	Weight%	Atomic %
C	5.52	31.42
Si	0.58	1.43
S	19.57	41.76
Au	5.47	1.90
Hg	68.86	23.49

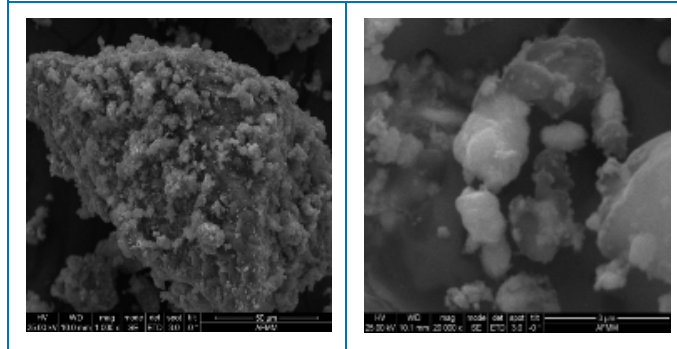
**Table 7: SEM-EDX of RGP**

Element	Weight%	Atomic%
C	5.68	24.26
Si	0.39	0.71
S	37.40	59.82
K	0.68	0.89

Au	6.34	1.65
Hg	49.51	12.66

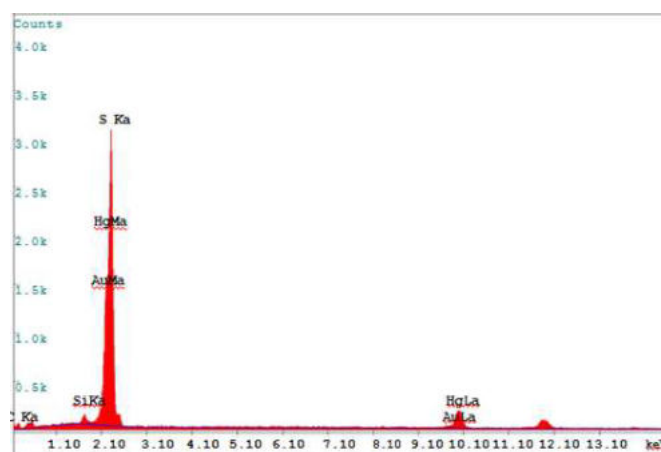


**Fig. 9: RGPK -SEM-EDX (A and B)**



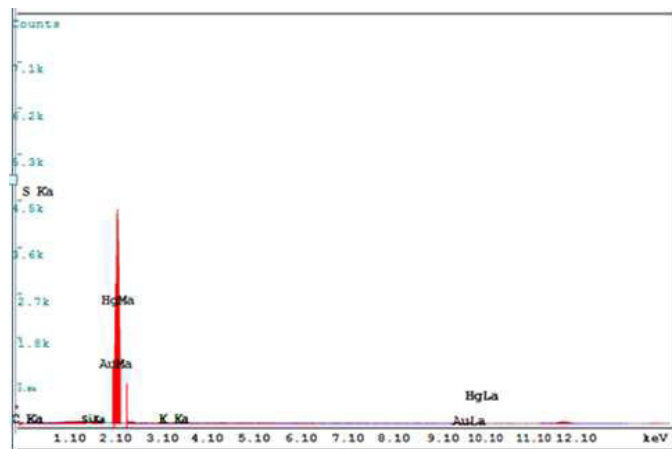
**Fig. 10: RGP-SEM-EDAX(A and B)**

**Fig. 11: RGPK- SEM-EDAX Graph**



**Particle Size:**<sup>[10]</sup> Particle size determination by ZETA PALS, Particles with diameters from 10nm to 30µm (depending on particle density) can be measured. Mean particle size of RGPK and RGP found to be 479.6 nm and 475.3nm respectively.

Fig. 12: RGP-SEM-EDAX Graph



**FTIR:**<sup>[11]</sup> FTIR helps in identifying organic functional groups, unknown materials, quality or consistency of a sample and amount of components in a mixture.

Table 8: Showing FTIR Peaks of RGPK

Sample - I (RGPK)				
Sample peaks Cm <sup>-1</sup>	Standard Peaks Cm <sup>-1</sup>	Bond	Specific type of bond	Functional groups
782.99	850-550	C-Cl stretch	Medium	Alkyl halides
	900-675	C-H "oop"	Strong	Aromatics
	1000-650	=C-H bend	Strong	Alkenes
	910-665	N-H wag	strong, broad	1°, 2° amines
1401.32	1500 - 1400	C-C Stretch( in ring )	Medium	Aromatics
1601.12	1650 - 1580	N-H bend	Medium	1° Amines
2808.37	2830-2695	H-C=O : C-H Stretch	Medium	Aldehydes

Table 9: Showing FTIR Peaks of RGP

Sample - I (RGP)				
Sample peaks Cm <sup>-1</sup>	Standard Peaks Cm <sup>-1</sup>	Bond	Specific type of bond	Functional groups
	850-550	C-Cl stretch	Medium	Alkyl halides

708.95	900-675	C-H "oop"	Strong	Aromatics
	1000-650	=C-H bend	Strong	Alkenes
	910-665	N-H wag	strong, broad	1°, 2° amines
1584.72	1650-1580	N-H bend	Medium	1° Amines
3682.78	3700 - 3500	N-H Stretch	Medium	Amides

Fig. 13. Graph of FTIR -RGPK

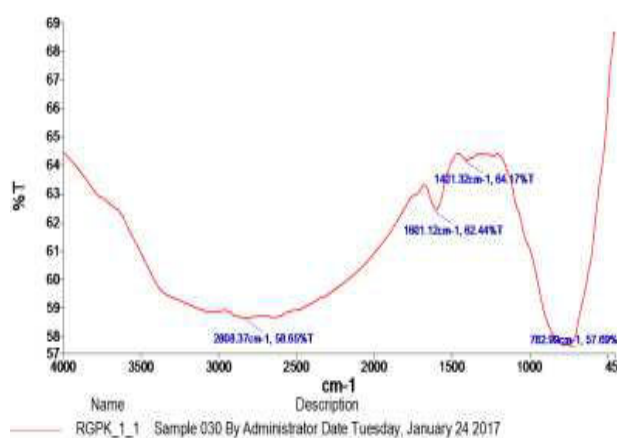
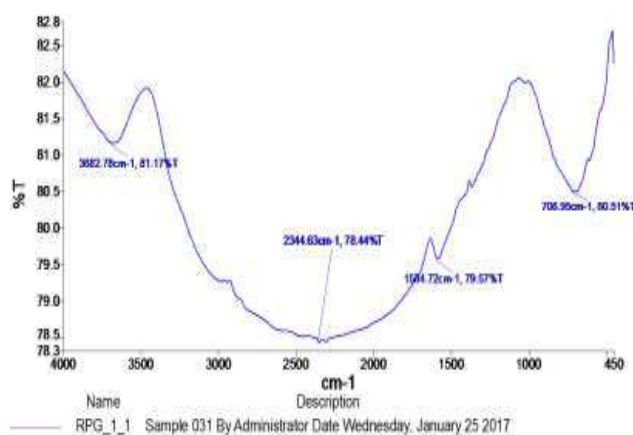


Fig. 14. Graph of FTIR -RGP



**DISCUSSION**

The *Gandhaka Drava Paka* used here has brought up a desired changes in the *Pottali* like critical analysis can be done in different steps.

**Paka Lakshana:** As per the colour of the *Gandhaka* the *Paka* of *Pottali* was considered perfect or complete when the color of the sulphur becomes *Vyoma Varna* or *Neelashyama* (Bluish black).

Upon heating, Sulphur melts to provide a yellow liquid that flows readily. As the temperature increases the color of Sulphur changes to red and eventually darkens further. The color is caused by the presence of a small amount of red S<sub>3</sub> and S<sub>4</sub> molecules. At about 160-195°C the color of Sulphur becomes dark red and its viscosity sharply increases. The reason for such a viscosity is that Sulphur rings open and combine to form long polymeric chains with more than 500,000-800,000 Sulphur atoms per chain.

In this preparation Sulphur may react with organic matters present in the silk cloth as well as in *Pottali* to form sulphur associated functional group, which may later added to compound formation of *Pottali* with functional group formation for maximum bioavailability. Sulphonation is an example of electrophilic substitution in which the electrophile is a sulphur trioxide molecule, SO<sub>3</sub>. Here *Vyoma Varna* of *Gandhaka* at 6<sup>th</sup> hr showed completion of *Paka*.

**Metallic Sound:** Metallic sound produced by the *Pottali* when banged against the container. This may be due to long polymerization of Sulphur to give hardness to *Pottali*. Along with components present in the *Pottali* and adding fibers of silk cloth. When RGP banged against mud pot and iron rod it showed a typical metallic sound.

**Burning of cloth:** During the processing the cloth containing *Pottali* was burnt, this sign was considered as one of the *Paka Lakshanas* of *Pottali*. Silk cloth was burnt due to prolong maintenance of heat and varied viscosity of sulphur and constant heat through sulphur media.

After *Gandhaka Paka*, there was gain in weight of RGP; this is because adhered Sulphur to *Pottali*.

**Organoleptic charaters:** The black colour is due to as major percentage of *Kajjali* in final product. *Varitara*, *Rekhapoornata*, *Nishchandrata* showed reduced particle size and lustreless after long duration of trituration of *Kajjali* with other ingredients.

#### Analysis of Physical Standards

On observing the values of the pH of both RGPK and RGP are weak acidic in nature.

According to pH- partition co-efficient concept, Favourable area of absorption for pH of 2-5 is stomach, 7-8.5 is Intestine and for pH ranging between 5-7 in whole of GI Tract. So RGPK and RGP being weak acidic of pH 5.84 is best absorbed throughout the Gastro Intestinal tract.

Total Ash value helps to know about inorganic constituents in the test drug. Organic substances are burned in the presence of the oxygen. Most of minerals are converted to oxides, sulphates, phosphates, chlorides or silicates. Here in RGPK and RGP lesser values of ash indicating presence of less Impurity

**Acid Insoluble Ash Value:** The human metabolic process and pharmacokinetics depends upon purity and human acceptable forms of drug. When RGPK and RGP is treated with Hydrochloric acid, 1% and 0.50% of acid insoluble ash was detected. Which signifies the genuinity of the product and suggests it is best in terms of solubility and absorption. Means 99.5% of bio-active drug components is soluble.

**Water soluble ash value:** Water soluble ash indicates selective media of drug administration. Water soluble ash value of RGPK and RGP is 1% and 0.50%. Here the salivary secretions, gastric enzymes play an important role in its dissolution.

**Loss on drying:** RGPK and RGP-0.00%. LOD at 110°C. Hence it can be stated that these samples have devoid of moisture content. Concurrently it can be stated that the shelf life of the drug will be more.

#### Chemical Parameters

**Mercury values:** The percentage of Mercury in RGPK is 15% and in RGP is 21.25%. As the product contains more amount of mercury in proportion and less amount of sulphur as leading to increase in mercury percentage with no bond and compound formation.

**Sulphur values:** The percentage of Sulphur in RGPK is 9.35% and 15.05% in RGP Increase of Sulfur in RGP may be due to *Gandhaka Drava Paka*.

**Gold values:** The percentage of Gold in RGPK is 0.15% and 0.12% in RGP



**SEM-EDAX:** Change in the percentage of elements in RGPK and RGP in both chemical analysis and SEM-EDAX may be due to the heat treatment, which causes breaking of bond and the formation of new bond with the evaporation of certain gases like SO<sub>2</sub> resulting in the increase and decrease of other elements.

**XRD:** Both the samples showed same structure as cubic with same compounds HgS and Au<sub>2</sub>S.

**Particle Size:** The particle size has an important influence on dissolution rate. Smaller the drug particle size larger the surface area, leads to faster dissolution. Nanoparticle size of RGPK and RGP will result in precise drug delivery and thereby increasing the bio availability of the drug in both the samples.

**FTIR:** RGPK and RGP Showed presence of Organic Compounds with functional groups. Amines which are the main functional group of all proteins might be present due to *Godugdha* and *Kumari* which are rich sources of proteins. Aldehydes are the functional groups of carbohydrates. Monosaccharides and polysaccharides present richly in *Kumari*.

**NPST:** The colour change is observed across 3 different phases of RGPK and RGP on potassium iodide treat papers where characteristic and corresponded to the standard description. N.P.S.T findings of 2 these samples are newly explored and can form in-house referral standards.

## CONCLUSION

*Rasagarbha Pottali* is black colored, tasteless, amorphous touch and with characteristic odour. All relevant analytical data of samples of RGPK and RGP have less difference in their physical and chemical values. RGP with nano particle size of 475.3nm reveal the drug is easily transported in to cells and nuclei and specificity to the target can be achieved as desired. XRD showed HgS and Au<sub>2</sub>S compounds with cubic structure in both samples. SEM EDAX proved the presence of C, Si, Au, S, K, Hg elements in RGP. In FTIR of RGP presence of Alkyl halides, Aromatics, Alkenes,

Primary and secondary amines, amides functional groups.

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